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Lycium barbarum Fruit (Goji) Attenuates the Adrenal Steroid Response to an Exercise Challenge and the Feeling of Tiredness: A Randomized, Double-blind, Placebo-controlled Human Clinical Study

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Abstract

We examined the effects of *Lycium barbarum* fruit (goji) intake on general well-being in a randomized, double-blind, placebo-controlled 30-day intervention trial. Plasma levels of cortisol, dehydroepiandrosterone (DHEA), glucose, urea nitrogen (BUN) and lactic acid followed by an exercise challenge were assessed at the pre- and post-intervention. Relative to the placebo group (n=19), tiredness and overall health were significantly improved in the *Lycium barbarum* group (n=20). Cortisol, DHEA and lactic acid levels were significantly increased by the exercise for the pre-intervention: However, at the post-intervention, *Lycium barbarum* intake significantly attenuated cortisol and DHEA levels. Lactic acid levels were comparable for both groups, and glucose and BUN levels were not altered. These results show that *Lycium barbarum* consumption attenuates the adrenal steroid response and reduces the feeling of tiredness.

Keywords: *Lycium barbarum*, Goji, Exercise, Cortisol, Dehydroepiandrosterone, Lactic acid, Tiredness, General Well-being

1. Introduction

Our previous randomized, double-blind, placebo-controlled human clinical studies showed that daily consumption of *Lycium barbarum*, in the form of fruit juice, GoChi®, significantly increased subjective feelings of general well-being and reduced fatigue (Amagase *et al*., 2008). *Lycium barbarum* consumption significantly enhanced *in vivo* immune functions as indicated by increased number of lymphocytes and blood concentrations of immunoglobulin G and interleukin (IL)-2 (Amagase *et al*., 2009a). *In vivo* anti-oxidant effects of *Lycium barbarum* include a significant increase in blood concentrations of superoxide dismutase and glutathione peroxidase and a significant reduction in lipid peroxidation (malondialdehyde) (Amagase *et al*., 2009b). *Lycium barbarum* intake has also been shown to increase metabolic rate/energy expenditure in a dose-dependent manner (Amagase *et al*., 2011b). *Lycium barbarum* juice used in these studies is standardized for its main active constituents, *Lycium barbarum* polysaccharide (LBP). *Lycium barbarum* is a Solanaceous defoliated shrubbery and has been a commonly prescribed traditional medicine in Asian countries for over 2,500 years (Amagase *et al*., 2008, 2011a; Bensky *et al*., 1993; Chang *et al*., 2001, 2008). Additional effects of *Lycium barbarum* include improved endurance, anti-agng, neuroprotection, anti-diabetic, anti-glaucoma, anti-tumor activity and cytoprotection have been reported (Amagase *et al*., 2011a).

Adrenal steroids, such as cortisol and prohormone, dehydroepiandrosterone (DHEA), regulate a variety of

cardiovascular, metabolic, immunologic and homeostatic functions (American Heart Association, 2009; Buford *et al*., 2008; Walker, 2007). These "stress hormones" are released in response to a variety of physical, metabolic and psychological stressors. DHEA is produced primarily in the adrenal glands and is released along with cortisol in response to stress (Dillon, 2005). Exercise is a potent stimulus for cortisol release and exercise increases DHEA production (Filaire *et al*., 1998; Tissandier *et al*., 2001; Copeland *et al*., 2002). Thus, measurement of DHEA and cortisol levels in response to an exercise challenge may provide an index of the physiological effects of *Lycium barbarum* on the response to a physical stressor.

To extend the analysis of the physiological actions of *Lycium barbarum* and to investigate possible mechanisms of action as the first step, we examined the effects of *Lycium barbarum* intake on exercise-induced adrenal steroid release as well as lactic acid, glucose and blood urea nitrogen (BUN) concentrations in plasma at pre- and post-30-day-intervention trial under a randomized, double-blind, placebo-controlled manner.

2. Materials and Methods

2.1 Lycium barbarum and placebo preparation

FreeLife International Inc, located in Phoenix, Arizona, supplied a commercially available, LBP-standardized *Lycium barbarum* fruit (goji) juice (GoChi; Lot No. ASA07351) which was produced from fresh ripe *Lycium barbarum* fruit. Description and standardization procedures of the test material were previously described (Amagase *et al*., 2008). In brief, the yield of juice as a percentage weight of the starting fresh plant material is approximately 35%. The juice was processed in an aseptic manner and kept refrigerated before use at 2 to 8 ºC. GoChi is standardized to contain a content of LBP equivalent to that found in at least 150 g of fresh fruit in 120 ml, the amount customarily consumed in traditional Chinese medicine (Yu *et al*., 2007; Amagase *et al*., 2011a). Based on our previous dose-seeking study on energy expenditure (Amagase *et al*., 2011b) and other various studies (Amagase *et al*., 2008, 2009a, 2009b), we used 120 ml of GoChi in the present studies as an established dose.

Placebo control material (Lot No. A198) was carefully prepared as previously described (Amagase *et al*., 2009a) to match the color, flavor, and taste of GoChi in a formulation of sucralose (10 mg), artificial fruit flavor (30 mg), citric acid (60 mg), and caramel color (12 mg) in 30 mL of purified water. It was packaged in the same type of container; however, it provided no nutritional value or LBP.

In addition, a novel trace amount of flavor was added to both active and placebo preparations to mask the differences, so no study participants had been exposed to the flavor-masked samples specifically prepared for the present study.

2.2 Clinical study

A 30-day intervention study was performed in a randomized, double-blind, placebo-controlled manner. To maintain high compliance with our first exercise challenge test and make the test under a similar physical activity condition in the office work, we recruited the participants from in-house. All randomized participants were healthy men and women, age 18 y and older (average age = 33.6 ± 1.9 y) (Table 1A). The CONSORT chart in Figure 1 shows the population including ethnic backgrounds. Recruitment was conducted to ensure that participants were serious about participating in these studies and well aware of its demands. All participants in the study were fully informed of the purpose of the study, and signed the Human Subjects Informed Consent forms approved by the Internal Review Board organized under the Helsinki Declaration. Exclusion and inclusion criteria were same as the previous studies (Amagase *et al*., 2008, 2009a, 2009b). Following enrollment, all participants completed a 2 to 4 week wash-out period during which time they discontinued use of any dietary supplements, including *Lycium barbarum* or *Lycium barbarum*-containing foods, if any, energy drinks, caffeinated beverages or tea, and these restrictions were continued throughout the study based upon the self-declaration in the daily dietary diary and verbal confirmation. Our previous studies have shown that there were no statistically significant differences after this wash-out period in various subjective indicators. A total of 39 healthy male and female adults were randomly assigned to either the *Lycium barbarum* treatment or placebo control group for this 30-day intervention study (Figure 1). Sixty-seven percent were women. Male and female participants were randomized separately to ensure an equal number of men and women in each treatment group. The participants, all investigators and staff involved in this study were blinded to the participant's treatment assignment. Tested products were assigned a number or letter code. This code remained unrevealed to the investigators involved in the study until after completion of the data analyses. No participants were pregnant during the study based upon standard urine pregnancy test (Kurkel Enterprises, HCG Lot No. 5-06257, Redmond, Washington). There were no statistical differences in demographic and clinical characteristics of the study population, and pre-study diet on the parameters of dietary intake (Table 1A), average *Lycium barbarum*

consumption history, if any, and consumption patterns for other beverages such as sweetened beverages (soda), coffee, tea and alcoholic beverages, or smoking history. Food intake was monitored throughout the study by means of daily dietary diaries maintained by the participants. We noticed that self-reported dietary diary may not be completely accurate in the clinical study. However, we believed that it was better than no food log to trace the food records, and may reflect rough caloric intake during the study, as participants were aware of what they eat. In fact, average caloric intake was somewhat realistic based on their diary (Table 1A). All participants reported regular intake of a Western style diet. Daily energy intake was calculated by combining macronutrients intake from the individual diary recorded in the evening with their entire food, snack and beverage intake. A review of the participants' daily diary, there appears to be no change in the participants' other juice intake during the intervention period compared to pre-intervention. All participants were monitored daily to ensure full compliance with the protocol including restriction of dietary intake. Upon randomization, participants consumed 120 ml of *Lycium barbarum* juice or placebo each morning shortly after a meal in front of the researcher on weekdays for a period of 30 days under free-living conditions. To monitor the weekend compliance, we recovered empty bottles on the following Monday. No dropouts occurred in either group during the intervention period. Based upon the previous studies (Amagase *et al*., 2008, 2009a, 2009b, 2011b), a sample size of 39 participants was deemed to be sufficient to detect effectiveness of *Lycium barbarum* alone with 95% confidence and 80% power.

At the pre- and post-intervention period (Day 1 or Day 30), all participants were given physical anthropometric measurements collected following an overnight 12 h fast and included: body weight and body mass index (BMI) (Seca 703, Hamburg, Germany) (Figure1). All participants were administered a written questionnaire with a rating scale (0-5) (Amagase *et al*., 2008) at the time of pre- (Day 1) and post-intervention (Day 30) immediately before the exercise challenge (Figure1). The questionnaire consisted of physical and psychological fatigue-related symptoms, such as fatigue, feelings of tiredness, musculoskeletal questions, cardiovascular questions, and questions regarding possible side effects.

To provide a comparable short and intense exercise challenge, each participant was tested on a ramp-type progressive electronically braked either upright (Schwinn, Model 126, Vancouver, WA) or recumbent cycle ergometer (Schwinn, Model 226-recumbent, Vancouver, WA) (Figure 1). This exercise challenge was performed only on Day 1 (pre-intervention) and Day 30 (post-intervention) as an acute intense physical stress. After resting on the cycle ergometer for 1 min of unloaded pedaling to confirm the resting heart rate, the work rate (WR) was increased by 20 to 30 watt/min adjusted according to the participant's age and fitness level by monitoring display of the heart rate. Workloads were individualized for each participant and were calculated to be equivalent to the WR corresponding roughly to 70% of age-adjusted maximum heart rate as determined non-invasively based on American Heart Association (2011) and Sharkey et al (2007). Participants were vigorously encouraged during the high-intensity phases of the exercise protocol equal 12 to 14 minutes (Radom-Aizik *et al*., 2008, 2009) until preset 200 kcal were burned. The initial result of the exercise challenge for all the participants at the pre-intervention time point on heart rate, watts and calorie burned did not show any statistically significant differences for the post-intervention challenge as shown in Table 1B. In the background of the participants, there were no statistical differences in the average exercise frequency or length between the groups (Table 1A). Changes in plasma concentrations of DHEA, cortisol, glucose and lactic acid were assessed pre- and post-intervention immediately before and after the exercise challenge (Figure 1). Hormone concentrations were measured by enzyme-linked immunosorbent assay (ELISA) (Diagnostic Systems Laboratories, Inc. Webster, Texas), lactic acid levels determined in a YSI 2300 Stat Plus analyzer (YSI, Inc., Yellow Springs, Ohio), glucose and BUN were determined by standard medical laboratory methods (LabExpress, Phoenix, Arizona).

2.3 Statistical analysis

Dietary intake data were analyzed with non-parametric Mann-Whitney *U*-Test (placebo vs *Lycium barbarum*). For all clinical symptom questions, each question was graded and the scores analyzed for changes between pre- and post-intervention with the nonparametric Wilcoxon matched pairs tests. A 2 x (2) mixed ANOVA (group x test) was used for body weight, BMI, and a 2 x (2) x (2) mixed ANOVA (group x test x time) was used for plasma levels of hormones, glucose, BUN concentrations. Descriptive statistics were calculated for placebo and *Lycium barbarum* for all dependent measures and summarized as means ± SEM. The data were processed using Statistica version 8 (StatSoft, Inc., Tulsa, Oklahoma). Differences were considered significant at $P<0.05$.

3. Results

Significant differences ($P<0.05$) between pre- and post-intervention were found in the *Lycium barbarum* group ($n = 20$) for questions in tiredness categories as shown in Table 2. The *Lycium barbarum* group showed

significant reductions in feelings of tiredness after exercise, poor circulation, and overall health conditions. Compared to the pre-intervention results, more than 65% of people who consumed *Lycium barbarum* reported they did not feel tiredness after exercise. In contrast to the *Lycium barbarum* group, the placebo group (n = 19) showed no statistically significant changes following the 30-day intervention period. There were no statistically significant changes in body weight, BMI or total body fat between the groups or pre- vs. post-intervention in both groups (Table 2).

DHEA, cortisol and lactic acid concentrations were all significantly increased in the placebo group after the exercise challenge for both pre- and post-treatment tests ($P<0.05$) (Table 3). Similarly, an increase in plasma levels of DHEA and cortisol was observed after the initial exercise challenge in the *Lycium barbarum* group before treatment; however, following *Lycium barbarum* treatment for 30 days the exercise-induced increase in DHEA and cortisol levels was significantly attenuated. The exercise-induced increase in lactic acid concentration was not changed by *Lycium barbarum* or placebo intake (Table 3). Blood glucose levels were comparable both pre- and post-intervention for both groups. BUN level post- intervention was increased in both groups, but no statistical difference was found after exercise (Table 3).

4. Discussion

The present study was the first human clinical study to show that *Lycium barbarum* intake significantly attenuated the increases in plasma DHEA and cortisol concentrations produced by a short and intense exercise challenge. Exercise represents a physical stress that challenges homeostasis. In response to this stressor, autonomic nervous system and the hypothalamic-pituitary-adrenal axis (HPA-axis) are known to react and to participate in the maintenance of homeostasis. This includes elevation of cortisol and cathecholamines in plasma (Mastorakos *et al*., 2005). The present study results suggest that consumption of *Lycium barbarum* may increase adaptability to a physical stressor such as exercise by either reducing production and function of glucocorticoids, or accelerating metabolism of these hormones. Some of the immunomodulatory actions of *Lycium barbarum* may be mediated in part through changes in the HPA-axis. *Lycium barbarum* intake may interact with cortisol by stimulating the immune system as reported in a previous clinical study (Amagase, 2009a). Likewise, the reported ability of *Lycium barbarum* to significantly reduce subjective feelings of tiredness or fatigue, especially after exercise, may also be related to changes in adrenal steroid regulation. Cortisol counteracts the action of insulin by increasing gluconeogenesis, promoting lipolysis and mobilizing extrahepatic amino acids and ketone bodies, which leads to increased circulating glucose concentrations in the blood (Freeman *et al*., 2004) and prolonged cortisol secretion causes hyperglycemia (Barseghian *et al*., 1982). LBP has been reported to enhance the storage of muscle and liver glycogen, to increase the activity of lactate dehydrogenase (LDH) before and after swimming, to inhibit the increase of blood urea nitrogen (BUN) after strenuous exercise, to accelerated the clearance of BUN after exercise, to increase adaptability to an exercise load, to enhance resistance to fatigue and to accelerate the elimination of lactic acid in mice (Luo *et al*., 2000). However, we found that plasma lactic acid and glucose concentrations were not influenced by *Lycium barbarum* intake. Nonetheless, participants reported an increase in endurance/energy in the daytime and reduced fatigue following 30 days of *Lycium barbarum* consumption. These effects were consistent with previous study results (Amagase *et al*., 2008, 2009a). There is evidence that elevated endogenous glucocorticoid activity can be associated with visceral obesity. This may be mediated centrally level via the HPA-axis and peripherally via increased conversion of cortisone to cortisol by 11-ß–hydroxysteroid-dehydrogenase type 1 in adipose tissue (Masuzaki 2001; Pasquali *et al*., 1993). Increased activity of the HPA-axis has been linked to metabolic syndrome or “Syndrome X”, and may contribute to the clustering of low HDL cholesterol, high triglycerides, insulin resistance, hypertension, and visceral obesity that characterize this syndrome, all of which represent major risk factors for cardiovascular disease, stroke, and diabetes mellitus type II (Brunner, 2002; Brotman *et al*., 2003). In female weanling mice, LBP was shown to enhance food conversion rate and to reduce body weight after 21 days of consumption (Zhang *et al*., 2002). We reported a similar reduction in weight gain in adult male rats given *Lycium barbarum* (Nance *et al*., 2009). These results suggest that LBP may modulate metabolism *in vivo*. In support, it was shown in a clinical trial that *Lycium barbarum* intake increased energy expenditure and/or metabolic rate in humans (Amagase, 2011b). The magnitude of the experimental effects of *Lycium barbarum* in the current study may in part reflect the fact that the test participants had normal body weights and BMIs in addition to the limited consumption time period. It is possible that the metabolic impact of *Lycium barbarum* juice would be more readily demonstrated in overweight or obese participants and could be utilized to prevent or treat metabolic syndromes, including glucocorticoid-related conditions. In support of this possibility, animal studies show dramatic effects of *L barbarum* on blood glucose and insulin levels in diabetic rats that are not observed in nondiabetic controls (Zhao *et al*., 2005).

In support of a role for the anti-oxidant properties of *Lycium barbarum* (Amagase *et al*., 2009b; Wu *et al*., 2004; Gong *et al*., 2005) in the attenuation of the effects of exercise on cortisol release are the results of Davison, et al (2007). Similar to the current results, they showed that dietary supplementation with anti-oxidants (daily vitamin C (L-ascorbic acid, 1000 mg) and vitamin E (RRR-alpha-tocopherol, 400 IU) supplementation) blunted the cortisol response to a single prolonged exercise challenge (Davison *et al*., 2007). Also, LBP has been shown to prevent oxidative stress following exhaustive exercise in rats (Shan *et al*., 2011) and the anti-inflammatory effect of *Lycium barbarum* consumption was demonstrated by Reeve et al (2010) in an animal model of UV radiation induced oxidative skin damage.

5. Conclusion

This is the first randomized trial to evaluate the effects of *Lycium barbarum* on exercise-induced adrenal steroid in humans. *Lycium barbarum* intake significantly attenuated the increases in plasma DHEA and cortisol concentrations produced by a short and intense exercise challenge. The *Lycium barbarum* group showed significant reductions in feelings of tiredness after exercise. Our results suggest that daily consumption of *Lycium barbarum* may attenuate stress-related reactivity and facilitate adaptation to physical stressors. Furthermore, as elevated endogenous glucocorticoid activity has been linked with visceral obesity and the metabolic syndrome, our findings suggest that altered HPA activity may be related to previously reported metabolic effects of *Lycium barbarum* and its active ingredients.

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References

Amagase, H., Sun, B., & Borek, C. (2009b). *Lycium barbarum* (goji) juice shows significant *in vivo* antioxidant effects in human serum in a randomized, double-blind, placebo-controlled clinical study. *Nutrition and Research*, *29*, 19-25. http://dx.doi.org/10.1016/j.nutres.2008.11.005

Amagase, H., Sun, B., & Nance, D. M. (2009a). Immunomodulatory Effects of a Standardized *Lycium barbarum* Fruit Juice in Chinese Older Healthy Human Subjects. *Journal of Medicinal Foods*, *12*(5), 1159-1165. http://dx.doi.org/10.1089/jmf.2008.0300

Amagase, H., & Farnsworth, N. R. (2011a). A Review of Botanical Characteristics, Phytochemistry, Clinical Relevance in Efficacy and Safety of *Lycium barbarum* Fruit (Goji). *Food Research International*, *44*, 1702–1717. http://dx.doi.org/10.1016/j.foodres.2011.03.027

Amagase, H., & Nance, D. M. (2008). A Randomized, Double-blind, Placebo-controlled, clinical study of the general effects of a standardized *Lycium barbarum* (Goji) Juice, GoChi™. *Journal of Alternative and Complementary Medicine, 14*, 403-412. http://dx.doi.org/10.1089/acm.2008.0004

Amagase, H., & Nance, D. M. (2011b). *Lycium barbarum* increases caloric expenditure and decreases waist circumference in healthy overweight men and women: Pilot Study. *Journal of the American College of Nutrition, 30*(5), 304-309.

American Heart Association. (2009). [Online] Available: http://www.americanheart.org/presenter.jhtml?identifier=3057643 (January 25, 2009)

American Heart Association. (2011). [Online] Available: http://www.heart.org/HEARTORG/Conditions/HighBloodPressure/PreventionTreatmentofHighBloodPressure/Physical-Activity-and-Blood-Pressure_UCM_301882_Article.jsp (December 13, 2011)

Barseghian, G., Rachmiel, L., & Epps, P. (1982). Direct Effect of Cortisol and Cortisone on Insulin and Glucagon Secretion. *Endocrinology, 111*, 1648. http://dx.doi.org/10.1210/endo-111-5-1648

Bensky, D., & Gamble, A. (1993). Gou Qi Zi. In *Chinese Herbal Medicine, Materia Medica*. (revised ed.), pp. 333-334. Seattle: Eastland Press, Inc.

Brotman, D. J., Girod, J. P., Tankó, L. B., Bagger, Y. Z., Alexandersen, P., Christiansen, C., & Larsen, P. J. (2003). Can glucocorticoid homeostasis explain the anti-atherogenic effect of peripheral adiposity? Reply. *Circulation*, *108*, e61. http://dx.doi.org/10.1161/01.CIR.0000086784.14560.C3

Brunner, E. J., Hemingway, H., Walker, B. R., *et al*. (2002). Adrenocortical, autonomic, and inflammatory causes of the metabolic syndrome: nested case-control study. *Circulation, 106*(21), 2659-2665. http://dx.doi.org/10.1161/01.CIR.0000038364.26310.BD

Buford, T. W., & Willoughby, D. S. (2008). Impact of DHEA(S) and cortisol on immune function in aging: a brief review. *Applied Physiology, Nutrtion, and Metabolism, 33*, 429-33. http://dx.doi.org/10.1139/H08-013

Chang, H. M., & But, P. P. H. (2001). Gouqizi. In *Pharmacology and Applications of Chinese Materia Medica, 2*, pp. 852-4. Singapore: World Scientific.

Chang, R. C., & So, K. F. (2008). Use of anti-aging herbal medicine, *Lycium barbarum*, against aging-associated diseases. What do we know so far? *Cellular and Molecular Neurobiology, 28*, 643-52. http://dx.doi.org/10.1007/s10571-007-9181-x

Copeland, J. L., Consitt, L. A., & Tremblay, M. S. (2002). Hormonal responses to endurance and resistance exercise in females aged 19-69 years. *Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 57*, B158-65. http://dx.doi.org/10.1093/gerona/57.4.B158

Davison, G., Gleeson, M., & Phillips, S. (2007). Antioxidant supplementation and immunoendocrine responses to prolonged exercise. *Medicine & Science in Sports & Exercise, 39*, 645-652. http://dx.doi.org/10.1249/mss.0b013e318031303d

Dillon, J. S. (2005). Dehydroepiandrosterone, dehydroepiandrosterone sulfate and related steroids: their role in inflammatory, allergic and immunological disorders. *Inflammation & Allergy - Drug Targets, 4*, 377-85. http://dx.doi.org/10.2174/1568010054022079

Filaire, E., Duché, P., & Lac, G. (1998). Effects of amount of training on the saliva concentrations of cortisol, dehydroepiandrosterone and on the dehydroepiandrosterone: cortisol concentration ratio in women over 16 weeks of training. *European journal of applied physiology and occupational physiology, 78*, 466-71. http://dx.doi.org/10.1007/s004210050447

Freeman, & Scott. (2004). *Biological Science*. (2nd Pkg ed.) Prentice Hall; Upper Saddle River, New Jersey, ISBN 0-13-218746-9.

Gong, H., Shen, P., Jin, L., Xing, C., & Tang, F. (2005). Therapeutic effects of *Lycium barbarum* polysaccharide (LBP) on irradiation or chemotherapy-induced myelosuppressive mice. *Cancer Biotherapy and Radiopharmacology, 20*, 155-62. http://dx.doi.org/10.1089/cbr.2005.20.155

Luo, Q., Yan, J., & Zhang, S. (2000). Isolation and purification of *Lycium barbarum* polysaccharides and its antifatigue effect. *Wei Sheng Yan Jiu (Journal of Hygiene Research), 29*, 115-7.

Mastorakos, G. Paylatou, M. Diamanti-Kandarakis, E., & Chrousos, G. P. (2005). Exercise and the Stress System. *Hormones, 4*(2), 73-89.

Masuzaki, H., Paterson, J., Shinyama, H., Morton, N. M., Mullins, J. J., Seckl, J. R., & Flier, J. S. (2001). A transgenic model of visceral obesity and the metabolic syndrome. *Science, 294*, 2166-2170. http://dx.doi.org/10.1126/science.1066285

Nance, D. M., Amagase, H., & Luczy-Bachman, G. (2009). Effects of *Lycium barbarum* on basal and lps-induced cytokine production. *Brain, Behavior, and Immunity*, 23(Suppl 2), S51. PNIRS 2009, PsychoNeuroImmunology Research Society Annual Meeting 2009.

Pasquali, R., Cantobelli, S., Casimirri, F., *et al*. (1993). The hypothalamic-pituitary-adrenal axis in obese women with different patterns of body fat distribution. *Journal of Clinical Endocrinology & Metabolism, 77*, 341-346. http://dx.doi.org/10.1210/jc.77.2.341

Radom-Aizik, S., Zaldivar, F. Jr,, Leu, S. Y., Galassetti, P., & Cooper, D. M. (2008). Effects of 30 min of aerobic exercise on gene expression in human neutrophils. *Journal of Applied Physiology, 104*, 236-243. http://dx.doi.org/10.1152/japplphysiol.00872.2007

Radom-Aizik, S., Zaldivar, F. Jr., Leu, S. Y., & Cooper, D. M. (2009). Brief bout of exercise alters gene expression in peripheral blood mononuclear cells of early- and late-pubertal males. *Pediatric Research, 65*(4), 447-52.

Reeve, V. E., Allanson, M., Arun, S. J., Domanski, D., & Painter, N. (2010). Mice drinking goji berry juice (*Lycium barbarum*) are protected from UV radiation-induced skin damage *via* antioxidant pathways. *Photochemical & Photobiological Sciences, 9*, 601-607. http://dx.doi.org/10.1039/b9pp00177h

Shan, X., Zhou J., Ma T., & Chai Q. (2011). *Lycium barbarum* Polysaccharides reduce exercise-induced oxidative stress. *International Journal of Molecular Sciences, 12*, 1081-1088. http://dx.doi.org/10.3390/ijms12021081

Sharkey, B. J., & Gaskill, S. E. (2007). *Fitness & Health*, (six ed.). (pp 55-56, and pp. 100-105). Champaign, IL: Human Kinetics.

Tissandier O., Péres, G., Fiet, J., & Piette, F. (2001). Testosterone, dehydroepiandrosterone, insulin-like growth factor 1, and insulin in sedentary and physically trained aged men. *European Journal of Applied Physiology, 85*(1-2), 177-84. http://dx.doi.org/10.1007/s004210100420

Walker, B. R. (2007). Glucocorticoids and cardiovascular disease. *European Journal of Endocrinology*, *157*.

545-59. http://dx.doi.org/10.1530/EJE-07-0455

Wu, S. J., Ng, L. T., & Lin, C. C. (2004). Antioxidant activities of some common ingredients of traditional Chinese medicine, *Angelica sinensis*, *Lycium barbarum* and *Poria cocos*. *Phytotheraoy Research, 18*, 1008-12. http://dx.doi.org/10.1002/ptr.1617

Yu, M. S., Lai, C. S., Ho, Y. S., Zee, S. Y., So, K. F., Yuen, W. H., & Chang, R. C. (2007). Characterization of the effects of anti-aging medicine Fructus *lycii* on beta-amyloid peptide neurotoxicity. *International Journal of Molecular medicine, 20*, 261-8.

Zhang, M., Wang, J., & Zhang, S. (2002). Study on the composition of *Lycium barbarum* polysaccharides and its effects on the growth of weanling mice. *Wei Sheng Yan Jiu (Journal of Hygiene Research)*, 31, 118-9.

Zhao, R., Qingwang, L. I., & Xiao, B. (2005). Effects of *Lycium barbarum* plysaccharide on the improvement of insulin resistance in NIDDM rats. *Yakugaku Zasshi, 125*, 981-8. http://dx.doi.org/10.1248/yakushi.125.981

Abbreviations: DHEA, dehydroepiandrosterone; ACTH, adrenocorticotropic hormone; *L. barbarum*, *Lycium barbarum*; LBP, *Lycium barbarum* polysaccharide; IL, interleukin; WR, work rate; ELISA, Enzyme-Linked Immuno Sorbent Assay; BMI, body mass index; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; RMR, resting metabolic rate; HPA, hypothalamic-pituitary-adrenal.

Table 1. (A) Baseline demographic variables of non-discriminated participants in the current randomized, double-blind, placebo-controlled human clinical study. (B) Parameters of maximal heart rate, watts and calorie burned during the short and intense exercise challenge at pre- and post-intervention in both *Lycium barbarum* (*L. barbarum*) and placebo groups. This exercise challenge was performed only on Day 1 (pre-intervention) and Day 30 (post-intervention) as physical stress.

A

Variable			n	Mean ± SEM
Age (years old)		**Placebo**	19	31.1 ± 2.5
		L. barbarum	20	36.0 ± 2.9
Female Population (%)		**Placebo**	19	63.2
		L. barbarum	20	70.0
Exercise (times/week)		**Placebo**	19	0.5 ± 0.1
		L. barbarum	19	0.3 ± 0.1
Average Daily Caloric Intake (kcal)*	Pre-intervention/wash-out period	**Placebo**	19	1,866 ± 158
		L. barbarum	20	1,972 ± 181
	during the 30-day intervention study period	**Placebo**	19	1,807 ± 134
		L. barbarum	20	1,839 ± 144

Each value indicates mean ± SEM except female population. Sample numbers varied due to missing answers. *calculated from daily food diary. Age and average daily caloric intake data were analyzed by ANOVA, and exercise frequency data were analyzed with nonparametric Mann-Whitney U Test (Placebo vs *Lycium barbarum*). There was no statistical significance between the groups.

B

		n	Pre-intervention	n	Post-intervention
Heart Rate (beat/min)	**Placebo**	19	139.1 ± 3.0	19	145.3 ± 3.2
	L. barbarum	20	141.1 ± 2.7	20	138.8 ± 2.7
Total Watts	**Placebo**	19	82.3 ± 5.6	19	98.9 ± 8.1
	L. barbarum	20	79.8 ± 4.5	20	91.2 ± 5.2
Calorie burned (kcal)	**Placebo**	19	204.7 ± 11.2	19	223.6 ± 17.1
	L. barbarum	20	190.7 ± 8.1	20	207.4 ± 11.9

Each value indicates mean ± SEM. N.S., not significant analyzed by ANOVA. There was no statistical significance between the groups, or pre- and post-intervention.

Table 2. Effect of *Lycium barbarum* (*L. barbarum*) or placebo on (A) anthropometric parameters and (B) subjective indications compared to pre-intervention in a randomized, double-blind, placebo-controlled human clinical study

A

		N	Pre-intervention	n	Post-intervention
Body weight (kg)	**Placebo**	19	82.3 ± 5.1	19	82.5 ± 5.1
	L. barbarum	20	82.4 ± 4.8	20	82.7 ± 5.0
Body mass index (BMI) (kg/m^2)	**Placebo**	19	29.3 ± 1.7	19	29.4 ± 1.7
	L. barbarum	20	29.5 ± 1.6	20	29.7 ± 1.6
B					
Tiredness	**Placebo**	19	2.2 ± 0.3	19	1.8 ± 0.4
	L. barbarum	20	2.4 ± 0.4	19	1.9 ± 0.4
Muscular complaints	**Placebo**	18	1.9 ± 0.3	19	1.9 ± 0.3
	L. barbarum	18	1.9 ± 0.3	20	1.8 ± 0.3
Physical discomfort	**Placebo**	19	1.7 ± 0.3	19	1.4 ± 0.3
	L. barbarum	20	1.9 ± 0.4	20	1.6 ± 0.4
Joint pain	**Placebo**	19	1.4 ± 0.3	19	1.6 ± 0.4
	L. barbarum	19	2.1 ± 0.4	19	1.8 ± 0.4
Stiff Shoulder	**Placebo**	19	1.6 ± 0.4	19	1.7 ± 0.4
	L. barbarum	20	2.4 ± 0.4	20	2.1 ± 0.4
Tiredness after exercise	**Placebo**	19	1.7 ± 0.3	19	1.9 ± 0.3
	L. barbarum	20	2.1 ± 0.3	20	**1.4 ± 0.2**[a]
Feeling of bad circulation	**Placebo**	19	0.1 ± 0.1	19	0.3 ± 0.1
	L. barbarum	20	1.3 ± 0.4	20	**0.5 ± 0.1**[a]
Overall health conditions	**Placebo**	19	60.0 ± 7.0	19	59.9 ± 8.5
(Total Scores)	***L. barbarum***	20	79.2 ± 8.3	20	**67.3 ± 8.7**[a]

Each value indicates mean ± SEM. Anthropometric parameters were analyzed by ANOVA. These subjective indications were collected from the questionnaire asked immediately before the short and intense exercise challenge on both pre- (Day 1) and post-intervention (Day 30). Sample numbers varied due to missing answers. Lower scores reflect improvements in subjective ratings. [a] indicates significant difference ($P<0.05$) from pre-intervention analyzed by the nonparametric Wilcoxon matched pairs tests.

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Table 3. Effect of *Lycium barbarum* (*L. barbarum*) or placebo on plasma dehydroepiandrosterone (DHEA), cortisol, lactic acid, glucose and blood urea nitrogen (BUN) concentrations at pre- and post-exercise in a randomized, double-blind, placebo-controlled human clinical study

		Pre-intervention				**Post-intervention**			
		n	**Pre-exercise**	**n**	**Post-exercise**	**n**	**Pre-exercise**	**n**	**Post-exercise**
DHEA (μg/dl)	**Placebo**	18	8.2 ± 1.1	18	**10.4 ± 1.2**^{b}	18	7.3 ± 0.8	18	**11.5 ± 1.0**^{b}
	L. barbarum	17	6.1 ± 1.1	18	**8.4 ± 1.0**^{b}	18	5.2 ± 0.9	18	5.7 ± 0.9
Cortisol (μg/dl)	**Placebo**	18	34.8 ± 6.2	18	**52.2 ± 12.4**^{b}	18	23.0 ± 2.1	18	**51.8 ± 15.5**^{b}
	L. barbarum	15	37.3 ± 4.3	18	47.6 ± 6.8	18	47.8 ± 11.1	18	42.4 ± 4.3
Lactic acid (mmol/l)	**Placebo**	18	2.5 ± 0.2	18	**6.1 ± 0.5**^{b}	18	2.3 ± 0.3	18	**6.1 ± 0.5**^{b}
	L. barbarum	18	2.4 ± 0.2	18	**5.8 ± 0.4**^{b}	18	2.2 ± 0.2	18	**5.8 ± 0.5**^{b}
Glucose (mg/dl)	**Placebo**	19	91.3 ± 2.1	19	90.8 ± 2.8	19	90.1 ± 1.8	19	92.5 ± 3.2
	L. barbarum	20	99.2 ± 10.3	20	95.3 ± 7.8	20	98.9 ± 9.5	20	96.1 ± 7.2
BUN (mg/dl)	**Placebo**	19	12.1 ± 0.9	19	12.4 ± 0.8	19	**13.5 ± 1.0**^{a}	19	**13.7 ± 0.9**^{a}
	L. barbarum	20	11.7 ± 0.6	20	11.9 ± 0.6	20	**13.5 ± 0.6**^{a}	20	**13.9 ± 0.6**^{a}

An exercise challenge on a cycle ergometer at 70% of age-adjusted maximum heart rate was given on each individual participant at pre- (Day 1) and post-intervention (Day 30). Parameters of maximal heart rate, watts and calorie burned during the exercise challenge were not statistically different in both pre- and post-intervention and also in both *Lycium barbarum* and placebo groups. This exercise challenge was performed only at the time of pre- and post-intervention. Intervention period of *Lycium barbarum* or placebo was 30 days. Each value indicates mean ± SEM. Sample numbers varied due to missing samples. ^{a}, ^{b} indicate significant difference ($P<0.05$) from pre-intervention and pre-exercise, respectively analyzed by ANOVA.

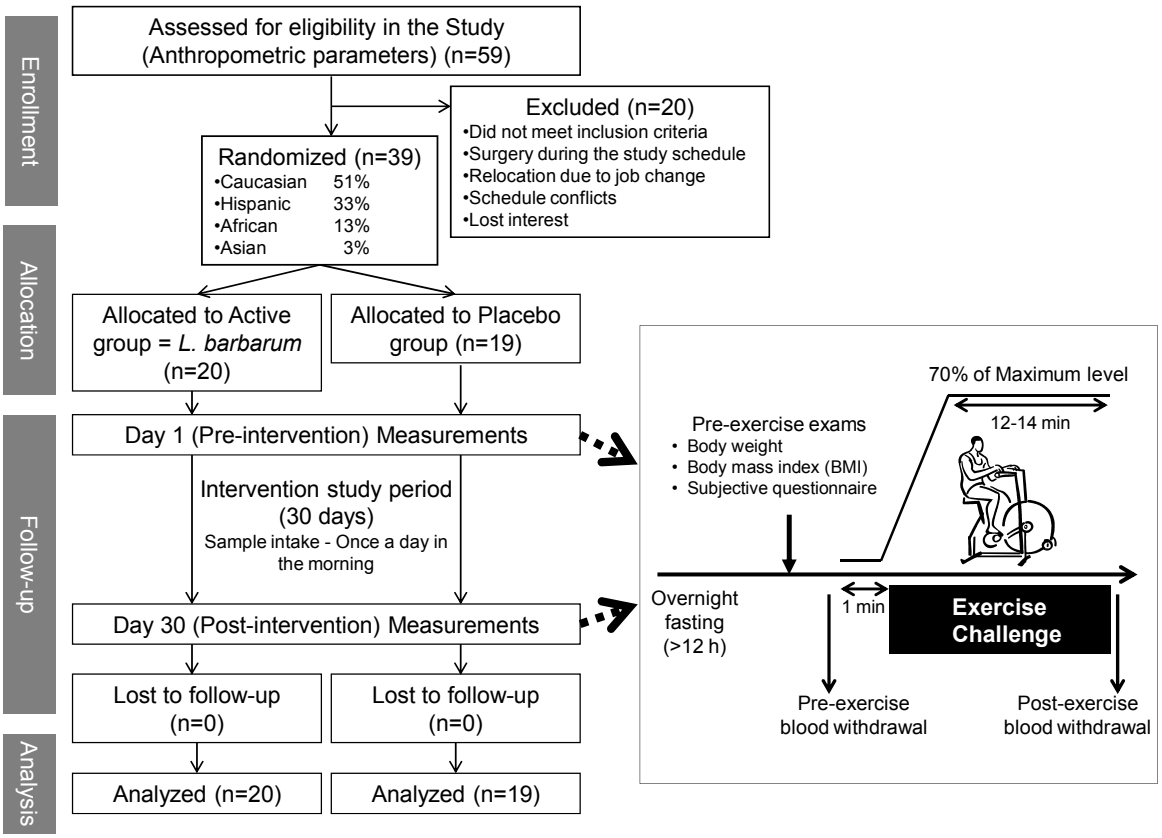

Figure 1. CONSORT flow diagram, study design and the experimental block on Day 1 (pre-intervention) and Day 30 (post-intervention). Participants completed pre-exercise examinations including anthropometric parameter measurements, subjective questionnaire in about 15 min, followed by a pre-exercise blood withdrawal, a short and intense exercise challenge on a cycle ergometer at 70% of age-adjusted maximum heart rate by loading ride for about 12-14 min and post-exercise blood withdrawal on both Day 1 and Day 30

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Nudging Customers towards Healthier Choices: An Intervention in the University Canteen

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Abstract

The notion of *nudge effects* was investigated in two field experiments which focused on influencing customers' purchases by manipulating the location and availability of food in a University canteen setting. Study 1 manipulated the location of fruit and confectionary. Study 2 restricted the types of bread (i.e. brown only not white) that customers could choose for their sandwiches**.** The results of the Study1 showed that the fruit sales increased when positioned away from the checkouts. The majority of interviewed customers bought fruit *intentionally* but those who bought confectionary did it *on impulse*. In the Study 2, a restricted choice of baguettes did not reduce sales, with customers simply buying more brown baguettes. This increase did not persist after the intervention. Simple changes in the location and availability of food items can *nudge* the customers' purchases towards the choice of healthier options. There may be different processes that guide the purchase of fruit (*intention/deliberation*) and confectionary (*impulse/reflex*).

Keywords: Dietary intervention, Microenvironment, Impulse buying, Location, Availability, Nudge effects

1. Introduction

Research indicates that when individuals are not required to commit to dietary change, they seem unable to change what they eat in a sustained way (Kumanyika, Bowen, Rolls *et al*., 2000). Similarly, the rise in obesity and dietary related chronic conditions such as diabetes and coronary heart disease (WHO, 2003; NHES I; NHAHES IV: NHS, 2009) illustrates how unhealthy dietary habits can be resistant to manipulation. Yet, this is not the whole picture. A recent review of dietary change within randomised control trials (Chapman, 2010) supports the view that successful dietary change is possible, provided that individuals are highly motivated, are given a large amount of support during the intervention and actively strive to achieve their goals. Furthermore, studies of a diverse non-clinical sample (Chapman & Ogden, 2009; Chapman & Ogden, 2010) indicate that dietary changes are highly prevalent, sometimes unintentional and often experienced without an individuals' active involvement. For example, participants reported frequently making small changes to their diet such as a reduction in sugar or an increase in fruit and vegetables with minimal effort and planning. This type of change finds reflection in the *nudge effects* described by Thaler and Sunstein (2008) where individuals are *nudged* inconspicuously towards making choices that protect their health.

The research that precedes the *nudge* literature has aimed to investigate the effectiveness of interventions, which target populations, rather than the individual. These interventions have been conducted in *microenvironments* such as schools, workplaces, restaurants, grocery stores and in *macroenvironments* e.g. fortification of breads

and grains with folic acid in the USA (Seymour, Yaroch, Serdula *et al*., 2004). A recent review of interventions in microenvironments (Stubenitsky, Aaron, Catt *et al*., 2002) categorized intervention strategies used in workplaces, universities, grocery stores and restaurants into *information* , *incentives*, *availability* and *access*. *Information* strategies included signs, posters, labels, shelf tags, menu symbols, flyers, booklets, TV, radio and newspaper advertisements. *Incentive* strategies included price reductions and promotions. *Availability* strategies included changing catering practices, recipes, and preparation methods, while *access* strategies included changing the location of items and bringing items to customers. The authors concluded that nutrition interventions had the greatest impact when they were conducted in 'limited access' sites, i.e. workplaces and universities, as opposed to grocery stores, as it was easier to maneuver customers towards making healthier choices. When perceived from the perspective of an individual, environmental interventions that focus on reduced availability and/or changed access to food items can be conceptualized as a result of nudging strategies developed purposefully by the local or a national authority.

One frequent focus of environmental interventions has been the promotion of fruit and vegetable consumption and reduction of confectionary intake (defined as food or drink that is calorie-dense and high in refined sugar) mainly through providing customers with nutrition information and offering them price reductions (e. g. Jeffery, French, Raether et al., 1994). However, there have also been interventions that used only availability and access to influence customers' choices (e. g. 13). In Hoerr and Louden's (1993) study, the latter strategies have been shown to be more effective than using information strategy. The authors tested the effect on sales in vending machines of the increased availability of high nutritional quality snacks in year 2 of the intervention versus providing additional nutrition information above each item in year 3. These interventions did not explore whether a strategy of changed location on it's own would influence customers' purchasing habits. For example, would changes in the location of fruit and confectionary make a difference in sales? The retail business has long been aware of the value of the space adjacent to checkouts as a place where the customer is 'forced' to look at the merchandise with the objective of tempting them to buy the offered items (Levy & Weitz, 2004). The notion that customers are prone to impromptu purchases at the checkout had been supported in literature (e.g. Rook & Fisher, 1995; Huddleston, Whipple & VanAuken, 2004). A substantial body of research has been devoted to the phenomenon of *impulse buying,* defined as making unplanned and sudden purchases, which are initiated on the spot, and are accompanied by powerful urges and feelings of pleasure and excitement (Rook, 1997). Impulsive purchases are often triggered by the shopping environment (Beatty & Farrell, 1998). The items sold at checkouts are positioned intentionally to trigger impulse buying. The items encountered most frequently at checkouts are magazines, chewing gum, confectionary, soft drinks and batteries (Front End Focus, 2008). These minor changes in the micro environment find reflection in the nudge theory and nudge strategies which usually consist of covert interventions that aim to change behaviour in subtle ways (Just & Payne, 2009), To date, however, there are no published studies testing the effect of nudging changes in eating behavior by locating fruit at the checkouts and removing the confectionary from the checkout area. This could be due to commercial interests that dictate maximizing profit from sales regardless of the long term consequences for the health of the population.

In line with this emphasis on population-based intervention, the present paper reports the results from two experimental studies, which highlighted the impact of nudging in dietary behaviour with a focus on two aspects of the environment: the location of food (close to or away from the checkout) and the availability of food. In particular, the study aimed to assess whether manipulating these variables impacted upon the sales of fruit, confectionary and type of baguette (white vs brown) and also explored whether purchases of confectionary and fruit, following the intervention, were governed by impulse or whether they were made intentionally.

2. Study 1. The Impact of Location on the Sales of Fruit and Confectionary

2.1 Method

Ethical approval for both studies was obtained through the University Ethics Committee and the intervention took place in January – February 2009.

2.1.1 Design

An experimental ABA design - repeated measures - was used with the intervention lasting 3 weeks (A: Week 1 / B: Week 2 / A: Week 3). During this time the location of selected food items in the university canteen was manipulated in order to influence the consumers' purchases (near the checkout vs away from the checkout). A qualitative component was included in the study in the form of interviews conducted with 12 customers each day of the intervention to explore the reasons for their food purchases.

2.1.2 Procedure

Week 1, Monday to Friday: Confectionary comprising of Kit Kats (chocolate biscuits) and assorted sweet biscuits were located by the checkouts; a basket with assorted fruit was located on a sideboard, away from the checkouts.

Week 2, Monday to Friday: Fruit basket was located by the checkouts; confectionary was located on the sideboard, away from the checkouts.

Week 3, Wednesday, Thursday, Friday, Monday and Tuesday (intervention was delayed due to university closure on Monday and Tuesday caused by heavy snow conditions): Confectionary were repositioned back by the checkouts and fruit was repositioned on the sideboard, away from the checkouts.

2.2 Analysis

Data was collected in the form of food items purchased on the basis of till data. The sales of fruit and confectionary were analysed using chi-square.

2.2.1 Outcome variable

Weekly sales of the targeted items: fruit and confectionary.

2.3 Qualitative component

2.3.1 Participants

For the qualitative component, 183 consumers in University canteen who bought Kit Kats, sweet biscuits, fruit or none of the above between 12noon and 2pm of every day of the intervention were approached and asked to participate in the study. Three customers declined, 180 customers agreed to take part in the study (98.4%). The researcher approached potential participants as they proceeded through checkout if they satisfied inclusion criteria. All students, visitors as well as academic, administrative and maintenance staff were included. The canteen employees were excluded. Customers who bought confectionary items or fruit as a snack rather than as a part of their lunch were not approached to give an interview, as their needs and intentions would have been different to the customers who bought fruit or confectionary in addition to another lunch item i.e. a sandwich or a hot meal option. The customers who bought both confectionary and fruit were not approached to give an interview either as they didn't have to make a choice between these two items.

2.3.2. Interview schedule

Twelve interviews were conducted on each day of the intervention during lunch time in one of the University of Surrey canteens, between 12noon and 2pm. Four consumers who bought confectionary, four customers who bought fruit and four customers who bought neither fruit or confectionary were asked to participate in the study. 120 participants were asked the following questions: 'Why did you buy Kit Kat/biscuits/fruit today?', 'Do you usually buy this item?' and 'What do you usually eat for lunch?' These questions were designed to establish whether the participants' purchase was made on *impulse* or *intentionally*. Participants were also asked whether they planned what they were going to eat for lunch: 'always', 'most of the time', 'sometimes', 'rarely' or 'never'. This question aimed to establish to what extend a participants' choice of lunch items was flexible. 60 participants who did not purchase fruit or confectionary item were asked whether they were tempted to buy any of these items.

2.3.3 Analysis

The interviews were analysed using content analysis. Participants' answers were coded and analysed using descriptive analysis, t-tests and one-way Anova. The differences in sales were analysed using Chi-square.

2.4 Results

In order to assess the impact of location of food on sales of food differences in sales of fruit vs confectionary according to location were assessed using chi square. The results are shown in Table 1.

(Table 1)

The weekly sales were compared: chi-sq (2) = 20.906, p = 0.0001. Post-hoc comparisons revealed statistically significant difference in sales between Week 1 and 2 (chi-sq (1) = 11.997, p = .0005) and week 2 and 3 (chi-sq (1) = 18.535, p = .0001). There was no statistically significant differences between Week 1 and 3 (chi-sq (1) = 0.883, p = .347). These results showed a significant effect of the intervention as the sales of fruit and confectionary changed in the second week. These changes however, were not in the predicted direction. The sales of both fruit and confectionary were lower when these items were positioned by the checkouts. The sales of

both fruit and confectionary increased when they were positioned on the sideboard, away from the checkouts. When the fruits were positioned by the checkouts in the second week, the sales decreased from 372 items to 275 items, which constitutes a difference of 26.1%. When the confectionary was moved away from the checkouts, sales in the second week increased from 197 items to 225 items, which constitutes the difference of 14.2%. Thus the observed effect was larger for fruit sales.

2.4.1 Qualitative data including content analysis of the interviews with the participating customers

96 female and 84 male customers were interviewed during the intervention. Most of them were students (62.2%) but members of staff were also well represented (34.4%). The large majority of participants were of white ethnic origin (73.3%) and a large minority was of Asian ethnic origin (16.1%). Most of participants declared their income per year as lower than £10 000 but 36.1% of participants declared their income as above £20 000. A large majority (64.4%) said that they were eating at the canteen several times a week. Full details are shown in Table 2.

(Table 2)

The results obtained from 120 participants who bought either a confectionary item or fruit showed that the majority of them when purchasing a confectionary item did it on impulse [*N*=36 (30%)] while the participants who purchased fruit, tended to do intentionally [N=41 (43.2%)]. The differences were statistically significant (chi-sq (1) = 9.701, p = .001). The results are shown in Figure 1.

(Figure 1)

Within the group of participants who did not purchase either a confectionary item or fruit with their lunch (*N* = 60). A large majority said that they were not tempted to buy a confectionary item or fruit. There were more participants who said they were tempted to buy a confectionary item (11.7%) that the participants who were tempted to buy fruit (6.7%) but the difference was not statistically significant. Full results are shown in Table 3.

(Table 3)

2.5 Discussion

The results of Study 1 confirmed the prediction that there will be a difference in sales figures of fruit and confectionary depending on their location. However, these results did not confirm a second prediction that the sales figures will rise when items are positioned close to the checkouts. The latter was particularly salient with the sales of fruit, which fell significantly when displayed by the checkouts and rose when displayed on the sideboard. This pattern was also evident but to a lesser extent in the sales of confectionary.

Retailers are aware of the value of checkout space and are keen to maximize their profits by reserving it for products that are known to elicit impulse buying. Confectionary and magazines, according to Front End Focus study (2008) based on sales data from 565 US leading retailers, are bought most frequently and are classed as 'high impulse' purchase by the industry. The study compared the sales of 18 categories of products sold at checkouts e.g. candy, mints, books, batteries, phone cards, cosmetics, etc. and listed 45 more unusual categories of items that had appeared by checkout stands, such as children's' toys, air fresheners, pizza cutters, windshield solvents, etc. Fruit did not feature among any of these categories and perhaps that is why there had been no studies conducted on the effect on sales of locating fruit in the checkout area.

A possible reason why fruit and confectionary sales declined when either of these items was located by the checkouts may have been due to the efficient cashier service in the university canteen. It meant that customers were not subjected to a long waiting time; therefore the possibility of indulging in an impulse buy was reduced. The time spent queuing at the checkout is an important variable in impulse buying: 5-7 minutes is an average - not too long to upset the customers but long enough to tempt them to purchase additional items found at the checkout (Front End Focus, 2008).

The results, showing that participants tended to buy confectionary items on impulse and to purchase fruit intentionally, supported data on impulse buying of confectionary at checkouts. A more surprising result was that the sales of fruit rose when it was positioned on the sideboard. One of the reasons could have been that the fruit was placed in an aesthetically appealing basket rather than split into separate boxes containing bananas, oranges or apples. This arrangement gave an impression of a home fruit bowl rather than the fruit display in a supermarket. Having all fruit in one basket was appealing but it also meant that the process of choosing a particular fruit needed additional time – a preferred fruit might have been underneath other fruit, the fruit quality had to be evaluated, a banana (most popular choice of fruit) had to be detached from a bunch, etc. These actions are relatively time consuming and cannot be performed if there is a pressure to proceed quickly in a queue.

These actions also require a degree of deliberation and by definition are at the other end of the spectrum to impulsive behaviour.

3. Study 2. The Impact of Availability on Sales of Baguettes

3.1 Design and procedure

An experimental, before and after design with repeated measures was used. The canteen removed for one day the choice of white baguettes from their sandwich bar. Instead, the customers were offered brown baguettes only. The sales of brown baguettes were recorded. There were no interviews conducted on the day when the brown baguettes were sold as the researcher did not want to influence the future choices that the customers might make relating to the kind of baguette they choose for their lunch.

3.2 Outcome variable

Sales of white and brown baguettes.

3.3 Analysis

Data was collected in the form of food items purchased on the basis of till data. The sales of white and brown baguettes were analysed using chi-square.

3.4 Results

The number of sales of both white and brown baguettes on different days is shown in the Table 4.

(Table 4)

These results showed that the sales of brown baguettes on the day of the intervention were almost as high as the number of total sales of white and brown baguettes on any other day indicating that restricting the availability of white baguettes resulted in a shift in purchases towards the healthier option. However, the intervention did not seem to change customers' preferences as this increase in the sales of brown baguettes did not persist after the intervention.

3.5 Discussion

This intervention aimed to test the possibility of nudging strategy on customers' dietary choices in the short term (on the day of the intervention) and in the long term (post intervention). The intervention showed that it was possible to guide customers towards making healthier choice when other options were not available. The customers, no doubt, were aware of this situation but they did not resist it. The sales of brown baguettes on the day of the intervention were almost as high as the combined sales of white and brown baguettes on other days. The customers did have other choices as the sandwich bar also offered ciabattas, focaccias and crusty rolls, so if they did not like brown baguettes they could ask to have their sandwich made in a different type of bread. And yet they didn't, which suggests that habits can be easily disrupted. The one day intervention was not enough; however, to change customers preferences in the long term as the sales of brown baguettes did not increase in the days following the intervention. Despite the fact that the canteen offered in their sandwich bar the choice of other breads in addition to baguettes and by implication the customers choices were not reduced radically, when the length of the intervention was being negotiated, the catering manager agreed to just one day of removing white baguettes from the menu as not to disrupt normal service for too long and not to risk the loss of revenue. However, irrespective of these restrictions the results demonstrated that nudging the customers towards healthier choices is a promising strategy in guiding customers' dietary behaviour. This conclusion supports findings of interventions in microenvironments that demonstrated availability and location to be effective strategies in influencing customers' purchases (e.g. Hoerr & Louden, 1993).

4. General Discussion

Study 1 and 2 aimed to influence consumers' purchases at the university canteen by manipulating the location of fruit and confectionary and by nudging the customers towards a healthier dietary choice (brown baguettes) during lunchtime. The results confirmed the predictions that there would be a difference in sales of fruit and confectionary depending on their location, and that the customers would purchase more brown baguettes if white baguettes are not available. However, contrary to predictions, there was a decrease in sales of items that were positioned by the checkouts and there was not an increase in sales of brown baguettes after the intervention.

The fact that the sales of fruit rose in the weeks when it was located away from the checkouts indicates that there may be different processes that guide the purchase of fruit and confectionary. Fruit belongs to a shopping category that requires greater 'elaboration' in decision making and by implication does not lend itself for purchasing at the checkout (Miranda, 2008). Confectionary, on the other hand belongs to a shopping category

that is characterized by 'impulse buying'. 'Impulse buying' has been studied extensively within marketing and psychology. Marketing has relied on impulse buying for the profit margins. Some US statistics claim that 62% of sales in supermarkets are a result of impulsive buying (Abrahams, 1997). Psychology has contributed to the understanding of this phenomenon by developing *reflective-impulsive model* (RIM), (Strack & Deutsch, 2002) which sees the behaviour as a joint outcome of two systems. The impulsive system guides quick and spontaneous behaviour while the reflective system guides intentional and deliberate actions. The ratio of impulsive to reflective component in behaviour depends on the regulatory strength of the reflective system. Reflective system has limited capacity and when it gets depleted through i.e. having to resist temptation, its regulatory power diminishes (Baumeister, 2002). The RIM has proved particularly useful in the study of consumer behaviour and there is now convincing evidence that having to make choice depletes the self-regulatory resource, especially when there are more than two options to choose from (Moller, Deci & Ryan, 2006; Vohn, Baumeister, Twenge et al., Unpublished manuscript). This suggests that customers would not necessarily feel better served when faced with multiple choices and that it would be easier for them to self-regulate if they had to chose from not more than a couple of options.

Study 2 also showed that a simple intervention can change dietary choices but indicated that although single event may disrupt habitual behaviour, it may not change it in the long term. In a recent study, Lally and colleagues (2008) endeavored to model the habit formation process by asking participants to repeat a novel behaviour (either eating or exercise) every day in the same context for 12 weeks. They found that it took on average 70 days to develop a habit and that a single failure to perform the behaviour had very little impact on the development of the habit. This evidence may explain why buying a brown baguette on a single occasion would not change a long ingrained habit. The fact that a single disruption is unlikely to produce sustained behaviour change should not be taken with resignation but it should be perceived as a prospective trigger which, if followed by continued changes in the environment, has a potential for long term dietary behaviour change.

The notion of nudging strategies as a viable way of health interventions may seem aversive in the light of widely held views that individuals should be free from the patronizing influence of the state. Yet the same individuals view the provision of state-provided health care (in the UK) for granted. In order to reconcile these two divergent expectations, Thaler and Sunstein (2008) propose 'libertarian paternalism' as an ideal that can be justified and viable for both the state and the individual. In their view, the individual can be guided to make choices that have been judged by society as beneficial to long term health. This is the task for 'choice architects'. Their role is to design an environment in which an individual is nudged to make choices that promote health and well being. Thaler and Sunstein (2008) argue that nudging people preserves the ethos of free will and freedom of choice, whilst gently steering them onto a path that benefits the individual's health.

A number of field studies reviewed by Just and Payne (2009), suggest a variety of nudge strategies that may be effective and acceptable to the consumer. These include the provision of small plates and utensils in 'all you can eat' restaurants, and positioning buffet food further away from the customers. Another idea is offering customers who purchase their food in school or work cafeterias the option of 'restricted debit cards'. The restriction refers to the limited budget available to spend on food and beverages deemed as unhealthy. This way, all customers can retain freedom of choice, while some customers will take up the restricted debit card option as a way of self-regulating.

5. Conclusion

Although literature indicates that dietary habits may be difficult to change, a recent study suggests that many changes occur across the life span and that some of these occur without effort or intentionality The present study aimed to explore the impact of two simple changes in the environment and show that manipulation of both the location and availability of food can influence customers' purchases towards the choice of healthier options. These findings contribute evidence to the growing momentum in embracing a new development of creating environments that 'nudge' customers into making beneficial choices for their long term health prospects.

References

Abrahams, B. (1997). It's all in the mind. *Marketing, 27*, 31-33.

Baumeister, R. (2002). Ego-depletion and self-control failure: An energy model of the self's executive function. *Self and Identity, 1*, 129-36. http://dx.doi.org/10.1080/152988602317319302

Beatty, S. E., & Ferrell, M. E. (1998). Impulse buying: Modelling its precursors. *Journal of Retailing, 74*, 169-91. http://dx.doi.org/10.1016/S0022-4359(99)80092-X

Chapman, K., & Ogden, J. (2009). How do people change their diet? An exploration into mechanisms of dietary change. *Journal of Health Psychology, 14*(8), 1229-42. http://dx.doi.org/10.1177/1359105309342289

Chapman, K., & Ogden, J. (2010). The prevalence of mechanisms of dietary change in a community sample. *Appetite, 55*, 447-53. http://dx.doi.org/10.1016/j.appet.2010.08.006

Chapman. K. (2010). Can people make healthy changes to their diet and maintain them in the long term? A review of the evidence. *Appetite, 54*, 433-41. http://dx.doi.org/10.1016/j.appet.2010.01.017

Choumenkovitch, S. F., Selhub, J., Wilson, P. W. F., Rader, J. I., Rosenberg, I. H., & Jacques, P. F., (2002). Folic acid intake from fortification in the United States exceeds predictions. *Journal of Nutrition, 132*, 2792-8.

Front End Focus. (2008). Best Practices for Superior Checkout Merchandising. https://www.frontendfocus.com/documents/publications/Front-End%20Focus%20Overall%20Study.pdf.

Hoerr, S. M., & Louden, V. A. (1993). Can nutrition information increase sales of healthful vended snacks? *Journal of School Health, 63*, 386-90. http://dx.doi.org/10.1111/j.1746-1561.1993.tb06167.x

Huddleston, P., Whipple, J., & VanAuken, A. (2004). Food store loyalty: Application of consumer loyalty framework. *Journal of Targeting, Measurements and Analysis for Marketing*. *12*, 213-30. http://dx.doi.org/10.1057/palgrave.jt.5740110

Jeffery, R. W., French, S. A., Raether, C. & Baxter, J. E. (1994). An environmental intervention to increase fruit and salad purchases in cafeteria. *Preventive Medicine, 23*, 788-92. http://dx.doi.org/10.1006/pmed.1994.1135

Just, D. R., & Payne, C. P. (2009). Obesity: Can Behavioral Economics Help? *Annals of Behavioral Medicine, 38*, Suppl(1), S47-S55.

Kumanyika, S. K., Bowen, D., Rolls, B. J., Van Horn, L., Perri, M. G., Czajkowski, S. M., & Schron, E. (2000). Maintenance of dietary behaviour change. *Health Psychology, 19*(Suppl.), 42-56. http://dx.doi.org/10.1037/0278-6133.19.Suppl1.42

Lally, P., van Jaarsveld, C., Potts, H., & Wardle, J. (2008). Can we model the habit formation process? *Annals of Behavioral Medicine,* Suppl (1), S212.

Levy, M., & Weitz, B. A. (2004). *Retailing Management*. McGraw-Hill, New York.

Miranda, M. (2008). Determinants of shoppers' checkout behaviour at supermarkets. Journal *of Targeting, Measurement and Analysis for Marketing, 16*, 312-21. http://dx.doi.org/10.1057/jt.2008.23

Moller, A. C., Deci, E. L., & Ryan, R. M. (2006). Choice and ego-depletion: The moderating role of autonomy. *Personality and Social Psychology Bulletin, 32*, 1024-36. http://dx.doi.org/10.1177/0146167206288008

NHANES IV. (1999-2000). Fourth National Health and Nutrition Examination Survey**.** U.S. Department of Health and Human Services**,** Centres for Disease Control and Prevention**,** National Centre for Health Statistics.

NHES I. (1959). First Health Examination Survey. U.S. Department of Health and Human Services. National Center for Health Statistics. Centers for Disease Control and Prevention.

NHS Information Centre, Lifestyle Statistics. (2009). Statistics on obesity, physical activity and diet: England, February 2009.

Rook, D. W. (1987). The buying impulse. *Journal of Consumer Research*, *14*, 189-99. http://dx.doi.org/10.1086/209105

Rook, D. W., & Fisher, R. (1995). Normative influences on impulsive buying behaviour. *Journal of Consumer Research*, *22*, 305-13. http://dx.doi.org/10.1086/209452

Seymour, J. D., Yaroch, A. L., Serdula, M. D., Blanck, H. M., & Khan, L. K.(2004). Impact of nutrition environmental interventions on point-of-purchase behaviour in adults: a review. *Preventative Medicine*, *39*, S108-S136. http://dx.doi.org/10.1016/j.ypmed.2004.04.002

Strack, F., & Deutsch, R. (2004). Reflective and impulsive determinants of social behavior. *Personality and Social Psychology Review, 8*, 220-47. http://dx.doi.org/10.1207/s15327957pspr0803_1

Stubenitsky, K., Aaron, J. L., Catt, S. L., & Mela, D. J. (2000). The influence of recipe modification and nutritional information on restaurant food acceptance and macronutrient intakes. *Public Health Nutrition, 3*, 2001-9. http://dx.doi.org/10.1017/S1368980000000239

Thaler, R. H., & Sunstein, C. A. (2008) *Nudge: improving decisions about health, wealth, and happiness.* New Haven: Yale University Press.

Vohs, K. D., Baumeister, R. F., Twenge, J. M., Nelson, N. M., Rawn, C. D., Schmeichel, B. J., & Tice, D. M. (2007). Making choices impairs subsequent self-control: A limited resource account of decision-making, self-regulation, and active initiative. Manuscript submitted for publication.

WHO (2003). World Health Organization. *Obesity and Overweight*. Geneva.

Table 1. Number of sales of fruit and confectionary at the university canteen during the three weeks of intervention

	Week 1	**Week 2**	**Week 3**
Fruit	372 (S)	275 (C)	360 (S)
Confectionary	197 (C)	225 (S)	169 (C)

(S) - location of food item on the sideboard

(C) - location of the food item by the checkouts

Table 2. Participants' demographic characteristics

Variable		**N**	**(%)**
Sex	Female	96	53.3
	Male	84	46.7
Age	≤23	66	36.7
	24-32	56	1.1
	33+	58	32.2
	Mean (standard deviation)	31.2	12.4
	Range	18-71	
Ethnic origin	White	132	73.3
	Asian	29	16.1
	Black	7	3.9
	Other	12	6.7
Affiliation	Student	112	62.2
	Staff	62	34.4
	Visitor	6	3.3
Income per year	Below £10 000	92	51.1
	£10 000 - £20 000	23	12.8
	Over £20 000	65	36.1
Frequency of eating at the canteen			
	Less than 1 x month	19	10.6
	Less than 1 x week	28	15.6
	Several times per week	116	64.4
	Nearly every day	17	9.4

Table 3. The group of participants who did not buy a confectionary item or fruit and who declared either feeling tempted or not tempted to buy either of these items

	Confectionary		**Fruit**		**Total**
	Tempted	**Not tempted**	**Tempted**	**Not tempted**	
	N° (%)	**N° (%)**	**N° (%)**	**N° (%)**	**N° (%)**
Participants	7 (11.7)	53 (88.3)	4 (6.7)	56 (93.3)	60 (100)

Table 4. A number of sales of white and brown baguettes on the day of the intervention, pre-intervention and post-intervention

	Thursday one week before the intervention day	**Wednesday** one day before the intervention day	**Thursday** **Intervention day**	**Friday** one day after the intervention day	**Thursday** one week after the intervention day
Brown baguettes	73	63	135	71	72
White baguettes	70	58	0	75	80
Total	**143**	**121**	**135**	**146**	**152**

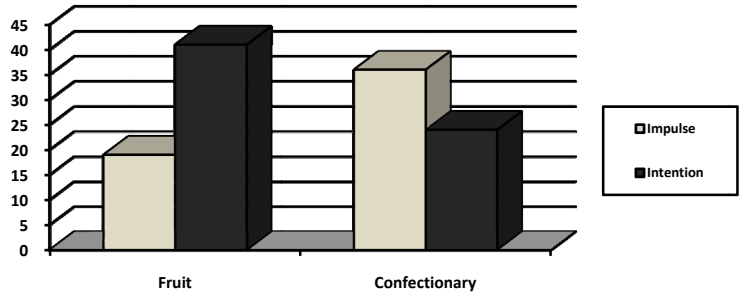

Figure 1. The number of sales of fruit and confectionary lead by impulse or intention during the studied 3-week period

Monitoring on the Presence of Ascorbic Acid in Not Prepacked Fresh Meat Preparations by a Validated HPLC Method

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Abstract

Ascorbic acid is a commonly-used food additive permitted in prepacked fresh meat preparations. Consequently, the addition of this antioxidant is subject to a specific packaging authorization. In this survey 180 samples of not prepacked fresh meat preparations have been analyzed in order to evaluate the diffusion of not authorized ascorbic acid additions. The ascorbic acid has been determined by a high performance liquid chromatography with diode array detection method, validated according to the European Legislation. Quantifiable concentrations of ascorbic acid (> LOQ = 20.1 mg · kg^{-1}) were registered in 33 samples confirming a wide diffusion of not authorized additions. Moreover, low concentrations not attributable to a additives additions were detected in 14 samples. Ascorbic acid sources (tomato) were declared on the label of these samples. Considering that the addition of this food additive is generally considered safe, these low concentrations registered may be useful for the determination of an allowable limit for the ascorbic acid in not prepacked fresh meat preparations.

Keywords: Ascorbic acid, Food additives, Meat preparations, Liquid chromatography, Validation

1. Introduction

The L-ascorbic acid and its salts (ascorbates) are commonly-used food additives permitted by the actual Normative in fresh meat preparations (hamburger, fresh sausages and minced meats) (Table 1). The ascorbic acid is a food antioxidant; the actual European Normative (European Commission, 2011) does not define legal limits for its employment, it makes reference to an ambiguous *"quantum satis"*.

This food additive is used in fresh meat preparations because it may exercise the following activities: meat colour development, inhibition of nitrosamines formation and prevention of oxidation and of colour fading also during product storage.

The characteristic cured meats colour is obtained by reduction of nitrosometamyoglobin to nitrosomyoglobin. The oxidation of ascorbic acid to dehydroascorbic acid that occurs when this additive is added to cured meats may accelerate this reduction (Watts & Lehmann, 1952; Izumi, 1992; Nam & Ahn, 2003). In addition, the ascorbic acid antioxidant action prevents the myoglobin oxidation to metamyoglobin which has a brown colour (Hughes, 2003).

The ascorbic acid may prevent the nitrosamines formation in meats through the reduction of nitrate to nitrogen oxide that does not react with secondary amines to form nitrosamines (Kalus & Filby, 1980; Hotchkiss & Cassens, 1987; Tannenbaum *et al.*, 1991).

Finally, the lipids oxidation may be retarded because the ascorbic acid addition and the consequent oxidation of this additive reduces the oxygen available for lipids breakdown (Roig *et al.*, 1993; Hillstrom *et al.*, 2003).

Is important to underline that this additive is permitted only in prepacked preparations of fresh minced meat. Consequently, a specific duty to obtain a food packaging authorization, released by the local health authority, subsists for the producer.

However, this duty is not respected in several cases. This is especially due to a law misinterpreting. In fact, the producers do not know the duty to obtain a packaging authorization and then they add this additive in prepacked products because ascorbic acid is permitted in minced meat preparations by the actual Normative (Paturzo & Bizzozero, 2001; Iammarino *et al.*, 2010).

In this work 180 samples of not prepacked fresh meat preparations (hamburger, fresh sausages and minced meats) were analyzed in order to evaluate the extent to which not authorized ascorbic acid addition is diffused.

The ascorbic acid has been determined by a high performance liquid chromatography with diode array detection method, validated according to the European Legislation.

In many samples low concentrations of ascorbic acid, not attributable to an additive addition, were detected. These concentrations were elaborated in order to evaluate a value that may be suggested as maximum admissible limit for ascorbic acid in not prepacked fresh meat preparations.

2. Materials and Methods

The investigation was performed on 180 samples of not prepacked fresh meats preparations (hamburger, fresh sausages and minced meats) composed of pork, cow, horse, chicken and mixed meats.

The samples were collected on several markets located in Foggia (Italy) during the period June 2010 – November 2011. Two replicates of each sample were analysed and the ascorbic acid contents were evaluated as mean of two measurements.

2.1 Chemicals and working standard solutions

L-Ascorbic acid (≥99.0%) and sodium acetate anhydrous were supplied by Sigma-Aldrich (Stenheim, Germany). Phosphoric acid (85.0%), acetic acid glacial and acetonitrile of HPLC grade were purchased from J.T. Baker (Deventer, Netherlands). Potassium phosphate monobasic (≥98.0%) and potassium phosphate bibasic (≥98.0%) were supplied by Carlo Erba Reagenti (Milan, Italy). All solutions were prepared in ultrapure water with a specific resistance of 18.2 MΩ-cm, supplied by a Milli-Q RG unit, Millipore (Bedford, MA, USA).

2.2 Sample preparation

A 4-g portion of sample (different types of fresh meat preparation), homogenized by blade homogenizer, was mixed with 40 mL of phosphate buffer 10^{-2} M pH 3.5, obtained dissolving 1.36g of potassium phosphate monobasic and 1.74g of potassium phosphate bibasic in 1000 mL of ultrapure water and then correcting the pH value to 3.5 by addition of phosphoric acid. The mixture was vortexed for one minute. After centrifugation for 5 min at 250 x g at room temperature, the supernatant was filtered through Whatman No. 40 filters (Whatman, Springfield Mill, UK) and then about 1.5 mL were filtered through Anotop 10 LC, 0.2 μm, 10 mm filters (Whatman, Springfield Mill, UK) directly in vial prior to chromatographic analysis.

2.3 Apparatus and method

The chromatographic method for the determination of ascorbic acid in meat products was optimized starting from a chromatographic separation proposed by Phenomenex (Torrance, CA) (Phenomenex Inc., 2011) that employs the Reversed Phase Liquid Chromatography coupled with UV-Diode Array Detection (UV-DAD) (Iammarino & Di Taranto, 2011). All the chromatographic separations were performed on a HPLC system, Waters™ 2690 Separations Module (Milford, MA) equipped with a Waters™ 996 PDA Detector (Milford, MA), a micro vacuum degasser, an autosampler and a column compartment. The chromatographic column was a Luna C18 column (250×4.6 mm i.d., particle size 5 μm. Phenomenex, Torrance, CA) equipped with a HILIC Security Guard Cartridge (4×3.0 mm. Phenomenex, Torrance, CA), operating at a flow-rate of 1.5 mL min^{-1} following the gradient elution of acetonitrile, water and acetate buffer 100 mM, pH 5.8 described in table 2.

Acetate buffer was obtained dissolving 7.708g of sodium acetate in 1000 mL of ultrapure water and then correcting the pH value to 5.8 by addition of glacial acetic acid.

The absorbance signal was detected at 260 nm. Setting the acquisition wavelength range from 200 to 500 nm, the diode array detector allows to obtain the ascorbic acid absorbance spectrum. It is possible to compare the spectrum of sample with the spectrum of a standard solution and increase the method selectivity though this comparison.

2.4 Validation procedures

The analytical method optimized for the ascorbic acid determination in meat products was validated by an *in-house* validation model, following the Thompson harmonized validation guidelines (Thompson *et al*, 2002) which describes the analytical parameters to appraise to assure the method reliability in agreement with Regulation 882/2004/EC (European Commission, 2004) and Decision 657/2002/EC (European Commission, 2002).

The parameters evaluated were linearity, specificity, detection and quantification limits (LOD and LOQ), accuracy, ruggedness and measurement uncertainty.

The method linearity was verified by the injection, on three different days, of three series of standard solutions at ascorbic acid concentrations of 12.5, 25, 50, 100 and 200 mg · L^{-1}. The linearity was verified for each calibration curve and for the mean curve verifying the determination coefficient values (r^2), higher than 0.990, where standard deviations of slope and intercept are estimated at the 95% confidance level (Table 3). A chromatogram of an ascorbic acid standard solution at a concentration of 50 mg · L^{-1} is reported in figure 1 with related absorbance spectrum.

The limit of detection (LOD) and quantification (LOQ) were elaborated according to the following equations: LOD= $3.3s_a/b$ and LOQ= $10s_a/b$ (Miller & Miller, 1993), where s_a is the standard deviation of the intercept and b is the slope of the linear regression. LOD and LOQ values of 6.6 mg · kg^{-1} and 20.1 mg · kg^{-1}, respectively, were obtained (Table 3).

The method specificity was demonstrated by analysing 20 fresh meat samples (5 cow, 5, pork, 5 horse and 5 chicken) and verifying the absence of interfering peaks in the retention time-window of interest (±2.5% of ascorbic acid retention time). In Figure 2 a chromatogram of a blank fresh meat sample is shown; the ascorbic acid is well separated from endogenous compounds under the optimized gradient elution, and the chromatogram is interference-free in the time-window of elution of the analyte.

Repeatability and recovery (accuracy) were evaluated by performing tests on three sets of blank cow fresh meat samples (six replicates each) fortified with ascorbic acid at concentrations of 50, 500 and 1000 mg kg^{-1}. The analyses were executed on different days by the same instrument but different operators (*Intermediate Precision*). The precision (expressed as CV%) and the recovery percentages obtained are reported in Table 4. By comparison with maximum standard deviations admitted by Horwitz equation, as reported in Decision (EC) No. 657/2002, the method precision was demonstrated. The recovery percentages, in the range 80-110% (reference range indicated in Decision (EC) No. 657/2002 for mass fraction ≥ 10 μg kg^{-1}) demonstrated the method trueness.

Method ruggedness under *major changes* conditions was evaluated by using the Youden factorial experimental design (Youden & Steiner, 1975). The seven factors chosen as variables for Youden test were the matrix and six fictitious factors. Consequently, the Youden experimental design requires twelve independent experiments: four with validation matrix (cow fresh meat) and four with each testing matrices. Different types of fresh meat samples: 4 pork, 4 horse and 4 chicken, fortified with 500 mg · kg^{-1} of ascorbic acid were analysed. The analyses of alternatives matrices (pork, horse and chicken fresh meats) gave a calculated standard deviation of difference (S_{Di}) lower than the estimated method precision (S_{Di}= 8.0), evaluated as twice the repeatability standard deviation of cow fresh meat samples at a fortification level of 500 mg · kg^{-1}. These results confirmed that the matrix variation has no effect on the analytical performances and consequently the method is also applicable to pork, horse and chicken fresh meat samples.

The estimation of the measurement uncertainty is compulsory for laboratories accredited ISO 17025 (International Organization for Standardization, 2000). Several approaches have been proposed for the determination of this parameter (EURACHEM/CITAC, 2000), in this work the bottom-up method using the data obtained from each step of the analytical procedure was used (Hund *et al*, 2001). The measurement uncertainty was calculated on the basis of the uncertainties propagation law, by the equation:

$$\bar{u} = \sqrt{(\bar{u}(C))^2 + (\bar{u}(V_f))^2 + (\bar{u}(w))^2}$$

Where ū indicates the relative uncertainty, C is the analyte concentration in matrix, V_f is the final extract volume and w is the sample weight. The determination of ū(C) was performed considering four uncertainty sources: (a) standards preparation; (b) method repeatability; (c) method recovery; (d) calibration curve. A relative expanded measurement uncertainty of 5.6% was obtained by using a coverage factor k of 2, corresponding approximately to a 95% confidence level. This parameter confirmed the method reliability and the laboratory technical competence in the quantitative determination of the ascorbic acid in meat products.

3. Results and Discussion

The results of the analyses performed on 180 samples of cow, pork, horse, chicken and mixed fresh meat preparations (hamburger, fresh sausages and minced meats), collected in local markets of Foggia (Italy) are reported in table 5. The samples resulted "positive" for ascorbic acid (> LOQ = 20.1 mg · kg^{-1}) (33 samples, equal to 18.3%) were classified into two categories in relation to the measured concentrations.

In the first category, of "*not-compliant*" (19 samples, equal to 10.6%), the samples with ascorbic acid concentrations higher than 160.0 mg kg^{-1} were inserted. These high ascorbic acid concentrations are surely to attribute to not allowed additive additions.

In the range 41.2 – 160.0 mg kg^{-1} there were no samples with quantifiable concentrations of ascorbic acid, so it was considerate a second category, of *"compliant"* (14 samples, equal to 7.8%), including the samples with ascorbic acid concentrations lower than 41.2 mg · kg^{-1}. It is important to underline the presence of tomato (fresh, sauce or tinned peeled), reported on the products labels of these samples,.

In Graphic 1 the subdivision into negative, "*compliant*", and "*not-compliant*" samples is shown.

A comparison between two chromatograms related of these two categories of positive samples is reported in Figure 2.

The fraudulent addition of ascorbic acid in the samples with low concentrations of ascorbic acid (*"compliant samples"*) seems not likely. In fact, the ascorbic acid cannot exercise a useful antioxidant activity at these levels. Reading the products ingredients reported on the labels, it seems more appropriate to attribute such ascorbic acid residues to the presence of tomato (fresh, sauce or tinned peeled).

Considering that the ascorbic acid addition to foodstuffs is generally considered as safe (FDA, 1979) it is possible to suggest a maximum admissible level in not prepacked fresh meat preparations, if ascorbic acid sources (such as tomato) are indicated on the product label.

In order to simplify the identification of this cutoff value, the distribution of the ascorbic acid concentrations registered is described in Figure 3, (the samples with ascorbic acid concentrations higher then 400 mg kg^{-1} are not included). It is possible to verify that there are no *"compliant"* samples that exceed 50 mg kg^{-1} of ascorbic acid.

Taking into account the distribution of the observed ascorbic acid concentrations, considering the measurement uncertainty of the method (5.6%) and an appropriate tolerance, it is possible to suggest a maximum allowable limit of 50.0 mg kg^{-1} in not prepacked fresh meat preparations (in the presence of ascorbic acid sources). Below this value the sample should be considered as *"compliant"*.

Numerous cases of not allowed ascorbic acid additions were verified during this survey.

Through this monitoring it was possible to verify that these irregularities are probably not attributable to a real necessity for the producer, but to a lacking disclosure of the legislation.

In fact, the most part of producers were not informed on the limitations related to the ascorbic acid use, and they proceed with this additive addition because it is permitted in minced meat preparations by the actual Normative.

Considering the high percentage of positive samples registered there is the necessity to more disclose this duty law.

4. Conclusions

From a survey of 180 not prepacked fresh meat preparations samples, ascorbic acid concentrations higher than the method limit of quantification (LOQ = 20.1 mg kg^{-1}) were registered in 33 samples. In 19 samples ascorbic acid concentrations from 160.0 mg kg^{-1} until to 4170.0 mg kg^{-1}, surely to attribute to not allowed additive additions, were registered. In 14 samples such concentrations were in the range 21.9 – 41.2 mg kg^{-1}. These low concentrations are probably to attribute to the presence of ascorbic acid sources (such as tomato) in the product.

Considering the high percentage of positive samples registered, there is the necessity to more disclose the duty law that foresees the obtainment of a packaging authorization for the producer that wants to add ascorbic acid in prepacked products.

In addition, taking into account the distribution and the percentages of ascorbic acid registered at low concentration, it is possible to suggest a cut-off value of 50.0 mg kg^{-1}. This value may be useful for a future estimation of a maximum allowable limit for the ascorbic acid in not prepacked fresh meat preparations, if ascorbic acid sources are present in the product formulation.

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References

EURACHEM/CITAC. (2000). GUIDE CG 4: *Quantifying Uncertainty in Analytical Measurement*. Ellison, S. L. R., Rosslein, M., Williams, A. (Eds). (2nd ed.), Teddington (UK).

European Commission. (2002). Decision (EC) No. 657/2002. 12 August 2002. *Official Journal of the European Union, 221*, 8-36.

European Commission. (2004). Regulation (EC) No. 882/2004. 29 April 2004. *Official Journal of the European Union, 165*, 1-141.

European Commission. (2011). Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. *Journal of the European Union, 295*, 98-99.

Food and Drug Administration. (1979). Database of Select Committee on GRAS Substances (SCOGS) Reviews, L-ascorbic acid, Report No. 59, ID Code 50-81-7.

Hillstrom, R. J., Yacapin-Ammons, A. K., & Lynch, S. M. (2003). Vitamin C inhibits lipid oxidation in human HDL. *J. Nutr., 133*(10), 3047-51.

Hotchkiss, J. H., & Cassens, R. G. (1987). Nitrate, nitrite and nitroso compounds in foods. *Food Technol, 41*(4), 127-134.

Hughes, H. (2003). Best Food Writing. New York: Marlowe & Company.

Hund, E., Massart, D. L., & Smeyers-Verbeke, J. (2001). Operational definitions of uncertainty. *Trends Analytcal Chemistry, 20*(8), 394-406. http://dx.doi.org/10.1016/S0165-9936(01)00089-9

Iammarino, M., & Di Taranto A. (2011). Preparazioni carni fresche: HPLC per la determinazione di sostanze antiossidanti non ammesse. *Laboratorio 2000,* 44-50.

Iammarino, M., Di Taranto, A., & Muscarella, M. (2011). Investigation on the natural presence of substances with preservative effects in minced meat preparations. *Ingredienti Alimentari,* 15-23.

International Organization for Standardization. (2000). General requirements for the competence of testing and calibration laboratories.

Izumi, K. (1992). Reaction of nitrite with ascorbic acid or ascorbic acid-2-derivatives. *J. Food. Sci., 57*, 1066. http://dx.doi.org/10.1111/j.1365-2621.1992.tb11264.x

Kalus, W. H., & Filby, W. G. (1980). Inhibition of nitrosamine formation by ascorbic acid: participation of free radicals in its anaerobic reaction with nitrite. *Cellular and Molecular Life Sciences., 36*(2), 147-149. http://dx.doi.org/10.1007/BF01953698

Miller, E. J. C. & Miller, J. N. (1993). *Statistics for Analytical Chemistry* (3rd ed.), New York, NY, USA: Ellis Horwood PTR Prentice Hall, p.115.

Nam, K. C., & Ahn, D. U. (2003). Effects of ascorbic acid and antioxidants on the color of irradiated ground beef. *Journal of food science, 68*(5), 1686-1690. http://dx.doi.org/10.1111/j.1365-2621.2003.tb12314.x

Paturzo, F., & Bizzozero, N. (2001). Risultati di un'indagine sul contenuto di additive in campioni commerciali di carne trita. *Ingegneria Alimentare, 3*, 15-16.

Phenomenex, Inc. (2011). Chromatography Product Guide 11/12. Vitamin Mix on Luna HILIC, p. 207.

Roig M. G., Rivera, Z. S., & Kennedy J. F. (1993). L-ascorbic acid: An overview. *International Journal of Food Sciences and Nutrition, 44*(1), 59-72. http://dx.doi.org/10.3109/09637489309017424.

Tannenbaum, S. R., Wishnok, J. S., & Leaf, C. D. (1991). Inhibition of nitrosamine formation by ascorbic acid. *Am. J. Clin. Nutr, 53*(1 Suppl). 247S-250S. http://dx.doi.org/10.1111/j.1749-6632.1987.tb23774.x

Thompson, M., Ellison, S. R. L., & Wood, R. (2002). Harmonized guidelines for single laboratory validation of methods of analysis. *Pure Appl. Chem, 74*(5), pp. 835–855. http://dx.doi.org/10.1351/pac200274050835

Watts, B. M., & Lehmann, B. T. (1953).The effect of ascorbic acid on the oxidation of hemoglobin and the formation of nitric oxide hemoglobin. *Journal of food science, 17*, 100-108. http://dx.doi.org/10.1111/j.1365-2621.1952.tb16744.x

Youden, W. J., & Steiner, E. H. (1975). *Statistical Manual of the AOAC - Association of the Official Analytical Chemists*. AOAC-I, Washington, DC, p. 35.

Table 1. Food additives permitted in prepacked preparations of fresh minced meat

E-number	Name	Maximum level
E 261	Potassium acetate	*quantum satis*
E 262	Sodium acetates	*quantum satis*
E 300	Ascorbic acid	*quantum satis*
E 301	Sodium ascorbate	*quantum satis*
E 302	Calcium ascorbate	*quantum satis*
E 325	Sodium lactate	*quantum satis*
E 326	Potassium lactate	*quantum satis*
E 330	Citric acid	*quantum satis*
E 331	Sodium citrate	*quantum satis*
E 332	Potassium citrate	*quantum satis*
E 333	Calcium citrate	*quantum satis*

Table 2. Gradient elution settings

Time (minutes)	%A (Acetonitrile)	%B (Water)	%C (Acetate buffer)
0.0	90	5	5
2.5	90	5	5
7.5	50	45	5
10.0	50	45	5
11.0	90	5	5
15.0	90	5	5

Table 3. Calibration parameters and decision limits

$y = a + bx$ [a]				
a ± SD	b ± SD	R^2 [b]	LOD[c]	LOQ[c]
$(3.5 \pm 4.8)10^4$	$(92.0 \pm 0.9)10^3$	0.9997	6.6	20.1

[a] y is the peak area of ascorbic acid and x is the concentration in mg · L^{-1}.

[b] Determination coefficient.

[c] Expressed as ascorbic acid in mg · kg^{-1} of sample.

Table 4. Precision and recovery of the validated method

Ascorbic Acid					
CV% (n = 6)			Recovery % (n = 6)		
Fortification level: 50 mg · kg^{-1}	Fortification level: 500 mg · kg^{-1}	Fortification level: 1000 mg · kg^{-1}	Fortification level: 50 mg · kg^{-1}	Fortification level: 500 mg · kg^{-1}	Fortification level: 1000 mg · kg^{-1}
3.8	4.0	5.3	100.9	97.7	104.0

Table 5. Results obtained by analysing 180 fresh meat preparations samples

Type of fresh meat preparation	Meat origin	Negative samples (<LOQ = 20.1 mg · kg^{-1})	*"Not-compliant"* samples Concentration range[a]: [160 – 4170]	*"Compliant"* samples Concentration range[a]: [21.9 – 41.2]
Hamburger (59 samples)	Cow (16)	12	1 190.0±10.6	3 22.6±1.3 23.7±1.3 36.6±2.0
	Pork (12)	8	2 330.0±18.5 223.0±12.5	2 25.6± 1.4 29.6±1.7
	Chicken (5)	5	0	0
	Cow/Pork (26)	19	4 1259.0±70.5 4170.0±233.5 1600.0±89.6 200.0±11.2	3 32.9±1.8 41.2±2.3 36.7±2.1
Fresh sausages (83 samples)	Cow (13)	11	0	2 33.1±1.9 38.7±2.2
	Pork (30)	25	3 340.0±19.0 160±9.0 860.0±48.2	2 34.2±1.9 30.0±1.7
	Chicken (10)	10	0	0
	Cow/Pork (19)	16	2 831.0±46.5 830.0±46.5	1 25.1±1.4
	Horse (7)	5	1 256.0±14.3	1 21.9±1.2
	Horse/Pork (4)	3	1 1015.0±56.8	0
Minced meat (38 samples)	Cow (5)	5	0	0
	Pork (15)	13	2 253.0±14.2 237.0±13.3	0
	Cow/Pork (13)	11	2 191.0±10.7 170.0±9.5	0
	Chicken (5)	4	1 331.0±18.5	0

[a] Expressed as mg · kg^{-1} of Ascorbic acid ± expanded measurement uncertainty percentage.

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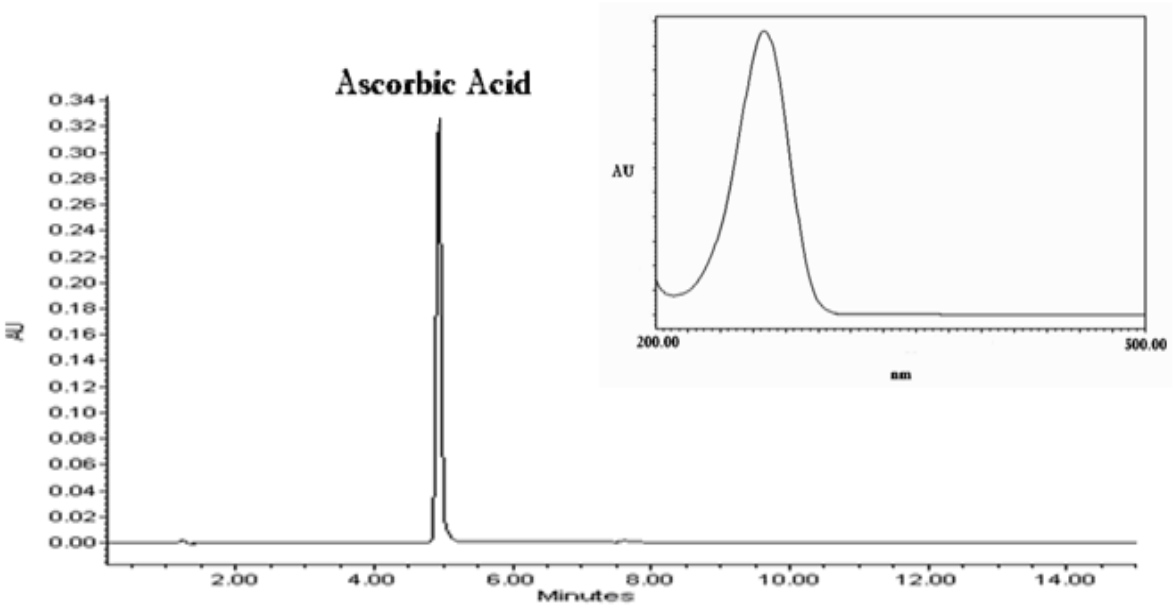

Figure 1. Chromatogram of an ascorbic acid standard solution at a concentration of 50 mg L^{-1}. The related absorbance spectrum is shown in the box

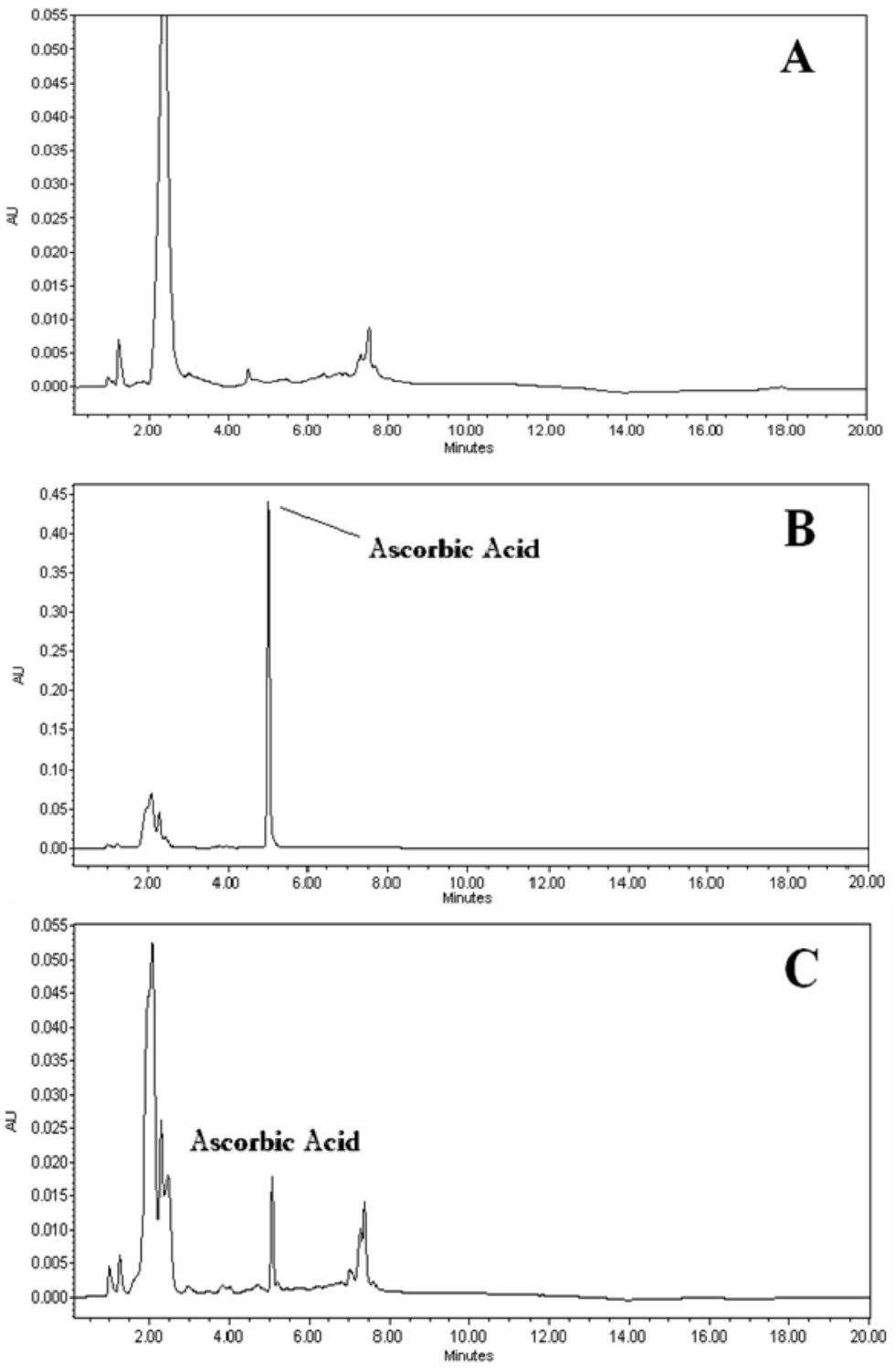

Figure 2. Chromatograms comparison: Blank cow fresh meat sample (A); "*Not-compliant*" sample: fresh cow/pork sausage with an observed ascorbic acid concentration of 831.0 mg kg^{-1} (B); "*Compliant*" sample: cow/pork hamburger with an observed ascorbic acid concentration of 32.9 mg kg^{-1} (C)

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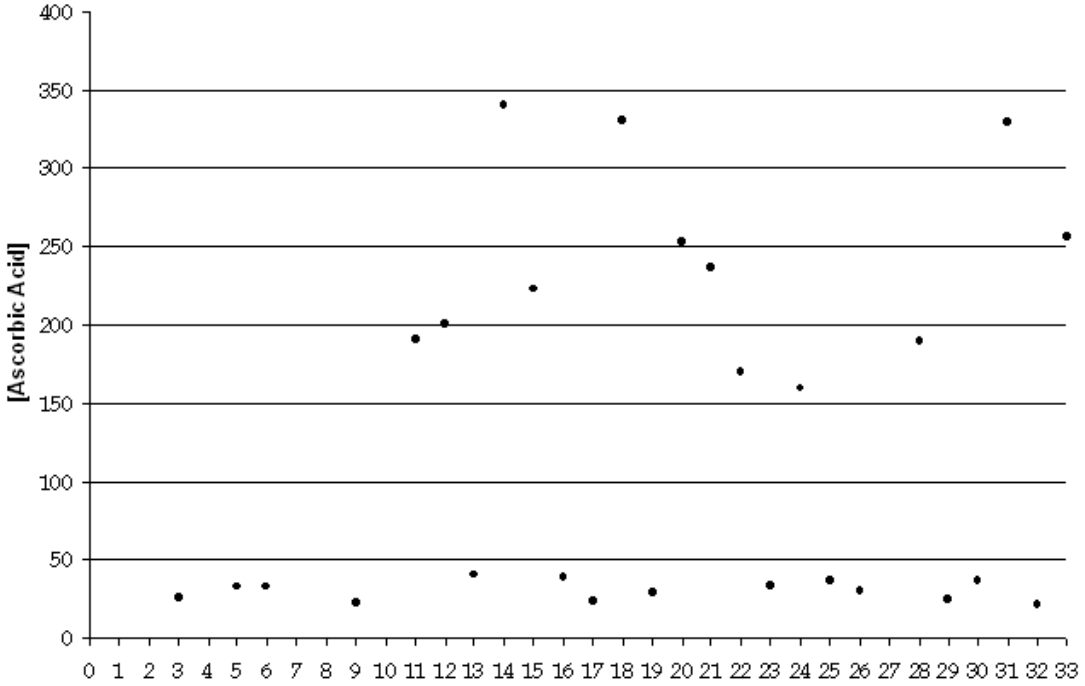
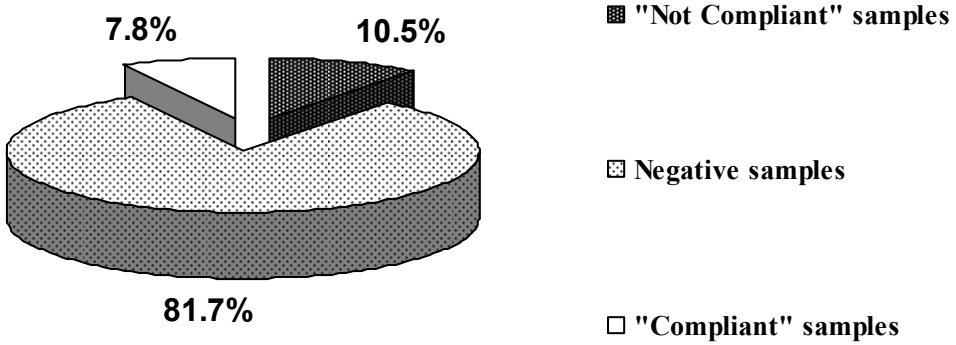


Figure 3. Distribution of ascorbic acid concentrations observed in positive samples. Values indicated as mg kg^{-1} of ascorbic acid



Graphic 1. Subdivision into negative, "*Not-compliant*" and "*Compliant*" samples

Negative Spillover Effect on Demand for US Pork and Vegetables Caused by BSE Outbreak

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Abstract

After the BSE outbreak in December 2003 in the United States, Japanese consumers refrained from buying US beef and other agricultural products produced in the US. We use a probit model to analyze how the BSE outbreak has affected demand for products other than beef and what household characteristics have an impact on decisions to purchase US agricultural products. We show that the BSE outbreak has had a significant impact on the purchasing decisions of Japanese consumers. Presence of children in the household and interest in country-of-origin information also significantly affected consumer attitudes and their purchases of US agricultural products.

Keywords: BSE, Probit model, Purchase decisions

1. Introduction

Consumers cannot fully assess the safety of agricultural products directly. Hence, consumers must rely upon other factors such as national image to evaluate the quality of products from a country (van der Lans *et al*., 2001; Beverland & Lindgreen 2002; van Ittersum *et al*., 2003x). They use this image as stereotypical information (Janda & Rao, 1997; Maheswaran, 1994; Hong & Wyer, 1989, 1990). Once a country's image is damaged by a food safety crisis, consumers may gain an impression of health risks from stereotypical information and change their purchasing behavior. That is, the crisis creates concerns of negative spillover effects to totally unrelated agricultural products from the same country. In fact, the outbreak of bovine spongiform encephalopathy (BSE) in December 2003 in the United States (US), led some Japanese consumers to refrain from buying not only US beef but also other agricultural products from the US. Such negative spillover effects result in economic losses to producers and distributors of agricultural products. Therefore, it is important to understand the negative spillover effect of the food safety crisis on other agricultural products from the same country.

Various studies have analyzed the impact of agricultural product safety crises on demand for related products. Verbeke and Ward (2001), Gould and Villarreal (2002), Marsh et al. (2004), Peterson and Chen (2005) and Saghaian et al. (2007) analyze the impacts of the BSE outbreak on demand for beef and on other meats that are substitutes for beef. Nevertheless, they do not focus on negative spillover effects on other agricultural products.

How then does the level of negative spillover effect vary according to household? Responses to a food safety crisis vary across consumers (Barrena & Sánchez, 2010). Therefore, we can consider the possibility that the level of negative spillover effect also varies across types of households. While this has an important implication for risk communication, there are no empirical research analyses of this question.

Inconsistent behavior of consumers in different types of households in the face of a product-related crisis can be explained not only by risk perception but also by risk attitude (Pennings *et al*., 2002). Risk perception is a consumers' assessment of the uncertainty of a risk. Risk attitude explains the extent to which consumers dislike the risk. Economic analyses of risk perception and risk attitude are based on the random utility model. In the random utility model, risk perception is a consumer's subjective view of the probability of risk (Zepeda *et al*., 2003). In addition, risk attitude is considered a combination of utility from consumption of products and disutility from health problems in the model. Specifically, we can identify consumers' purchase decisions regarding US agricultural products by comparing the expected utility when the product is "safe" and that when it is hazardous to health

We analyze how the negative spillover effect caused by the BSE outbreak in the US varies according to the attributes of Japanese households by means of a random utility model. We deal with this case for the following two reasons. First, Japanese consumers reacted strongly to the BSE outbreak because it is well known in Japan that BSE is a serious disease of cattle and is potentially transmissible to humans through eating infected meat. Second, it is easy to detect changes in consumer behavior before and after the BSE outbreak in Japan because many Japanese consumers had purchased US agricultural products before it.

We choose pork, which is perceived as a substitute for beef, and vegetables, which are not believed to be related directly to demand for beef to analyze the difference between the impacts of the BSE outbreak on agricultural products. Because there may be a difference between utility functions for pork and for vegetables, we consider the expected utility functions for the respective agricultural products.

We also note that the level of negative spillover effects may vary with the subjective probability of and disutility from health hazards, and utility from the US agricultural products, which depend on household characteristics. Therefore, we conducted a questionnaire survey of randomly sampled households to discover these factors. In this study, we formulate the purchase decisions for US pork and vegetables using a probit model. A summary of our results is as follows. (1) Households that buy US beef tend not to refrain from buying US pork and vegetables. (2) Households that consume less beef have a tendency not to reduce their demand for US pork and vegetables. (3) Households that care about the origin of their agricultural products tend to refrain from buying US agricultural products. (4) Households without children are apt to refrain from buying US pork compared with households with children.

This paper consists of six sections. In the second section, we give an outline of the questionnaire survey conducted in this study. We explain the model in the third section, and show the empirical results in the fourth section. In the fifth section, we estimate the probabilities of purchasing US agricultural products. In the final section, we summarize this paper and mention some remaining issues.

2. Survey Overview

We conducted a questionnaire survey in November 2006 to observe the impact of the BSE outbreak in the US on Japanese consumers' demand for US agricultural products. We constructed a sample of 1000 subjects from households in Osaka and Tokyo to compare consumer attitudes in these areas. In Japan, there are areas where consumers favor beef over pork and vice versa. Osaka is a notable area where residents consume more beef than pork, and in Tokyo, residents consume more pork than beef. We can see this difference by comparing expenditure on beef and pork in Figure 1. Figure 1 shows that households in Osaka purchase more beef than do households in Tokyo, and households in Tokyo purchase more pork than do households in Osaka. Consumers who prefer beef may perceive a higher probability of health hazards from beef (Renner *et al*., 2008). We assume that the subjective probability of suffering health hazards from US agricultural products is higher in areas where households consume more beef than pork. We chose subjects at random from the telephone directory and sent them questionnaires with gift cards worth 500 yen as a reward for cooperating in the survey.

In the questionnaire, we asked whether respondents had consumed US beef "before" the BSE outbreak occurred

in the US and about their willingness to buy US beef "after" the event. The Japanese government banned imports of US beef until December 2005, after which all imports of US beef were again banned from January 2006. Moreover, some retail outlets in Japan hesitate to sell US beef even after the resumption of trade in July 2006. The question in our questionnaire was thus not whether the respondents had bought US beef, but whether it was acceptable for them to buy it. We asked whether they bought US beef at the time when the US had not introduced blanket testing of cows for BSE. If the answer was "no," we also asked whether they would buy US beef if blanket testing were introduced. Moreover, we asked whether respondents had consumed US pork (and US vegetables) before the BSE outbreak, and whether they had refrained from buying US pork (and US vegetables) after the event, to detect shifts in consumer attitudes before and after the BSE outbreak.

We also asked households whether they were concerned about where their agricultural products came from before the BSE outbreak. We consider that the interest of consumers in country-of-origin information was one of the most important factors influencing the demand for agricultural products. That is, consumers who are concerned about country-of-origin information may have a greater negative impact on the demand for US agricultural products because of the BSE outbreak. It is also noted that family structure and income of households affect the demand for agricultural products with potential health hazards (Lin 1995; Dosman *et al*., 2002). Therefore, we asked households about their family structure and income, which may affect the demand for US agricultural products. In terms of family structure, we asked whether households had any members aged under 20 or over 60 years.

We had 489 surveys returned from Tokyo and 525 from Osaka. In this study, we focus on the households that had purchased all three US agricultural products (beef, pork and vegetables) before the BSE outbreak, because it was assumed that the other households never bought US agricultural products. There were 193 respondents in Tokyo and 190 in Osaka who had purchased all three US agricultural products before the BSE outbreak.

Table 1 shows the percentages of households that did not avoid US pork and vegetables in each area (Tokyo and Osaka), and, in each case, the willingness of consumers to buy US beef with or without blanket testing. We obtain three interesting results. First, households that were willing to buy US beef (regardless of blanket testing) tended not to avoid either of the other US agricultural products compared with those who were unwilling to do so even if blanket testing were to be introduced. Second, households in Tokyo that consumed less beef tended not to avoid the other two US agricultural products compared with households in Osaka that preferred beef. Finally, some households avoided US pork rather than vegetables because of the BSE outbreak. These results indicate that the probability of avoiding US agricultural products varies according to both consumer attitudes toward US beef and frequency of households' consumption of beef. Moreover, we note that the BSE outbreak had different impacts on the demands for US pork and vegetables.

3. Model

In this paper, we analyze the types of households that tended to avoid US agricultural products other than beef in response to the BSE outbreak according to the results of the questionnaire survey. We also clarify the differences in the impact of BSE on these products. Viscusi (1990) analyzes behavior of consumers faced with risk using a discrete choice demand model and assumes that consumers maximize their expected utility under uncertainty. According to Viscusi (1990), we construct a consumer decision-making model for US pork and vegetables as follows.

We suppose that households decide whether to avoid an agricultural product ($y_j = 0$) or not ($y_j = 1$) in accordance with the magnitude of their expected utility. The subscript j indicates US pork and vegetables and takes $pork$ or $vegetable$, respectively. We assume that if consumers bought US agricultural products, they might suffer a health hazard. Let π_j be the subjective probability of suffering a health hazard from the agricultural products. We also assume that consumers have a utility function $U_j(safe)$ if they do not suffer a health hazard and have $U_j(\mathrm{h}azard)$ if they do. We specify that the expected utility goes to zero if households refrain from buying agricultural products. Households do not avoid US agricultural products if:

$$(1-\pi_j)U_j(safe)+\pi_j U_j(\text{h}azard)>0 \tag{1}$$

and otherwise they do. We can rewrite (1) as:

$$U_j(safe)+\pi_j\left(U_j(\text{h}azard)-U_j(safe)\right)>0\,. \tag{2}$$

The second term of the left-hand side, $U_j(\text{h}azard)-U_j(safe)$, indicates disutility when consumers suffer health hazards from US agricultural products.

We measure the consumer decisions to purchase US agricultural products using a probit model based on equation (2). The probit model is one of the discrete choice models that estimate the effects of explanatory variables on the binary outcomes. For example, Verbeke *et al*., (2000) use such a model to analyze the factors that affected consumer purchase decisions for beef after the BSE crisis.

First, let y_i^* be a continuous variable that represents the latent preference of individual i. Based on equation (2), we set the latent variable y_i^* as:

$$y_j^{\ *}=U_j(safe)+\pi_j\left(U_j(\text{h}azard)-U_j(safe)\right)+u \tag{3}$$

where u is the error term that follows a standard normal distribution. We suppose that the observed choice y_i is related to the latent variable y_i^* in accordance with the following equation:

$$\begin{aligned} y_j&=1 \quad if \quad y_j^{\ *}>0,\\ y_j&=0 \quad if \quad otherwise. \end{aligned} \tag{4}$$

We represent the probability $\text{Prob}\,(y_j=1)$ that households do not refrain from buying each US agricultural product after the BSE outbreak as follows:

$$\text{Prob}\,(y_j=1)=\Phi\left[U_j(safe)+\pi_j\left(U_i(\text{h}azard)-U_j(safe)\right)+u\right] \tag{5}$$

and the probability $\text{Prob}\,(y_j=0)$ that households are discouraged from buying US products is expressed by:

$$\text{Prob}\,(y_j=0)=1-\Phi\left[U_j(safe)+\pi_j\left(U_j(\text{h}azard)-U_j(safe)\right)+u\right] \tag{6}$$

where $\Phi[\bullet]$ is the cumulative distribution function of the standard normal distribution. Then the log likelihood function is denoted as:

$$\begin{aligned} \log L=&\sum_{y_j=1}\log\left[\Phi\left\{U_j(safe)+\pi_j\left(U_j(\text{h}azard)-U_j(safe)\right)\right\}\right]\\ &+\sum_{y_j=0}\log\left[1-\Phi\left\{U_j(safe)+\pi_j\left(U_j(\text{h}azard)-U_j(safe)\right)\right\}\right] \end{aligned} \tag{7}$$

Therefore, the difference in degree of negative spillover effect is explained by the difference in π_j, $U_j(hazard)-U_j(safe)$ and $U_j(safe)$ among households. In this model, risk perception is indicated by π_j and risk attitude by $U_j(hazard)-U_j(safe)$ and $U_j(safe)$. Next, we specify these variables as follows.

Consumers' beef purchasing behavior after the BSE outbreak may be linked to the level of damage to the national image of US agricultural products. Specifically, it may be considered that consumers who avoid US beef because of the BSE outbreak also perceive high risks in other agricultural products from the US. Therefore, we classify consumers into three categories depending on their willingness to buy US beef: (1) consumers who are willing to buy US beef at this time, when the US has not yet introduced blanket testing; (2) consumers who would be willing to buy US beef if blanket testing were introduced; and (3) consumers who would not be willing to buy it even if blanket testing were introduced. We assume that the subjective perception of the probability of a health hazard from US agricultural products, π_j, depends on the willingness of consumers to buy US beef after the BSE outbreak and where they live.

Area of residence may also affect the level of perceived risk. Tonsor et al. (2009) show that perceived risk of BSE differs among countries. Purchasing behavior, which includes frequency of purchase, is dependent on country or area of residence. Frequency of purchase of beef affects the ability to assess its safety (Henson and Northen, 2000). The more beef a consumer buys, the higher the probability of encountering a perceptible health hazard (Renner *et al*., 2008). Therefore, consumers who had purchased more US beef may have perceived greater risk, not only in US beef, but also in other US agricultural products. Thus, we distinguish between households in Tokyo, where residents tend to consume pork rather than beef, and those in Osaka where residents prefer beef. Then, we formulate π_j as follows:

$$\pi_j = \theta_{1j} + \tau_{11j} beef_A + \tau_{12j} beef_B + \tau_{13j} tokyo \tag{8}$$

The variable $beef_A$ is a dummy variable that takes a value of one if households are willing to buy US beef, and zero otherwise. The variable $beef_B$ is a dummy variable that takes the value of one if households would be willing to buy US beef if blanket testing were introduced, and zero otherwise. The variable $tokyo$ is a dummy that takes the value of one if households are in Tokyo, and zero if they are in Osaka. We also note that θ_{1j} is a constant term and τ_{11j}, τ_{12j}, and τ_{13j} are parameters.

The interest of consumers in country-of-origin information may affect the level of risk they accept. Consumers who estimate greater disutility from US agricultural products may pay more attention to the source of the agricultural products. Therefore, we specify the disutility from health hazard $U_j(\text{hazard}) - U_j(safe)$ using variables that indicate the level of awareness of country-of-origin information. That is:

$$U_j(hazard) - U_j(safe) = \theta_{2j} + \tau_{21j} country_j \tag{9}$$

The variable $country_j$ is a dummy that takes the value of one if households pay attention to country-of-origin information of agricultural products, and zero otherwise; θ_{2j} is a constant term; and τ_{21j} is a parameter.

Family structure and household income affect the preference for US pork and vegetables. Therefore, we classify households into two categories according to age of family members: (1) households with one or more members aged under 20 years, and (2) households with one or more members aged over 60 years. We also take the logarithm of household income and use it as an explanatory variable. Then, we formulate $U_j(safe)$ as:

$$U_j(safe) = \theta_{3j} + \tau_{31j} chi + \tau_{32j} old + \tau_{33j} \log(inc) \tag{10}$$

Where $j = pork, vegetable$. The variable chi is a dummy variable that takes the value of one if a member of the household is under 20, and zero otherwise. The variable old is a dummy variable that takes the value of

one if a member of the household is over 60, and zero otherwise. The variable $\log(inc)$ is the logarithm of income; θ_{3j} is a constant term; and τ_{31j}, τ_{32j}, and τ_{33j} are parameters.

Substituting equations (8), (9), and (10) into equation (3), we represent the latent variable y_i^* as:

$$\begin{aligned} y_j^* &= \theta_{3j} + \tau_{31j} chi + \tau_{32j} old + \tau_{33j} \log(inc) \\ &\quad + \left(\theta_{1j} + \tau_{11j} beef_A + \tau_{12j} beef_B + \tau_{13j} tokyo\right)\left(\theta_{2j} + \tau_{21j} country_j\right) + u \end{aligned} \tag{11}$$

If we express $y^* = f(x) + u$, we can rewrite equation (7) as:

$$\log L = \sum_{y_j=0} \log\left[1 - \Phi\{f(x)\}\right] + \sum_{y_j=1} \log\left[\Phi\{f(x)\}\right] \tag{12}$$

The definitions and the means of explanatory variables by area are shown in Table 2. To discuss the representativeness of the sample in terms of demographics, family structure and income, we compare the means shown in Table 2 with those of the population. The sample means of the demographic variables are as follows. The proportion of households with children under 20 years old is 0.24 in Tokyo and 0.30 in Osaka. The proportion of households with a person over 60 years old is 0.64 in Tokyo and 0.58 in Osaka. Average annual household income is 7.22 million yen in Tokyo and 6.63 million yen in Osaka. The figures for the population in each area are as follows. According to census data, the proportion of households with children under 18 years old is 0.18 in Tokyo and 0.24 in Osaka. The census also shows that the proportion of households with an elderly person over 65 years old is 0.28 in Tokyo and 0.32 in Osaka. According to a national survey of family income and expenditure, average annual household income is 7.80 million yen in Tokyo and 6.44 million yen in Osaka. We cannot make a simple comparison between the sample and the population, because in the census, children are defined as those under 18 years old and elderly people are defined as those over 65 years old. Even so, we note that sampling is biased toward households with elderly people. Therefore, we must consider how demographics affect consumer decision making below.

4. Results

We estimate a probit model using the maximum likelihood method. First, we set constant terms θ_{1j} and θ_{2j} equal to one to identify θ_{3j}. We choose a model that minimizes Akaike's information criterion (AIC) among all the combinations of the candidates for explanatory variables. The criterion is expressed as:

$$AIC = -2\ln(L) + 2k$$

Where L is the likelihood of the model and k is the number of parameters. The empirical results of the full model, including all explanatory variables, and the minimum AIC model are reported in Table 3.

Table 3 shows that the estimated coefficient of $beef_A$ is positive and statistically significant in the decision-making model for both pork and vegetables. This indicates that households that are willing to buy US beef without blanket testing tend not to avoid US pork or vegetables. We also note that the coefficient of $beef_B$ is estimated as positive and statistically significant in the decision-making model for pork and the value is smaller than that for $beef_A$. The difference in magnitude of the coefficient for $beef_A$ and $beef_B$ implies that the BSE outbreak impacted the demand, not only for US beef, but also for other agricultural products produced in the US; however, households that estimate their own level of health risk as being lower do not appear to avoid US pork.

We also note that the estimated coefficient of $tokyo$ is positive in the decision-making model for both products and statistically significant in the model for pork. That is, households in Tokyo, where residents are less fond of beef, tend not to avoid US agricultural products compared with those in Osaka. This means that households that

prefer beef, in other words, households that have more opportunities to consume it, may perceive a higher probability of a health hazard from US agricultural products, as Renner et al. (2008) point out.

Both of the estimated coefficients of $country_{pork}$ and $country_{vegetable}$ are negative and statistically significant.

That is, households that pay more attention to country-of-origin information for agricultural products tend to avoid US agricultural products. This implies that households that are nervous about the origin of agricultural products may estimate their disutility of health hazard to be higher than that of others.

We show that the coefficient for chi is estimated as positive and statistically significant in the decision-making model for pork. In other words, households with children under 20 are less likely to avoid US pork than those without. This may be because of a difference in dietary patterns in households with or without children. That is, we consider that households with children obtain greater utility from consuming US pork compared with other households.

5. Analysis of Purchase Probability

In this section, we assess the probabilities of refraining from buying US agricultural products in terms of household characteristics. In this section, we describe the probabilities of avoiding products as "hesitation probabilities." To examine avoidance in more detail, we conduct the following two analyses using the results obtained in the previous section. First, to verify what characteristics of households have a significant impact on the decisions of households to purchase US agricultural products, we clarify the characteristics of households according to the results of the estimated variables in the minimum AIC model shown in Table 3. Table 3 shows that the variables $tokyo$ and $country_j$ are selected in the minimum AIC decision-making model for both agricultural products. Therefore, we split the characteristics of households into two types: households living in Tokyo (i.e., the case where $tokyo = 1$); and households in Osaka (i.e., the case where $tokyo = 0$). Moreover, we also split the characteristics of households into two groups: households that pay attention to country-of-origin information for agricultural products (i.e., the case where $country_j = 1$); and households that do not (i.e., the case where $country_j = 0$).

In terms of a willingness to buy US beef, both $beef_A$ and $beef_B$ are selected in the decision-making model for pork, and $beef_A$ is selected in the model for vegetables. To estimate the probability of buying US pork, we divide the characteristics of households into three types: (1) households willing to buy US beef without blanket testing (i.e., the case where $beef_A = 1$ and $beef_B = 0$); (2) households unwilling to buy US beef if blanket testing were introduced (i.e., the case where $beef_A = 0$ and $beef_B = 1$); and (3) households unwilling to buy US beef even if blanket testing were introduced (i.e., the case where $beef_A = 0$ and $beef_B = 0$). For US vegetables, we also divide the characteristics of households into two types: (1) households willing to buy at this time, when the blanket testing has not yet been introduced (i.e., the case where $beef_A = 1$); and (2) others (i.e., the case where $beef_A = 0$).

The variable chi, which indicates whether the household has children, is selected in the decision-making model for pork. Therefore, we estimate the probability of buying US pork according to another two household characteristics: (1) households with one or more members aged under 20 years (i.e., the case where $chi = 1$); and (2) others (i.e., the case where $chi = 0$).

Thus, we estimate the probabilities of buying US pork separately for 24 types of household ($2 \times 2 \times 3 \times 2$) and estimate probabilities of buying US vegetables separately for eight types ($2 \times 2 \times 2$). From equation (6), the probability of avoiding US pork can be estimated by:

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$$\text{Prob}(y_{pork} = 0)$$
$$= 1 - \Phi\left[\hat{\theta}_{3pork} + \hat{\tau}_{31pork} chi + (1 + \hat{\tau}_{11pork} beef_A + \hat{\tau}_{12pork} beef_B + \hat{\tau}_{13pork} tokyo)(1 + \hat{\tau}_{21pork} country_{pork})\right]$$

and the probability of avoiding US vegetables is estimated by:

$$\text{Prob}(y_{vegetable} = 0)$$
$$= 1 - \Phi\left[\hat{\theta}_{3vegetable} + (1 + \hat{\tau}_{21vegetable} country_{vegetable})(1 + \hat{\tau}_{11vegetable} beef_A + \hat{\tau}_{13vegetable} tokyo)\right]$$

where $\hat{\theta}_{3j}$, $\hat{\tau}_{31j}$, $\hat{\tau}_{21j}$, $\hat{\tau}_{11j}$, $\hat{\tau}_{12j}$ and $\hat{\tau}_{13j}$ ($j = pork, vegetable$) are the estimated parameters.

Figure 2 is a bar chart that shows the probabilities of refraining from buying US pork and vegetables in each type of household. The black bar shows this probability for households willing to buy immediately without blanket testing. The gray bar shows the probability for households willing to buy US beef if blanket testing were introduced. The white bar shows the probability for households unwilling to buy US beef even with blanket testing.

The "hesitation probabilities" of households willing to buy US pork if blanket testing were introduced are around 20 to 40 points higher than those for households willing to buy US beef without blanket testing. (Compare the black and the gray bars for US pork in Figure 2.) In addition, the hesitation probabilities of households unwilling to buy even with blanket testing are 10 to 20 points higher compared with those of households willing to buy if blanket testing were introduced. (Compare the gray and the white bars of US pork in Figure 2.) In terms of probability of avoiding US vegetables, the hesitation probabilities of households willing to buy US beef without blanket testing are around 20 points higher than those of others. (Compare the black and the white bars of US vegetables in Figure 2.) Compared with the probabilities of avoiding both US pork and vegetables according to location of households, the hesitation probabilities in Osaka, where residents prefer beef, are around 5 to 10 points higher than those in Tokyo. We also show that households that pay attention to country-of-origin information have higher probabilities of avoiding US agricultural products. The hesitation probabilities are around 10 to 25 points higher for US pork, and around 20 points higher for US vegetables. Moreover, the hesitation probabilities for households without children are 5 to 15 points higher than those for households with children. These results show that the willingness of households to buy US beef and their interest in country-of-origin information have a more significant impact on households' purchase decisions of US agricultural products compared with frequency of consumption and family structure.

We next discuss the impact of willingness of households to buy US beef and their interest in country-of-origin information in more detail. To verify the differences in the impact on consumer attitudes toward US pork and US vegetables, we calculate the probabilities of avoiding each product according to the willingness of households to buy US beef and their interest in country-of-origin information. In terms of willingness of households to buy US beef, the average probability of avoiding US pork is 19% for households willing to buy US beef without blanket testing, 31% for households willing to buy US beef if blanket testing were introduced and 64% for households unwilling to buy US beef. That is, the hesitation probability of households willing to buy US beef with blanket testing is 31 points higher and that of households unwilling to buy US beef even with blanket testing is 45 points higher than that of households willing to buy US beef without blanket testing.

In terms of the interest of households in country-of-origin information, the hesitation probability for pork when households pay attention to county of origin is estimated as 53%, which is 17 points higher than that for households who do not. The hesitation probability for US vegetables among households that pay attention to country-of-origin information is estimated as 33%, which is 21 points higher than that for households that do not.

We conclude that both households' purchase attitudes toward US beef and the interest of households in country-of-origin information have a significant impact on the purchasing decisions of households regarding US agricultural products. In addition, concerning the purchasing decisions about US pork, consumer attitudes toward US beef are more influential than the interest of households in country-of-origin information, although the impact on their purchasing decisions about US vegetables are at the same level. This indicates that it is easy for households to suspect the relationship between safety of pork and BSE because pork is a meat similar to beef, which is related to BSE.

6. Conclusion

In this paper, we use a probit model based on a questionnaire survey to analyze what types of households tend to avoid US agricultural products. We show that: (1) households willing to buy US beef after the BSE outbreak are less likely to avoid US pork and vegetables; (2) households in Tokyo, which consume less beef, have a tendency not to reduce their demand for US pork and vegetables; (3) households that care about country-of-origin information tend to avoid US agricultural products; and (4) households without children are apt to avoid US pork compared with households with children.

Additionally, we ascertain what characteristics of households have a significant impact on the purchasing decisions of households of US agricultural products, and find a difference in the impact on demand for US pork and vegetables. As a result, the willingness of consumers to buy US beef and the interest of households in country-of-origin information have more significant impacts on the decisions of households to purchase US agricultural products. We also show that the attitudes of consumers toward US beef have a greater influence on the decisions of households to purchase US pork than they do on purchases of US vegetables because households may perceive a higher probability of a health hazard from pork, which is regarded as being almost the same product as beef.

We conclude the paper with the following outstanding issues. First, it is difficult to evaluate such a dynamic effect with one questionnaire survey, such as the one conducted in this study. If we would like to collect these data, it would have to be panel data on the same sample individuals. Second, we selected the subjects from a telephone directory. Sampling from a telephone directory may cause sampling bias because many households refuse to list their phone numbers in a telephone directory. However, we are unable to discuss how the bias affects our results at this time. These two issues are to be considered in further research.

References

Barrena, R., & Sánchez, M. (2010). Differences in Consumer Abstraction Levels as a Function of Risk Perception. *Journal of Agricultural Economics, 61*(1), 34–59. http://dx.doi.org/10.1111/j.1477-9552.2009.00224.x

Beverland, M., & Lindgreen, A. (2002). Using Country of Origin in Strategy: The Importance of Context and Strategic Action. *Brand Management, 10*(2), 147-167. http://dx.doi.org/10.1057/palgrave.bm.2540112

Dosman, D. M., Adamowicz, W. L., & Hrudey, S. E. (2002). Socioeconomic Determinants of Health- and Food Safety-Related Risk Perceptions. *Risk Analysis, 21*(2), 307–318. http://dx.doi.org/10.1111/0272–4332.212113

Gould, G. W., & Villarreal, H. J. (2002). Adult Equivalence Scale and Food Expenditures: An Application to Mexican Beef and Pork Purchases. *Applied Economics, 34*(9), 1075–1088. http://dx.doi.org/10.1080/00036840110074141

Henson, S., & Northen, J. (2000). Consumer Assessment of the Safety of Beef at the Point of Purchase: A Pan-European Study. *Journal of Agricultural Economics, 51*(1), 90–105. http://dx.doi.org/10.1111/j.1477-9552.2000.tb01211.x

Hong, S. T., & Wher, R. S. Jr. (1990). Determinants of Product Evaluation: Effects of the Time Interval between Knowledge of a Product's Country of Origin and Information about its Specific Attributes. *The Journal of Consumer Research, 17*(3), 277–288. http://dx.doi.org/10.1086/208557

Hong, S. T., & Wyer, R. S. Jr. (1989). Effects of Country-of-Origin and Product Attribute Information on Product Evaluation: An Information Processing Perspective. *The Journal of Consumer Research, 16*(2), 175–187. http://dx.doi.org/10.1086/209206

Ishida, T., Ishikawa, N., & Fukushige, M. (2010). Impact of BSE and Bird Flu on Consumers' Meat Demand in Japan. *Applied Economics, 56*(1), 49–56. http://dx.doi.org/10.1080/00036840701564392

Janda, S., & Rao, C. P. (1997). The Effect of Country-of-Origin Related Stereotypes and Personal Beliefs on Product Evaluation. *Psychology and Marketing, 14*(7), 689–702. http://dx.doi.org/10.1002/(SICI)1520-6793(199710)14:7<689::AID-MAR4>3.0.CO;2-C

Lin, C. T. J. (1995). Demographic & Socioeconomic Influences on the Importance of Food Safety in Food Shopping. *Agricultural and Resource Economics Review, 24*(2), 190–198.

Maheswaran, D. (1994). Country of Origin as a Stereotype: Effects of Consumer Expertise and Attribute Strength on Product Evaluations. *The Journal of Consumer Research, 21*(2), 354–65. http://dx.doi.org/10.1086/209403

Marsh, T. L., Schroeder, T. C., & Mintert, J. (2004). Impact of Meat Product Recalls on Consumer Demand in the USA. *Applied Economics, 36*(9), 897–909. http://dx.doi.org/10.1080/0003684042000233113

Pennings, J. M. E., Wansink, B., & Meulenberg, M. T. G. (2002). A Note on Modeling Consumer Reactions to a Crisis: The Case of the Mad Cow Disease. *International Journal of Research in Marketing, 19*(1), 91–100. http://dx.doi.org/10.1016/S0167-8116(02)00050-2

Peterson, H. H., & Chen, Y. -J. (2005). The Impact of BSE on Japanese Retail Meat Demand. *Agribusiness, 21*, 313–327. http://dx.doi.org/10.1002/agr.20050

Renner, B., Schüz, B., & Sniehotta, F. F. (2008). Preventive Health Behavior and Adaptive Accuracy of Risk Perceptions. *Risk Analysis, 28*(3), 741–748. http://dx.doi.org/10.1111/j.1539-6924.2008.01047.x

Saghaian, S. H., Maynard, L.J., & Reed, M. R. (2007). The Effects of *E. coli* 0157:H7, FMD and BSE on Japanese Retail Beef Prices: A Historical Decomposition. *Agribusiness, 23*(1), 131–147. http://dx.doi.org/10.1002/agr.20106

Tonsor, G. T., Schroeder, T. C., & Pennings, J. M. E. (2009). Factors Impacting Food Safety Risk Perceptions. *Journal of Agricultural Economics, 60*(3), 625–644. http://dx.doi.org/10.1111/j.1477-9552.2009.00209.x

van der Lans, I. A., van Ittersum, K. , De Cicco, A., & Loseby, M. (2001). The Role of the Region of Origin and EU Certificates of Origin in Consumer Evaluation of Food Products. *Food Policy, 28*(4), 451–477. http://dx.doi.org/10.1093/erae/28.4.451

van Ittersum, K., Candel, M. J. J. M., & Meulenberg, M. T. G. (2003). The Influence of the Image of a Product's Region of Origin on Product Evaluation. *Journal of Business Research,* 56(3), 215–226. http://dx.doi.org/10.1016/S0148-2963(01)00223-5

Verbeke, W., & Ward, R. W. (2001). A Fresh Meat Almost Ideal Demand System Incorporating Negative TV Press and Advertising Impact. *Agricultural Economics, 25*(2–3), 359–374. http://dx.doi.org/10.1111/j.1574-0862.2001.tb00215.x

Verbeke, W., Ward, R.W., & Viaene, J. (2000). Probit Analysis of Fresh Meat Consumption in Belgium: Exploring BSE and Television Communication Impact. *Agribusiness, 16*(2), 215–234. http://dx.doi.org/10.1002/(SICI)1520-6297(200021)16:2<215::AID-AGR6>3.0.CO;2-S

Viscusi, W. K. (1990). Do Smokers Underestimate Risks? *Journal of Political Economy, 98*(6), 1253–1269. http://dx.doi.org/10.1086/261733

Zepeda, L., Douthitt, R., & You, S. Y. (2003). Consumer Risk Perceptions toward Agricultural Biotechnology, Self-protection, and Food Demand: The Case of Milk in the United States. *Risk Analysis, 23*(5), 973–984. http://dx.doi.org/10.1111/1539-6924.00374

Data

Ministry of Internal Affairs and Communications. (2002–2006). Family Income and Expenditure Survey.

Ministry of Internal Affairs and Communications. (2005). Census.

Ministry of Internal Affairs and Communications. (2004). National Survey of Family Income and Expenditure.

Table 1. Summary statistics of the questionnaire results

Area	Consumers' willingness to buy US beef With or without blanket testing	Total		Households that do not refrain from buying US pork*		Households that do not refrain from buying US vegetables**	
Tokyo (fewer prefer beef)	Willing to buy without blanket testing	97	(50%)	88	(91%)	92	(95%)
	Willing to buy if blanket testing were introduced	48	(25%)	30	(63%)	41	(85%)
	Not willing to buy even if blanket testing were introduced	48	(25%)	16	(33%)	33	(69%)
	Total	193	(100%)	134	(69%)	166	(86%)
Osaka (more prefer beef)	Willing to buy without blanket testing	84	(44%)	71	(85%)	79	(94%)
	Willing to buy if blanket testing were introduced	69	(36%)	30	(43%)	46	(67%)
	Not willing to buy even if blanket testing were introduced	37	(19%)	14	(38%)	24	(65%)
	Total	190	(100%)	115	(61%)	149	(78%)
Total	Willing to buy without blanket testing	181	(47%)	159	(88%)	171	(94%)
	Willing to buy if blanket testing were introduced	117	(31%)	60	(51%)	87	(74%)
	Not willing to buy even if blanket testing were introduced	85	(22%)	30	(35%)	57	(67%)
	Total	383	(100%)	249	(65%)	315	(82%)

* The figures in parentheses indicate the proportion of households that do not avoid US pork according to their willingness to buy US beef with or without blanket testing.

** The figures in parentheses indicate the proportion of households that do not avoid US vegetables according to their willingness to buy US beef with or without blanket testing.

Table 2. Definitions and means of explanatory variables

Description	Dummy variables	Tokyo	Osaka	Total
Consumers' willingness to buy US beef				
Willing to buy US beef without blanket testing	$beef_A$=1 & $beef_B$=0	0.50	0.44	0.47
Willing to buy US beef if blanket testing were introduced	$beef_A$=0 & $beef_B$=1	0.25	0.36	0.31
Not willing to buy even if blanket testing were introduced	$beef_A$=0 & $beef_B$=0	0.25	0.19	0.22
Level of awareness toward country-of-origin information				
Pay attention to country-of-origin information for pork	$country_{pork}$=1	0.17	0.16	0.17
Pay no attention to country-of-origin information for pork	$country_{pork}$=0	0.83	0.84	0.83
Pay attention to country-of-origin information for vegetables	$country_{vegetable}$=1	0.23	0.22	0.23
Pay no attention to country-of-origin information for vegetables	$country_{vegetable}$=0	0.77	0.78	0.77
Family structure				
Households with one or more members aged under 20 years	chi=1	0.24	0.30	0.27
Households without one or more members aged under 20 years	chi=0	0.76	0.70	0.73
Households with one or more members aged over 60 years	old=1	0.64	0.58	0.60
Households without one or more members aged over 60 years	old=0	0.36	0.42	0.40
Household income				
Income (million yen)	inc(continuous)	7.22	6.63	6.93
Sample size		193	190	383

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Table 3. Empirical results

	Pork				Vegetable			
	Full model		Minimum AIC model		Full model		Minimum AIC model	
variables	Coefficient	t-statics	Coefficient	t-statics	Coefficient	t-statics	Coefficient	t-statics
constant term	–0.62	(–2.10) *	–1.59	(–8.95) **	0.36	(1.09)	-0.40	(-3.02) **
tokyo	0.44	(2.73) **	0.37	(2.41) *	0.49	(2.43) *	0.36	(1.84)
$beef_A$	1.65	(8.42) **	1.58	(8.08) **	1.49	(5.80) **	1.16	(5.17) **
$beef_B$	0.50	(2.54) *	0.46	(2.33) *	0.41	(1.93)		
$country_{pork}$	–0.45	(–2.68)	–0.29	(–3.01) **	—		—	
$country_{vegetable}$	—		—		–0.76	(–4.58) **	–0.51	(–4.76) **
chi	0.37	(2.03) *	0.37	(2.21) *	–0.35	(–0.17)		
old	–0.39	(–0.23)			–0.05	(–0.27)		
log(inc)	–0.02	(–0.18)			–0.05	(–0.35)		
Log-L	–195.9		–194.8		–148.2		–148.1	
AIC	407.9		401.6		313.3		304.2	
Hit or Lose Ratio	0.67		0.75		0.82		0.82	
n	383		383		383		383	

Note: ** and * mean significant at the 1% and 5% levels, respectively.

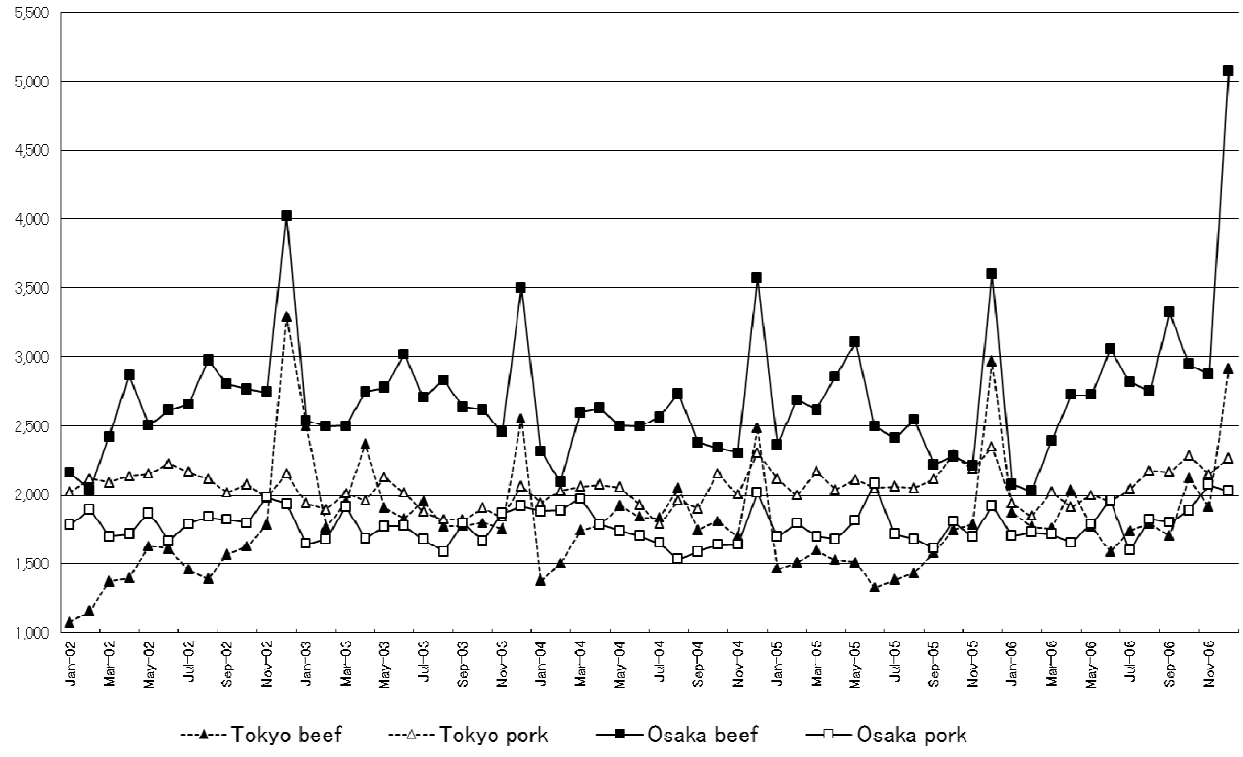

Figure 1. Shift of expenditure on beef and pork

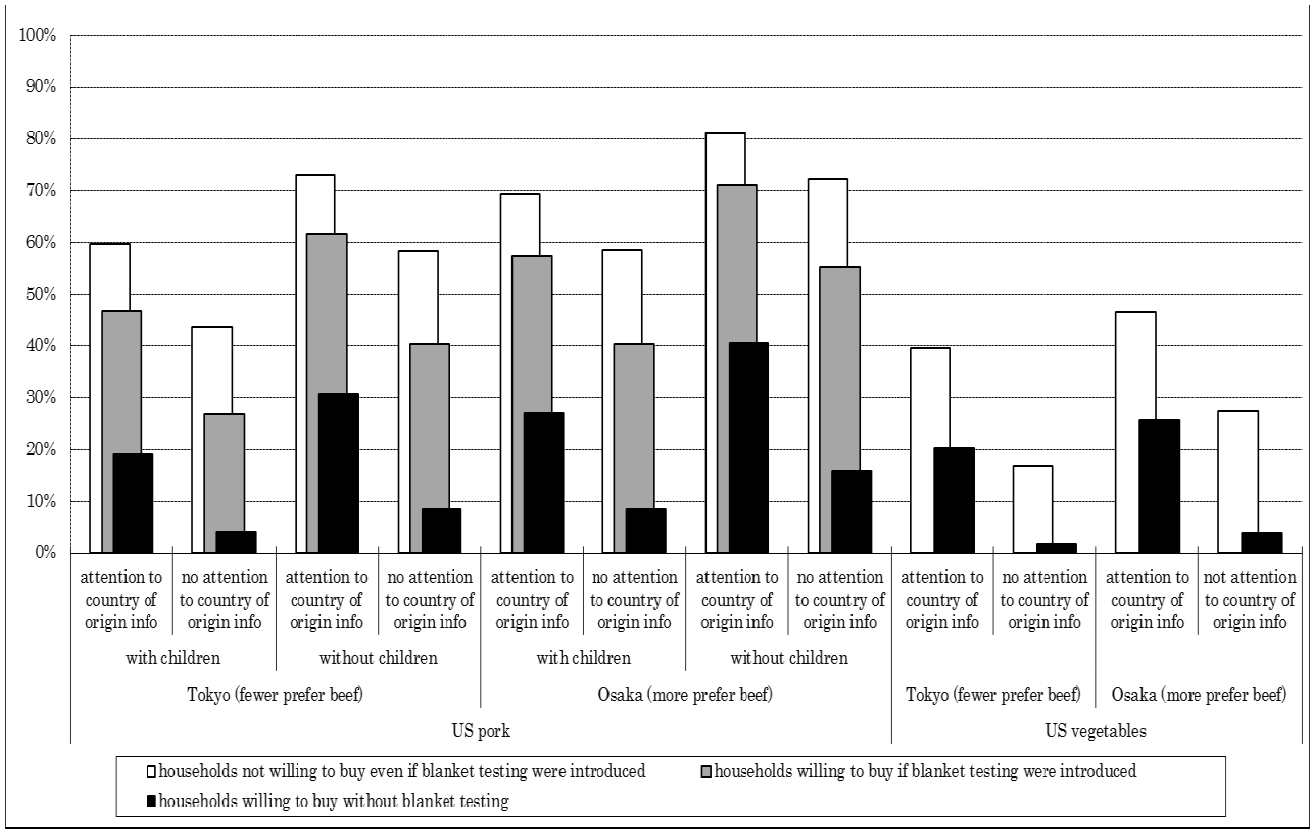

Figure 2. Estimated probabilities of refraining from buying US products for each type of household

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Polycyclic Aromatic Hydrocarbons (PAH) and Phenolic Substances in Cold Smoked Sausages Depending on Smoking Conditions Using Smouldering Smoke

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Abstract

The contents of polycyclic aromatic hydrocarbons (PAH) and phenolic substances in mini-salamis were investigated depending on cold smoking conditions (smouldering smoke). Three different smoke densities (light, medium, and intensive smoke) and ventilator velocities (750, 1500, and 3000 rpm) as well as wood chips of four different moisture contents (12%, 19%, 24%, and 30%) were tested in a total of 24 smoking experiments. During the smoking process, the concentrations of oxygen, carbon dioxide and carbon monoxide, the humidity and the

temperature in the smoking chamber as well as the smoke generation temperature were continuously determined. The chemical analysis included benzo[a]pyrene, PAH4, and six other PAHs with contents above 0.1 µg/kg as well as the phenolic substances guaiacol, 4-methylguaiacol, syringol, eugenol, and trans-isoeugenol. The smoke density had influence on the PAH contents. Sufficient amounts of phenolic substances (sum contents above 30 mg/kg) were detected in all the experiments.

Keywords: Polycyclic aromatic hydrocarbons, Phenolic substances, Mini-salamis, Smoking conditions, Smouldering smoke, GC/MS

1. Introduction

Polycyclic aromatic hydrocarbons (PAH) consist of two or more condensed aromatic carbon rings and are formed during the incomplete combustion of organic material (Smith, 1984). About 660 different compounds belong to the PAH group (Sander & Wise, 1997), some of them showing carcinogenic properties (IARC, 1987; IARC, 2010). Due to the carcinogenic properties, the Scientific Committee on Food (SCF) recommended that the PAH contents in food should be "as low as reasonably achievable" in adherence to the so-called ALARA-principle (SCF, 2002). Furthermore, the Codex Alimentarius Commission recommended the investigation and the identification of optimal smoking conditions for minimizing PAH contents (CAC, 2008).

In a previous study (Pöhlmann et al., 2012) it was shown that a minimization of the PAH compounds in hot smoked sausages using glow smoke is possible. The most important parameter influencing the PAH contents was the smoke generation temperature, however, the ventilator velocity also had a noticeable influence on the PAH contents. Lowering the contents of the PAH compounds did not necessarily lead to a decrease in the amounts of phenolic substances which are of considerable importance for the organoleptic properties of smoked meat products (Bratzler et al., 1969; Kjallstrand & Petersson, 2001) and show antimicrobial (Davidson & Branden, 1981) and antioxidative (Toth, 1982; Wittkowski, 1985) properties.

The main objective of this study was to investigate the correlations between the PAH contents and the phenolic substances in raw sausages depending on cold smoking conditions using smouldering smoke. Within the group of PAH compounds the investigations did not only focus on benzo[a]pyrene (BaP) as the EFSA had concluded that BaP was not a suitable indicator for the occurrence of PAHs in food, assessing that the sum of the four PAH compounds BaP, chrysene (CHR), benzo[a]anthracene (BaA), and benzo[b]fluoranthene (BbF) (PAH4) was the most suitable indicator for PAHs in food (EFSA, 2008). New maximum levels for PAH4 in smoked meat products of 30 µg/kg (1/9/2012 to 31/08/2014) and, later, of 12 µg/kg were established in Commission Regulation (EU) No 835/2011. Consequently, the contents of BaP and PAH4 and, additionally, the contents of the six other PAHs: benzo[c]fluorene (BcL), cyclopenta[c,d]pyrene (CPP), benzo[k]fluoranthene (BkF), benzo[j]fluoranthene (BjF), indeno[1,2,3-c,d]pyrene (IcP), and benzo[g,h,i]perylene (BgP) with contents above 0.1 µg/kg were considered in order to draw conclusions concerning the influence of the smoking parameters on the PAH contents.

Within the group of phenolic substances the dominant compounds guaiacol, 4-methylguaiacol, syringol, eugenol, and trans-isoeugenol were analyzed. The smoking experiments were performed with mini-salamis having the advantage of a large surface/mass ratio and a short ripening period. Since it was shown that the PAH contents increased with smoking time (Djinovic et al., 2008a; Djinovic et al., 2008b), the same smoking time was selected for all the smoking experiments. All in all, 24 smoking experiments were performed.

2. Materials and Methods

2.1 Preparation of mini-salamis

The mini-salamis were made of 76.9% frozen pork, 19.4% frozen back fat, 2.4% salt (containing 0.4% sodium nitrite ($NaNO_2$)), 0.4% glucose, 0.4% spice mix "Salami Mild French-Style" from Raps (Kulmbach, Germany), and at least 2.5 x 10^{10} active microorganisms "Optistart Sprint" from Raps (Kulmbach, Germany). Due to raising activity of undesirable microorganisms in less smoked mini-salamis, the content of starter cultures was increased to a minimum of 10^{11} active microorganisms (for smoking experiments 6 a, b to 12 a, b) and 0.4 – 0.5% glucono delta-lactone (GDL) were added (for smoking experiments 8 a, b to 12 a, b). Sheep casings made from the sub-mucosa of the small intestine were used. A batch weighed approx. 8 kg and was used for two smoking experiments (first smoking experiment = a; second smoking experiment = b) and as matrix blank.

2.2 Smoking experiments

Before smoking, the mini-salamis were dried and cured for two days in a climatic chamber at a temperature of 22 °C. On the third day they were smoked in a T 1900 Ratio smoking chamber combined with an RZ 325 smoke generator from Fessmann (Winnenden, Germany) at 22 °C for 30 min, using beech wood chips (size: 4.0 – 12.0

mm; KL 2-16) obtained from J. Rettenmaier & Söhne (Rosenberg, Germany). The smoke generator coupled with the smoking chamber was able to produce three different smoke densities: intensive, medium, and light smoke. The process of the smoke generation started with fresh air, which was injected into a TOP-device at a rate of 40 m^3/h, where the volume of air was split into smouldering air and additive air. The smouldering air flowed to the smouldering area through a tube and caused the beech wood chips to glow. The inlet of smouldering air depended on the additive air parameters. The dilution of smoke also depended on the additive air which was led to the ash area via two tubes of different inner diameters. Opening both tubes resulted in light smoke. Medium smoke was produced by closing the tube with the smaller inner diameter while opening the tube with the larger diameter. This way, intensive smoke was generated by opening the tube with the smaller inner diameter while closing the tube with the larger inner diameter to obtain less dilution. The ventilator velocity in the smoking chamber was also variable between 750, 1500, and 3000 rpm. The smoking time was set to 30 min for each smoking experiment. The moisture of the wood chips was only measured but not changed for experiments 1 a, b to 9 a, b and averaged out at about 12.0%. For the experiments 10 a, b to 12 a, b the moisture of wood chips was adjusted by adding distilled water. The changes in the smoke density, ventilator velocity and moisture of the wood chips resulted in 24 experiments (12 experiments performed as duplicates) (Table 1). After two additional days of drying and curing at 20 °C, about 1 kg of the mini-salamis were homogenized in a 5 L bowl chopper and stored in the dark at -18 °C until chemical analysis.

2.3 Measurement of smoke generation temperature and gases

A NiCrNi sensor from Testo (Lenzkirchen, Germany) was positioned 2-3 cm above the heating rod grooves in the smoke generator for measuring the temperature of the wood combustion. The data were collected by a 350-S flue gas analyzer from Testo (Lenzkirch, Germany). For monitoring the smoke composition during the smoking process, the smoke-air mixture was received from the smoking chamber at a flow rate of about 1 L/min. It passed through a tube (length: approximately 1 m; inner diameter: 13 mm), a wash bottle (filled with 400 mL distilled water) and finally a condensate trap before being analyzed by the flue gas analyzer mentioned above. The concentrations of carbon dioxide and oxygen were measured in volume percent while the concentrations of carbon monoxide were measured in ppm. A data point was created by averaging five values of every second recorded gas concentration.

2.4 Measurement of pH value

The pH-value of the mini-salamis was measured at the end of the ripening period using a Portamess Type 911 pH meter from Knick (Berlin, Germany).

2.5 Sensory evaluation

The production of the mini-salamis required five days. The sensory evaluation was performed after an additional two days of storage at 4 °C. The following aspects were evaluated: color, texture, odor and flavor. If a sample fulfilled the expectations of a panelist, 0 points were given to a category. For deviations from the expectations positive points for “too high” and negative points for “too low” were given. Five to six panelists evaluated the mini-salamis.

2.6 Reagents

The solvents n-hexane, iso-octane, and ethylacetate were purchased from LGC Standards (Wesel, Germany) in Picograde® quality. The drying material used in the PLE cells (poly (acrylic acid), partial sodium saltgraft-poly (ethylene oxide), cross-linked, 90–850 μm) was obtained from Sigma-Aldrich (Steinheim, Germany), and the glass microfiber filters were purchased from Büchi (Flawil, Switzerland). The extracts were filtered through 1.0 μm PTFE syringe filters purchased from Grace (Deerfield, USA) or 0.45 μm PTFE OPTI-Flow syringe filters obtained from Wicom (Heppenheim, Germany). The GPC column was filled with Bio-Beads S-X3 (200–400 mesh) purchased from Bio-Rad Laboratories (Munich, Germany). The last clean-up step was performed by means of Supelclean™ LC-Si SPE Tubes, 6 mL (1 g), obtained from Supelco (Bellefonte, USA). A standard mixture of the isotope labeled or fluorinated PAH i.e. benzo[a]anthracene-$^{13}C_6$, chrysene-$^{13}C_6$, benzo[b]fluoranthene-$^{13}C_6$, benzo[k]fluoranthene-$^{13}C_6$, benzo[a]pyrene-$^{13}C_4$, indeno[1,2, 3-cd]pyrene-d_{12}, benzo[g,h,i]perylene-$^{13}C_{12}$, and 5-fluorobenzo[c]fluorene was prepared in iso-octane, mixing the solutions of the single compounds purchased from LGC Standards [(^{13}C and ^{2}H labeled compounds) Wesel, Germany] and the Biochemical Institute for Environmental Carcinogens [(fluorinated compounds) Grosshansdorf, Germany], respectively. The PAH recovery standard mixture consisted of benzo[a]anthracene-d_{12}, benzo[a]pyrene-d_{12}, and benzo[g,h,i]perylene-d_{12} (LGC Standards, Wesel, Germany) in iso-octane. For the response factor calibration the reference standard PAH-Mix 183 containing all 15+1 EU priority PAHs obtained from Dr. Ehrenstorfer (Augsburg, Germany) was applied.

The standards of guaiacol (2-methoxyphenol), 4-methylguaiacol (2-methoxy-4-methylphenol), syringol (2,6-dimethoxyphenol), eugenol (4-allyl-2-methoxyphenol), and isoeugenol (cis/trans; 2-methoxy-4-(1-propenyl)phenol) were obtained from Alfa Aesar (Karlsruhe, Germany). Isotope labeled $^{13}C_6$-guaiacol was obtained from LGC Standards (Wesel, Germany) and guaiacol-d_4 from Dr. Ehrenstorfer GmbH (Augsburg, Germany). All phenolic standards were dissolved in ethyl acetate. Lithium chloride was purchased from J.T. Baker (Deventer, Netherlands), sodium sulphate and sodium hydrogencarbonate were both purchased from Merck (Darmstadt, Germany). Ethyl acetate was obtained from LGC Standards (Wesel, Germany) and diethyl ether from Acros organics (New Jersey, USA). BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) was obtained from Supelco (Bellefonte, USA) and Hypersep Si SPE cartridges from Thermo Fisher Scientific (Bellefonte, USA).

2.7 Analysis of PAH contents

2.7.1 Pressurized liquid extraction (PLE)

Approx. 3 g of homogenized mini-salamis were mixed with an equal amount of the drying material poly(acrylic acid), partial sodium salt-graft-poly(ethylene oxide). The resulting material was transferred into 40 mL cells filled with 3 g drying material equipped with disposable glass-fiber filters. Then, 50 µL of a PAH standard mixture containing isotope labeled (^{13}C and ^{2}H) and fluorinated PAH compounds was added as internal standard. After the addition of a further 9 g of drying material, the extraction was performed with a Speed Extractor E-916 from Büchi (Flawil, Switzerland) and n-hexane as solvent. Two static cycles were accomplished (operating conditions: 70 °C, 70 bar, static time 10 min and purge time 120 s). The solvent of the extract was evaporated using a Multivapor P-12 from Büchi (Flawil, Switzerland; water bath: 40 °C).

2.7.2 Gel permeation chromatography (GPC)

The evaporated PLE extract was dissolved in 4.5 mL cyclohexane/ethylacetate (1:1, v/v) and filtered through a polytetrafluoroethylene (PTFE) syringe filter of pore size 1 µm. The GPC column (25 mm i.d.) was filled with 60 g Bio-Beads S-X3. The samples were eluted at a flow rate of 5 mL/min applying cyclohexane/ethylacetate (1:1, v/v). The GPC solvent was removed by a rotary evaporator. Afterwards, the eluate was dried in a nitrogen stream. The waste time was 0–36 min and the collect time 36–65 min. The dried GPC eluate was dissolved in 1 mL cyclohexane (Jira, 2004).

2.7.3 Solid phase extraction (SPE)

The samples were transferred onto silica gel SPE cartridges conditioned with 3 mL cyclohexane and eluted with 10 mL cyclohexane.

2.7.4 Preparation for GC/MS analysis

The dried SPE eluate was dissolved in 1 mL iso-octane and 50 µL of the PAH-recovery standard mixture and transferred to a 1 mL tapered vial. The remaining sample was carefully concentrated in a nitrogen stream to about 50 µL (Jira et al., 2008).

2.7.5 Fast-GC/HRMS analysis

Fast-GC/HRMS was performed using a Trace-GC chromatograph (ThermoFisher Scientific, Milan, Italy) equipped with a split/splitless injection port. A chromatographic separation of the PAHs (with the exception of a separation of CHR and triphenylene (TP)) was performed on a TR-50MS column (10 m x 0.1 mm x 0.1 µm) (ThermoFisher Scientific, Bremen, Germany). Injection temperature was 260 °C, injection volume 1.5 µL (splitless). Helium with a constant flow of 0.6 mL/min was used as carrier gas. The following temperature program was applied: isothermal at 140 °C for 1 min, at 10 °C/min to 240 °C, at 5 °C/min to 270 °C, at 30 °C/min to 280 °C, at 4 °C/min to 290 °C, at 30 °C/min to 315 °C and at 3 °C/min to 330 °C. The identification of the PAHs by GC/HRMS was performed using a sector mass spectrometer DFS (Thermo Fisher Scientific, Bremen, Germany) working in the electron impact (EI) positive ion mode, applying an electron energy of 45 eV. The temperature of the source and the transfer line was heated up to 280 °C and 300 °C, respectively. The resolution of the MS was tuned to 8000 (10% valley definition) (Ziegenhals et al., 2008).

2.8 Analysis of phenolic compounds

2.8.1 Steam distillation and extraction

The five phenolic compounds were determined using a modified method based on a previously published method (Pöhlmann et al., 2012). The smoked sausage was homogenized and 1g of the mini-salamis was filled in an insert of an Antonacopoulos apparatus (Antonacopoulos, 1960; Toth, 1982). 10 µl of internal standard ($^{13}C_6$-guaiacol; 0.1 mg/mL in ethyl acetate) and 50 mL aqueous LiCl solution (30%) were added and boiled until

400 mL were distilled. With a $NaHCO_3$ solution, the distillate was adjusted to pH 5, extracted three times with 100mL diethyl ether, and dried over Na_2SO_4. The solvent was removed with a rotary evaporator at 40 °C and 850 mbar.

2.8.2 Solid phase extraction (SPE)

The residue was dissolved in 1 mL ethyl acetate and applied to a conditioned silica gel SPE cartridge (1 g/ 6 mL). The phenolic compounds were eluted with 6 mL ethyl acetate.

2.8.3 Trimethylsilylation

10 µL recovery standard (guaiacol-d_4; 0.1 mg/mL in ethyl acetate) was added to the SPE-eluate. An aliquot of 1 mL of this solution was derivatized with 100 µL BSTFA for 2h at 70 °C.

2.8.4 GC/MS analysis

The trimethylsilylated phenolic extract was analyzed by GC/MS using an Agilent 7890A GC coupled with an Agilent 5975C inert mass spectrometric detector. The GC was equipped with a DB-5MS capillary column (30 m x 0.25 mm i.d. x 0.25 µm) obtained from Agilent (Waldbronn, Germany). Helium with a constant flow of 1.5 mL/min was used as carrier gas. The injection volume was 1 µL, using a PTV injection port with the following injection temperature program: isothermal at 70 °C for 0.03 min, at 600 °C/min to 280 °C, held 4 min, at 600 °C/min to 320 °C; held for 3 min and subsequently cooled down to 70 °C. The following oven temperature program was used: isothermal at 70 °C for 1 min, at 25 °C/min to 120 °C, at 5 °C/min to 155 °C, at 30 °C/min to 320 °C and held for 5 min. The mass spectrometer was operated in a selected ion monitoring (SIM) mode combined with the SCAN mode with an electron impact ionization of 70 eV using the positive ion mode. The source and the transfer line were heated up to 230 °C and 280 °C, respectively. The temperature of the quadrupole was 150 °C.

2.9 Statistical analysis

The statistical analysis was performed by ANOVA using the software Statistica 7.1 (StatSoft Inc., 2005).

3. Results and Discussion

3.1 pH and weight loss analysis

The pH values and weight losses after the process of ripening are shown in Table 2 as arithmetic means and standard deviations. The pH values varied in a range of 4.7 to 5.3 for the smoked mini-salamis and between 5.1 and 5.9 for the non-smoked sausages. Due to the changes in the ingredients i.e. the addition of GDL as acidifier in the course of the investigations, it was not possible to correlate the pH values and the smoking conditions. The weight losses of the smoked mini-salamis varied between 38.3 and 48.5% and for the non-smoked mini-salamis between 37.7 and 48.5%.

3.2 Sensory evaluation

Mini-salamis produced with intensive smoke obtained ratings closer to the expectations of the panelists concerning flavor and odor, than the lightly smoked mini-salamis. Medium smoked mini-salamis were assessed between these two classifications. Sausages smoked at a ventilator velocity of 1500 rpm obtained ratings closer to the expectations of the panelists concerning flavor and odor than the sausages smoked at a ventilator velocity of 750 rpm. Mini-salamis smoked at 3000 rpm were assessed between these two ventilator velocities.

3.3 Smoke generation temperature

The profile of the smoke generation temperature was in general described by a period of a temperature increase at the beginning of the smoking process. After this period the temperature fluctuated within a nearly constant range. For the determination of the maximal smoke generation temperature and the mean smoke generation temperature of a smoking experiment, the complete time period of the smoking process was considered.

There were differences between the first and the second smoking experiment in the temperature profile. The first smoking experiment started at room temperature and needed 12 to 20 min to reach a relatively constant level. The second smoking experiment however started at a temperature between 90 °C and 160 °C as the smoke generator was already heated up. Consequently, the time required to reach a constant temperature was shorter (5 to 15 min). The mean smoke generation temperature of the second experiment was always higher than the mean smoke generation temperature of the first experiment. This tendency was not necessarily true for the smoke generation temperature maxima as, in some experiments; the maximum of smoke generation temperature of the first experiment succeeded the maximum of the second experiment for a short period of time.

The different smoke densities were produced as mentioned above. For intensive smoke the higher supply of

smouldering air and less dilution via additive air resulted in higher smoke generation temperature maxima and mean temperatures. For medium smoke with a lower supply of smouldering air and higher dilution via additive air the smoke generation temperature maxima and mean temperatures were lower. Light smoke resulted in the lowest smoke generation temperature maxima and mean temperatures (intensive smoke: maximum temperature: 845 ± 31 °C, mean temperature: 598 ± 34 °C; medium: maximum temperature: 680 ± 34 °C, mean temperature: 434 ± 35 °C and light: maximum temperature: 514 ± 80 °C, mean temperature: 324 ± 47 °C). Previous experiments with the same smoke generator (Pöhlmann et al., 2012) showed a direct proportionality between the maximum of the smoke generation temperature and the ventilator velocity. In the present study, such dependencies could not be observed. The main differences between the previous and the present experiments were the smoking times and the temperature in the smoking chamber. In the previous study (Pöhlmann et al.), Frankfurter-type sausages were smoked at 58 °C until comparable smoking colors were obtained and therefore variable smoking times (10–30 min) were used. In contrast, in all experiments of the present study, the mini-salamis were smoked for 30 min at 22 °C.

The mean smoke generation temperature varied from 268 °C (light smoke, ventilator velocity: 1500 rpm, first experiment) to 626 °C (intensive smoke, ventilator velocity: 1500 rpm, second experiment). The smoke generation temperature maxima ranged from 417 °C (light smoke, ventilator velocity: 750 rpm, first experiment) to 887 °C (intensive smoke, ventilator velocity: 1500 rpm, second experiment).

The mean and the maximum of the smoke generation temperature were also influenced by the moisture of the beech wood chips. A higher moisture of the wood chips (experiment 1a, 10a and 11a: 11.7, 18.3 and 24.4%; experiment 1b, 10b, 11b and 12b: 12.0, 19.7, 24.0 and 29.5%) resulted in lower mean smoke generation temperatures (experiment 1a, 10a and 11a: 367 - 585 °C, R^2 = 0.96; experiment 1b, 10b, 11b and 12b: 346 – 624 °C, R^2 = 0.97). The mean smoke generation temperature and smoke generation temperature maximum (196 °C and 369 °C, respectively) of experiment 12a with a moisture of wood chips of 29.8% showed that the wood chips did not smoulder correctly and, consequently, the results were not discussed.

3.4 Correlations between the PAH contents, gas concentrations, and smoking conditions

The contents of the investigated PAHs were quantified by isotope dilution analysis. Fluorinated standards were used for compounds for which ^{13}C–labelled or deuterated standards were not commercially available. Under the gas chromatographic conditions described above, a separation of CHR and triphenylene (TP) was not possible (Ziegenhals et al., 2008). Consequently, a sum parameter of CHR and TP was used. TP was assessed to be not classifiable as to its carcinogenicity to humans (group 3) (IARC, 2010).

The main focus of this study was on BaP and PAH4 (sum content of BaP, BaA, CHR and BbF). Also the contents of BcL, BgP, BkF, BjF, CPP, and IcP were analyzed. The contents of these PAH compounds are shown in Table 3.

For experiments 1 a, b to 9 a, b the original wood chips with a moisture between 10.7 and 12.9% (mean 11.9 ± 0.5%) were used. The smoke densities influenced the smoke generation temperature whereas the ventilator velocities did not show an obvious dependency. As described above, intensive smoke resulted in the highest mean smoke generation temperatures and temperature maxima. Consequently, the PAH4 contents and the contents of the individual PAHs were higher for intensive smoke (BaP: 0.47 ± 0.17; PAH4: 3.02 ± 0.77 µg/kg; N = 6), than for medium (BaP: 0.25 ± 0.07; PAH4: 2.07 ± 0.19 µg/kg; N = 6; p (intensive / medium) < 0.05) and light (BaP: 0.19 ± 0.09; PAH4: 1.81 ± 0.30 µg/kg; N = 6; p (intensive / light) < 0.01) smoke, respectively (Figure 1) The same tendency was observed between medium and light smoke, but could not be proofed statistically (p > 0.05). The different ventilator velocities did not influence the contents of BaP and PAH4. The mean BaP and PAH4 contents for the ventilator velocities 750, 1500, and 3000 rpm were very similar to each other (3000 rpm: BaP: 0.31 ± 0.07 µg/kg, PAH4: 2.25 ± 0.19 µg/kg; 1500 rpm: BaP: 0.28 ± 0.20 µg/kg, PAH4: 2.27 ± 0.86 µg/kg; 750 rpm: BaP: 0.31 ± 0.22 µg/kg, PAH4: 2.37 ± 0.95 µg/kg; N = 6). Yet the percentage standard deviations of BaP and PAH4 contents were significantly lower for a ventilator velocity of 3000 rpm (BaP: 24%, PAH4: 8%) than for 1500 (BaP: 70%, PAH4: 38%) and 750 rpm (BaP: 69%, PAH4: 40%) (Figure 2). Non-smoked mini-salamis were also analysed (BaP: 0.09 ± 0.06; PAH4: 1.39 ± 0.27 µg/kg; N = 13). A differentiation between the first and the second smoking experiment showed that the mean smoke generation temperatures of the first experiment were always lower than those of the second experiment. Yet the contents of PAH4 and the other analyzed PAHs were higher in the first experiment than in the second one. Consequently, the higher PAH contents detected in the first smoking experiment compared to the second experiment could not be explained by higher smoke generation temperatures. It should be noted that the concentrations of carbon monoxide (CO) in the smoking chamber showed the same tendency as the PAH contents: For experiments 1 a, b to 7 a, b the CO

maximum concentrations of the first smoking experiment were on average about 6% higher (first experiment: 2547 – 10953 ppm) than the CO maximum concentrations of the second experiment (2396 – 10320 ppm) (Figure 3). For the experiments 8 a, b and 9 a, b, the CO maximum concentrations of the first and the second experiment were very similar to each other (8 a: 2915 ppm, 8 b: 2925 ppm; 9 a: 3339 ppm, 9b: 3352 ppm), and the PAH4 contents (8 a: 1.91 µg/kg, 8 b: 1.60 µg/kg; 9 a: 1.56 µg/kg, 9 b: 1.48 µg/kg) were not much higher than those of the non-smoked salamis (1.39 ± 0.27 µg/kg). The higher CO maximum concentrations of the first experiments 1 a to 7 a indicate a less complete combustion of the wood chips compared to the second experiment. The conditions of a less complete combustion possibly favor the formation of the PAHs. The ratios between the maxima of CO and carbon dioxide (CO_2) concentrations were on average 0.8 for intensive, 0.7 for medium, and 0.5 for light smoke. There were no differences in these ratios between the first and the second smoking experiment, but the mean smoke generation temperature in the second experiment was on average 54 °C higher for intensive smoke, 48 °C higher for medium smoke, and 83 °C higher for light smoke. The minimum concentrations of oxygen (O_2) (19.2 – 20.6%) did not show any differences between the first and the second smoking experiments. As mentioned in section 3.3, the temperature profiles of the first and the second smoking experiment were different. The first experiment resulted in higher PAH contents, but the mean smoke generation temperatures were lower. On the other hand, in the first smoking experiments a longer time period was needed to reach a constant temperature level than in the second experiments.

The influence of the moisture of the wood chips was also investigated applying a constant smoke density (intensive smoke) and ventilator velocity (3000 rpm). As described above, the higher moisture of the wood chips resulted in lower mean smoke generation temperatures. But a higher moisture of the wood chips also resulted in higher PAH4 contents in a linear relationship (experiment 1a, 10a and 11a: BaP 0.41 – 0.53 µg/kg (R^2 = 0.99), PAH4 2.60 – 3.26 µg/kg (R^2 = 0.99); experiment 1b, 10b and 11b: BaP 0.32 – 0.47 µg/kg (R^2 = 0.98), PAH4 2.30 – 3.09 µg/kg (R^2 = 0.98)) (Figure 4). The temperature data of experiment 12a with a moisture of the wood chips of 29.8% showed that these wood chips did not smoulder properly. Consequently, the PAH4 content of this experiment did not fit in the linear correlation and was not discussed any further. However, the PAH4 content of smoking experiment 12b (moisture of wood chips 29.5%), with a common temperature profile showed also a lower PAH4 content than expected and was not included in the calculation of the correlation. Consequently, the moisture of the wood chips of 30% caused such a low smoke generation temperature that the other effects, which had usually led to higher PAH4 contents, were compensated.

In a previous study it was shown that the percentage contributions of BaA, BbF, CHR+TP, and BaP to PAH4 were not constant (Pöhlmann et al., 2012). An increasing absolute content of PAH4 resulted in higher percentage contributions of BaP and BbF and lower contributions of BaA and CHR+TP. In this study, the same tendencies were observed. The percentage contribution of BaA decreased from 48 to 29% and for CHR+TP from 43 to 31%. The percentage contribution of BbF increased from 5 to 17% and for BaP from 6 to 18% (Figure 5). The formation of PAHs with a higher molecular weight like BbF and BaP are favored under higher temperatures, which is also a condition that causes higher absolute PAH4 contents (Mücke et al., 1991; Pöhlmann et al. 2012).

3.5 Correlations between the contents of phenolic substances and the smoking conditions

The contents of the phenolic compounds in the cold smoked sausages are shown in Table 4. The samples were all analyzed in duplicate. The relative standard deviation of the analyzed samples was below 20%, and the recovery in spiked matrix ranged from 72% to 84%. The quantification was performed by a response factor calibration. All the compounds were quantified using $^{13}C_6$-guaiacol as internal standard and guaiacol-d_4 as recovery standard.

The highest sum content of the five phenolic compounds (76.2 mg/kg) was detected in intensively smoked sausages applying a ventilator velocity of 750 rpm. The lowest contents with an identical moisture level of the wood chips (11.9%) were detected in lightly smoked sausages (29.6 mg/kg; ventilator velocity: 3000 rpm).

The mean content of eugenol in smoked mini-salamis was only 2.2 mg/kg. The eugenol content in non-smoked salamis was half that (1.1 mg/kg). Furthermore, the content of trans-isoeugenol was about three times higher than the content in non-smoked sausages. Consequently, for the interpretation of the contents of the individual phenolic compounds, the contents of eugenol and trans-isoeugenol were not considered. The contents of guaiacol, 4-methylguaiacol, and syringol in non-smoked sausages were about ten times lower than the contents in smoked sausages.

Guaiacol was the main compound of the five analyzed phenolic compounds showing contents between 8.8 mg/kg (light smoke; 3000 rpm) and 24.5 mg/kg (intensive smoke; 750 rpm). The contents of syringol ranged between 8.9 mg/kg (light smoke; 3000 rpm) and 20.3 mg/kg (intensive smoke; 750 rpm), and the contents of

4-methylguaiacol were measured between 6.5 mg/kg (light smoke; 3000 rpm) and 20.3 mg/kg (light smoke; 1500 rpm).

The correlation between the smoke density, the ventilator velocity, and the sum content of the five phenolic compounds is shown in Figure 6. The mean value of the two smoking experiments was used for the interpretation.

The ventilator velocity influenced the content of phenolic compounds in intensively smoked mini- salamis. The sum content of the five phenolic compounds increased from 45.8 mg/kg at a ventilator velocity of 3000 rpm to 74.6 mg/kg at a ventilator velocity of 750 rpm. The content of medium smoked sausages showed no dependency on different ventilator velocities. The highest sum content of phenolic compounds in lightly smoked sausages was analyzed in mini-salamis smoked at a ventilator velocity of 1500 rpm (60.9 mg/kg). The lowest content was detected in lightly smoked sausages applying a ventilator velocity of 3000 rpm (35.3 mg/kg).

The smoke density showed a great influence on the maximum of the smoke generation temperature. The phenolic content of mini-salamis smoked at a ventilator velocity of 3000 rpm and 1500 rpm showed no direct correlation to the smoke density. At 3000 rpm sum contents of the five phenolic compounds between 35.3 mg/kg (light smoke) and 52.6 mg/kg (medium smoke) were detected, at 1500 rpm the sum content ranged between 53.5 mg/kg (medium smoke) and 67.8 mg/kg (intensive smoke). In contrast, the sum content of phenolic compounds in mini-salamis smoked at 750 rpm decreased from 74.6 mg/kg (intensive smoke) to 59.5 mg/kg (medium smoke) and up to 50.6 mg/kg (light smoke).

As already mentioned, the moisture of the wood chips influenced the maximum of the smoke generation temperature. The contents of phenolic compounds in the mini-salamis that were smoked with moistened wood chips also showed a dependency on the different moisture of the wood chips. The sum content of the phenolic compounds increased from 41.2 mg/kg (original wood moisture: 11.7%; maximum of smoke generation temperature: 827 °C) to 68.2 mg/kg (wood moisture: 29.5%; maximum of smoke generation temperature: 544 °C).

A correlation between the sum content of the five phenolic compounds and the content of the PAH4 was only observable for the experiments using wood chips that had a different moisture. The PAH4 contents of the smoked mini-salamis with the highest phenolic contents were analyzed at about 3.1 µg/kg. Therefore, the desired objective of lowering the PAH contents without lowering the contents of the phenolic compounds was not achieved by moistening of wood chips.

4. Conclusions

Mini-salamis with low PAH contents were produced in all of the smoking experiments. The contents of BaP were in the range of 0.1 to 0.7 µg/kg (mean: 0.3 µg/kg), and the contents of PAH4 were between 1.5 and 4.1 µg/kg (mean: 2.5 µg/kg). Therefore the mean contents of BaP and PAH4 are more than a factor of 10 below the maximum levels established by the EU legislation. A minimization of the PAH compounds in cold smoked sausages using smouldering smoke is possible. The most important parameter influencing the PAH contents is the smoke generation temperature which is influenced by the smoke density. Therefore, an accurate control of the smoke generation temperature to avoid the maxima of smoke generation temperature above 800 °C ($p < 0.05$) and to achieve mean smoke generation temperatures below 500 °C ($p < 0.05$) is a promising approach for lowering the PAH contents in cold smoked sausages.

The minimization of these low PAH contents in cold smoked mini-salamis is limited as the lowest observed PAH contents in smoked sausages were nearly at the same level as in unsmoked sausages. The reason for this contamination, which cannot be attributed to PAH-containing spices, is probably a contamination by the air in which PAHs occur ubiquitously in the climatic chamber since the samples are stored in the chamber for about four days. This kind of contamination cannot be excluded with justifiable efforts, consequently, the PAH contents at this low level are a limitation to the minimization strategies for PAHs in cold smoked raw sausages.

As it was observed for hot smoked sausages (Pöhlmann et al., 2012), an increase in the moisture content of the wood chips also does not seem to be a reasonable approach for reducing the PAH contents in cold smoked sausages using smouldering smoke. This proved to be counterproductive as even smoking with wood chips of a higher moisture content led to higher PAH contents.

For PAH minimization strategies it does not seem to be necessary to consider the contents of phenolic compounds since sufficient amounts of phenolic compounds (sum contents above 30 mg/kg) were detectable in all of the smoking experiments.

The risk of the formation of PAH compounds during the cold smoking process of meat products is higher for the

first smoking experiment when starting the smoking process with a cold smoke generator as in the smoking experiments performed in this study, slightly, but not significantly ($p > 0.05$) lower PAH contents were detected in the second experiment.

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References

Antonacopoulos, N. (1960). Verbesserte Apparatur zur quantitativen Destillation wasserdampfflüchtiger Stoffe. Zeitschrift für Lebensmittel-Untersuchung und –Forschung, *13*, 113-160. http://dx.doi.org/10.1007/BF01858931

Bratzler, L. J., Spooner, M. E., Weatherspoon, J. B., & Maxey, J. A. (1969). Smoke Flavor as Related to Phenol, Carbonyl and Acid Content of Bologna. *Journal of Food Science, 34*, 146-148. http://dx.doi.org/10.1111/j.1365-2621.1969.tb00906.x

CAC. (2008), Codex Alimentarius Commission. Proposed Draft Code of Practice for the Reduction of Contamination of Food with Polycyclic Aromatic Hydrocarbons (PAH) from Smoking and Direct Drying Processes: ftp://ftp.fao.org/codex/alinorm08/al31_41e.pdf.

Commission Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. *Official Journal of the European Union L, 215*, 4-8

Davidson, P. M. & Branden, A. L. (1981). Anti-microbial activity of non-halogenated phenolic-compounds. *Journal of Food Protection, 44*, 623-632.

Djinovic, J., Popovic, A., & Jira, W. (2008a). Polycyclic aromatic hydrocarbons (PAHs) in different types of smoked meat products from Serbia. *Meat Science, 80*, 449-456. http://dx.doi.org/10.1016/j.meatsci.2008.01.008

Djinovic, J., Popovic, A., & Jira, W. (2008b). Polycyclic aromatic hydrocarbons (PAHs) in traditional and industrial smoked beef and pork ham from Serbia. *European Food Research & Technology, 227*, 1191-1198. http://dx.doi.org/10.1007/s00217-008-0836-8

EFSA. (2008). Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on Polycyclic Aromatic Hydrocarbons in Food. *EFSA Journal, 724*, 1-114

IARC. (1987). Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Overall Evaluation of Carcinogenity: An Updating of IARC Monographs, Supplement 7, International Agency for Research on Cancer, Lyon, France.

IARC. (2010). Monographs on the Evaluation of Carcinogenic Risks to Humans. International Agency for Research on Cancer, Lyon, France. http://monographs.iarc.fr/ENG/Monographs/vol92/mono92.pdf.

Jira, W. (2004). A GC/MS method for the determination of carcinogenic polycyclic aromatic hydrocarbons (PAH) in smoked meat products and liquid smokes. *European Food Research & Technology, 218*, 208-212. http://dx.doi.org/10.1007/s00217-003-0827-8

Jira, W., Ziegenhals, K., & Speer, K. (2008). Gas chromatography-mass spectrometry (GC-MS) method for the determination of 16 European priority polycyclic aromatic hydrocarbons in smoked meat products and edible oils. *Food Additives & Contaminants: Part A, 25* (6), 704-713. http://dx.doi.org/10.1080/02652030701697769

Kjallstrand, J., & Petersson, G. (2001). Phenolic antioxidants in wood smoke. *Science of the Total Environment, 277*, 69-75. http://dx.doi.org/10.1016/S0048-9697(00)00863-9

Mücke, W., Steinmetzer, H. C., Stumpp, J., Baumeister, W., Boneberg, R., & Vierle, O. (1991). PAK-Immisionskonzentrationen – Ergebnisse mehrjähriger Messungen polycyclischer aromatischer Kohlenwasserstoffe in Bayern. Umweltwissenschaften und Schadstoff-Forschung – Zeitschrift für Umweltchemie und Ökotoxikologie, *3*, 176-179.

Pöhlmann, M., Hitzel, A., Schwägele, F., Speer, K., & Jira, W. (2012). Contents of polycyclic aromatic hydrocarbons (PAH) and phenolic substances in Frankfurter-type sausages depending on smoking conditions using glow smoke. *Meat Science, 90*, 176-184. http://dx.doi.org/10.1016/j.meatsci.2011.06.024

Sander, L. C., & Wise, S. A. (1997). Polycyclic Aromatic Hydrocarbon Structure Index. NIST Special Publication 922 (revised 2011), Gaithersburg: National Institute of Standards and Technology.

http://www.nist.gov/mml/analytical/organic/sp922page.cfm.

SCF, Scientific Committee on Food. (2002). Opinion of the Scientific Committee on Food on the Risks to Human Health of Polycyclic Aromatic Hydrocarbons in Food. http://ec.europa.eu/food/food/chemicalsafety/contaminants/out153_en.pdf

Smith, I. M. (1984). PAH from coal utilization. Emissions and effects, Report No ICTIS/TR29, IEA Coal Research London.

Toth, L. (1982). Chemie der Räucherung. Weinheim, Germany.

Wittkowski, R. (1985). Phenole im Räucherrauch. VCH Verlagsgesellschaft Weinheim.

Ziegenhals, K., Hübschmann, H. J., Speer, K., & Jira, W. (2008). Fast-GC/HRMS to quantify the EU priority PAH. *Journal of Separation Science, 31*, 1779-1786. http://dx.doi.org/10.1002/jswsc.200700641

Table 1. Different process parameters for the smoking experiments

Experiment	Smoking time [min]	Smoke density	Ventilator velocity [rpm]	Moisture of beech wood [%]
1 a	30	intensive	3000	11.7
1 b	27*	intensive	3000	12.0
2 a	30	intensive	1500	11.7
2 b	30	intensive	1500	12.9
3 a	30	intensive	750	12.0
3 b	30	intensive	750	11.9
4 a	30	medium	3000	10.7
4 b	30	medium	3000	11.5
5 a	30	medium	1500	11.7
5 b	30	medium	1500	11.6
6 a	30	medium	750	12.2
6 b	30	medium	750	12.6
7 a	30	light	3000	11.9
7 b	30	light	3000	12.0
8 a	30	light	1500	12.1
8 b	30	light	1500	12.2
9 a	30	light	750	11.5
9 b	30	light	750	12.2
10 a	30	intensive	3000	18.3
10 b	30	intensive	3000	19.7
11 a	30	intensive	3000	24.4
11 b	30	intensive	3000	24.0
12 a°	30	intensive	3000	29.8
12 b	30	intensive	3000	29.5

* Smoking experiment 1 b was interrupted after 27 min

° For the interpretation of experiment 12 the results from experiment 12b were used, because the smoking experiment 12 a failed (the maximum of smoke generation temperature was only 370°C).

Table 2. Results of pH value and weight loss (arithmetic mean and standard deviation)

	smoked mini salamis	Light smoke	Medium smoke	Intensive smoke	Non-smoked mini salamis
pH value	5.1 ± 0.2	5.1 ± 0.1	5.2 ± 0.2	5.0 ± 0.2	5.5 ± 0.3
Weight loss [%]	43.9 ± 3.6	43.1 ± 4.1	45.2 ± 3.5	43.6 ± 3.6	44.1 ± 3.6

Table 3. PAH contents [µg/kg] in smoked mini salamis applying different smoking conditions

Experiment	BaA [µg/kg]	CHR +TP [µg/kg	BbF [µg/kg]	BaP [µg/kg]	BcL [µg/kg]	CPP [µg/kg]	BjF [µg/kg]	BkF [µg/kg]	IcP [µg/kg]	BgP [µg/kg]	PAH4 [µg/kg]	Total [µg/kg]
1 a	0.94	0.91	0.34	0.41	1.86	0.64	0.23	0.18	0.23	0.42	2.60	6.16
1 b*	0.89	0.87	0.21	0.32	1.61	0.41	0.21	0.15	0.17	0.23	2.30	5.07
2 a	1.57	1.34	0.35	0.65	3.24	1.02	0.38	0.25	0.35	0.37	3.91	9.52
2 b	1.05	0.92	0.22	0.30	2.59	0.54	0.17	0.13	0.16	0.16	2.49	6.23
3 a	1.58	1.26	0.51	0.71	3.19	1.24	0.35	0.26	0.37	0.32	4.06	9.78
3 b	1.15	0.95	0.24	0.40	2.66	0.59	0.19	0.13	0.19	0.19	2.74	6.69
4 a	0.64	0.87	0.37	0.35	0.85	0.30	0.22	0.20	0.25	0.45	2.23	4.49
4 b	0.94	0.81	0.17	0.19	2.55	0.56	0.12	0.08	0.11	0.21	2.10	5.73
5 a	0.75	0.72	0.20	0.28	1.78	0.31	0.13	0.11	0.15	0.23	1.94	4.66
5 b	0.77	0.68	0.14	0.17	1.99	0.29	0.09	0.06	0.11	0.21	1.76	4.50
6 a	0.88	0.82	0.18	0.25	2.47	0.55	0.13	0.11	0.14	0.19	2.12	5.71
6 b	0.97	0.92	0.15	0.23	2.35	0.33	0.14	0.12	0.16	0.33	2.28	5.71
7 a	0.87	0.90	0.10	0.31	1.27	0.30	0.20	0.20	0.21	0.26	2.18	4.61
7 b	0.79	0.90	0.14	0.28	1.21	0.22	0.20	0.21	0.22	0.31	2.10	4.48
8 a	0.84	0.75	0.15	0.17	2.07	0.43	0.10	0.09	0.11	0.21	1.91	4.91
8 b	0.76	0.63	0.11	0.10	2.15	0.34	0.06	0.05	0.05	0.11	1.60	4.36
9 a	0.67	0.64	0.11	0.13	1.47	0.32	0.08	0.07	0.07	0.10	1.56	3.67
9 b	0.61	0.60	0.12	0.15	1.14	0.16	0.09	0.08	0.08	0.16	1.48	3.20
10 a	1.19	0.99	0.35	0.47	2.34	0.89	0.24	0.18	0.26	0.41	2.99	7.31
10 b	1.18	1.01	0.31	0.40	2.24	0.72	0.21	0.16	0.20	0.33	2.90	6.76
11 a	1.25	1.10	0.38	0.53	2.00	0.88	0.26	0.20	0.26	0.25	3.26	7.10
11 b	1.15	1.03	0.44	0.47	1.91	0.65	0.25	0.20	0.23	0.31	3.09	6.65
12 a°	1.04	1.34	0.20	0.26	2.02	0.77	0.18	0.13	0.14	0.12	2.83	6.19
12 b	1.34	1.13	0.17	0.34	2.56	0.92	0.19	0.14	0.17	0.13	2.98	7.08
smoked (N=23)	0.99 ± 0.27	0.90 ± 0.19	0.24 ± 0.12	0.33 ± 0.16	2.07 ± 0.62	0.55 ± 0.29	0.18 ± 0.08	0.15 ± 0.06	0.18 ± 0.08	0.26 ± 0.10	2.46 ± 0.70	5.85 ± 1.66
non-smoked (N=13)	0.60 ± 0.12	0.60 ± 0.12	0.10 ± 0.04	0.09 ± 0.06	1.63 ± 0.41	0.31 ± 0.12	0.07 ± 0.03	0.05 ± 0.03	0.07 ± 0.05	0.12 ± 0.06	1.39 ± 0.27	3.64 ± 0.68
p-value(smoked/ non-smoked)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.05	< 0.01	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

* Smoking experiment 1 b was interrupted; the smoking time was only 27min

° For the interpretation of experiment 12 the results from experiment 12b were used, because the smoking experiment 12 a failed (the maximum of smoke generation temperature was only 370°C).

Table 4. Contents of phenolic substances [mg/kg] in smoked mini-salamis applying different smoking conditions

Experiment	Guaiacol [mg/kg]	4-Methylguaiacol [mg/kg]	Syringol [mg/kg]	Eugenol [mg/kg]	*Trans*-isoeugenol [mg/kg]	Sum of 5 phenols [mg/kg]
1 a	14.9	9.4	10.2	1.7	4.9	41.2
1 b*	16.7	12.3	13.9	1.8	5.7	50.4
2 a	22.2	17.1	16.2	2.8	10.1	68.4
2 b	22.9	17.9	16.8	2.6	7.0	67.2
3 a	22.9	17.7	20.3	3.1	9.0	73.0
3 b	24.5	19.1	19.1	2.9	10.6	76.2
4 a	15.3	9.6	12.8	1.6	5.4	44.8
4 b	17.7	15.5	16.2	2.6	8.4	60.4
5 a	14.8	12.2	11.1	1.9	6.0	46.0
5 b	19.1	16.1	14.7	2.4	8.5	60.9
6 a	16.8	14.0	14.9	2.2	7.9	55.9
6 b	19.1	15.8	17.0	2.5	8.7	63.0
7 a	8.8	6.5	8.9	1.4	4.1	29.6
7 b	11.6	9.8	12.6	1.9	5.2	41.0
8 a	14.5	12.6	12.7	2.0	6.4	48.2
8 b	22.3	20.3	19.0	2.9	9.2	73.6
9 a	14.4	12.0	10.9	1.9	6.7	45.8
9 b	17.3	14.9	14.6	2.3	6.2	55.3
10 a	15.8	11.5	11.7	2.3	7.2	48.6
10 b	23.7	17.5	17.8	2.5	8.2	69.7
11 a	16.7	14.0	16.7	2.7	11.5	61.6
11 b	17.1	14.6	17.5	2.9	9.7	61.9
12 a°	11.6	9.2	12.3	1.3	7.4	41.8
12 b	21.7	17.2	16.5	2.4	10.3	68.2
smoked (N=23)	17.9 ± 4.1	14.2 ± 3.5	14.9 ± 3.1	2.3 ± 0.5	7.7 ± 2.0	57.0 ± 12.4
non-smoked (N=13)	2.2 ± 0.7	1.5 ± 0.5	1.8 ± 0.5	1.1 ± 0.2	2.5 ± 0.5	9.0 ± 2.0
p-value (smoked / non-smoked)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

* Smoking experiment 1 b was interrupted; the smoking time was only 27min

° For the interpretation of experiment 12 the results from experiment 12b were used, because the smoking experiment 12 a failed (the maximum of smoke generation temperature was only 370°C).

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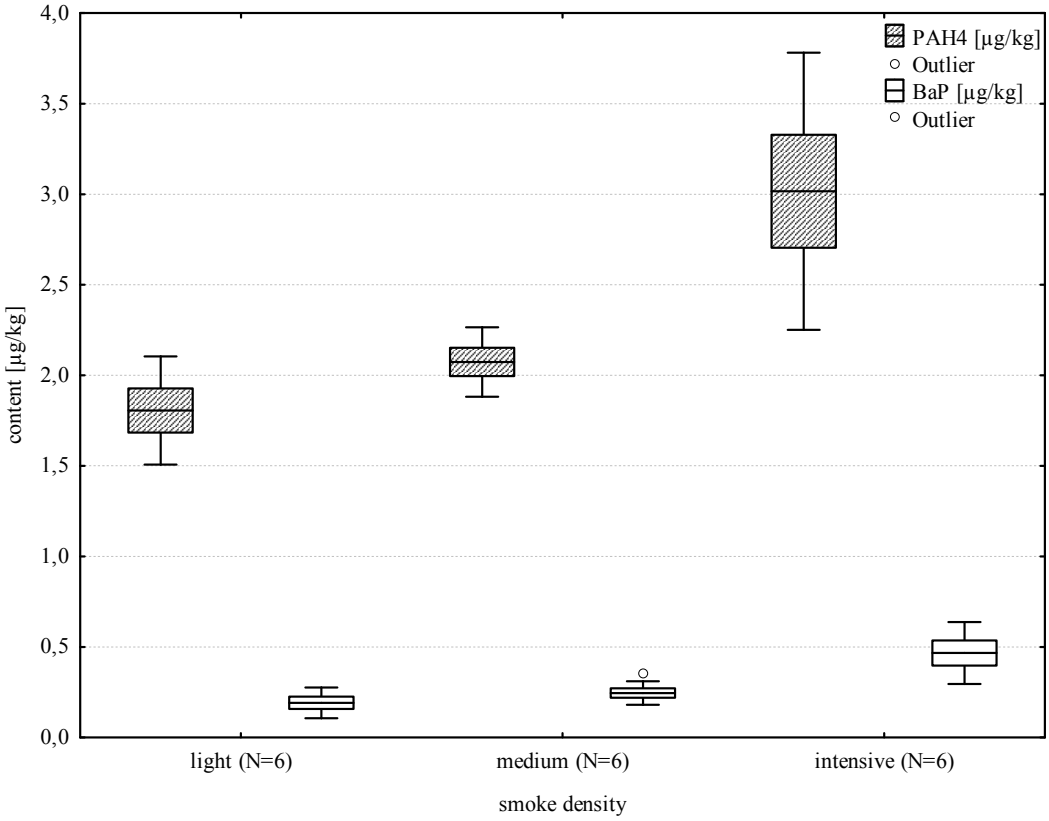

Figure 1. Correlation between smoke density and contents of BaP and PAH4 [µg/kg] in mini-salamis

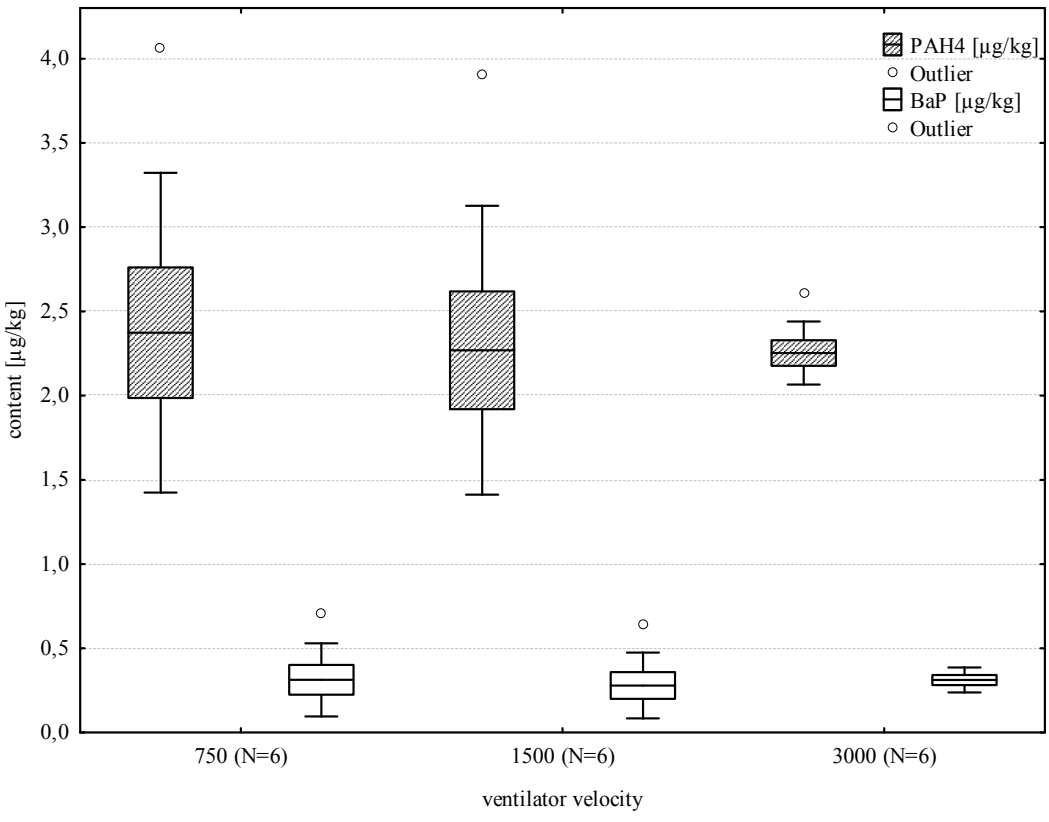

Figure 2. Correlation between ventilator velocity [rpm] and contents of BaP and PAH4 [µg/kg] in mini-salamis

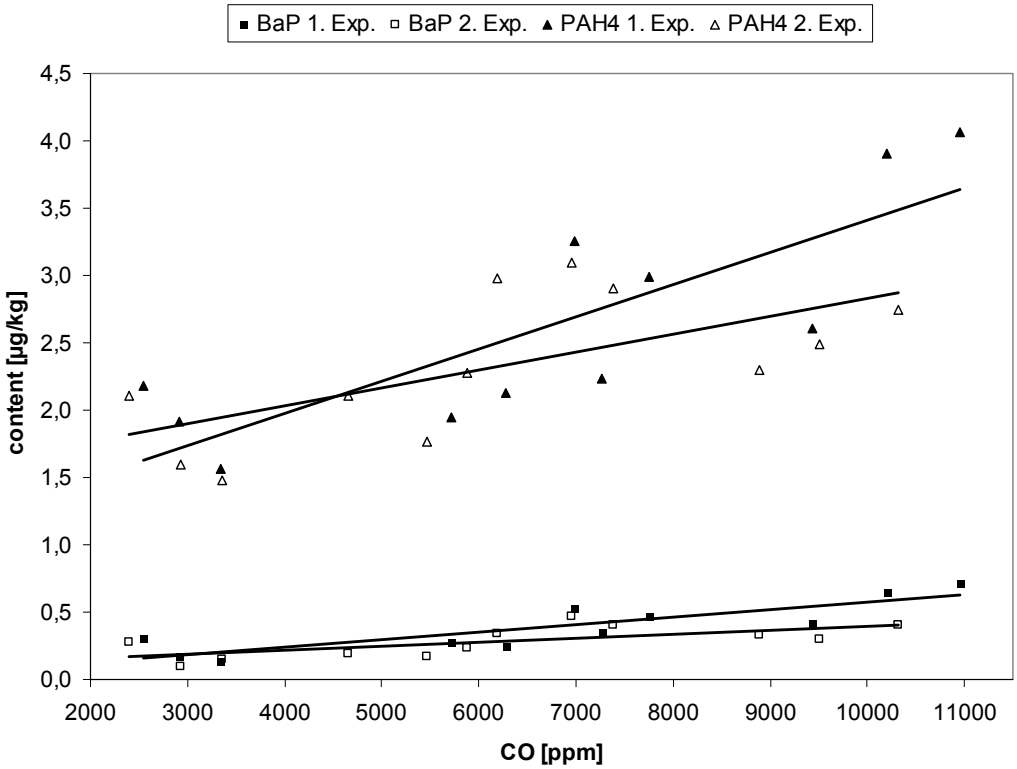

Figure 3. Correlations between maximum concentrations of CO and contents of BaP and PAH4 [µg/kg] in mini-salamis (N=23)

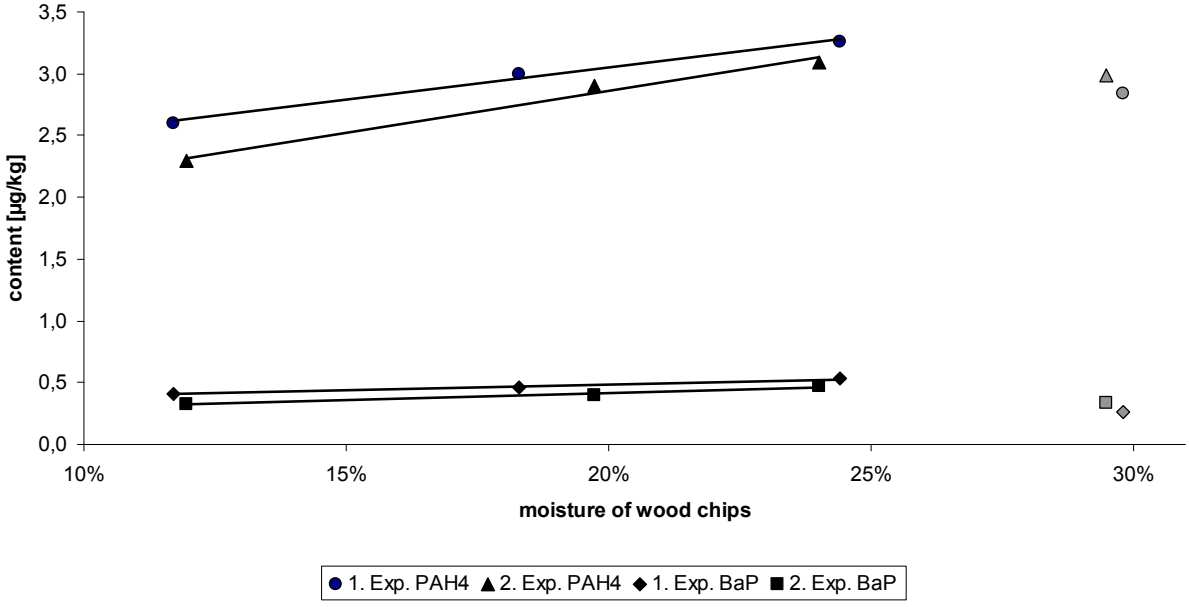

Figure 4. Correlations between moisture of wood chips [%] and contents of BaP and PAH4 [µg/kg] (N=4)

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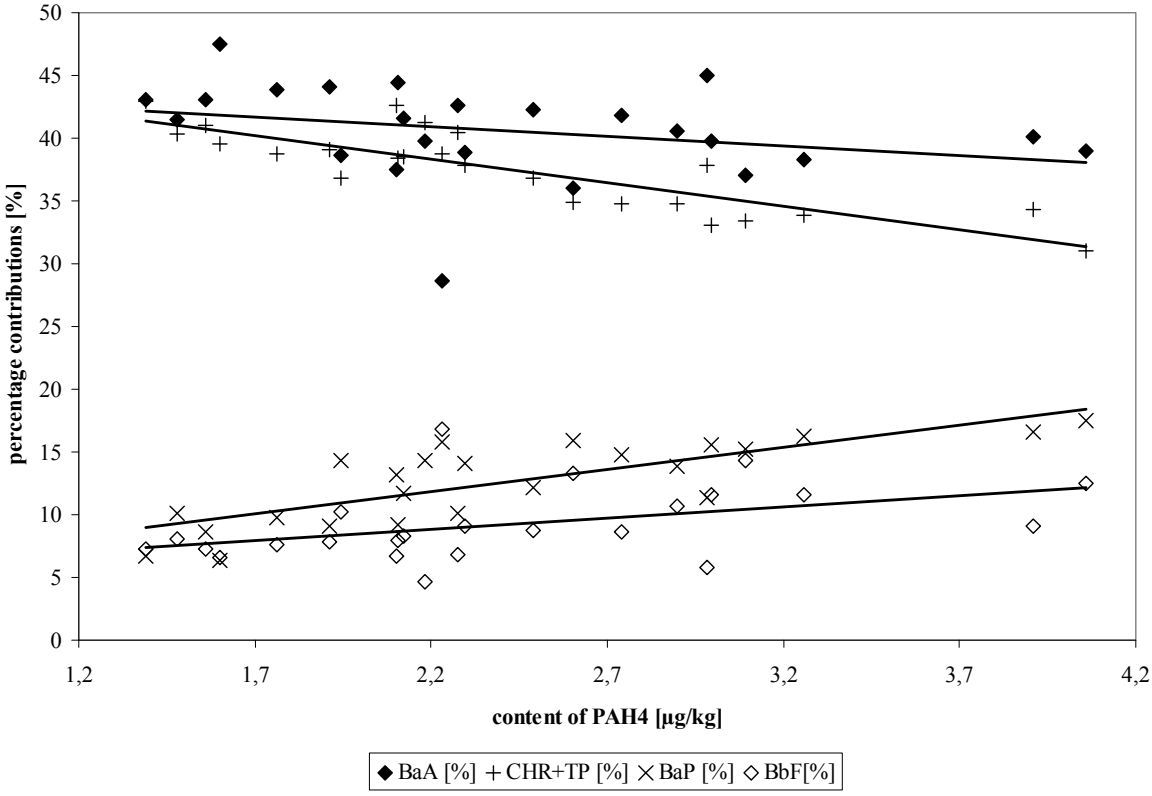

Figure 5. Percentage contributions [%] of single PAH to the PAH4 content [µg/kg] in dependence of the PAH4 level (N=23)

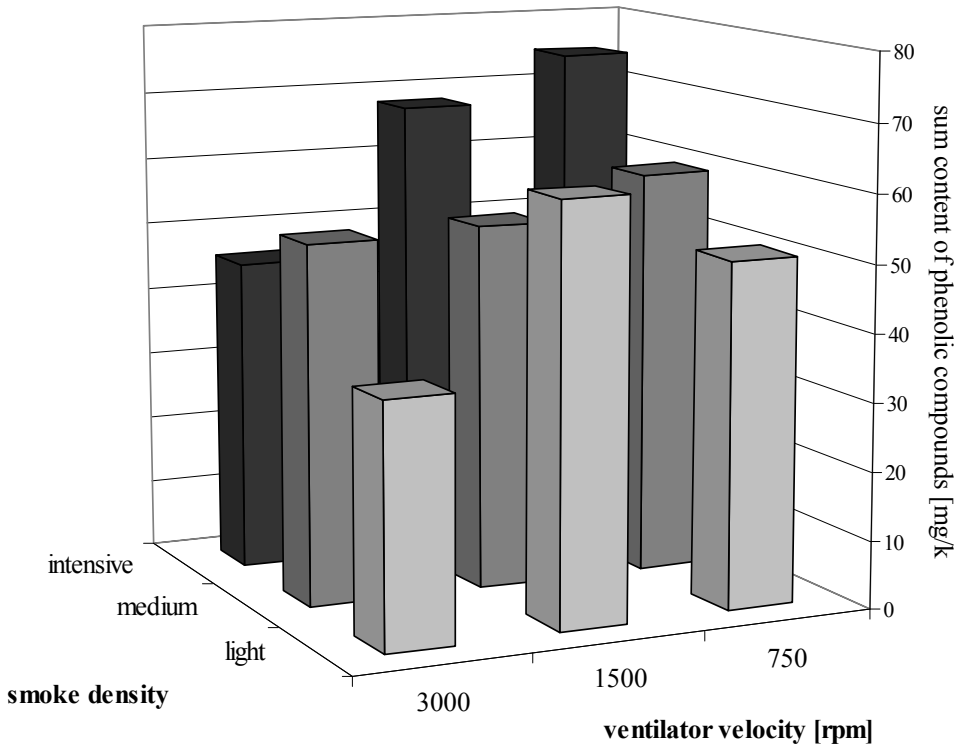

Figure 6. Correlations between smoke density, ventilator velocity [rpm] and sum content of phenolic compounds [mg/kg] in smoked mini-salamis

Ultraviolet-C Light Effect on Pitaya (*Stenocereus griseus*) Juice

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Abstract

Pitaya (*Stenocereus griseus*) juice, obtained from fresh pitayas, was processed using a continuous ultraviolet-C (UV-C) light (57 μW/cm^2) system. Juice was processed at five flow rates (0.46, 3.28, 6.57, 16.49 and 30.33 mL/s) and five treatment times (5, 10, 15, 20, and 25 min). Fresh juice was used as control. Some physicochemical (pH, total soluble solids, color, and betalains), antioxidant (total phenolic compounds and antioxidant activity), and microbiological (aerobic mesophylls bacteria and yeasts plus molds) characteristics were assessed in fresh and UV-C processed juices. It was observed that the UV-C treatments did not affect pH and total soluble solids in juice. The total change in color (ΔE) increased as treatment times increased; however, ΔE values were reduced at high flow rates. The betalains and total phenolic compounds contents were reduced as flow rates and treatment times increased; consequently, the antioxidant activity lessened in juice. A maximum reduction of 2.11 and 1.14 log cycles was observed for mesophylls and yeasts plus molds, respectively, in the UV-C light treated pitaya juice.

Keywords: Antioxidant activity, Betalains, Phenolic compounds, Pitaya juice, UV-C light

1. Introduction

Emerging technologies such as high hydrostatic pressure, pulsed electric fields, ultrasound, and ultraviolet light have been used to obtain food products with characteristics similar to fresh products that consumers are demanding today. The short-wave ultraviolet-C light is a physical method that does not generate chemical residues in the food and today is used for water and surfaces disinfection (Quek & Hu, 2008). The UV-C light (254nm) is easy to use for disinfection purposes of liquid foods. It has lethal effects on micro organisms such as bacteria, viruses, protozoa, yeasts, and molds (Begum, Hocking-Ailsa & Miskelly, 2009). The germicidal effect of UV-C light on micro organismsis at the DNA level. The absorption of UV-C light generates electronic changes that may cause breaking of the DNA bonds; therefore, microbial cells could be compromised. The photoproducts (pyrimidine nucleotide bases), generated by the application of UV-C light, block the DNA transcription and replication; even more, inhibits cell functions that may cause the cell death (Guerrero-Beltrán & Barbosa-Cánovas, 2004).

Fruit juices are usually pasteurized in order to inactivate micro organisms and enzymes responsible for undesirable changes. However, the sensory characteristics of the pasteurized food productcould be damaged by the high temperatures used for processing (Ibarz & Barbosa-Cánovas, 2002). In 2000, the Food and Drug Administration (FDA) approved the use of UV-C light as a method for “cold pasteurization”; however, it was

also advised that the reduction of resistant pathogens should be at least of 5 log cycles (FDA, 2001) to ensure the effectiveness of the process.

The UV-C lighth as recently been used for researching in the fruit juices processing area, but this has been mainly focused on the inactivation of microorganisms (Guerrero-Beltrán & Barbosa-Cánovas, 2005; Gabriel & Nakano, 2009; Lu *et al.*, 2010), enzymes (Barka*et al.*, 2000; Guerrero-Beltrán & Barbosa-Cánovas, 2006), and changes in color (Keyser *et al*, 2008). Few researchers have comprehensively assessed the UV-C lighte ffects (Pala & Tocluku, 2010; Falguera *et al.*, 2011) on other fruit juice components. The UV-C light treatment of liquid fruit products has to be performed carefully since some requirements should be accomplished. Among these requirements is to sustain aturbulent flow to warranty that the whole liquid is reached by the UV-C light to deliver a microbiologically safe food product (FDA, 2000). Despite of this and due to the presence of colored compounds, organic compounds, and suspended matter, characteristic that may affect the UV-C light penetration into the liquid food product, the light transmission might be attenuated. As a result theUV-C light efficiency could be reduced (Caminiti *et al*., 2010).

Pitaya (*Stenocereus griseus*) is a red-peel fruit produced by a species of the *cactaceae* family. The fruit is surrounded of large prickles similar to small needles. It has an oval or spherical shape. Pulp fruit possesses weetened delicate flavor; it is also juicy and has small black seeds that crunches “pleasantly” when masticate them (Ayala *et al.*, 2007). The fresh fruits weight ranges from 85.9 to 398.5g. Pulp and peel make 76-84 and 16-24%, respectively (Luna, 2006). The total soluble solids content, pH, and titratable acidity (as citric acid) range10-11, 3.9-5.0, and 14.0-0.5 %, respectively (Luna, 2006). One of themain problems of fresh pitaya is the shortshelf-life (3 to 5 days); therefore, it is important to use the appropriate technologies to increase its shelf-lifein a fresh fashion or to obtain pitaya processed products such as juices and nectars.

The aim of this study was to evaluate the physicochemical, microbiological, and antioxidant characteristics of pitaya juice treated with ultraviolet-C light.

2. Materials and Methods

2.1 Pitaya juice

“Pitaya of May” (*Stenocereus griseus*) was obtained from the municipalities of Cuauhtémoc Huitziltepec and Tepeyahualco, Puebla, Mexico. Fruits were sorted and chosen free from physical and microbiological damages. Fruits were disinfected with a solution of sodium hypochlorite (150 ppm). Pitayas were peeled and homogenize dusing a Black and Dekker domestic food processor (Towson, Maryland, USA). Afterward, juice was sieved (0.297 mm) to remove seeds and some large particles suspended in pulp.

2.2 UV-C light equipment

Pitaya juice was processed using an ultraviolet light system, similar to a double-walled heat exchanger, assembled at the University of the Americas Puebla. The UV-C lamps, acquired from Light Sources, Inc. (Orange, Connecticut, USA) were 303 and15 mm in length and diameter, respectively. Lamps were of 17 W in intensity to deliver a dose of 57μW/cm^2. The UV-C flowing system hosts a volume of 430 mL into the double-walled system. The system has an inner quartz tube with an outer diameterof 2.2 cm and a stainless still external tube with an inner diameter of 4.8 cm.

2.3 UV-C light treatment

Pitaya juice (600 mL) was placed in a double-walled vessel which was kept at 4° Cusinga Cole Parmer Cooling Polistat Circulator system (Vernon, Illinois, USA). The juice was pumped and recirculated in the UV-C system using a 75553-71 Master Flex peristaltic pump (Vernon, Illinois, USA) at 5 different flow rates (0.46, 3.28, 6.57, 16.49, and 30.33 mL/s). The processing time of juice, for each flow rate, was 5, 10, 15, 20, and 25 minutes corresponding to doses of 0.171, 0.342, 0.513, 0.684, and 0.86 kJ/m^2, respectively (Guerrero-Beltrán & Barbosa-Cánovas, 2006). Untreated juice was used as control. The UV-Clight treatment was performed in duplicate.

2.4 Physicochemical characteristics

Total solublesolids and pH were evaluated according to the 932.12 and 981.12 AOAC (2000) methods, respectively.

2.5 Color

Ten milliliters of pitaya juice were placed in a small petri dish (6 cm in diameter and 1.5 cm in height) to measure the *L* (luminosity, white-black), *a* (green-red), and *b* (yellow-blue) color parameters, in the Hunter scale,

using a Gardner Colorgard® System 05 (Geretsried, Germany) colorimeter in the transmittance mode. The total change in color (ΔE) was calculated using the next equation:

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2}$$

Where L_0, a_0, and b_0 and L, a, and b are the color parameters before and after the UV-C light treatment, respectively. The hue angle (H) and the chroma (C, intensity) color parameters were calculated using the next equations:

$$H = Tan^{-1}\left(\frac{b}{a}\right) \qquad C = \sqrt{(a)^2 + (b)^2}$$

2.6 Phenolic compounds

Phenolic compounds in pitaya juice were determined using the Gao, Ohlander, Jeppsson, Bjork & Traljkovski (2000) method with modifications. Two mL of distilled water were placed in an amber glass tube; then, 200 µL of the Folin and Ciocalteu´s phenol reagent (Sigma-Aldrich, Toluca, Mexico) and 100 µL of pitaya juice were added. Mixture was totally homogenized and then incubated for 3 minutes at room temperature (25 °C). Afterward, 1 mL of 20% (p/v) Na_2CO_3 was added and thoroughly mixed. This blend was incubated for 1 hour at room temperature in a dark environment. The absorbance was measured at 765 nm using an UV-visible spectro photometer model 2800 H (UNICO, NJ, EUA). The calculation of the content of phenolic compounds was performed using a standard curve of Gallic acid:

$$GA = \left(\frac{A - b}{m}\right) * 100$$

Where GA is the Gallic acid content (mg Gallic acid/mL), A is the absorbanceof the sample, b is the intercept (-0.01), and m is the slope (4.108 abs/mg GA/mL).

2.7 Betalains

Betalains were assessed according to the Stintzing, Schieber & Carle (2002) method. Pitaya juice was diluted with McIlvaine buffer (pH 6.5, citrate-phosphate) to obtain absorption values in the range 0.9-1.0. The betanin and indicaxanthin contents were measured at wave lengths of 538 and 480 nm, respectively. The betalains content is the sum of the betanin and indicaxanthin contents and was calculated according to the next equation:

$$CB = \frac{A * DF * M * 1000}{\varepsilon * l}$$

Where CB is the betalains content (mg/L), A is the absorbance, DF is the dilution factor, l is the quartz cell pathway (1 cm), ε is the molar extinction coefficient (for $\varepsilon_{betanin}$ is 60,000 mole/L·cm, and for $\varepsilon_{indicaxanthin}$ is 48,000 mole/L·cm), and M is the molecular weight (550 and 308 g/mole for betanin and indicaxathin, respectively).

2.8 Antioxidant activity

The antioxidant activity in juice was determined according to the Kuskoski, Asuero, Parrilla, Troncoso, & Fett (2004) methodology. The $ABTS^+$ radical was formed placing 5 mL of distilled water, 3.3 mg of potassium persulfate, and 19.4 mg of the ABTS reagent into an amber glass flask. Reagents were totally mixedand let stand for 16 hours in a dark environment. Afterward, absolute ethanol was mixed with the $ABTS^+$ radical (ABTS radical solution) until reaching an absorbance of 0.70 ± 0.02 at 754 nm. Eighty µL of pitaya juice were mixed with 3,920 L of the ABTS radical solution, totally mixed and the initial absorbance measure (A_i). Mixture was let react for 7 minute and the final absorbance measured (A_f). The amount of the antioxidant activity was calculated using the trolox (T) standard curve as follow:

$$UI = \frac{A_i - A_f}{A_i} * 100$$

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$$UT = \frac{UI - b}{m} * 100$$

Where *UT* is the amount of trolox (mg T/mL), *UI* is the percentage of inhibition, *b* is the intercept(3.52) and *m* is the slope (371.5 abs/mg T/mL).

2.9 Total counts

Aerobic mesophyll bacteria (AMB) and molds plus yeasts (MY) were counted using the standard plate count agar and the acidified (10% tartaric acid) potato dextrose agar, respectively. Petri plates for the AMB were incubated in an oven at 35±2 °C and the number of colony forming units per mL (CFU/mL) were counted in a period of 24-48 hours, while the petri plates for the ML were incubated during 5 days at 25±2 °C.

2.10 Mathematical modeling

The decimal reduction time (D_{uv}) values were calculated using the first-order kinetics model for the survivors in pitaya juice after the UV-C light treatment (Stermer, Lasater-Smith & Brasington, 1987) as follow:

$$Log\left(\frac{N_t}{N_o}\right) = -kIt = -kF = -kD$$

$$D = F = I * t$$

$$D_{UV} = -\frac{1}{k}$$

Where N_t is the survivors microbial load (CFU/mL) after UV-C light treatment, *No* is the initial microbial load (CFU/mL), *k* is the inactivation constant rate (min^{-1} or m^2/kJ), $F = D$ is the dose or fluence (kJ/m^2), *I* is the intensity of the UV-C lamp (W/m^2), and *Duv* is the decimal reduction time required to inactivate 90% of the microorganisms at constant dose or fluence.

2.11 Statistical analysis

All results were evaluated by analysis of variance (ANOVA) using the Minitab 14 program (Minitab Inc., PA, USA). A *p* value of 0.05 was used for deciding significant differences among averages according to the Turkey's test.

3. Results and Discussion

3.1 Juice characteristics

Table 1 presents the physicochemical and antioxidant characteristics of fresh pitaya juice. The total soluble solids (Bx) content was lower than that reported for other types of pitayas. Luna (2006) reported values of total soluble solids in the range 10-17.25% (w/w). pH and phenolic compounds content in pitaya juice were similar to those reported for cacti fruits (Nurliyana, Syed, Mustapha, Aisyah & Kamarul, 2010) other than pitaya. Ochoa & Guerrero (2012) reported a phenolic compounds content of 42.01±8.06 mg of GA/100 mL of red prickle pear juice. Nurliyana *et al*. (2010), on the other hand, reported values of 3.75-36.12 mg of GA/100 g of pitaya pulp. The content of betalains in pitaya juice was higher than the amount reported for other red-pigmented fruits (Repo de Carrasco & Encina 2008; Castellanos & Yahia, 2008). This may probably explain the high antioxidant activity found in pitaya juice.

It can also be observed that the *L* color parameter indicates that juice is dark in lightness. The *a* value is in the red side and the *b* value is in the blue side of the color space chart. Pitaya juice has a dark red-purple color; this was corroborated by thehuevalue which is in the red color side of the color space chart. This could be probably because pitaya juice contains a higher amount of betanin (red-purple color) than indicaxanthin (yellow-orange color). The value of chroma (intensity) indicates that pitaya juice has an intense dark red-purple color.

3.2 UV-C light effect on pitaya juice

3.2.1 Physicochemical characteristics

Neither pH nor total soluble solids of pitaya juice were significantly affected (P>0.05) by the UV-C light at the selected flow rates and treatment times. The average values of the total soluble solids and pH, after UV-C light

treatment, were 6.79±0.04 and 5.93±0.07 %, respectively. A number of researchers have reported that UV-C light did not have effect on pH, total soluble solids, and titratable acidityof fruit juices (Noci, Riener, Walkling, Cronin, Morgan & Lying, 2008; Caminiti *et al*., 2010; Pala & Toklucu, 2011).

3.2.2 Color

Table 2 presents the *L*, *a*, *b*, and *ΔE* color parameters of pitaya juice treated with UV-C light for 25 minutes. It is observed that the UV-C light significantly affected ($P < 0.05$) color parameters at different flow rates. The higher the flow rate, the lower the change in color of pitaya juice. When comparing the lowest (0.46 mL/s) and highest (30.33 mL/s) flow rates, the highest flow rate make the least change in color of pitaya juice. Guerrero-Beltrán, Welti-Chanes, & Barbosa-Cánovas (2009) reported that increasing the flow rate, for treating grape juice with UV-C light, a lower contact between UV-C light and the liquid food product may occur; therefore, juice could be less affected in its color parameters. Moreover, although data are not presented here, no effect of UV-C light was observed on the color of pitaya juice when increasing treatment time. This could be probably due to the relationship between retention time and flow rate; therefore, further damage to pigments in juice at low flow rates may occur. However, this change in color is too small to make it visible to the naked eye.

3.2.3 Phenolic compounds

Figure 1 presents the phenolic compounds content in UV-C light treated pitaya juice. The phenolic compounds content significantly decreased ($P < 0.05$) as the UV-C light processing time increased. However, no effect ($P > 0.05$) was observed regarding the phenolic compounds content when increasing flow rates to treat pitaya juice in the UV-C light system. The reduction of phenolic compounds could be due to the UV-Clight effect on the structure of phenolics (Koutchma, 2009). Piga, Del Caro, Pinna, & Agabbio (2003) and Bakowska, Kucharska, & Oszmianski (2003) pointed out that phenolic compounds may protect pigments, ascorbic acid, and antioxidant activity in fruits and juices against environmental injuries. Pala & Toklucu (2011) treated pomegranate juice with UV-C light in a flow range of 12.5 to 62.4 J/mL. They reported no significant differences ($P > 0.05$) in the phenolic compounds content of the UV-C light treated, heat processed, and fresh pomegranate juices. Caminiti*et al*. (2010) also reported that the content of phenolic compounds in apple juice was not affected by the UV-C light when juice was treated at 5.31 and 53.10 J/cm^2 during 30 and 300 s, respectively. Noci *et al*. (2008) reported that phenolic compounds decreased in apple juice treated with UV-C light during 30 minutes. However, their experiment consisted in exposing 800 mL of juice, in a Pyrex plate (25 mm in diameter), to the light of an UV-C mercury lamp (30 W) placed at a distance of 30 cm on top of the juice.

3.2.4 Betalains

Figure 2 presents the betalains content in pitaya juice processed with UV-C light. It is observed that increasing flow rates and treatment times significantly decreased ($P < 0.05$) the betalains content; the betalains reduction ranged 3.89-20.21%. This betalains reduction could be probably due to the photons producedby UV-C light. Photons could be absorbed by organic molecules such as betalains which possess conjugated bonds and aromatic rings responsible for color (Woo, Ngou, Ngo, Soong & Tang, 2011; Koutchma, 2009). Guerrero-Beltrán *et al*. (2009) reported that long periods of UV-C light to treat fruit products may undergo discoloration reactions of the pigments. Bakowska *et al*. (2003) reported that phenolic compounds may act as inhibitors of the degradation of anthocyanins during the exposition of fruit and, or vegetable products to the UV-C light. Likely, phenolic compounds might protect pitaya betalains for short periods of time. Pala & Tocluku (2011) reported that the anthocyanins content of pomegranate juice decreased gradually with increasing the UV-C light dose; they reported a decrease in the anthocyanin content of 1.8, 3.9, and 8.4% at UV-C exposure doses of 12.5, 34.4, and 62.4 J/mL, respectively.

3.2.5 Antioxidant activity

Figure 3 presentsthe antioxidant activity in pitaya juice treated with UV-C light. Substantial antioxidant activity was expected since phenolic compounds and betalains may function as antioxidants. The antioxidant activity was reduced by the UV-C light at both treatment times and flow rates. The antioxidant activityde creaseds ignificantly ($P < 0.05$) in juice as processing time increased. However, no significant differences ($P > 0.05$) were observed in the antioxidant activity content within flow rates. Caminiti *et al*. (2010) pointed out that the antioxidant activity decreased as the UV-C dose was increased for processing apple juice; however, Pala & Tocluku (2011) reported no significant difference in the antioxidant activity content in fresh and UV-C light treated pomegranate juice using different doses.

It has been reported that UV-C light may affect compounds with high antioxidant activity. For example, Sabliov, Fronczek, Astete, Khachaturyan, Khachatryan & Leonardi (2009) reported that the initial content of α-tocopherol,

dissolved in hexane or methanol, was significantly reduced when increasing the UV-C light treatment. Cvetkovic, Markovic, Cvetkovic & Radovanovic (2011), on the other hand, reported that UV-A, UV-B, and UV-C may reduce the antioxidant activity of phenolic compounds such as rutin and quercentin; the damage of these compounds was increased as the UV-C exposure time was increased. They pointed out that the reduction of compounds with antioxidant activity could be due to the combination of UV-C light and oxygen.

3.2.6 Microbial inactivation

Figures 4 and 5 present the aerobic mesophyll bacteria and molds plus yeasts inthe UV-C light treated pitaya juice, respectively. The number of colony forming units per milliliter, in both types of microorganisms, decreased as the flow rate and treatment time increased. Pitaya juice possesses a dark red-purple color and this could avoid the penetration of light; however, turbulence regime, formed by increasing the speed, is enough for making all the liquid be in contact with the UV-C light and obtain a greater microbial inactivation (Li, Deng & Nyung, 2010). Caminiti *et al*. (2010) and Guerrero-Beltrán & Barbosa-Cánovas (2005) pointed out that the transparency and soluble and insoluble solids of the liquid food product, or medium, are critical factors in the microbial inactivation with UV-C light; both color and turbidity may block the pathway of light and prevent to microorganisms to be reached by the UV-C light. Li *et al*. (2010) reported that the penetration of UV-C light in the product is of utmost importance since better results, regarding microbial inactivation, are obtained on the surface of the food. Recent investigations have been performed to explore the effect of UV-C light in the inactivation of microorganisms inoculated in different fruit juices. Guerrero-Beltrán & Barbosa-Cánovas (2005) and Guerrero *et al*. (2009) reported a log reduction of 1.34 ± 0.35 and 1.3 for *S. cerevisiae* inoculated in apple and grape juice, respectively. These results agree with those obtained in this research for yeasts plus molds in pitaya juice.

Table 3 presents the microbial counts and the log reductions for AMB and MY for the selected flow rates after 25 minutes of UV-C light treatment of pitaya juice. It can be observed that the AMB load is higher than the MY load; however, both types of microorganisms were reduced by the UV-C light. Li *et al*. (2010) pointed out that the efficiency of the UV-C light effect on microorganisms is a function of the initial microbial load. It is also observed that the higher the flow rate, the higher the inactivation effect for both types of microorganisms. The greater inactivation for AMB (2.11 log cycles reductions) and MY (1.14 log cycles reductions) was reached after 25 min of UV-C light treatment. This could be because bacterial cells are smaller than molds and yeasts; therefore, bacteria could be more easily reached and compromised by the UV-C light (Montgometry, 1985). In addition, bacterial cells are constituted by large levels of pyrimidine in the DNA; this may increase the probability of generating more cross-linkages between thymine and cytosine (Torkamani & Niakousari, 2011) by the UV-C light. Oteiza, Giannuzzi & Zaritzky (2010) reported that orange juice, inoculated with *S. cerevisiae*, decreased the ability of UV-C light for inactivating five *E. coli* O157: H7 strains. They concluded that yeasts, due to their size, increased the coefficient of absorption of the UV-C light. Therefore, high doses might be requiredto obtainthe same inactivation effect on the *E. coli* trains. López-Malo, Guerrero, Santiesteban & Alzamora (2005) processed apple juice, inoculated with *L. monocytogenes* and *S. cerevisiae*, with UV-C light. They reported that bacteria are more sensitive (> 5 log reduction) than yeasts (4 log reductions) to UV-C light.

Table 4 presents the first-order kinetics parameters for the AMB and MY inactivation in pitaya juice treated with UV-C light. The first order kinetics representation provided good fittings of the inactivation (R^2> 0.95) of AMB. This representation is not entirely appropriate for MY. D_{uv} values decreased as flow rates increased, this means that the inactivation of the microbial load increased as flow rate increased.Guerrero-Beltrán & Barbosa-Cánovas (2005) reported D_{uv} values of 5.9, 7.0, and 22.4 minutes for *E. coli*, *L. innocua*, and *S. cerevisiae* inactivation in apple juice treated with UV-C light at a flow rate of 9.13 mL/s. Torkamani & Niakosari (2011), on the other hand, reported D_{uv} values of 0.82 and 1.05 kJ/m^2 for total counts and yeasts plus molds, respectively, in not inoculated orange juice. Both sets of values are similar to those found in this study for same types of microorganisms.

4. Conclusions

The use of UV-C light to treat pitaya juice is a feasible alternative to deliver a microbiologically safe juice to consumers. Despite of not accomplish the 5 log reductions required by the FDA, pitaya juice maintains its quality because other physicochemical attributes remained barely unchanged. The decline in the quality attributes of pitaya juice is mainly a function of time or dose of treatment with the UV-C light. Maximum log cycle reductions of 2.11 and 1.14 for AMB and MY, respectively, were obtained after 25 minutes of UV-C light treatment at a flow rate of 30.33 mL/s. Under these conditions of treatment, changes in color were practically non-existent compared to fresh juice. The phenolic compounds, antioxidant activity, and betalains content

decreased by 13.27, 3.55, and 26.02%, respectively, in comparison to the content in fresh juice. It is important to take into account that the actual effect of the UV-C light on microbial inactivation in pitaya juice may depend on the UV-C dose used to treat juice as well as the type and amount of microorganisms.

References

AOAC. (2000). Official methods of analysis. 14th ed., *Association of Official Analytical Chemists*. Washington, DC, USA.

Ayala, K., Beltrán, C., & Cruz, T. (2007). Determinación de la actividad enzimática de extractos crudos de cuatro variedades de pitaya *Stenocereus griseus*H. In *XII Congreso Nacional de Biotecnología y Bioingeniería*, 25-29.

Bakowska, A., Kucharska, A. Z., &Oszmianski, J. (2003). The effects of heating, UV irradiation, and storage on stability of the anthocyanin-polyphenol copigment complex. *Food Chemistry, 81*, 349-355. http://dx.doi.org/10.1016/S0308-8146(02)00429-6

Barka, E. A., Kalantari, S., Makhlouf, J., & Arul, J. (2000). Impact of UV-C irradiation on the cell wall-degrading enzymes during ripening tomato (*Lycopersicon esculentum* L) fruit. *Journal of Agricultural and Food Chemistry, 48*, 667-671. http://dx.doi.org/10.1021/jf9906174

Begum, M., Hocking-Ailsa, D., & Miskelly, D. (2009). Inactivation of food spoilage fungi by ultra violet (UVC) irradiation. *International Journal of Food Microbiology, 129*(*1*), 74-77. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.11.020

Caminiti, I., Palgan, I., Muñoz, A., Noci, F., Whyte, P., Morgan, D., Cronin, D., & Lyng, J. (2010). The effect of ultraviolet light on microbial inactivation and quality attributes of apple juice. *Food Bioprocess Technology*. http://dx.doi.org/10.1007/s11947-010-0365-x.

Castellanos, E., & Yahia, E. M. (2008). Identification and quantification of betalains from the fruits of 10 Mexican prickly pear cultivars by high performance liquid chromatography and electrospray ionization mass spectrometry. *Journal of Agricultural and Food Chemistry, 56*, 5758-5764. http://dx.doi.org/10.1021/jf800362t

Cvetkovic, D., Markovic, D., Cvetkovic, D., & Radovanovic, B. (2011). Effects of continuous UV-irradiation on the antioxidant activities of quercetin and rutin in solution in the presence of lecithin as the protective target. *Journal of the Serbian Chemical Society, 76*(*7*), 973–985. http://dx.doi.org/10.2298/JSC101123089C

Falguera, V., Pagán, J., & Ibarz, A. (2011). Effect of UV irradiation on enzymatic activities and physicochemical properties of apple juices from different varieties. LWT - *Food Science and Technology, 44*, 115-119. http://dx.doi.org/10.1016/j.lwt.2010.05.028

FDA. (2000). 21 CFR Part 179. Irradiation in the production, processing and handling of food. *Federal Register, 65*, 71056–71058.

FDA. (2001). Hazard Analysis and Critical Point (HACCP); Procedures for the safe and sanitary processing and importing of juice; Final rule. *Federal Register, 66*, 6137-6202.

Gabriel, A., & Nakano, N. (2009). Inactivation of *Salmonella*, *E. coli* and *Listeria monocytogenes* in phosphate-buffered saline and apple juice by ultraviolet and heat treatments. *Food Control*, *20*, 443-446. http://dx.doi.org/10.1016/j.foodcont.2008.08.008

Gao, X., Ohlander, M., Jeppsson, N., Bjork, L., & Traljkovski, V. (2000). Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea L. (*Hippophae rhamnoides*L) during maturation Buckthorn. *Journal of Agricultural and Food Chemistry*, *48*, 1485-1490. http://dx.doi.org/10.1021/jf991072g

Guerrero-Beltrán, J. A., & Barbosa-Cánovas, G. V. (2004). Advantages and limitations on processing foods by UV light. *Food Science and Technology International*, *17*(*1*), 137-147.

Guerrero-Beltrán, J. A., & Barbosa-Cánovas, G.V. (2005). Reduction of *Saccharomyces cerevisiae*, *Escherichia coli* and *Listeria innocua* in apple juice by ultraviolet light. *Journal of Food Process Engineering, 28,* 437-452. http://dx.doi.org/10.1111/j.1745-4530.2005.00040.x

Guerrero-Beltrán, J. A., & Barbosa-Cánovas, G. V. (2006). Inactivation of *Saccharomyces cerevisiae* and polyphenoloxidase in mango nectar treated with UV light. *Journal of Food Protection*, *69*(*2*), 362-368.

Guerrero-Beltrán, J. A., Welti-Chanes, J. S., & Barbosa-Cánovas, G. V. (2009). Ultraviolet-C light processing of grape, cranberry and grapefruit juices to inactivate *Saccharomyces cerevisiae. Journal of Food Process Engineering*, *32*(6), 916-932. http://dx.doi.org/10.1111/j.1745-4530.2008.00253.x

Ibarz, A., & Barbosa-Cánovas, G. V. (2002). Thermal processing of foods.In A. Ibarz, & G. V. Barbosa-Cánovas (Eds.), *Unit Operations in Food Engineering* (491-534), Washington: CRC Press.

Keyser, M., Muller, I. A., Cilliers, F. P., Nel, W., & Gouws, P. A. (2008). Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. *Innovative Food Science & Emerging Technologies, 9*(*3*), 348–354.http://dx.doi.org/10.1016/j.ifset.2007.09.002

Koutchma, T. (2009). Advances in ultraviolet light technology for non-thermal processing of liquid foods. *Food and Bioprocess Technology, 2*(*2*), 138–155. http://dx.doi.org/10.1007/s11947-008-0178-3

Kuskoski, M., Asuero, A., Parrilla, M., Troncoso, A., & Fett, R. (2004). Actividad antioxidante de pigmentos antociánicos. *Ciencia y Tecnología de Alimentos* (Campinas, Brazil), *24*, 691-693.

Li, C., Deng, B., & Nyung, C. (2010). A numerical prediction on the reduction of microorganisms with UV disinfection. *Journal of Mechanical Science and Technology*, *24*(*7*), 1465-1473. http://dx.doi.org/10.1007/s12206-010-0502-5

López-Malo, A., Guerrero, S. N., Santiesteban, A., & Alzamora, S. M. (2005). Inactivation kinetics of *Saccharomyces cerevisiae* and *Listeria monocytogenes* in apple juice processed by novel technologies. In *2nd Mercosur Congress on Chemical Engineering, 4th Mercosur Congress on Process Systems Engineering*, Rio de Janeiro, Brazil, August, 14-18.

Lu, G., Li, C., Liu, P., Cui, H., Xia, Y., & Wang, J. (2010). Inactivation of microorganisms in apple juice using an ultraviolet silica-fiber optical device. *Journal of Photochemistry and Photobiology B: Biology*, *100*, 167-172. http://dx.doi.org/10.1016/j.jphotobiol.2010.06.003

Luna, C. (2006). Clasificación y ordenación morfológica del fruto de variantes cultivadas de pitaya [*Stenocereus Pruinosus* (otto) Buxb.] en la Mixteca baja, México. *Revista Chapingo Serie Horticultura, 12*(*2*), 245-251.

Montgometry, J. M. (1985). *Water treatment: Principles and design*. New York: John Wiley and Sons.

Noci, F., Riener, J., Walkling, M., Cronin, D. A., Morgan, D. J., & Lying, J. G. (2008). Ultraviolet irradiation and pulsed electric fields (PEF) in a hurdle strategy for the preservation of fresh apple juice. *Journal of Food Engineering, 85*, 141-146. http://dx.doi.org/10.1016/j.jfoodeng.2007.07.011

Nurliyana, R., Syed, I., Mustapha, K., Aisyah, M. R., & Kamarul, K. (2010). Antioxidant study of pulps and peels of dragon fruits: a comparative study. *International Food Research Journal, 17*, 367-375.

Ochoa, C. E., & Guerrero, J. A. (2012). Efecto del almacenamiento a diferentes temperaturas sobre la calidad de tuna roja (*Opuntia ficus indica* (L.) Miller). *Información Tecnológica, 23*(*1*), 117-128.

Oteiza, J., Giannuzzi, L., & Zaritzky, N. (2010). Ultraviolet treatment of orange juice to inactivate *E. coli* O157:H7 as affected by native microflora. *Food and Bioprocess Technology, 3*(4), 603-614. http://dx.doi.org/10.1007/s11947-009-0194-y

Pala, C., & Tocluku, A. (2011). Effect of UV-C light on anthocyanin and other quality parameters of pomegranate juice. *Journal of Food Composition and Analysis, 24*(6), 790-795. http://dx.doi.org/10.1016/jh.jfca.2011.01.003

Piga, A., Del Caro, A., Pinna, I., & Agabbio, M. (2003). Changes in ascorbic acid, polyphenol content and antioxidant activity in minimally processed cactus pear fruits. *Lebensmittel-Wissenschaft und-Technologie*, *36*, 257-262.

Quek, P., & Hu, J. (2008). Indicators for photoreactivation and dark repair studies following ultraviolet disinfection. *Journal of Industrial Microbiologyand Biotechnology*, *35*, 533-541. http://dx.doi.org/10.1007/s10295-008-0314-0

Repo de Carrasco, R., & Encina, C. (2008). Determinación de la capacidad antioxidante y compuestos bioactivos de frutas nativas peruanas. *Revista de la Sociedad Química del Perú, 74*(*2*), 108-124.

Sabliov, C., Fronczek, C., Astete, C., Khachaturyan, M., Khachatryan, L., & Leonardi, C. (2009). Effects of temperature and UV light on degradation of α-tocopherol in free and dissolved form. *Journal of the American Oil Chemists Society, 86*, 895-902.http://dx.doi.org/10.1007/s11746-009-1411-6

Stermer, R. A., Lasater-Smith, M., & Brasington, C. F. (1987).Ultraviolet radiation an effective bactericide for fresh meat. *Journal of Food Protection*, *50*(*2*), 108-111.

Stintzing, F. C., Schieber, A., & Carle, R. (2002). Identification of betalains from yellow beet (*Beta vulgaris* L.) and cactus pear (*Opuntiaficus-indica*(L.) Mill by HPLC-electrospray ionization mass spectrometry. *Journal of Agricultural and Food Chemistry, 50*, 2302-2307. http://dx.doi.org/10.1021/jf011305f

Torkamani, A. E., & Niakousari, M. (2011). Impact of UV-C light on orange juice quality and shelf life. *International Food Research Journal, 18*(*4*), 1265-1268.

Woo, K., Ngou, F., Ngo, L., Soong, W., & Tang, P. (2011). Stability of betalain pigment from red dragon fruit (*Hylocereus polyrhizus*). *American Journal of Food Technology*, *6*(*2*), 140-148. http://dx.doi.org/10.3923/ajft.2011.140.148.

Table 1. Physicochemical and antioxidant characteristics of fresh pitaya (*Stenocereus griseus*) juice

Characteristic		Quantity
Total soluble solids (%)		6.75±0.08
pH		5.91±0.08
Color	*L*	30.52±0.10
	a	61.91±0.65
	b	18.78±0.06
	Hue	16.87±0.08
	Chroma	64.13±0.13
Phenolic compounds (mg GA/100 mL)		39.00±0.09
Betalains	Betanin (mg/L)	61.05±2.22
	Indicaxanthin (mg/L)	52.48±0.18
Antioxidant activity (mg de T/100 mL)		100.60±1.17

Table 2. Color parameters of pitaya juice after 25 min of UV-C light treatement[1]

	Color parameters			
Flow rate (mL/s)	*L*	*a*	*b*	ΔE
0.00	$30.52\pm0.10_a$	$61.91\pm0.65_a$	$18.78\pm0.06_a$	0.00_a
0.46	$28.26\pm0.14_b$	$58.16\pm0.14_b$	$17.50\pm0.07_b$	3.38_b
3.28	$29.27\pm0.08_c$	$59.88\pm0.16_c$	$18.21\pm0.06_c$	1.63_c
6.57	$29.47\pm0.10_c$	$59.67\pm0.12_c$	$18.31\pm0.08_c$	1.70_c
16.49	$28.57\pm0.08_d$	$58.68\pm0.09_d$	$17.63\pm0.08_d$	2.88_d
30.33	$30.43\pm0.04_a$	$62.22\pm0.14_a$	$18.67\pm0.04_a$	0.87_a

[1]: Same litters within columns indicate no significant differences (P > 0.05).

Table 3. Log cycles reduction for aerobic mesophyll bacteria and yeasts plus molds in UV-C light treated pitaya juice for 25 minutes

	Aerobic mesophyll bacteria		Yeasts plus molds	
Flow rate (mL/s)	(CFU/mL) ($x10^{-3}$)	log (*N*/*No*)	(CFU/mL)	log (*N*/*No*)
0.00	38.0±0.079	0	850±21	0
0.46	18.0±0.079	-0.33	630±57	-0.12
3.28	12.0±0.078	-0.52	460±35	-0.27
6.57	9.6±0.020	-0.6	430±14	-0.29
16.49	1.0±0.014	-1.58	165±21	-0.71
30.33	0.5±0.028	-2.11	62±11	-1.14

Table 4. First order kinetics data from the aerobic mesophyll bacteria and yeasts plus molds inactivation in UV-C light treated pitaya juice

	Aerobic mesophyll bacteria					Yeasts plus molds				
Flow rate (mL/s)	m (1/min)	b	R^2	D_{uv} (min)	D_{uv} (kJ/m^2)	m (1/min)	b	R^2	D_{uv} (min)	D_{uv} (kJ/m^2)
0	--	--	--	--	--	--	--	--	--	--
0.46	-0.013	0.005	0.97	80.1	2.74	-0.003	-0.042	0.50	375	12.8
3.28	-0.022	0.022	0.98	46.7	1.59	-0.012	0.019	0.93	87.1	2.98
6.57	-0.025	0.031	0.99	40.8	1.39	-0.011	0.049	0.75	101	3.45
16.49	-0.068	0.175	0.98	14.9	0.51	-0.029	0.014	0.89	35.9	1.23
30.33	-0.073	-0.130	0.96	13.9	0.47	-0.04	-0.26	0.91	23.5	0.80

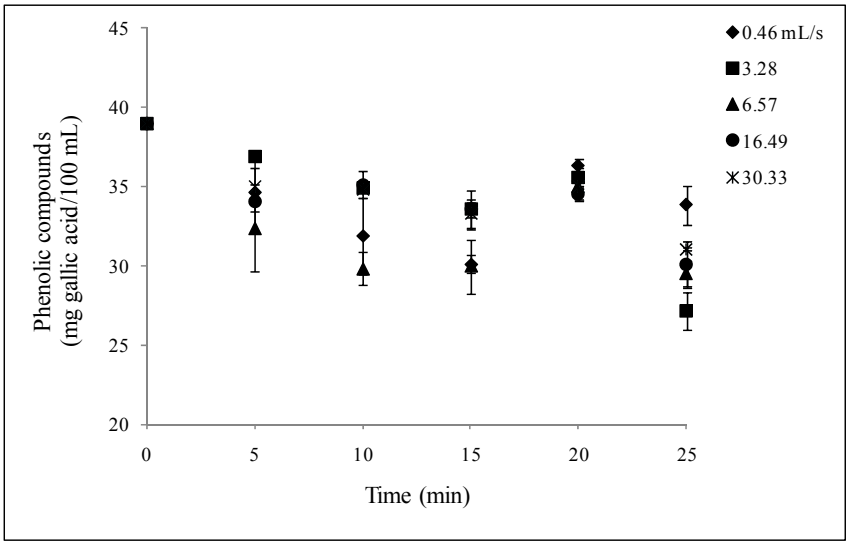

Figure 1. Phenolic compounds in UV-Clight treated pitaya juice

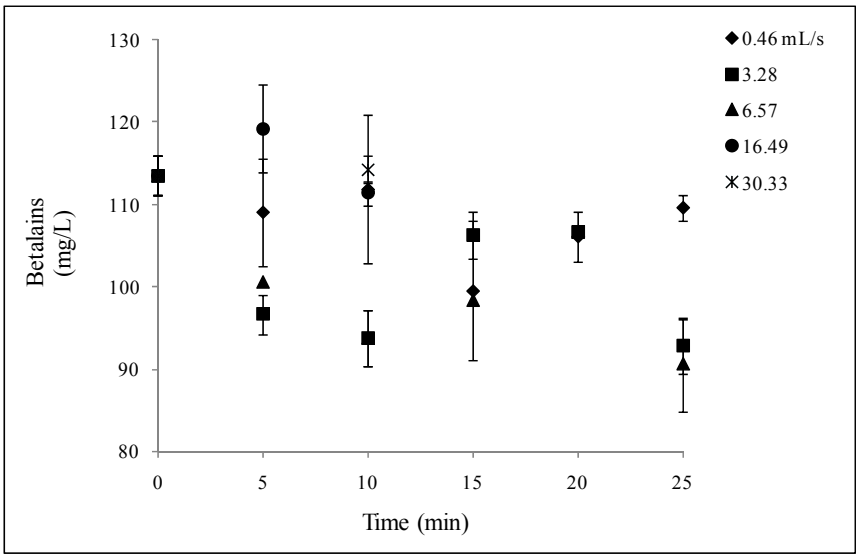

Figure 2. Betalains content in UV-C light treated pitaya juice

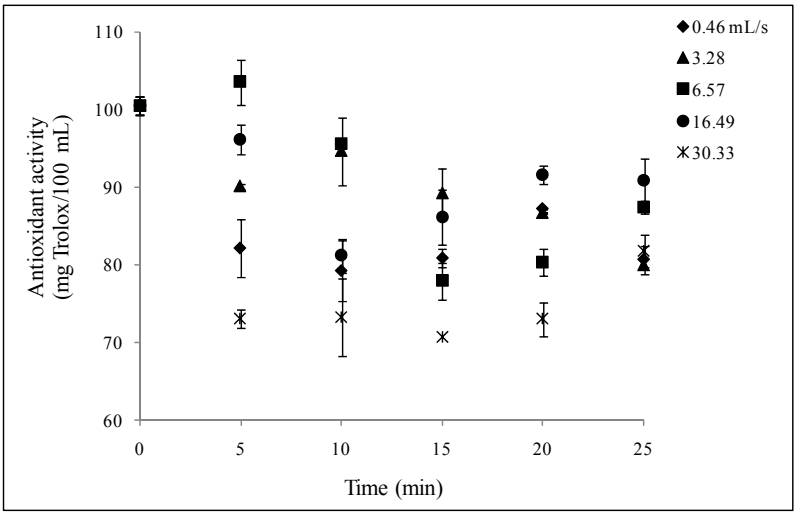

Figure 3. Antioxidant activity in UV-C light treated pitaya juice

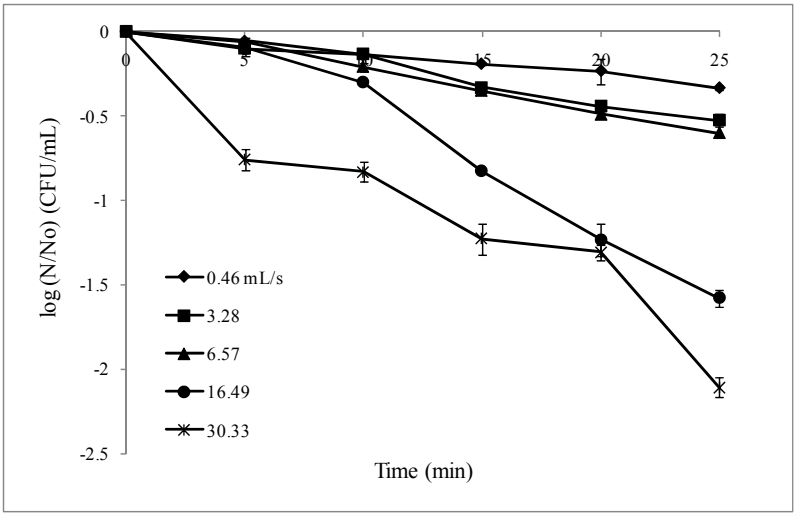

Figure 4. Aerobic mesophyll bacteria in UV-C light treated pitaya juice

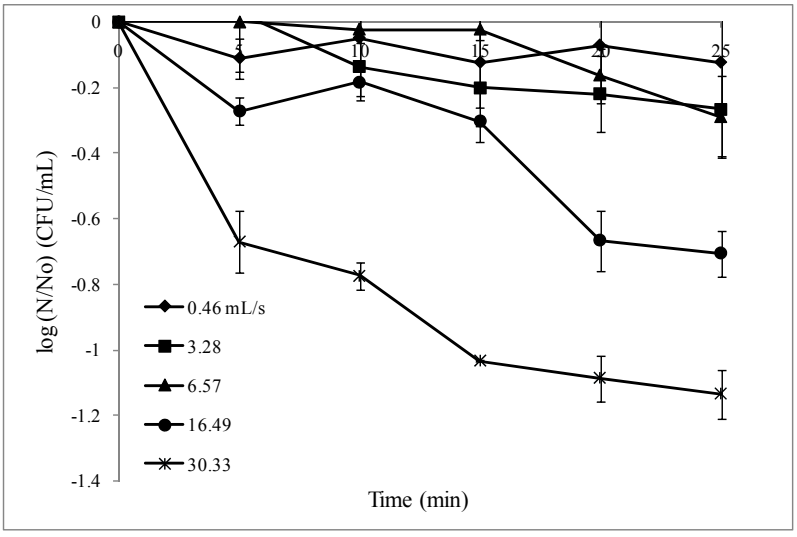

Figure 5. Molds plus yeasts in UV-C light treated pitaya juice

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Attitude of Working Mothers to Exclusive Breastfeeding in Calabar Municipality, Cross River State, Nigeria

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Abstract

Attitude of working mothers on breastfeeding was studied between December, 2011 and February, 2012 in Calabar Municipality, Cross River State, Nigeria. One hundred willing mothers were interviewed using self-administered questionnaires, for literate mothers and oral administration of questionnaires to illiterate mothers. Data were analyzed using student's t-test and chi-square statistics. Our results have confirmed that some factors such as age, tribe, religion, occupation, marital status, educational status, health status, socio-economic status, attendance at anti-natal clinics, number of children and diverse opinions have affected the attitudes of working mothers towards EBF. We concluded that attitude of working mothers to EBF is influenced by some demographic and socio-economic factors.

Keywords: Exclusive Breastfeeding, Working Mothers

1. Introduction

Breastfeeding has been accepted as the most vital intervention for reducing infant mortality and ensuring optimal growth and development of children (Gupta & Arora, 2007). Breastfeeding is the ideal method suited for the physiological and psychological needs of an infant (Subbiah, 2003). It is estimated that sub-optimal breastfeeding, especially non-exclusive breastfeeding in the first 6 months of life, results in 1.4 million deaths and 10% of the disease burden in children younger than 5 years of age (WHO, 2009). Exclusive breastfeeding (EBF) for the first 6 months of life improves the growth, health and survival status of newborns (WHO, 2003) and is one of the most natural and best forms of preventive medicine (WHO, 2001). EBF plays a pivotal role in determining the optimal health and development of infants, and is associated with a decreased risk for many early life diseases and conditions, including otitis media, respiratory tract infection, diarrhoea and early childhood obesity (Ip *et al.,* 2007).

It has been estimated that EBF reduces infant mortality rates by up to 13% in low-income countries (Jones *et al.,* 2003). A large cohort study undertaken in rural Ghana concluded that 22% of neonatal deaths could be prevented if all infants were put to breast within the first hour of birth (Edmund *et al.,* 2006). Reviews of studies from developing countries show that infants who are not breastfed are 6 to 10 times more likely to die in the first months of life than infants who are breastfed (WHO, 2000; Bahl *et al.,* 2005).

Some researchers have proposed that lack of suitable facilities outside of the home, inconvenience, conflicts at work, family pressure and ignorance adversely affect the willingness of women to practise EBF (Ogbonna *et al.,*

2000; Forbes *et al.,* 2003). The need to return to work has also been implicated as a factor interfering with EBF (Mahgoub *et al.,* 2002).

The Nigerian government established the Baby-Friendly Hospital Initiative (BFHI) in Benin, Enugu, Maiduguri, Lagos, Jos and Port Harcourt with the aim of providing mothers and their infants a supportive environment for breastfeeding and to promote appropriate breastfeeding practices, thus helping to reduce infant morbidity and mortality rates. Despite these efforts, child and infant mortality continue to be major health issues affecting Nigeria. The infant mortality rate for the most recent five-year period (1999-2003) is about 100 deaths per 1,000 live births. EBF rates in Nigeria continue to fall well below the WHO/UNICEF recommendation of 90% EBF in children less than 6 months (WHO, 2009).

A more detailed understanding of the attitude of working mothers to exclusive breastfeeding EBF in Nigeria is needed to develop effective interventions to improve the rates of EBF and thus reduce infant mortality. The objective of the present study is to investigate the attitude of working mothers to exclusive breastfeeding (EBF) in Calabar municipality, Cross River State, Nigeria.

2. Materials and Methods

The study was carried out from December, 2011 to February 2012 in Calabar municipality, Cross River State. Ethical clearance was obtained from the Ethical committee of the Cross River State Ministry of Health before the study was conducted. Verbal consent was obtained from willing mothers.

A total of 100 mothers were randomly interviewed. A structured questionnaire was self-administered by literate mothers and interviewer-administered for those who are not literate. Information contained in the questionnaire include age, tribe, religion, occupation, marital status, educational status, socio-economic status, attendance of ante-natal clinic, extended family presence, health status, number of children and breastfeeding attitude. Data were analysed using the Statistical Package for Social Sciences (SPSS), version 17.0. Means and proportions were compared using Student's *t*-test and the chi-square test.

3. Results

Out of the 100 mothers interviewed, 24 women practised exclusive breastfeeding (EBF), 66 were mixed feeding mothers (MFM) and 10 were non-breastfeeding mothers. Age distribution showed that 10 women were in the age range of 16-26, 65 women were in the age range of 27-37 and 25 women in the range of 38-48 respectively. Exclusive breastfeeding rate was highest in age range of 27 -37 with 66.67% and lowest in age range of 16-26 with 8.33%.

Exclusive breastfeeding rate evaluated according to tribe was highest amongst the Efiks and Ibibios with 10 (41.66%) mothers practising EBF followed by Hausa tribe with 7(29.17%), Igbos with 4(16.67%) while the Yoruba tribe had the lowest 3(12.5%). EBF evaluated according to religion was highest amongst the Christians with 18 (75%) mothers practising EBF followed by Muslims with 4(16.67%), while those who were neither Christians nor Muslims had the lowest EBF of 2(8.33%). Exclusive breastfeeding rate evaluated according to occupation was highest in self-employed mothers with 16 (66.67%) practising while civil and public servants had the same EBF of 4(16.67%) each. EBF evaluated according to marital status was highest in married mothers19 (79.17%), followed by divorced mothers with 3(12.5%), while single mothers had the lowest EBF of 2(8.33%).

EBF evaluated according to educational status was highest in tertiary institutions with 10 (41.66%) mothers followed by primary and secondary institutions with 6(25%) each while illiterate mothers had the lowest EBF of 2(8.33%). For socio-economic status, lower class mothers had the highest EBF of 14 (58.33%) followed by middle class mothers with 6(25%), while upper class mothers had the lowest EBF of 4(16.67%). EBF was higher in mothers who attended ante-natal clinics with 20(83.33%) than mothers who did not attend ante-natal clinics with EBF of 4(16.67%). EBF was higher in mothers who enjoyed the presence of extended family members with 18(75%) than mothers without the presence of extended family members with EBF of 6(25%). EBF was only found in healthy mothers with 24(100%) while HIV positive mothers, mothers on certain medication and mothers with active and untreated ailment did not practise EBF 0(0%). Finally, mothers with 1-2 children had the highest EBF of 18(75%), followed by mothers with 3-4 children with EBF of 4(16.67%) while mothers with 5 children and above had the lowest EBF of 2(8.33%). Table 1 shows Frequency and percentage of mothers practising EBF by some selected Socioeconomic and Demographic Characteristics in Calabar municipality.

(Table 1)

4. Discussion

The EBF of 24% in this study was low but agrees with reports by other workers in Nigeria (Otaigbe *et al.,* 2005); this is in spite of the high level of knowledge about EBF, indicating that some detracting factors may be at play. Mothers between the ages of 27-37 years showed the highest EBF in this study which was significantly ($P<0.05$) different from mothers in 16-26 and 38-48years. This may be attributed to inexperience on the part of the younger women, who are also more easily influenced by family pressure. Although mothers from the Efik and Ibibio tribes had the highest EBF in this study, there was no significant difference ($P>0.05$) from mothers in other tribes. Smaller family size (1-2 children) had a positive influence on EBF among women than large families (3-5 children). However, this is an indication that mothers can cope better with the demands of EBF when they have fewer babies who are well spaced out; this reduces the likelihood of 'burnout' and maternal exhaustion.

A higher maternal educational level was observed to favour EBF significantly ($P<0.05$) as compared to illiterate mothers. This may be informed by their understanding of the health implications of EBF on child's health. This is in support of the findings of Ogbonna et al, 2000.

Attendance of ante-natal clinic enhances mothers' understanding and appreciation of the demands and benefits of EBF, and empowers them to resist external interferences and pressures.

Although extended family members had a higher value of EBF in the study, there was no significant difference ($P>0.05$) with mothers without extended family members. Women who were self employed showed the highest EBF as compared to women who were public and civil servants. This shows that the nature of occupation can interfere with EBF since women who were public and civil servants were distracted by their occupation than self employed mothers. This is also in support of Ogbonna *et al.,* 2000.

In our study, 10% of the women never practised EBF believing that their breast milk was insufficient for babies need. They misinterpreted excessive crying by babies to be an indication of hunger. However, 66% of mothers who practised mixed feeding believed that their breast milk needs to be supplemented with artificial formulae for fast growth and health of their babies.

Married women were observed to have the highest EBF than singles and divorced mothers. This is an indication that the presence of a husband can have positive influence on EBF. The restraint of few unhealthy mothers from EBF as observed in this study is a reflection of counselling by health workers to safeguard their babies from cross-infections.

The few non-breastfeeding mothers and mixed feeding mothers encountered in this study were having negative opinions towards breastfeeding, seeing it as embarrassing in the public while a few are not willing to carry fallen breast which are likely to come after exclusive breastfeeding and a few are afraid of pains associated with breastfeeding. For these last categories, there are needs for encouragements and support from friends, family members and proper counselling towards attitudinal change in favour of EBF (Kloeblen et al., 2002; Persad & Mensinger, 2008; Hurley et al., 2008).

The opinions of mothers who are not willing to embark on EBF and those who are partially supporting it should be respected by subtle intervention through proper counselling by health workers. Moreover, government and non governmental agencies should establish crèche close to offices where nursing mothers could have access to breastfeed their babies during official hours.

Government policies towards maternity leave should reflect the need to provide reasonable time for nursing mothers to breastfeed their babies before returning to work.

Referenes

Bahl, R., Frost, C., Kirkwood, B. R., Karen, E., Martines, J., & Bhandari N. (2005). Infant feeding patterns and risks of death and hospitalization in the first half of infancy: multicentre cohort study. *World Health Organization, 83*, 418-426.

Edmond, K. M., Zandoh, C., Quigley, M. A., Amenga-Etego, S., Owusu-Agyei, S., & Kirkwood, B. R. (2006). Delayed Breastfeeding Initiation Increases Risk of Neonatal Mortality. *Pediatrics, 117*, e380-e386. http://dx.doi.org/10.1542/peds.2005-1496

Forbes, G. B, Adams-Curtis, L. E., Hamm, N. R., & White, K. B (2003). Perceptions of the woman who breastfeeds: the role of erotophobia, sexism, and attitudinal variables. *Sex Roles: A Journal of Research, 49*, 379-388.

Gupta, A., & Arora, V. (2007). The State of World's Breastfeeding -Tracking Implementation of the Global Strategy for Infant and Young Child Feeding. International Baby Food Action Network (IBFAN), Asia Pacific. South Asia report.

Hurley, K. M., Black, M. M., & Papas, M. A. (2008). Variation in breastfeeding behaviours, perceptions, and experiences by race/ethnicity among a low-income statewide sample of Special Supplemental Nutrition Program for Women, Infants and Children (WIC) participants in the United States. *Maternal Child Nutrition 4*, 95-105. http://dx.doi.org/10.1111/j.1740-8709.2007.00105.x

Ip, S., Chung, M., Raman, G., Chew, P., Magula, N., DeVine, D., Trikalinos, T., & Lau, J. (2007). Breastfeeding and maternal and infant health outcomes in developed countries. Rockville, MD; US Department of Health and Human Services.

Jones, G., Steketee, R., Black, R., Bhutta, Z., & Morris, S. (2003). The Bellagio Child Survival Study Group: How many child deaths can we prevent this year? *Lancet, 362*(19), 65-71. http://dx.doi.org/10.1016/S0140-6736(03)13811-1

Kloeblen-Tarver, A. S., Thompson, N. J., & Miner, K. R. (2002). Intent to breast-feed: The impact of attitudes, norms, parity and experience. *American Journal of Health and Behaviour, 26*, 182-187. http://dx.doi.org/10.5993/AJHB.26.3.3

Mahgoub, S. E. O., Bandeke, T., & Nneypi, M. (2002). Breastfeeding in Botswana: Practices, attitudes, patterns, and the socio-cultural factors affecting them. *Journal of Tropical Paediatric, 48*, 195-199. http://dx.doi.org/10.1093/tropej/48.4.195

Ogbonna, C., Okolo, A. A., & Ezeogu, A. (2000). Factors influencing exclusive breastfeeding in Jos, Plateau State, Nigeria. *West African Journal of Medicine, 19*, 107-110.

Otaigbe, B. E., Alikor, E. A. D., & Nkanginieme, K. E. O. (2005). Growth pattern of exclusively breastfed infants. *Nigerian Journal of Medicine, 14*, 137-145.

Persad, M. D., & Mensinger, J. L. (2008). Maternal breastfeeding attitudes: Association with breastfeeding intent and socio-demographics among urban primiparas. *Journal of Community Health,* 33, 53–60. http://dx.doi.org/10.1007/s10900-007-9068-2

Subbiah, N. (2003). A Study to assess the Knowledge, Attitude, Practice and Problems of Postnatal Mothers regarding Breastfeeding. *Nursing Journal of India, 94*(8), 177-179.

World Health Organization (2000). WHO Collaborative Study Team on the Role of Breast-feeding on the Prevention of Infant Mortality. Effect of breastfeeding on infant and childhood mortality due to infectious diseases in less developed countries: a pooled analysis. *Lancet, 355*, 451-455. http://dx.doi.org/10.1016/S0140-6736(99)06260-1

World Health Organisation (2003). *The Global Strategy for Infant and Young Child Feeding*. Geneva.

World Health Organization (2009). Infant and young child feeding Model Chapter for textbooks for medical stu-dents and allied health professionals.

Table 1. Frequency and percentage of mothers practising EBF by Some Selected Socioeconomic and Demographic Characteristics in Calabar municipality

FACTOR	FREQUENCY	EBF(%)	Chi -square	P-Value
AGE				
16-26	10	2(8.33)		
27-37	65	16(66.67)	13.00	0.002
38-48	25	6(25)		
	100	**24**		
TRIBE				
Igbo	30	4(16.67)		
Efik/Ibibio	50	10(41.66)	5.00	0.172
Yoruba	15	3(12.5)		
Hausa	5	7(29.17)		
	100	**24**		
RELIGION				
Christian	90	18(75)		
Muslim	5	4(16.67)	19.00	0.000
None of the above	5	2(8.33)		
	100	**24**		
OCCUPATION				
Civil servant	53	4(16.67)		
Public servant	30	4(16.67)	2.667	0.102
Self employed	17	16(66.67)		
	100	**24**		
MARITAL SERVICE				
single	15	2(8.33)		
married	6	19(79.17)	22.75	0.000
divorced	9	3(12.5)		
	100	**24**		
EDUCATIONAL STATUS				
Illiterate	20	2(8.33)		
Primary	5	6(25)		
Secondary	35	6(25)	7.0	0.300
Tertiary	40	10(41.66)		
	100	**24**		
SOCIO-ECONOMIC STATUS				
Lower class	28	14(58.33)		
Middle class	52	6(25)	7.0	0.300
Upper class	20	4(16.67)		
	100	**24**		
ATTENDANCE OF ANTE-NATAL CLINIC				
Yes	86	20(83.33)		
No	14	4(16.67)	10.67	0.001
	100	**24**		
EXTENDED FAMILY PRESENCE				
Yes	77	18(75)		
No	23	6(25)	6.0	0.014
	100	**24**		
HEALTH STATUS				
Healthy mothers	70	24(100)		
HIV positive mothers	15	0(0)	-	-
Mothers on certain medication	5	0(0)		
Others with active, untreated ailment	10	0(0)		
	100	**24**		
NO. OF CHILDREN				
1-2	60	18(75)		
3-4	32	4(16.67)	19.00	0.000
≥5	8	2(8.33)		
	100	**24**		
BREASTFEEDING ATTITUDE				
EBF from 0-6months	24			0.000
MFM from 0-6months	66		50.94	
Non- breastfeeding mothers	10			
	100			

EBF=Exclusive Breast Feeding; MFM=Mixed Breast Feeding.

Interaction between Food-borne Pathogens (*Campylobacter jejuni*, *Salmonella* Typhimurium and *Listeria monocytogenes*) and a Common Soil Flagellate (*Cercomonas* sp.)

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Abstract

Free-living protozoa may harbor, protect, and disperse bacteria, including those ingested and passed in viable form in feces. The flagellates are very important predators on bacteria in soil, but their role in the survival of food-borne pathogens associated with fruits and vegetables is not well understood. In this study, we investigated the interactions between a common soil flagellate, *Cercomonas* sp., and three different bacterial pathogens (*Campylobacter jejuni*, *Salmonella* Typhimurium, and *Listeria monocytogenes*). Rapid growth of flagellates was observed in co-culture with *C. jejuni* and *S.* Typhimurium over the time course of 15 days. In contrast, the number of *Cercomonas* sp. cells decreased when grown with or without *L. monocytogenes* for 9 days of co-culture. Interestingly, we observed that *C. jejuni* and *S.* Typhimurium survived better when co-cultured with flagellates than when cultured alone. The results of this study suggest that *Cercomonas* sp. and perhaps other soil flagellates may play a role for the survival of food-borne pathogens on plant surfaces and in soil.

Keywords: *Cercomonas* sp., *C. jejuni*, *L. monocytogenes*, *S.* Typhimurium, Flagellate

1. Introduction

Outbreaks of food-borne disease caused by *Campylobacter*, *Salmonella* or *Listeria* associated with the consumption of contaminated vegetables have recently been reported and received worldwide attention (Beuchat, 1996; Crook et al., 2003; Pakalniskiene et al., 2009; Gajraj, Pooransingh, Hawker & Olowokure, 2011; Gardner et al., 2011). Fresh produce consumed raw or minimally processed, such as fruits and vegetables, provide an ideal route for the transmission of certain enteric pathogenic bacteria including *Salmonella* spp., *Escherichia coli*, *Campylobacter jejuni*, and *Listeria monocytogenes* (Beuchat, 2002; Islam et al., 2004; Berger et al., 2010; Newell et al., 2010; Brassard, Guévremont, Gagné & Lamoureux, 2011). Primary sources of pre-harvest contamination include soil-improvement with untreated or improperly composted manure and contaminated irrigation water (Buck, Walcott & Beuchat, 2003; Islam et al., 2004; Berger et al., 2010; McLaughlin, Casey, Cotter, Gahan & Hill, 2011). It has been reported that the microbiota of soil-grown fruits and vegetables may be reflecting the microbiota of soils in which they grow (Jay, Loessner & Golden, 2005).

Protozoa, traditionally divided on the basis of their means of locomotion into four broad categories ciliates, flagellates, sporozoans, and amoebae, are the primary bacterial predators in soil. Of these groups, flagellates and amoebae are thought to be the most abundant and are able to enter soil pore necks as small as 3 μm (Ekelund & Rønn, 1994; Gaze, Burroughs, Gallagher & Wellington, 2003). Flagellates as well as amoebae are important bacterial grazers, and flagellates have been shown to change the composition of the bacterial community in a different manner than the soil amoebae *Acanthamoebae* spp. They play an important role in microbial degradation processes and nutrient flow in soil (Pedersen, Nybroe, Winding, Ekelund & Bjørnlund, 2009). Recent studies have suggested that free-living amoebae are important players in the evolution of obligate and facultative bacterial pathogens (Zhou, Elmose & Call, 2007). Although it has been shown that amoebae can prolong the survival of food-borne pathogens (Gaze et al., 2003; Zhou et al., 2007; Baré et al., 2010), relatively little is known about the role of flagellates in the epidemiology of food-borne diseases. Furthermore, it has been reported that flagellates are present in high numbers on vegetables such as lettuce and spinach (Gourabathini, Brandl, Redding, Gunderson & Berk, 2008; Vaerewijck, Sabbe, Baré & Houf, 2011). These protists ingest only a few bacteria at a time and their role in the survival of food-borne pathogens on plant surface and in soil remains to be investigated (Gourabathini et al., 2008). Accordingly, we investigated the ability of three different food-borne pathogens (*C. jejuni*, *S.* Typhimurium, and *L. monocytogenes*) to survive in co-culture with *Cercomonas* sp. - a common soil flagellate. These bacterial pathogens were selected because they have caused recent outbreaks (Beuchat, 1996; Crook et al., 2003; Gajraj, Pooransingh, Hawker & Olowokure, 2011; Gardner et al., 2011). Although flagellates are the most abundant and widespread soil mesofauna, relatively little is known regarding the impact of this free-living protozoan on fresh produce.

2. Materials and Methods

2.1 Bacteria and conditions

The reference strains of *C. jejuni* NCTC 11168, *L. monocytogenes* VDL 148, and *S.* Typhymurium NCTC 12023 were used in this study to investigate the interactions of these pathogens with a common soil flagellate, *Cercomonas* sp. Before each experiment, *C. jejuni* was grown under microaerobic conditions for 24 h on blood agar (BA) plates (Tryptic soy agar containing 5% [vol/vol] whole sheep blood, 10 μg/ml vancomycin and 5 μg/ml trimethoprim) at 37ºC. *L. monocytogenes* and *S.* Typhimurium were grown on BA plates for 16 h in aerobic conditions.

2.2 Protozoan

The flagellate *Cercomonas* sp. reference strain ATCC 50334 was used as an axenic culture and is maintained at 15ºC on a mixture of heat-killed cells of a soil isolate *Pseudomonas putida* reference strain ATCC 17426. as *Pseudomonas* spp. can be a food source of *Cercomonas* sp. as previously described (Pedersen et al., 2009). and a nutrient medium (ATCC medium 802). The bacteria were harvested and washed twice with modified Neff's Amoeba Saline (AS) buffer (Lekfeldt & Rønn, 2008) and then killed at 80ºC for 15 min. The heterotrophic flagellate *Cercomonas* sp. cells from an actively growing axenic culture was washed three times with AS buffer and subsequently added to 25 cm^2 cell culture flask (Nunc, Roskilde, Denmark) containing 5 ml of ATCC medium 802 to reach the final concentration of 2×10^3 flagellate cells/ml.

2.3 Co-culture experiments

An inoculum of each food-borne pathogen was added to separate flagellate flask with an estimated starting concentration of 10^8 CFU/ml. For control experiments, 100 μl of 5×10^9 CFU/ml heat-killed *P. putida* was added to a flagellate flask as a positive control, while 100 μl of AS buffer was added to another flagellate flask as a

negative control. All flasks were incubated at 15ºC in aerobic conditions. The number of bacterial cells and flagellates were determined at day 3, 6, 9, 12, and 15 of the co-cultures.

2.4 Survival of bacteria and flagellate

The growth of the flagellate was measured by counting the concentration of flagellates (cells/ml) at different time points in the cell culture flasks using an inverted light microscope with LED illumination at ×200 magnification (Leica DM IL LED, Leica Microsystems GmbH, Wetzlar, Germany). For *C. jejuni*, aliquots of 100 µl of 10-fold serial dilutions of co-culture medium were spotted on BA plates and incubated at 37ºC in microaerobic conditions for 36 h until bacterial colonies formed. For *S.* Typhimurium and *L. monocytogenes*, aliquots of 100 µl of 10-fold serial dilutions of co-culture were spread on BA plates and incubated at 37ºC in aerobic conditions for 16 and 24 h, respectively.

2.5 Statistical analysis

A Student's *t*-test was used to compare the numbers of bacteria in co-culture. *P*-values of < 0.05 were considered statistically significant.

3. Results and Discussion

To investigate the interaction of food-borne pathogens with flagellates, we first determined whether these bacteria have an effect on the growth of *Cercomonas* sp. As shown in Figure 1, the flagellate *Cercomonas* sp. did not grow in the co-culture with *L. monocytogenes* and lost the viability after day 3 and decreased more after 6 days until no cells were detectable by day 12. There was no significant difference in the number of *Cercomonas* sp. cells when cultivated with or without *L. monocytogenes* for flagellate cells decreased rapidly in both cases (Figure 1). Interestingly, the rapid growth of flagellates was observed in the co-culture with *C. jejuni* and *S.* Typhimurium as well as in the positive control. The numbers of flagellates counted in flasks cultivated with *C. jejuni* and *S.* Typhimurium were almost equal to numbers of flagellate cells obtained in positive control flasks (where heat-killed *P. putida* was added) over the time course of 15 days. These results are in agreement with a previous study that described Gram-negative bacteria including *Pseudomonas* spp. as a good food source for the growth of *Cercomonas* sp. (Lekfeldt & Rønn, 2008; Pedersen et al., 2009).

The effect of flagellates on survival of food-borne pathogens in co-culture was determined by conventional bacterial plate counting (CFU) at different time points. As shown in Figure 2, no significant difference was obtained with the number of *L. monocytogenes* cultivated with or without *Cercomonas* sp. after 12 days (Figure 2). This corresponded well to the decreased number of *Cercomonas* sp. cells, suggesting that this bacterium is not a food source and may be toxic for the flagellates. Cytotoxicity of haemolytic *Listeria* spp. in protozoa was originally demonstrated by Ly & Muller, (1990). They have shown that haemolytic *L. monocytogenes* and *L. seeligeri* induce lysis of *Tetrahymena pyriformis* and *Acanthamoeba castellanii* during 8-15 days, while only few protozoa underwent lysis in the presence of non-haemolytic *L. innocua*. Interestingly, the number of *C. jejuni* cells in co-culture with *Cercomonas* sp. decreased slowly and remained approximately 2×10^2 CFU/ml at day 15. This corresponded well with the higher final number of flagellate cells when grown with this bacterium of apparent high food source (Figure 1). In contrast, in the absence of flagellates, CFU number of *C. jejuni* decreased rapidly and 2.6×10^4 and 3.4×10^2 CFU/ml were obtained at day 3 and day 6, respectively. The number of *S.* Typhimurium cells obtained in the co-culture with *Cercomonas* sp. was significantly higher ($P<0.05$) than those obtained in the culture without flagellates on day 9, 12 and 15 (Figure 2). This bacterium seems to be a good food source for the flagellate as a higher number of *Cercomonas* sp. was observed over the time course of 15 days. Although flagellates ingest *C. jejuni* and *S.* Typhimurium in the co-cultures, these bacteria still seem to survive longer in the presence of this protozoan than when cultivated without protozoan. Our data suggest that the flagellates use *C. jejuni* and *S.* Typhimurium as food sources, but there seems to be a mutual benefit in the relationship. By enhancing bacterial survival, the protozoa do not run out of food, while the bacteria "enjoy" the more favorable conditions generated by the flagellates and use the flagellates as temporary protective structures and vehicles for dissemination. It has been reported that flagellates ingest only a few bacteria at a time (Gourabathini et al., 2008), and thus they do not hinder the survival of *C. jejuni* and *S.* Typhimurium, which are in agreement with our data. Our data suggest that flagellates may play a role in the transmission of food-borne pathogens as they may enter the human food chain following the application of animal manures to agricultural land with raw consumed crops such as salads, fruit and vegetables. Furthermore, it has been reported that food-borne pathogens originating from animal manures could survive for a long time in soil after application (Nicholson, Groves & Chambers, 2005). Alongside amoebae which have been demonstrated to promote the survival of these pathogens (Gaze et al., 2003; Baré et al., 2010); our study suggests that flagellates may also play a similar role as amoebae.

Observations reported here demonstrate that *Cercomonas* sp., a common soil flagellate, is strongly attracted to and consumes both *C. jejuni* and *S.* Typhimurium which can be introduced into agricultural soil through the deposition of animal faeces, untreated irrigation water, or runoff water from livestock feeding lots (Islam et al., 2004; Berger et al., 2010). Our data indicate that *Cercomonas* sp. consumed *C. jejuni* and *S.* Typhimurium as food sources but not *L. monocytogenes*. Furthermore, *Cercomonas* sp. not only consumes but also significantly prolonged the survival of both *C. jejuni* and *S.* Typhimurium in co-culture up to 15 days while *L. monocytogenes* died after 3-6 days. We did not determine the internal location of bacterial pathogens inside *Cercomonas* sp., but our data support and suggest that by prolonging the survival of bacterial pathogens when cultivated with *Cercomonas* sp. can open a window for the possibility of a cross contamination of these pathogens from soil to the human food chains. The cross contamination could be due to *Cercomonas* sp. itself acting as a vector for carrying the bacteria, but it needs to be proved and examined by different methods. In addition, prolonging the survival of food-borne pathogens in soil by *Cercomonas* sp. could increase the risk of other protozoa, insects, worms or wild birds to be a vector for the pathogens. Also, it is very interesting to study what factors contribute to prolonging the survival of the bacterial pathogens in co-culture with *Cercomonas* sp. The experiments in this direction are in progress. Furthermore, the results of this study could open a new direction for studying the interaction between protozoa and bacterial pathogens from the environments such as fertilized soil, water and animal manures to human foods, specially the consumption of raw crops.

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References

Baré, J., Sabbe, K., Huws, S., Vercauteren, D., Braeckmans, K., Van Gremberghe, I. Favoreel, H., & Houf, K. (2010). Influence of temperature, oxygen and bacterial strain identity on the association of *Campylobacter jejuni* with *Acanthamoeba castellanii*. *FEMS Microbiology Ecology, 74*, 371-381. http://dx.doi.org/10.1111/j.1574-6941.2010.00955.x

Berger, C. N., Sodha, S. V., Shaw, R. K., Griffin, P. M., Pink, D., Hand, P., & Frankel, G. (2010). Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environmental Microbiology, 12*, 2385-2397. http://dx.doi.org/10.1111/j.1462-2920.2010.02297.x

Beuchat, L. R. (1996). *Listeria monocytogenes*: incidence on vegetables. *Food Control, 7*, 223-228. http://dx.doi.org/10.1016/s0956-7135(96)00039-4

Beuchat, L. R. (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes and Infection, 4*, 413-423. http://dx.doi.org/10.1016/s1286-4579(02)01555-1

Brassard, J., Guévremont, É., Gagné, M.-J., & Lamoureux, L. (2011). Simultaneous recovery of bacteria and viruses from contaminated water and spinach by a filtration method. *International Journal of Food Microbiology, 144*, 565-568. http://dx.doi.org/10.1016/j.ijfoodmicro.2010.11.015

Buck, J. W., Walcott, R. R., & Beuchat, L. R. (2003). Recent trends in microbiological safety of fruits and vegetables. *Plant Health Progress*. http://dx.doi.org/10.1094/PHP-2003-0121-01-RV.

Crook, P. D., Aguilera, J. F., Threlfall, E. J., *et al*. (2003). A European outbreak of *Salmonella enterica* serotype Typhimurium definitive phage type 204b in 2000. *Clinical Microbiology and Infection, 9*, 839-845. http://dx.doi.org/10.1046/j.1469-0691.2003.00655.x

Ekelund, F., & Rønn, R. (1994). Notes on protozoa in agricultural soil with emphasis on heterotrophic flagellates and naked amoebae and their ecology. *FEMS Microbiology Reviews, 15*, 321-353. http://dx.doi.org/10.1016/0168-6445(94)90068-x

Gajraj, R., Pooransingh, S., Hawker, J. I., & Olowokure, B. (2011). Multiple outbreaks of *Salmonella braenderup* associated with consumption of iceberg lettuce. *International Journal of Environmental Health Research*, 1-6. http://dx.doi.org/10.1080/09603123.2011.613114

Gardner, T. J., Fitzgerald, C., Xavier, C., Klein, R., Pruckler, J., Stroika, S., & McLaughlin, J. B. (2011). Outbreak of campylobacteriosis associated with consumption of raw peas. *Clinical Infectious Diseases*, *53*, 26-32. http://dx.doi.org/10.1093/cid/cir249

Gaze, W. H., Burroughs, N., Gallagher, M. P., & Wellington, E. M. H. (2003). Interactions between *Salmonella typhimurium* and *Acanthamoeba polyphaga*, and observation of a new mode of intracellular growth within contractile vacuoles. *Microbial Ecology, 46*, 358-369. http://dx.doi.org/10.1007/s00248-003-1001-3

Gourabathini, P., Brandl, M. T., Redding, K. S., Gunderson, J. H., & Berk, S. G. (2008). Interactions between food-borne pathogens and protozoa isolated from lettuce and spinach. *Applied and Environmental Microbiology, 74*, 2518-2525. http://dx.doi.org/10.1128/aem.02709-07

Islam, M., Morgan, J., Doyle, M. P., Phatak, S. C., Millner, P., & Jiang, X. (2004). Persistence of *Salmonella enterica* serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Foodborne Pathogens and Disease, 1*, 27-35. http://dx.doi.org/10.1089/153531404772914437

Jay, J. M., Loessner, M. J., & Golden, D. A. (2005). Taxonomy, role, and significance of microorganisms in foods modern food microbiology, pp. 13-37. In: Springer US, http://dx.doi.org/10.1007/0-387-23413-6_2

Lekfeldt, J. D. S., & Rønn, R. (2008). A common soil flagellate (*Cercomonas* sp.) grows slowly when feeding on the bacterium *Rhodococcus fascians* in isolation, but does not discriminate against it in a mixed culture with *Sphingopyxis witflariensis*. *FEMS Microbiology Ecology, 65*, 113-124. http://dx.doi.org/10.1111/j.1574-6941.2008.00486.x

Ly, T. M. C., & Muller, H. E. (1990). Ingested *Listeria monocytogenes* survive and multiply in protozoa. *Journal of Medical Microbiology, 33*, 51-54. http://dx.doi.org/10.1099/00222615-33-1-51

McLaughlin, H., Casey, P., Cotter, J., Gahan, C., & Hill, C. (2011). Factors affecting survival of *Listeria monocytogenes* and *Listeria innocua* in soil samples. *Archives of Microbiology, 193*, 775-785. http://dx.doi.org/10.1007/s00203-011-0716-7

Newell, D. G., Koopmans, M., Verhoef, L., *et al*. (2010). Food-borne diseases -The challenges of 20 years ago still persist while new ones continue to emerge. *International Journal of Food Microbiology*, *139*, S3-S15. http://dx.doi.org/10.1016/j.ijfoodmicro.2010.01.021

Nicholson, F. A., Groves, S. J., & Chambers, B. J. (2005). Pathogen survival during livestock manure storage and following land application. *Bioresource Technology, 96*, 135-143. http://dx.doi.org/10.1016/j.biortech.2004.02.030

Pakalniskiene, J., Falkenhorst, G., Lisby, M., Madsen, S. B., Olsen, K. E. P., Nielsen, E. M., Mygh, A., Boel, J., & Mølbak, K. (2009). A foodborne outbreak of enterotoxigenic *E. coli* and *Salmonella* Anatum infection after a high-school dinner in Denmark, November 2006. *Epidemiology and Infection, 137*, 396-401. http://dx.doi.org/10.1017/S0950268808000484

Pedersen, A., Nybroe, O., Winding, A., Ekelund, F., & Bjørnlund, L. (2009). Bacterial feeders, the nematode *Caenorhabditis elegans* and the flagellate *Cercomonas longicauda*, have different effects on outcome of competition among the *Pseudomonas* biocontrol strains CHA0 and DSS73. *Microbial Ecology, 57*, 501-509. http://dx.doi.org/10.1007/s00248-008-9455-y

Vaerewijck, M. J. M., Sabbe, K., Baré, J., & Houf, K. (2011). Occurrence and diversity of free-living protozoa on butterhead lettuce. *International Journal of Food Microbiology, 147*, 105-111. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.03.015

Zhou, X., Elmose, J., & Call, D. R. (2007). Interactions between the environmental pathogen *Listeria monocytogenes* and a free-living protozoan (*Acanthamoeba castellanii*). *Environmental Microbiology, 9*, 913-922. http://dx.doi.org/10.1111/j.1462-2920.2006.01213.x

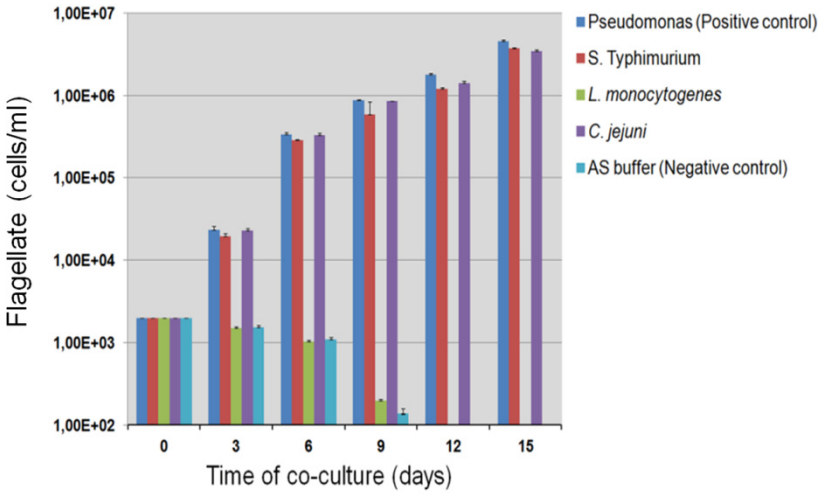

Figure 1. Growth of flagellates in co-culture with or without bacteria at different time points at 15ºC in aerobic conditions. Data are means and standard errors of at least three independent experiments

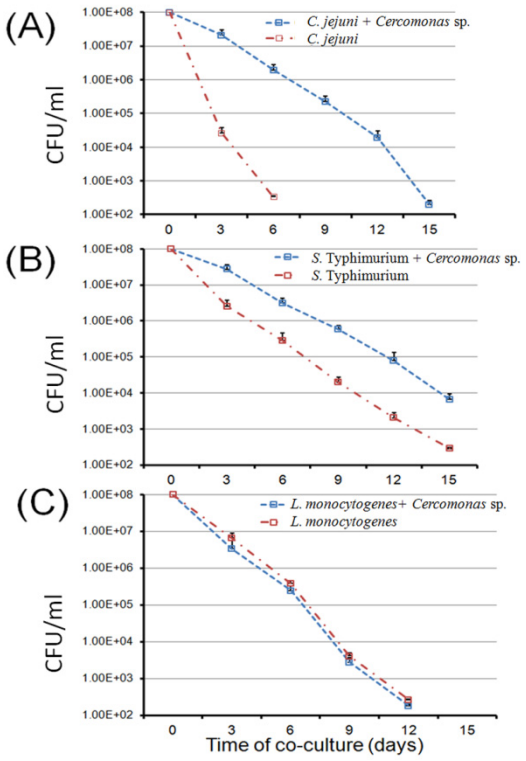

Figure 2. The survival of food-borne pathogens in co-culture with or without *Cercomonas* sp. at different time points at 15ºC in aerobic conditions. CFU counts are present as (A) *C. jejuni*, (B) *S.* Typhimurium, and (C) *L. monocytogenes*. Data are means and standard errors of at least three independent experiments

The Hygroscopic Properties and Sorption Isosteric Heats of Different Chinese Wheat Types

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Abstract

The moisture sorption isotherm data of fourteen Chinese wheat varieties were determined using the static gravimetric method at five different temperatures (10, 20, 25, 30 and 35 °C) and relative humidity ranging from 11.3 to 96%. Eight models, namely Brunauer-Emmett-Teller, CAE, Chen-Clayton, Modified-Chung-Pfost (MCPE), Modified-Henderson, Modified-Guggenheim-Anderson-deBoer, and Modified-Oswin and Strohman-Yoerger, were used to fit the sorption data. MCPE shows the best fitting results. A significant hysteresis effect was found between wheat desorption and adsorption isotherm at lower ERH, but the similar hygroscopic properties remained for different wheat types like hard vs. soft, red vs. white, and winter vs. spring, respectively. The experimental results show that the isosteric heats for both wheat adsorption and desorption, and all the sorption heats for different wheat types decrease rapidly with increasing seed moisture initially, however, after the moisture is more than 15% w.b. they decrease tardily with increasing moisture content. The isosteric heats of wheat desorption were considerably higher than those of adsorption below 17.5% m.c., but the similar sorption isosteric heats were found for wheat types like hard vs. soft, red vs. white, or winter vs. spring, respectively. It is concluded that the wheat grains from different types have similar hygroscopic properties and sorption isosteric heats and can be synchronously dealt with during physical control in storage.

Keywords: Wheat (*Triticum aestivum*), Hygroscopicity, Sorption, Hysteresis effect, Isosteric heat of sorption, Thermodynamics

1. Introduction

Wheat (*Triticum aestivum*) is the major grain in China, with its annual production being around 100 million metric tons in recent years. In China, a portion of the wheat is stored for a longer period of time (3 to 5 years) than that in the developed countries with deterioration controlled largely through moisture content and temperature. In order to maintain the quality of the wheat during this storage time period, it is important and interesting to know the relationship between the storage condition (temperature and humidity) and the quality of the wheat. In other words, knowing the relationship between equilibrium moisture content (EMC) and equilibrium relative humidity (ERH) of the cereal grains is essential. This relationship has been widely studied and different models/equations have been introduced (Nellist & Dumont, 1979; Van den Berg & Bruin, 1981; Sun & Woods, 1994; Blahovec, 2004; de Carvalho Lopes et al., 2006). Among 77 isotherm equations compiled by Van den Berg & Bruin (1981), only ten equations are commonly used to fit EMC-ERH relationship for wheat data (Sun & Woods, 1993). Among these ten equation, Chen-Clayton (CCE), Day-Nelson, Henderson, Modified Chung-Pfost (MCPE), Modified Henderson (MHE), and Strohman-Yoerger (SYE) equations are used to describe the sorption behavior of wheat chaff and unthreshed kernels in wheat heads (Duggal, Muir & Brooker, 1982). Based on the results, it was known that among these equations, the smallest residual sums of squares of ERH were obtained using the SYE for wheat kernels and the CCE for chaff, and the minimum standard error of estimate of ERH by the MCPE, respectively. All these conclusions were made based on the study of one or two

source sets of wheat data. Nellist & Dumont (1979) collected wheat data from thirteen sources and fitted the data using five common equations in order to obtain isotherm equations for general drying applications. This work indicated that the MCPE results in the best fit. As the authors mentioned, if more and better data became available, the coefficients of MCPE equation should be updated. Sun & Woods (1994) analyzed 29 source sets of wheat EMC/ERH data using five models (i.e. CCE, MCPE, MHE, Modified-Oswin (MOE), and SYE). It was concluded that the MCPE and the MOE are the preferred equations due to the fact that these two equations can describe each and all the individual data set and are three-coefficient invertible equations. Understandingly, the variety of the wheat has some influence on the results and all these studies did not include the Chinese wheat varieties. In this paper, the moisture sorption isotherm data of fourteen Chinese wheats are reported.

In respect of kernel hardness, wheat is divided into several types: durum, hard, soft, and mixed wheat residing between soft and hard wheat. Pfost et al. (1976) used MCPE analyzed the EMC/ERH data for hard, soft, and durum wheats. The results indicate that there is some variation in the resulting coefficients of the MCPE among these three types of wheat. Sun & Woods (1994) compared the MCPE curves for the three types of wheat (eight hard wheat varieties, six soft wheat varieties, and three durum wheat varieties) at three temperatures of 0, 30 and 60 $^{\circ}$C. At the temperature of 0 $^{\circ}$C, the hard-wheat curve lies well above the others and the durum wheat results in the lowest curve. At 30 $^{\circ}$C the difference in the hygroscopic properties of the three types of wheat become very small due to the three curves coming closer. However at 60 $^{\circ}$C, these three curves depart from each other again in the reverse manner, the curve for durum wheat lying above the curve for soft wheat, with the curve for hard wheat at the bottom. Recently, we studied the moisture sorption data of some Chinese wheat varieties (one mixed, two hard, two soft wheats). By using six equations, the Brunauer-Emmett-Teller (BET), CCE, MCPE, MHE, MOE and SYE, it is found that these hard and soft wheat varieties show similar hygroscopic properties and sorption isosteric heats (Li et al., 2011). In the literature, few reports are about the hygroscopic properties and sorption isosteric heats between winter and spring wheat, as well as between red and white wheat.

Thermodynamic, structural and dynamic approaches have been used to understand the properties of water and calculate the energy requirements of heat and mass transfer in biological systems (Fasina, Ajibola & Tyler, 1999). Thermodynamic functions/variables of the isotherm sorption deepen the understanding of experimental results. These thermodynamic functions/variables include isosteric heat of sorption, integral enthalpy, and integral entropy. The knowledge of sorption isotherms at different temperatures enables an evaluation of the heat of sorption, which determines the interaction between an adsorbent and adsorbate. All these provide a guideline for the drying process (Iglesias, Chirife & Viollaz, 1976). The level of material moisture content at which the net isosteric heat of sorption approaches the latent heat of vaporization of water is often taken as an indication of the amount of 'bound water' existing in the product (Kiranoudis et al., 1993). The heat of vaporization of sorbed water may increase to values well above that for the vaporization of pure water as food is dried to low moisture levels (Rizvi, 1986).

In this paper, a systematical study of 14 Chinese wheat varieties collected from different regions is reported. The EMC/ERH data is analyzed using eight equations in order to determine the most suitable EMC/ERH model for grain moisture sorption isotherms of fourteen Chinese wheat varieties, and compare the fitted sorption isotherms and isosteric heat of water sorption between different wheat types classified in respect of hardness, color and seedtime, providing theoretic basis for wheat treatments after harvest.

2. Materials and Methods

2.1 Wheat samples and experimental procedures

Fourteen varieties of wheat (*Triticum aestivum*) used in this work were collected from eight regions in China in 2007 and 2008. These include twelve winter wheat and two spring wheat as shown in Table 1. The hardness of wheat samples were measured with an SKCS 4100 (Perten Instruments AB, Sweden). Of these fourteen varieties, seven varieties are hard wheat, five varieties are soft wheat, and the remaining two varieties are mixed type wheat, or seven varieties are white wheat, the others are red wheat. The wheat seeds used for this study were intact, clean and plump. For adsorption experiment, the wheat seeds were dried to a wet bulb moisture content (m.c.) of 7-8% wet basis (w.b.) at 40.5 $^{\circ}$C in an oven, and then dehydrated by P_2O_5 solid in a dessicator to below 5% w.b. For the samples of desorption experiment, the wheat seeds were re-moisturized to the m.c. of 23% w.b., and equilibrated at 4 $^{\circ}$C for two weeks.

The static gravimetric method, with nine saturated salt solutions to maintain constant vapor pressure (Jayas & Mazza, 1991; Li et al., 2011), was used to obtain nine equilibrium moisture contents at each of five constant temperatures (10, 20, 25, 30, and 35 $^{\circ}$C). The saturated salt solutions included lithium chloride, potassium acetate, magnesium chloride, potassium carbonate, magnesium nitrate, cupric chloride, sodium chloride, potassium

chloride, and potassium nitrate. Twenty-seven wide mouth glass bottles (250-mL) each contained 65 mL salt solution, and were kept in one temperature controlled cabinet to maintain nine groups of different relative humidity (r.h.) levels ranging from 11.3 to 96% ERH. Every relative humidity at one temperature was triplicated and a total of 135 bottles was used in the experiment for five sorption isotherms of a wheat variety. The temperature of cabinets was monitored using a standard thermometer and controlled with an accuracy of ±0.5 °C. Each sample of wheat seeds (4-5 g) was placed into a small bucket (3 cm diameter × 4 cm length) made from copper wire gauze, and hung into the glass bottle on a copper wire pothook under a rubber plug, 2-3 cm above saturated salt solutions. The rubber plug was tightly pushed into the bottle mouth. From three weeks after exposing the samples in the saturated vapour at 35 °C, the copper wire buckets with samples were weighed every other day until the change in mass between two successive readings was less than 2 mg. When the sample was exposed to a lower temperature, the sample was left longer to equilibrate. However, the wheat seeds exposed over the saturated potassium nitrate solution for 3-4 days at higher temperatures were susceptible to molds growth, and removed immediately mould was observed on any seed. The moisture content of the sample at this constant stage was defined to be the EMC and was determined by the oven method (AOAC, 1980). The sample was dried to constant weight under 103.0±0.5 °C for 22-28 h.

2.2 Analysis of the adsorption and desorption data

Eight equations were used to fit the EMC data of wheat adsorption and desorption as given in Table 2. The fitting was conducted using the non-linear regression procedure in SPSS 13.0 for Windows (SPSS Inc., 2006), which minimizes the sum of squares of deviations between experimental and predicted data in a series of iterative steps. The determination coefficient (R^2), residue sum of squares (RSS), the standard error (SE), and mean relative percentage error (MRE) as defined below are used as the criteria to determine the best equation for the data analysis.

$$R^2 = \sqrt{1 - \frac{\sum_{i=1}^{n}(m_i - m_{pi})^2}{\sum_{i=1}^{n}(m_i - m_{mi})^2}} \quad (1)$$

$$RSS = \sum_{i=1}^{n}(m_i - m_{pi})^2 \quad (2)$$

$$SE = \sqrt{\frac{\sum_{i=1}^{n}(m_i - m_{pi})^2}{(n-1)}} \quad (3)$$

$$MRE = \frac{100\sum_{i=1}^{n}\left|\frac{m_i - m_{pi}}{m_i}\right|}{n} \quad (4)$$

Where m_i is the experimental value, m_{pi} the predicated value, m_{mi} the average of experimental values, and n the number of observations. The determination coefficient (R^2) was one of the primary criteria for selecting the best equation to fit the experimental data. In addition to R^2, the other statistical parameters, MRE as a percentage, RSS and SE were used to determine the quality of the fit. The equations (1) - (4) were used for calculating R^2, RSS, SE, and MRE, respectively. The fit of an equation is good enough for practical purposes when MRE is less than 10% (Aguerre, Suarez & Viollaz, 1989).

2.3 Determination of the isosteric heat of sorption

The total energy required to remove a unit mass of water from wheat kernels, i.e. the differential heat of sorption (h_s), is conveniently partitioned into two components, namely the latent heat of vaporization of free water (h_v) and the differential heat of wetting (h_w). The h_s of adsorption and desorption of wheat grains were respectively

calculated by the following six equations according to Thorpe (2001).

$$\frac{h_s}{h_v} = 1 + \frac{p_s}{r.h.} \times \frac{dT}{dP_s} \times \left.\frac{\partial r.h.}{\partial T}\right|_{m.c.} \tag{5}$$

$$h_v = 2501.33 - 2.363 \times t \tag{6}$$

$$P_s = \frac{6 \times 10^{25}}{(273.15 + t)^5} \times \exp\left(-\frac{6800}{t + 273.15}\right) \tag{7}$$

$$\frac{dP_s}{dT} = \frac{P_s}{(t + 273.15)} \times \left(\frac{6800}{t + 273.15} - 5\right) \tag{8}$$

$$\left.\frac{\partial r.h.}{\partial T}\right|_{m.c.} = \frac{C_1 \times r.h.}{(t + C_2)^2} \times \exp(-C_3 \times m.c.) \tag{9}$$

$$\left.\frac{\partial r.h.}{\partial T}\right|_{m.c.} = \frac{-1}{\left\{1 + \left(\frac{C_1 + C_2 \times t}{m.c.}\right)^{C_3}\right\}^2} \times \left\{\frac{C_2 \times C_3}{m.c.} \times \left(\frac{C_1 + C_2 \times t}{m.c.}\right)^{(C_3 - 1)}\right\} \tag{10}$$

The equation (5) enables one to calculate h_s/h_v, provided dP_s/dT and $\left.\partial r.h./\partial T\right|_{m.c.}$ can be evaluated by equations (8) and (9), respectively. The h_v of free water in equation (6) is dependent on temperature. The saturated vapor pressure, P_s, can be calculated by equation (7). The derivative of *r.h.* with respect to t, $\left.\partial r.h./\partial T\right|_{m.c.}$ depends on the sorption isotherm equation used, and the Modified Chung-Pfost (MCPE) in equation (9), or Modified Oswin (MOE) in equation (10) used in this study.

3. Results

3.1 Fitting of sorption equations to experimental sorption data

The results of fitting the sorption equations to the experimental data of adsorption and desorption isotherms by nonlinear regression analysis were respectively evaluated with the statistical indices such as RSS, SE, R^2 and MRE. Of eight equations, namely BET, CAE, CCE, MCPE, Modified Guggenheim-Anderson-deBoer (MGAB), MHE, MOE, and SYE (Table 2), seven equations such as CAE, CCE, MCPE, MGAB, MHE, MOE, and SYE gave the better fit to the experimental data of adsorption and desorption isotherms in a wide range of 11.3 to 96.0% ERH, but the BET equation gave the better fit in the range of 11.3 to 49.9% ERH. The further comparisons of the sorption equations in a form of $r.h. = f(M,t)$ or $M = f(r.h.,t)$ for twenty-eight sets of isotherm data were given in Table 3. The average values of R^2 and error parameters (RSS, SE, and MRE) were calculated for the twenty-eight sets of isotherm data. In the form of $r.h. = f(M,t)$, the equations for desorption were ranked for accuracy in an order: CAE, SYE, MCPE, MOE, CCE, MHE and MGAB, but for adsorption the order was: CAE, SYE, MCPE, CCE, MHE, MOE and MGAB. The CAE model being used in Chinese stored grain aeration gave the least standard error of estimate, least mean relative percentage deviation, and explained variation on the ERH, thus it could be taken as the best model among the seven $r.h. = f(M,t)$ models because the residual plots showed a random deviation. In case of a form of $M = f(r.h.,t)$, the equations for desorption were ranked in an order: MCPE, CCE, BET, MHE, MOE, and MGAB, the order for adsorption equations were MCPE, CCE, MHE, BET, MOE, and MGAB. However, CAE is five-coefficient, temperature dependent equation, and it can be not easily inverted to give EMC as a function of ERH. SYE is four-coefficient, temperature independent

equation, and also cannot be explicitly invertible. CCE is a four-coefficient, temperature dependent and explicitly invertible equation. The other commonly used equations, such as MCPE, MHE, MOE and MGAB all are three-coefficient, temperature dependent and easily invertible equations (Table 2). MCPE fitted the data reasonably well. MHE, MOE and MGAB equations were again less effective in fitting the data. Thus, the MCPE in a form of $r.h. = f(M,t)$, or $M = f(r.h.,t)$ was considered to best describe the equilibrium moisture data of fourteen wheat varieties in a wide range of 11.3 to 96.0% ERH.

3.2 Comparison of hygroscopic properties of different wheat samples

The best fitted MCPE parameters for desorption and adsorption isotherms of different wheat samples were summarized in Table 4. For MCPE model in a form of $r.h. = f(M,t)$, the parameters C_1 and C_2 in adsorptive isotherm equation were significantly different from those corresponding to desorptive isotherm equation, respectively. In contrast, there was slight difference in corresponding parameters C_1, C_2 and C_3 between hard and soft wheat, as well as between red and white wheat, or between winter and spring wheat, respectively.

The experimental sorption data of wheat samples were fitted with MCPE and the predicted data were compared between wheat types. Figure 1 shows the fitted sorption isotherms of 14 data sets at 20 and 30 °C. The isotherms of desorption and adsorption for wheat samples were sigmoidal in shape. At a constant ERH, both types of EMC decreased with an increase in temperature. A substantial difference was observed between the adsorption and desorption data at the same temperature. The desorption data was higher than the adsorption data except at high *r.h.*, and the moisture sorption hysteresis effect was more significant at lower ERH. Both width and span of the hysteresis effect tended to decrease with an increase in temperature.

Figure 2 compared the predicted sorption isotherms between wheat types at 20 and 30 °C, respectively. The moisture sorption data of soft wheat were insignificantly higher than those of hard wheat at these two temperatures. The very similar moisture sorption data were also observed between red and white wheat, as well as between winter and spring wheat, respectively.

In a form of $M = f(r.h.,t)$, the deduced MCPE of each wheat variety was used to calculate the moisture content for grain safe storage with ERH equal to 70% (Table 5). At a borderline condition of 70% r. h., the average moisture contents of fourteen wheat varieties at different temperatures of 10, 15, 20, 25, 30, and 35 °C were 14.90%, 14.57%, 14.26%, 13.97%, 13.71%, and 13.46% w.b., respectively. At 20-25 °C the safe storage m.c. for desorption was 13.93-14.21%, these m.c. were 13.58-13.88%, 14.38-14.69%, 14.40-14.60% for hard, soft and mixed wheat, respectively. The safe storage m.c. (14.03-14.30%) of red wheat at 20-25 °C is similar to that (13.95-14.25%) of white wheat. Furthermore, the safe storage m.c. (14.08-14.35%) of winter wheat at 20-25 °C was slightly higher than that (13.48-13.84%) of spring wheat.

3.3. Comparison of isosteric heats of sorption between different wheat samples

3.3.1 The isosteric heats between wheat adsorption and desorption

The isosteric heat of sorption (h_s) was calculated from the equations (6) to (9). The coefficients C_1, C_2, and C_3 of MCPE equation with a form of $r.h. = f(M,t)$ in Table 4 were used as the coefficients in equation (9). Figure 3A shows the influence of grain moisture content ranging from 4 to 24% w.b. on the isosteric heats of wheat desorption and adsorption. The isosteric heats of both wheat desorption and adsorption decreased rapidly with an increase in seed moisture content until the m.c. of 15% w.b. was reached, but after the moisture is more than 15% w.b. they decreased slowly with increasing moisture content. At lower moisture contents below 15%, both isosteric heats of wheat desorption and adsorption at lower temperatures were higher than those at higher temperatures. The isosteric heats of wheat desorption were significantly higher than those of adsorption below 15% m.c., but above 15% m.c. there was no difference found between them.

The influence of another moisture sorption model such as MOE on the calculated isosteric heats of sorption was also compared in Figure 3B. The coefficients C_1, C_2, and C_3 of MOE equation (Table 6) with a form of $r.h. = f(M,t)$ were respectively used as the coefficients in equation (10). When the MOE model was employed to predict, the isosteric heats of both wheat desorption and adsorption decreased rapidly with an increase in seed moisture content from 7.5 to 17.5% w.b., but above 17.5% m.c. they decreased slowly with increasing moisture content (Figure 3B). The isosteric heats of wheat desorption were significantly higher than those of adsorption in the moisture range of 4 to 20% w.b. At lower moisture contents below 15%, both isosteric heats for wheat desorption and adsorption at lower temperatures tended to be similar to those at higher temperatures. However, above 15% m.c., both isosteric heats for wheat desorption and adsorption at lower temperatures were slightly higher than those at higher temperatures.

3.3.2 The sorption isosteric heats of sorption between hard and soft wheat

Figures 3C and 3D show both sorption isosteric heats of hard and soft wheat at different temperatures predicted by MCPE and MOE models, respectively. The isosteric heats for both sorption of hard and soft wheat were decreased rapidly with an increase in seed moisture content until the moisture content of 17.5% w.b. was reached, and thereafter they decreased slowly with increasing moisture content. The sorption isosteric heats of soft wheat were slightly higher than those of hard wheat under all moisture contents at a constant temperature. For the sorption isosteric heats predicted by MCPE (Figure 3C), at lower moisture contents below 17.5%, the sorption isosteric heats for both hard and soft wheat at lower temperatures were slightly higher than those at higher temperatures. However, the effect of temperature on the sorption isosteric heats of both hard and soft wheat was depleted when the MOE model was employed to calculate (Figure 3D).

3.3.3 The sorption isosteric heats between red and white wheat

Figures 4A and 4B show both sorption isosteric heats of red and white wheat estimated by the MCPE and MOE models, respectively. Similarly to both sorption isosteric heats of hard and soft wheat, it seems that both sorption isosteric heats of red and white wheat were decreased rapidly with increase in seed moisture content until a moisture content of 17.5% w.b. was reached, but above 17.5% they decreased slowly with increasing moisture content. Additionally, the sorption isosteric heats of red wheat were very similar to those of white wheat under all moisture contents at a constant temperature. For the sorption isosteric heats calculated by MCPE (Figure 4A), at lower moisture contents below 17.5%, the isosteric heats for the sorption of both red and white wheat at lower temperatures were slightly higher than those at higher temperatures, but the effect of temperature on the sorption isosteric heats of red and white wheat was depleted when MOE model was used (Figure 4B).

3.3.4 The sorption isosteric heats between winter and spring wheat

Figures 4C and 4D give both sorption isosteric heats of winter and spring wheat predicted by the MCPE and MOE models, respectively. In parallel to the changes in sorption isosteric heats for both hard and soft wheat, as well as for both red and white wheat, the sorption isosteric heats for both winter and spring wheat were decreased rapidly with increase in seed moisture content until the moisture content of 17.5% w. b. was reached, and thereafter they decreased slowly with increasing moisture content. The sorption isosteric heats of spring wheat were slightly higher than those of winter wheat under all moisture contents at a constant temperature. For the sorption isosteric heats calculated by MCPE (Figure 4C), at lower moisture contents below 17.5%, the isosteric heats for the sorption of both winter and spring wheat at lower temperatures were slightly higher than those at higher temperatures, but the influence of temperature on the sorption isosteric heats of winter and spring wheat was eliminated when MOE model was adopted (Figure 4D).

4. Discussion

The theoretical implications of moisture sorption hysteresis range from a depiction of the irreversibility of the sorption process to the question of validity of thermodynamic functions determined from such a system (Kapsalis, 1987). It has been accepted that there was significant hysteresis effect between wheat desorption and adsorption at lower ERH (Pfost et al., 1976; Sun & Woods, 1993; 1994). Sun & Woods (1994) analyzed thirty-three source sets of wheat EMC/ERH data with the preferred equations MCPE and MOE, and considered that the wheat hysteresis effect was not greatly influenced by temperature. However, in this study for the average fitted sorption data of fourteen wheat varieties, both width and span of the hysteresis effect tended to decrease with an increase in temperature. It was noted by Kapsalis (1987) that the span of hysteresis loop always decreases with increasing temperature, but the width of hysteresis loop could increase, remain unchanged, or reduce with increasing temperature. The hysteresis loops of wheat grains at 10-35 $^{\circ}$C are of type three classified by Kapsalis (1987). In agreement with these results, the coefficients of MCPE and the isosteric heats for wheat desorption and adsorption were different. So far our knowledge on elucidation of hysteresis phenomenon of cereal moisture sorption is rather limited. The practical implications of moisture sorption hysteresis in wheat can deal with the effect on storage stability.

A study by Chen & Morey (1989) indicated the necessity to choose the most appropriate moisture sorption equation for a specific crop. In this study, among the all eight acceptable moisture isotherm equations (BET, CAE, CCE, MCPE, MGAB, MHE, MOE and SYE) tested, the MCPE with a form of $r.h. = f(M,t)$, or a form of $M = f(r.h.,t)$ both best describe the EMC data of the fourteen wheat varieties in a wide range of 11.3 to 96.0% ERH. The obtained parameters of MCPE in a form of $r.h. = f(M,t)$ were similar to those of Sun & Woods (1994) though they considered MOE in a form of $M = f(r.h.,t)$ to best fit for the wheat sorption data. Thus, the MCPE in a form of $M = f(r.h.,t)$ was used to calculate the moisture contents of wheat safe storage at different temperatures. The safe storage m.c. for fourteen wheat varieties was predicted to be 13.46-14.90% w.b. at temperatures ranging from 10 to 35 $^{\circ}$C. These values agree to the safe moisture level,

usually taken as that in equilibrium with a maximum of 70% r.h. is about 14% w.b. for the starch cereal grains (Pixton, 1982). At six temperatures ranging from 10 to 35 °C, the standard deviation of m.c. for safe storage of fourteen wheat samples is around 0.7% w.b., close to standard deviation of 0.5% w.b. that the different methods for cereal grain moisture determination should not be beyond (AOAC, 1980).

To our knowledge, few studies have compared the hygroscopic properties between wheat types. We found that the very similar safe storage m.c. remained between red and white wheat. Considered the 0.5% standard deviation of moisture measurement, the similar safe storage m.c. were also found between hard and soft wheat, and between winter and spring wheat. Sun & Woods (1994) compared the fitted curves of MCPE for eight hard wheat varieties and six soft wheat varieties at three temperatures of 0, 30 and 60 °C. At the temperature of 0 °C, the curve for hard wheat lied well above that of soft wheat, but the difference in hygroscopic properties of these two types wheat become very small at 30 °C, then at 60 °C the curve of soft wheat lied above that of soft wheat. In this study, the fitted MCPE isotherms of soft wheat slightly lied above those of hard wheat at the temperatures of 20 and 30 °C. The similar hygroscopic properties between hard and soft wheat might be due to the overall effects of hygroscopic properties of their respective protein and starch (Li et al., 2011). The reason for the similar hygroscopic properties between red and white wheat, as well as between winter and spring wheat, needs further study on microstructural or morphologic characteristic of different wheat varieties.

The isosteric heats for both wheat desorption and adsorption, and for the sorption of both hard and soft wheat, decreased rapidly with an increase in seed moisture content till up to 15% w.b., but thereafter they decreased slowly with increasing moisture content. As mentioned above, similar trends of sorption isosteric heats were also observed for both red and white wheat, as well as for both winter and spring wheat. The isosteric heats of wheat desorption were dramatically higher than those of adsorption from 4.0 to 17.5%, but above 17.5% m.c. there was no difference found between them. These results were different from those reported by Öztekin & Soysal (2000) that the isosteric heats of wheat desorption were higher than those of adsorption from 9.1 to 13.0% w.b., but from 13.1 to 20% the isosteric heats of desorption were lower than those of adsorption. Their difference between desorption and adsorption isosteric heats below 13.0% is much smaller than ours. In their study, they also compared the sorption isosteric heats between hard and soft wheat in the moisture range from 9.1 to 16.7% w.b. The sorption isosteric heats of soft wheat were much higher than those of hard wheat from 9.1 to 12.3% w.b., but from 12.31 to 16.7% the sorption isosteric heats of soft wheat were lower than those of hard wheat. In this study, the sorption isosteric heats of soft wheat were slightly higher than those of hard wheat from 4 to 15% w.b., but above 15% no difference was found between the sorption isosteric heats of soft wheat and hard wheat. The same trends of the sorption isosteric heats were observed for winter and spring wheat, as well as for red and white wheat. In the study of Öztekin & Soysal (2000), the heat of sorption of wheat grains approached that of pure water at the moisture content of about 16.7% wet basis, but in this study the m.c. is around 15.0% w.b., close to those of melon seed, cassava, alfalfa pellets, gari, winged bean seed, and tea at moisture contents of about 11.5, 26.5, 13.8, 13.0, 13.0, and 13.0% w.b., respectively (Arslan & Toğrul, 2006). The rapid increase in the heat of sorption at low moisture content might be due to the existence of highly active polar sites on the surface of wheat grains, which were covered with water molecules forming a mono-molecular layer (Tsami, 1991). The decrease in the isosteric heats with higher amounts of sorbed water can be quantitatively explained by considering that sorption initially occurs on the most active available sites giving rise to high interaction energy. As these sites become occupied, sorption occurs on the less active ones, resulting in lower heats of sorption (Wang & Brennan, 1991). In low moisture contents, the values of the isosteric heats were higher than the latent heat of vaporization of water, indicating that the energy of binding between the water molecules and the sorption sites was higher than the energy which holds the molecules of pure water together in the liquid phase (Al-Muhtaseb, McMinn, & Magee, 2004). At high moisture contents, there was no significant difference between the sorption isosteric heat and the latent heat of vaporization of water over a broad range of moisture contents. Similar findings were reported for the isosteric heats of melon seeds and cassava (Aviara & Ajibola, 2002), starch powder (Al-Muhtaseb, McMinn, & Magee, 2004), and Brussels sprouts (Irzyniec & Klimczak, 2003). Comparison of the adsorption and desorption data shows that, at a specific moisture content, the isosteric heat of desorption was higher than the corresponding adsorption data. This indicates that there are more polar sites on the surface of the solid, and the energy of binding between the water molecules and the surface is higher (Tsami, 1991). Heat values for desorption give a measure of the energy that needs to be supplied to dehydrate the foodstuff.

The sorption isosteric heats for both winter and spring wheat, as well as for both red and white wheat were also compared in this study. The minor difference between the sorption isosteric heats of winter and spring wheat at a particular temperature was very similar to that of soft wheat and hard wheat. No difference was found between the sorption isosteric heats of red and white wheat at all moisture contents from 4.0 to 24% w.b. at a constant

temperature. These results suggest the similar hygroscopic properties and sorption isosteric heats occur for different wheat types, i.e. hard vs. soft wheat, red vs. white wheat, or winter vs. spring wheat, respectively.

When MCPE was used to calculate the wheat heat of sorption, at lower moisture contents below 17.5% w.b., the isosteric heats of both desorption and adsorption, of both sorption of hard and soft wheat, as well as of both sorption of winter and spring wheat, and those of both sorption of red and white wheat under lower temperatures all were higher than those under higher temperatures. However, when MOE was used for the calculation of the heat of sorption, regardless of either desorption or adsorption, either hard or soft wheat, as well as either winter or spring wheat, and either red or white wheat, there was no difference found in the isosteric heats of sorption at different temperatures at an EMC below 15%, but above 15% the isosteric heats of sorption at lower temperatures were slightly higher than those at higher temperatures. It has been noted that h_s/h_v was calculated to be dependent on temperature, but the dependence was small (Thorpe, 2001). In this study for two models MCPE and MOE employed respectively to calculate the wheat heat of sorption, MOE can eliminate the dependence of h_s on temperature. Thus, in contrast to a big difference in isosteric heats of wheat desorption and adsorption below the m.c. of 15% w.b., we consider that the similar sorption isosteric heats occur between hard and soft wheat, between red and white wheat, as well as between winter and spring wheat, and the grains from different wheat types could be concordantly treated after harvest.

5. Conclusion

This study determined the moisture sorption isotherms of wheat grains for fourteen Chinese varieties. It is found that MCPE model results in the best fitting to the sorption data. A significant hysteresis effect was found between wheat desorption and adsorption at lower ERH, but the similar hygroscopic properties remained between wheat types, i.e. hard and soft wheat, red and white wheat, or winter and spring wheat, respectively. The isosteric heats for wheat adsorption and desorption, and the sorption heats for hard and soft wheat, winter and spring wheat, as well as red and white wheat, all decreased rapidly with an increase in seed moisture up to 15% w.b., thereafter they decreased slowly with increasing moisture content. The isosteric heats of desorption were higher than those of adsorption below 15% m.c., but above 15 % m.c. there was no difference between the desorption and adsorption. The similar isosteric heats of grain sorption found between hard and soft wheat, as well as between red and white wheat, or between winter and spring wheat indicate that the wheat grains from different types have similar hygroscopic properties and sorption isosteric heats, and can be synchronously dealt with during drying, storage and aeration.

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References

Aguerre, R. J., Suarez, C., & Viollaz, P. E. (1989). New BET type multilayer sorption isotherms: Part II. Modelling water sorption in foods. *Lebensmittel-Wissenschaft und-Technologie, 22*, 192-195.

Al-Muhtase, A. H., McMinn, W. A. M., & Magee, T. R. A. (2004). Water sorption isotherms of starch powders. Part 2: thermodynamic characteristics. *Journal of Food Engineering, 62*(2), 135-142. http://dx.doi.org/10.1016/S0260-8774(03)00202-4

AOAC. (1980). Official Methods of Analysis, thirteenth ed. Association of Official Analytical Chemists, Washington DC.

Arslan, N., & Toğrul, H. (2006). The fitting of various models to water sorption isotherms of tea stored in a chamber under controlled temperature and humidity. *Journal of Stored Products Research, 42*(2), 112-135. http://dx.doi.org/10.1016/j.jspr.2005.01.001

Aviara, N. A., & Ajibola, O. O. (2002). Thermodynamics of moisture sorption in melon seed and cassava. *Journal of Food Engineering, 55*(2), 107-113. http://dx.doi.org/10.1016/S0260-8774(02)00023-7

Blahovec, J. (2004). Sorption isotherms in materials of biological origin mathematical and physical approach. *Journal of Food Engineering, 65*(4), 489-495. http://dx.doi.org/10.1016/j.jfoodeng.2004.02.012

Brunauer, S., Emmett, P. H., & Teller, E. (1938). Adsorption of gases in multi-molecular layers. *Journal of the American Chemical Society, 60*(2), 309-319.

Chen, C. C., & Morey, R. V. (1989). Comparison of four EMC/ERH equations. *Transactions of the ASAE, 32*, 983-990.

Chen, C. S., & Clayton, J. T. (1971). The effect of temperature on sorption isotherms of biological materials. *Transactions of the ASAE, 14*, 927-929.

de Carvalho Lopes, D., Helvecio Martins, J., de Castro Melo, E., & de Barros Monteiro P. M.(2006). Aeration simulation of stored grain under variable air ambient conditions. *Postharvest Biology and technology, 42*(1), 115-120. http://dx.doi.org/10.1016/j.postharvbio.2006.05.007

Duggal, A. K., Muir, W. E., & Brooker, D. B. (1982). Sorption equilibrium moisture contents of wheat kernels and chaff. *Transactions of the ASAE, 25*, 1086-1090.

Fasina, O., Ajibola, O. O., & Tyler, R. (1999). Thermodynamics of moisture sorption in winged bean seed and gari. *Journal of Food Process Engineering, 22*(6), 405-418.

Iglesias, H. A., Chirife, J., & Viollaz, P. (1976). Thermodynamic of water vapor sorption by sugar beet root. *Journal of Food Technology, 11*, 91-101.

Irzyniec, Z., & Klimczak, J. (2003). Effect of temperature on sorption isotherms of Brussels sprouts. *Nahrung/Food, 47*(1), 24-27.

Jayas, D. S., & Mazza, G. (1991). Equilibrium moisture characteristics of safflower seeds. *Transactions of the ASAE, 34*, 2099-2103.

Jayas, D. S., & Mazza, G. (1993). Comparison of five three-parameter equations for the description of adsorption data of oats. *Transactions of the ASAE, 36*, 119-125.

Kapsalis J. G. (1987). Influence of hysteresis and temperature on moisture sorption isotherms. In: Rockland, L.B., & Beuchat, L.R. (Eds.). Water acitivity: Theory and Applications to Foods. Marcel Dekker Inc, New York, USA, pp. 173-213.

Kiranoudis, C. T., Maroulis, Z. B., Tsami, E., & Marinos-Kouris, D. (1993). Equilibrium moisture content and heat of desorption of some vegetables. *Journal of Food Engineering, 20*(1), 55-74. http://dx.doi.org/10.1016/0260-8774(93)90019-G

Li, X. J., Cao, Z. Y., Wei, Z. Y., Feng, Q. Y., & Wang, J. S. (2011). Equilibrium moisture content and sorption isosteric heats of five wheat varieties in China. *Journal of Stored Products Research, 47*(1), 39-47. http://dx.doi.org/10.1016/j.jspr.2010.10.001

Nellist, M. E., & Dumont, S. (1979). Desorption isotherms for wheat. Departmental Note No. DN/CDV/983/06010. National Institute Agricultural Engineering, Silsoe, Bedford, UK.

Öztekin, S., & Soysal, Y. (2000). Comparison of adsorption and desorption isosteric heats for some grains. *Agricultural Engineering International: the CIGR Journal of Scientific Research and Development, 2*, 1-17. http://www.cigrjournal.org/index.php/Ejounral/issue/view/14

Pfost, H. B., Maurer, S. G., Chung, D. S., & Milliken, G. A. (1976). Summarizing and reporting equilibrium moisture data for grains, vol. 76-3520. American Society of Agricultural Engineers, St. Joseph, MI, USA.

Pixton, S. W. (1982). The importance of moisture and equilibrium relative humidity in stored products. *Tropical Stored Products Information, 43*, 16-29.

Rizvi, S. S. H. (1986). Thermodynamic properties of food in dehydration. In: Rao M.A., & Rizvi S.S.H. (Eds), Engineering Properties of Food, Marcel Dekker Inc., New York, pp. 133-214.

SPSS Inc. (2006). SPSS for Windows, Release 13.0.1. SPSS Inc., Chicago, USA.

Strohman, R. D., & Yoerger, R. R. (1967). A new equilibrium moisture content equation. T*ransaction of the ASAE, 10*, 675-677.

Sun, D. W., & Woods, J. L. (1993). The moisture contact relative humidity relationship of wheat-A review. *Drying Technology, 11*(7), 1523-1551.

Sun, D. W., & Woods, J. L. (1994). The selection of sorption isotherm equations for wheat based on the fitting of available data. *Journal of Stored Product Research, 30*(1), 27-43. http://dx.doi.org/10.1016/0022-474X(94)90270-4

Thompson, T. L., Peart, R. M., & Foster, G. H. (1986). Mathematical simulation of corn drying-a new model. *Transactions of the ASAE, 11*, 582-586.

Thorpe, G. R. (2001). Physical basis of aeration. In: Navarro, S., & Noyes, R. (Eds). The Mechanics and Physical of Modern Grain Aeration Management. CRC Press, Boca Raton, pp. 135-144, pp.186.

Tsami E. (1991). Net isosteric heat sorption in dried fruits. *Journal of Food Engineering, 14*(4), 327-335. http://dx.doi.org/10.1016/0260-8774(91)90022-K

Van den Berg, C., & Bruin, S. (1981). *Water activity and its estimation in food systems-theoretical aspects*. In Rockland, L.B., & Stewards, G.F. (Eds.). Water activity: Influence on Food Quality. Academic Press, New York, pp. 1-43.

Wang, N., & Bremmam, J. G. (1991). Moisture sorption isotherm characteristics of potatoes at four temperatures. *Journal of Food Engineering, 14*(4), 269-287. http://dx.doi.org/10.1016/0260-8774(91)90018-N.

Wu, Z. D., Li, X. J., Guo, D. L., & Wang, S. L. (2011). The use of a moisture sorption equation CAE for depot aeration of shelled corn in China. An oral presentation at the 2011 CIGR section VI international symposium on towards a sustainable food chain, food process, bioprocessing and food quality management. Nantes, France, April 18-20.

Nomenclature

a_1, a_2, b_1, b_2, d	the coefficients of CAE equation	h_w	differential heat of wetting (KJ/Kg)
C_1, C_2, C_3, C_4	equation coefficients	R^2	coefficient of determination
d.b.	dry basis	P_s	saturate vapor pressure (Pa)
EMC	equilibrium moisture content	*r.h.*	relative humidity
ERH	equilibrium relative humidity	RSS	residue sum of squares
m.c.	moisture content	SE	standard error
m_i	experimental value	T	absolute temperature (K)
m_{mi}	average mean of experimental value	t	temperature (°C)
m_{pi}	predicated value	vs.	versus
MRE	mean relative percentage error (%)	w.b.	wet basis
n	number of observations		
h_s	isosteric heat of sorption (KJ/Kg)		
h_v	latent heat of vaporization of free water (KJ/Kg)		

Table 1. The characteristics of fourteen wheat samples adopted in this study

Sample No.	Wheat variety	SKCS Hardness	Wheat Classes	Producing region	Harvest time
1	Nanduan	68	Hard white winter	Shandong	June, 2008
2	Longyuan 2	74	Hard white winter	Shaanxi	June, 2007
3	Changwu 3297	73	Hard white winter	Shaanxi	June, 2007
4	Henan Bai	59	Hard white winter	Henan	June, 2008
5	Nongda Hong	50	Mixed red winter	Beijing	June, 2007
6	Sanyuan	51	Mixed red winter	Beijing	June, 2007
7	Shunyi 8433	40	Soft white winter	Beijing	June, 2008
8	Nongda 5177	40	Soft white winter	Beijing	June, 2007
9	Xuzhou Bai	36	Soft white winter	Jiangsu	May, 2008
10	Zhaozhuang 1	24	Soft red winter	Beijing	June, 2008
11	Lumai 1	18	Soft red winter	Shandong	June, 2007
12	Hebei Yongqing	74	Hard red winter	Hebei	June, 2007
13	Neimeng Chun	77	Hard red spring	Neimenggu	June, 2007
14	Longjiang Chun	69	Hard red spring	Heilongjiang	June, 2007

Table 2. The isotherm equations used in this study

Models	Equation[a]	Reference
Brunauer-Emmett-Teller (BET)	$M = \frac{(C_1 + C_2 \times t) \times C_3 \times r.h.}{(1 - r.h.) \times (1 - r.h. + C_3 \times r.h.)}$ ($r.h.$ <50%)	Brunauer et al., 1938
CAE	$r.h. = \exp\{\{\frac{d}{222}[\exp(\frac{b_1 - M}{a_1}) - \exp(\frac{b_2 - M}{a_2})](1737.1 - \frac{474242}{273 + t}) + d[1 - \exp(\frac{b_1 - M}{a_1})] + 202\}/87.72\}$	Wu et al., 2011
Chen-Clayton (CCE)	$M = \frac{1}{-C_3 \times (t + 273.15)^{C_4}} \ln[\frac{(t + 273.15)^{C_2} \ln(r.h.)}{-C_1}]$ or $r.h. = \exp\{\frac{-C_1}{(t + 273.15)^{C_2}} \exp[-C_3 \times (t + 273.15)^{C_4} \times M]\}$	Chen & Clayton, 1971
Modified Chung-Pfost (MCPE)	$r.h. = \exp[-\frac{C_1}{t + C_2} \exp(-C_3 \times M)]$ or $M = -\frac{1}{C_3} \times \ln[-\frac{(t + C_2) \times \ln(r.h.)}{C_1}]$	Pfost et al., 1976
Modified Guggenheim-Anderson-deBoer (MGAB)	$r.h. = \frac{2 + \frac{C_3}{t} \times (\frac{C_1}{M} - 1) - \{[2 + \frac{C_3}{t} \times (\frac{C_1}{M} - 1)]^2 - 4 \times (1 - \frac{C_3}{t})\}^{\frac{1}{2}}}{2 \times C_2 \times (1 - \frac{C_3}{t})}$ or $M = \frac{C_1 \times C_2 \times (\frac{C_3}{t}) \times r.h.}{(1 - C_2 \times r.h.) \times (1 - C_2 \times r.h. + \frac{C_3}{t} \times C_2 \times r.h.)}$	Jayas &Mazza, 1993
Modified Henderson (MHE)	$r.h. = 1 - \exp[-C_1 \times (t + C_2) \times M^{C_3}]$ or $M = [-\frac{\ln(1 - r.h.)}{C_1 \times (C_2 + t)}]^{\frac{1}{C_3}}$	Thompson et al., 1986
Modified Oswin (MOE)	$r.h. = \frac{1}{1 + (\frac{C_1 + C_2 \times t}{M})^{C_3}}$ or $M = \frac{C_1 + C_2 \times t}{(\frac{1}{r.h.} - 1)^{\frac{1}{C_3}}}$	Chen & Morey, 1989
STYE	$r.h. = \exp[C_1 \times \exp(-C_2 \times M) \times \ln(P_s) - C_3 \times \exp(-C_4 \times M)]$	Strohman & Yoerger, 1967

[a] $r.h.$, relative humidity; M, equilibrium content, percentage wet basis; t,temperature (°C); P_s, saturated vapor pressure; C_1, C_2, C_3, and C_4; a_1, a_2, b_1, b_2 and d are coefficients in the equations.

Table 3. Summary of the results of fitting equations to the 28 data sets

Sorption type	Model function	Models	Statistical parameters				Order
			RSS[a]	SE	R^2	MRE%	
Desorption	$r.h. = f(M,t)$	CCE	0.02884	0.00070	0.98714	4.93836	4
		MCPE	0.02166	0.00052	0.99331	4.59279	2
		MGAB	0.03934	0.00094	0.98785	5.51814	6
		MHE	0.03870	0.00077	0.98805	6.43200	5
		MOE	0.03528	0.00060	0.98911	7.02714	3
		STYE	0.01912	0.00047	0.99339	4.07907	1
	$M = f(r.h.,t)$	BET	42.12103	1.03414	0.98417	4.82737	3
		CCE	12.86221	0.31371	0.99078	3.76914	2
		MCPE	11.89029	0.28314	0.98875	3.23164	1
		MGAB	40.27414	0.95891	0.96213	7.10179	6
		MHE	24.61518	0.58607	0.97685	4.89171	4
		MOE	24.72369	0.58866	0.97661	5.47250	5
Adsorption	$r.h. = f(M,t)$	CCE	0.01369	0.00033	0.99218	3.56929	3
		MCPE	0.01349	0.00032	0.99584	3.64493	2
		MGAB	0.03183	0.00076	0.99017	6.49014	6
		MHE	0.01819	0.00043	0.99442	4.27700	4
		MOE	0.02627	0.00047	0.99190	5.78950	5
		STYE	0.01291	0.00031	0.99600	3.58414	1
	$M = f(r.h.,t)$	BET	29.78256	0.72409	0.98333	4.87981	4
		CCE	7.99557	0.19501	0.99151	3.03871	2
		MCPE	8.45751	0.20136	0.99281	2.97600	1
		MGAB	32.94837	0.78454	0.97197	9.32607	6
		MHE	11.96531	0.28596	0.98966	4.03821	3
		MOE	25.92843	0.61734	0.97829	6.80193	5

[a]RSS, residue sum of squares, SE, the standard error, R^2, correlation coefficient, and MRE, mean relative percentage error.

Table 4. The best fitted MCPE parameters for the moisture sorption of wheat samples

Data set	Total wheat varieties	Coefficients of MCPE in a form of $r.h. = f(M,t)$			Statistical parameters			
		C_1	C_2	C_3	RSS	SE	R^2	MRE%
Desorption	14	529.932	41.687	0.223	2.31E-02	2.40E-04	0.9969	3.1117
Adsorption	14	920.105	150.246	0.206	1.98E-02	1.79E-04	0.9977	2.4708
Average[a]	14	622.365	72.117	0.214	1.91E-02	1.78E-04	0.9977	2.0693
Hard wheat	7	601.664	72.665	0.214	2.12E-02	1.89E-04	0.9975	2.323
Soft wheat	5	580.889	61.186	0.213	1.76E-02	1.69E-04	0.9978	2.052
Mixed wheat	2	930.351	118.819	0.217	1.96E-02	2.58E-04	0.9967	2.748
Red wheat	7	644.263	74.867	0.215	1.99E-02	1.95E-04	0.9975	2.412
White wheat	7	602.627	69.642	0.214	1.88E-02	1.76E-04	0.9977	2.017
Winter wheat	12	634.661	73.938	0.215	1.94E-02	1.87E-04	0.9976	2.164
Spring wheat	2	557.89	62.448	0.213	2.11E-02	2.27E-04	0.9971	3.141

[a]Average is the mean of desorption values and adsorption values of 14 wheat varieties.

Table 5. Estimation of the moisture content for wheat safe storage with the MCPE isotherm

Sample no.	Parameters of MCPE in a form of $M = f(r.h.,t)$			Moisture contents (% w.b.) of wheat grains at 70% ERH					
	C_1	C_2	C_3	10°C	15°C	20°C	25°C	30°C	35°C
1	689.488	53.566	0.224	15.24	14.91	14.59	14.30	14.02	13.76
2	1006.036	109.277	0.224	14.12	13.94	13.76	13.59	13.43	13.27
3	438.700	51.009	0.220	13.65	13.30	12.96	12.65	12.36	12.09
4	585.597	52.761	0.216	15.11	14.76	14.43	14.12	13.83	13.56
5	2394.201	296.777	0.219	14.09	14.02	13.94	13.87	13.80	13.73
6	744.465	45.296	0.228	15.92	15.55	15.20	14.87	14.57	14.29
7	521.849	48.853	0.220	14.61	14.24	13.89	13.57	13.28	13.00
8	475.356	36.445	0.204	16.45	15.95	15.50	15.08	14.70	14.34
9	665.515	55.459	0.221	15.16	14.83	14.52	14.23	13.95	13.69
10	987.527	75.072	0.231	15.08	14.83	14.60	14.37	14.16	13.96
11	722.930	55.033	0.220	15.63	15.30	14.98	14.69	14.41	14.15
12	688.704	63.555	0.231	14.15	13.86	13.59	13.34	13.10	12.88
13	385.071	39.355	0.214	14.42	13.97	13.56	13.18	12.83	12.50
14	478.517	45.377	0.214	14.89	14.49	14.12	13.77	13.45	13.15
Average value[b]				14.90± 0.78	14.57± 0.73	14.26± 0.70	13.97± 0.68	13.71± 0.67	13.46± 0.67
Desorption[a]	635.479	57.093	0.221	14.84	14.51	14.21	13.93	13.66	13.41
Hard wheat	564.602	54.757	0.22	14.53	14.19	13.88	13.58	13.31	13.05
Soft wheat	638.063	51.729	0.219	15.37	15.02	14.69	14.38	14.09	13.82
Mixed wheat	1015.397	89.640	0.223	15.03	14.81	14.60	14.40	14.21	14.03
Red wheat	689.899	60.829	0.222	14.90	14.59	14.30	14.03	13.78	13.54
White wheat	588.872	53.871	0.218	14.92	14.57	14.25	13.95	13.67	13.40
Winter wheat	684.633	60.503	0.221	14.95	14.64	14.35	14.08	13.82	13.58
Spring wheat	428.858	42.234	0.214	14.66	14.23	13.84	13.48	13.14	12.83

[a]Desorption is the average of desorption data of fourteen wheat varieties. [b]Average value is the means of samples from no. 1 to 14 plus standard deviation.

Table 6. The fitted MOE parameters for the moisture sorption of wheat samples

Data set	Total wheat varieties	Coefficients of MOE in a form of $r.h. = f(M,t)$			Statistical parameters			
		C_1	C_2	C_3	RSS	SE	R^2	MRE%
Desorption	14	12.638	-0.071	3.529	0.02311	5.50E-04	0.9929	6.1188
Adsorption	14	10.333	-0.029	2.879	0.01979	4.71E-04	0.9939	5.2387
Average[a]	14	11.492	-0.049	3.191	0.01912	4.55E-04	0.9941	5.078
Hard wheat	7	11.288	-0.048	3.134	0.0212	5.05E-04	0.9934	5.371
Soft wheat	5	11.978	-0.057	3.267	0.01758	4.19E-04	0.9946	4.901
Mixed wheat	2	10.961	-0.032	3.193	0.01956	4.66E-04	0.994	4.919
Red wheat	7	11.474	-0.048	3.202	0.01993	4.75E-04	0.9938	5.177
White wheat	7	11.507	-0.051	3.181	0.01883	4.48E-04	0.9942	5.177
Winter wheat	12	11.456	-0.048	3.191	0.01943	4.63E-04	0.994	5.122
Spring wheat	2	11.706	-0.056	3.194	0.02108	5.02E-04	0.9935	5.814

[a]Average is the mean of desorption and adsorption data of 14 wheat varieties.

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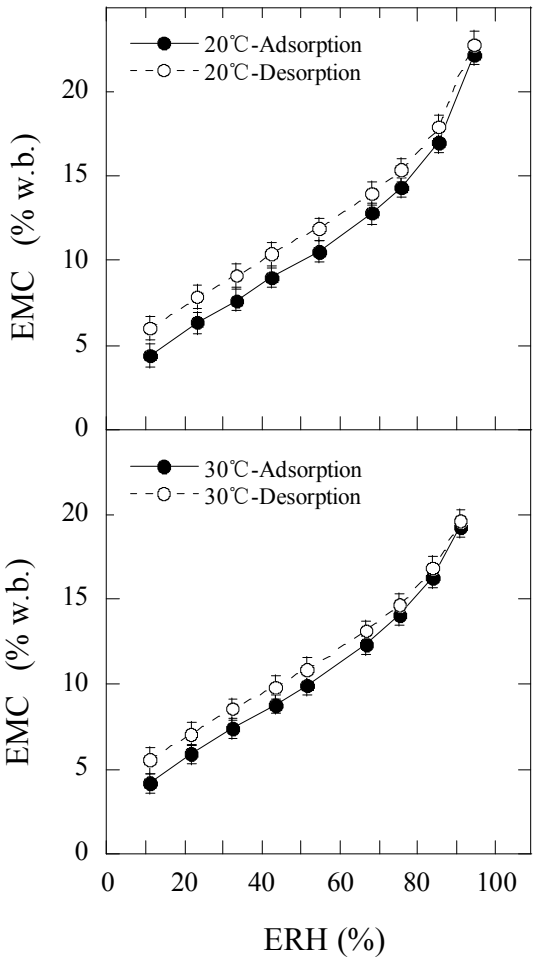

Figure 1. Comparison of the wheat desorption and adsorption isotherm at 20 and 30 °C predicted by the Modified Chung-Pfost equation (MCPE)

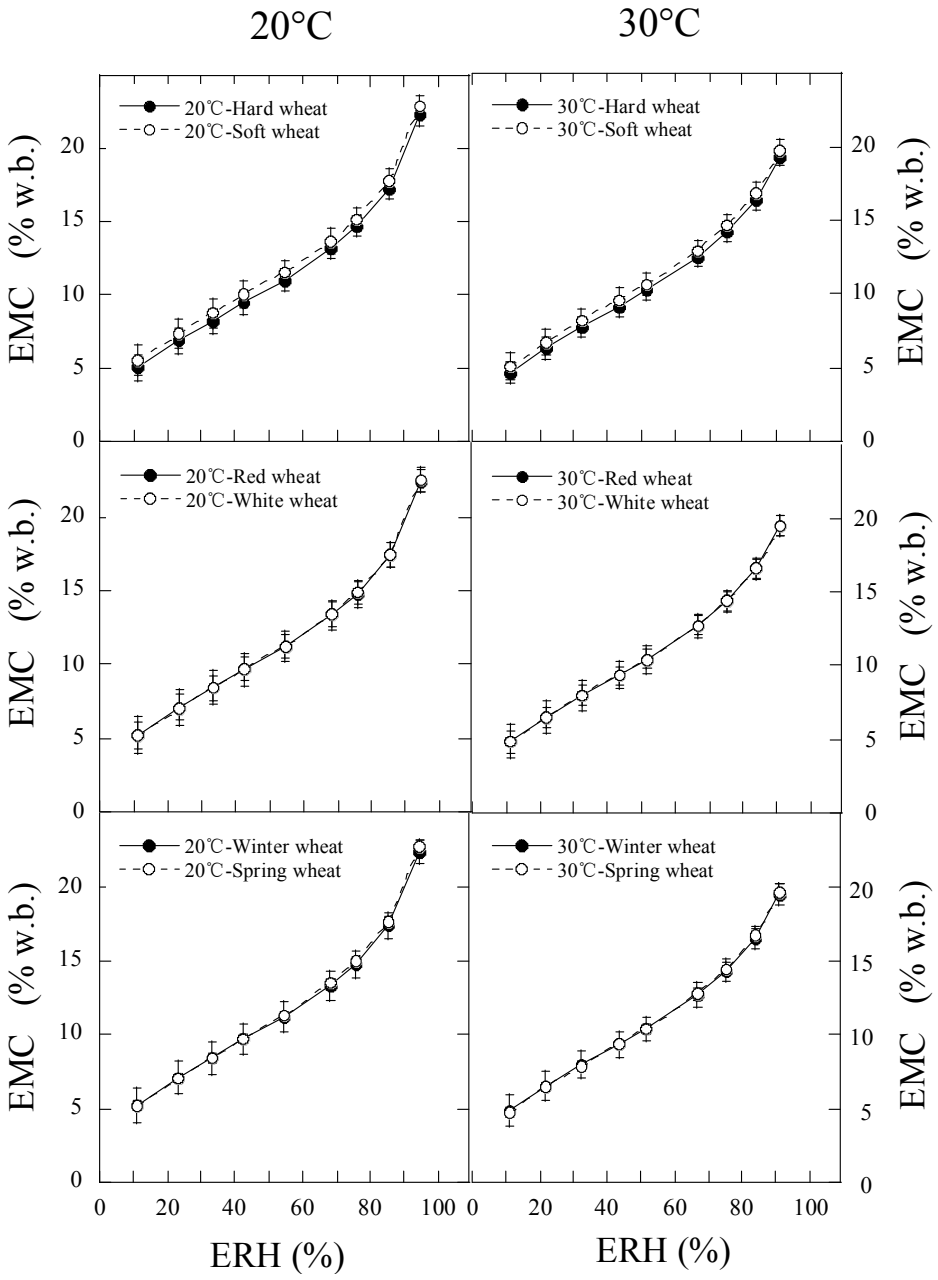

Figure 2. Comparison of the sorption isotherm of hard cv. soft wheat, red cv. white wheat, and winter cv. spring wheat at 20 and 30 °C predicted by the Modified Chung-Pfost equation

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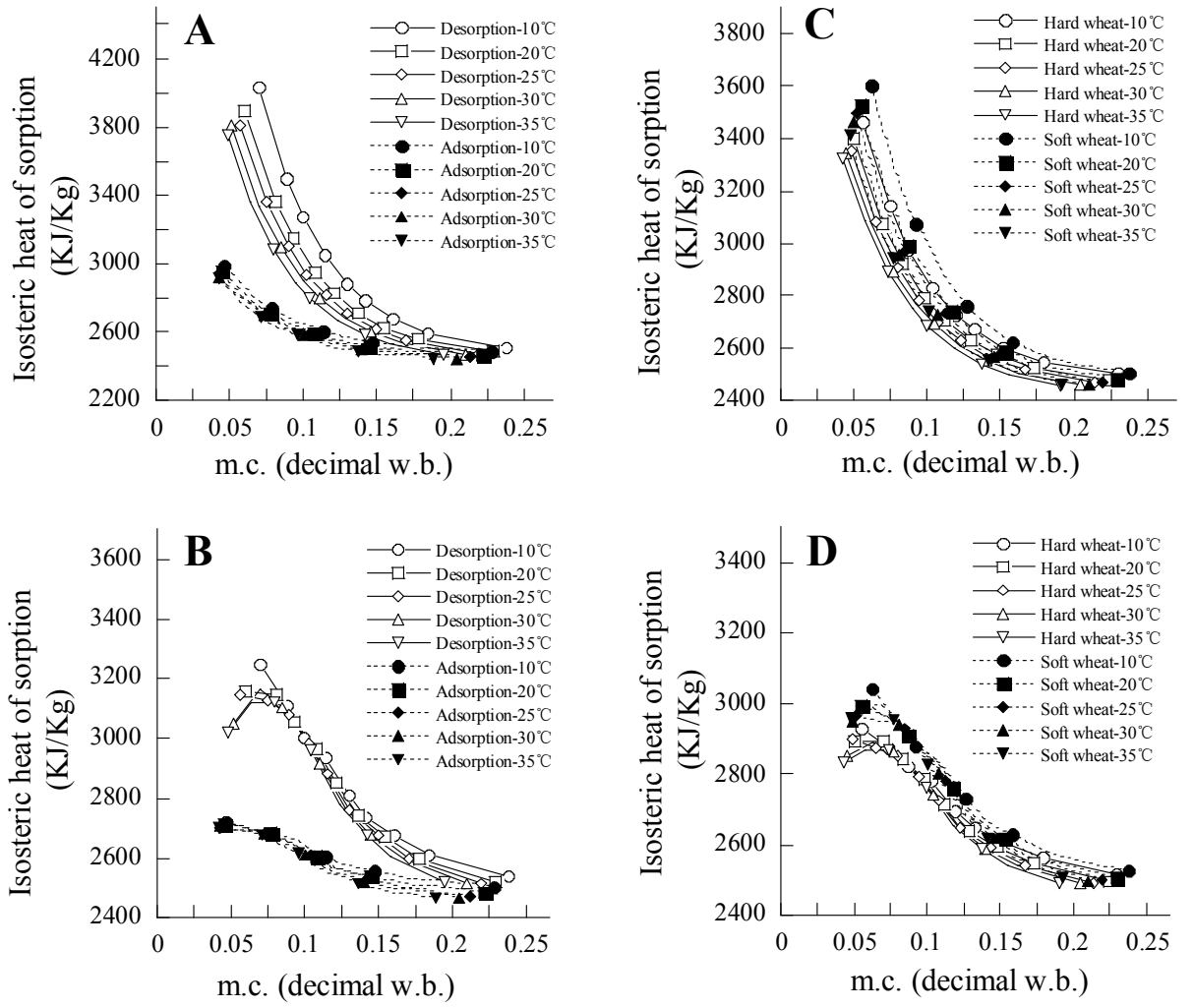

Figure 3. Comparison of isosteric heats of wheat desorption and adsorption, and the sorption isosteric heats of hard and soft wheat at different temperatures predicted by two models of MCPE (A and C), and MOE (B and D), respectively

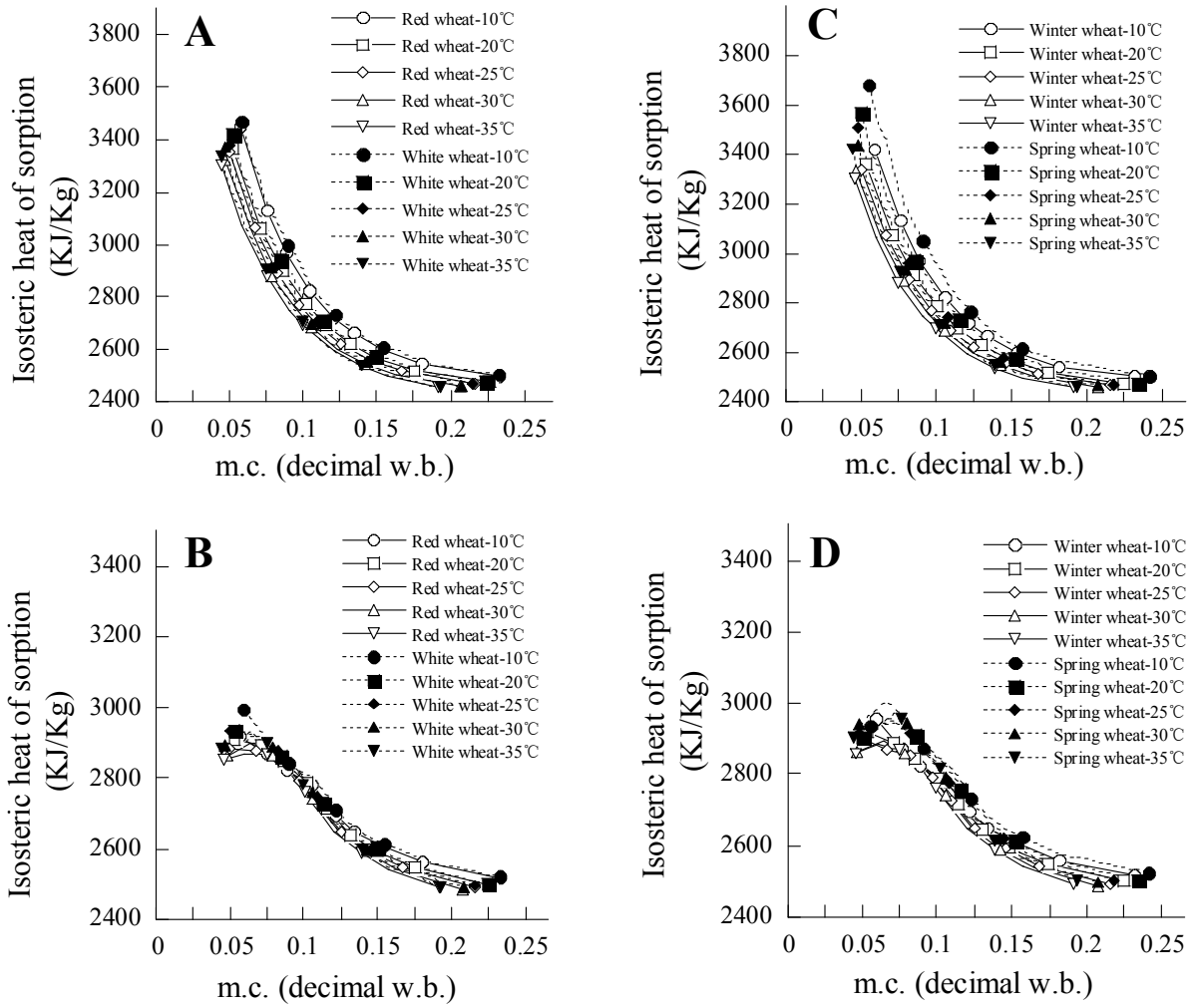

Figure 4. Comparison of the sorption isosteric heats between red and white wheat, and between winter and spring wheat at different temperatures predicted by two models of MCPE (A and C) and MOE (B and D), respectively

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The Effect of Thawing and Storage Temperature on the Microbial Quality of Commercial Frozen Ready Meals and Experimental Reduced Salt Frozen Ready Meals

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Abstract

The effect of thawing at 4ºC or ambient temperature (~20ºC) on the indigenous microflora of commercial regular salt (0.6-1.3% w/w) frozen ready meals was investigated. In a separate trial, the microbial quality of regular salt frozen ready meals was compared with reformulated reduced salt (0.2-0.54%, w/w) counterparts stored at 4ºC, 10ºC or ambient temperature over 8 days. All samples were analysed for psychrophilic, mesophilic, thermophilic and sporeforming bacteria, *Pseudomonas*, *Staphylococcus* and for the presence of *Listeria* species. During storage, psychrophiles, mesophiles, coliforms, *Pseudomonas*, sporeformers and *Listeria* were detected in the commercial regular salt ready meals while mesophiles, thermophiles, coliforms and *Pseudomonas* were detected in the reduced salt counterparts. Levels of mesophilic bacteria ranged from ~3-4 $\log_{10}$ in commercial regular salt meals and ~2-5 $\log_{10}$ in experimental lower salt meals. Overall, a substantial reduction in salt content (50 – 66%) did not appear to adversely impact on the microbial quality of the reduced salt meals.

Keywords: Ready-meals, Salt, Bacteria, Temperature

1. Introduction

Composite consumer foods which do not require significant further processing other than re-heating or completion of a cooking process are designated as ready-to-eat meals by the Food Safety Authority of Ireland (FSAI, 2001). Within the ready meals market, frozen foods comprise the largest sector which has seen a rapid expansion in product range (Department Of Agriculture, 2003). However, in terms of public health issues, excess dietary salt intake has been associated with consumption of foods containing high levels of non-discretionary salt or salt which has added during processing and outside of the control of the consumer. Processed foods such as frozen ready meals may contribute up to 70-80% of an excess dietary salt intake. Excess daily salt intake (~10-12 g) has been strongly linked with an increase in blood pressure (≥ 140mmHg systolic or ≥ 90 mmHg diastolic) leading to hypertension a major causative factor in the onset of cardiovascular disease (CVD). In Ireland, CVD is the most common cause of death according to the Irish Heart Foundation (Central Statistics Office, 2002; Durack et al., 2008) . It is now recommended by public health agencies that adults reduce their daily salt intake to a target of 6g (FSAI, 2003; Scientific Advisory Committee on Nutrition, 2003). To assist in reaching this target, various initiatives are underway worldwide involving public health agencies and food manufacturers who have been encouraged to reduce salt in their products (Consensus Action on Salt & Health, 2008; Food Standards Agency, 2008; FSAI, 2003). However, removal of salt from complex food formulations has implications for both sensory and microbiological quality. Salt is routinely used in these product

formulations and acts as both a flavouring agent and a preservative. Salt acts as a preservative by lowering of water activity (a_w) below the minimum values required for bacterial growth (Betts et al., 2007). Following a reduction in a_w, bacterial cells experience osmotic shock and plasmolysis and in order to resume growth, a_w must return to values which allow cells to recover (Davidson, 1997).

Freezing as a means of preservation is used throughout the food industry (O'Leary et al., 2000) and generally frozen ready meals benefit from a long shelf life (McAteer et al. 1995; Nissen et al. 2002; Redmond et al. 2005). Typically, frozen ready meals consist of a complex formulation of ingredients and because of the variation and complexity in these formulations it is likely that varying effects are exerted on bacterial growth and survival during thawing and subsequent storage. Indeed, bacterial species exhibit a variable response to the freezing process while freeze-thawing has been shown not to result in death of all bacteria but merely effect a reduction in population (Doyle & Schoeni, 1984). Surprisingly few studies have been carried out on the microbial quality of frozen ethnic ready meals especially under various thawing and storage conditions. This contrasts with more extensive published studies for other ready meal types including chilled meals, freeze-chilled meals and sous-vide products (McAteer et al., 1995; Nissen et al., 2002; O'Leary et al., 2000; Redmond et al., 2005). Hence, the objectives of this study were to determine the effect of thawing and storage temperature on a range of commercial regular salt frozen ready meals and subsequently to compare the microbial quality of commercial regular salt with experimental reduced salt frozen ready meals stored at various temperatures.

2. Materials and Methods

2.1 Manufacture of commercial regular salt or experimental reduced salt ready meals

The commercial regular salt frozen ready meals, chilli con carne, meat lasagne and chicken curry, were obtained from a leading manufacturer and held at -18ºC until analysis. Experimental reduced salt chilli con carne, meat lasagne and chicken curry meals were manufactured by the commercial supplier using the same proprietary formulations as the commercial regular salt meals but omitting added salt, including low sodium spice blends and in the case of lasagne, the use of reduced salt Cheddar cheese (1.8% w/w, compared with regular salt cheese, 2.7% w/w salt). The experimental reduced salt meals were manufactured on the same processing line as the commercial regular salt products. Typically, the manufacturing process involves cooking of a 600kg batch of Chicken curry or chilli con carne, a 600kg batch of Bolognese mix and a 300kg batch of Béchamel sauce. All products were heated to 90º – 100ºC followed by immediate cooling in a vacuum cooler to 10ºC over 45 mins, a final freezing step involved passage through a spiral freezer for 120 mins to give a final product temperature of -10º to -18º C. In the manufacture of chicken curry and chilli con carne, products are directly hot-filled into bags which are film sealed before freezing. Lasagne manufacture involved filling of plastic trays by personnel at depositing stations where individual layers were added to the tray. Lasagne meals were assembled in the following format: bolognese sauce (77g), pasta (10g), bolognese sauce (77g), pasta (10g), bolognese sauce (77g), pasta (10g), béchamel sauce (109g) and topped with cheddar cheese (5g). The final portion sizes were Chicken Curry 375g, Chilli con carne 350g and Lasagne 375g. The experimental reduced salt meals were manufactured specifically for this study and are not currently available for retail.

2.2 Compositional analysis of all meals

Compositional analysis of products was carried out on day 1 after overnight thawing at ambient temperature for 16 hours. A Jenway (Analytica, Dublin, Ireland) pH meter 3310 was used to measure pH in thawed ready meals. Samples were prepared as follows: 10g of food was homogenized with 90 ml of sterile distilled water, and stomached at high speed for 2 minutes, after which pH was measured directly on the homogenate. Water activity (a_w) in ~5g samples was measured at 23ºC using an Aqualab Series 3 water activity meter (Model TE - Labcell Ltd., Alton, Hants, UK). Moisture content was determined on a 50 g composite food sample prepared by blending using a Moulinex hand blender (Moulinex, Berkshire, UK); a 10 g sub-sample was then weighed into aluminium weighing dishes and placed in an oven at 105ºC for 18-24 hours. After this time period, samples were re-weighed until there was no further drop in weight and differences from initial weights were expressed as percentage dry matter, this figure was subtracted from 100 to give percentage moisture content. Salt content of regular salt ready meals and low salt equivalent meals was measured as follows: 10ml of concentrated nitric acid was added to 0.4-0.5g samples of each meal in acid digester tubes. Samples were digested using a Mars Xpress microwaveable acid digester (CEM Corporation, Matthews, North Carolina, USA). Samples were heated to 200ºC and held at this temperature for 15 minutes. Samples were analyzed using a Varian Atomic Absorption Spectrophotometer (JVA, Dublin, Ireland). Results were converted from ppm and expressed as % NaCl (w/w) for all samples.

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2.3 Effect of thawing temperatures on the microbial quality of commercial regular salt frozen ready meals

Commercial regular salt ready meals were removed from storage at -18ºC and thawed up to 24 hours at 4ºC or ambient temperature (~20ºC). Microbiological analysis was carried out in triplicate on samples taken after 4, 8, 16 and 24 hours: 10g of product was weighed into a sterile stomacher bag to which 90 ml of sterile 0.1% peptone water (Oxoid CM0009, Basingstoke, Hants, UK) at 30ºC was added, this ensured that all samples were completely thawed at time of analysis. Samples were then homogenised for 120 seconds in a Seward Stomacher 400 (AGB, Dublin, Ireland) at the high power setting. Serial dilutions from this homogenate were prepared using 0.1% peptone water and inoculated onto media described below. Results are taken from duplicate plates containing between 30 to 300 colonies and expressed as Colony Forming Units (CFU) per gram of product.

2.4 Effect of storage temperature on the microbial quality of commercial regular salt frozen ready meals and experimental low salt frozen ready meals

Frozen commercial regular salt ready meals and experimental reduced salt ready meals were thawed at ambient temperature for 16 hours. After analysis of thawed products on day 1, products were then stored at 4°C, 10°C or ambient temperature (~20°C) over 8 days. Microbiological analysis was carried out on day 3, 5 or 8 at each storage temperature as described below. After 8 days storage at the various temperatures, all meals were microwaved at full power (800 W) for 8 min according to manufacturer's instructions and samples were analysed for total aerobic viable counts as described below.

2.5 Microbiological analysis

On each sampling day, 10g portions of each meal were weighed and mixed with 90 ml of sterile 0.1% peptone water in sterile stomacher bags and homogenised for 120 seconds in a Seward Stomacher 400 at the high power setting. Serial dilutions were prepared from this homogenate using 0.1% peptone water and were inoculated in duplicate using standard spread-plate technique onto a range of media sourced from Oxoid. In the case of coliforms, an overlay pour-plate method was used. Total psychrophilic, mesophilic and thermophilic aerobic populations were estimated on Plate Count Agar (PCA - Oxoid CM0325) incubated at 4ºC, 37ºC or 55ºC, respectively. PCA plates at 37ºC and 55ºC were incubated for 48 hours and plates at 4ºC were incubated for 7 days. Total coliform counts were estimated on Violet Red Bile Agar (VRBA) (Oxoid CM0107): 1ml of serial dilutions and 10 ml of molten agar was added into sterile petri dishes and mixed to ensure uniform distribution of sample. After plates had solidified, a layer of ~ 10ml molten VRBA was poured over the set agar. Plates were then incubated at 37ºC for 24 hours. Positive colonies were identified as dark red-purple colonies with a red halo (lactose fermenters) greater than 0.5mm in diameter. Positive colonies were also verified using oxidative and fermentative (O/F) carbohydrate metabolism determination. Representative colonies were inoculated into O/F basal media (AGB, Dublin, Ireland) using a needle which was stabbed through the media to about 1/4 inch of the bottom of 2 tubes. One of the tubes was overlayed with 1-2 ml of mineral oil. All tubes were incubated at 37ºC for 24 hours. Positive results for coliforms produced yellow colour in both tubes after incubation (Becton Dickinson and Company, 2008). Staphylococci were estimated on Baird-Parker Medium (Oxoid CM0275) incubated at 37ºC for 48 hours. Growth of *Pseudomonas* spp. were estimated on Pseudomonas Agar Base (Oxoid CM0559), supplemented with C-F-C supplements (CM0559) and incubated at 25ºC for 48 hours. Total sporeformers and heat resistant bacteria were estimated on Nutrient agar (Oxoid CM0003) from samples of all meals including the separate rice portion which accompanies the chicken curry product. Sporeformer enumeration involved heating of diluted samples at 90ºC for 10 minutes using an Eppendorf Thermomixer comfort (Eppendorf, Hamburg, Germany) heating block and subsequent plating onto Nutrient agar followed by incubation at 30ºC for 24 hours. Novel Enrichment Broth (Oxoid CM1066) and Listeria Selective Agar (Oxoid CM0856) were used for detection of *Listeria* species. The procedure involved taking 25 g of sample and diluting with 225 ml of novel enrichment broth. Samples were then homogenised in a stomacher at the medium speed setting for 30 seconds. These samples were then incubated at 30ºC for 24 ±2 hours. Subsequently, 10 µl of this sample was streaked onto Listeria Selective Agar. Plates were incubated at 37ºC for 24 and/or 48 hours and checked for growth, indicated by the presence of black, shiny, convex colonies. After day 8, all samples stored at various temperatures were microwaved as per manufacturer's instructions (full power in an 800W microwave oven for 8 minutes). After microwave cooking, samples were diluted in sterile 0.1% peptone water, plated onto PCA and incubated at 37°C for 48 hours. Results are taken from duplicate plates containing between 30 to 300 colonies and expressed as Colony Forming Units (CFU) per gram of product. Where no colonies were present on a spread plate, the estimated count is reported as less than (<) one time the corresponding dilution i.e. no colonies on a 10^{-1} plate are reported as <100 estimated (Swanson et al., 2001).

2.6 Statistics

All graphs and bar charts including means and standard deviations were prepared using GraphPad Prism Version 5.0 (Graph Pad, San Diego, CA, USA). Analysis was carried out using two-way ANOVA comparing bacterial growth in commercial regular salt or in experimental reduced salt frozen ready meals.

3. Results

3.1 Compositional analysis of products

Salt content of commercial regular salt meals ranged from 0.66% to 1.13% and in experimental reduced salt meals ranged from 0.20% to 0.54%. The pH values of all meals were similar at pH, ~ 5.5. Water activity (a_w) of all meals was ~ 0.99, reflecting that of highly perishable foods. Products had moisture contents ranging from ~ 65- 85% (Table 1).

3.2 Effect of thawing at 4ºC or ambient temperature on microbial quality of commercial regular salt ready meals

3.2.1 Aerobic plate count at 4°C or 55°C

Growth was not detected on PCA plates incubated at 4º C or 55ºC for any of commercial regular salt meals when defrosted over 24 hours at 4ºC or at ambient temperature.

3.2.2 Aerobic plate count at 37°C

Meals analysed after 4, 12, 16 and 24 hours had differing aerobic populations. After 4 hours of thawing at both temperatures, the chicken curry product had the lowest population, ~3 $\log_{10}$ CFU/g, followed by chilli con carne, ~4 $\log_{10}$ CFU/g, while the lasagne had the highest population, ~5 $\log_{10}$ CFU/g (Figure 1). For chicken curry ready meals, populations remained constant up to 16 h, when thawed at either 4ºC or ambient temperature, with a slight increase to ~4 - 5 $\log_{10}$ CFU/g noted after 24 h thawing at ambient temperature. Similar trends were noted for chilli con carne, populations increased slightly at 24 h to ~3 $\log_{10}$ CFU/g at ambient temperature thawing. Results for lasagne over 24 h indicated that populations remained constant at either 4ºC or ambient temperature however populations were higher than in the other 2 meals, ~5 $\log_{10}$ CFU/g.

3.2.3 Staphylococci

Staphylococci were detected only in the commercial regular salt chilli con carne meal at ~ 2-3 $\log_{10}$ CFU/g at both thawing temperatures over 24 h period (Figure 2).

3.2.4 *Pseudomonas*

Pseudomonas species were not detected in commercial regular salt lasagne meals thawed at 4ºC or ambient temperature. For chilli con carne and chicken curry, only one sample thawed at ambient temperature at 24 h contained ~3.8 $\log_{10}$ CFU/g or ~3.5 $\log_{10}$ CFU/g colonies, respectively (Data not shown).

3.2.5 *Listeria* species

Of the three commercial regular salt ready meals, only lasagne thawed at 4ºC after 24 h showed positive growth of *Listeria* species and using an API *Listeria* kit (Biomerieux, Basingstoke, Hampshire, UK) these colonies were putatively identified as *L. seeligeri*.

3.2.6 Total coliform count

Coliforms were absent in commercial regular salt meals over 24 hours thawing at either 4ºC or ambient temperature.

3.3 Effect of storage temperature on the microbial quality of commercial regular salt ready meals or experimental reduced salt ready meals

3.3.1 Aerobic plate count at 4°C

Growth was not detected in commercial regular salt lasagne or chilli con carne meals or in any of the experimental reduced salt ready meals at any of the storage temperatures (4ºC 10ºC or ambient temperature). However, the commercial regular salt chicken curry meal contained 4.5 $\log_{10}$ CFU/g on day 8 at 4ºC storage.

3.3.2 Aerobic plate count at 37°*C*

Initially, aerobic populations after thawing, at day 1, in commercial regular salt ready meals or experimental reduced salt meals were ~3-4 $\log_{10}$ CFU/g and ~2-5 $\log_{10}$ CFU/g, respectively (Figure 3 a, b, c). Of the commercial regular salt meals, chicken curry stored at 4°C developed the lowest population over the 8 day storage ~ 4 $\log_{10}$ CFU/g (Figure 3a). The commercial regular salt chicken curry meal stored at 10°C developed final populations of ~6 $\log_{10}$ CFU/g, an overall increase of 3 $\log_{10}$ CFU/g from day 1. When this product was

stored at ambient temperature, populations increased rapidly, reaching ~ 8 $\log_{10}$ CFU/g by day 3 and remaining at this level to day 8. Populations in experimental reduced salt chicken curry meal stored at 4ºC did not increase over the 8 days storage. During storage at 10ºC, for the experimental reduced salt chicken curry meal there was an increase at day 5 to ~4 $\log_{10}$ CFU/g and the final population reached at day 8 was ~6 $\log_{10}$ CFU/g. However, at ambient storage, populations in the experimental reduced salt ready meal increased to levels comparable to the commercial regular salt meals, ~ 8 $\log_{10}$ CFU/g after 8 days storage. Initial aerobic populations in commercial regular salt chilli con carne were ~4 $\log_{10}$ CFU/g (Figure 3b) and during storage at 4°C increased to ~6 $\log_{10}$ CFU/g at day 8. During storage at 10°C, populations increased to ~7-8 $\log_{10}$ CFU/g at day 5 and remained at this level up to day 8. Commercial regular salt chilli con carne products stored at ambient temperature showed a substantial increase in bacterial populations between day 1 and day 8 with final populations at day reaching ~9 $\log_{10}$ CFU/g. Populations in the experimental reduced salt chilli con carne meals stored at 4ºC reached ~4 $\log_{10}$ CFU/g by day 8 (Figure 3b). When stored at 10ºC the final population in these meals reached ~7 $\log_{10}$ CFU/g. At ambient storage temperature, by day 8 the experimental reduced salt chilli con carne meals contained ~9 $\log_{10}$ CFU/g slightly exceeding populations detected in their commercial regular salt counterparts. In the commercial regular salt lasagne meal, the aerobic bacterial population increased to ~ 5-6 $\log_{10}$ CFU/g after 8 days at 4°C (Figure 3c). Storage at 10°C of the commercial regular salt lasagne products resulted in an increase in populations to ~ 8 $\log_{10}$ CFU/g by day 8. During storage at ambient temperature, bacterial populations increased to ~8 $\log_{10}$ CFU/g by day 3 and remained at this level to day 8. In the experimental reduced salt lasagne meals, initial populations at day 1 were higher than comparable commercial regular salt meals at ~5 $\log_{10}$ CFU/g (Figure 3c). During storage at 4ºC or 10ºC of the experimental reduced salt lasagne there appeared to be a reduction in populations after day 1 to ~3 $\log_{10}$ CFU/g and at 10ºC storage, after an initial decline the final population was ~5 $\log_{10}$ CFU/g while at 4ºC storage the final population was ~3 $\log_{10}$ CFU/g. During storage at ambient temperature of the experimental reduced salt lasagne, a final population of ~ 9 $\log_{10}$ CFU/g was reached after 8 days, ~1 $\log_{10}$ cycle higher than the commercial regular salt counterpart. Final bacterial populations reached in either commercial regular salt or in experimental reduced salt meals were highest at ambient temperature storage.

Generally, aerobic bacterial populations increased slowly in all thawed commercial regular salt ready meal products stored at 4°C over 8 days. However at 4ºC storage, for the experimental reduced salt meals, an increase in microbial populations was found only in the chilli con carne meal which increased by ~ 1 $\log_{10}$ over 8 days. At 10°C storage, bacterial populations in commercial regular salt chicken curry and chilli con carne meals increased by ~3 $\log_{10}$ CFU/g over 8 days while commercial regular salt lasagne showed a 4 $\log_{10}$ increase in population over this period. Storage of the experimental reduced salt meals at 10ºC resulted in an overall 4 $\log_{10}$ increase in both chicken curry and chilli con carne, while no increase in bacterial populations was found in experimental reduced salt lasagne meals. At ambient temperature storage, for both commercial regular salt or reduced salt products, final populations were 8-9 $\log_{10}$ CFU/g with overall increases between day 1 and day 8 of ~4-6 $\log_{10}$ cycles.

3.3.3 Aerobic plate count at 55°C

Growth was not detected in any of the commercial regular salt meals stored at 4°C**,** 10°C or ambient temperature on PCA plates incubated at 55ºC. Growth was detected in all experimental reduced salt meals but was present on agar plates as a film and consequently was uncountable; a number of isolates were subsequently identified as *Leuconostoc mesenteroides* using an API 50CH kit (Biomerieux, Basingstoke, Hants, UK).

3.3.4 Total coliform count

Coliforms were not detected in the commercial regular salt chilli con carne meals at any storage temperature. Coliforms were detected in one of the commercial regular salt chicken curry meals and one of the commercial regular salt lasagne meals at day 3 only when stored at ambient temperature, ~3 $\log_{10}$ CFU/g or ~4 $\log_{10}$ CFU/g, respectively. Coliforms were not detected in any of the experimental reduced salt chicken curry meals at any of the storage temperatures. At 4ºC storage, growth was not detected in the experimental reduced salt chilli con carne meals. At 10ºC storage growth was detected at day 5, at ~3 $\log_{10}$ CFU/g which remained at this level up to 8 days storage. When stored at ambient temperature, growth was not detected until day 5 where ~6 $\log_{10}$ CFU/g were detected and which remained at this level up to day 8. Growth was not detected in the experimental reduced salt lasagne meal at 4ºC or 10ºC storage. However at ambient temperature storage, ~ 2 $\log_{10}$ CFU/g were detected at day 3 or 5 which increased to ~3 $\log_{10}$ CFU/g by day 8.

3.3.5 Staphylococci

Staphylococci were not detected in any of the commercial regular salt or experimental reduced salt ready meals stored at 4°C**,** 10°C or ambient temperature.

3.3.6 *Pseudomonas*

Pseudomonas spp. were not detected in any of the commercial regular salt meals stored at 4°C. Growth was not detected in commercial regular salt chicken curry when stored at 4 or 10 ºC. Storage of commercial regular salt chicken curry meals at ambient temperature resulted in growth of *Pseudomonas* spp. to ~4 $\log_{10}$ CFU/g at day 3 with final populations of ~7.5 $\log_{10}$ CFU/g at day 8 (Figure 4a). In the case of commercial regular salt chilli con carne, *Pseudomonas* spp. were detected at ambient temperature storage at day 3, ~4 $\log_{10}$ CFU/g, and these populations reached > 6 $\log_{10}$ CFU/g at day 8 (Figure 4b). Growth of *Pseudomonas* species was detected in commercial regular salt lasagne stored at 10°C at day 5 with a final population of ~ 4 $\log_{10}$ CFU/g at day 8. Growth of *Pseudomonas* species was detected at ambient temperature storage of the commercial regular salt lasagne meal. This population increased from ~4 $\log_{10}$ CFU/g at day 5 to a final population of ~6 $\log_{10}$ CFU/g at day 8. Overall, populations of *Pseudomonas* species detected in commercial regular salt meals stored at ambient temperature decreased in the order: chicken curry > chilli con carne > lasagne. In the case of the experimental reduced salt chicken curry, growth of *Pseudomonas* spp was not detected at 4ºC over the 8 day period. At 10ºC growth was only detected at day 8 at ~3 $\log_{10}$ CFU/g. At ambient temperature storage, growth was detected at day 3 at 5 $\log_{10}$ CFU/g, decreasing at day 5 to < 4 $\log_{10}$ CFU/g with a final population at day 8 of ~ 4 $\log_{10}$ CFU/g. Growth was detected in experimental reduced salt chilli con carne at all storage temperatures (Figure 4b). At 4ºC storage, populations detected were 5 $\log_{10}$ CFU/g at day 8. At 10ºC storage, growth was detected at day 3 at 3 $\log_{10}$ CFU/g, and increased thereafter to ~6 $\log_{10}$ CFU/g at day 8. At ambient storage ~7 $\log_{10}$ CFU/g were detected at day 3 but appeared to decrease to a final population of ~ 6 $\log_{10}$ CFU/g at day 8. Growth was not detected in the experimental reduced salt lasagne meal at any of the storage temperatures

3.3.7 Sporeformers and heat resistant bacteria

Spore forming or heat resistant bacteria were not detected in the experimental reduced salt meals or in the commercial regular salt chilli con carne at any storage temperature. Colonies were not detected in commercial regular salt chicken curry meal at day 1 at any of the storage temperatures. However, on day 3 at ambient temperature storage, a number of colonies were present in the commercial regular salt chicken curry, which, on further investigation were determined as Gram negative, catalase-negative cocci and not *Bacillus* species. In rice samples stored at 10°C, a small number of colonies were detected at day 5 of storage and were subsequently determined to be Gram positive bacilli with a slow catalase reaction and were putatively identified as *Lactobacillus* species using API 50CHL (Biomerieux). A number of colonies, <30 per plate, were detected on day 3 in the commercial regular salt lasagne meal stored at 10°C, further examination of these colonies including gram staining and identification using API 50CHL (Biomerieux) determined them to be Gram positive cocci, not *Bacillus* spp.

3.3.8 *Listeria* species

Growth was detected only in commercial regular salt chicken curry meals after 8 days storage at 4°C and using an API Listeria kit, colonies were putatively identified as *Listeria grayii*. *Listeria* spp. were not detected in any of the experimental reduced salt meals.

3.3.9 Total bacterial count after microwaving

Growth was not detected in either of the commercial regular salt or experimental reduced salt meals following microwaving.

4. Discussion

To date, little published information exists on the effects of thawing temperature and storage time and temperature on the microbial quality of frozen commercial ethnic ready meals. Additionally, to our knowledge, a comparison of the effects of salt reduction on the microbiological quality of frozen ready meals during storage has not been undertaken. Despite manufacturers' instructions or public health safety information, consumers may thaw out frozen foods for inappropriate times and temperatures prior to cooking. Similarly, subsequent storage by the consumer of thawed ready meals may occur for prolonged periods at temperatures likely to allow microbial growth to exceed recommended levels. Consumer surveys have consistently revealed a lack of knowledge of correct refrigeration temperatures which may be due to the absence of thermometers in domestic refrigerators and this may lead to temperature abuse by the consumer (Worsfold & Griffith, 1997). Hence the temperatures selected in this study were designed to simulate thawing/storage either in a domestic refrigerator

operating at 4º to 10ºC or in a consumer temperature abuse scenario where the thawed meals were held at ambient temperature. All commercial regular salt or experimental reduced salt ready meals contained an aerobic population at day 1, (~2 - 5 $\log_{10}$ CFU/g) within FSAI recommendations (aerobic plate count of 4 - < 5 and ≥ 5 $\log_{10}$ CFU/g) (FSAI, 2001) with subsequent outgrowth on extended storage at various temperatures.

Data from the thawing trial provides evidence of an indigenous microbial flora present in the products after processing and which were detectable after 4 h thawing of all commercial regular salt products. Generally, the duration of frozen storage and food composition affects bacterial survival. Survival of bacteria following freezing is also dependant on the species and strain, the growth phase of the cells and the physiological condition of the cells prior to freezing (Dykes & Moorhead, 2001; Uljas & Ingham, 1999). Available water, aw, in the food matrix also affects growth, respiration and enzyme synthesis (Blondeaux et al., 1999) and the freezing process of food products reduces the available water content for the microorganisms present (Leistner & Gorris, 1995). This may be due to the fact that freezing of foods leads to formation of ice crystals which affects the mechanical structure of the food and also moisture migration (Durack et al., 2011)

The microbial load detected in the three regular salt ready meals during the 24 h thawing trial indicates a tolerance and an ability of bacterial strains to survive following a relatively severe industrial manufacturing regime. However, in this study it is not clear at what stage during the industrial process bacterial survival occurs.

Regarding the effects of storage temperature on microbial quality, all thawed commercial regular salt ready meals stored at 4ºC developed substantial bacterial populations over 8 days. Of the regular salt meals, bacterial numbers in lasagne and chilli con carne meals reached unacceptable levels at 8 days storage at 4ºC. Elevation of storage temperature to 10ºC resulted in higher developed bacterial populations which by day 3 in lasagne and chilli con carne meals exceeded recommended levels (FSAI, 2001). Continued elevations of storage temperature resulted in rapid increases in bacterial numbers in all meals by day 3, and were well in excess of recommended levels. Microbial counts of the experimental reduced salt meals differed from the commercial regular salt meals. In the case of aerobic plate counts (APC), initial microbial levels at 4ºC storage for both experimental reduced salt chicken curry and chilli con carne populations were much lower than in the commercial regular salt meals, 1 or 3 $\log_{10}$ cycle, respectively. However, final populations after 8 days at 4ºC storage in all three experimental reduced salt meals were significantly lower ($P < 0.001$) than those in commercial regular salt meals. The populations of these experimental reduced salt meals at 4ºC storage were within FSAI microbiological guidelines (APC of 4 - < 5 and ≥ 5 $\log_{10}$ CFU/g) (FSAI, 2001). Experimental reduced salt meals and commercial regular meals salt meals stored at ambient temperature and 10ºC storage contained similar final bacterial populations. Evidence of the ability of bacteria to survive freeze-chilling was reported by O' Leary et al. (2000). These workers found that salmon blast frozen to -35ºC for 2.5h, stored at -25ºC for 7 days, thawed overnight at 4ºC and subsequently stored at 4ºC for 5 days had higher total viable counts in comparison with freshly prepared products (cooked and analysed on the same day). These authors proposed that the thawed product may have had a more open structure due to freeze damage, with more free liquid containing cell nutrients making it more susceptible to microbial growth during subsequent chilling.

During the current study, the removal of a substantial level of salt (~50%) from the commercial regular salt products did not appear to adversely impact on microbial safety, as populations in the reduced salt experimental meals were similar to commercial regular salt meals. The use of salt as a method for food preservation has been well established (Desmond, 2006; Guinee & Fox, 2004). Boziaris et al., (2007) found that salt had a slightly inhibitory affect on bacteria especially at initial exposure but after adaptation, salt may actually stimulate bacterial growth. In contrast to the noted salt tolerance of *Listeria* spp, Conner et al., (1986) found that cabbage juice containing 1% salt caused an initial decrease in populations of *Listeria*. In ready meals the presence of *Enterobacteriaceae* may indicate inadequate heat processing or post pasteurization contamination. Indeed, while certain coliform species i.e. *E.coli*, *Yersinia enterocolitica* may experience some damage during freezing they are also capable of withstanding freezing and subsequent thawing cycles (Gurtler & Beuchat, 2005; Koujitani et al., 2006; Raccach et al., 2002; Warseck et al., 1973). In the present study, except for experimental low salt chilli con carne stored at ambient temperature, coliform populations were within FSAI recommendations for *Enterobacteriaceae* of ≤4 $\log_{10}$ CFU/g (FSAI, 2001).

Psychrophilic bacteria were detected in the commercial regular salt chicken curry meal stored at 4ºC at day 8 only. Psychrophiles or psychrotrophs generally have the ability to grow in temperature environments below ~ 15ºC to 20ºC (Cousin et al., 2001). Conversely, *Pseudomonas* spp. was detected in all commercial regular salt meals stored at ambient temperature but growth was not detected in any of these meals stored at 4ºC. In the experimental low salt meals *Pseudomonas* was only detected in the experimental low salt chilli con carne at 4ºC. The spoilage problem posed by growth of *Pseudomonas* spp. especially in meat products at refrigeration

temperatures has been highlighted a number of workers (Liu et al., 2006; Rajmohan, 2002). Another bacterial species well known for its ability to survive and grow at refrigeration temperatures (3-7ºC) is *Listeria monocytogenes* (Carlin et al., 1995; Carlin & Nguyen-The, 1994; Francis & Beirne, 1997; Rosso et al., 1996). Leistner (2000) found that *Listeria innocua* had a higher survival in margarine when stored at 7ºC compared with an ambient temperature of 25ºC. In this study, *Listeria* species were detected during thawing of regular salt lasagne at 4ºC or in the regular salt chicken curry meal stored at 4ºC on day 8. Indeed the commercial regular salt chicken curry meal was the only meal where general growth of psychrotrophs was evident. Although *L. grayii* is regarded as non-pathogenic it may indicate deterioration in hygiene standards or inadequate process conditions, leading to an increased risk of contamination by other pathogenic *Listeria* species (Greenwood et al., 2005). Lekroengsin et al. (2007) demonstrated that cooking processes, i.e. roasting, steaming and frying, are sufficient to destroy any *Listeria* species present in raw materials. Hence, the presence of *Listeria* spp. in the thawed ethnic ready meals may indicate some post-heat processing contamination.

In the manufacture of chicken curry and chilli con carne, products are directly hot-filled into bags which are film sealed and thereafter enter a spiral freezer. This process minimises human contact with the food after heating and therefore a lower risk of possible contamination by *Staphylococcus* species is present. However, lasagne manufacture involves minimal contact with personnel at depositing stations where individual layers are added to the tray. Hence, this particular process is potentially more susceptible to entry of S*taphylococcus* species. Staphylococci were detected in the commercial regular salt chilli meal only during thawing trials. It is quite possible for packs to be contaminated by Staphylococci post processing through human contact or processing vessels or equipment. In the storage trials, Staphylococci were absent in all of the ready meals indicating a good standard of general personal hygiene of workers in contact with the products and adequate cleaning of utensils and work surfaces (Lancette & Bennett, 2001).

The main ingredient of ready meals potentially susceptible to contamination by sporeforming bacteria is the cooked rice portion, which on extended storage may allow germination and outgrowth. Bacterial spores e.g. *Bacillus cereus*, are capable of withstanding refrigeration temperatures and can germinate and grow in foods stored under inappropriate conditions (Collado et al., 2003). However, in this study only a small number of possible sporeforming or heat resistant bacteria were detected in commercial regular salt lasagne, chicken curry and in the separate rice portion which accompanies the chicken curry meal and were not *Bacillus* spp.

Growth of bacterial species on plates incubated at 55ºC was detected in all experimental low salt meals but not in the commercial regular salt meals. Some isolates were putatively identified as *Leuconostoc mesenteroides,* a species involved in vegetable fermentation (Eom et al., 2007), cheesemaking (Clementi & Rossi, 1984), but which are also commonly associated with spoilage of cooked meat products (García-Gimeno et al., 2005).

In agreement with Aziz et al. (2002), Canumir et al. (2002) and Pucciarelli and Benassi (2005), the effectiveness of microwaving as a means of bacterial inactivation was demonstrated even in the heavily contaminated products after 8 days storage. However, this heat treatment does not inactivate pre-formed toxins or preclude the survival of pathogens or spoilage microorganisms in the viable-but-non-culturable state in these products. Indeed, Woo et al. (2000) noted that while microwaving of suspensions of *E. coli* or *B. subtilis* resulted in a significant reduction in cell viability, cell density, as measured by optical density at 600nm, did not decrease. These authors suggested that after exposure to microwave radiation, cells despite becoming non-viable, were not autolysed**.**

All of the ready meals in this study have slightly acidic pH values with relatively high moisture levels. The moisture levels in the experimental reduced salt chilli con carne and chicken curry were similar to their commercial regular salt counterparts, however, moisture levels in low salt lasagne were ~20% lower. The addition of salt to foods containing meat can increase water holding capacity. Conversely, removal of salt from meat-containing products such as lasagne may release water which is subsequently evaporated during heat processing at 100ºC (Aktas et al., 2003; Cheng & Sun, 2008). Regarding water activity, the majority of microorganisms grow at a_w values >0.90 (FSAI, 2005) and both commercial and experimental meals had a_w values in excess of this, making them suitable for bacteria growth.

In order for a food to bear a claim that it is low in sodium/salt, it must contain no more than 0.12g of sodium, (equivalent to 0.3g of salt, per 100g or per 100ml). To bear a claim of Very Low Sodium/Salt the product must contain no more than 0.04g of sodium (equivalent to 0.1g salt, per 100g or per 100ml) and in order to make a claim of Sodium-Free or Salt-Free the product must contain no more than 0.005g of sodium (equivalent to 0.0125g salt per 100g) according to EU Directive 80/777/EEC. Likewise in order for a product to claim that sodium/salt has been reduced there must be a 25% reduction difference compared with a similar product under the EU Directive 90/496/EEC (FSAI, 2007). In this study, the commercial regular salt meals contained ~1.75g –

2.0g salt per serving, providing up to 30% of recommended 6g daily salt intake. In the experimental meals salt was reduced substantially (50-66%) enabling experimental reduced salt chicken curry meal to be declared a low salt product while the experimental reduced salt chilli con carne or lasagne meals could bear a reduced salt claim.

In order to respond to calls for salt reduction in their products and to achieve ambitious salt reduction targets set by worldwide public health agencies the food industry urgently requires scientific data on the consequent effects of substantial salt reduction on food safety. This report attempts to provide such information, overall, it would appear that salt reduction of the order of 50% does not adversely impact on the microbiological quality of a range of ready meals. However, this study also highlights the short shelf life of thawed frozen ready meals even after the relatively severe manufacturing processes imposed on them. It is also clear from the data, of the requirement for adequate refrigerated storage conditions and the need for strict adherence to manufacturer's instructions regarding microwaving by the consumer.

References

Aktas, N., Aksu, M., & Kaya, M. (2003). The Influence of Marination with Different Salt Concentrations on the Tenderness, Water Holding Capacity and Bound Water Content of Beef. *Turkish Journal of Veterinary & Animal Sciences, 27*(5), 1207-1211,

Aziz, N. H., Mahrous, S. R., & Youssef, B. M. (2002). Effect of gamma-ray and microwave treatment on the shelf-life of beef products stored at 5 ºC. *Food Control, 13*(6-7), 437-444. http://dx.doi.org/10.1016/S0956-7135(01)00049-4

Becton Dickinson and Company. (2008). OF Medium with Carbohydrates. [Online] Available: http://www.bd.com/ds/technicalCenter/inserts/OF_Basal_Medium.pdf (02/10/2008)

Betts, G., Everis, L., & Betts, R. (2007). Microbial Issues in Reducing Salt in Food Products. In D. Kilcast & F. Angus (Eds.), *Reducing Salt in Foods: Practical Strategies,* pp. 174-200.

Blondeaux, A., Hamel, J. F., Widehem, P., & Cochet, N. (1999). Influence of water activity on the ice-nucleating activity of *Pseudomonas syringae*. *Journal of Industrial Microbiology & Biotechnology, 23*(6), 514-519. http://dx.doi.org/10.1038/sj.jim.2900758

Boziaris, I. S., Skandamis, P. N., Anastasiadi, M., & Nychas, G. J. E. (2007). Effect of NaCl and KCl on fate and growth/no growth interfaces of *Listeria monocytogenes* Scott A at different pH and nisin concentrations. *Journal of Applied Microbiology, 102*(3), 796-805.

Canumir, J. A., Celis, J. E., de Bruijn, J., & Vidal, L. V. (2002). Pasteurisation of apple juice by using microwaves. *Food Science and Technology, 35*(5), 389-392. http://dx.doi.org/10.1006/fstl.2001.0865

Carlin, C., Nguyen-The, C., & Abreu da Silva, A. (1995). Factors affecting the growth of *Listeria monocytogenes* on minimally processed fresh endive. *Journal of Applied Bacteriology, 78*, 636-646. http://dx.doi.org/10.1111/j.1365-2672.1995.tb03110.x

Carlin, F., & Nguyen-The, C. (1994). Fate of *Listeria monocytogenes* on Four Types of Minimally Processed Green Salads. *Letters in Applied Microbiology, 18*, 222-226. http://dx.doi.org/10.1111/j.1472-765X.1994.tb00852.x

Central Statistics Office, (2002). Annual Report. [Online] Available: http://www.oic.gov.ie/en/Publications/AnnualReports/2002/AnnualReport2002TextVersion/Name,2868,en.htm (25/06/2008)

Cheng, Q., & Sun, D. (2008). Factors Affecting the Water Holding Capacity of Red Meat Products: A Review of Recent Research Advances. *Critical Reviews in Food Science and Nutrition, 48*(2), 137-159. http://dx.doi.org/10.1080/10408390601177647

Clementi, F., & Rossi, J. (1984). Effect of Drying and Storage Conditions on Survival of *Leuconostoc oenos*. *American Journal of Enology and Viticulture, 35*(3), 183-186.

Collado, J., Fernández, A., Rodrigo, M., Camats, J., & Lopez, A. M. (2003). Kinetics of Deactivation of *Bacillus cereus* Spores. *Food Microbiology, 20*(5), 545-548. http://dx.doi.org/10.1016/S0740-0020(02)00158-2

Conner, D. E., Brackett, R. E., & Beuchat, L. R. (1986). Effect of temperature, sodium chloride, and pH on growth of *Listeria monocytogenes* in cabbage juice. *Appl. Environ. Microbiol., 52*(1), 59-63.

Consensus Action on Salt & Health, (CASH). (2008). National Salt Awareness Week 2008 - Salt and Children. [Online] Available: http://www.actiononsalt.org.uk/awareness/salt_awareness_week_2008.htm (15/01/2008)

Cousin, M. A., Jay, J. M., & Vasavada, P. C. (2001). Psychrotrophic Microorganisms. In F. P. Downes & K. Ito (Eds.), *Compendium of Methods for the Microbiological Examination of Foods,* pp. 159-160. Washington D.C.: American Public Health Society.

Davidson, M. P. (1997). Chemical Preservatives and Natural Antimicrobial Compounds. In M. P. Doyle, L. R. Beuchat & T. J. Montville (Eds.), *Food Microbiology: Fundamentals and Frontiers*, pp. 520-556. Washington DC: ASM Press.

Department Of Agriculture. (2003). Continued Success Predicted for Prepared Consumer Foods Strategy 2003-2006. [Online] Available: http://www.agriculture.gov.ie/index.jsp?file=pressrel/2003/123-2003.xml (02/02/2007)

Desmond, E. (2006). Reducing salt: A challenge for the meat industry. *Meat Science, 74*(1), 188-196. http://dx.doi.org/10.1016/j.meatsci.2006.04.014

Doyle, M. P., & Schoeni, J. L. (1984). Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. *Appl. Environ. Microbiol., 48*(4), 855-856.

Durack, E., Alonso-Gomez, M. M., & Wilkinson, M. G. (2011). Growth of *Escherichia coli* and *Staphylococcus aureus* in individual lasagne layers and evidence for migration of *E. coli* throughout the product. *Food Control, 22*(12), 2000-2005. http://dx.doi.org/10.1016/j.foodcont.2011.05.019

Durack, E., Alonso, M. M., & Wilkinson, M. G. (2008). Salt: A Review of its Role in Food Science and Public Health. *Current Nutrition and Food Science., Forthcoming*. http://dx.doi.org/10.2174/157340108786263702

Dykes, G. A., & Moorhead, S. M. (2001). Survival of Three *Salmonella* Serotypes on Beef Trimmings During Simulated Commerial Freezing and Frozen Storage. *Journal of Food Safety, 21*(2), 87-96. http://dx.doi.org/10.1111/j.1745-4565.2001.tb00309.x

Eom, H. -J., Seo, D. M., & Han, N. S. (2007). Selection of psychrotrophic *Leuconostoc* spp. producing highly active dextransucrase from lactate fermented vegetables. *International Journal of Food Microbiology, 117*(1), 61-67. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.02.027

Food Standards Agency, (2008). Salt: Is your food full of it? [Online] Available: http://www.salt.gov.uk/index.html (25/09/2008)

Francis, G. A., & Beirne, D. O. (1997). Effects of gas atmosphere, antimicrobial dip and temperature on the fate of *Listeria innocua* and *Listeria monocytogenes* on minimally processed lettuce. *International Journal of Food Science & Technology, 32*(2), 141-151. http://dx.doi.org/10.1046/j.1365-2621.1997.00390.x

FSAI. (2001). *Guidelines for the interpretation of results of microbiological analysis of some ready to eat foods sampled at point of sale.* (No. Guidance note No. 3).

FSAI. (2003). *Salt and Health: Review of the Scientific Evidence and Recommendations for Public Policy in Ireland*.

FSAI. (2005). *Determination of Product Shelf Life*.

FSAI. (2007). *General Labelling of Pre-Packaged Food*.

García-Gimeno, R. M., Hervás-Martínez, C., Rodríguez-Pérez, R., & Zurera-Cosano, G. (2005). Modelling the growth of *Leuconostoc mesenteroides* by Artificial Neural Networks. *International Journal of Food Microbiology, 105*(3), 317-332. http://dx.doi.org/10.1016/j.ijfoodmicro.2005.04.013

Greenwood, M., Willis, C., Doswell, P., Allen, G., & Pathak, K. (2005). Evaluation of chromogenic media for the detection of *Listeria* species in food. *Journal of Applied Microbiology, 99*(6), 1340-1345. http://dx.doi.org/10.1111/j.1365-2672.2005.02734.x

Guinee, T. P., & Fox, P. F. (2004). Salt in Cheese: Physical, Chemical and Biological Aspects. *Cheese: Chemistry, Physics and Microbiology: General Aspects* (Vol. 1, pp. 207-259). London: Elsevier.

Gurtler, J. B., & Beuchat, L. R. (2005). Performance of Media for Recovering Stressed Cells of *Enterobacter sakazakii* as Determined Using Spiral Plating and Ecometric Techniques. *Appl. Environ. Microbiol., 71*(12), 7661-7669. http://dx.doi.org/10.1128/aem.71.12.7661-7669.2005

Koujitani, E., Horisaka, T., Nomura, Y., Hara-Kudo, Y., Okatani, A. T., Iwata, T. et al. (2006). Immuno-Magnetic Separation and Agar Layer Methods for the Isolation of Freeze-Injured *Yersinia enterocolitica* O:8 from Water. *Journal of Veterinary Medical Science 68*(3), 195-199. http://dx.doi.org/10.1292/jvms.68.195

Lancette, G. A., & Bennett, R. W. (2001). *Staphylococcus aureus* and Staphylococcal Enterotoxins. In F. P. Downes & K. Ito (Eds.), *Compendium of Methods for the Microbiological Examination of Foods*, p. 387. Washington D.C.: American Public Health Society.

Leistner, L. (2000). Basic Aspects of Food Preservation by Hurdle Technology. *International Journal of Food Microbiology, 55*, 181-186, doi:10.1016/S0168-1605(00)00161-6, http://dx.doi.org/10.1016/S0168-1605(00)00161-6

Leistner, L., & Gorris, L. (1995). Food Preservation by Hurdle Technology. *Trends in Food Science & Technology, 6*, 41-46. http://dx.doi.org/10.1016/S0924-2244(00)88941-4.

Lekroengsin, S., Keeratipibul, S., & Trakoonlerswilai, K. (2007). Contamination Profile of *Listeria* spp. in Three Types of Ready-to-Eat Chicken Meat Products. *Journal of Food Protection, 70*(1), 85-89.

Liu, F., Guo, Y. Z., & Li, Y. F. (2006). Interactions of microorganisms during natural spoilage of pork at 5 ºC. *Journal of Food Engineering, 72*(1), 24-29. http://dx.doi.org/10.1016/j.jfoodeng.2004.11.015

McAteer, N. J., Grant, I. R., Patterson, M. F., the late Stevenson, H. M., & Weatherup, C. S. T. (1995). Effect of irradiation and chilled storage on the microbiological and sensory quality of a ready meal. *International Journal of Food Science & Technology, 30*, 757-771. http://dx.doi.org/10.1111/j.1365-2621.1995.tb01423.x

Nissen, H., J. T. Rosnes, Brendehau, J. G., & Kleiberg, G. H. (2002). Safety evaluation of sous vide-processed ready meals. *Letters in Applied Microbiology, 35*(5), 433-438. http://dx.doi.org/10.1046/j.1472-765X.2002.01218.x

O'Leary, E., Gormley, T. R., Butler, F., & Shilton, N. (2000). The Effect of Freeze-chilling on the quality of ready-meal components. *Lebensmittel-Wissenschaft und-Technologie, 33*, 217-224. http://dx.doi.org/10.1006/fstl.2000.0645

Pucciarelli, A. B., & Benassi, F. O. (2005). Inactivation of *Salmonella enteritidis* on raw poultry using microwave heating. *Brazilian Archives of Biology and Technology, 48*(6), 939-946. http://dx.doi.org/10.1590/S1516-89132005000800010

Raccach, M., Bamiro, T., Clinch, J., Combs, G., Gierczynski, A., & Karam, R. (2002). Frozen storage of *Escherichia coli* O157: H7 in vegetable broth. *Food Control, 13*(6-7), 381-385. http://dx.doi.org/10.1016/S0956-7135(02)00008-7

Rajmohan, S., Dodd, C. E. R., & Waites, W., M. (2002). Enzymes from isolates of *Pseudomonas fluorescens* involved in food spoilage. *Journal of Applied Microbiology, 93*, 205-213. http://dx.doi.org/10.1046/j.1365-2672.2002.01674.x

Redmond, G. A., Gormley, T. R., & Butler, F. (2005). Effect of short and long term frozen storage with MAP on the quality of freeze-chilled lasagne. *Lebensmittel-Wissenschaft und-Technologie, 38*(1), 81-87. http://dx.doi.org/10.1016/j.lwt.2004.03.012

Rosso, L., Bajard, S., Flandrois, J. P., Lahellec, C., Fournaud, J., & Veit, P. (1996). Differential growth of *Listeria monocytogenes* at 4 and 8 degrees C: Consequences for the shelf life of chilled products. *Journal of Food Protection, 59*(9), 944-949.

Scientific Advisory Committee on Nutrition. (2003). Salt and Health. [Online] Available: http://www.sacn.gov.uk/pdfs/sacn_salt_final.pdf (24/07/2007)

Swanson, K. M. J., Petran, R. L., & Hanlin, J. H. (2001). Culture Methods for Enumeration of Microorganisms. In F. P. Downes & K. Ito (Eds.), *Compendium of Methods for the Microbiological Examination of Foods* (pp. 56). Washington D.C.: American Public Health Society.

Uljas, H. E., & Ingham, S. C. (1999). Combinations of intervention treatments resulting in 5-log(10)-unit reductions in numbers of *Escherichia coli* O157 : H7 and *Salmonella typhimurium* DT104 organisms in apple cider. *Applied and Environmental Microbiology, 65*(5), 1924-1929.

Warseck, M., Ray, B., & Speck, M. L. (1973). Repair and Enumeration of Injured Coliforms in Frozen Foods. *Appl. Environ. Microbiol., 26*(6), 919-924.

Woo, I.-S., Rhee, I.-K., & Park, H.-D. (2000). Differential Damage in Bacterial Cells by Microwave Radiation on the Basis of Cell Wall Structure. *Appl Environ Microbiol, 66*(5), 2243-2247. http://dx.doi.org/10.1128/AEM.66.5.2243-2247.2000

Worsfold, D., & Griffith, C. (1997). Food Safety Behaviour in the home. *British Food Journal, 99/3*, 97-104. http://dx.doi.org/10.1108/00070709710168932

Table 1. Salt content, pH, a_w and moisture content of commercial regular salt and experimental low salt chilli con carne, chicken curry and lasagne. Values are means ±standard deviations

	Salt Content (%)		pH			Moisture		Water Activity	
	Regular	**Low salt**	**Regular**		**Low salt**	**Regular**	**Low salt**	**Regular**	**Low salt**
Chilli Con Carne	0.66 ± 0.06	0.20 ± 0.01	5.46	0.03	5.30 0.02	79.62 1.28	76.22 ±1.27	0.992 0.00	0.996 ± 0.00
Chicken Curry	1.13 ± 0.15	0.47 ± 0.02	5.84	0.02	5.49 0.03	74.54 0.30	78.01 ±0.42	0.990 0.00	0.996 ± 0.00
Meat Lasagne	1.01 ± 0.14	0.54 ± 0.02	5.58	0.09	5.37 0.09	84.60 0.15	66.09 ±0.49	0.984 0.00	0.977 ± 0.00

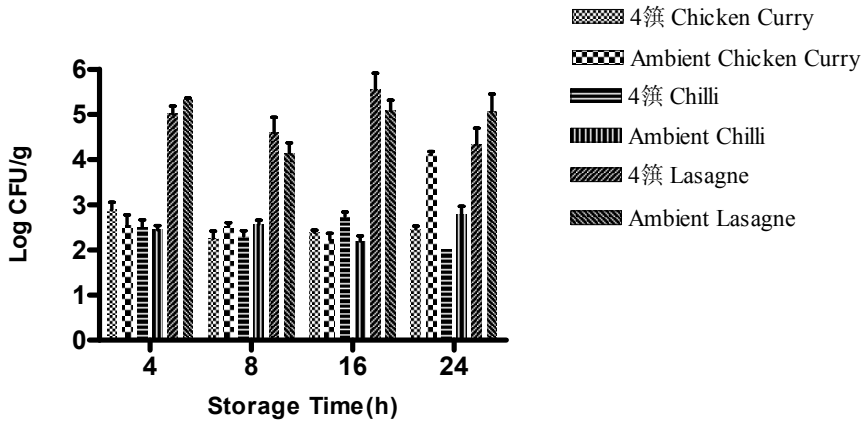

Figure 1. Total Mesophilic Aerobic Plate count in commercial regular salt chicken curry, chilli con carne, and lasagne meals when thawed over 24 hours at ambient temperature and 4°C. Values are means ± standard deviations

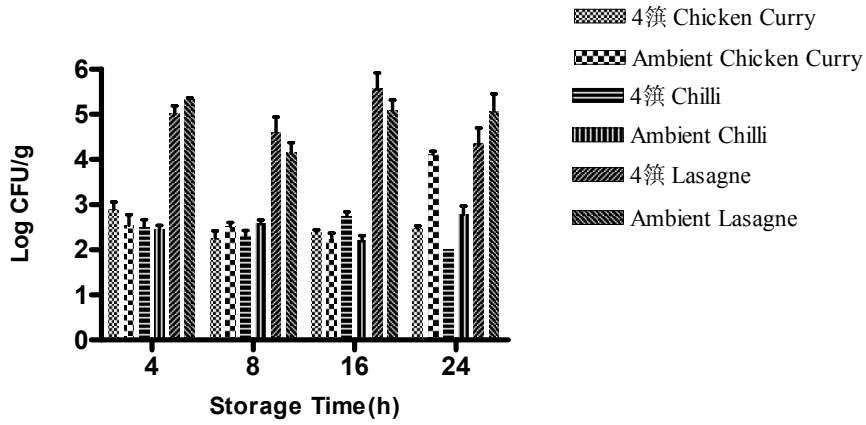

Figure 2. Total Staphylococci count in commercial regular salt chilli con carne meals when thawed over 24 hours at ambient temperature and 4°C storage. Values are means ± standard deviations

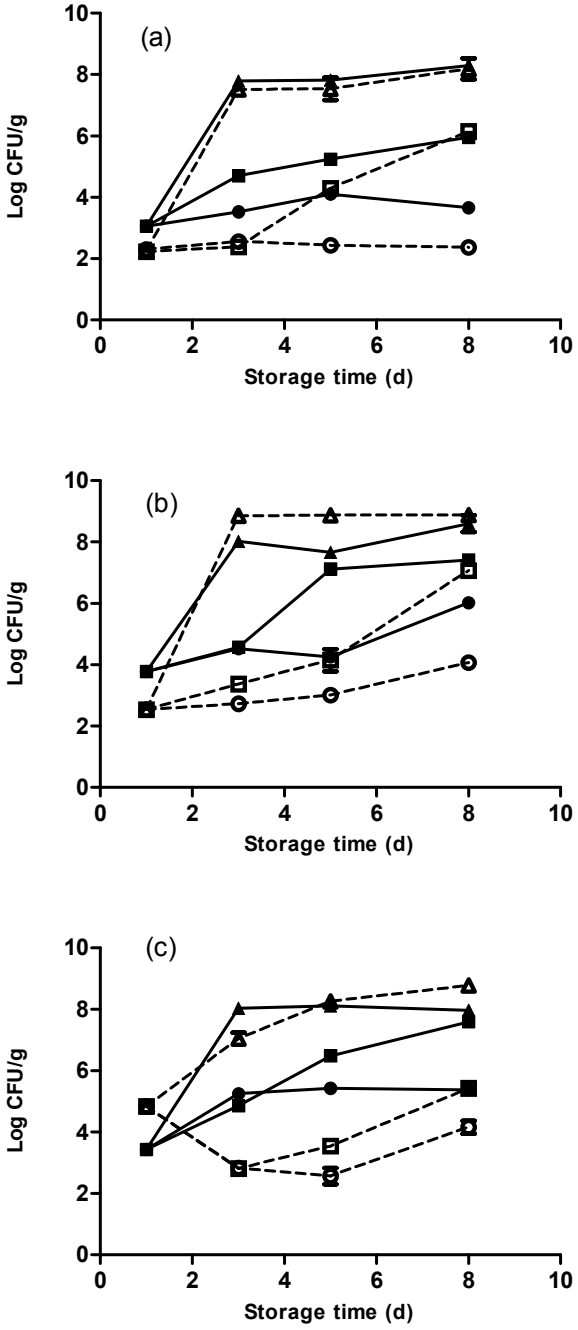

Figure 3. Total Mesophilic Aerobic Plate count in commercial regular salt (___) and experimental low salt (-----) (a) Chicken Curry (b) Chilli Con Carne and (c) Lasagne meals stored over 8 days at various temperatures; 4°C storage (● regular and ○low salt), 10°C (■ regular and □ low salt) or ambient temperature(▲regular and Δ low salt). Values are means ± standard deviations

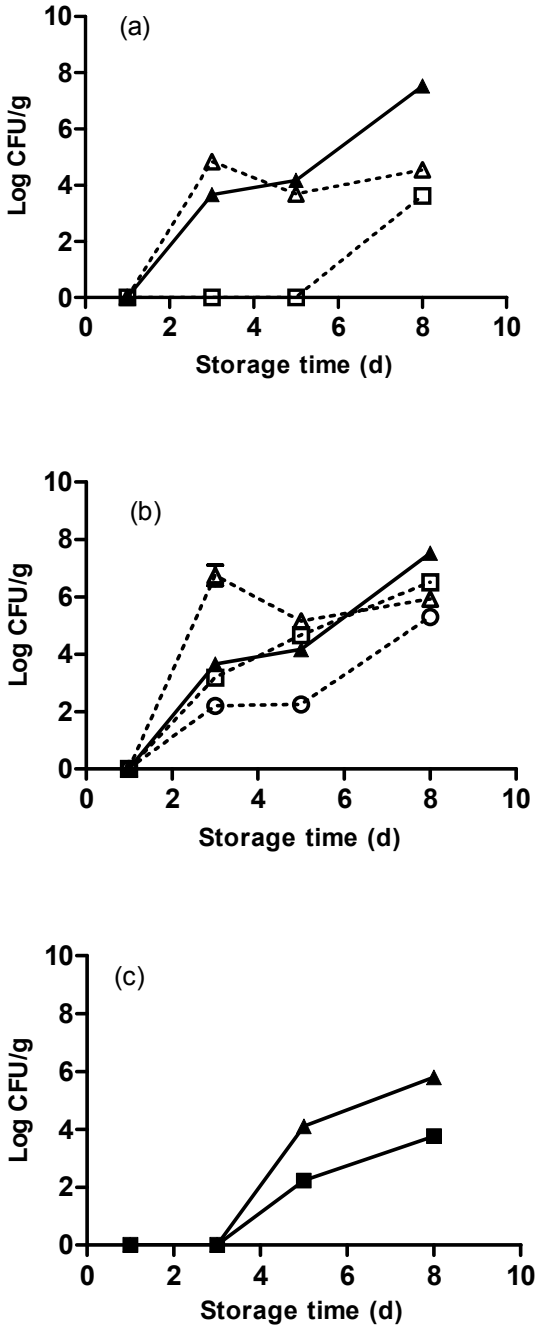

Figure 4. Total *Pseudomonas* count in commercial regular salt (____) and experimental low salt (----) (a) Chicken Curry (b) Chilli Con Carne and (c) Lasagne meals stored over 8 days at various temperatures; 4°C storage (● regular and ○low salt), 10°C (■ regular and □ low salt) or ambient temperature(▲regular and Δ low salt). Values are means ± standard deviations

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Nitrogen Supplementation on the Productivity and the Chemical Composition of Oyster Mushroom

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Abstract

Mushrooms have been used for food and medicinal purposes since ancient period. The β-glucans found in mushrooms are currently considered to be responsible for some of the medicinal properties of mushrooms, thereby motivating studies on characterization of these compounds. In our study, we tested the effect of nitrogen supplementation on the productivity and nutritional composition of *Pleurotus ostreatus* mushrooms. The fungi were grown in various substrates supplemented with urea or rice bran, and the biological efficiency, mineral composition, protein and β-glucan content were evaluated. The growth of *P. ostreatus* in substrates with nitrogen supplementation increases the mushroom's productivity and nutritional value. Our results also indicate that nitrogen supplementation, a simple technique, efficiently increases the β-glucan content of mushrooms.

Keywords: Rice bran, Urea, Biological Efficiency, Bioactive polysaccharides

1. Introduction

Mushrooms are becoming increasingly important and common in human diets, due to their nutritional (Barros, Cruz, Baptista, Estevinho & Ferreira, 2008; Bernaś, Jaworska & Lisiewska, 2006) and medicinal characteristics (Jedinak, Dudhgaonkar, Jiang, Sandusky & Sliva, 2010). The nutritional advantages of mushrooms include a low content of calories and a high content of proteins, minerals and dietary fiber (Beluhan & Ranogajec, 2011). As dietary food, mushrooms are comparable to vegetables. Also, mushrooms are a good source of protein, containing all of the essential amino acids, which allows mushrooms to serve as a meat substitute. Moreover, mushrooms have high vitamin B content and a low lipid content, which renders them nutritionally ideal for people who have heart problems (Ghorai, Banik, Verma, Chowdhury, Mukherjee & Khowala, 2009).

In the last decades, an increasing number of studies on mushrooms have been conducted, wich have been demonstrated the powerful properties of compounds extracted from mushrooms. Among these compounds, β-glucan have received considerable attention because of their medicinal properties, such as their ability to enhance macrophage functions, activate nonspecific immune responses, reduce cancer occurrences and developments (Jedinak et al., 2010; Wasser , 2011).

To improve the productivity and nutritional value of mushrooms several techniques, substrates, cultivation conditions and strains have been tested. Some studies show that supplementation with nitrogen source increase the biomass and mushroom's productivity (Curvetto, Figlas, Devalis & Delmastro, 2002; Buswell, Cai & Chang, 1995; Shashirekha, Rajarathnam & Bano, 2005). However, in the literature no studies related to increase β-glucans content in mushrooms was found. In this study we compare the addition of organic and inorganic nitrogen source on the productivity, β-glucan content, protein concentration and mineral composition of *P. ostreatus* mushrooms.

2. Material and Method

2.1 Microorganisms and inoculum production (spawn)

Pleurotus ostreatus strains (PLO 6 and PLO 2) were obtained from the Federal University of Viçosa and were maintained in Petri dishes containing potato dextrose agar (PDA) medium at 22 ºC. Wheat grains were used as a substrate for the spawn. The grains were cooked for 30 min in water at a 1:3 ratio of wheat grains:water (w/w).

After cooking, the grains were drained and supplemented with 0.35% $CaCO_3$ and 0.01% $CaSO_4$. These grains (70 g) were packed into small glass jars and were sterilized in an autoclave at 121 ºC for 90 min. After cooling, each jar was inoculated with 4 agar discs, 5 mm diameter, containing mycelium, and the jars were incubated in the dark, at room temperature, for 15 d.

2.2 Substrates and environmental conditions for mushroom production

The following substrates were obtained from local farms and were used for this study: eucalypt sawdust, corncobs, eucalypt bark, coffee husks and sugarcane bagasse (Table 1). Except for the control substrate, which lacked supplementation, all of the substrates were supplemented with 20% rice bran (w/w) or 0.5% urea (w/w). All of the substrates except the coffee husks were crushed and passed through a 0.5-mm sieve. The coffee husks were boiled for 2 h and were centrifuged, 1800 rpm x 5 min (Silva et al., 2012). The substrates were humidified until a moisture content of 70% was reached. The substrates were packed into polyethylene bags and were sterilized twice in an autoclave at 121 ºC for 90 min. After sterilization, each bag was inoculated using 70 g of spawn and was incubated in the dark at room temperature for 20 d.

2.3 Mushroom harvesting

After incubation period, the bags were transferred to a cultivation chamber at 25 ± 2 ºC and 90% relative air humidity in the presence of light throughout the entire harvesting period. The mushrooms were harvested at maximum development but with the hat closed. The mushrooms were weighed to determine their biological efficiency (BE), which was calculated with the following equation: BE = (fresh mass of mushroom / dry mass of substrate) x 100.

2.4 Nutritional composition

For mineral content determination, the dried mushrooms were triturated and submitted to nitroperchloric digestion (Tedesco, Gianello, Bissani, Bohnen, & Volkweiss; 1995). Phosphorus content was determined by a colorimetric method (Murphy & Riley, 1962). The mushrooms' calcium and magnesium contents were determined by atomic absorption spectrometry, and potassium content was measured by flame spectrometry (Thiers & Hviid, 1962). Total protein content was determined by the Kjeldahl method using a conversion factor of 4.38 (Guo, Lin & Lin, 2007). Soluble protein content was determined with a colorimetric method (Bradford, 1976) using bovine serum albumin as a standard. All analyses were performed in duplicate.

β-glucan content was performed in triplicate according to the methodology employed by Park, Ikegakim, Alencar, & Aguiar (2003). The concentration of β-glucan in the mushrooms was calculated with the following equation: β-glucan (g 100 g-1) = glucose (100 g-1) x 0.9. The correction factor of 0.9 takes into account the structural differences between free glucose and β-glucan.

2.5 Statistical analysis

The experiment was designed in completed and randomized blocks with five replicates. The data were subjected to analysis of variance and mean values were compared by Tukey's test ($p < 0.05$) using Saeg software (version 9.1, Federal University of Viçosa).

3. Results

Regardless of the substrate used for cultivation, supplementation with nitrogen increased the mushrooms' BEs (Figure 1). The highest BE was achieved with rice bran supplementation, especially when the mushrooms' substrates were based on sugarcane bagasse and eucalypt bark. When rice bran and urea supplementation were compared, the best results were yielded by rice bran supplementation, especially when sugarcane bagasse, corncobs and coffee husks were used as substrates (Figure 1).

The influence of nitrogen supplementation on the level of mineral absorption was directly related to the composition of the substrate (Table 2). The supplementation of substrates with rice bran affected positively the level of phosphorus absorption while urea supplementation affected negatively.

For most substrates, nitrogen supplementation did not affect the percentages of protein in the mushrooms (Table 3). However, urea supplementation decreases the protein content in both strains when they were cultivated in eucalypt sawdust. Furthermore, the protein content of PLO 2 strain grown in sugarcane bagasse supplemented with urea increased 33.60% compared to the control, and the protein content of the PLO 6 strain grown in sugarcane bagasse or eucalypt bark supplemented with rice bran decreased 32.77 and 19.05%, respectively, compared to the control (Table 3).

Urea supplementation increased the soluble protein content increased 8.72% compared to the control, and the β-glucan content increased 20.87% with rice bran and 17.65% with urea supplementations (Table 4).

4. Discussion

Nitrogen supplementation enhanced mushroom BE, especially when organic sources were used (Figure 1). The supplementation of the substrates with various sources of organic nitrogen, such as wheat bran, rice bran, maize wastewater, soya cake powder and rice, has increased the BEs of various species of basidiomycetes (Loss, Royer, Barreto-Rodrigues, & Barana, 2009, Moonmoon, Shelly, Khan, Uddin, Hossain, Tania, & Ahmed, 2011). Organic sources of nitrogen can be easily used by fungi because the absorption of these molecules is more energetically efficient than synthesizing the molecules, which allow the fungi to obtain more energy for mycelial growth and mushroom formation. The BE increase can be due to the high availability of water in substrate add with rice bran (Figure 1), since addition of rice bran decreases the granulometry of substrate, which improve the moisture retention (Özçelik & Peksen, 2007). For mushroom formation, the fungus requires a considerable amount of water, due to the high content of water in mushrooms (Tewari, 1986).

The BE values observed in our study (Figure 1) were similar to observed in other basidiomycetes, which ranges from 18.9 to 100% (Jafarpour, Zand, Dehdashtizadeh & Eghbalsaied, 2010; Loss, et al., 2009; Wang, Sakoda & Suzuki, 2001).

In general, supplementation of the substrates with rice bran did not affect the mineral content of the mushrooms (Table 2). However, the uptake of phosphorus increased for most substrates, which may be a result of the elevated levels of phosphorus in the rice bran (Özçelik & Peksen, 2007). Thus, the increase in the mushrooms' productivity did not impair the mineral composition of the mushrooms (Table 2 and Figure 1). Furthermore, addition of urea inhibits the assimilation of phosphorus, potassium and magnesium in some substrates (Table 2). Such downregulation has also been reported for the assimilation of Mg and K in plants (Khan, Watanabe, & Watanabe, 2000). Urea may act as a chelator, decreasing the availability of minerals. Further, nitrogen supplementation did not affect calcium concentrations, which is in agreement with reports that calcium does not bioacumulated in mushrooms (Kalac, 2009).

The protein content of mushrooms depends on several factors, such as substrate chemical composition, pileus size, cultivation time and strain (Bernaś, et al., 2006). The mushroom protein contents that were found in this study (Table 3) are in agreement with the range of mushroom protein contents reported in the literature (Bernaś, et al., 2006; Papaspyridi, Katapodis, Gonou-Zagou, Kapsanaki-Gotsi & Christakopoulos, 2010; Tshinyangu & Hennebert, 1996) varying between 17 and 42.5%, dependent on the correction factor (4.38, 6.25 or 6.38). These values were influenced by chemical composition of substrate, which reinforces the necessity of selecting suitable substrates and, in some cases, suitable nitrogen supplementation (Table 3).

Similar to the protein content, soluble protein in fungi also depend on the chemical composition of the substrate and fungus strain, varying between 5 and 14 mg g-1 (Membrillo, Sánchez, Meneses, Favela & Loera, 2011; Paul, Singh, Tyagi, Singh & Dubey, 2010). The protein contents found in our study were slightly greater than the previously published values (Table 4). In both strains, urea supplementation increased the soluble protein content. These results were in keeping with theoretical predictions stating that increase in the availability of nitrogen may enable increase protein contents of plants, animals and fungi (Chandel, Banerjee, See, Meena, Sharma & Verulkar, 2010; Ferrise, Triossi, Stratonovitch, Bindi & Martre, 2010; Janicki, Holter & Hayes, 1985; Membrillo, Sánchez, Meneses, Favela & Loera, 2008).

The percentages of β-glucans found in this study were less (Table 3) than reported in the literature (Carbonero, Gracher, Smiderle, Rosado, Sassaki, Gorin, et al., 2006; Manzi & Pizzoferrato, 2000; Papaspyridi, et al., 2010), which range around 38%, 39% and 53% , in *P. ostreatus*, *P. eryngii* and *P. pulmunarius*, respectively. However, values lower than 30% have been found in *P. ostreatus* (Papaspyridi, et al., 2010). The low value observed in our study can be attributed to the physiological characteristic of our isolates.

Functional foods represent one of the most interesting areas of research and innovation in the food industry (Arias-Aranda & Romerosa-Mártinez, 2010). According to these authors, functional foods may help to prevent disease, reduce the risk of developing disease, or enhance health. Mushrooms represent an unlimited source of polysaccharides, mainly β-glucans, with antitumor and immunostimulating properties (Wasser, 2002). Thus, studies about β-glucans content in mushrooms are important, since these compounds are beneficial to health. Our results indicate that nitrogen supplementation, a simple technique, efficiently increases the β-glucan content in the mushrooms. So, *P. ostreatus* mushroom cultivated in substrate supplemented with nitrogen is a good food source containing protein, minerals and bioactive compound, such as β-glucans.

5. Conclusions

This study shows that the cultivation of *P. ostreatus* in substrates supplemented with nitrogen is a simple technique to increases the productivity and β- glucans content in mushrooms. The use of rice bran increases the productivity of mushrooms more than urea supplementation does.

Acknowledgments

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References

Arias-Aranda, D., & Romerosa-Martínez, M. M. (2010). Innovation in the functional foods industry in a peripheral region of the European Union: Andalusia (Spain). *Food Policy, 35*, 240-246. http://dx.doi.org/10.1016/j.foodpol.2009.12.008

Barros, L., Cruz, T., Baptista, P., Estevinho, L. M., & Ferreira, I. C. F. R. (2008). Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Food and Chemical Toxicology, 46*, 2742-2747. http://dx.doi.org/10.1016/j.fct.2008.04.030

Beluhan, S., & Ranogajec, A. (2011). Chemical composition and non-volatile components of Croatian wild edible mushrooms. *Food Chemistry, 124*, 1076-1082. http://dx.doi.org/10.1016/j.foodchem.2010.07.081

Bernaś, E., Jaworska, G., & Lisiewska, Z. (2006). Edible Mushrooms as a Source of Valuable Nutritive Constituents. *Acta Scientiarum Polonorum – Technologia Alimentaria, 1*, 5-20.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248-254. http://dx.doi.org/10.1016/0003-2697(76)90527-3

Buswell, J.; Cai, Y. J. & Chang, S.T. (1995). Effect of nutrient nitrogen and manganese or manganese peroxidase and laccase production by *Lentinula (Lentinus) edodes*. *Fems Microbiology Letter, 128*, 81-88. http://dx.doi.org/10.1016/0378-1097(95)00087-L

Carbonero, E. R., Gracher, A. H. P., Smiderle, F. R., Rosado, F. R., Sassaki, G. L., Gorin, P. A. J., & Iacomini, M. (2006). A [beta]-glucan from the fruit bodies of edible mushrooms *Pleurotus eryngii* and *Pleurotus ostreatoroseus*. *Carbohydrate Polymers, 66*, 252-257. http://dx.doi.org/10.1016/j.carbpol.2006.03.009

Chandel, G., Banerjee, S., See, S., Meena, R., Sharma, D. J., & Verulkar, S. B. (2010). Effects of different nitrogen fertilizer levels and native soil properties on rice grain Fe, Zn and protein contents. *Rice Science, 17*, 213-227. http://dx.doi.org/10.1016/S1672-6308(09)60020-2

Ferrise, R., Triossi, A., Stratonovitch, P., Bindi, 222 M., & Martre, P. (2010). Sowing date and nitrogen fertilisation effects on dry matter and nitrogen dynamics for durum wheat: An experimental and simulation study. *Field Crops Research, 117*, 245-257. http://dx.doi.org/10.1016/j.fcr.2010.03.010

Curvetto, N. R., Figlas, D., Devalis, R., & Delmastro S. (2002). Growth and productivity of different *Pleurotus ostreatus* strains on sunflower seed hulls supplemented with $N–NH_4^+$ and/or Mn(II). *Bioresource Technology, 84*, 171–176. http://dx.doi.org/10.1016/S0960-8524(02)00013-5

Ghorai, S., Banik, S. P., Verma, D., Chowdhury, S., Mukherjee, S., & Khowala, S. (2009). Fungal biotechnology in food and feed processing. *Food Research International, 42*, 577-587. http://dx.doi.org/10.1016/j.foodres.2009.02.019

Guo, L.-Q., Lin, J.-Y., & Lin, J.-F. (2007). Non-volatile components of several novel species of edible fungi in China. *Food Chemistry, 100*, 643-649. http://dx.doi.org/10.1016/j.foodchem.2005.09.087

Jafarpour, M., Zand, A. J., Dehdashtizadeh, B., & Eghbalsaied, S. (2010). Evaluation of agricultural wastes and food supplements usage on growth characteristics of *Pleurotus ostreatus. African Journal of Agricultural Research, 5*, 3291-3296.

Janicki, F. J., Holter, J. B., & Hayes, H. H. (1985). Varying protein content and nitrogensolubility for *Pluriparous*, Lactating Holstein Cows: Digestive performance duringearly lactation. *Journal of Dairy Science, 68*, 1995-2008. http://dx.doi.org/10.3168/jds.S0022-0302(85)81061-4

Jedinak, A., Dudhgaonkar, S., Jiang, J., Sandusky, G., & Sliva, D. (2010). *Pleurotus ostreatus* inhibits colitis-related colon carcinogenesis in mice. *International Journal of Molecular Medicine, 26*, 643-650. http://dx.doi.org/10.3892/ijmm_00000509

Kalac, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry, 113*, 9-16. http://dx.doi.org/10.1016/j.foodchem.2008.07.077

Khan, N. K., Watanabe, M., & Watanabe, Y. (2000). Effect of partial urea application on nutrient absorption by hydroponically grown *Spinach* (*Spinacia oleracea* L.). *Soil Science Plant Nutrition, 46*, 199-208.

Loss, E., Royer, A. R., Barreto-Rodrigues, M., & Barana, A. C. (2009). Use of maize wastewater for the cultivation of the *Pleurotus* spp. mushroom and optimization of its biological efficiency. *Journal of Hazardous Materials, 166*, 1522-1525. http://dx.doi.org/10.1016/j.jhazmat.2008.11.014

Manzi, P., & Pizzoferrato, L. (2000). Beta-glucans in edible mushrooms. *Food Chemistry, 68*, 315-318. http://dx.doi.org/10.1016/S0308-8146(99)00197-1

Membrillo, I., Sánchez, C., Meneses, M., Favela, E., & Loera, O. (2008). Effect of substrate particle size and additional nitrogen source on production of lignocellulolytic enzymes by *Pleurotus ostreatus* strains. *Bioresource Technology, 99*, 7842-7847. http://dx.doi.org/10.1016/j.biortech.2008.01.083

Membrillo, I., Sánchez, C., Meneses, M., Favela, E., & Loera, O. (2011). Particle geometry affects differentially substrate composition and enzyme profiles by *Pleurotus ostreatus* growing on sugar cane bagasse. *Bioresource Technology, 102*, 1581-1586. http://dx.doi.org/10.1016/j.biortech.2010.08.091,

Moonmoon, M., Shelly, N. J., Khan, M. A., Uddin, M. N., Hossain, K., Tania, M., & Ahmed, S. (2011). Effects of different levels of wheat bran, rice bran and maize powder supplementation with saw dust on the production of shiitake mushroom (*Lentinus edodes* (Berk.) Singer). *Saudi Journal of Biological Sciences, 18*, 323-328 . http://dx.doi.org/10.1016/j.sjbs.2010.12.008

Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta, 27*, 31-36. http://dx.doi.org/10.1016/S0003-2670(00)88444-5

Özçelik, E., & Peksen, A. (2007). Hazelnut husk as a substrate for the cultivation of shiitake mushroom (*Lentinula edodes*). *Bioresource Technology, 98*, 2652-2658. http://dx.doi.org/10.1016/j.biortech.2006.09.020

Papaspyridi, L.-M., Katapodis, P., 266 Gonou-Zagou, Z., Kapsanaki-Gotsi, E., & Christakopoulos, P. (2010). Optimization of biomass production with enhanced glucan and dietary fibres content by *Pleurotus ostreatus* ATHUM 4438 under submerged culture. *Biochemical Engineering Journal, 50*, 131-138. http://dx.doi.org/10.1016/j.bej.2010.04.008

Park, Y. K., Ikegakim, M., Alencar, S. M., & Aguiar, C. L. (2003). Determination of β- glucan concentration in *Agaricus blazei Murill* mushroom by enzymatic method. *Ciência e Tecnologia de Alimentos, 23*, 312-316. http://dx.doi.org/10.1590/S0101-20612003000300003

Paul, R., Singh, V., Tyagi, R., Singh, A., & Dubey, D. (2010). Micro-Elements work for the growth and total soluble protein production in *Aspergillus niger* at different concentrations. *Journal of Pure and Applied Microbiology, 4*, 293-296.

Shashirekha, M. N., Rajarathnam, S., & Bano, Z. (2005). Effects of supplementing rice straw growth substrate with cotton seed on the analytical characteristics of the mushroom, *Pleurotus florida* (Block & Tsao). *Food Chemistry, 92*, 255-259. http://dx.doi.org/10.1016/j.foodchem.2004.07.034

Silva, M. C. S., Naozuka, J., da Luz, J. M. R., de Assunção, L. S., Oliveira, P.V., Vanetti, M. C. D., Bazzolli, D. M. S., Kasuya, M. C. M. (2012). Enrichment of *Pleurotus ostreatus* mushrooms with selenium in coffee husks. *Food chemistry, 131*, 558-563. http://dx.doi.org/10.1016/j.foodchem.2011.09.023

Tedesco, M. J., Gianello, C., Bissani, C. A., Bohnen, H., & Volkweiss, S. J. (1995). *Análises de solo, plantas e outros materiais* (2th ed.). Porto Alegre: Departamento de Solos, UFRGS.

Tewari, R. P. (1986). Mushroom cultivation. Extension Bulletin. *Indian Institute of Horticulture Research, 8*, 36.

Thiers, R. E., & Hviid, K. (1962). Interference--free flame photometry of Calcium in serum and urine. *Clinical Chemistry, 8*, 35-46.

Tshinyangu, K. K., & Hennebert, G. L. (1996). Protein and chitin nitrogen contents and protein content in *Pleurotus ostreatus* var. columbinus. *Food Chemistry, 57*, 223-227. http://dx.doi.org/10.1016/0308-8146(95)00202-2

Wang, D., Sakoda, A., & Suzuki, M. (2001). Biological efficiency and nutritional value of *Pleurotus ostreatus* cultivated on spent beer grain. *Bioresource Technology, 78*, 293-300. http://dx.doi.org/10.1016/S0960-8524(01)00002-5

Wasser, S. P., (2002) medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiology and Biotechnology, 60*, 258-274. http://dx.doi.org/10.1007/s00253-002-1076-7

Wasser, S. (2011). Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. *Applied Microbiology and Biotechnology, 89*, 1323-1332. http://dx.doi.org/10.1007/s00253-010-3067-4

Table 1. Materials used as substrates for the production of *Pleurotus ostreatus* mushrooms

Agroindustrial residues (substrates)	
Es	Eucalypt sawdust
EsRb	Es + Rice bran (20% w/w)
EsUr	Es + Urea (0.5 % w/w)
Co	Corncob
CoRb	Co + Rice bran (20% w/w)
CoUr	Co + Urea (0.5 % w/w)
Eb	Eucalypt bark
EbRb	Eb + Rice bran (20% w/w)
EbUr	Eb + Urea (0.5 % w/w)
Ch	Coffee husk
ChRb	Ch + Rice bran (20% w/w)
ChUr	Ch + Urea (0.5% w/w)
Sb	Sugarcane bagasse
SbRb	Sb + Rice bran (20% w/w)
SbUr	Sb + Urea (0.5% w/w)

Table 2. Mineral composition (as a percentage of dry mass) of *P. ostreatus* mushrooms grown in various substrates that were supplemented with rice bran or urea

Substrates*	Percentage of minerals in mushrooms								
	Magnesium**			Potassium					
	PLO 2 and PLO 6			PLO 2			PLO 6		
	Control	Rice bran	Urea	Control	Rice bran	Urea	Control	Rice bran	Urea
Es	0.17 ^{A}	0.16 ^{A}	0.14 ^{B}	2.63 ^{A}	2.23 ^{A}	1.86 ^{B}	2.42 ^{A}	2.41 ^{A}	1.39 ^{B}
Co	0.13 ^{A}	0.13 ^{A}	0.13 ^{A}	2.10 ^{A}	1.92 ^{B}	1.95 ^{A}	2.02 ^{A}	1.82 ^{A}	1.81 ^{A}
Eb	0.16 ^{A}	0.14 ^{B}	0.13 ^{B}	2.13 ^{A}	2.48 ^{A}	1.63 ^{B}	2.00 ^{AB}	2.07 ^{A}	1.75 ^{B}
Ch	0.13 ^{B}	0.15 ^{A}	0.11 ^{B}	2.27 ^{AB}	2.34 ^{A}	2.14 ^{B}	2.23 ^{A}	2.42 ^{A}	2.15 ^{A}
Sb	0.17 ^{A}	0.16 ^{A}	0.16 ^{A}	2.48 ^{A}	2.24 ^{A}	2.43 ^{A}	2.70 ^{A}	2.14 ^{B}	2.36 ^{B}
	Phosphorus**			Calcium***					
Es	1.38 ^{A}	1.14 ^{B}	0.91 ^{C}	0.0159					
Co	0.82 ^{B}	1.50 ^{A}	0.58 ^{C}	0.0043					
Eb	1.00 ^{B}	1.25 ^{A}	0.65 ^{C}	0.0131					
Ch	0.64 ^{B}	1.50 ^{A}	0.57 ^{B}	0.0056					
Sb	1.64 ^{A}	1.61 ^{A}	1.32 ^{B}	0.0054					

Means followed by different letters within the same line differ at Tukey's test ($P<0.05$).

* See table 1, ** Not significantly different between the strains ($P< 0.05$), *** Not significantly different between the strains and nitrogen supplementation ($P< 0.05$). Control = without nitrogen supplementation.

Table 3. Total protein (as a percentage of dry weight) of *P. ostreatus* mushrooms grown in various substrates that were supplemented with rice bran or urea

Substrates*	Total protein**					
	PLO2			PLO6		
	Control	Rice bran	Urea	Control	Rice bran	Urea
Es	28.60 ^{A}	22.86 ^{B}	22.56 ^{B}	27.38 ^{A}	27.68 ^{A}	18.75 ^{B}
Co	20.28 ^{A}	20.45 ^{A}	17.48 ^{A}	20.02 ^{A}	21.81 ^{A}	20.63 ^{A}
Eb	19.40 ^{A}	20.98 ^{A}	19.80 ^{A}	21.16 ^{A}	17.13 ^{B}	21.64 ^{A}
Ch	23.35 ^{A}	21.90 ^{A}	25.32 ^{A}	23.83 ^{A}	21.81 ^{A}	25.40 ^{A}
Sb	22.29 ^{B}	20.15 ^{B}	29.78 ^{A}	27.16 ^{A}	18.26 ^{B}	26.50 ^{A}

Means followed by different letters within the same line differ at Tukey's test ($P<0.05$).

Control = without nitrogen supplementation. * See table 1, ** Nitrogen correction factor used = 4.38.

Table 4. Soluble protein (mg g^{-1} dry weight) and β-glucan (percentage of dry weight) concentrations in *P. ostreatus* mushrooms grown in various substrates that were supplemented with rice bran or urea

Nitrogen supplementation	Soluble protein*	β-glucans*
Control	8.558 ^{B}	22.99 ^{B}
Rice Bran	8.419 ^{B}	27.79 ^{A}
Urea	9.305 ^{A}	27.05 ^{A}

Means followed by different letters within the same line differ at Tukey's test ($P<0.05$).

* Not significantly different between the strains and substrates ($P < 0.05$).

Control= without supplementation.

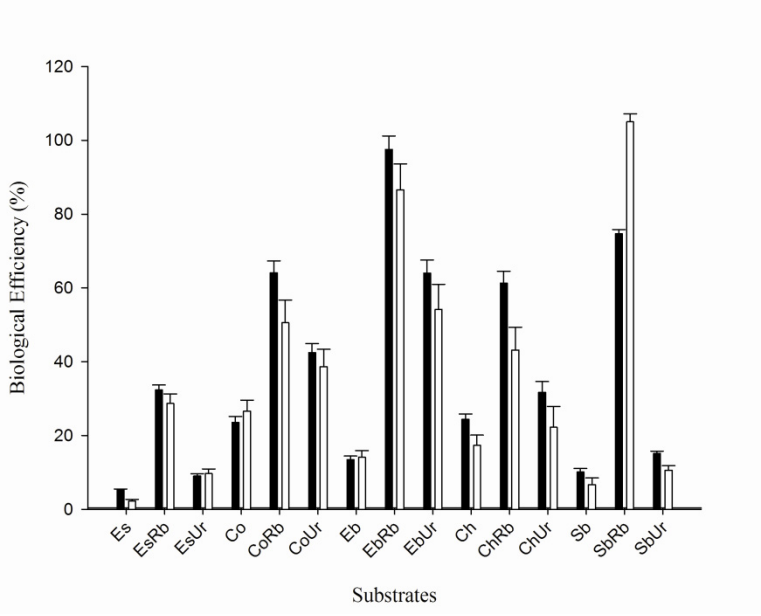

Figure 1. Biological efficiency of *P. ostreatus* (PLO 2 strain in black and PLO 6 strain in white) strains grown in different substrates supplemented with rice bran or urea. The following abbreviations are used: Es, eucalypt sawdust; Co, corncob; Eb, eucalypt bark; Ch, coffee husk; Sb, sugarcane bagasse; Rb, rice bran supplementation; and Ur, urea supplementation. These data represent the means ± sd (n = 5)

Food Security, Conservation Agriculture and Pulses: *Evidence from Smallholder Farmers in Zambia*

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Abstract

Food insecurity is common place among 44% (six million) of Zambian population. Conservation agriculture (CA) is an option being promoted to address this problem. There is little evidence showing whether CA adopters are better than non-CA adopters in terms of food security. Using a four years panel data, focus group discussions, key informant interviews, informal discussions and personal observations, this study documents the differences in household food security between CA adopters and non-CA adopters in relation to pulses. Results showed that most common pulses grown among smallholder farmers were groundnuts, cowpeas, soya beans and other beans. A tendency for the percentage of households growing pulses to be significantly higher among CA-adopters than among non-CA adopters was recorded. Cash income from pulses as percentage of total pulses production was significantly higher among CA adopters than among non-CA adopter in all the four years. Similar results were obtained for crop diversity and mean number of meals with pulses eaten in a day. Cases of women increasing their cash income from pulses because of CA practices were also reported. Focus group discussants explained that CA had reduced the intensity of food shortage during the peak hunger period because of early green harvest. With reference to pulses, it is concluded from this study that, among sampled smallholder farmers, CA adopters are relatively more food secure than non-CA adopters. Factors contributing to increased food security included farmer trainings in CA, increased access to planting seed, early land preparation and planting, and revitalisation of the practice of crop rotation.

Keywords: Livelihood security, Climate change, Gender, Food security, Climate smart agriculture

1. Introduction

Food insecurity is a day to day experience for hundreds of millions of people in the world. About 13 % of the world population are undernourished (FAOSTAT, 2011). This means about one billion people continuously experience food deprivation. The hot spots of food insecurity are in South Asia and Sub-Saharan Africa marked by favourable progress in Asia but sluggish progress in Sub-Saharan Africa (Wiesmann, 2006). Percentage of undernourishment is higher in Sub-Saharan Africa (35% and above) compared to most regions in the world (UN, 2011). Zambia is among countries in Sub-Saharan Africa with highest levels of undernourishment. About 44 % of the Zambian population experience food deprivation (FAOSTAT, 2011; UNPFA, 2011). The country has recorded almost no progress towards meeting the millennium development goal of reducing hunger. The proportion of undernourishment has increased from about 35% in the early 1990s to 38% (3 million) in the mid 1990s, 43% (3.6 million) in the early 2000 and 44% (6 million) towards 2011(FAO, 2010a).

The *state of food insecurity in the world* report defines food security as: "exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life" (FAO, 2010b:8). The key dimensions of food security are food availability, food access, food utilization and stability.

Food availability relates to physical presence of sufficient quantity and appropriate quality of food from own production, stocks, markets, food aid and communal resources (Siamwalla & Valdes, 1980; Ziervogel & Ericksen, 2010). Food availability at higher aggregated levels is a necessary but not sufficient condition for attaining food security at segregated levels (Devereux & Edwards, 2004; Renzaho & Mellor, 2010). This is because of limitation in physical, social, institutional and economic access to food by households and individuals.

The access dimension of food security includes sub components of physical access (ability to physically acquire food), economic access (ability to buy food) and social access (ability to acquire food through social capital) (World Food Programme, 2004). However, food access is not a guarantee for food security without addressing the food utilization dimension (Webb et al., 2006).

The dimension of food utilisation has two components: consumption and nutrition. The former refers to the quantity and quality of dietary intake while the latter refers to the extent to which the body is able to make best use of the nutrients from the consumed food in order to have an active and healthy life (Renzaho & Mellor, 2010; Riely, Mock, Cogill, Bailey, & Kenefick, 1999). Alternatively, food utilisation consists of ability to have physical means to use food available and ability of the body to use the nutrients effectively once the food is consumed (Renzaho & Mellor, 2010). The food culture, peoples' preferences, knowledge, assets, disease and sanitation are among cardinal elements of food utilisation dimension of food security concept.

The stability dimension addresses the "at all times" part of the food security definition in relation to the above three dimensions. This considers seasonality and vulnerability context of food security, thus need for stability in food availability, stability in access to adequate quantity and quality of preferred food and stability in food utilisation (Burchi, Fanzo & Frison, 2011). The stability dimension of food security requires asset creation and institutional building (Renzaho & Mellor, 2010).

1.1 Causes of food insecurity

Literature below shows that there are multiple layers of causes of food insecurity. No single factor is sufficient to explain adequately food insecurity situation in most of the developing countries. This section shows several factors that often interact to cause food insecurity in most developing countries.

1.1.1 Governance, structural, policy and institutional failures

von Braun (2010) contends that food insecurity is due to failures in governance of agriculture, food and nutrition. In order to address food insecurity there is need for the establishment of a global legitimate body to address the following: Research and innovation for improving agricultural productivity and ensuring food security; food emergencies through monitoring, responding to and preventing crises; health through improving food safety and setting health and nutrition standards; effective climate change adaptation and mitigation; prevention of excessive speculation in food markets and wild food price volatility; trade and investment by setting policies for trade of food reserves and standards for foreign investment that protect the poor; and promote environmental sustainability by protecting soils against degradation, promoting biodiversity and improving water use (von Braun, 2010).

Several structural and institutional failures contribute to food insecurity. These include increased inequalities in access to and control over resources; policy failures regarding smallholder producers; under-investment in agriculture, rural development and infrastructure; lack of consistency in effective operation of markets; and inadequate safety nets and social protection systems (High-Level Task Force on Global Food Security, 2010). Unhelpful or inconsistent government policies and programs also contribute towards food insecurity in most of the developing countries (Devereux, 2000; Tschirley & Jayne, 2010).

von Braun et al. (2012), point out that the lack of inter sectoral cooperation in generating solutions to the complex problem of food insecurity compromises efforts to enhance food security. In order to increase agricultural production and productivity, there is need for a healthy human capital and agriculture is a major source of valuable food needed for an active and health life (Asenso-Okyere, Chiang, Thangata & Andam, 2012). This literature suggests need for a collaborative involvement of the health sector, nutrition sector and agricultural sector in development interventions addressing food insecurity.

1.1.2 Gender gaps and poverty

Between 60 and 70% of the food in developing countries is produced by women yet they face more constraints than men in accessing technology, training and extension services, education, marketing and credit services (High-Level Task Force on Global Food Security, 2010). Quisumbing & Meinzen-Dick (2001) suggest that improving women's education is one of the single policy instruments that results into increased poverty

reduction by lowering child mortality and improving health, nutrition, and educational outcomes for children. Reducing gender gaps in agriculture could improve food security by 12 to 17% among hungry people (FAO, 2011d) because of a resulting 20 to 30% production increase from women's farms (FAO, IFAD & ILO, 2010). Empowering women by strengthening their asset (all forms of capital) and by providing legal and institutional measures to guarantee women's access and command over resources is key to achieving food security (Quisumbing & Meinzen-Dick, 2001). However, targeting women in isolation from men and the society in reducing gender gaps and food insecurity is insufficient (Quisumbing, Meinzen-Dick & Smith, 2004).

Devereux (2000) indicates that poverty and lack of a robust livelihood base were both a cause and consequence of food insecurity. Food insecurity leads to compromised human well-being at the same time poverty is the most prevalent underlying driver of food insecurity in Southern Africa (Misselhorn, 2005). Harrigan (2008) also points out that the poor are often exposed to food insecurity because they have fragile and limited entitlements and capabilities. Food insecurity in turn can reinforce poverty in terms of poor nutrition, poor health and erosion of assets (Harrigan, 2008). Poverty and food insecurity are intertwined.

1.1.3 Population, food losses, production and productivity failures

The state of world population 2011 report indicates that the world population has risen to seven billion (UNPFA, 2011). Population increase is perceived to be exacerbating food insecurity, poverty and threatening the environment (Premanandh, 2011). Long-term trends of slower growth in agricultural production and rapid growth in food demand also contribute to global food insecurity through a sharp downward trend in world aggregate food stocks and increase in food prices (Trostle, 2008).

Food losses contribute to food insecurity in various ways depending on commodities involved, production areas and seasons (Kader, 2005). Globally, about one third of food produced for human consumption is lost or wasted (FAO, 2011b). Food losses refer to reductions in both quality and quantity of food (Premanandh, 2011). Causes of food losses include premature harvesting; lack of effectiveness during mature harvesting; inadequacies in postharvest handling resulting in spillage and degradation; inadequacies in storage and processing; and diseases and pests (FAO, 2011b). This implies that reducing food losses could increase food security both at a global and household level.

Waddington et al. (2010) show that production constraints (abiotic, biotic, management and socio-economic) contribute to food insecurity through reduction in yields and crop productivity. Factors contributing to production constraints vary according to location, farming system, crops, socio-economic factors, and policy and institutional environments. Constraints to production and productivity are well documented and include limited access to quality seed, soil fertility depletion, insufficient access to agricultural information and training, limited access to effective and efficient equipment, weed competition, diseases and pests, limited access to financial services, limited economic access to chemical fertiliser, extreme weather events (climate change), poor crop rotations, inappropriate use of inputs, labour shortage, decreased public expenditure and investment in agriculture, and physical soil degradation (Hesselberg & Yaro, 2006; Maxwell, Webb, Coates & Wirth, 2010; Torero, 2011; Umar, Aune, Johnsen & Lungu, 2011).

1.2 Options for addressing food insecurity

Options for addressing household food insecurity are as many as their corresponding causes. These options include reducing losses and wastage, agro-forestry, hydroponics, transgenics, food loss prevention and control, policy reform and regulation, infrastructural development, change in dietary and consumption patterns, cash transfers and climate smart agriculture like conservation agriculture (FAO, 2011c; Popkin, Adair, & Ng, 2012; Premanandh, 2011; Sabates-Wheeler & Devereux, 2010).

1.2.1 Conservation agriculture (CA) and conservation agriculture project (CAP) in Zambia

Conservation agriculture (CA) is increasingly promoted as an option for addressing food insecurity (FAO, 2011c). CA is commonly defined as a set of agricultural practices with three interrelated core principles: minimum mechanical soil disturbance, permanent organic soil cover and diversified crop species in rotation or associations including legumes (FAO, 2011c; Govaerts et al., 2009; Hobbs, Sayre & Gupta, 2008). To this concept, Conservation Farming Unit (CFU) added planting of *Faidherbia albida* for soil fertility improvement and dry season early land preparation (CFU, 2011:78). There are two main variants of CA among smallholder farmers in Zambia: hand hoe based CA and animal draft powered (ADP) ripping.

Figure 1 shows a hand hoe based CA. Hand hoe based CA involves digging of planting basins (CA basins) spaced at 0.7 meters along the rows and 0.9 meters between rows using a *Chaka* hoe (CFU, 2009b). Crop residues and other vegetative matter are supposed to be retained on surface as permanent organic soil cover in

the area between basins. Recommended dimensions of a basin are 0.2 meters in depth, 0.3 meters in length and the same width as that of the blade of the *Chaka* hoe (CFU, 2009b). A *Chaka* hoe has an elongated thick strong blade and a long handle compared to a traditional hand hoe. These features of a *Chaka* hoe account for its heaviness (4 to 5 kg) relative to a traditional hoe. On the contrary, hand hoe based conventional agriculture involves tilling the whole field using a traditional hand hoe or making ridges resulting in maximum soil disturbance.

The animal draft powered CA (ADP ripping) shown in Figure 2 involves using a *Magoye* ripper instead of a conventional mould board plough. Instead of complete soil inversion as in conventional agriculture with a plough, famers practising ADP ripping (Figure 2) make at least 0.15-0.20 meters deep ripped furrows at 0.9 meters spacing in CA and retain crop residues and other vegetative matter between ripped lines (CFU, 2009a). Minimum tillage restricts soil disturbance to the precise area where the crop is sown resulting into a minimum soil disturbance of around 10% of the area in both CA basins and ADP CA ripping (FAO, 2011a).

Figure 3 shows a third variant of CA, tractor based form of CA. This mechanised kind of CA (Figure 3) is not common among smallholder farmers because of lack of capital, knowledge and access to the machinery.

Since the mid 1990s, the Conservation Farming Unit (CFU) of the Zambia National Farmers Union (ZNFU) has championed the training of both institutions and individual farmers on CA in the country. Conservation Agriculture Project (CAP) was a donor funded project implemented by CFU from 2007 to 2011 (CFU, 2006). CA is claimed to offer benefits of increased soil organic matter, improvements in water harvesting, reduction in the risk of crop failure, increased and stabilised yields, reduction in soil erosion, improvement in soil structure, reduced pests and diseases, reduced weed pressure, increased productivity and enhancing food security (Derpsch, Friedrich, Kassam, & Hongwen, 2010; Li et al., 2011; Marongwe et al., 2011). Despite these potential benefits, there is hardly a study in Zambia that has shown the differences between CA adopters and non-CA adopters in relation to food security from the context of pulses. Hence, the objective of this study was to document the differences in food security between CA adopters and non-CA adopters with reference to pulses. The following research questions were addressed:

1. What are the common pulses grown among smallholder farmers?
2. What are the differences in the spread of growing pulses between CA adopters and non-CA adopters over a period of four consecutive farming seasons?
3. Is there any association between practices of CA (minimum tillage and crop rotation) and access to a meal with pulses in a day?
4. How do smallholder farmers perceive the preference of various legumes promoted under CA project?
5. What are the differences in food security between CA adopters and non-CA adopters in terms of:
    a. Cash income from pulses as percentage of total pulses production?
    b. Crop diversity?
    c. Number of meals with pulses eaten in a day?
6. What are experiences of women regarding conservation agriculture and food security in relation to pulses?

2. Methods

Data was collected between June and October each year from 2007 to 2010. This period was chosen because most parts of the study areas were easily accessible by road at that time. Both qualitative and quantitative methods were used in collection and analysis of data.

2.1 Study area and sampling

Figure 4 shows the study areas.

The study areas were chosen because CAP was operating in these areas. In this study, a smallholder farmer is defined as households farming on less than 20 hectares (Haggblade & Tembo, 2003). CFU provided extension services to smallholder farmers in the study areas. Most farmers in the study areas practised mixed farming involving crops like maize, sweet potatoes, cassava, numerous kinds of beans, cowpeas, cotton, sorghum, millet, sweet stalks, pumpkins, water melons and cucumbers. Major types of livestock included cattle, goats, pigs and poultry. The agricultural system is mainly rain-fed and farmers seldom practice irrigation (Siegel & Jeffrey, 2005).

CAP had targeted 120,000 smallholder farmers to adopt CA from 2007 to 2011. Using updated CAP registers, 640 farmers were randomly sampled in 2007 for the questionnaire survey. Information was collected from the same households for four consecutive farming seasons 2006/2007 to 2009/2010. The sample size reduced from 640 to 535 in 2008, 486 in 2009 and 440 in 2010 due to deaths, migration, some respondents declining to be interviewed and others simply being absent at the time of the survey. Purposive sampling was used in the selection of key informants and focus group discussants in order to have participants who were known to have opinions and experiences on the topics for discussions.

2.2 Data collection and analysis

Questionnaires, focus group discussions, key informant interviews, informal discussions, direct observation, and review of literature were used to collect data. The survey questionnaire was used to collect most of the data on quantitative aspects and the other methods collected mostly data on qualitative aspects.

In addressing the first research question, farmers were asked to name the types of crops that they had grown in a particular season. This data was analysed using percentages to show the common pulses grown among smallholder farmers.

On the second research question percentage of households engaged in growing pulses and a two sample proportional test were used. Data on area under pulses was also collected and a t-test was used to compare the means of CA adopters (indicated by having area under minimum tillage) to non-CA adopters. Mean number of trainings on CA attended in each year were also compared for CA adopters and non-CA adopters to add to the accounting of results.

For the third research question, firstly, the association between minimum tillage (CA principle) and access to a diet with pulses (food security indicator) and secondly association between crop rotation (CA principle) and access to a meal with pulses were done using chi-square test. A binary logistic regression was used to determine the direction of the association and odds ratio.

Content analysis of information from key informants and focus group discussants on their experiences and concerns raised regarding legumes promoted by the CAP was used to answer the fourth research question on food preference.

Cash income from pulses as percentage of total pulse production was used as an indicator of food security in answering the fifth research question. This indicator was chosen because most smallholder farmers primarily grow crops for home consumption and excess for sale. In this way, percentage of pulses cash income to total pulses production (at market price) is a proxy indicator for food adequacy. It is also a proxy indicator for economic access to other food security requirements. This assumption was checked by simple linear regressions with number of food secure months as a response variable and percentage of pulses cash income as an explanatory variable.

Crop diversity (number of crops grown each farming season) was also used as a proxy indicator for food security assuming a positive relationship between food security and crop diversity. This assumptions was also checked by simple linear regressions with number of food secure months as a response variable and crop diversity as an explanatory variable. Proxy indicators are mostly used to measure food security because no perfect single measure that captures all dimensions of food security concept has yet been found (FAO, 2002; Webb et al., 2006).

T-test was used to assess the differences between CA adopters and non-CA adopters in the means for share of pulse cash income and crop diversity. Results were also triangulated with the differences between CA adopters and non-CA adopters in mean the number of times a meal with pulses was eaten in a day.

Content analysis of information from focus group discussants, key informants, informal discussions and review of literature relating to women's experiences towards food security in relation to pulses was explored to address the sixth research question.

3. Results and Discussion

For succinct purposes the farming seasons 2006/2007, 2007/2008, 2008/2009 and 2009/2010 will be referred to as 2007, 2008, 2009 and 2010 in the presentation and discussion of results.

3.1 Common food legumes grown among smallholder farmers

Most common pulses grown among sampled smallholder farmers were groundnuts (*Arachis hypogaea),* cowpeas *(Vigna unguiculata),* other beans and soya beans *(Glycine max)* in the order of decreasing prevalence (Figure 5).

Odendo, Bationo & Kimani (2011) also report that groundnuts, cowpeas, soya beans and common beans (*Phaseolus vulgaris)* were the most important pulses in Sub-Saharan Africa.

As shown in Figure 5 it was common for farmers to grow more than one pulse. This is shown by the total percentage of more than 100 in each farming season. Other beans included diversified varieties of common beans (*Phaseolus vulgaris),* sesame (*Sesamum indicum)*, mbambara beans (*Vigna subterranean)* and pigeon peas (*Cajanus cajan).* These pulses were not among the prime food legumes promoted under CAP as pointed out by focus group discussant. Legumes promoted by CFU under CAP included groundnuts, cowpeas, soya beans, velvet beans (*Mucuna pruriens)*, guar (*Cyamopsis tetragonoloba)* and pigeon peas (CFU, 2006).

Figure 5 also shows an overall increase in the spread of growing pulses over the four farming seasons. This increase came from increased access to extension support through farmer trainings on CA and distribution of legume seeds (mostly cowpeas and groundnuts). The steady and near six fold increase in the spread of growing other beans suggests a positive effect of CFU's extension support to farmers. Key informants confirmed that farmer training on CA conducted under CAP emphasised, among others, on practising crop rotation that includes legumes. These results show that emphasis of the principle of practising crop rotation with legumes was being taken up by farmers to the extent that some farmers had started growing pulses that were not directly promoted by the project. Direct observation during an informal visit in two villages in Chibombo district in 2012 showed that it was more common to find legume fields than at the beginning of Conservation Agriculture Project (CAP). Some informal discussions indicated that men were increasingly getting involved in the process of growing legumes. However, there is need for further investigation on the dynamics of gender relation in line with conservation agriculture and pulses.

3.2 Extent of growing pulses among CA adopters and non-CA adopters

The variable, extent of growing pulses, was chosen as a proxy indicator of food security because it allowed analysis of the differences between CA adopters and non-CA adopters in two scale dimensions. The first dimension was the horizontal dimension (spread) measured as percentage of households engaged in growing pulses. The second dimension was a vertical dimension (intensity) of growing food legumes measured as area under legumes.

3.2.1 Spread of growing food legumes

In general, the percentage of households having area under legumes showed an increase from 69% in 2007 to 83% in 2008; 87% in 2009 and 95% in 2010. This is because of improved access to extension support and access to legumes seeds for planting. Results (Table 1) show, an overall tendency for the percentage of households engaged in growing pulses among CA adopters to be higher than among non-CA adopters.

The overall differences (all pulses) between CA adopters and non-CA adopters shown in Table 1 were highly significant in the first and fourth farming seasons but marginally significant in the third season. The lack of significance in the second farming season could be due to wide spread flooding that was reported by respondents.

A further analysis of differences between CA adopters and non-CA adopters in individual pulses also gave consistent of results. The spread of growing cowpeas had almost doubled for both CA adopters and non-CA adopters over the four farming seasons despite a decrease recorded in 2008. The spread of growing groundnuts had steadily increased by about 30% among CA adopters and about 40% among non-CA adopters. About a threefold steady increase in the spread of other pulses was recorded among CA adopters and a near ninefold steady increase for non-CA adopters. Marginal changes were noted in the case of soya beans. The results show that the spread in growing of pulses was not limited to crops promoted by CA project but extended also to pulses that were important for food security from smallholder farmers' perspective. The overall increase in households growing pulses over time for both CA adopters and non-CA adopters shows that non-CA adopters could have also benefited from CAP extension system. Key informants reported that trainings in CA were open to all farmers. Some non-CA adopters could also have accessed seeds from the CA project though the CA adopters were a priority group.

Cross checking with simple logistic regression with area under legumes as a binary response (1=having area under legumes and 0=having no area under legumes) and CA adoption as a binary explanatory variable, similar results were obtained. Positive coefficients: 0.45 (p-value=0.022) in 2007; 0.27 (p-value=0.236) in 2008; 0.49 (p-value=0.073) in 2009; and coefficient of 1.45 (p-value=0.005) in 2010 were obtained with 1.57, 1.31, 1.64, and 4.29 odds ratios respectively. These results mean that in each of the four farming seasons a CA adopter was more likely to grow legumes than non-CA adopter.

3.2.2 Area under food legumes

Area under legumes was used to assess the differences in intensity of growing pulses between CA adopters and non-CA adopters.

For both CA adopters and non-CA adopters, from 2007 to 2008 there was a reduction in mean area followed by a marginal increase in 2009 and a substantial increase in 2010 farming season. Results (Table 2) showed no statistical difference between CA adopters and non-CA adopters. These results indicate that a CA adopter and non-CA adopter generally grow pulses to a similar extent. These findings are coherent to the finding from an adoption study on the same farmers that indicated that practising crop rotation with legumes was a very common practice to both CA adopters and non-adopters (Nyanga, *in press*). Increased dependency and demand on the project as a source of free seed was noted as it was very common for farmers to complain of not having received enough or not having received any cowpea seeds and/or groundnut seeds from the CA project. These results indicate the need to have sustainable means of access to seed by smallholder farming communities. The substantial increase recorded in 2010 season (Table 2) mostly came from groundnuts and cowpeas (Figure 6).

Figure 6 shows the mean areas and corresponding standard error bars for households engaged in growing respective pulses. Results show significant difference between CA adopters and non-CA adopters in the mean area of other pulses in 2009 season. This could be due to distribution of legumes seeds especially pigeon peas mostly among CA adopters. A few CA adopters reported having got some pigeon peas from the project. Direct observation also showed that some key informants such as lead farmers had small areas under pigeon peas near homesteads in the last two seasons. Discussions with key informants indicated that pigeon pea may not be part of a common diet among smallholder farmers. This was also evident from some farmers who were asking such as: *Is this crop* (pigeon pea) *for food or not? How do you prepare it?* This was not the case for cowpeas.

Substantial reductions in mean area for most legumes for both CA adopters and non-CA adopters during 2008 farming season were recorded due to wide spread floods during that farming season. In the case of other pulses, stoppage in growing of velvet beans also contributed to the decrease recorded in 2008. CAP project staffs confirmed the wide distribution of velvet beans in 2007 season and getting complaints from farmers afterwards that the crop could not be easily cooked or sold. A female farmer reported that:

> *This bean (velvet beans) does not get cooked. It is very hard... needs a lot of water and firewood...The other problem is that there are no people to buy the crop.*

The above experience indicate that Velvet beans could increase the burden of women in terms of time spent cooking the pulse and also in terms of increased requirement for water and firewood collection. These results show that there is need to take the smallholder farmers' food preferences and food utilisation dimensions seriously in CA projects.

The mean area for cowpeas did not reduce but instead increased during the farming season with floods. This could have been due to increased access to seed and the growing of the crop twice within a single farming season. Most farmers reported that the variety of cowpeas that they had got from CAP was maturing much earlier than the conventional type. Cases of farmers growing cowpeas twice in a single farming season were also reported.

3.3 Association between CA practices and consumption of pulses

Smallholder farmers practising CA were also expected to have one third of their cultivated land under legumes (CFU, 2006). The underlying assumption was that promotion of growing of legumes in CA would translate to increased food security through increased consumption of pulses and cash income. Hence, it was necessary to assess the association, firstly, between minimum tillage and consumption of pulses and secondly, between crop rotation and pulses intake.

Using 24 hrs recalls, on whether a household had eaten a meal with pulses, results showed no significant association between CA principle of minimum tillage and intake of pulses in all the four years (Table 3).

A cross tabulation furthers showed significant association between crop rotation and intake of pulses for the last three seasons (Table 3). The lack of significant association during the first year of CAP implementation, 2007, is partly due to the emphasis of growing velvet beans a legume without common place in the diet of smallholder farmers and also sceptical behaviour of smallholder farmers towards new varieties of food legume seeds.

A binary regression with two categorical explanatory variables; CA adoption (where 1= CA adopter and 0= non-CA adopter) and crop rotation (1=yes and 0= no) was used to determine the direction and likelihood effect on food security. Food security was measured as a dichotomy response variable of access to a meal with pulses (1= accessed, 0= not accessed).

As shown in Table 4 there was positive and significant effect of crop rotation on likelihood of pulses intake for all the last three seasons. A smallholder household practising crop rotation had an odd about twice higher of having a diet with pulses than a smallholder household not practising crop rotation (assuming common values for other variables).

These results show that access to a diet with pulses is more influenced by the CA principle of crop rotation than the principle of minimum tillage. This suggests that farmers are growing legumes not only on fields under minimum tillage but under conventional tillage as well. Farmers could be using either conventional agriculture or conservation agriculture depending on the type of pulse grown.

Table 4 further shows that minimum tillage especially basins during a season with wide spread floods could have led to decreased likelihood of accessing a meal with pulses. This is indicated by the negative coefficient in 2008 a season with wide spread floods. The negative coefficient for crop rotation in 2007 season is indicative of a possible immediate negative effect of promoting velvet beans on the likelihood of a household having a meal with pulses because it could not be eaten nor sold.

3.4 Share of cash income to total pulses production

The differences between CA adopters and non-CA adopters in terms of food security with respect to pulses was also assessed using cash income from pulses as percentage of total pulses production. A simple linear regression showed a significant positive relationship between share of pulses cash income and food secure months (Table 5). The results in Table 5 imply that increase in pulses production increases household food security.

Figure 7 shows mean percentage of pulse cash income to total production with corresponding standard error bars. These results (Figure 7) show firstly, that CA adopters had a tendency to have a higher share of pulses cash income than non-CA adopters. Secondly, results show the negative effect of extreme weather event of flooding in 2008, a one third reduction in pulses cash income as percentage of total pulses produced. This implied increased food insecurity due to extreme weather event. Thirdly, the results indicate a steady two fold increase in the share of pulses cash income over the last three farming seasons. This implies that both CA adopters and non-adopters had similar pattern of improvements in food security vis-a-vis pulses, despite CA adopters having a relatively higher food security status than non-CA adopters. Fourthly, CA adopters and non-CA adopters consumed more than 50% of their pulses produced. This shows that food insecurity is still a huge challenge. This is because about 70% for a CA household and 80% for non-CA adopters of their total pulses produced is mostly shared between household food consumption, post harvest losses, seed for the next farming season, remittances and paying of loans.

3.5 Crop diversity

Crop diversity as number of crops grown was also used as an indicator of household food security. Firstly, this indicator was chosen because CA principle of crop rotation does not only stress on the need to involve legumes but also diversified crops. Secondly, the indicator was chosen because of the assumption of existence of a positive relationship between household food security and crop diversity. Crops grown among smallholder farmers included cassava, cotton, cowpeas, maize, soya beans, groundnuts, sunflower, sweet potatoes and other pulses. These nine crops were used in the assessment of crop diversity, in spite that marginal crops such as millet, sorghum, sun hemp and sweet stalks were also grown by sampled farmers. There was also an underestimation because crops like pumpkins, water melons and various types of cucumbers were grown mixed with the major crops. These results show that smallholder farmers have a diversified portfolio of crops that they grow. Regression results confirmed the assumption of a positive relationship between crop diversity and food security (Table 6).

Simple linear regression results (Table 6) show that an increase in crop diversity increases household food security among smallholder farmers, holding other factors constant.

In terms of differences in crop diversity between CA adopters and non-CA adopters, results (Figure 8) show a tendency for the mean number of crops grown to be higher among CA adopters than among non-CA adopters. These differences where significant ($p<0.001$ in 2007; $p=0.009$ in 2008; $P= 0.025$ in 2009; and marginally significant in 2010, $p=0,050$). These results are consistent with findings presented earlier supporting the argument that CA adopters have a tendency to be more food secure than non-CA adopters in relation to pulses. Results were triangulated with number of meals with pulses eaten last 24 hours (Figure 9). Results from 24 hours recall also showed a consistent tendency of CA adopters having relatively accessed more pulses in their diet than non-CA adopters.

3.6 Green harvest and women

Respondents in most focus group discussions noted that households that were practising CA started eating green harvest from CA plots much earlier than those that were not practicing minimum tillage. Discussion with smallholder farmers indicated that most households had CA basin fields often located near homesteads for strategic food security purposes. Management of such fields was often spearheaded by women. Farmers often urged that such fields were an important source of green harvest thus contributing to food security during the usual hunger peak period (October to April). One of the female respondents noted that:

> *The advantage of CA especially basins is that you start eating the crop from the field earlier than from crops under conventional agriculture...you can also plant this new variety of cow peas twice within one farming season. ...The type of cowpeas from CFU is nice because it does not have "mpengele"* (seeds that cannot get cooked).

Another farmer reported that early planting of pulses had offered her opportunity to earn some cash income during the peak food shortage period. This was largely dependent on timely access to output market and individual commitment. The farmer in her 60s explained that:

> *Previously* (before CAP project) *I was used to waiting for my groundnuts field to be ploughed after the family main maize field and sometimes after cotton fields* (mostly male domain crop) *had been planted. As a result, I used to plant late, often planting behind a plough such that germination was not as good as it is when I plant in CA basins. Now, I have* (additional) *benefits, I plant early in my small CA basin field and later plant my groundnuts on a large ploughed field. The fresh groundnuts* (planted early) *are very profitable when taken to town* (urban market) *during January and February*.

Cases of men getting more involved in growing legumes were also reported. Men often in informal discussions claimed that women were happy with conservation agriculture because of the emphasis of growing their legumes. These statements indicated that women had an extra voice, the CA trainings, which had influenced men to put relatively more priority on growing of pulses now than before CA project. Generally, legumes were perceived by farmers as a vital means for soil fertility improvement, important for food security purposes, source of fodder, important for reducing soil erosion and suppression of weeds when used as cover crops. Similar results have been reported by Odendo et al. (2011). Among women, pulses were an important source of income. A general perception of respondents was that women were more likely than men to spend their income from pulses on the family consistent with argument for improving food security through reducing gender gaps in agriculture (Momsen 2010).

It was a common view among smallholder farmers that early land preparation in CA plots enabled them to plant earlier than in conventional agricultural fields. Triangulation with results from questionnaires showed that land preparation on average started in the third week of August (21) for CA basins and CA ripping started 30 days later than basins. On the other hand, land preparation on average in conventional hand hoe plots started 68 days later than in CA basins. Similarly, conventional ploughing on average started 94 days later than in CA basins. Each of these mean dates was significantly different ($p<0.05$) from others. This supports the reports from farmers that land preparation in CA plots is done much earlier than in convention fields. However, the benefits of early land preparation in CA were dependent upon farmer's timely access to planting seed and rainfall distribution that particular season. There is need to investigate further on seasonality and the extent of contribution of CA to household food security through green harvest under varied rainfall distribution taking into account the intra household gender dimensions.

Both women and men expressed knowledge of the importance of pulses as a source of valuable proteins. However, women seemed to be more knowledgeable than men on the diverse ways of preparing pulses for consumption. It was pointed out that some women kept part of the pulses in order to exchange with maize during the frequent hunger peak period (October to April). One of the women explained that:

> *It is very profitable to exchange groundnuts with maize during the regular period with food shortage. A meda* (5 litre container of about 4 to 5 kg) *of groundnuts is exchanged for at least 20 litres* (20 kg) *tin of maize.*

The above results show that food security with respect to pulses is mediated by gender. However, storage of pulses was a challenge because most respondents pointed out that rats and other pests were contributing to post harvest losses.

3.7 Influential factors

An interaction of trainings in CA given to farmers and access to seed was a major reason for the differences between CA adopters and non-CA adopters in this study. Among CA-adopters, the percentage of farmers that attended at least one CA training in a year increased from 66% in the first year to 90% the following year followed by a slight decrease to 83% and 80% for the last two seasons. Among non-CA adopters, the percentage of farmers attending at least one CA training in a year has also increased from 37% in 2007 to 67% in 2008; 61% in 2009 and 56% in 2010.

The mean number of trainings attended by CA adopters was significantly higher than mean number of trainings attended by non-CA adopters during each of the four seasons (Figure 10). Simple linear regression of number of CA training on crop diversity also showed significant positive effect in all seasons, coefficient=0.09 and p-value 0.009 in 2007; coefficient=0.05 and p-value 0.033 in 2008; marginally significant in 2009 (coefficient =0.05, p-values=0.081) and significant in 2010 (coefficient=0.08, p-value 0.016). A correlation between the number of crops involved in rotation and number of CA trainings attended also indicated positive significant (p-values<0.05) relationship in each of the four seasons though the strength of the relationship was weak, not more than a correlation coefficient of 0.2 each year. Similar results were obtained with a simple linear regression of training on cash income from pulses as share of the total pulses production: coefficient=0.02, p-value=0.004 in 2007; in 2008 coefficient=0.01, p-value=0.014; in 2009 coefficient=0.01, p-value=0.306; and coefficient=0.01, p-value=0.015 in 2010. The R-square in each case was not more than 4% implying that other factors such as access to seed, household demography, assets, education and access to output markets could further explain the results in this study. Nonetheless, these results support the argument for the positive effect of CAP extensions system on household food security in relation to pulses. Other studies have also shown a positive and significant effect of CA trainings on adoption of CA practices among smallholder farmers in Zambia (Nyanga, *in press*).

4. Conclusion

This study has shown that the most common pulses grown among sampled smallholder farmers in Zambia were groundnuts, cowpeas, other beans and soya beans in the order of decreasing prevalence. Other beans included diversified varieties of common beans, sesame, mbambara beans and pigeon peas. An increase in percentage of households growing pulses over four farming seasons has also been shown. There was a tendency for the percentage of households growing pulses to be significantly higher among CA-adopters than among non-CA adopters. This is because of a combined effect of CA trainings and increased access to seed that was higher among CA adopters than among non-CA adopters. This study further showed significant association between CA principle of crop rotation and access to a meal with pulses for the last three years. The study has also shown that farmers preferred growing legumes that could be eaten and/or easily sold thus pulses familiar to smallholders' diet were preferred to alien pulses. It has also been shown in this study that cash income from pulses as percentage of total pulses production was higher among CA adopters than among non-CA adopter. Similar results were recorded in the case of crop diversity and mean number of meals with pulses eaten in a day. Cases of women increasing their cash income from pulses because of CA practices and reduction in the intensity of food shortage during the peak hunger period because of early green harvest from CA have also been reported in this study. With reference to pulses, it is concluded from this study that CA adopters are relatively more food secure than non-CA adopters among sampled smallholder farmers in Zambia. Factors contributing to increased food security included farmer trainings in CA, increased access to planting seed, early land preparation and planting made possible by practising minimum tillage, and revitalisation of the practice of crop rotation.

The following concerns are raised: Efforts to increase seed access should further be promoted. There must be political will by donors, national government and international development agencies to promote food legumes that are part of preferred diet instead of promotion of alien species among smallholder farmers. Building from existing and working systems could increase efficiency and effectiveness of CA projects in reducing food insecurity. Setting the CA development agenda right, not sacrificing the livelihood needs of smallholder farmers, is important for any meaningful reversal of household food insecurity through conservation agriculture. Investment in linking farmers to output markets must seriously be addressed if CA projects are to be of much more impact in reducing food insecurity than is currently. Pragmatic ways of reducing gender gaps among smallholder farmers have to be further sought and implemented in CA projects.

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References

Asenso-Okyere, K., Chiang, C., Thangata, P., & Andam, K. S. (2012). Two-Way Links between Health and Farm Labor. In S. Fan & R. Pandya-Lorch (Eds.), *Reshaping agriculture for nutrition and health,* pp. 121-127. Washington, DC: IFPRI.

Burchi, F., Fanzo, J., & Frison, E. (2011). The Role of Food and Nutrition System Approaches in Tackling Hidden Hunger. *International Journal of Environmental Research and Public Health, 8*(2), 358-373. http://dx.doi.org/10.3390/ijerph8020358

CFU. (2006). *Reversing Food Insecurity and Environmental Degradation in Zambia through Conservation Agriculture*. Lusaka: Conservation Farming Unit.

CFU. (2009a). *Conservation Farming & Conservation Agriculture Handbook for Ox Farmers in Agro-Ecological Regions I & II*. Lusaka: Conservation Farming Unit, (Chapter E).

CFU. (2009b). *Conservation Farming and Conservation Agriculture Hand Book for Hoe Farmers in Agro-Ecological Regions I & IIa-Flat Culture*. Lusaka: Conservation Farming Unit, (Chapter C).

CFU. (2011). *The practice of Conventional and Conservation Agriculture in Eastern and Southern Africa*. Lusaka: Conservation Farming Unit.

Derpsch, R., Friedrich, T., Kassam, A., & Hongwen, L. (2010). Current status of adoption of no-till farming in the world and some of its main benefits. *International Journal of Agricultural and Biological Engineering, 3*(1), 1-25. http://dx.doi.org/10.3965/j.issn.1934-6344.2010.01.001-025

Devereux, S. (2000). Food insecurity in Ethiopia *A discussion paper for DFID*. Brighton: IDS Sussex.

Devereux, S., & Edwards, J. (2004). Climate Change and Food Security. *IDS Bulletin, 35*(3), 22-30. http://dx.doi.org/10.1111/j.1759-5436.2004.tb00130.x

FAO. (2002). Measurement and assessment of food deprivation and under-nutrition *Proceedings of the International Scientific Symposium (June 26-28, 2002),* pp. 301-304. Rome, Italy: Rome: FAO.

FAO. (2010a). Country Profile: Food Security Indicators. Country: Zambia Retrieved 16. 04. 2012, http://www.fao.org/fileadmin/templates/ess/documents/food_security_statistics/country_profiles/eng/Zambia_E.pdf

FAO. (2010b). *The State of Food Insecurity in the World*. Rome: FAO.

FAO. (2011a). Climatic Risk Analysis in Conservation Agriculture in Varied Biophysical and Socio-economic Settings of Southern Africa *Network paper 03*. Rome: FAO.

FAO. (2011b). Global food losses and food waste: extent, causes and prevention. Rome: FAO.

FAO. (2011c). Socio-Economic Analysis of Conservation Agriculture in Southern Africa *Network No. 2*. Rome: FAO.

FAO. (2011d). *The State of Food and Agriculture. Women in Agriculture. Closing the gender gap for development*. Rome: FAO.

FAO, IFAD, & ILO. (2010). Gender dimensions of agricultural and rural employment: Differentiated pathways out of poverty. Status, trends and gaps. Rome: FAO, IFAD & ILO.

FAOSTAT. (2011). Prevalence of undernourishment in total population Retrieved 16. 04. 2011, from http://www.fao.org/economic/ess/ess-fs/fs-data/ess-fadata/en/

Govaerts, B., Verhulst, N., Castellanos-Navarrete, A., Sayre, K. D., Dixon, J., & Dendooven, L. (2009). Conservation Agriculture and Soil Carbon Sequestration: Between Myth and Farmer Reality. *Critical Reviews in Plant Science, 28*, 97-122. http://dx.doi.org/10.1080/07352680902776358

Haggblade, S., & Tembo, G. (2003). Conservation Farming in Zambia *EPTD Discussion paper no.108*: International Food Policy Research Institute.

Harrigan, J. (2008). Food insecurity, poverty and the Malawian Starter Pack: Fresh start or false start? *Food Policy, 33*(3), 237-249. http://dx.doi.org/10.1016/j.foodpol.2007.09.001

Hesselberg, J., & Yaro, J. A. (2006). An assessment of the extent and causes of food insecurity in northern Ghana using a livelihood vulnerability framework. *Geojournal, 67*(1), 41-55. http://dx.doi.org/doi:10.1007/s10708-006-9007-2

High-Level Task Force on Global Food Security. (2010). Food and nutrition security: Updated Comprehensive Framework for Action. Rome: FAO.

Hobbs, P. R., Sayre, K., & Gupta, R. (2008). The role of conservation agriculture in sustainable agriculture. *Philosophical transactions of the royal society, 363*, 543-555. http://dx.doi.org/10.1098/rstb.2007.2169

Kader, A. (2005). Increasing food availability by reducing postharvest losses of fresh produce. *Acta Hort, 682*, 2169-2175. http://www.actahort.org/books/682/682_296.htm

Li, L., Huang, G., Zhang, R., Bill, B., Guangdi, L., & Kwong, Y. C. (2011). Benefits of Conservation Agriculture on Soil and Water Conservation and Its Progress in China. *Agricultural Sciences in China, 10*(6), 850-859. http://dx.doi.org/10.1016/S1671-2927(11)60071-0

Marongwe, L. S., Kwazira, K., Jenrich, M., Thierfelder, C., Kassam, A., & Friedrich, T. (2011). An African success: the case of conservation agriculture in Zimbabwe. *International Journal of Agricultural Sustainability, 9*(1), 153-161. http://dx.doi.org/10.3763/ijas.2010.0556

Maxwell, D., Webb, P., Coates, J., & Wirth, J. (2010). Fit for purpose? Rethinking food security responses in protracted humanitarian crises. *Food Policy, 35*(2), 91-97. http://dx.doi.org/10.1016/j.foodpol.2009.10.002

Misselhorn, A. A. (2005). What drives food insecurity in southern Africa? a meta-analysis of household economy studies. *Global Environmental Change, 15*(1), 33-43. http://dx.doi.org/10.1016/j.gloenvcha.2004.11.003

Nyanga, P. H. (*In press*). Factors influencing adoption and area under conservation agriculture: A mixed methods approach. *Sustainable Agriculture Research, 1*(2), *in press*.

Odendo, M., Bationo, A., & Kimani, S. (2011). Socio-Economic Contribution of Legumes to Livelihoods in Sub-Saharan Africa In M. Odendo, A. Bationo, S. Kimani, A. Bationo, B. Waswa, J. M. Okeyo, F. Maina, J. Kihara & U. Mokwunye (Eds.), *Fighting Poverty in Sub-Saharan Africa: The Multiple Roles of Legumes in Integrated Soil Fertility Management* (pp. 27-46). Netherlands: Springer

Popkin, B. M., Adair, L. S., & Ng, S. W. (2012). Global nutrition transition and the pandemic of obesity in developing countries. *Nutrition Reviews, 70*(1), 3-21. http://dx.doi.org/10.1111/j.1753-4887.2011.00456.x

Premanandh, J. (2011). Factors affecting food security and contribution of modern technologies in food sustainability. *Journal of the Science of Food and Agriculture, 91*(15), 2707-2714. http://dx.doi.org/10.1002/jsfa.4666

Quisumbing, A. R., & Meinzen-Dick, R. S. (2001). Empowering women to achieve food security *Focus 6. Policy brief 1 of 12*. Washington, DC: IFPRI.

Quisumbing, A. R., Meinzen-Dick, R. S., & Smith, L. C. (2004). Increasing the effective participation of women in food and nutrition security in Africa *2020 Africa conference brief 4*. Washington, DC: IFPRI.

Renzaho, A. M. N., & Mellor, D. (2010). Food security measurement in cultural pluralism: Missing the point or conceptual misunderstanding? *Nutrition, 26*(1), 1-9. http://dx.doi.org/10.1016/j.nut.2009.05.001

Riely, F., Mock, N., Cogill, B., Bailey, L., & Kenefick, E. (1999). *Food Security Indicators and Framework for Use in the Monitoring and Evaluation of Food Aid Programs*. Washington DC: U.S. Agency for International Development.

Sabates-Wheeler, R., & Devereux, S. (2010). Cash transfers and high food prices: Explaining outcomes on Ethiopia's Productive Safety Net Programme. *Food Policy, 35*(4), 274-285. http://dx.doi.org/10.1016/j.foodpol.2010.01.001

Siamwalla, A., & Valdes, A. (1980). Food insecurity in developing countries. *Food Policy, 5*(no 4, November), 258-272.

Siegel, B. P., & Jeffrey, A. (2005). Poverty Reducing Potential of Smallholder Agriculture in Zambia: Opportunities and Constraints *Working paper Series No. 85*. Washington, DC: World Bank.

Torero, M. (2011). Alternative mechanisms to reduce food price volatility and price spikes *Science review: SR 21*. London: The Government Office for Science.

Trostle, R. (2008). Global Agricultural Supply and Demand: Factors Contributing to the Recent Increase in Food Commodity Prices *Outlook Report WRS-0801*. Washington, DC: ERS, USDA.

Tschirley, D. L., & Jayne, T. S. (2010). Exploring the Logic Behind Southern Africa's Food Crises. *World Development, 38*(1), 76-87. http://dx.doi.org/10.1016/j.worlddev.2009.09.008

Umar, B. B., Aune, J. B., Johnsen, F. H., & Lungu, O. I. (2011). Options for Improving Smallholder Conservation Agriculture in Zambia. *Journal of Agricultural Science 3*(3), 50-62. http://dx.doi.org/doi:10.5539/jas.v3n3p50

UN. (2011). The Millennium Development Goals Report 2011 (pp. 6-15 Goal 11). New York: UN.

UNPFA. (2011). *The State of World Population 2011: People and possibilities in a world of 7 billion*. New York: UNPFA.

von Braun, J. (2010). Strategic body needed to beat food crises. *Nature, 465*(7298), 548-549. http://dx.doi.org/doi:10.1038/465548a

von Braun, J., Ruel, M. T., & Gillespie, S. (2012). Bridging the Gap between the Agriculture and Health Sectors. In S. Fan & R. Pandya-Lorch (Eds.), *Reshaping Agriculture for Nutrition and Health* (pp. 183-190). Washington, DC: IFPRI.

Waddington, S., Li, X., Dixon, J., Hyman, G., & de Vicente, M. (2010). Getting the focus right: production constraints for six major food crops in Asian and African farming systems. *Food Security, 2*(1), 27-48. http://dx.doi.org/10.1007/s12571-010-0053-8

Webb, P., Coates, J., Frongillo, E. A., Rogers, B. L., Swindale, A., & Bilinsky, P. (2006). Measuring Household Food Insecurity: Why It's So Important and Yet So Difficult to Do. *The journal of nutrition, 136*(5), 1404S-1408S.

Wiesmann, D. (2006). A Global Hunger Index: Measurement Concept, Ranking of Countries, and Trends *FCND Discussion Paper 212*. Washington, DC: IFPRI.

World Food Programme. (2004). *Baseline Food Security Analysis in Iraq*. Rome: United Nations World Food Programme.

Ziervogel, G., & Ericksen, P. J. (2010). Adapting to climate change to sustain food security. *Wiley Interdisciplinary Reviews: Climate Change, 1*(4), 525-540. http://dx.doi.org/10.1002/wcc.56

Table 1. Spread of growing pulses by CA adoption over four seasons

	Farming season	2007	2008	2009	2010
Cowpeas	CA adopters, % (n)	28.6 (54)	20.1 (56)	30.2 (95)	51.8 (128)
	Non-CA adopters , % (n)	17.6 (72)	8.7 (22)	27.9 (46)	34.2 (41)
	Z-value	2.91	3.82	0.52	3.26
	p-value	0.004	0.000	0.600	0.001
Groundnuts	CA adopters, % (n)	67.6 (127)	80.2 (223)	81.9 (258)	88.8 (221)
	Non-CA adopters , % (n)	61.4 (251)	77.5 (196)	74.6 (123)	85.5 (100)
	Z-value	1.48	0.77	1.83	0.86
	p-value	0.139	0.439	0.074	0.390
Soya beans	CA adopters, % (n)	15.3 (29)	8.27 (23)	16.5 (52)	11.3 (28)
	Non-CA adopters , % (n)	8.3 (34)	7.11 (18)	8.5 (14)	12.8 (15)
	Z-value	2.39	0.50	2.66	-0.42
	p-value	0.017	0.616	0.008	0.678
Other pulses	CA adopters, % (n)	7.9 (15)	9.7 (27)	18.8 (59)	26.3 (65)
	Non-CA adopters , % (n)	3.4 (14)	6.7 (17)	10.3 (17)	26.1 (31)
	Z-value	2.10	1.26	2.62	0.05
	p-value	0.036	0.207	0.009	0.957
All pulses	CA adopters, % (n)	75.7 (143)	84.5 (235)	88.9 (280)	97.5 (234)
	Non-CA adopters , % (n)	66.5 (274)	80.6 (204)	83.0 (137)	90.1 (100)
	Z-value	2.35	1.18	1.71	2.46
	p-value	0.019	0.237	0.087	0.014

Table 2. Area (ha) under legumes by CA adoption

Farming season	2007		2008		2009		2010	
	Mean	Std error	Mean	Std error	Mean	Std error	Mean	Std error
CA adopters	0.36	0.05	0.32	0.02	0.34	0.02	0.54	0.03
Non-CA adopters	0.45	0.03	0.32	0.03	0.33	0.05	0.57	0.06
T-value	1.58		0.03		0.06		-0.52	
p-value	0.116		0.980		0.955		0.602	

Table 3. Association between CA practices (minimum tillage and crop rotation) and intake of pulses

CA practices	Pulses intake 2007 (n=639)		Pulses intake 2008 (n=534)		Pulses intake 2009 (n=478)		Pulses intake 2010 (n=371)	
	Yes	No	Yes	No	Yes	No	Yes	No
Used minimum tillage (%)	15.5	16.0	29.6	22.8	38.5	27.6	43.9	24.0
Minimum tillage not used (%)	29.8	38.8	26.6	20.9	17.1	16.8	18.3	13.9
Pearson Chi-square	1.735		0.015		2.738		2.112	
P-value	0.188		0.904		0.098		0.146	
Crop rotation practised (%)	42.2	47.3	44.9	30.9	44.1	29.7	56.3	32.4
Crop rotation not done (%)	6.7	5.8	11.1	13.1	11.5	14.6	5.4	5.9
Pearson Chi-square	0.002		7.261		9.306		3.989	
P-value	0.963		0.007		0.002		0.046	

Table 4. Binary regression results of the effect of minimum tillage (MT) and crop rotation (CRT) on access to a meal with pulses

Season	2007 (n=601)		2008 (n=530)		2009 (n=475)		2010 (n=367)	
	MT=1	CRT=1	MT=1	CRT=1	MT=1	CRT=1	MT=1	CRT=1
Coefficient	0.23	-0.16	-0.02	0.56	0.25	0.62	0.31	0.65
SE of coefficient	0.18	0.25	0.18	0.20	0.19	0.21	0.23	0.33
Odds ratio	1.26	0.85	0.98	1.75	1.28	1.87	1.37	1.92
P-value	0.184	0.526	0.908	0.006	0.206	0.003	0.172	0.048

Table 5. Estimates of effect of share pulses cash income on food secure months

Season	2007 (n=639)	2008 (n=528)	2009 (n=481)	2010 (n=412)
Coefficient	0.75	1.52	1.18	1.30
SE of coefficient	0.35	0.46	0.40	0.34
P-value	0.035	0.001	0.004	< 0.001

Table 6. Estimates of effect of crop diversity on number of food secure months

Season	2007 (n=639)	2008 (n=532)	2009 (n=481)	2010 (n=361)
Coefficient	0.23	0.39	0.41	0.15
SE of coefficient	0.08	0.10	0.09	0.07
P-value	0.004	<0.001	<0.001	0.045

Figure 1. Conservation agriculture basins in Zambia 2010

Figure 2. Animal draft powered conservation agriculture (ripping) in Zambia 2010

Figure 3. Tractor based conservation agriculture in Zambia 2010

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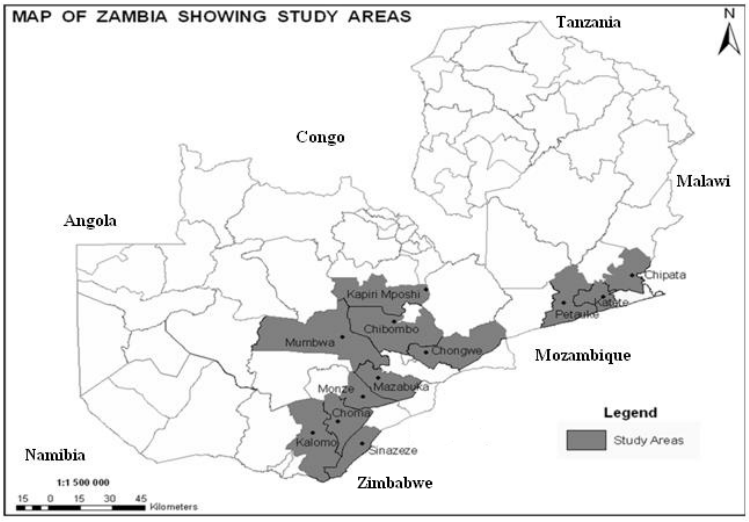

Figure 4. Map of Zambia showing study areas

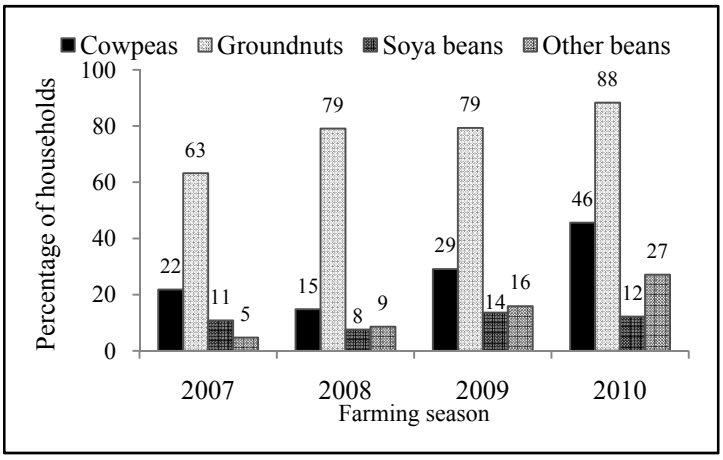

Figure 5. Common pulses grown by farming season

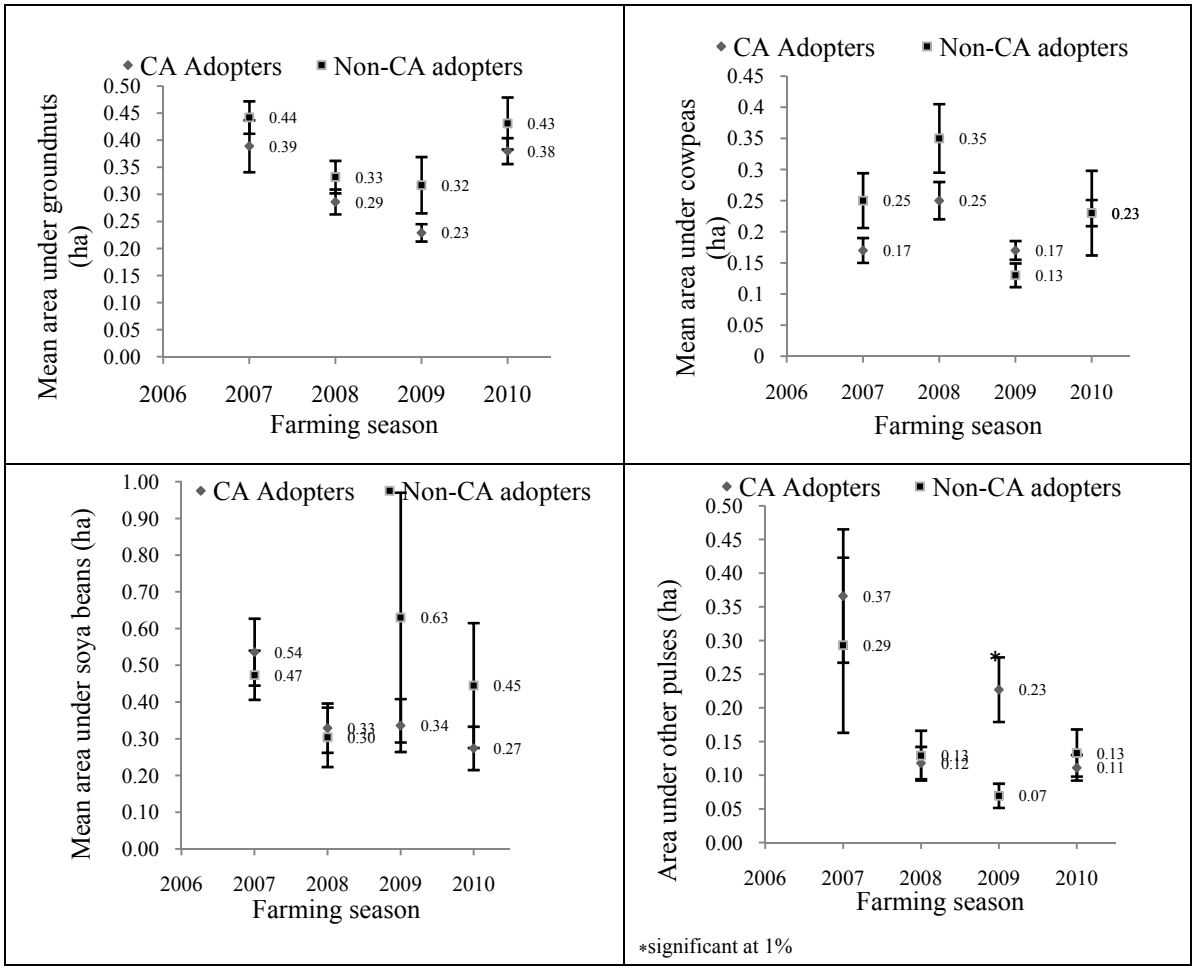

Figure 6. Area under respective pulses by CA adoption

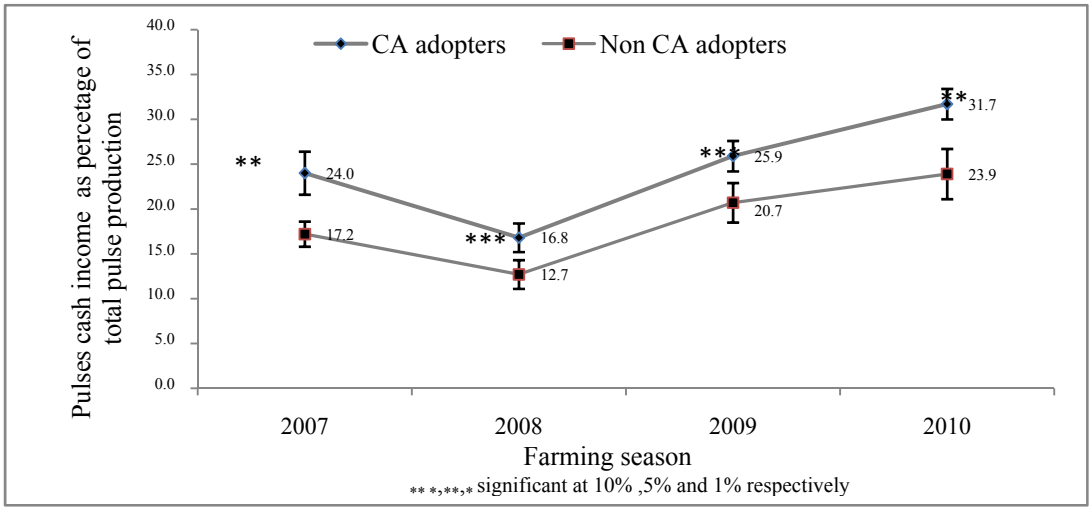

Figure 7. Pulses cash income as percentage of total pulse production

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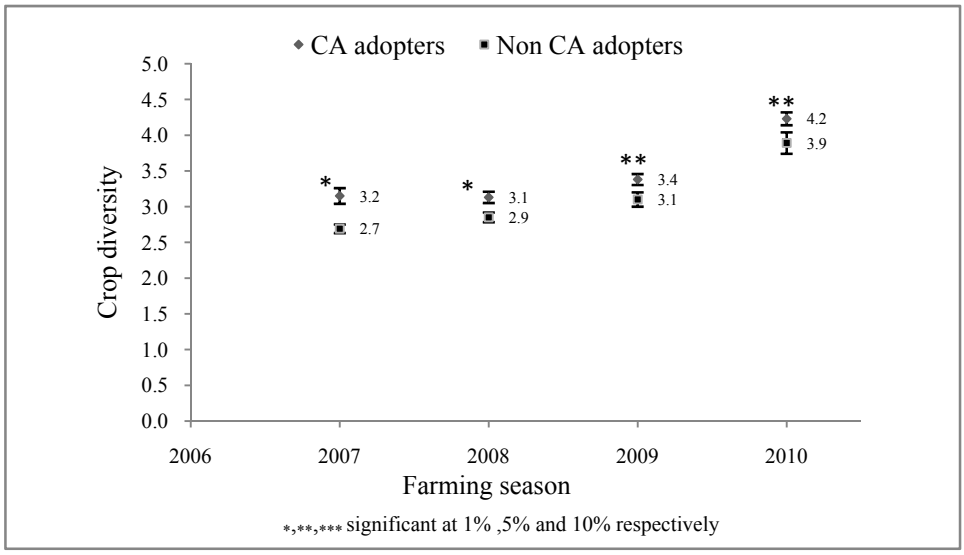

Figure 8. Average number of crops grown by CA adoption

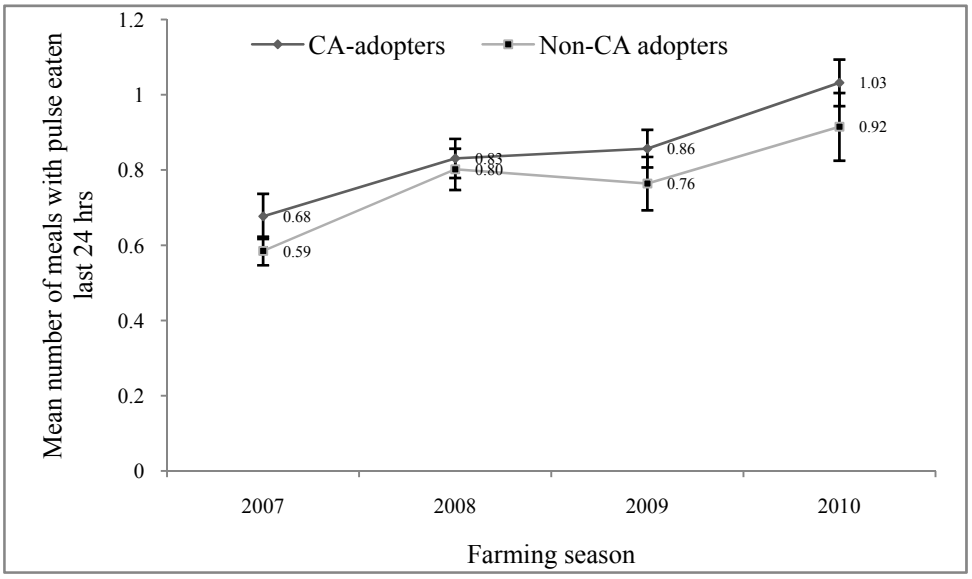

Figure 9. 24 hours recall of meals with pulses eaten by CA adoption

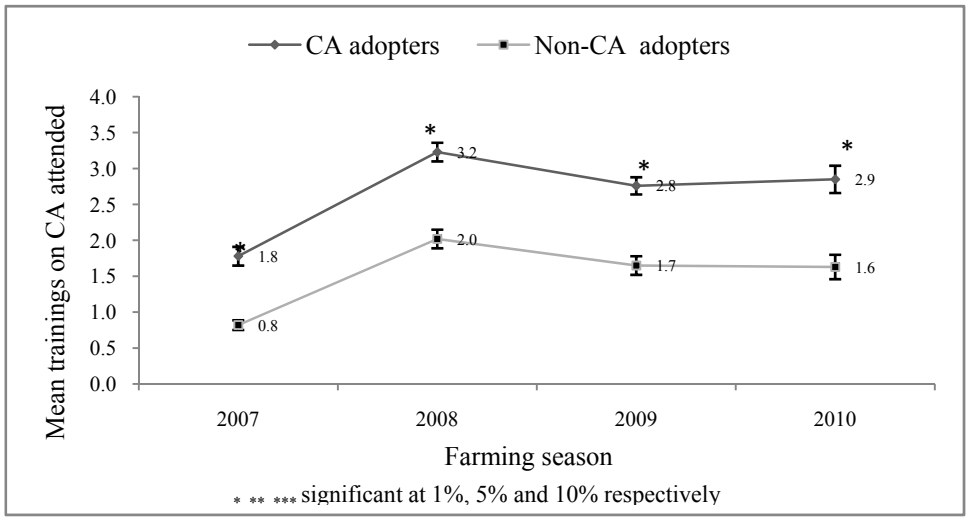

Figure 10. Farmer trainings on Conservation agriculture

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A Cafeteria-based Tasting Program Improved Elementary School Children's Fruit Preferences and Self-efficacy to Consume Fruits and Vegetables

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Abstract

This quasi-experimental study examined the influence of a cafeteria-based tasting program on children's fruit and vegetable (FV) preferences and self-efficacy, social norms and outcome expectations to consume FV. Third and fifth graders in the intervention schools were offered tastes of eightFV for eight weeks followed by two weeks of tasting four months post-intervention (follow-up). Preferences for 38 FV and self-efficacy, social norms and outcome expectations to consume FV were assessed at baseline, post-intervention and after follow-up tastings. Sixty-eight percent of the intervention-group children tasted fruits at least eight times and vegetables at least 20 times during the 8-week tasting program and were included in the analysis (52% third graders, 48% boys). Children who participated in tasting reported increased preferences for less commonly served fruits from baseline to post-intervention and frombaseline to follow-up and greater self-efficacy to consume FV from baseline to follow-up when compared to control-group children.

Keywords: Children, Fruits, Vegetables, Preference, Tasting program, Self-efficacy

1. Introduction

Children who prefer and who eat fruits and vegetables (FV) compared to energy-dense foods may have a lower risk for overweight or obesity (Lakkakula, Zanovec, Silverman, Murphy, & Tuuri, 2008;Ledoux, Hingle, & Baranowski, 2011). More than 35% of elementary school-aged children in the United States are overweight or obese and only about 25% of them consume the recommended amounts of FV (United States Department Health and Human Services and United States Department of Agriculture, 2005). Two antecedents of children's consumption of FV include the strength of their preferences for FV and confidence that they can change personal eating behaviors i.e., the social norms of their context and sense of self-efficacy (Baxter & Thompson, 2002; Domel et al., 1996; Loewen & Pliner, 1999). A predisposition to prefer sweet and salty foods and to avoid unfamiliar foods is a normative childhood characteristic (Birch, 1999) but in a supportive environment children can learn to like foods that they initially reject. When FV are available and children are encouraged to experience their flavors, children can learn to like and consume them (Loewen & Pliner, 1999; Lakkakula , Geaghan, Zanovec, Pierce, & Tuuri, 2010; Lakkakula et al., 2011; Wardle, Herrera, Cooke, & Gibson, 2003; He et al., 2009). It is particularly important for children from low-income households, who may lack access to FV (Champagne et al, 2004; Larson, Story & Nelson, 2009), to experience eating these nutrient-rich foods (Drewnowski & Spector, 2004). Fruits and vegetables are available to children who participate in the U. S. National School Lunch Program (United States Department of Agriculture, 2011). Fresh apples and oranges, and canned peaches and pineapples are the most commonly served fruits in school lunches while lettuce salads, French fries and cooked carrots are the most commonly offered vegetables (United States Department of Agriculture, 2007).

Schools are locations where children can learn and practice healthful behaviors (Bergman & Gordon, 2010). School-based interventions that are based upon accepted theoretical models such as Bandura's (2004) social cognitive theory (SCT) have increased children's nutrition knowledge (Tuuri et al., 2009), their preferences for FV (Wardle et al., 2003), and both positively (Tuuri et al., 2009; Thompson, Bachman, Baranowski, & Cullen, 2007; Cullen, Eagan, Baranowski, Owens, & de Moor, 2000) and negatively (He et al., 2009) influenced psychosocial predictors of children's FV consumption. School-based tasting programs intended to familiarize children with the flavors of FV and encourage FV consumption in a social setting surrounded by their peers have increased children's liking of these foods (Lakkakula et al., 2011; Lakkakula, Geaghan, Zanovec, Pierce, & Tuuri, 2010; Wardle, Herrera, Cooke, & Gibson, 2003). However, it is unknown if a change in children's liking of FV is accompanied by a change in other antecedents of actually eating FV, such as children's food preferences, self-efficacy, social norms and outcome expectations (Thompson, Bachman, Baranowski, & Cullen, 2007) or if a tasting program that promotes a limited number of FV increases preferences for a variety of similar items.

The purpose of the Building Preferences for Fruits and Vegetables Program was to introduce low-income elementary school-aged children to the flavors of four fruits and four vegetables offered by the National School Lunch Program and to increase their FV liking, visual preferences, and psychosocial predictors of FV consumption. Children's willingness to taste the FV that were offered to them was tracked to identify non-compliant children. The program's positive impact on the children's liking of the tasted FV reported immediately after tasting the foods has been previously reported (Lakkakula et al., 2011). It was hypothesized that the program would also increase the children's self-described preferences for a variety of FV after viewing a picture of each item and their psychosocial predictors of FV consumption. The primary objective of the present study was to examine the influence of participatingin this school-based FV tasting program on children's self-reported preferences for a variety of FV and their self-efficacy, social norms, and outcome expectations to consume FV. A secondary

objective was to examine the relationship between children's visual preference for FV and their risk for being overweight or obese.

2. Methods

2.1 Participants

Third- and fifth-grade children attending four low-income public elementary schools (Cullen et al., 2004) in southeastern Louisiana were recruited to participate in a seven-month quasi-experimental study. All children were invited to participate in the program but underweight children were excluded from the analysis. Prior to participation, parent consent and personal assent were obtained. The program was approved by the Louisiana State University Agricultural Center Institutional Review Board.

2.2 Design

Two intervention schools and two control schools were matched based upon the percentage of students eligible to receive free or reduced-price lunch (57%-80% of students) and racial/ethnic representation. Children enrolled in each of the four schools were measured for height and weight and completed questionnaires estimating their FV preferences and psychosocial variables associated with FV consumption at baseline. Following this initial data collection, children in the intervention schools began a FV tasting program. Four vegetables (bell peppers, carrots, peas and tomatoes) and four fruits (apricots, cantaloupe, peaches and pears) were chosen for tasting based upon results from a previous study with a similar population of children who reported that these FV were neither greatly liked nor disliked (Lakkakula, Zanovec, Silverman, Murphy & Tuuri, 2008). The children tasted small pieces of either the four vegetables or the four fruits twice a week on a rotating basis for eight weeks. Four months following the completion of the 8-week tasting program children again tasted the four vegetables or the four fruits twice a week for two weeks. Details of the tasting protocol and evaluation of the children's liking of these eight tasted items are described in a previously published manuscript (Lakkakula et al., 2011). Children who tasted the four fruits an average of two times and the four vegetables an average of five times were considered to have experienced sufficient tasting exposures to be included in the present study (Lakkakula et al., 2011). Children in the control schools did not participate in tasting but were exposed to a variety of brightly colored posters promoting FV that were displayed in the cafeteria on a weekly basis. Both intervention- and control-group children completed questionnaires at baseline, post-8-week intervention and after follow-up tastings.

2.3 Questionnaire

The child's self-reported preferences for 17 fruits and 21 vegetables, and self-efficacy, social norms, and positive and negative outcome expectations to consume FV were obtained from a questionnaire administered by the researchers in the classroom using a standard protocol. The FV preferences and SCT constructs questions had been previously validated with a similar population group (Baranowski et al., 2000; Cullen et al., 2003) but the FV preference questions were modified to include a color picture of each food item. Fruits were presented in alphabetical order and were followed by vegetables also listed alphabetically. To the right of each food picture there were the following columns seeking the child's response: 1) a broadly smiling cartoon face and the words "I like it a lot," 2) a smiling cartoon face with the words "I like it a little," 3) frowning cartoon face with the words "I do not like it," and 4) neutral cartoon face with the words "I have never tasted it." The FV items included were those commonly consumed by a nationally representative sample of children (United States Department of Agriculture, 1998). The four social norms questions that estimated the child's normative beliefs about "What do you think about eating FV?" included the following possible responses: 1) a very good thing, 2) a good thing, 3) not important, and 4) I don't know. Children rated their positive and negative outcome expectations about eating FV everyday using a 5- point Likert scale that ranged from "I disagree very much" to "I agree very much." Self-efficacy to consume FV estimated from questions asking "How sure are you that you can consume FV?" were similarly evaluated using a 5-point Likert scale that ranged from "I am sure I cannot" to "I am sure I can."

2.4 Anthropometry

Children's heights and weights were collected at all schools under the supervision of the school nurse using a standard protocol. Standing heights and weights were measured with portable digital scales (Seca 880, Seca Co. Hanover, MD) and stadiometers (Shorr Productions Inc. Olney, MD). Each child's gender-specific BMI-for-age percentile was determined, and he/she was categorized as underweight, healthy weight, overweight or obese (Centers for Disease Control & Prevention, 2011).

2.5 Statistical analysis

Data were examined using Statistical Analysis Software (SAS, Version 9.1.3; Cary, NC, 2003). Descriptive statistics for gender, grade and weight status were collected. The internal reliability of the constructs of

self-efficacy, social norms, and outcome expectations derived from the questionnaire data were examined using Cronbach's alpha tests. A reliability score of ≥ 0.7 was considered to be an acceptable measure of behavior (Cooper, 1983).

A factor analysis with a varimax rotation was used to determine the number and nature of underlying factors affecting the relationship between each set of variables (fruit preference, vegetable preference, self-efficacy, social norm, positive outcome expectations and negative outcome expectations). Mixed-model analyses of variance (PROC MIXED) evaluated change in children's FV preferences and psychosocial variables at the three study phases (baseline, post-intervention and follow-up). Using multi-level modeling, fixed effects included treatment and test; children and school were considered a random effect. To examine the differences between and within groups in each factor, a post-hoc analysis with a Tukey-Kramer adjustment of the least square means for each factor was conducted.

Each child's average FV preference score at baseline was determined based upon his/her responses to the 38 FV items (I don't know what this is = 0; I don't like it = 1; I like it a little = 2; I like it a lot = 3) and then compared to the his/her weight classification. Children were categorized as healthy weight (BMI-for-age 5^{th} to < 85^{th} percentile) or overweight/obese (BMI-for-age ≥ 85^{th} percentile) (Centers for Disease Control and Prevention, 2011). Underweight children were excluded from the analysis. The relationship of FV preference with weight category was examined using logistic regression. The level of significance was set at $p < 0.05$.

3. Results

3.1 Demographics

Two hundred sixty-nine children were measured for height and weight and completed the baseline questionnaire. One hundred fifty-five children (57.6%) were considered to be a healthy weight (BMI ≥ 5^{th} and < 85^{th} percentile), 104 children (38.7%) were overweight or obese (BMI ≥ 85^{th} percentile) and 10 children (3.7%) were underweight (BMI < 5^{th} percentile). After excluding the 10 underweight children, the remaining 259 students included 141 fifth graders (54%) and 136 boys (53%). Racial/ethnic representation included: 138 white (53%), 116 black (45%), and two Hispanic, one Asian, and two biracial (2%) children. The children's grade, gender, and race/ethnicity were not associated with their weight status.

Two hundred sixteen of the 259 children who completed the baseline questionnaire (79% intervention group; 52% 3^{rd} grade children; 52% girls) also completed the same survey following the 8-week-intervention and after the four-month follow-up tastings (follow-up). Sixty-eight percent of the children in the intervention group (116 of 171children) completed the required number of tastes to be included in the data analysis. Fifty-two percent of the children ($n = 83$) were in the third grade and 48% ($n = 78$) were boys. Participants included 57% ($n = 91$) white, 39% ($n = 63$) black, 2% ($n = 4$) Hispanic, 1% ($n = 1$) Asian and 1% ($n = 2$) "other" (bi-racial backgrounds) children. Table 1 presents the characteristics of the intervention and control group participants.

3.2 Questionnaire

Survey questions describing fruit preferences, vegetable preferences, self-efficacy and positive outcome expectations to consume FV were acceptable based upon their Cronbach's Alpha scores (Table 2). The questions examining social norms and negative outcome expectations were considered to be unreliable measures of behavior because the reliability scores were less than 0.7 and wereomitted from further analysis. The number of factors extracted to measure preferences, self-efficacy, and positive outcome expectations were determined by the eigenvalues approximating one. Rotated factor matrix items with loading scores greater than or equal to 0.4 were loaded on each given factor (Munro, 2001).

A four-factor fruit preference pattern explained 78% of the variance and a five-factor vegetable grouping explained 35% of the variance. The fruit grouping included the following four factors: Factor 1, apricots, avocados, cantaloupe, kiwi, mangos, and papayas; Factor 2, bananas, peaches, pears, pineapple, strawberries, and watermelon; Factor 3, oranges, plums and tangerines; and Factor 4, apples and grapes. Vegetable factors included the following five factors: Factor 1, bell peppers, broccoli, cauliflower, celery, cabbage, cucumbers, lettuce, spinach, and tomatoes; Factor 2, baked potatoes, green beans, and potato salad; Factor 3, carrots, corn and peas; Factor 4, garlic and onions; and Factor 5, greens and sweet potatoes.

A three-factor description of self-efficacy to consume FV explained 30% of the variance and two positive outcome expectations factors explained 97% of the variance. Self-efficacy Factor 1 included items describing drinking fruit juice and choosing fruit instead of a usual dessert, and Factor 2 dealt with consuming raw vegetables instead of chips and cookies. Self-efficacy Factor 3 responses included adding fruit to cereal, eating a green salad or vegetable served at lunch at school, consuming a salad or big serving of vegetables for dinner and eating three or

more servings of vegetables and five or more servings of fruit and vegetables each day. Positive Outcome Expectation Factor 1 suggested that the child perceived FV consumption as promoting beauty, friendship and strength while Factor 2 described having more energy, less risk for becoming fat, and greater opportunity to make the family proud.

This school cafeteria-based FV tasting program positively influenced children's preferences for Fruit Preference Factor 1 and Self-efficacy Factor 3 (Table 3). In the intervention group, preferences for apricots, avocados, cantaloupe, kiwi, mangos and papaya increased from baseline to post-intervention (p=0.04) and from baseline to follow-up (p = 0.01), but did not change in the control group. Children in the intervention group also reported greater confidence that they could consume fruits, vegetables and salad (Self-efficacy Factor 3) between baseline and follow-up (p = 0.01) although there were no significant differences between the intervention and control groups (p = 0.07). Preferences for vegetables and positive outcome expectations did not change in either the intervention or control groups.

3.3 FV preferences and risk of being overweight and obese

In order to examine the association between children's self-described preferences for the 38 FV pictured in the questionnaire and their weight status, children were placed into one of three FV preference groups and one of two weight categories (healthy weight or overweight/obese). The FV preference groups were as follows: Group 1, low preference (mean score 1.0 to 1.6, n = 8), Group 2, moderate preference (mean score 1.7 to 2.3, n = 97), and Group 3, high preference (mean score 2.4-3.0, n = 154). No differences were observed between the low and high (p = 0.96) and moderate and high preference groups (p = 0.37) and the risk of being overweight or obese. This lack of association was in contrast to findings from a similar study with low-income black children residing in the same geographical location (Lakkakula, Zanovec, Silverman, Murphy & Tuuri, 2008). Both groups of children attended low-income schools, but the present study included a majority of white children while the former study included only black children. In addition, the FV questionnaire used in the present study included a picture of each FV item while the study by Lakkakula and colleagues did not, and the children may have responded differently because they had a visual image of the food.

4. Discussion

The Building Preferences for Fruits and Vegetables school cafeteria-based tasting program positively impacted known predictors of food consumption including preferences for fruits less commonly served by the United States National School Lunch Program and self-efficacy to consume FV. Because food preferences are strong predictors of intake (Baxter & Thompson, 2002; Neumark-Sztainer, Wall, Perry, & Story, 2003; Loewen & Pliner, 1999) these findings suggest that the children became more accepting of less familiar fruits and began consuming more of these nutrient-rich items. The tasting program appeared to decrease the children's neophobia and made them more willing to consume a variety of fruits. The "learned safety" hypothesis (Kalat & Rozin, 1973) would suggest that the children found eating less familiar fruits to be acceptable to their gastrointestinal systems and pleasurable to their palates. The fruits may have elicited an endogenous response to the sweet tastes and encouraged a new-found preference for these foods (Gosnell & Levine, 2009). The lack of change in opinion for vegetables may reflect a less pleasurable response after tasting and consuming these items and supports the fact that vegetables have been reported to be one of children's least liked foods (Perez-Rodrigo, Ribas, Serra-Majem & Aranceta, 2003; Skinner, Carruth, Bounds & Ziegler, 2002; Nu, MacLeod & Barthelemy, 1996). A lack of change in self-reported preference for vegetables, however, may not preclude an increase in actual consumption. A recent study by Zeinstra and colleagues observed that offering children a choice between vegetables did not impact their self-described liking or consumption of these items (Zeinstra, Renes, Keolen, Kok & de Graaf, 2010). However, without comparing self-reported information about food preferences with actual food consumption it is not possible to determine if the FV tasting program impacted consumption of these items.

The program's positive impact on self-efficacy to consume FV also suggested that the children responded to participating in the cafeteria-based tasting program by eating more FV served by the National School Lunch program and consuming greater amounts of these foods served at home (Thompson, Bachman, Baranowski & Cullen, 2007). Self-efficacy is one of the key constructs of the Social Cognitive Theory (Bandura, 2004) and describes a person's confidence in his/her ability to take action and overcome barriers to performing a behavior. It is one of the key factors that affect the likelihood that a person will change a behavior. The program helped the children change their self-efficacy toward consuming FV by encouraging them to taste small bites of selected FV on a consistent basis surrounded by their peers in the social setting of the school cafeteria.The lack of preference change for vegetables and other fruits reported by the intervention-group children on the FV questionnaire contrasted with their positive opinions expressed immediately aftertasting the eight FV items (Lakkakula et al.,

2011). These findings suggest that while the children began to like the flavors of the tasted FVs they may not have beenwilling to choose them when given an alternative. A lack of willingness to choose a fruit or vegetable may be problematic in today's environment that is rich in highly palatable and heavily marketed foods high in calories, sugar, fat and salt(Institute of Medicine, 2004; Institute of Medicine, 2005). Marketing and availability are known to influence children's food preferences, purchasing requests and dietary intake (Institute of Medicine, 2005). Perhaps the abundance of these highly marketed and flavorful items including candies, cookies, ice cream and fruit-flavored beverages (Williams, 2005) interfere with preference change for FV.

The study had several limitations. The lack of random assignment of schools limited the strength of the argument for a causal effect. The study was conducted with low-income third- and fifth-grade public elementary students in the southern United States and may not represent the general elementary school population. However, because schools that participate in the U. S. Child Nutrition Program follow standard procedures and purchase similar items to be offered to children, the program should be appropriate for children in other geographical regions and socio-economic groups. This study was also limited by the fact that data were self-reported and validity wasdependent upon the self-knowledge and truthfulness of the children.

5. Conclusion

Children who participated in a school cafeteria-based FV tasting program described greater preferences for less commonly served fruits and more self-efficacy to consume FV. The program can be adopted by teachers and parents as a way to increase children's acceptance of a variety of nutrient-rich foods and has the potential to change behavior and improve the diet. Future research should focus on ways to incorporate FV tasting programs into routine practice in the school setting and increase the participation rate of children. Furthermore, additional research is needed to determine if the children's self-described increase in preferences and self-efficacy resulted in greater FV consumption. Future behavioral interventions should also address factors in the child's environment that may interfere with preference change and FV consumption including developed preferences for highly marketed and palatable foods. Comprehensive approaches that encourage new foods while addressing barriers that limit preference change are needed so as to positively impact children's food choices and encourage healthful dietary behaviors.

References

Bandura, A. (2004). Health Promotion by Social Cognitive Means. *Health Education & Behavior, 31*(2), 143-164. http://dx.doi.org/10.1177/1090198104263660

Baranowski, T., Davis, M., Renicow, K., Baranowski, J., Doyle, C., Lin, L. S., et al. (2000). Gimme 5 fruit, juice, and vegetables for fun and health: outcome evaluation. *Health Education & Behavior, 27*(1), 96-111. http://dx.doi.org/10.1177/109019810002700109

Baxter, S., & Thompson, W. O. (2002). Fourth-grade children's consumption of fruit and vegetable items available as part of school lunches is closely related to preferences. *Journal of Nutrition Education and Behavior, 34*, 166-171. http://dx.doi.org/10.1016/S1499-4046(06)60086-9

Bergman, E. A., & Gordon, R. W. (2010). Position of the American Dietetic Association: local support for nutrition integrity in schools. *Journal of American Dietetic Association, 110*, 1244-1254. http://dx.doi.org/10.1016/j.jada.2010.06.014

Birch, L. L. (1999). Development of food preferences. *Annual Review of Nutrition, 19*, 41-62. http://dx.doi.org/10.1146/annurev.nutr.19.1.41

Centers for Disease Control and Prevention. (2011). *Children overweight and obesity*. Retrieved from http://www.cdc.gov/obesity/childhood/basics.html

Champgne, C. M., Bogle, M. L., McGee, B. B., Yadrick, K., Allen, H. R., Kramer, T. R., et al. (2004). Dietary intake in the lower Mississippi delta region: results from the Foods of our Delta Study. *Journal of the American Dietetic Association, 104*, 199-207. http://dx.doi.org/10.1016/j.jada.2003.11.011

Cooper, J. C. (1983). Factor Analysis: An Overview. *The American Statistician, 37*(2), 141-147. http://dx.doi.org/10.2307/2685875

Cullen, K. W., Baranowski, T., Owens, E., Marsh, T., Rittenberry, L., & de Moor, C. (2003). Availability, accessibility, and preferences for fruit, 100% fruit juice, and vegetables influence children's dietary behavior. *Health Education & Behavior, 20*, 615-626. http://dx.doi.org/10.1177/1090198103257254

Cullen, K. W., Eagan, J., Baranowski, T., Owens, E., & de Moor, C. (2000). Effect of a la carte and snack bar foods at school on children's lunchtime intake of fruits and vegetables. *Journal of American Dietetic Association, 100*, 1482-1486. http://dx.doi.org/10.1016/S0002-8223(00)00414-4

Cullen, K. W., Zakeri , L., Pryor, E. W., Baranowski, T., Baranowski, J., & Watson, K. (2004). Goal setting is differentially related to change in fruit, juice, and vegetable consumption among fourth-grade children. *Health Education & Behavior, 31*(2), 258-259. http://dx.doi.org/10.1177/1090198103260518

Domel, S. B., Thompson, W. O., Davis, H. C., Baronowski, T., Leonard, S. B., & Baronowski, J. (1996). Psychosocial predictors of fruit and vegetable consumption among elementary school children. *Health Education Research, 11*(3), 299-308. http://dx.doi.org/10.1093/her/11.3.299

Drewnowski, A., & Spector, S. E. (2004). Poverty and obesity: the role of energy density and energy costs. *Journal of Clinical Nutrition, 79*, 6-16. http://dx.doi.org/10.1017/S1368980009992989

Gosnell, B. A., & Levine, A. S. (2009). Reward systems and food intake: role of opioids. *International Journal of Obesity, 33*, S54-S58. http://dx.doi.org/10.1038/ijo.2009.73

He, M., Beynon, C., Bouck, M. S., Onge, R. S., Stewart, S., Khoshaba, L., et al. (2009). Impact evaluation of the Northern Fruit and Vegetable Pilot Programme - a cluster-randomised controlled trial. *Public Health Nutrition, 12*(11), 2199-2208. http://dx.doi.org/10.1017/S1368980009005801

Horne, P. J., Tapper, K., Lowe, C. F., Hardman, C. A., Jackson, M. C., & Woolner, J. (2004). Increasing children's fruit and vegetable consumption: a peer-modeling and rewards-based intervention. *European Journal of Clinical Nutrition, 58*, 1649-1660. http://dx.doi.org/10.1038/sj.ejcn.1602024

Institute of Medicine. (2004, September 29). *Preventing childhood obesity: Health in the Balance.* Retrieve from http://www.iom.edu/Reports/2004/Preventing-Childhood-Obesity-Health-in-the-Balance.aspx

Institute of Medicine. (2005, December 5). *Food Marketing to children and youth: Threat or opportunity?* Retrieved from http://www.iom.edu/Reports/2005/Food-Marketing-to-Children-and-Youth-Threat-or-Opportunity.aspx

Kalat, J. W., & Rozin, P. (1973). "Learned safety" as a mechanism in long delay taste aversion learning in rats. *Journal of Comparative Physiological and Psychology, 83*, 198-207. http://dx.doi.org/10.1037/h0034424

Lakkakula, A. P., Zanovec, M., Silverman, L., Murphy, E., & Tuuri, G. (2008). Black children with high preferences for fruits and vegetables are at less risk of being at risk of overweight or overweight. *Journal of American Dietetic Association, 108*, 1912-1915. http://dx.doi.org/10.1016/j.jada.2008.08.019

Lakkakula, A., Geaghan, J. P., Wong, W., Zanovec, M., Pierce, S. H., & Tuuri, G. (2011). A cafeteria-based tasting program increased liking of fruits and vegetables by lower, middle and upper elementary school-age children. *Appetite, 57*(1), 299-302. http://dx.doi.org/10.1016/j.appet.2011.04.010

Lakkakula, A., Geaghan, J., Zanovec, M., Pierce, S., & Tuuri, G. (2010). Repeated taste exposure increase liking for vegetables by low-income elementary school children. *Appetite, 55*, 226-231. http://dx.doi.org/10.1016/j.appet.2010.06.003

Larson, N. I., Story, M. T., & Nelson, M. C. (2009). Neighborhood environments disparities in access to healthy foods in the U.S. *American Journal of Preventative Medicine*, 36 (1), 74-81. http://dx.doi.org/10.1016/j.amepre.2008.09.025

Ledoux, T. A., Hingle, M. D., & Baranowski, T. (2011). Relationship of fruit and vegetable intake with adiposity; A systematic review. *Obesity Reviews, 12*, e143-e150. http://dx.doi.org/10.1111/j.1467-789X.2010.00786.x

Loewen, R., & Pliner, P. (1999). Effects of prior exposure to palatable and unpalatable novel foods on children's willingness to taste other novel food. *Appetite, 32*, 351-366.http://dx.doi.org/10.1006/appe.1998.0216

Munro, B. H. (2001). *Statistical methods for health care research* (4th ed.). Philadelphia: Lippincott, Williams and Wilkins.

Neumark-Sztainer, D., Wall, M., Perry, C., & Story, M. (2003). Correlates of fruit and vegetable intake among adolescents: findings from Project EAT. *Preventive Medicine, 37*, 198-208. http://dx.doi.org/10.1016/S0091-7435(03)00114-2

Nu, C. T., MacLeod, P., & Barthelemy, J. (1996). Effects of age and gender on adolescent's food habits and preferences. *Food Quality Preference, 7*, 251-262. http://dx.doi.org/10.1016/S0950-3293(96)00023-7

Perez-Rodrigo, C., Ribas, L., Serra-Majem, L. L., & Aranceta, J. (2003). Food preferences of Spanish children and young people: the enKid study. *European Journal of Clinical Nutrition, 57*(Suppl 1), S45-S48. http://dx.doi.org/10.1038/sj.ejcn.1601814

Skinner, J. D., Carruth, B. R., Bounds, W., & Ziegler, P. J. (2002). Children's food preferences: a longitudinal analysis. *Journal of American Dietetic Association, 102*, 1638-1647. http://dx.doi.org/10.1016/S0002-8223(02)90349-4

Strecher, V. J., McEvoy DeVellis, B., Becker, M. H., & Rosenstock, I. M. (1986). The role of self-efficacy in achieving health behavior change. *Health Education Quarterly, 13*, 73-91. http://dx.doi.org/10.1177/109019818601300108

Thompson, V. J., Bachman, C. M., Baranowski, T., & Cullen, K. W. (2007). Self-efficacy and norm measures for lunch fruit and vegetable consumption are reliable and valid among fifth grade students. *Journal of Nutrition Education and Behavior, 39*(1), 2-7. http://dx.doi.org/10.1016/j.jneb.2006.06.006

Tuuri, G., Zanovec, M., Silverman, L., Geaghan, J., Solmon, M., Holston, D., et al. (2009). "Smart Bodies" school wellness program increased children's knowledge of healthy nutrition practices and self-efficacy to consume fruit and vegetables. *Appetite, 52*, 445-451. http://dx.doi.org/10.1016/j.appet.2008.12.007

United States Department Health and Human Services and United States Department of Agriculture. (2005). *Dietary Guidelines for American* (6th ed.). Washington, DC: Government Printing Office.

United States Department of Agriculture. (1999). *ARS Food Surveys Research Group: CSFII 1994-1996, 1998 Data Set*, available on CD-ROM.

United States Department of Agriculture. (2012). *Food and Nutrition Service.* National School Lunch Program: Program Fact Sheet. Retrieved from HYPERLINK "http://www.fns.usda.gov/cnd/Lunch/" http://www.fns.usda.gov/cnd/Lunch/

United States Department of Agricultute. (2012). *Food and Nutrition Service*. School Nutrition Dietary Assessment Study - III: Volume 1: School Food Service, School Food Environment, and Meals Offered and Served. Retrieved from HYPERLINK "http://www.fns.usda.gov/ora/" http://www.fns.usda.gov/ora/

Wardle, J., Cooke, L. J., Gibson, E. L., Sapochnik, M., Sheiham, A., & Lawson, M. (2003). Increasing children's acceptance of vegetables; a randomized trial of parent-led exposure. *Appetite, 40*, 155-162. http://dx.doi.org/10.1016/S0195-6663(02)00135-6

Wardle, J., Herrera, M.-L., Cooke, L., & Gibson, E. L. (2003). Modifying children's food preferences: the effects of exposure and reward on acceptance of an unfamiliar vegetable. *European Journal of Clinical Nutrition, 57*, 341-348. http://dx.doi.org/10.1038/sj.ejcn.1601541

Williams, J. (2005). *Product proliferation analysis for new food and beverage products targeted to children 1994-2004.* University of Texas at Austin Working Paper.

Zeinstra, G. G., Renes, R. J., Keolen, M. A., Kok, F. J., & de Graaf, C. (2010). Offereing choice and its effect on Dutch children's liking and consumption of vegetables: a randomized controlled trial. *American Journal of Clinical Nutrition, 91*, 349-356. http://dx.doi.org/10.3945/ajcn.2009.28529

Table 1. Demographics of children in the study sample

Characteristic	Intervention (n=116)	Control (n=45)
Grade		
Third	58	25
Fifth	58	20
Gender		
Boy	63	15
Girl	53	30
Race-Ethnicity		
White	66	25
Black	45	18
Hispanic	3	1
Asian	1	0
Other	1	1

Table 2. Sample questionnaire items and standardized Cronbach's alpha reliability scores

Measure	# of items	Response scale	Sample item	Alpha reliability		
				Pre	Post	Follow-up
Fruit preferences	17	a	How much do you like an apple?	0.94	0.91	0.91
Vegetable preferences	21	a	How much do you like a bell pepper?	0.88	0.91	0.90
Self-efficacy	18	b	For dinner, I think I can eat a green salad	0.92	0.94	0.93
Outcome expectations	13	c				
Positive	9	c	If I eat fruits and vegetables every day, I will be healthier	0.84	0.80	0.83
Negative	4	c	If I eat fruits and vegetables every day, my friends will make fun of me	0.52	0.36	0.53
Social norms	4	d	Most kids my age think that eating 2 or more servings of fruit juice each day is a good thing	0.66	0.66	0.67

The possible response scales included the following:

[a] 1 = I do not like it, 2 = I like it a little, 3 = I like it a lot, 4 = I have never tasted it.

[b] 1 = I am sure I cannot, 2 = I don't think so, 3 = I am not sure, 4 = I think so, 5 = I am sure I can.

[c] 1 = I disagree very much 2 = I disagree a little 3 = I am not sure 4 = I agree a little 5 = I agree very much.

[d] 1 = A very good thing, 2 = A good thing, 3 = Not important, 4 = I don't know.

Table 3. Least square means for treatment by test interactions (n = 161)

Survey Construct	**Intervention**			**Control**		
	Baseline	Post-Intervention	Follow-up	Baseline	Post-Intervention	Follow-up
Fruit Preference Factor 1[a]	-0.242	0.208*	0.220*	-0.154	-0.389	-0.499
Self-efficacy Factor 3[b]	-0.187	0.046	0.170†	-0.024	-0.236	0.028

Identification of Traditional Foods with Public Health Potential for Complementary Feeding in Western Kenya

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Abstract

The diversity of traditional foods in Kisumu West District of Western Kenya was assessed with an aim to identify the foods with a potential for complementary feeding. Leaves were the most consumed plant part amongst vegetables, while a few fruits were consumed together with their seeds. *Amaranthus cruentus* L. was found to be consumed as a leafy vegetable while another variety, *Amaranthus hybridus* L. was found to be consumed as a grain. Four species of winged termites, a grasshopper, black ant and *dagaa* fish were also identified. Twelve of the traditional foods were found to be associated with nutritional and health benefits as perceived by the locals. Traditional food processing methods such as boiling, fermentation and sun drying were identified. Thus exploitation of the species possessing nutrient, health and processing benefits needs to be explored in complementary feeding.

Keywords: Traditional, Nutritional, Health, Availability, Acceptability

1. Introduction

Kenya has a diverse traditional food system with important dietary contributions and therefore the importance of wild and semi-domesticated food sources for subsistence farmers cannot be over-emphasized (Ogoye-Ndegwa & Aagaard-Hansen, 2003). Persistent use of a large number of these traditional foods plays an important role in the continued adaptation of the communities of Western Kenya, to the particular economic and ecological conditions (Orech *et al.,* 2007a).

Traditional foods may be described as a large and heterogeneous group of raw and processed foods which include; wild indigenous plants and animals collected from uncultivated land and forest (e. g. leafy plants, roots, berries, small rodents and insects) and from aquatic environments (e. g. fish, frogs and snails); semi-domesticated indigenous plants and animals, for example gardening of indigenous plant species such as amaranth in Africa and culture of indigenous fish species in rice fields in Asia; locally available staple foods processed using traditional processing technologies (Ogoye-Ndegwa & Aagaard-Hansen, 2003). Traditional foods have some features in common; they are culturally acceptable, may be an integral part of local food habits, may have no or low commercial value and may either be collected for consumption or traded locally.

Plants provide most of the foodstuffs consumed by peasant farming communities in tropical countries. Communities in Western Kenya, depend on many plant products for subsistence and for generation of cash income. Sizeable areas are dedicated to the cultivation of dietary staples and crops, many of which are not traditional (Johns & Kokwaro, 1990). Traditional foods may contribute to a nutritionally balanced diet by supplying essential vitamins and minerals (FAO, 1988). Moreover, traditional foods adapted to local conditions contribute to the diet in periods of seasonal scarcity (Ogoye-Ndegwa & Aagaard-Hansen, 2003; Orech *et al.,* 2007a), thus contributing to an important traditional buffer against periodic famines that are becoming increasingly prevalent in other areas of the tropics.

Traditional foods may play a very crucial role of providing macronutrients and micronutrients (Ogoye-Ndegwa & Aagaard-Hansen, 2003; Kinyuru *et al.,* 2009; Kinyuru *et al.,* 2010a; Kinyuru *et al.,* 2010b) and may more importantly be utilized to alleviate childhood malnutrition (Roos *et al.,* 2007). This is in addition to their ecological, agronomical, cultural (Geissler *et al.*, 2002; Ogoye-Ndegwa *et al.,* 2002) and medicinal values (Lindeberg *et al.,* 2003).

Although traditional foods have huge benefits to human health, they are generally uncultivated and underutilized (Abukutsa-Onyango, 2003; Kiambi & Atta-Krah, 2003). This study was aimed at identifying and documenting traditional foods with public health potential for complementary feeding in Western Kenya.

2. Methodology

2.1 Study area and population

The study was conducted in the rural areas of Kisumu West District namely: Kanyoto, Kapuonja "A", Karateng West, Marera, Ong'io, Meronda, and Karateng' East villages of Maseno Division and Seme, Reru and Kaura villages of Kombewa Division. Kisumu West District lies between a latitude of -0.25 (0° -15' 0 S) and a longitude of 34.92 (34° 55' 0 E), with a land area of 919 km^2, with an approximate population of 1 million according to the 2009 population and housing census (KNBS, 2010). Majority of the inhabitants in the District are people with Luo ethnic background whose main economic activities are fishing and subsistence farming; maize, sorghum, and fresh vegetables. With an approximate altitude of 1580 meters above sea level, Kisumu West District is part of the extension of the humid tropical zone of central Africa into western Kenya. Oral consent to participate in the study was sought from all the participants.

2.2 Study design

It was a cross-sectional study with random sampling used in order to allow the generalization of conclusions. The methodological protocol closely followed a multidisciplinary approach combining botanical inventorying; collection of voucher plant specimens and taxonomic assessment; semi-structured and informal interviews (Martin 1995; Grenier 1998) which came up with a list of Luo (vernacular) names of the different foods mentioned. Secondary data on the nutritional potential of the foods was collected from food composition tables was also evaluated.

2.3 Data collection methods

2.3.1 Field survey

The field survey targeted women in child bearing age and fathers. Farm plots of some interviewees were surveyed for food plant species and samples collected. Older persons were sought for historical use aspects and some few individuals encountered by chance were also considered in the course of the field study.

2.3.2 Key informant interviews

Six (6) key informants were purposively selected with the help of interviewees, elders and the chiefs (local administrators). The interviewer read out names of foods and sought explanation why those foods were eaten. The survey tool included a list of all plant and animal foods originating from the field survey described above. The objective was to find reasons why the earlier listed foods were consumed and determine whether the consumption of these foodstuffs was linked to nutritional and/or health benefits. Common phrases such as, 'increase of blood levels', 'addition of energy', 'enhancement of breast milk by lactating mothers' from the informants were noted. Availability of the foods was also probed. Cooking methods of the foods was also probed.

2.3.3 Secondary data

Literature on iron and zinc content of the food was sought. The Kenya food composition database (Sehmi, 1993) was used to ascertain the iron content of the foods. Since the Kenya food composition database lacks zinc content values for the foods tabulated, Tanzania food composition tables (Lukmwaji *et al*., 2008) were used to ascertain the zinc content of the mentioned foods.

2.4 Data analysis

Vernacular names of the foods were annotated with scientific names. Foods were separated according to food groups namely; fruits and vegetables, cereal grains, pulses, seeds, tubers/roots and animal foods. Foods with perceived nutritional and health benefits were tabulated.

3. Results

3.1 Traditional food groups and the edible parts

Among the plant source traditional foods, 25 fruits and vegetables (Table 1), 6 cereal/legumes, 1 starchy root/tuber (Table 2) were identified. Traditional plant species were collected from vegetation types such as scrubs, thickets, grasslands as well as from kitchen gardens, farmlands, built-up areas, hedges and wastelands. Leaves were the most consumed amongst the traditional vegetables while a few fruits were consumed together with their seeds. One (1) vegetable, *Odielo* (*Commelina Africana)* is consumed as a whole plant. *Ododo* (*Amaranthus hybridus* L.) was found to be consumed as a leafy vegetable (Table 1) while its grain species (*Amaranthus cruentus* L.) was found to be consumed as a grain (Table 2). *Amaranthus cruentus* L. was found to be a tall plant to the height of an adult man while *Amaranthus hybridus* L. grows only up to knee height of an average adult man. Cassava leaves were consumed as a vegetable while the tubers were also found to be consumed. Some of the fruits were consumed together with the seeds.

Most of the vegetables and fruits presented in Table 1 grow abundantly and are easy to procure in large quantities from various habitats of the local environment, during both short and long rainy seasons. Some of the species such *as Mussaenda arcuata, Biden pilosa, Amaranthus hybridus, Asystasia mysorensis, Bidens pilosa, Commelina africana, Corchorus trilocularis, Launaea cornuta* and *Solanum nigrum* are invasive weeds in cultivated fields, and thus are uprooted and burnt or fed to domesticated animals by the local farmers. They are abundant all year round and hence, are important food resource during periods of drought or poor harvests. The vegetables *Amaranthus* spp., *Asystasia mysorensis* and *Gynandropsis gynandra* are self sustaining and once planted, persist in the field through self-seeding for many years.

According to the interviews informants, *Sorghum bicolor, Eleusine coracana, Zea mays and Amaranthus cruentus* were mentioned as the cereal grains traditionally utilized in the area (Table 2). However, there reported promotion interests of *A. cruentus* grains as a nutritious by the Government of Kenya for complementary feeding and even for lactating women *vis a vis* the previous traditional utilization of the leaves alone. *Sorghum bicolor, Manihot esculentum* are perennials of local abundance. None of the cereal grains reported are harvested from the wild, they are all cultivated. *Vigna unguiculata* was mentioned as pulse while *Ipomea batatas* and *Manihot esculentum* were adversely mentioned as tubers with special attachment to the community. *M. esculentum* (cassava) was mentioned *as* an important food especially during dry spells due its ability to withstand harsh environmental conditions. Simsim (*Sesamum indicum*) is consumed as a seed and it was considered to be very nutritious.

Among the traditional animal source foods, 6 edible insects and 1 fish (Table 3) were identified. Of the edible insects, four (4) species were edible termites; one (1) was of long-horned grasshoppers (*Ruspolia differens*) and the winged black ants (*Calebara vidua)*. The edible insects are collected from the wild. The termites were reported to be harvested during the rainy seasons. Some of the species were reported to make termite moulds and thus harvesting is done near the moulds while others simply emerge from the ground while it rains. Some traditional harvesters are able to detect possible emergence sites for the insects.

Some interviewees could not differentiate the different species of termites with some referring to them as *ng'wen,* a common Luo name for termites in general. However, the common termite species were *Macrotermes subhylanus* and *Macrotermes bellicosus* while *Pseudocanthotermes militaris* and *Pseudocanthotermes spiniger* were considered scarce. The locals considered *M. subhyalinus* as the most delicious followed by *P. militaris*. Although the *M. bellicosus* are generally larger than those of *P. militaris,* the former was reported to cause running stomach. All the insects are consumed whole with some people de-winging them except *C. vidua* whose fatty abdomen is the preferred delicacy. However, some respondents mentioned that it is also consumed whole or de-winged. It was revealed that *C. vidua* is currently facing extinction with very few emergencies reported with only a few interviewees confirming to have consumed the insect.

Dagaa fish (*Rastrineobola argentea*) is harvested from Lake Victoria in large numbers. *R. argentea* was reported to be very popular food around the study area since it borders the lake. Harvesting of *dagaa* is a demanding activity and the methods used include scoop net, seine net and catamaran or lift net. The harvesting of *dagaa* employs a lot of people along the lake carrying activities of fishing, preservation and selling. The fish is consumed whole with no de-gutting reported by the interviewees.

3.2 Nutritional, health benefits and availability of selected traditional foods

Key informants interviews, 12 traditional foods were selected based on the health and nutritional benefits that the local people associated with consumption of the foods by mothers and their infants (Table 4).The most common benefits included provision of energy as well as increasing blood in the human body. Other health benefits included prevention of stomach pains, bloating and constipation. Amaranth (*Amaranth sp.*) was said to increase breast milk in lactating women. The winged termite (*Macrotermes subhylanus*) and *dagaa* fish (*Rastrineobola argentea*) were said to have a body building function and enhancing good health.

Availability of most of the foods mentioned ranged from moderate to high, however, availability for jute (*Corchurus olitorius)* and oxalis *(Oxalis latifolia)* was rated to be low and therefore amassing enough amounts for product development would be a challenge. The winged termites were also reported to be widely available during different seasons of the year with *dagaa* fish (*R. argentea*) being highly available. Traditionally termites were never fed to weaning children but rather eaten by grown, walking, children. A key informant lamented a decline in availability of the termites and associated decline of this food resource with increased farming especially with a surge in use of insecticides.

3.3 Food preparation/processing methods

Different food preparation methods were recorded (Table 5). Traditionally, food was prepared by the mother alone who is acquainted with good cooking skills and maintains high hygienic standards. The interviewees argued that the father has no skills and knowledge on good preparation of food. Most vegetables were consumed after boiling. Most cereals were traditionally prepared into porridges while seeds and legumes were pounded. The selection of flour for porridge preparation depends on available food types, largely influenced by the agro-ecological zone. As an example, millet was mentioned as the basic weaning porridge while maize flour is used in later weaning stages. The preference for millet is attributed to its dark color which is attributed to a rich nutritional value. Traditionally one food was prepared alone; it was common that a mother would stick to one cereal millet, sorghum or even maize. Preparation of termites for consumption involves mostly frying or

sun-drying fresh harvests, which are dried for consumption as snacks or sauces of various types. Consumption of live termites with or without salt, and preparing a sauce of fresh (not dried) alates were also reported to be common.

4. Discussion

Secondary data indicated that among the cereal grains, amaranth (*Amaranthus sp*.) had the highest iron content (21mg/100g) while among the leafy vegetables, spider plant (*Gynandropsis gynandra*) had the highest (30mg/100g). Winged termites (*M. subhylanus*) had the highest iron content (21mg/100g) among the animal source foods. Amaranth grain has been promoted as a cereal grain in Nyanza region for the past five years by the Kenya Government (Kinyuru & Muchui, 2009) and has been widely accepted as a nutritious food especially for infants and immune depressed persons. White maize has significant iron content (4.76mg/100g) and high energy (Sehmi, 1993) as well as its availability. Termites have high iron content (Sehmi, 1993), zinc content (Lukmwaji *et al*., 2008). Termites emerged as widely consumed, among target communities with distinct availability seasons associated with long and short rains. They are available in local markets when in season and harvesting is easily done when in and out of season. *Dagaa* (*R. argentea*), a small pelagic fish species, most popularly known as *omena* in the Luo language, has significant iron content, 7 mg/100g (Sehmi, 1993), zinc content (Lukmwaji *et al*., 2008) in addition to high availability and consumption within the study area.

The communities in Western Kenya are small-scale farmers who adhere mostly to traditional subsistence agricultural practices and therefore most families continue to depend on crops for subsistence (Johns & Kokwaro, 1991; Orech *et al*., 2007a). The retention of knowledge by the local people on the use of edible traditional foods attests to the continuing importance of these resources for subsistence and as part of the cultural heritage of the Luo. However, in the past, most locals were knowledgeable about traditional foods both domesticated and wild. Unfortunately, this trend is slowly changing (Abukutsa-Onyango 2003; Ogoye-Ndegwa & Aagaard-Hansen 2003) with fewer people able to recognize the foods leave alone cook and consume them. Encouraging production and consumption of the traditional vegetables may help tackle the problem of dwindling popularity (Ruel & Levin, 2000).

In general, people in Luo areas are well versed in the native names of traditional plants used for both food and medicine (Johns & Kokwaro, 1991; Orech *et al*., 2007b). Traditional food plants are particularly important in the driest regions, which are most vulnerable to drought (Orech *et al*., 2007a). According to Johns & Kokwaro (1991), the vegetable species namely *Amaranthus hybridus*, *Basella alba, Gynandropsis gynandra, Cucurbita maxima, Solanum nigrum,* and *Vigna unguiculata* are of critical importance both in the days of food shortage and days of abundance. Unfortunately, the vegetable species have continued to become rare. For example, *G. gynandra* is presently rare, but if found only prefers fertile loamy and clay soil around abandoned homes, home gardens, farmlands or cattle pastures.

Currently, there are efforts to domesticate some of the plants to avoid extinction and at the same time help fight food insecurity and malnutrition. This has been boosted by numerous research conducted around them. Traditional crops in this area have received a considerable evaluation related to their nutritional, pharmacological, and toxicological properties (Abukutsa-Onyango, 2003; Ogoye-Ndegwa & Aagaard-Hansen 2003; Orech *et al*., 2007b). Most of them are a major source of micro-nutrients and are, at the same time, toxicologically safe. According to Orech *et al.,* (2005), some traditional vegetables contain possible agents that can cause acute or chronic toxicities when consumed in large quantities or over a long period of time. However, it was reported that Luo women know such species and prepare them using traditional cooking methods to make consumption of the vegetables safe.

Similarly, traditional animal foods have received their fair share of interest in the scientific world. Edible insects have been evaluated for their distribution and abundance in Lake Victoria region of Western Kenya (Nyeko & Olubayo, 2005; Ayieko *et al*., 2010) in addition to their consumption and associated cultural values (Ayieko & Oriaro, 2008; Ayieko & Nyambuga, 2009). A modern trap to harvest *agoro* winged termites has also been developed and tested (Ayieko *et al.*, 2011). Recently, a lot of interest has also been directed at evaluating the nutritional potential of some insects consumed among the Luo of Western Kenya. Some insects have been found to be a rich source of minerals, fat soluble vitamins and even highly digestible proteins (Christensen et al., 2006; Ayieko, 2007; Kinyuru *et al.,* 2010a; Kinyuru *et al.,* 2010b). Use of edible insects as a novel ingredient in conventional food products has also been documented. Kinyuru *et al.,* (2009) developed baked wheat-termite buns with a significant portion of the ingredients being edible termites while other varied products have been developed by Ayieko *et al*., (2010). Research has shown that consumption of edible larva of *Cirina forda* (Westwood) in Nigeria does not pre-dispose neurotoxicity or hepatotoxicity to study animals (Akinawo *et al.,*

2005). This can be further complemented by the fact that no insects consumption related toxicity disaster has been reported by the consuming communities in Kenya.

The *dagaa* (*R. argentea*) has played a very important role in job creation, nutrition, income generation and food security, especially during the dry seasons when agricultural activities are reduced to a minimum (Bille & Shemkai, 2006). There are, however, considerable quality losses of these tiny fish as a result of the lack of modern fish processing and preservation methods (Abila & Jansen, 1997). They are still processed and preserved by the traditional sun drying method (Bille & Shemkai, 2006). Consumption of *dagaa* could be greatly enhanced if they are processed using modern and improved preservation and processing methods. Their use in complementary foods will have to be carefully considered because of the fact that they quickly lose quality leading to a bitter and soapy taste and as such contributing to low acceptability (Dampha *et al*., 1995). Their superiority in micro-nutrient density, fatty acids and proteins makes them a suitable food for complementary feeding. Currently, cottage industries have gone into blending *dagaa* with other flours for use by infants.

Grain amaranth has the potential to contribute to the nutritional needs of vulnerable individuals because of its high protein content, superior protein quality, high content of essential fatty acids and micronutrients (Tagwira *et al.,* 2006). Amaranth has been associated with aiding recovery of severely acutely malnourished children and an increase in the body mass index of people formerly wasted by HIV/AIDS (SRLP, 2005; Tagwira *et al.,* 2006). Consumption of amaranth has also been associated with higher milk production among breast feeding mothers (Muyonga *et al*., 2008).

In conclusion, the Luo of Kisumu West District are small-scale farmers who adhere mostly to traditional subsistence agricultural practices. In the past, most village folks were knowledgeable about both domesticated and wild traditional leafy vegetables. However, presently only a few individuals are likely to recognize all traditional leafy vegetables. The study showed traditional foods diversity, with nutritional and health potential to the local communities. Traditional food processing methods identified that could be further improved and exploited. In order to exploit the species possessing potential to provide nutrient, health and economic benefits, further analysis on nutrients and anti- nutrients needs to be done.

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References

Abila, R. O., & Jansen, E. G. (1997). From local to global market-the fish exporting and fishmeal industries of Lake Victoria: Structure, strategies and social economic impact in Kenya. Report No. 2 In: Social Economics of Lake Victoria Fisheries. IUCN East Africa Program, Nairobi, Kenya.

Abukutsa-Onyango, M. A. (2003). The role of African indigenous vegetables in poverty alleviation in Kenya. Proceedings of the 1st PROTA International Workshop, 23-25 September 2002, Nairobi, Kenya. Prota Foundation. Wageningen University, The Netherlands. pp 269-270.

Akinnawo, O. O., Abatan, M. O., & Ketiku, A. O. (2002). Toxicological study on the edible larva of *Cirina forda* (Westwood). *African Journal of Biomedical Research, 5*, 43-46.

Ayieko M. A., Ndong'a F. O., & Tamale A. (2010). Climate change and the abundance of edible insects in the Lake Victoria Region. *Journal of Cell and Animal Biology, 4*(7), 112-118.

Ayieko, A. M. (2007). Nutritional value of selected species of reproductive *Isoptera* and *Ephemeroptera* within the ASAL of Lake Victoria basin. *Journal of Discovery and Innovations, 19*(2), 126-130.

Ayieko, M. A., & Nyambuga, I. A. (2009). Termites and lake flies in the livelihood of households within the Lake Victoria region: Methods for harvesting and utilization. Technical report for the National Museums of Kenya, Research Program on Sustainable Use of Dryland Biodiversity, National Museums of Kenya, Nairobi pp 1-11.

Ayieko, M. A., & Oriaro, V. (2008). Consumption, indigenous knowledge and cultural values of lake flies species within the Lake Victoria region. *African Journal of Environment and Technology, 2*(10), 282-286.

Ayieko, M. A., Obonyo, G. O., Odhiambo, J. A., Ogweno, P. L., Achacha, J., & Anyango, J. (2011). Constructing and using a light trap harvester: rural technology for mass collection of *agoro* termites (*Macrotermes subhylanus*). *Research Journal of Applied Sciences, Engineering and Technology, 3*(2), 105-109.

Ayieko, M. A., Oriamo, V., & Nyambuga, I. A. (2010). Processed products of termites and lake flies: Improving entomophagy for food security within the Lake Victoria region. *African Journal of Food Agriculture Nutrition and Development, 10*(2), 2085-2098. http://dx.doi.org/10.4314/ajfand.v10i2.53352

Bille, P. G., & Shemkai, R. H. (2006). Process development, nutrition and sensory characteristics of spiced-smoked and sun-dried dagaa (*Rastrineobola argentea*) from Lake Victoria, Tanzania. *African Journal of Food, Agriculture, Nutrition and Development, 6*(2), 1-12.

Christensen, D. L., Orech, F. O., Mungai, M. N., Larsen, T., Friss, H., & Aagaard-Hansen, J. (2006). Entomophagy among the Luo of Kenya: A potential mineral source? *International Journal of Food Sciences and Nutrition, 57*(3/4), 198 - 203. http://dx.doi.org/10.1080/09637480600738252

Dampha, N. J., Kishe M., & Shemkai, R. H. (1995). Mwongozo wa ubanikaji wa dagaa wa ziwa Victoria kwa moshi, chumvi na pilipili. (Procedure for smoking Dagaa of Lake Victoria with Salt and Pepper). UNDP/FAO-Mradi wa Uvuvi Kagera (Fishery project at Kagera)URT/90/005, Kagera, Tanzania.

FAO. (1988). Traditional food plants. FAO Food and Nutrition Paper 42, Food and Agriculture Organization of the United Nations, Rome.

Geissler, P. W., Harris, S. A., Prince, R. J., Olsen, A., Odhiambo, R. A., Oketch-Rabah, H., Madiega, P. A., Anderson, A., & Mølgaard, P. (2002). Medicinal plants used by Luo mothers and children in Bondo District, Kenya. *Journal Ethnopharmacology, 83*, 39-54. http://dx.doi.org/10.1016/S0378-8741(02)00191-5

Grenier, L. (1998). Working with Indigenous Knowledge: A Guide for Researchers. IDRC, Ottawa.

Johns T., & Kokwaro, J. O. (1991). Food Plants of the Luo of Siaya District, Kenya. *Economic Botany*, *45*(1), 103-113. http://dx.doi.org/10.1007/BF02860055

Kiambi, D., & Atta-Krah, K. (2003). Plant genetic resources in the global and African setting. Proceedings of the 1st PROTA International Workshop, 23-25 September 2002, Nairobi, Kenya. Prota Foundation. Wageningen University, The Netherlands. pp 269-270.

Kinyuru, J. N., & Muchui, M. N. (2009). Post harvest handling of African Leafy Vegetables in Kenya: Progress, constraints, opportunities and way forward. Proceedings: 9th workshop on sustainable horticultural production in the tropics. Value chains of vegetables for local market, export and processing. Snowcrest Hotel, Arusha, Tanzania, 2nd – 5th December, 2009.

Kinyuru, J. N., Kenji, G. M., & Njoroge, M. S. (2009). Process development, nutrition and sensory qualities of wheat buns enriched with edible termites (*Macrotermes subhylanus*) from Lake Victoria region, Kenya. *African Journal of Food Agriculture, Nutrition and Development, 9*(8), 1739-1750.

Kinyuru, J. N., Kenji, G. M., Njoroge, S. M., & Ayieko, M. (2010a). Effect of processing methods on the in-vitro protein digestibility and vitamin content of edible winged termite (*Macrotermes subhylanus*) and grasshopper (*Ruspolia differens*). *Food and Bioprocess Technology, 3*(5), 778-782. http://dx.doi.org/10.1007/s11947-009-0264-1

Kinyuru, J. N., Kenji, G. M., Njoroge, S. M., & Ayieko, M. (2010b). Nutritional potential of longhorn grasshopper (*Ruspolia differens*) consumed in Siaya District. *Journal of Agriculture, Science and Technology, 12*(1), 32-46.

KNBS. (2010). Kenya National Bureau of Statistics. 2009 population and housing census highlights. http://www.knbs.or.ke/Census%20Results/KNBS%20Brochure.pdf (Accessed on 4th August 2010).

Lindeberg, S., Cordain, L., & Eaton, S. B. (2003). Biological and clinical potential of a paleolithic diet. *Journal of Nutrition and Environmental Medicine, 13*(3), 149-160. http://dx.doi.org/10.1080/13590840310001619397

Lukmwaji, Z., Hertzmark, E., Mlingi, N., Assey, V., Ndossi, G., & Fawzi, W. (2008). Tanzania Food Composition Tables. MUHAS- TFNC, HSPH, Dar es Salaam, Tanzania.

Martin, G. J. (1995). Ethnobotany: A methods manual. WWF, UNESCO & RBG, Kew, Chapman and Hall.

Muyonga, J. H., Nabakabya, D., Nakimbugwe, D. N., & Masinde, D. (2008). Chapter 8: Using Food Science and Technology to Improve Nutrition and Promote National Development. Efforts to promote amaranth production

and consumption in Uganda to fight malnutrition. Robertson, GL Lupien JR (Eds), International Union of Food Science & Technology.

Nyeko, P., & Olubayo, F. M. (2005). Participatory assessment of farmers' experiences of termite problems in agroforestry in Tororo District, Uganda. *Agricultural Research and Extension Network, 143*, 1-10.

Ogoye-Ndegwa, C., Abudho, D., & Aagaard-Hansen, J. (2002). New learning in old organizations': Children's participation in a school-based nutrition project in western Kenya. *Development Practice, 12*(3&4), 449-460. http://dx.doi.org/10.1080/0961450220149799

Ogoye-Ndegwa, C., & Aagaard-Hansen, J. (2003). Traditional gathering of wild vegetables among the luo of western kenya - A nutritional anthropology project. *Ecology of Food and Nutrition, 42,* 69-89. http://dx.doi.org/10.1080/03670240303114

Orech, F. O., Aagaard-Hansen, J., & Friis, H. (2007). Ethnoecology of traditional leafy vegetables of the Luo people of Bondo district, western Kenya. *International Journal of Food Sciences and Nutrition, 58*(7), 522-530. http://dx.doi.org/10.1080/09637480701331163

Orech, F. O., Akenga, T, Ochora, J., Friis, H., & Aagaard-Hansen, J. (2005). Potential toxicity of some traditional leafy vegetables consumed in Nyang'oma Division, Western Kenya. *Journal of Food Agriculture, Nutrition and Development, 5*(1), 1-14.

Orech, F. O., Christensen, D. L., Larsen, T., Friis, H., Aagaard-Hansen, J., & Estambale, B. A. (2007). Mineral content of traditional leafy vegetables from Western Kenya. *International Journal of Food Sciences and Nutrition, 58*(8), 595-602. http://dx.doi.org/10.1080/09637480701350288

Roos, N., Thorseng, H., Chamnan, C., Larsen, T., Gondolf, U. H., Bukhave, K., & Thilsted, S. H. (2007). Iron content in common Cambodian fish species: Perspectives for dietary iron intake in poor, rural households. *Food Chemistry, 104,* 1226–123. http://dx.doi.org/10.1016/j.foodchem.2007.01.038

Ruel, M., & Levin, C. (2000). Assessing the potential for food-based strategies to reduce vitamin a and iron deficiencies: A review of recent evidence. Food Consumption and Nutrition Division. International Food Policy Research Institute 2033 K Street, N.W. Washington, D.C. 20006 U.S.A.

Sehmi, K. J. (1993). National Food Composition Tables and the Planning of Satisfactory Diets in Kenya. Government Printers, Nairobi.

Sorensen, A. D., Hilmer, S., Charlotte, B., Keld, E. A., Ib, S., Susanne, S., & Klaus, B. (2007). Matrix effects of lupine (*Lupinus leteus* L.) and rape seed (*Brassica napus* L.) products on *in-vitro* non-heme iron availability from pork meat. *Journal of Food Composition and Analysis, 20,* 515-522. http://dx.doi.org/10.1016/j.jfca.2006.12.007

SRLP. (2005). Sustainable Rural Livelihoods Program. Annual Report, 2004-2005.

Tagwira, M., Tagwira, F., Dugger, R., & Okum, B. (2006). Using grain amaranth to fight malnutrition. RUFORUM working document, No. 1, pp. 201-206.

Wanink, J. H. (1999). Prospects for the fishery on the small pelagic *Rastrineobola argentea* in Lake Victoria. *Hydrobiologia, 407*, 183–189. http://dx.doi.org/10.1023/A:1003708624899

Table 1. Vegetables, fruits and their edible parts according to interviewees

Local name	Common name	Scientific name	Edible parts
Anyuka	Forest star	*Mussaenda arcuata*	Fruit with seed
Apilo	Pepper	*Capsicum frutescens L.*	Leaves & fruits
Nderma	Climbing spinach	*Basella alba* L.	Leaves
Onyiego	Black jack	*Bidens pilosa* L.	Leaves
Oganda	Common bean	*Phaseolus vulgaris* L.	Leaves
Ododo	Amaranth	*Amaranthus hybridus* L.	Leaves
Ododo	Amaranth	*Amaranthus cruentus* L.	Leaves
Awayo	Oxalis	*Oxalis latifolia* Kunth	Leaves
Onyulo	Sesame	*Sesamum calycimum* Welw. *var. angustifolium (Oliv.)*	Leaves
Ochuoga	Bush plum	*Carissa spinarum L.*	Fruit with seed
Atipa	-	*Asystasia mysorensis* T. Anderson	Leaves & fruits
Akeyo/Dek	Spider plant	*Gynandropsis gynandra* (L.) Briq.	Leaves
Omwogo	Cassava	*Manihot esculentum* Crantz.	Leaves
Budho	Cucumber	*Cucumis africanus* L.	Leaves
Apoth	Jute, Jew's mallow	*Corchurus olitorius* L.	Leaves
Susa budho	Pumpkin	*Curcurbita maxima* Duchesne ex. Lam.	Leaves & fruits
Osao	Egyptian riverhemp	*Sesbania sesban* L.	Leaves
Kandhira	Wild Mustard, Ethiopian cabbage	*Brassica juncea* (L.) Czern.	Leaves
Mitoo	Slender leaf rattlebox	*Crotalaria ochroleuca* G. Don.	Leaves
Osuga	African nightshade	*Solanum nigrum* L.	Leaves
Bo	Cowpeas	*Vigna unguiculata* (L.) Walp.	Leaves
Mapera	Guava	*Psidium guajavum* L.	Fruit with seed
Rabuon nyaluo	Sweet potato	*Ipomoea batatas* (L.) Lam.	Leaves
Odielo	Wandering jew	*Commelina Africana L.*	Whole plant
Nyim	Simsim	*Sesamum indicum* L.	Leaves
Achak	Wild lettuce	*Launaea cornuta* (Hochst. Ex Oliv. & Hiern) C. Jeffrey	Leaves

- [a] : Common names lacking

Table 2. Cereal grains, pulses, seeds, tuber/root foods and their edible parts to interviewees

Local name	Common name	Scientific name	Part consumed
Bel	Sorghum	*Sorghum bicolor* (L.) Moench	Grains
Omwogo	Cassava	*Manihot esculentum* Crantz.	Tubers
Kal	Finger millet	*Eleusine coracana* Gaertn.	Grains
Rabuon	Sweet potato	*Ipomoea batatas* (L.) Lam.	Tubers
Rabuon nduma	Yam	*Dioscorea alata* L.	Tubers
Oduma	White maize	*Zea mays* L.	Grains
Nyim	Simsim	*Sesamum indicum* L.	Seeds
Ng'or	Pigeon peas	*Vigna unguiculata*	Pulses
Ododo	Amaranthus	*Amaranthus cruentus* L.	Grains

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Table 3. Traditional animal source foods and their edible parts to interviewees

Local name	Common name	Scientific name	Edible parts
Sisi	Winged termite	*Pseudacanthotermes militaris* (Hagen)	Whole;de-winged
Riwo	Winged termite	*Macrotermes bellicosus* (Smeathman)	Whole; de-winged
Agoro	Winged termite	*Macrotermes subhylanus* (Rambur)	Whole; de-winged
Oyala	Winged termite	*Pseudacanthotermes spiniger (*Sjostedt*)*	Whole; de-winged
Onyoso	Winged black ant	*Carebara vidua* (Smith)	Abdomen; de-winged; whole
Tsenesene	Long-horned grasshopper	*Ruspolia differens* (Serville)	Whole; de-winged
Omena	Dagaa fish	*Rastrineobola argentea* (Pellegrin)	Whole

Table 4. Nutritional and health benefits, availability of selected foods according to key informants

Common name	Scientific name	Nutritional & health benefits	Availability of the food	Iron[1] (mg/100g)	Zinc[2] (mg/100g)
Finger millet	*Leusine coracana* Gaertn.	Source of energy	High	20.0	1.2
Sorghum	*Sorghum bicolor* L.	Source of energy	High	13.0	0.8
Amaranth grain	*Amaranthus cruentus* L.	Increases breast milk in mothers; Increases energy & blood; Reduces constipation	High	21.0	-
Simsim	*Sesamum indicum* L.	Body building & source of energy	High	9.5	7.8
White maize	*Zea mays* L.	Source of energy	High	4.5	1.8
Jute	*Corchurus olitorius* L.	Increases blood and energy	Low	25.0	-
Oxalis	*Oxalis latifolia* Kunth	Enhances appetite; Source of energy	Low	28.0	-
Cassava leaves	*Manihot esculenta* Crantz	Source of energy	Moderate	7.7	0.4
Spider plant	*Gynandropsis gynandra* (L.) Briq.	Prevents stomach pains and bloating; Prevents difficult deliveries in pregnant women; Increases blood and energy	High	30.0	-
Commelina	*Commelina africana* L.	Source of energy & increases blood; Reduces constipation	Moderate	28.0	-
-	*Asystasia mysorensis* T. Anderson	Prevents stomach pains and bloating; Reduces joints pains & increases blood	Moderate	6.0	-
Winged termite	*Macrotermes subhylanus* (Rambur)	Body building; Increases blood	Moderate	21.0	2.5
Dagaa fish	*Rastrineobola argentea* (Pellegrin)	Body building ; Promotes good health	High	7.0	5.2

[1]Source: National food composition tables and the planning of satisfactory diets in Kenya (Sehmi 1993); [2]Source: Tanzania food composition tables (Lukmwanji *et al*., 2008); - : Common names or values missing

Table 5. Traditional food preparation methods of food groups according to interviewees

Food group	Preparation method
Vegetables	Boiling
Cereals	Milling, boiling to a porridge
Roots/tubers	Pounding, fermenting
Legumes	Pounding
Seeds	Pounding
Insect	Sun-drying, frying
Fish	Boiling

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Assessment of Physico-chemical Characteristics, Viability and Inhibitory Effect of Bifidobacteria in Soymilk

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Abstract

The study investigated viability of *Bifidobacteria* isolates from breast-fed infants in soymilk with the aim of producing a beverage with health- improving potentials from an affordable source.

Strains of *Bifidobacteria* spp isolated from breast-fed babies were identified on the basis of morphological and biochemical characteristics. The pure isolates were inoculated into sterile soymilk and their viability was studied at ambient (28 ± 2^{o}C) and refrigeration temperatures (4 ± 1^{o}C) The bifidobacteria isolates were identified as *B. adolescentis* and *Bifidobacterium longum*. The inhibitory effects of the *Bifidobacteria* isolates were tested on *Escherichia coli* and *Staphylococcus aureus. B. longum* was found to be viable for 35 days and inhibited *Escherichia coli* better than *Staphylococcus aureus*. Changes in the physico-chemical characteristics of the inoculated soymilk were also monitored over the storage period.

Crude protein increased from 1.76 to 2.14% and 1.96% in soymilk samples stored at ambient and refrigeration temperatures respectively.

The study has shown that *B. longum* which is supported by soymilk exhibits antimicrobial activity against tested food borne pathogens – indicating that probiotic soymilk could be produced from *B. longum* and soymilk.

Keywords: Ambient, Refrigeration, *Bifidobacterium*, Inhibitory, Soymilk, Viability

1. Introduction

Probiotics are live microorganisms which when ingested enhance the well-being of the host through their effects on the intestinal microflora (Havenaar & Veld, 1992; Aunty *et al.,* 2001). Other reported positive effects of probiotics include digestion of lactose, synthesis of vitamins and lowering of serum cholesterol levels (Aramide *et al.,* 2009). They contribute to biological defense mechanisms, prevention of diseases, recovery from diseases, and control of physical conditions and influence the ageing process (Patidar & Prajapati, 1997). From the foregoing, incorporation of probiotic bacteria with scientifically supported health claims in foods has great potentials for improving the quality of life.

The most widely used probiotics belong to the genera *Lactobacillus* and *Bifidobacterium* (Bowley, 2005). *Bifidobacterium* is the dominant bacteria in the intestinal microflora of breast-fed infants (Abe, 2004). For a probiotic bacteria to benefit human health it must be able to withstand the manufacturing process without losing its viability and functionality when incorporated into food products (Saarela *et al.*, 2000). Dairy products have been employed in the food industry and embraced by many consumers as vehicle of delivering probiotic microorganisms. Various types of fermented milk products including bifidus milk, and bifidus-acidophilus milk are examples of popular *probioticated* products obtained with Bifidobacteria (Kurman & Rasic, 1993; Patidar & Prajapati, 1997).

Currently, efforts are being made to use plant sources such as roselle extract, ginger and garlic extract as substrates for cultivating probiotics (Aramide *et al.*, 2009, Adeniran *et al.*, 2010). Soy products have been suggested as the best substitute for dairy products (Tsangali *et al.*, 2002). Among the reasons for this are low cost, being lactose-free, non-allergenecity and low technology required. It is reported that soymilk can be produced for about one third to one half the cost of cow's milk and a unit area of the land can produce about 10 times as much soymilk per year as dairy milk (Samona, 1993). Thus it is important that the developing countries begin to think of partially

replacing the increasingly expensive dairy products with high quality soymilk (Nsofor & Osuji, 1997). In addition, lactose intolerant people feel discomfort and pain, usually accompanied by diarrhea when they consume dairy milk (Poskitt, 1993). Soymilk can be consumed by these groups of people since it contains no lactose and has been reported to be an excellent dairy milk substitute (Nsofor & Osuji, 1997; Omoni & Aluko, 2005).

Furthermore, the other documented health advantage of consuming soymilk is its being cardio protective. That is, it contains beneficial cholesterol, not the bad cholesterol that can cause thrombosis and high blood pressure. A diet high in soy foods has been associated with reduced risk of several types of cancer, such as breast, endometrial and prostate. Soy isoflavones' antioxidant properties likely contribute to soy foods' anti-cancer effects by reducing free radical damage to fats (lipid peroxidation) and DNA. Soy isoflavones may also induce Phase II liver enzymes (such as glutathione-s-transferase and quinone reductase), which help the body eliminate the toxic byproducts (aromatic hydrocarbons) produced when meats are charbroiled (Azadbakht *et al*., 2003). Soymilk has also been found to be capable of reducing Type 2 diabetes, kidney and health diseases (Villegas *et al*., 2008)

This study therefore examined the viability of *Bifidobacteria* species isolated from breast-fed babies in soymilk for five weeks. The isolates were earlier characterized and their inhibitory effects on potentially pathogenic organisms in soymilk evaluated. Accompanying changes in physic-chemical characteristics of the inoculated soymilk were also monitored over a period of storage.

2. Materials and Methods

Soybeans were purchased from the main market, Ile-Ife, Nigeria. The pathogenic organisms, *Escherichia coli* (type strain NCIB 86) and *Staphylococcus aureus* (type strain NCIB 8588) used were obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

2.1 Methods

The experiment included isolation of *Bifidobacteria* and *Lactobacilli* spp from faecal samples from breast-fed babies, characterization of the microbes, preparation of soymilk, inoculation of the milk with the bacterial isolates and determination of inhibitory effect of probiotic microbe with pathogenic organisms.

2.2 Preparation of soymilk

Soybeans were processed into milk using the method shown in flow chart of Figure 1. Subsequently, 27ml of the prepared soymilk was dispensed into 30ml screw capped test tubes and heat treated in an autoclave at 115 ^{0}C for 20*mins*.

2.3 Microbiological analyses

Media for cultivation, isolation and identification of bacterial isolates were of analytical grade (Oxoid, England); and were prepared using standard methods. All media for bacteria cultivation and identification were sterilized by autoclaving at 121 ^{0}C for 15*mins*.

2.3.1 Isolation of *Bifidobacterium* and *lactobacillus* species from faecal samples

Faecal samples (20 samples) from healthy breast fed babies were collected from the Children's immunization section of the Obafemi Awolowo University, Teaching Hospital complex, IleIfe, Nigeria into sterile McCartney bottles and immediately transported to the laboratory.

Isolation of *Bifidobacteria* was done by mixing one gram of each of the faecal samples with 9ml of reinforced clostridial medium (a diluent for anaerobes) (Harrigan & McCance, 1976; Harrigan, 1998). Serial dilutions were done and pour plates were made with a selective medium, trypticase peptone yeast extract agar (TYP) supplemented with 0.1% sodium thioglycholate and 0.5% propionic acid (Olutiola *et al*., 1991; Yusof *et al*., 2000). Plates were incubated at 37 ^{0}C for 72*h* under anaerobic condition using anaerobic jar and gas generating kit (Oxoid; Basing stoke, U.K). From the diluted samples, Lactobacilli were isolated by mixing 1 ml of samples with de Man Rogosa and Sharpe agar, (MRS) containing 1% Sodium azide. The 1% sodium azide provided micro aerophilic condition (Harrigan & McCance, 1976; Brankovi & Baras, 2001). Plates were incubated at 37 ^{0}C for 72*h* under aerobic conditions.

2.3.2 Identification of bacterial isolates

Representative colonies on trypticase peptone yeast extract agar and de Man Rogosa and Sharpe agar were picked on the basis of their cultural characteristics, and purified by repeated streaking on the fresh sterile media plates and incubated. Pure cultures obtained were coded and transferred to agar stabs and slants respectively and stored in the refrigerator until required for identification. The bacteria isolates were identified based on their cultural, morphological and biochemical characteristics following the schemes of Harrigan and McCance (1976) and

Sgorbati *et al.* (1995). Characterization was based on cultural and microscopic morphology, Gram's reaction, growth characteristics, and selected biochemical tests including catalase test, nitrate reduction, ammonia reduction, and sugar fermentation test.

2.3.3 Preparation of test samples for inoculation

(a) Prepared sterile soymilk (27ml) was dispensed into each of the test tubes and each tube was inoculated with 3ml of pure *Bifidobacteria* culture at 10% (v/v) concentration. A set of the samples was stored at ambient temperature (28 ± 2 ^{o}C) while the other set was refrigerated (4 ± 1^{o}C) for viability test.

(b) For the antagonism test, equal number of tubes containing 28ml of sterile soymilk was each inoculated with 1ml of the *Bifidobacteria* culture and 1ml of 24*h* old culture of *E. coli.* The test samples were stored at ambient temperature.

(c) Another set of tubes containing 28ml of sterile soymilk were each inoculated with1ml of the *Bifidobacteria* culture and 1ml of 24*h* old culture of *Staphylococcus aureus*. Samples were kept at ambient temperature.

(d) Equal number of tubes as in (b and c) above containing the sterile soymilk was each inoculated with 1ml 24*h* old culture of *E. coli.* This served as control samples for the test samples in (b) above. The control samples were also stored at ambient temperature.

(e) The same number of tubes with each containing 28ml of sterile soymilk as in (d) above was each inoculated with 1ml of 24*h* culture of *Staphylococcus aureus.* This served as control for (d) above. Samples were kept at ambient temperature.

2.3.4 Determination of the viability of *Bifidobacteria* isolates in soymilk

Counts of the *Bifidobacteria* were determined weekly for 5 weeks from inoculated soymilk samples stored at both ambient and refrigeration temperatures. From each tube, 1ml was pipetted into 9ml of reinforced clostridial medium, mixed thoroughly and serial dilutions of the mixture were made and the microbe enumerated using pour plate method. Diluted samples were poured trypticase peptone yeast extract agar supplemented with 0.1% ascorbic acid and 0.5% propionic acid (Hoover, 1999). The plates were incubated at 37 ^{0}C under anaerobic condition for 72*h* and the colony count of the *Bifidobacteria* colonies was done using a Gallenkamp colony counter (CNW-300 model) (Harrigan & McCance, 1976; Harrigan, 1998)

2.3.5 B*In vitro* antagonism of *Bifidobacteria* isolates and pathogenic strains in soymilk

The inhibiting effect of the selected *Bifidobacteria* species on food borne bacterial pathogens was assayed by the broth culture method described by Visser & Holzapfel (1992) and Kobayashi *et al.,* (2003). Samples were taken from each test tube of soymilk inoculated with 1ml of *Bifidobacteria* culture and 1ml each of 24*h* cultures of *E. coli* and *S. aureus* respectively, and from each tube of the soymilk samples inoculated with 1ml each of the pure cultures of the pathogenic organisms all stored on the shelf at room temperature, weekly for 5 weeks for counts of the pathogens.

2.3.5.1 *Escherichia coli*

Counts for *E. coli* was accomplished by mixing 1ml of the soymilk (containing Bifidobacteria culture and 1ml each of 24*h* cultures of *E. coli*) into 9ml of 0.1% peptone water diluent (Harrigan & McCance, 1976) and mixed thoroughly. Serial dilution of each of the inoculated samples was carried out. From each diluted sample, 1ml was pipetted into each plate and about 20ml of MacConkey agar was introduced into each (Harrigan and McCance, 1976; Olutiola *et al*., 1991). The plates were incubated aerobically at 37 ^{0}C and observed after 24*h* (Yusof *et al.*, 2000). Counts were achieved using a Gallenkamp colony counter (CNW-300 model).

2.3.5.2 *Staphylococcus aureus*

Counts of *S. aureus* was achieved by mixing 1ml of the sample (containing *Bifidobacteria* culture and 1ml each of 24*h* cultures of *S. aureus* overnight culture of *S. aureus* into 9ml of a 0.1% peptone water diluent (Harrigan and McCance, 1976; Harrigan, 1998) and mixed thoroughly. Serial dilution of the inoculated samples was carried out and pour plates made with Mannitol Salt agar. The plates were incubated aerobically at 37 ^{0}C and observed after 24*h*. Counts were done using a Gallenkamp colony counter (CNW-300 model).

2.4 Determination

2.4.1 pH, Titratable acidity and total solids determination

The method of AOAC (2000) was employed in determination of pH, Titratable acidity (TTA) and Total Solids

2.4.2 Determination of Amino Nitrogen

Amino nitrogen was determined using the Formol titration method (Frais, 1972). The formaldehyde used in this analysis was first neutralized with standardized 0.1M sodium hydroxide solution. Five ml of each soymilk sample were put in conical flasks and two to three drops phenolphthalein added. These were then neutralized with standardized 0.1M sodium hydroxide solution, adding the alkali dropwise until the faintest permanent pink color was produced. Six milliliters of the neutralized formaldehyde solution were added to each of the neutralized soymilk samples. The disappearance of the pink color was observed, and then the contents of the flasks were titrated against the standard sodium hydroxide solution until a pink colour was produced as before, the titre values were recorded and used in calculating the amino nitrogen present in each sample.

2.4.3 Determination of total reducing sugar.

Total reducing sugar was determined by the use of Dinitrosalicylic reagent (Miller, 1959 & Adeniran *et al*., 2008). The soymilk samples were diluted to 4 times its volume with distilled water and filtered through a paper filter placed in a funnel. One ml of each of the sample's filtrate was put in test tubes and 1ml distilled water was put in another test tube as blank after which 1ml of DNSA was added to each of the test tubes and boiled for 5mins. After boiling, 10 mls of distilled water were added to each tube and the absorbance read at 540 nm in a Spectrophotometer (CECIL CE 3041 model). The reducing sugar concentrations of the samples were read from a calibration curve prepared by measuring the absorbance of varying concentrations of glucose and DNSA reagent at 540nm.

2.4.4 Determination of Proximate Composition of inoculated soymilk

AOAC (2000) method was used to determine the proximate composition of the soymilk after inoculation with *B. longum* and during subsequent storage at ambient and refrigerated temperatures.

2.5 Statistical analysis

The values obtained from each of the analyses were means of triplicate readings. Origin Pro (1999-20020) computer package was employed to analyze the experimental data generated.

3. Results and Discussion

3.1 Identification of human intestinal probiotic strains

Strains of *Bifidobacteria* isolated from fecal samples of breast fed babies were characterized as shown in Table 1. They were identified on the basis of their unique morphology of V- or Y-shape or being multiple branched rods. This is an important differentiating property between the two related genera *Bifidobacterium* and *Lactobacillus* as (Mitsuoka, 1992; Sgorbati *et al*., 1995; Brankovi & Baras, 2001). The bifidobacteria isolates were identified as *Bifidobacterium longum* and *Bifidobacterium adolescentis* based on their fermentation characteristics. Both species have been reported to be a part of the faecal bacteria population in breast fed babies (Mitsuoka, 1992; Sgorbati *et al*., 1995). Most of the Bifidobacteria isolates in this study were identified as *Bifidobacterium longum.* It is considered the commonest species of *Bifidobacteria* found both in infant and adult faecal material (Sgorbati *et al.*, 1995).

The DNA of *B. longum* is said to be 50 – 76% related to that of *Bifidobacterium infantis*. This close genetic relationship is reflected in their similar fermentation activity (Sgorbati *et al.*, 1995) and the identification of strains belonging to one of the two species is reported to remain a difficult task (Sgorbati *et al*., 1995). For identification, Scardovi (1986) suggested that strains able to ferment arabinose and melezitose be regarded as *B. longum* while strains that cannot ferment arabinose be held as *B. infantis.*

The *Lactobacillus* strains isolated were characterized on basis of their morphology and fermentation profile as *Lactobacillus fermentum, Lactobacillus plantarum* and *Lactobacillus salivarius* (Mitsuoka, 1992).

3.2 Viability of the Bifidobacteria isolates in soymilk.

Bifidobacterium longum, which was inoculated into the sterile soymilk, proliferated in soymilk as shown in Figure 2. An initial increase in population was observed in samples stored at both storage temperatures; this was followed by a decrease from the 21st day onward. This observation agrees with the finding of Gilliland *et al.* (2002) in which the growth of *B. longum* was monitored in a fermented milk product over a period of 35 days. This decrease after 21 days could be explained as being the result of diminishing nutrient supply (Tsangalis *et al*., 2002) and decreasing pH below values required for optimum growth (Hoover, 1999). Aramide *et al.,* (2009) found *Lactobacillus acidophilus* and *L. plantarum* viable in roselle extract after 27 days of storage at ambient temperature while Adeniran *et al.,* (2010) also reported that after 5 weeks of storage of inoculated samples at

ambient temperature, *L. bulgaricus* and *L. plantarum* were still viable in ginger extract. Finding of the present study is in agreement with similar studies on *Lactobacillus* spp on plant-based beverages.

The rate of population reduction was lower in the refrigerated samples as expected. A slight decrease in bacteria population during the first seven days of refrigeration was also observed. This could be due to the sudden cold storage (Hoover, 1999; Prescott *et al*., 1999, Adeniran *et al.*, 2010) or cold shock effects on the microbial cells, a phenomenon called the lag phase. This also accounted for the lower Bifidobacteria counts observed when compared to samples stored at room temperature especially at periods of maximum microbial growth, day 14 and 21. Generally, the growth of microorganisms and in particular Bifidobacteria is known to slow down at low temperatures (Hoover, 1999; Gilliland *et al*., 2002).

The higher counts in the refrigerated samples on the 28th and 35th day could be as a result of residual nutrients in the soymilk which was being slowly used up by the growing microbial population and also as the result of the slightly higher pH values which were within the pH values for the optimum growth of Bifidobacteria (Hoover, 1999; Gilliland *et al*., 2002). The values were further subjected to statistical analysis and the correlation between storage at room and refrigeration temperatures were found not to be statistically significant at 5% level. This suggests that the soymilk *probioticated* with *B. longum* could be stored at both temperatures without significant reduction in the bacterial counts.

B. *adolescentis*, on the other hand, was observed to be viable in the inoculated soymilk for less than 3 days at ambient and refrigeration temperatures. No viable count of the isolate was recorded at 3rd day of incubation in the soymilk samples. The reason for the non-proliferation of this organism in soymilk could be due to its inability to metabolize the predominant sugars in the soymilk, which were mainly raffinose and stachyose requiring α-galactosidase activity for metabolism (Hoover, 1999; Tsangalis *et al*., 2002). Also, this particular isolate was found to be a strict anaerobe. This could have caused its non-proliferation in the soymilk since steps were not taken to ensure a low oxidation – reduction potential in the medium during storage (Hoover, 1999; Gilliland *et al*., 2002).

3.3 Antimicrobial effect of the isolate Bifidobacterium longu

Presented in Figure 3 is the result of viable counts of food borne pathogens which were cultured with B. longum in soymilk, stored at ambient temperature. This is the temperature at which pathogens grow and produce toxins (Prescott et al., 1999). This result reflects the inhibitory effects of the test probiotic strain against food borne pathogens E. coli and S. aureus. According to earlier findings, Bifidobacteria have been known to help in maintaining healthy functioning of the intestinal tract directly through antimicrobial activity (Danone, 1997). The results obtained in this study show that the pathogens E. coli and S. aureus had higher counts when grown as pure cultures in the soymilk than when grown in combination with the probiotic isolate, throughout the duration of storage. This is in agreement with the results of a study which showed a reduction, and finally the extinction of a plant pathogen treated with the probiotic strain L. plantarum (Visser & Holzapfel, 1992).

Figure 3 also shows that the growth of E.coli was better inhibited compared to the growth of S. aureus. Escherichia coli has been reported to be strongly inhibited by acetic acid, especially by those produced by some strains of the probiotic species B. longum (Kobayashi *et al.*, 2003; Ibrahim *et al.*, 2005) and could be inferred that the inhibitory effect on E.coli observed in Figure 3 could be due to the presence of organic acid (likely acetic acid) produced by the probiotic strain in the soymilk. As with other short chain fatty acids the undissociated form of the molecule acetic acid mediates the antimicrobial effect by collapsing the electrochemical proton gradient causing bacteriostasis and eventual death of susceptible bacteria. Acetic acid and propionic acid have higher pKa values than lactic acid and therefore a higher undissociated ratio at a given pH. This is reported to be one reason for their increased antimicrobial efficacy compared to lactic acid (Earnshaw, 1992).

For sample containing the probiotic strain and the pathogen *S. aureus,* the degree of inhibition by the probiotic strain was observed to be lower and the pathogen remained viable throughout storage duration while for the sample containing the probiotic strain and the pathogen *E*. *coli*, the pathogen remained viable until the 21st day of storage when it was completely inhibited. Findings of Bowley (2005); Ibrahim *et al.* (2005); Aramide *et al.* (2009) and Adeniran *et al*. (2010) affirmed that different probiotic strains exhibit different degree of inhibition against different types of pathogens.

3.4 pH and titratable acidity values of B. longum inoculated soymilk

From Table 2, it can be seen that the pH values of samples stored at ambient temperature showed a decrease from 5.72 on the first day of storage to pH 4.58 on the 7th day compared to refrigerated samples which showed a gradual decrease from 5.72 on the first day of storage to 5.63 on the 7th day. At the end of storage period, the pH was 4.36 and 5.35 respectively for samples stored at ambient and refrigeration temperatures. There was no appreciable

change in the pH of the control samples by the 35th day. The decrease in pH of the test samples was probably due to the production of short chain carboxylic acid such as acetic acid and lactic acid, the major fermentation products of the genus *Bifidobacterium* (Yusof *et al*., 2000; Kobayashi *et al*., 2001; Brankovi & Baras, 2001; Tsangalis *et al*., 2002). The difference in the rate of decrease in the pH of samples stored at ambient temperature compared to those refrigerated was due to relatively higher level of microbial growth and hence increased acid production (Prescott *et al*., 1999). The pH was not low enough to cause a permanent change in the consistency of the soymilk by the end of the storage period.

Result of TTA indicates that that the acidity of the inoculated samples increased with storage time.

3.5 Total solid content

The total solids decreased from 5.16 to 4.43% at the end of the storage period for samples stored at ambient temperature and from 5.16 to 4.50 for samples stored at refrigeration temperature (Table 2). This could be attributed to the uptake of these solids by the growing microbial population (Nsofor *et al*., 1997; Rani & Khetarpaul, 1999).

3.6 Amino Nitrogen and crude protein content

Table 3 shows the changes in amino nitrogen and crude protein content of soymilk samples inoculated with *B. longum* stored at ambient and refrigeration temperatures. An initial increase of the amino nitrogen content was observed from 0.18 to 0.22 mg ml^{-1} in the two sets of *probioticated* soymilk samples. This could be due to partial breakdown of protein to peptide and amino acid (Rani & Khetarpaul, 1999). Thereafter, the amino nitrogen level was observed to be quite stable. It is likely that the amino nitrogen taken up by the multiplying *B. longum* population was being replaced via autolysis of some of the microbial cells in the soymilk. Since nitrogen is required for growth and biosynthesis of components of the bacterial cells, inadequate quantity and poor quality of free amino acids could activate bacterial proteolytic enzymes so that the amino acids might increase as a result of hydrolysis of the milk substrate (Brankovi & Baras, 2001). One of the beneficial effects of *Bifidobacteria* is the preservation of nitrogen content by preventing putrefaction thus it has been reported that the adaptation of *Bifidobacterium* species to gastrointestinal tract enhances nitrogen retention (Gibson *et al*., 1997).

The crude protein content for samples stored at ambient temperature also increased gradually from 1.76 to 2.14% and from 1.76 to 1.96% for samples stored at refrigeration temperature (Table 3). This could have been caused by increase in the production of proteins from the multiplication of microbial cells, which are mainly proteins. Amino nitrogen and crude protein were not significantly different at ambient and refrigeration temperatures of storage ($p > 0.05$).

3.7 Total reducing sugar and carbohydrate content

The total reducing sugar content of the test samples (Table 3) showed an increase by the slowly multiplying *Bifidobacteria* population in the samples from 1.28 to 3.77% for samples stored at ambient temperature and from 1.28 to 2.49% for samples stored at refrigeration temperature by the 7th day of storage. This increase in reducing sugar content of the test samples was possibly due to the production of reducing sugars via hydrolysis of the predominant α – galactosyl oligosaccharides including raffinose and stachyose present in the soymilk (Tsangalis *et al*., 2002). These sugars have been found to be satisfactory fermentable substrate for the growth of bifidobacteria in soymilk (Garro *et al*., 1999; Tsangalis *et al.,* 2002). This is because it has been shown from experimental studies that *Bifidobacterium* species produce intracellular α – galactosidase required to metabolize these sugars (Tsangalis *et al*., 2002). The subsequent reduction in the sugar content of the test samples is suggested to be due to its utilization (Garro *et al*., 1999; Brankori & Baras, 2001). The slightly higher values observed in the refrigerated test samples were due to slower utilization.

The initial sharp reduction in carbohydrate values observed between the 1st and the 7th day of storage from 3.75 to 1.67% for samples stored at ambient temperature could be related to hydrolysis of the carbohydrate by amylolytic enzymes of the probiotic bacteria resulting in the increased reducing sugar production observed during the same period (Rani & Khetarpaul, 1999; Tsangalis *et al*., 2002) and thus starch digestibility.

4. Conclusion

It can be concluded that of the two *Bifidobacteria* species isolated from breast-fed babies, *B. longum* was found to proliferate in soymilk for 35 days. Also, the microbe exhibited antimicrobial activity against *E. coli* and *S. aureus* though to a greater degree in the former than the latter. In addition, soymilk which supported the potentially probiotic *B. longum,* has also been shown to have great potentials in the formulation of probiotic beverages.

References

Abe., F. (2004). Bifidobacteria Promote Infant Health. *Journal of Food Technology International, 1*, 36 – 37.

Adeniran, H. A., Abiose, S. H., & Ogunsua, A. O. (2008). Production of Fungal ∃- amylase and Amyloglucosidase on some Nigerian Agricultural Residues. *Food Bioprocess Technology, 3*(5), 693-698. http://dx.doi.org/10.1007/s11947-008-0141-3

Adeniran, H. A., Abiose, S. H., & Ukeyima, M. T. (2010). Microbiological Assessment of *probioticated* ginger-based beverages. *Nutrition and Food Science, 40*(2), 209-220. http://dx.doi.org/10.1108/00346651011029246

Aramide, A. A., Abiose, S. H., & Adeniran, H. A. (2009). Microbial Evaluation of Probiotic Beverage from Roselle extract. *African Journal of Food Science, 1*(12), 385-392.

Auty, M. A. E., Gardiner, G. E., Mc Brearty, S. J., O'sullivan, E. O., Mulvihill, D. M., Collins, J. K., Fitzgerald, G. F., Stanton, C., & Ross, R. P. (2001). Direct In Situ Viability Assessment of Bacteria in Probiotic Dairy Products Using Viability Staining in Conjunction with Confocal Scanning Laser Microscopy. *Journal of Applied and Environmental Microbiology, 67*(1) 420-425. http://dx.doi.org/10.1128/AEM.67.1.420-425.2001

Azadbakht, L., Shakerhosseini, R., Atabak, S., Jamshidian, M., Mehrabi, Y., & Esmaill-Zadeh, A. (2003). Beneficiary effect of dietary soy protein on lowering plasma levels of lipid and improving kidney function in type II diabetes with nephropathy. *European Journal of Clinical Nutrition, 57*(10), 1292-1294. http://dx.doi.org/10.1038/sj.ejcn.1601688

Bowley, A. (2005). Probiotics. *Nutriview, 1*, 8.

Brankovi, S. D., & Baras, J. (2001). Comparative Study on Biochemical Activity of the Intestinal Isolates *Lactobacillus* Species V3 and *Bifidobacterium* species A71 in Different Substrates. *Journal of Serbian Chemical Society, 66*(9), 581-589.

Danone. (1997) Bifidobacteria. *Danone World Newsletter*, 16.

Earnshaw, R. G. (1992). *Antimicrobial Action of Lactic Acid Bacteria: Natural Food Preservation Systems*. In B. J. B. Wood (Ed.), *The Lactic Acid Bacteria*. Vol1. London: Elsevier Science Publishers. http://dx.doi.org/10.1007/978-1-4615-3522-5_9

Frais, F. (1972). *Introductory Biochemistry*: An Introductory Course. London: Butterworth and Co Ltd. Pub.

Garro, M. S., Valdez, G. F., de Oliver, G., & Giori, G. S. de. (1999). Hydrolysis of soymilk oligosaccharides by *Bifidobacterium longum* CRL849. *Food Research and Technology, 208* (1), 57 -59.

Gibson, G. R., Saavedra, J. M., Macfarlane, S., & Macfarlane, G. T. (1999). Probiotics and Intestinal infections. In R. Fuller (Ed.), *Probiotics* 2: Applications and Practical Aspects. London: Chapman and Hall.

Gilliland, S. E., Reilly, S. S., Kim, G. B., & Kim, H. S. (2002). Viability during storage of selected Probiotic Lactobacilli and Bifidobacteria in Yoghurt- like Product. *Journal of Food Science, 67* (8), 3091-3095. http://dx.doi.org/10.1111/j.1365-2621.2002.tb08864.x

Harrigan, W. F., & McCance, M. E. (1976). *Laboratory Methods in Food and Dairy Microbiology*. London: Academic Press Publishers.

Harrigan, W. F. (1998). *Laboratory Methods in Food Microbiology* (pp 155-230). San Diego: Academic Press.

Havenaar, R., & Veld, J. H. J. (1992). Probiotics: A General view, In B.J.B. Wood (Ed), *The Lactic Acid Bacteria* ,Vol. 1. London: Elsevier Science Publishers.

Hoover, G. (1999) *Bifidobacterium* In *Encyclopedia of Food Microbiology,* Vol 1, (pp 210-212). London : Academic Press. http://dx.doi.org/10.1006/rwfm.1999.0170

I. I. T. A. (1989). Soybeans for Good Health: How to Grow and Use Soybeans in Nigeria International Institute for Tropical Agriculture, Ibadan, Nigeria.

Ibrahim, S. A., Dharmavaram, S. R., Seo, C. W., & Shahbazi, G. (2005) Antimicrobial activity of *Bifidobacterium longum* (NCFB 2259) as influenced by Spices. *Internet Journal of Food Safety, 2*, 6-8.

Kobayashi, I., Muraoka, H., Saika, T., Nishida, M., Akita, H., Iwata, S., Sato, Y., & Sunakawa, K. (2003). Inhibitory Effect of Bifidobacteria on the *In vitro* Production of Verotoxins by *Escherichia coli* 0157:H7. *Japan Bifidus Foundation*, 81-84.

Kurmann, J. A., & Rasic, J. L. (1993). *Bifidobacteria in Foods*. In *Encyclopedia of Food Science, Food Technology and Nutrition* Vol. 1, (pp 374-378). London: Academic Press.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry, 31*, 426-428. http://dx.doi.org/10.1021/ac60147a030

Mitsuoka, T. (1992). The Human Gastrointestinal Tract. In B.J.B Wood. (Ed). *The Lactic Acid Bacteria,* Vol. 1, London: Elsevier Science Publishers.

Nsofor, L. M., & Osuji C. M. (1997). Stability, Rheology and Chemical Properties of Soymilk Concentrates Developed from Sprouted Soybeans. *Journal of Food Science and Technology, 34*,(1), 33-40.

Olutiola, P. O., Famurewa, O., & Sonntag, H. G. (1991). *An Introduction to General Microbiology: A Practical Approach*. Germany: Heidelberg Publishers.

Omoni, A. O., & Aluko, R. E. (2005). Soybean foods and their benefits: potential mechanisms of action. *Nutrition Reviews, 63*(8), 272-283. http://dx.doi.org/10.1111/j.1753-4887.2005.tb00141.x

Patidar, S. K., & Prajapati, J. B. (1997). Methods for Assessing the Immunostimulating Properties of Dietary Lactobacilli – A critical Appraisal. *Journal of Food Science and Technology*, *34* (3), 181-194.

Poskitt, E. M. E. (1993). *Infant: Feeding Problems. In the Encyclopedia of Food Science, Food Technology and Nutrition,* Vol. 4, (pp 2014-2017). London: Academic Press.

Prescott, L. M., Harley, J. P., & Klein, D. A. (1999). Microbiology 4th edition. Toronto: WCB/Mc Graw-Hill.

Rani, B., & Kheterpaul, N. (1999). Probiotics fermentation of indigenously developed RSMT mixture: Effect of antinutrients and digestibility of starch and proteins. *Journal of Food Science and Technology, 36* (1), 71 – 73.

Saarela, M., Mogensen, G., Matto, J., & Matilda-Sandholm, T. (2000). Probiotic bacteria: safety, functional and technological properties. *Journal of Biotechnology, 84*, 197 – 215. http://dx.doi.org/10.1016/S0168-1656(00)00375-8

Samona, A. (1993). Soymilk. *In the Encyclopedia of Food Science, Food Technology and Nutrition*. Vol. 6, (pp 4239- 4242). London: Academic Press.

Scardovi, V. (1986). *Genus Bifidobacterium Orla-Jesen*. In R. E. Buchanan & N. S. Gibbons (Ed.) (8th ed.) Sneath, Bergey's Manual of Determinative Bacteriology (pp. 669-676). Baltimore: Williams and Wilkins Pub.

Sgorbati, B., Biavati, B., & Palenzona, D. (1995). The Genus *Bifidobacterium*, In: B.J.B. Wood & W.H. Holzapfel, (Ed.), *The Genera of Lactic Acid Bacteria*, Vol 2, London: Blackie Academic and Professional Pub. http://dx.doi.org/10.1007/978-1-4615-5817-0_8

Tomasik, P. J., & Tomasik P. (2003). Probiotics and Prebiotics. *Journal of Cereal Chemistry, 80* (2), 113 – 117. http://dx.doi.org/10.1094/CCHEM.2003.80.2.113

Tsangalis, D., Ashton, J. F., McGill, A. E. J., & Shah, N. P. (2002). Enzymic Transformation of Isoflavone Phytoestrogens in Soymilk by β-glucosidase – producing *Bifidobacteria*. *Journal of Food Science*, *67* (8), 3104-3113. http://dx.doi.org/10.1111/j.1365-2621.2002.tb08866.x

Villegas, R., Gao, Y. T., & Yang, G. (2008). Legume and soy food intake and the incidence of type 2 diabetes in the Shanghai Women's Health Study. *American Journal of Clinical Nutrition, 87*(1), 162-167.

Visser, R., & Holzapfel, W. H. (1992). *Lactic Acid Bacteria in the Control of Plant Pathogen*s, In: B.J.B Wood. (Ed.). *The Lactic Acid Bacteria* Vol. 1. London: Elsevier Science Publishers.

Yusof, R. M., Haque, F., Ismail, M., & Hassan, Z. (2000). Isolation of *Bifidobacterium infantis* and its antagonistic activity against ETEC 0157 and *Salmonella typhimurium* S-285 in weaning foods. *Asia Pacific Journal of Clinical Nutrition*, *9*(2), 130-135. http://dx.doi.org/10.1046/j.1440-6047.2000.00154.x

Table 1. Identification of human intestinal *Bifidobacterium* isolates

TESTS	ISOLATES					
	1	2	3	4	5	6
Morphology	Club shaped, Long thin rods	Club shaped, V-, and Y-shaped	Club shaped long rods	Club shaped long rods	Club shaped long rods,	Club shaped long rods,
Gram Stain	+	+		+	+	+
Catalase test	-		-			-
Sugar Fermentation	g.x 1000)le 4. soymilk samples. protein ash, moisture, fatolids 167167167167 167167167167 167167167167 167167167167 167167167167 167167167167 167167167167 167167+	W	+	+	+	+
Xylose		+	+	+	+	+
Fructose		+	+	+	+	+
Maltose		-	-	-	-	-
Rhamnose		-	-	-	-	-
Sorbose		+	+	+	+	+
Glycerol		+	+	+	+	+
Erythritol		+	ND	ND	ND	ND
Arabinose		ND	+	+	+	+
Adonitol		+	+	+	+	+
Dulcitol		-	-	-	-	-
Starch		+	+	+	+	+
Melizitose		-	-	-	-	-
Salicin		-	-	-	-	-
		-	-	-	-	-
		+	+	+	+	+
		-	-	-	-	-
		+	+	+	+	+
		-	-	-	-	-
		+	ND	ND	ND	ND
		ND	+	+	+	+
		+	-	-	-	-
		-				
		+				
		-				
		W				
		+				
		-				
Probable identity of organism	B. longum	B. adolescentis	B. longum	B. longum	B. longum	B. longum

Symbol: + = Positive, - = Negative, W = Weak, ND = Not determined

Table 2. pH, TTA and Total solids of inoculated soymilk in storage (%)

Storage Period (Weeks)	pH		TTA (% Lactic acid))		Total Solids content (%)	
	Ambient Temp.	Refrigerated Temp.	Ambient Temp.	Refrigerated Temp.	Ambient Temp.	Refrigerated Temp.
0	5.72±0.12	5.72±0.12	0.17±0.02	0.17±0.02	5.16±0.21	5.16±0.21
1	4.58±0.15	5.63±0.13	0.43±0.03	0.19±0.01	4.62±0.20	4.78±0.19
2	4.51±0.18	5.62±0.12	0.45±0.02	0.20±0.04	4.26±0.22	4.60±0.18
3	4.47±0.13	5.64±0.14	0.51±0.01	0.21±0.01	4.23±0.21	4.57±0.22
4	4.39±0.12	5.44±0.15	0.46±0.04	0.20±0.02	4.53±0.19	4.73±0.17
5	4.36±0.12	5.44±0.19	0.52±0.04	0.18±0.01	4.43±0.19	4.50±0.09

Table 3. Amino Nitrogen, Crude protein and Carbohydrate contents of inoculated soymilk in storage

Storage Period (Weeks)	Amino Nitrogen (mg/ml)		Crude Protein Content (%)		Total Reducing Sugar Content (mg/ml)		Carbohydrate Content (%)	
	Ambient Temp.	Refrig. Temp.	Ambient Temp.	Refrig. Temp.	Ambient Temp.	Refrig. Temp.	Ambient Temp.	Refrig. Temp.
0	0.18±0.02	0.18±0.02	1.76±0.05	1.76±0.5	1.28±0.06	1.28±0.06	3.75±0.13	3.75±0.13
1	0.22±0.01	0.22±0.01	1.84±0.11	1.76±0.07	3.77±0.19	2.49±0.20	1.67±0.09	2.23±0.10
2	0.22±0.01	0.22±0.02	1.90±0.07	1.77±0.11	0.98±0.04	1.78±0.03	1.50±0.07	1.91±0.06
3	0.22±0.02	0.20±0.02	1.95±0.02	1.79±0.08	0.98±0.02	1.25±0.02	1.43±0.10	1.19±0.05
4	0.22±0.01	0.20±0.03	2.14±0.07	2.08±0.04	0.87±0.01	1.09±0.05	1.21±0.04	1.19±0.06
5	0.22±0.02	0.22±0.01	2.14±0.09	1.96±0.02	0.75±0.02	1.09±0.02	1.21±0.06	1.24±0.07

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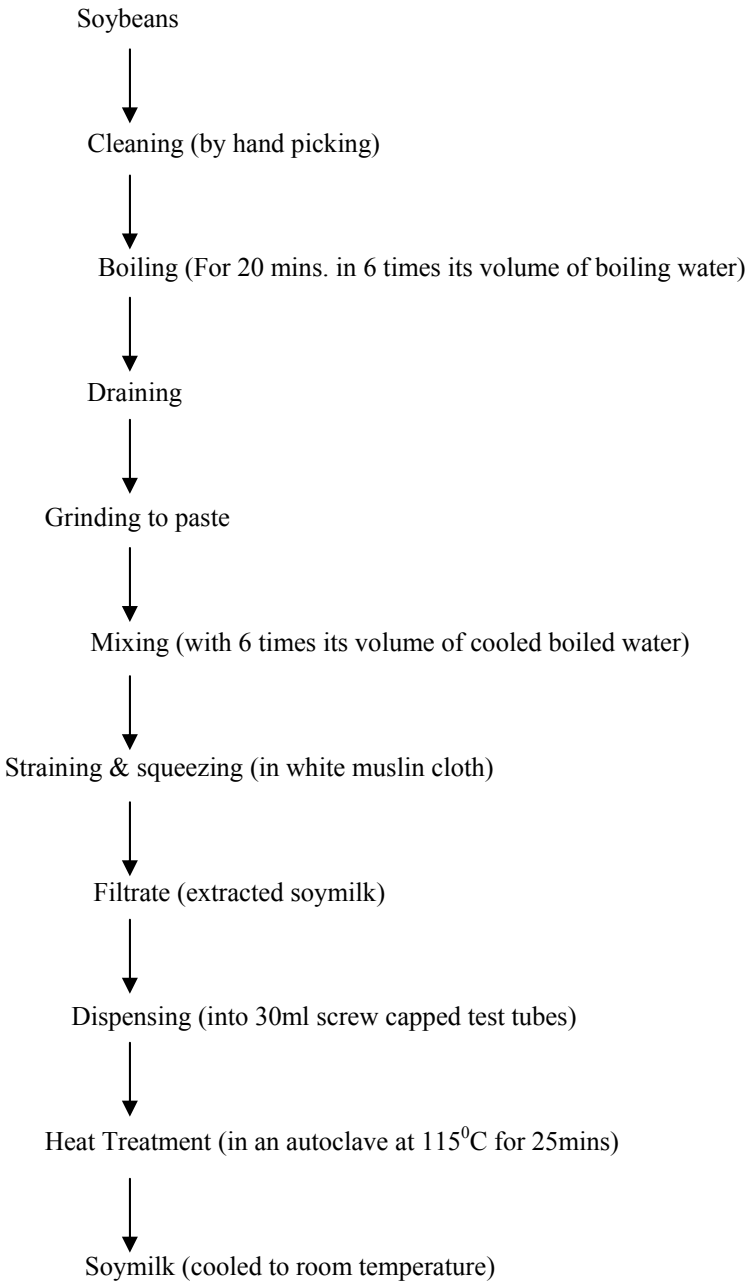

Figure 1. Flow chart for the preparation of soymilk (IITA, 1989)

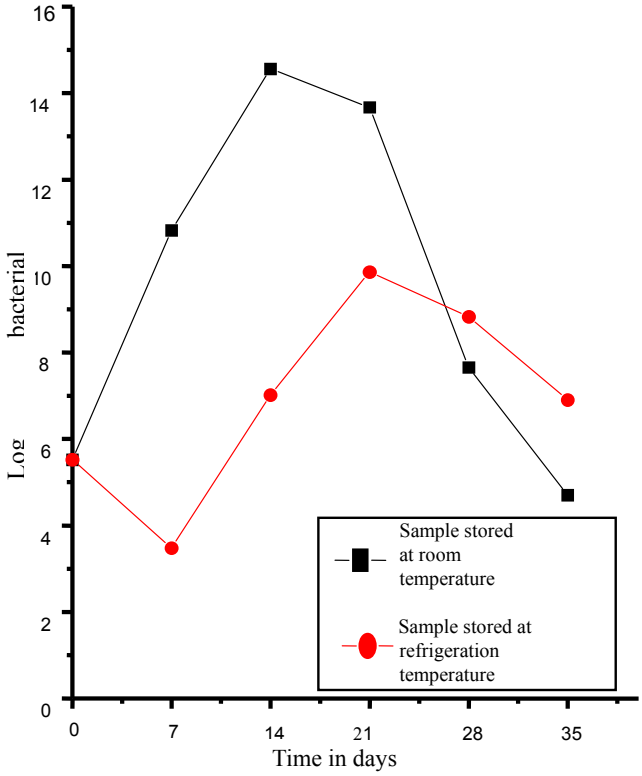

Figure 2. Viable count of B. longum in soymilk

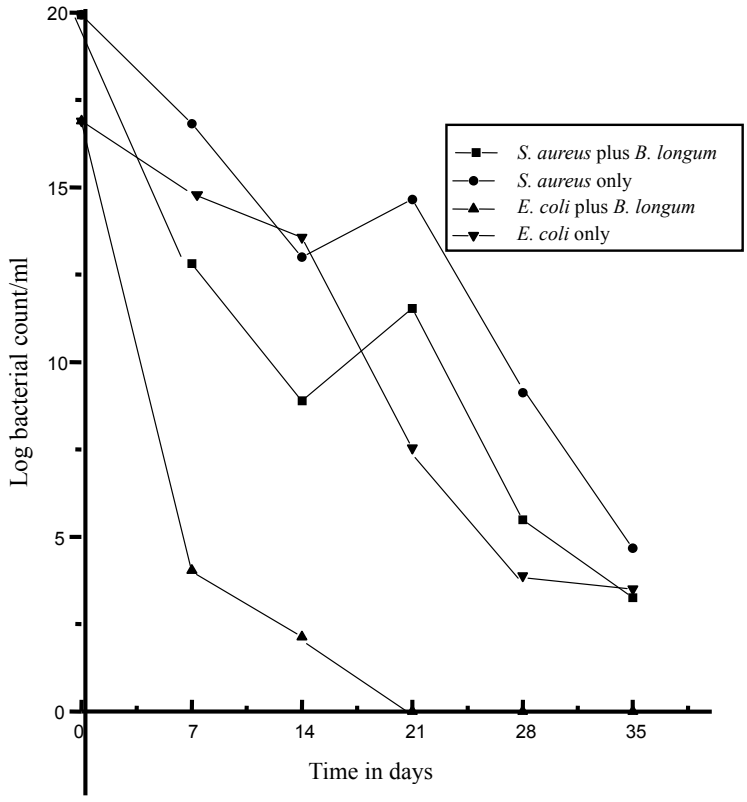

Figure 3. Antimicrobial effects of *B. longum on Escherichia coli* and *Staphylococcus aureus* in soymilk

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Randomized Controlled Trial on the Effects of Tualang Honey and Hormonal Replacement Therapy (HRT) on Cardiovascular Risk Factors, Hormonal Profiles and Bone Density Among Postmenopausal Women: A Pilot Study

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Abstract

Results of recent trial have shown some negative effects of HRT on postmenopausal women. Therefore, there has been a need to search for an alternative treatment and honey is one of the well known traditional remedies used in minimizing postmenopausal problems. The objectives of the study were to investigate the effects of Tualang honey on the cardiovascular risk factors, changes in hormonal profiles and also effect on the bone. A randomized controlled trial comparing the effects of Tualang honey 20 g/day and HRT for a 4-month intervention period among healthy postmenopausal Malay women aged 45-60 years old was conducted. The primary outcome measures were changes from baseline on the cardiovascular risk profiles, hormonal profiles and effect on bone. Tualang honey compared with low dose HRT, consumed for 4 months by postmenopausal women had no demonstrable effects on the parameters examined such as blood pressure measurement, body mass index and waist circumference. There was no significant difference in the lipid profile, blood sugar profile and bone density between the two groups at the end of the study period.

Keywords: Tualang honey, HRT, Postmenopausal, cardiovascular risk factor, hormonal profiles, bone densitometry

1. Introduction

Menopause is a natural progression of women's physiology. Hormone replacement therapy (HRT) has been the basis of the treatment of menopausal state. The high incidence in cardiovascular disease is due to the increase in the risk factors associated with menopausal state. It is also associated with the emergence of features of metabolic syndrome which includes increase in central or intra abdominal body fat, a change toward more atherogenic lipid profile, with increased low density lipoprotein particles and increased insulin resistance (Knopp, 2002; Eaton & Anthony 2002). Postmenopausal state is associated with a decrease in the bone mineral density

which leads to osteoporosis. There is a widely held belief that the decrease in the estradiol level directly contribute to this. Estradiol directly acts on osteoclast by reducing the rate of bone resorption (Steinweg, 2002).

Despite the proven benefit of hormone replacement therapy (HRT), only 15% of postmenopausal women currently use HRT (Amato & Sylvie, 2002) and of those who started HRT nearly 30% subsequently stopped (Ryan, Harrison & Blake, 1992). The main reasons for not taking HRT were concern over its side effects, safety and efficacy of the treatment. Among the common side effects experienced by women are breast tenderness, edema and breakthrough bleeding. However the major concern regarding HRT relates to the risk of cancer.

The Women's Health Initiative (WHI) Study stated an increase risk in breast cancer, cardiovascular disease, stroke and thromboembolic disease with conjugated equine estrogen plus medroxyprogesterone acetate compared with placebo (Prestwood, 2003). In view of this problem many women are increasingly turning to alternative medicine in an effort to manage their menopausal symptoms (Amato & Sylvie, 2002). However, there are still questions regarding the effectiveness of available alternative medicines in managing menopausal symptoms and complications. A recent result from a RCT indicated that herbal supplements namely black cohosh and soy foods did not relieve vasomotor symptom among menopausal women (Newton, Reed, LaCroix, Grothaus, Ehlrich & Guiltinan, 2006).

The use of bee products including honey, pollen, propolis, royal jelly, bee venom and wax in treating illness is known as apitherapy and it can be traced back more than 6000 years to ancient Egypt. The Greeks and the Roman also used bee products for medicinal purpose. This is described by Hippocrates (460–370 BC), Aristotle (384–332 BC) and Galen (130–200 AD), who prescribed the use of honey and bee venom as a cure for baldness (Hellner, Winter, von Georgi & Munstedt, 2007).

Honey is also mentioned in many cultures and religions as an important medicinal product. In Islamic teaching, one chapter in the holy book Quran is named after the bees and the verses in the chapter describe the importance of honey in men's life. In Indian medicine, ayurveda, honey has been used for many centuries in their method for healing and treating illness. Honey in ayurveda is "the highest food" and is referred to as "food for the Gods" (Subrahmanyam, 2007). Honey is also used widely in the Chinese medicine either singly or in combination with other herbs. In Malaysia honey is widely used for treating a multitude of ailments from simple respiratory tract infection to treatment of complicated diabetic foot ulcer. Postmenopausal women are also known to consume honey since it gives them a sense of general wellbeing. However there is limited data regarding the effectiveness of honey in the treatment of specific menopausal symptoms.

Tualang honey is one type of honey which can be found in Malaysia along with, among others Gelam, Belimbing, Durian, and Kelapa. Tualang honey got its name from the Tualang tree where the bees which produce the honey built their nests. The Tualang tree or Koompassia excelsa is Asia's largest tree (growing up to 80 metres) and can found in the lowland rainforests of southern Thailand, Peninsular Malaysia, northeastern Sumatra, Borneo and Palawan. Their habitat is the primary tropical rainforest. They prefer damp locations along rivers, in valleys, and the lower slopes of hills. Although common in these forests the Tualang is not naturally abundant therein; they tower above the canopy, their initial branches not occurring until about 30 meters above ground. It is the choice for Apis dorsata bees which are the world's largest bees to build their nests. It is presumed that the towering trees afford the bees with safety from the rest of the habitat. Another feature of the Tualang tree is its slippery trunk which prevents the sun bears to climb up the trees and reach the honey combs. A single Tualang tree may contain about 100 Apis dorsata nests, and each nest, with the profile of a half-moon and up to 1.5 meters across, may contain about 30,000 bees. Collectively, these bees can produce up to 450 kg of honey from one tree (Oldroyd, Osborne & Mardan, 2000; Itioka , Inoue, Kaliang, Kato & Nagamitsu, 2001).

Research looking at the effect of Tualang honey has shown that it is comparable to manuka honey in terms of its antibacterial activity (Tan et al, 2009). This result suggests that Tualang honey could potentially be used as an alternative therapeutic agent against certain microorganisms, particularly A. baumannii and S. maltophilia. Other areas where the use of Tualang honey are under investigations are in treatment of radiation mucositis (Biswal, Zakaria & Ahmad, 2003), allergic rhinitis and human immunodeficiency virus (HIV) infection, among others.

There is limited data regarding the use of honey for management of menopausal women. An animal study revealed that administration of honey to ovariectomised rats improves the endometrial and vaginal thickness (Siti Sarah & Siti Amrah, 2010). The vaginal epithelium of honey treated rat showed proliferation and mucination with lack of keratinization. There was also increase in the serum testosterone and progesterone level in the honey treated rats. This study was a pilot study to look at the cardiovascular parameters (blood pressure, waist circumference, total cholesterol, high density lipoprotein, low density lipoprotein, fasting blood sugar), hormonal profiles (follicle stimulating hormone, luteinizing hormone, testosterone and estradiol) and also for bone

densitometry of four months administration of Tualang honey on postmenopausal women.

Ethical consideration:

The study protocol was reviewed and approved by the Universiti Sains Malaysia Human Ethics Committee (USMKK/PPP/JEPeM (198.3(11)).

2. Methods

2.1 study subjects

This was a randomized, prospective, clinical study to evaluate the effects of Tualang honey in comparison with HRT. Subjects will be confined to healthy postmenopausal women who were naturally menopause for more than one year. The study period was four months. A total of 79 patients were recruited.

Group 1: Subjects receiving 20 g/day of Tualang honey. The honey used was from a single batch honey supplied by Federal Agricultural Marketing Authorities (FAMA), Malaysia, evaporated by FAMA to achieve a water content of about 20%, submitted to Sterile Gamma company at Shah Alam, Selangor for sterilization at 25 kGy and packed in 20 g sachet in collaboration with School of Pharmaceutical Sciences laboratory.

Group 2: Subjects receiving hormonal replacement therapy (Femoston®), also known as Femo conti 1/5 (contain 1 mg Estradiol valerate and 5 mg Dydrogesterone) supplied by Solvay Pharma Malaysia.

The choice of the dose of the honey used in the study is based on the animal study using the ovariectomised rat (Siti Sarah & Siti Amrah, 2006). The optimal dose shown to increase the testosterone level was 200 mg/kg/day in the animal model. After taking the average human weight as 60 kg, the dose calculated for human was 12 g (200 mg/kg x 60 kg (average human weight) = 12g). Twenty gram is considered to be medium dose, chosen to study the effect of honey in human being. Furthermore, the dose of the Femo conti is the optimal dose used in the treatment of postmenopausal problem.

Sample size was calculated based on animal study (Siti Sarah and Siti Amrah, 2010) using 2 proportion formula. After considering 20% drop-out and power of 80%, the subjects for each treatment group were 35.

Inclusion criteria were age 45 - 60 years old and naturally menopause for more than one year. No present active medical, surgical and gynecological problems, body mass index 18-35 kg/m^2, not on HRT for more than 3 months

Exclusion criteria were women whose taking any form of herbal extract in the last 3 months before study entry, history of drug or alcohol abuse, following ovariectomy, history of breast or cervical carcinoma, taking medication that affect bone metabolism, including glucocorticoid, anticonvulsant and methotrexate, clinical relevant cardiovascular, gastrointestinal, hepatic, neurologic, endocrine, hematologic or other major diseases making implementation of the protocol or other interpretation of the study result difficult, endometrial thickness more than 0.5 cm detected from pelvic ultrasonography and mental condition rendering the subject unable to understand the nature, scope and possible consequences of the study.

Informed consent was obtained and the study was explained to the subject by the investigator.

Demographic details including weight and height, physical examination and investigations including pelvic ultrasonography, brief medical history relating to past and current illnesses and concomitant medication was obtained. About 5 mL of fasting blood samples were collected for at baseline and at the end of the study. Subjects were thoroughly examined by Medical Specialists who were part of the Clinical Trial Team at every 2-monthly visits. Randomization was computer-generated .

The investigator may cease study treatment and withdrew the subject or the subject may withdraw herself from participation in the study at any time. Possible reasons for patient withdrawal include the need to take medication, which may interfere with study measurement, patient experiences an intolerable/unacceptable adverse event, patient exhibits non-compliance with the protocol. Patient unwilling to proceed and/or consent was withdrawn and investigator withdraws patient for reasons unrelated to the study drug (e.g., undercurrent illness).

2.2 Safety assessment

2.2.1 Protocol specific clinical assessment

Demographic details were recorded at screening. The subject's body weight and blood pressure were measured at 2 monthly periods. At the screening visit, a physical examination was conducted to determine the patient's current medical conditions and past clinically significant events. This includes all events that have occurred within the last three months and any other earlier event related to the inclusion and exclusion criteria or the subject's disease. This data was recorded at the screening visit. Throughout the study period, subject was directly

questioned about the occurrence of any new signs and symptoms and any changes from baseline was recorded as an adverse event. A physical examination was repeated at the end of the treatment and exit evaluation to assist in determining if there had been any changes to the patient's health during the study period.

2.3 Vital signs

Supine blood pressure and pulse rate was recorded at every visit. Patient was supine or semi-recumbent for five minutes prior to evaluating vital signs.

2.4 Concomitant medication

Concomitant medication included all co-administered drugs and treatment such as analgesics, tonics, herbals or traditional medicines and vitamin and/or mineral supplements. All concomitant medication taken within 7 days prior to commencement of study drug administration and for the duration of the study were recorded in the Clinical Record Form (CRF), including indication, dose, frequency, date and route administered.

2.5 Safety reporting of adverse event and serious adverse event were done

Subjects who are found to be less than 75% compliant with test article usage at any study visit will be withdrawn. Subjects who are found to be taking prohibited medications or supplements without the knowledge of the principal investigator will also be withdrawn. Any major protocol deviations (i.e., those that increase the risk to subjects and/or compromise the integrity of the study or its results) will result in subject discontinuation.

2.6 Statistical analysis

Data was entered, cleaned and analyzed using SPSS version 12. Means and standard deviations for numerical variables and frequency and proportion for categorical variables were reported along with histogram or bar chart when necessary. Level of significance was set at 5% and results were presented with 95% confidence intervals. Repeated measure ANOVA and Multiple Logistic Regression confirmatory analysis were used for analyses.

3. Results

Overall 82 subjects were screened and found to be eligible for the study and were then randomized into two treatment group. However three of them withdraw from the study, leaving a total of 79 participants who actually completed the study. Table 1 and Table 2 showed baseline sociodemographic data.

(Table 1 & Table 2)

The mean age of menopause in this study sample was 49.7 with the overall mean duration of menopause was 5.8 years, with the mean duration in the honey treated group of 5.3 years and 6.3 years in the HRT group. There was no significant difference between the age at menopause and the duration of menopause between the two groups ($p>0.1$).

The mean parity of these participants was 4.9 with 50.6% having 5 or more children. In term of parity, the honey treated group generally had more children, with 22 of them have more than 5 children compared to 18 with less than 5 children. However when compared with the HRT group the difference was not significant ($p > 0.1$).

In term of gynecological problem, majority of them (64.4%) reported no significant gynecological problem. Those who have positive gynecological and obstetrics history (35.4%) were mainly due to history of miscarriages and caesarean section for delivery (Table 1).

All of the study participant had an educational background, with 25.3% attained tertiary education, 54.4% up to secondary school level and the rest, 20.3% up to primary school level. In term of household income slightly more than half of the participant (53.1%) had an income of less than RM 1000 per month, while 34.2% had an income of between RM 1000-3000 per month, the rest (12.7%) had an income of more than RM 3000 per month. There was a significant differences between the educational level and the income between the two groups with p-value of <0.05 (Table 2)

Majority of the study (63.3%) participant were healthy with no medical problem, while 36.7% have medical problems but stable or controlled on treatment. Among the medical illness cited were osteoarthritis, hypertension and asthma. There was no statistically significant difference between the honey and the HRT group in term of past medical history (Table 2).

(Table 3 & Table 4)

The baseline clinical findings are presented in Tables 3 and 4 showed the difference between the groups in term the baseline clinical examination findings and baseline biochemical results respectively. The mean systolic and diastolic blood pressures were 132.7 mmHg and 82.0 mmHg respectively, which were within normal limit. There

was a significant difference of systolic blood pressure between the two groups with those treated with honey having a lower reading (127.9 mmHg versus 137.6 mmHg) (p value = 0.002). The mean body mass index and the waist circumference were 27.7 kg/m^2 and 86.1cm respectively. The BMI was considered to be in the overweight range, while the waist hip circumference was also increase. There was no significant difference between the two groups (p value = 0.907 and 0.717 respectively).

The lipid profile of the participants generally showed dyslipidaemic feature with a mean total cholesterol of 5.7 mmol/L and LDL 3.7 mmol/L. The HDL and TG were in normal range (Table 4). There was no significant difference of all the lipid profile between the groups except for LDL level. There was a significant difference between the groups in term of the LDL -C level, with the group treated with honey having lower LDL level (3.4 mmol/L vs 3.9 mmol/L) ($p < 0.05$).

(Table 5)

The hormonal profile of the study participants was comparable at baseline, with a very high mean FSH value of 73.7 μIU/ml which indicate late menopausal stage (Table 5). There was no significant difference between the two groups in term of the hormonal profile ($p > 0.05$).

(Table 6)

Table 6 showed the result of the bone densitometry at baseline for both groups. The mean bone densitometry of the study participants was 0.96477 g/cm^2 and the BMD at the lumbar spine was 1.02565 g/cm^2. There was no difference between the two groups in term of the baseline bone densitometry at the femur ($p = 0.906$) and at the lumbar spine ($p = 0.625$).

(Table 7 & Table 8)

There is a significant increase in the waist circumference at 4 months in the HRT group when compared with the baseline value after analysis with paired t- test (86.56 cm versus 89.13 cm) ($p<0.05$). No other significant changes were seen in any of the groups in the other parameters (Table 7).There was no significant difference between the two groups in term of the clinical findings at four months of the study even after performing ANCOVA test and controlling the baseline values (Table 8). The adjusted mean of the blood pressure in HRT group was 134.5 mmHg and 132.5 mmHg in the honey group ($p = 0.587$). In term of the body mass index, the adjusted value in the HRT and honey group was 27.9 and 27.3 kg/m2 ($p = 0.246$).

(Table 9)

Referring to Table 9, there was a minimal but significant increase in the level of total cholesterol and LDL-C in the honey treated group after 4 months of treatment ($p<0.05$). The level of the cholesterol was 5.52 mmol/l and 5.68 mmol/l at baseline and at 4 months respectively. The level of LDL-C was 3.39 mmol/l and 3.66 mmol/l respectively. The was also a significant increase in the level of fasting blood sugar in the honey treated group with a level of 5.25 mmol/l at baseline and 5.68 mmol/l at 4 months ($p<0.05$).

(Table 10)

There was no significant difference in the lipid profile and blood sugar profile between the two groups at the end of the study period even after analyzing with ANCOVA test and controlling the baseline values (Table 10). The adjusted mean LDL value of the HRT and the honey group were 3.8 and 3.9 mmol/L respectively ($p=0.487$). Even though there was a significant difference in the baseline value of the LDL between the two groups, the same pattern was not seen at the end of the four months period. There was also no significant change between the blood sugar levels between the groups at the end of the study. The adjusted mean blood sugar reading of the HRT and honey group were 5.4 and 5.8 respectively ($p=0.218$).

(Table 11)

There were significant changes in the FSH, LH, estradiol and testosterone level in the hormone treated group at 4 months of treatment compared with baseline, while in the honey treated group the only significant change was observed in the LH level (Table 11).

(Table 12)

In Table 12, using the ANCOVA analysis, a significantly lower level of the FSH and LH levels were seen in the HRT group at 4 months of treatment compared with the honey group, with FSH value of 45.3μIU/L and LH of 21.7 μIU/L ($p < 0.001$). The estradiol level meanwhile was significantly elevated with a mean level of 244.0 pmol/L ($p < 0.001$). The findings were still significant after controlling for baseline values, age, BMI, waist circumference and duration of menopause.

(Table 13 & Table 14)

There was no significant changes seen in the bone density between the two groups at four months of study at both femoral and lumbar spine ($p>0.05$) (Table 13). The adjusted mean of bone density at the femoral site in the HRT and Tualang honey group was 0.960 and 0.938 g/cm^2 respectively (Table 14). Meanwhile the adjusted mean of bone density at the lumbar spine in the HRT and Tualang honey was 0.999 and 0.996 g/cm^2 respectively.

4. Discussion

4.1 Sociodemographic characteristic

The mean age at menopause of the study subjects were 49.7 years. A similar result was also attained in a recent study done in Kelantan, which found that the mean age of menopause to be 49.7 years (Dhillon, Singh, Shuib, Hamid & Mahmood, 2006). The age of menopause was also comparable to other countries in the region and the rest of the world. The mean age of menopause of study sample in Singapore was 49.0 years (Foo-Hoe, Lay-Wai, Seang-Mei, Lee & Ken., 2005) and 48.0 years in United Arab Emirates (Rizk, Bener, Ezimokhai, Hassan & Micallef, 1998). In multiethnic sample of US midlife women, the mean age of menopause was around 51.4 years (Gold *et al.*, 2001). Although there is not much difference in the mean age of menopause, there is still variability seen.

The age of menopause is important in term of its association with cardiovascular disease and stroke. There was overall significant association between younger age at menopause and higher risk of coronary heart disease among women who experienced natural menopause and never used hormone therapy (Hu *et al.*, 1999). Similar association between stroke risk was seen with earlier onset of menopause, where age at natural menopause before age 42 was associated with increased ischemic stroke risk (Lisabeth *et al.*, 2009).

The mean duration of menopause of the study subjects were 5.8 years. Based on the Stages of Reproductive Aging Workshop (STRAW) classification for menopausal stage (Soules *et al.*, 2001), this is considered as late menopause. Menopausal women in this stage of menopause are more at risk of the long term sequelae of menopause such as osteoporosis and cardiovascular disease.

The majority of the study participants had a mean household income of less than RM 1000, denoting low socioeconomic income. The socioeconomic status of the participants was also reflected in their educational level, where majority of them (74.7%), did not continue their education until tertiary level. This is comparable to a study looking at sexual function in postmenopausal women in Kelantan which showed 23% had no education, 25% only had primary school education with only 10% were from professional groups (Dhillon *et al.*, 2006).

This is also similar to a study done in Sarawak looking at menopausal symptoms in menopausal women (Syed Alwi, Siti Rubiah & Verna, 2010). However, a study done in urban area showed a different finding where a majority of the women were working (Nik Nasri, 1994). This may reflect on the distribution of the country's population.

4.2 Cardiovascular parameters and risk factors

Cardiovascular parameters measured in this study include both the clinical examination and blood investigations for the lipid profile and fasting blood sugar. The average BMI of the study participants was 27.7 kg/m^2 which was considered to be in the overweight range. This is in keeping with the finding of the Malaysian Adults Nutrition Survey (MANS) carried out between 2002 and 2003. This survey found that nearly one third of nearly 11,000 of the survey sample were overweight (Azmi *et al.*, 2009). This survey also found that significantly more women was obese compared to the men.

In term of the waist circumference, the mean waist circumference was 86.1 cm which was high according to the International Diabetes Federation criteria for Asian women (IDF, 2006). The mean waist circumference of this study participant was also in line with the finding of the Third National Health Morbidity Survey 2006 which quoted a high prevalence (17.4%) of abdominal obesity in Malaysian population especially in women (Kee *et al.*, 2008).The finding of the current study was in contrast with the Pan Asia Menopause (PAM) study, which found a lower mean BMI of between 23.4 to 24.0 in Asian women (Haines *et al.*, 2005). The difference between these two studies could be due to differences in life style, dietary and socio economic factors.

The result for baseline lipid profile of the study participants generally showed dyslipidaemic pattern with a mean total cholesterol of 5.7 mmol/L and LDL 3.7 mmol/L. The normal value stated by the Third Report of the Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (ATP III) for total cholesterol is less than 5.2 mmol/L, LDL of less than 2.6 mmol/L, HDL of more than 1.1 mmol/L and TG of less than 1.7 mmol/L (NCEP, 2001). The HDL and the TG values of the study participants were within normal limit. There

was no significant difference between the two groups at baseline. This result was in line with the current knowledge regarding the association of menopause with dyslipideamia (Knopp, 2002). The increase at menopause is partly because of advancing age and partly because of the loss of estrogen. Postmenopausal status is also known to be associated with the emergence of metabolic syndrome which is characterized by central adiposity, insulin resistance and lipid abnormality (Carr, 2003). This is due to the changes in the body composition, characterized by an increase in weight and fat mass and a decrease in lean body mass. Metabolic syndrome is well known risk factor for cardiovascular disease.

The result of the clinical examination which includes blood pressure, waist circumference and BMI showed no significant differences between the two groups at the end of the study period. However, there was a statistically significant increase in total cholesterol, LDL-C, and fasting blood sugar level in the honey treated group at 4 months of study compared with baseline.

The result of this study differed from a few studies which looked at the effect of honey on the lipids and glucose level. A study looking at the effect of natural honey on cardiovascular parameters involving 55 overweight or obese, but otherwise healthy individuals showed beneficial effect of honey on the cholesterol, LDL and TG level (Yaghoobi *et al.*, 2008). Even though the findings were statistically significant the clinical relevance was questionable since the changes from baseline were generally between 3 – 6% only. Another study looking at the effect of honey on diabetic patients also showed an improvement in the level of the lipid profile after 2 months of treatment (Mohsen *et al.*, 2009).

The differences between this study and the previous studies could be explained by a few factors.

First, these studies used different study populations which limit the comparability with the recent study. The trials involving men might not hold the same result for postmenopausal women. Second, the type of honey used was different from honey used in the current study. As discussed earlier there are differences in the honey component based on the surrounding flora and fauna from where the bees fed on. It is highly possible that this difference could account for the different effect seen in the different studies. Thirdly, the differences in the dosage used. The previous study used higher dose of 70 g (Yaghoobi *et al.*, 2008) compared with current study which only use 20g. The dose chosen for the current study was based on the previous animal study and even then it was nearly double the dose calculated.

The increase in the fasting blood sugar in the honey treated group raises concern regarding the use of honey especially in diabetic patient. One of the ways to assess the suitability of certain food for diabetic patient is by looking at the glycaemic index. Glycaemic index (GI) is the measurement of the body blood glucose response after a carbohydrate load. According to the international table of glycemic index, honey has a GI value ranging between 32 and 87 (Foster & Miller, 2002). The GI value of table sugar is around 68. Hence, honey can be substituted for table sugar because of its additional health benefits. A study looking at the glycaemic index of Tualang honey however, has categorized the honey as intermediate GI food having a value of 65 (Roberta & Al-Safi, 2009) which was slightly lower than the average table sugar. The study which looked at the effect of honey on diabetic women showed no differences in the fasting blood glucose level of the honey treated group (Mohsen et al., 2009). However this study noted an increase in the HbA1C level in the participant. Therefore caution still need to be practiced in the use of honey in diabetic patient.

Investigations on the effects of other herbal treatments used in the management of menopause showed fairly similar result on the cardiovascular parameters. A randomized controlled trial using soy isoflavones in postmenopausal women showed no significant beneficial effect on the plasma concentrations of lipids, glucose, or insulin (Hall *et al.*, 2006). Other studies which used black cohosh (Wuttke, Gorkow & Seidlov-Wuttke., 2006) and wild yam (Komesaroff, Black, Cable, & Sudhir, 2001) in menopausal symptoms also failed to show any additional beneficial effect on the lipid profile.

It would be remissing if the effects of hormonal replacement therapy particularly Femo conti on the cardiovascular parameters are not discussed. This study showed no significant changes in the lipid profile or other cardiovascular parameters after the four month use of the hormone therapy. However previous studies showed beneficial effect of the combined low dose hormone therapy 17β estradiol plus 5 mg dydrogesterone especially on increasing the HDL level (de Kraker *et al.*, 2004, Stevenson, Teter & Lees., 2001). The possible difference of the result could be due to the study population and the duration of the study. Previous studies were conducted for longer duration, at least 1 year compared with this study. The duration of this study might not be sufficient to see the effect of the treatment on the cardiovascular parameters.

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4.3 Hormonal level

The baseline value of the hormonal level of the study participants showed very high FSH and LH level which indicated late menopause. This was supported by the mean duration of menopause of the study participants which was on average of 6 years. The findings of the hormonal level in this study was similar to the pattern of hormonal changes observed during the late postmenopause stage in various studies (Sowers et al., 2008a; Hall, 2004). The estradiol level was also noted to be low in this study participant as observed in a study looking at the changes in estradiol level in relation to the final menstrual period in a population-based cohort of women (Sowers *et al.*, 2008b).

There was a significant change in the hormonal level in the hormone treated group at four months of treatment with reduction in the FSH, LH and increment of the estradiol level. Similar decrease in the LH and testosterone was seen in the honey treated group. However no significant changes was observed in the FSH and estradiol level. The significance of this finding needs further clarification. Further studies need to be done to see the effect of honey on the hormonal profile especially with the inclusion of vaginal and endometrial cytology. As mentioned previously, the vaginal and endometrial cytology of the honey treated ovariectomised rats showed improvement in the endometrial and vaginal epithelial thickness even though there was no effect on the estradiol and FSH level (Siti Sarah & Siti Amrah, 2006).

4.4 Bone density

The mean bone density measurement at baseline for the study participant was 0.9477 g/cm^2 and the BMD at the spine was 1.02565 g/cm^2. This result was comparable to the finding of a study done locally to see the relationship of body composition and bone mineral density in healthy postmenopausal women in Malaysia (Siew Swee *et al.*, 2009). In this study 8% of the study population had osteoporotic changes in the vertebral spine. The major difference with these two studies was a significantly higher percentage (80%) of the study population had osteopenic changes compared with 34.2% in the current study. The difference could be attributed to the difference in the race of study population where our study was predominantly of Malay race while the other study was done among Chinese women only. Another possible factor was the higher BMI of the study population. Our study participants were on average belongs to the overweight group. It is well known that high BMI is a protective factor for bone loss. Other lifestyle factors which played a role in the onset of osteoporosis in postmenopausal women are the smoking status and exercise level. None of these factors were evaluated in the current study.

The result at four months of treatment showed no significant change in term of bone mineral density when compared at baseline and between the two groups. This study was unable to produce the same result seen in animal study where there was a significant change in the bone mass and weight after treatment with honey (Siti Sarah, 2006). The difference with the animal study could be due to the species difference in the metabolism of the honey. The timing of the supplementation could also play a role. In our study, the average duration of menopause was around 6 years; in the rat model honey was given shortly after ovariectomy. Late supplementation may not restore bone loss or prevent further loss of bone. It is well known that prevention of bone loss is easier than reversing them when the loss had already occurred. Another probable reason why this study failed to show any effect on the bone is the study duration, which was only for four months. Previous studies using different treatment modalities either hormonal treatment or alternative treatment were conducted for longer duration, at least 6 months to see the beneficial effect on the bone.

There was conflicting result regarding the bone protective effect of other alternative treatment. A meta analysis concluded that using soy isoflavone for at least 6 months showed reduction in bone loss at the spine of post menopausal women (De-Fu, Li-Qiang, Pei-Yu & Ryohei., 2008). However, a randomized clinical trial conducted following the meta analysis failed to show the protective effect even after one year of use (Brink *et al.*, 2008). Effects of the other alternative treatment on bone metabolism for example black cohosh were less well documented.

5. Conclusions

Tualang honey compared with low dose HRT, consumed for 4 months by postmenopausal women had no demonstrable effects on the parameters examined such as blood pressure measurement, body mass index and waist circumference. There was no significant difference in the lipid profile and blood sugar profile between the two groups at the end of the study period. A significantly lower level of the FSH and LH and increase estradiol level were seen in the HRT group at 4 months of treatment compared with the honey group. There was no significant changes seen in the bone density between the two groups at four months of study.

Recommendation

(1). A larger study should be done involving all the various ethnics in Malaysia with a proper randomized sampling method to ensure higher generalizability and a more significant effects.

(2). Multiple doses strength of honey should have been used instead of just one dose, with the inclusion of higher doses as appropriate.

(3). An attempt should be made to devise a method whereby honey can be formulated in ways which allow blinding of the procedure for example in tablet form. However attempts should be made beforehand to ensure the formulation does not change the honey major active constituents.

(4). Duration of the study should be lengthened to at least 6 months to 1 year to ensure effect on the bone density is maximized.

(5). A future study on use of honey in post menopausal women should include the effect of honey on postmenopausal symptoms, an area where alternative medicine is highly studied.

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References

Amato, P, Sylvie, C., & L. M. P. (2002). Estrogenic activity of herbs commonly used as remedies for menopausal symptoms. *The J. of the North American Menopause Society, 2*, 145-150.

Azmi, M., Junidah, R., Siti Mariam, A., Safiah, M., Fatimah, S., Norimah, A., Poh, B., Kandiah, M., Zalilah, M., & Wan Abdul, M. (2009). Body Mass Index (BMI) of Adults: Findings of the Malaysian Adult Nutrition Survey (MANS). *Mal J Nutr, 15*(2), 97-119.

Biswal, B. M., Zakaria, A., & Ahmad, N. M. (2003). Topical application of honey in the management of radiation mucositis. A Preliminary study. *Supportive Care in Cancer, 11*(4), 242-248.

Brink, E., Coxam, V., Robins, S., Wahala, K., Cassidy, A., & Branca, F. (2008). Long-term consumption of isoflavone-enriched foods does not affect bone mineral density, bone metabolism, or hormonal status in early postmenopausal women: a randomized, double-blind, placebo controlled study. *Am J Clin Nutr, 877*, 61-70.

Carr, M. (2003). The emergence of the metabolic syndrome with menopause. *Journal of Clinical Endocrinology and Metabolism, 88*(6), 2404-2411. http://dx.doi.org/10.1210/jc.2003-030242

de Kraker, A. T., Kenemans, P., Smolders, R. G. V., Kroeks, M. V. A. M., & van der Mooren, M. J. (2004). The effects of 17[beta]-oestradiol plus dydrogesterone compared with conjugated equine oestrogens plus medroxyprogesterone acetate on lipids, apolipoproteins and lipoprotein(a). *Maturitas, 49*(3), 253-263. http://dx.doi.org/10.1016/j.maturitas.2004.05.006

De-Fu, M., Li-Qiang, Q., Pei-Yu, W., & Ryohei, K. (2008). Soy isoflavone intake increases bone mineral density in the spine of menopausal women: Meta-analysis of randomized controlled trials. *Clinical Nutrition*, 2757-64.

Dhillon, H. K., Singh, H. J., Shuib, R., Hamid, A. M., & Mahmood, N. M. Z. N. (2006). Prevalence of menopausal symptoms in women in Kelantan, Malaysia. *Maturitas, 54*(3), 213-221. http://dx.doi.org/10.1016/j.maturitas.2005.11.001

Eaton, C. B., & Anthony , D. (2002). Cardiovascular Disease and the maturing woman. *Clinics In Family Practice, Volume , 4*(1), 71-88. http://dx.doi.org/10.1016/S1522-5720(03)00052-7

Foo-Hoe, L., Lay-Wai, K., Seang-Mei, S., Lee. J. J. M., & Ken, G. (2005). The age of menopause and the menopause transition in a multiracial population: a nation-wide Singapore study. *Maturitas,* 52169-180.

Foster, P., Holt, S., & Brand Miller, J. (2002). International table of glycemic index and glycemic load values. *American Journal of Clinical Nutrition,* 765-56.

Gold, E. B., Bromberger, J., Crawford, S., Samuels, S., Greendale, G. A., Harlow, S. D., & Skurnick, J. (2001). Factors Associated with Age at Natural Menopause in a Multiethnic Sample of Midlife Women. *Am. J. Epidemiol., 153*(9), 865-874. http://dx.doi.org/10.1093/aje/153.9.865

Haines, C. J., Xing, S. M., Park, K. H., Holinka, C. F., & Ausmanas, M. K. (2005). Prevalence of menopausal symptoms in different ethnic groups of Asian women and responsiveness to therapy with three doses of conjugated estrogens/medroxyprogesterone acetate: The Pan-Asia menopause (PAM) study. *Maturitas, 52*(3-4), 264-276. http://dx.doi.org/10.1016/j.maturitas.2005.03.012

Hall, W. L., Vafeiadou, K., Hallund, J., *et al*. (2006). Soy-isoflavone-enriched foods and markers of lipid and glucose metabolism in postmenopausal women: interactions with genotype and equol production. *Am J Clin Nutr, 83*(3), 592-600.

Hellner, M., Winter, D., von Georgi, R., & Mu nstedt, K. (2007). Apitherapy: Usage And Experience In German Beekeepers. *eCAM*, 1-5.

Hu, F. B., Grodstein, F., Hennekens, C. H., Colditz, G. A., Johnson, M., Manson, J. E., Rosner, B. Stampfer, M. J. (1999). Age at Natural Menopause and Risk of Cardiovascular Disease. *Arch Intern Med, 159*(10), 1061-1066. http://dx.doi.org/10.1001/archinte.159.10.1061

International Diabetes Federation. (2006). The IDF consensuses worldwide definition of the metabolic syndrome.(available: http://www.idf.org/webdata/docs/IDF_Meta_def_final.pdf. Accessed on 25July 2010).

Itioka, T., Inoue, T., Kaliang, K., Kato, M., & Nagamitsu, T. (2001). Six-Year population fluctuation of the giant honey bee Apis dorsata (Hymenoptera: Apidae) in a tropical lowland Dipterocarp Forest in Sarawak. *Annals of the Entomological Society of America, 94*(4), 546-549. http://dx.doi.org/10.1603/0013-8746(2001)094%5B0545:SYPFOT%5D2.0.CO;2

Kee, C., Jamaiyah, H., Noor Safiza, M., Geeta, A., Khor, G., Suzana, S., Jamalludin, A., & Rahmah, R. (2008). Abdominal Obesity in Malaysian Adults: National Health and Morbidity Survey III (NHMS III, 2006). *Mal J Nutr, 14*(2), 125-135.

Knopp, R. H. (2002). Risk factors for coronary artery disease in women. *Am J Cardiol, 89*(suppl)28E-35E. http://dx.doi.org/10.1016/S0002-9149(02)02409-8

Komesaroff, P. A., Black, C. V. S., Cable, V., & Sudhir, K. (2001). Effects of wild yam extract on menopausal symptoms, lipids and sex hormones in healthy menopausal women. *Climacteric, 4*(2), 144-150.

Lisabeth, L. D., Beiser, A. S., Brown, D. L., Murabito, J. M., Kelly-Hayes, M., & Wolf, P. A. (2009). Age at Natural Menopause and Risk of Ischemic Stroke: The Framingham Heart Study. *Stroke, 40*(4), 1044-1049. http://dx.doi.org/10.1161/STROKEAHA.108.542993

Mohsen, B., Asal, A., Saeed, H., Mohammad Hasan, F., Mazaher, R., & Mohammad, P. (2009). Effects of natural honey consumption in diabetic patients: an 8-week randomized clinical trial. *International Journal of Food Sciences and Nutrition,* 60(7), 618-626. http://dx.doi.org/10.3109/09637480801990389

Newton, K., Reed, S., LaCroix, A., Grothaus, L., Ehrlich, K., & Guiltinan, J. (2006). Treatment of vasomotor symptoms of menopause with black cohosh,multibotanicals, soy, hormone therapy, or placebo:a randomized trial. *Ann Intern Med, 145*, 869-879.

Nik Nasri, I. (1994). A study on the menopause in Malaysia. *Maturitas, 19*(3), 205-209. http://dx.doi.org/10.1016/0378-5122(94)90073-6

Oldroyd, B., Osborne, K., & Mardan, M. (2000). Colony relatedness in aggregations of Apis dorsata Fabricius (Hymenoptera,Apidae*). Insectes soc*, 4794-95.

Prestwood, K. (2003). The search for alternative therapies for menopausal women : estrogenic effects of herbs. *J. Clin of Endocri & Metab, 88*, 4075-76. http://dx.doi.org/10.1210/jc.2003-031277

Rizk, D. E. E., Bener, A., Ezimokhai, M., Hassan, M. Y., & Micallef, R. (1998). The age and symptomatology of natural menopause among United Arab Emirates women. *Maturitas* 29197-202.

Roberta, S., & Al-Safi, A. (2009). Two varieties of honey that are available in Malaysia gave intermediate glycemic index values when tested among healthy individuals. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub,153*(2), 145-148.

Ryan, P. J., Harrison, R., & Blake, G. M,. (1992). Compliance with hormone replacement therapy after screening for postmenopausal osteoporosis. *Br J Obstet Gynaecol, 99*(325-8). http://dx.doi.org/10.1111/j.1471-0528.1992.tb13732.x

Siew Swee, W., Ting, G., Tan, S., Chan, S., Zaitun, Y., & Suriah, A. (2009). The relationship between body composition and bone mineral density in healthy postmenopausal Chinese women in *Malaysia. Jurnal. Sains*

Kesihatan Malaysia, 5(2), 29-38.

Sirola, J., Kroger, H., Honkanen, R., Jurvelin, J. S., Sandini, L., Tuppurainen, M. T., & Saarikoski, S. (2003). Factors affecting bone loss around menopause in women without HRT: a prospective study. *Maturitas, 45,* 45159-167. http://dx.doi.org/10.1016/S0378-5122(03)00150-6

Siti Amrah, S. (2006). Honey and reproductive hormones. *Proceedings from 1st International conference on the medicinal uses of honey,* 26-28, August 2006. Kota Bharu, Kelantan.

Siti Sarah, M., Siti Amrah, S., Rohaizan, Y., Sirajudeen, K., & Norhayati, O. (2006). Effect of tualang honey on reproductive organs in ovariectomised rats. *Proceedings from 1st International conference on the medicinal uses of honey*, 26-28 August 2006. Kota Bharu, Kelantan.

Soules, M. R., Sherman, S., Parrott, E., Rebar, R., Santoro, N., Utian, W., & Woods, N. (2001). Stages of reproductive aging (STRAW). *Journal of Women's Health & Gender-Based Medicine, 10*(9), 834-848. http://dx.doi.org/10.1080/cmt.4.4.267.272

Sowers, M. R., Zheng, H., McConnell, D., Nan, B., Harlow, S. D., & Randolph, J. F. Jr. (2008b). Estradiol Rates of Change in Relation to the Final Menstrual Period in a Population-Based Cohort of Women. *J Clin Endocrinol Metab, 93*(10), 3847-3852. http://dx.doi.org/10.1210/jc.2008-1056

Sowers, M. R., Zheng, H., McConnell, D., Nan, B., Harlow, S., & Randolph, J. F. Jr. (2008a). Follicle Stimulating Hormone and Its Rate of Change in Defining Menopause Transition Stages. *J Clin Endocrinol Metab, 93*(10), 3958-3964. http://dx.doi.org/10.1210/jc.2008-0482

Steinweg, K. K. (2002). Menopause, bone physiology, and osteoporosis prevention. *Clinics In Family Practice, 4*(1), 89-111. http://dx.doi.org/10.1016/S1522-5720(03)00053-9

Stevenson, J. C., Teter, P., & Lees, B. (2001). 17[beta]-Estradiol (1 mg/day) continuously combined with dydrogesterone (5, 10 or 20 mg/day) increases bone mineral density in postmenopausal women. *Maturitas, 38*(2), 197-203. http://dx.doi.org/10.1016/S0378-5122(00)00219-X

Subrahmanyam, M. (2007). Topical application of honey for burn wound treatment - an overview.*Annals of Burns and Fire Disasters, 20*(3), 44-48.

Syed Alwi, S., Siti Rubiah, Z., & Verna, L. (2010). Assessment of menopausal symptoms using modified Menopause Rating Scale (MRS) among middle age women in Kuching, Sarawak, Malaysia. *Asia Pac Fam Med, 9*(1), 5. http://dx.doi.org/10.1186/1447-056X-9-5

Tan, H., Rahman, R., Gan, S., Halim, A., Hassan, S., Sulaiman, S., & Bs, K.-K. (2009). The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. *BMC Complementary and Alternative Medicine, 9*(1), 34. http://dx.doi.org/10.1186/1472-6882-9-34

Thomas, F., Renaud, F., Benefice, E., De Meeus, T., & Guegan, J. (2001). International variability of ages at menarche and menopause:patterns and main determinants. *Human Biology, 73*(2), 271-290. http://dx.doi.org/10.1353/hub.2001.0029

Wuttke, W., Gorkow, C., & SeidlovÃ¡-Wuttke, D. (2006). Effects of black cohosh (Cimicifuga racemosa) on bone turnover, vaginal mucosa, and various blood parameters in postmenopausal women: a double-blind, placebo-controlled, and conjugated estrogens-controlled study. *Menopause, 13*(2), 185-196. http://dx.doi.org/10.1097/01.gme.0000174470.44822.57

Yaghoobi, N., Al-Waili, N., Ghayour-Mobarhan, M., *et al*. (2008). Natural Honey and Cardiovascular Risk Factors; Effects on Blood Glucose, Cholesterol, Triacylglycerole, CRP, and Body Weight Compared with Sucrose. *The Scientific World Journal*, 8463-469.

Table 1. Baseline demographic data

Characteristics	All[a] (n=79)		Trial groups[a] HRT (n=39)		Tualang honey (n=40)		*P*-value[b]
Demographic data							
Age (years)	55.4	(3.15)	55.3	(3.04)	55.6	(3.29)	0.735
Age of menarche (years)	13.6	(1.62)	13.9	(1.66)	13.3	(1.56)	0.135
Duration of menopause (years)	5.8	(3.96)	6.3	(4.54)	5.3	(3.28)	0.273
Mean age at menopause (years)	49.7	(4.03)	49.2	(4.90)	50.2	(2.93)	0.265
Number of parity							
< 5	39	(49.4)	21	(53.8)	18	(45.0)	0.432[c]
≥ 5	40	(50.6)	18	(46.2)	22	(55.0)	
Past obstetrics and gynaecology history							
Absent	51	(64.6)	26	(66.7)	25	(62.5)	0.699[c]
Present	28	(35.4)	13	(33.3)	15	(37.5)	

[a] Values are expressed as mean (standard deviation, SD) unless otherwise specified

[b] Independent t test

[c] Chi-squared test.

Table 2. Baseline Demographic data and past medical history

Characteristics	All[a] (n=79)		Trial groups[a] HRT (n=39)		Tualang honey (n=40)		*P*-value[b]
Race							
Malay	78	(98.7)	35	(100)	39	(97.5)	0.320[d]
Non Malay	1	(1.3)	0	(0.0)	1	(2.5)	
Education level							
Primary school	16	(20.3)	9	(23.1)	7	(17.5)	0.041[c]
Secondary school	43	(54.4)	25	(64.1)	18	(45.0)	
Institution/University	20	(25.3)	5	(12.8)	15	(37.5)	
Income per month							
≤ RM 1000	42	(53.1)	25	(64.1)	17	(42.5)	0.019[d]
RM 1000 – 3000	27	(34.2)	13	(33.3)	14	(35.0)	
≥ RM 3000	10	(12.7)	1	(2.6)	9	(22.5)	
Past medical history							
Absent	50	(63.3)	28	(71.8)	22	(55.0)	0.122[c]
Present	29	(36.7)	11	(28.2)	18	(45.0)	

[a] Values are expressed as mean (standard deviation, SD) unless otherwise specified

[b] Independent t test

[c] Chi-squared test ,[d] Fisher Exact test.

Table 3. Baseline clinical findings of women treated with HRT and Tualang honey

Characteristics	All[a] (n=79)		Trial groups[a] HRT (n=39)		Tualang honey (n=40)		*P*-value[b]
Clinical examinations							
Systolic BP (mmHg)	132.7	(13.8)	137.6	(13.9)	127.9	(12.10)	0.002
Diastolic BP (mmHg)	82.0	(8.73)	83.6	(9.48)	80.4	(7.75)	0.111
Body mass index (kg/m^2)	27.7	(4.35)	27.6	(4.56)	27.3	(4.20)	0.907
Waist circumference (cm)	86.1	(11.6)	85.6	(11.3)	86.5	(12.07)	0.717

[a] Values are expressed as mean (standard deviation, SD) unless otherwise specified

[b] Independent t test.

Table 4. Baseline fasting lipids and blood sugar level in HRT and Tualang honey groups

Characteristics	All[a] (n=79)		Trial groups[a] HRT (n=39)		Tualang honey (n=40)		*P*-value[b]
TC (mmol/L)	5.7	(1.62)	5.9	(1.77)	5.4	(1.42)	0.108
TG (mmol/L)	1.7	(1.03)	1.8	(1.13)	1.6	(0.92)	0.511
LDL-C (mmol/L)	3.7	(1.04)	3.9	(0.98)	3.4	(1.04)	0.025
HDL-C (mmol/L)	1.5	(0.37)	1.6	(0.38)	1.4	(0.36)	0.154
FBS (mmol/L)	5.4	(1.78)	5.6	(2.13)	5.3	(1.36)	0.405

[a] Values are expressed as mean (standard deviation, SD) unless otherwise specified

[b] Independent t test.

Table 5. Baseline hormonal profiles in HRT and Tualang honey groups

Characteristics	All[a] (n=79)		Trial groups[a] HRT (n=39)		Tualang honey (n=40)		*P*-value[b]
FSH (μIU/ml)	73.7	(28.99)	72.2	(29.12)	75.1	(29.17)	0.665
LH (μIU/ml)	31.8	(13.08)	30.2	(12.45)	33.4	(13.63)	0.270
Estradiol (pmol/L)	58.4	(97.72)	57.4	(75.44)	59.4	(116.40)	0.927
Testosterone (nmol/L)	0.9	(0.53)	0.8	(0.48)	0.9	(0.58)	0.723

[a] Values are expressed as mean (standard deviation, SD) unless otherwise specified

[b] Independent t test.

Table 6. Baseline value of bone densitometry between HRT and Tualang honey

Bone densitometry	All[a] (n=79)		Trial groups[a] HRT (n=39)		Tualang honey (n=40)		*P*-value[b]
Femur (g/cm^2)	0.9477	(0.1603)	0.9625	(0.1386)	0.9667	(0.1807)	0.906
Lumbar (g/cm^2)	1.0256	(0.1663)	1.0163	(0.1547)	1.0346	(0.1785)	0.625

[a] Values are expressed as mean (standard deviation, SD) unless otherwise specified

[b] Independent t test.

Table 7. Cardiovascular outcome in term of clinical findings at baseline and 4 months of intervention in both treatment groups

	Mean (SD)			
Trial group	**Baseline**	**4 months**	**t statistics (DF)**	**P value[a]**
BMI (kg/m^2)				
Honey	27.73 (4.20)	27.34 (4.13)	1.13 (39)	0.264
HRT	27.62 (4.60)	27.83 (4.56)	9.83 (38)	0.332
WC (cm)				
Honey	86.53 (12.07)	89.18 (9.20)	1.734 (39)	0.91
HRT	85.56 (11.39)	89.13 (11.37)	3.02(38)	**0.04**
SBP (mmHg)				
Honey	127.95 (12.10)	130.88 (14.04)	1.153 (39)	0.256
HRT	137.63(13.99)	136.18 (10.03)	0.539 (38)	0.593
DBP (mmHg)				
Honey	80.43 (7.75)	79.75 (10.03)	0.378 (39)	0.708
HRT	83.56 (9.48)	81.08 (8.92)	1.283 (38)	0.207

[a] Paired t test.

Table 8. Cardiovascular parameters in term of clinical findings at 4 months of study

Study variables	**Crude mean (SD[a])**		**Adjusted mean (95% CI[b])**		**Adjusted mean difference (95% CI[b])**	***F stat*[c]**	***P*-value[d]**
	Placebo (n=39)	**Tualang honey (n=40)**	**Placebo (n=39)**	**Tualang honey (n=40)**			
Cardiovascular disease risk factors							
Systolic BP (mmHg)	136.2 (14.10)	130.9 (14.04)	134.5 (129.85, 139.09)	132.5 (127.94, 137.03)	-1.92 (-8.96, 5.11)	0.29	0.587
Diastolic BP (mmHg)	81.1 (8.92)	79.7 (10.03)	80.2 (77.03 (83.43)	80.6 (77.42, 83.73)	0.35 (-4.53, 5.23)	0.02	0.886
BMI (kg/m^2)	27.8 (4.56)	27.3 (4.13)	27.9 (27.23, 28.54)	27.3 (26.64, 27.94)	-0.59 (-1.59, 0.42)	1.37	0.246
WC (cm)	89.1 (11.37)	89.2 (9.20)	88.9 (86.87, 91.05)	89.3 (87.28, 91.40)	0.38 (-2.80, 3.57)	0.05	0.812

[a] Standard deviation

[b] Confidence interval

[c] *F statistic*

[d] Analysis of covariance (ANCOVA) after adjusted for baseline values, age, BMI, WC and duration of menopause.

Table 9. Biochemical profile for cardiovascular findings at baseline and 4 months of intervention for both treatment groups

	Mean (SD)			
Trial group	**Baseline**	**4 months**	**t statistics (DF)**	**P value[a]**
TC (mmol/L)				
Honey	5.52 (1.35)	5.68 (1.45)	-2.39 (39)	**0.021**
HRT	5.92 (1.77)	6.28 (1.30)	-1.16 (38)	0.252
TG (mmol/L)				
Honey	1.65 (0.91)	1.58 (1.11)	0.52 (39)	0.606
HRT	1.80 (1.13)	1.70 (1.00)	0.73 (38)	0.472
LDL-C (mmol/L)				
Honey	3.39 (1.01)	3.66 (1.18)	-2.21 (39)	**0.033**
HRT	3.98 (1.05)	4.01 (1.280	-0.16 (38)	0.874
HDL-C (mmol/L)				
Honey	1.45 (0.35)	1.46 (0.27)	-0.34 (39)	0.739
HRT	1.58 (0.38)	1.56 (0.37)	0.22 (38)	0.822
FBS (mmol/L)				
Honey	5.25 (1.35)	5.68 (1.45)	-3.69 (39)	**0.001**
HRT	5.61 (2.12)	5.38 (1.70)	0.77 (38)	0.442

[a] Paired t test.

Table 10. Cardiovascular in term of biochemical profile at 4 months of intervention

	Crude mean (SDa)		Adjusted mean (95% CIb)		Adjusted mean difference		
Study variables	HRT	Tualang honey	HRT	Tualang honey	(95% CIb)	F statc	P-valued
	(n=39)	(n=40)	(n=39)	(n=40)			
TC (mmol/L)	6.0 (1.64)	5.7 (1.35)	5.7 (5.43, 6.06)	5.9 (5.69, 6.31)	0.25(-0.23,0.74)	1.1	0.298
TG (mmol/L)	1.6 (0.92)	1.6 (1.12)	1.6 (1.26, 1.85)	1.6 (1.31, 1.89)	0.04(-0.40,0.49)	0.03	0.848
LDL-C (mmol/L)	4.1 (1.24)	3.7 (1.14)	3.8 (3.54, 4.06)	3.9 (3.69, 4.20)	0.14 (-0.260.53)	0.48	0.487
HDL-C (mmol/L)	1.6 (0.37)	1.5 (0.27)	1.6 (1.48, 1.66)	1.5 (1.36, 1.55)	-0.12(0.26,0.03)	2.59	0.112
FBS (mmol/L)	5.5 (1.68)	5.7 (1.45)	5.4 (4.96, 5.79)	5.8 (5.36, 6.19)	0.40(-0.24,1.04)	1.54	0.218

[a] Standard deviation

[b] Confidence interval

[c] *F statistic*

[d] Analysis of covariance (ANCOVA) after adjusted for baseline values, age, BMI, WC and duration of menopause.

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Table 11. Hormonal profile at baseline and 4 months of intervention for both treatment groups

	Mean (SD)			
Trial group	**Baseline**	**4 months**	**t statistics (DF)**	**P value[a]**
FSH (μIU/ml)				
Honey	75.07 (29.17)	78.76 (28.90)	-1.29 (39)	0.204
HRT	72.22 (29.16)	45.31 (29.03)	4.86 (38)	**0.000**
LH (μIU/ml)				
Honey	33.42 (13.62)	36.55 (12.71)	-2.86 (39)	**0.007**
HRT	30.16 (12.45)	21.75 (13.96)	3.14 (38)	**0.003**
Estradiol (pmol/L)				
Honey	59.39 (116.4)	41.53 (23.87)	1.02 (39)	0.314
HRT	57.36 (75.44)	244.02 (300.5)	-3.39 (38)	**0.000**
Testosterone (nmol/L)				
Honey	0.87 (0.57)	0.64 (0.49)	3.88 (39)	**0.000**
HRT	0.85 (0.47)	0.48 (0.34)	5.19 (38)	**0.000**

[a] Paired t-test.

Table 12. Hormonal profile at 4 months of intervention

Study variables	**Crude mean (SD[a])**		**Adjusted mean (95% CI[b])**		**Adjusted mean difference (95% CI[b])**	***F stat*[c]**	***P*-value[d]**
	Placebo (n=39)	**Tualang honey (n=40)**	**Placebo (n=39)**	**Tualang honey (n=40)**			
Hormonal profile							
FSH (μIU/ml)	45.3 (29.04)	78.8 (28.91)	46.1 (38.10, 54.05)	78.0 (70.15, 85.89)	31.94 (20.48, 43.41)	300.88	0.000
LH (μIU/ml)	21.7 (13.96)	36.6 (12.71)	22.7 (18.86, 26.53)	35.6 (31.85, 39.41)	12.94 (7.42, 18.45)	21.92	0.000
Estradiol (pmol/L)	244.0 (300.51)	41.5 (23.88)	242.3 (170.63, 314.00)	43.2 (-27.54, 113.94)	-199.12 (-302.18, -96.05)	14.85	0.000
Testosterone(nmol/L)	0.5 (0.34)	0.6 (0.49)	0.5 (0.40, 0.61)	0.6 (0.52, 0.73)	0.12 (-0.03, 0.27)	2.48	0.120

[a] Standard deviation

[b] Confidence interval

[c] *F statistic*

[d] Analysis of covariance (ANCOVA) after adjusted for baseline values, age, BMI, WC and duration of menopause.

Table 13. Bone density at baseline and 4 months of intervention for both treatment groups

Trial group	Mean (SD) Baseline	4 months	t statistics (DF)	P value[a]
Femur (g/cm^2)				
Honey	0.9669 (0.1807)	0.9421 (0.1324)	1.222 (39)	0.229
HRT	0.9629 (0.1283)	0.9559 (0.1283)	0.867 (38)	0.391
Lumbar spine (g/cm^2)				
Honey	1.0347 (0.1785)	0.9999 (0.1528)	1.970 (39)	0.056
HRT	1.0163 (0.1547)	0.9941 (0.2259)	0.698 (38)	0.490

[a] Paired t test.

Table 14. Bone density at 4 months of intervention

	Crude mean (SD[a])		Adjusted mean (95% CI[b])		Adjusted mean difference		
Study variables	**HRT**	**Tualang honey**	**HRT**	**Tualang honey**	**(95% CI[b])**	***F stat*[c]**	***P*-value[d]**
	(n=39)	**(n=40)**	**(n=39)**	**(n=40)**			
Femur (g/cm^2)	0.9559 (0.1283)	0.9420 (0.1324)	0.960 (0.937,0.984)	0.938 (0.915, 0.962)	0.022 (-0.011, 0.056)	1.849	0.178
Lumbar spine(g/cm^2)	0.9941 (0.2259)	0.9999 (0.1528)	0.999 (0.949,1.049)	0.996 (0.9464, 1.0464)	0.03 (-0.68, 0.074)	0.007	0.943

[a] Standard deviation

[b] Confidence interval

[c] *F statistic*

[d] Analysis of covariance (ANCOVA) after adjusted for baseline values, age, BMI, and duration.

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The Effect of γ-irradiation and Cooking on the Physicochemical Properties of African Oil Bean Seed (Pentaclethra macrophylla benth) and Its Oil Extract

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Abstract

Irradiation is a potentially useful technology for ensuring the safety and extending the shelf-life of food products. The effects of γ-irradiation (5kGy, 10kGy), cooking and combined treatment of 10 kGy irradiation and cooking on the chemical and functional properties of African oil bean seed (Pentaclethra macrophylla Benth) and physicochemical properties of its oil were investigated. The irradiated seeds and un-irradiated seeds were mechanically dehulled and milled into flour using an attrition mill. 'Raw seed', 'cooked seed', 'irradiated (5kGy) seed', 'irradiated (10kGy) seed' and 'irradiated (10kGy) and cooked seed' flour samples were analyzed for proximate composition, mineral content, functional properties and antinutritional factors, and the seed oils were analyzed for peroxide value, iodine value, acid value and free fatty acid content. The results show that the combined effect of γ-irradiation and cooking gave highest increase in nutritional quality, reducing protein from 32.91g/100g DM in the raw seed to 30.55g/100g DM and increasing fat content from 47.43g/100g DM to 51.19g/100g DM. Combined treatment led to the retention of sodium, calcium, zinc and iron more than the single treatments, but the same process reduced magnesium from 0.52mg/100g to 0.47mg/100g and phosphorus from 0.43mg/100g to 0.35 mg/100g. Least gelation concentration and emulsion capacity increased with combined γ-irradiation and cooking, whereas foaming capacity and water absorption capacity decreased significantly ($p<0.05$). Gamma-irradiation and cooking reduced the concentration of all the antinutritional factors. Combined γ-irradiation and cooking increased the peroxide value significantly ($p<0.05$) from 7.03 mg/g oil to 16.50 mg/g oil and increased the acid value, iodine value and free fatty acid of P. macrophylla seed oil. Combination of γ-irradiation and cooking increased nutrient bioavailability in the seed; it led to improvement in the functional properties and increased the susceptibility of the oil to rancidity.

Keywords: Gamma irradiation, Cooking, Oil bean seed, Physicochemical properties, Oil extract

1. Introduction

The ionizing radiation of the electromagnetic spectrum is the radiation of primary interest in food preservation, and comprises of gamma ray, X-ray and rays from high energy electron beams. Irradiation can be used as a method for preserving many types of food. The principal process which involves the use of gamma irradiation involves exposing the food to a specific dose of ionizing radiation for example, cobalt 60 (60Co) which acts by effecting the alteration of cell structure, DNA damage etc. for the purpose of disinfestations, sprouting inhibition, achieving partial or incomplete inactivation of cells of specific pathogens or of potential spoilage microorganisms, that may naturally be present on unprocessed food and other benefits.

The method employed in food preservation via γ-irradiation is done through the use of normal radioactive decay which involves the use of radioisotopes, generally 60Co and caesium 137 (137Cs). It is the cheapest source of

radiation because the source element i.e. cobalt and caesium can be obtained by atomic fission or atomic waste product. One of the most important properties of gamma ray is that it carries no mass/charge but discrete amounts (quanta) of energy. It can behave like a wave or particle (photons), has excellent penetrating power by producing large amount of energy as high as 100Kev, and has the shortest wavelength. The major concern in fatty foods is the acceleration of oxidative rancidity. Oxidative rancidity causes the formation of hydroperoxyl radicals which gives rise to unstable hydroperoxides that form carbonyl compounds leading to rancidity in unsaturated fatty acid. Irradiation is more effective in the reduction of protease inhibitors, oligosaccharides and phytic acid and other antinutrients in legumes and cereals than other domestic and industrial processing techniques.

Some products require irradiation special conditions such as low temperature, or in an oxygen free atmosphere with combination treatments such as heat and irradiation (Farkas, 1990; Grant & Patterson, 1995). Combined food processing is a strategy that permits effective processing while minimizing the severity of treatment. To maximize the effectiveness of combination treatment, mild irradiation treatment between 1 and 10 KGy is stated as the best (Campbell–Platt & Grandson, 1990). The use of cooking and γ-irradiation as forms of preservation are both proven technologies and the effectiveness of both could be maximized if they are combined.

The benefit of combining irradiation with cooking can be deduced by comparing an irradiated cooked seed and irradiated uncooked seed and also irradiated seed and irradiated cooked seed all at the same dosage level. The effect of this combined treatment method was studied in the present research work using African oil bean seed.

The African oil bean tree Pentaclethra macrophylla Benth (Leguminosae, subfamily mimosoidae) is a large leguminous, nodule-forming multipurpose tree species occurring naturally in the humid lowlands and some parts of the sub-humid zones of West and Central Africa (Enujiugha, 2008). The oil bean seed serves as a good source of edible protein and calories as the seeds are made edible by fermenting for 3-5 days. In parts of sub-Saharan Africa, the fermented product is eaten with stockfish, garden egg and sliced tapioca in a mixed vegetable salad; this is the extremely popular 'African salad', a favourite snack at masquerade and other festivals (Achinewhu, 1982). It serves as a spice and flavouring agent (Enujiugha & Agbede , 2000). The oil content of the African oil bean seed was found to be 52.28% (Enujiugha & Ayodele-Oni, 2003) with the following percent by weight of the different components: triglyceride, 39.9%; diglyceride, 2.1%; monoglyceride, 1.6%; free fatty acids (FFA), 32.9%; and sterol, 23.5%. In the fermented seed oil, the other components with exception of sterol are hydrolyzed to the free fatty acids (triglycerides become 4.89% while free fatty acids become 73.85%). African oil been seed is high in major mineral nutrients such as phosphorus, calcium, magnesium and sodium (Enujiugha & Akanbi, 2002) making it perform important functions in the body when consumed. The African oil bean seed is also known to contain high concentrations of lectins (Enujiugha & Agbede, 2000), trypsin inhibitors (Kingsley, 1995), phytates, tannins and oxalates (Enujiugha & Ayodele-Oni, 2003). The objective of this study was to assess the effect of different γ-irradiation doses and combination treatment with cooking (hurdle technique) on the nutritional quality and functional properties of the seed and the physicochemical properties of its oil extract.

2. Materials and Methods

2.1 Materials

Freshly harvested African oil bean seeds (Pentaclethra macrophylla Benth) were obtained from local farmers in Imo State of Nigeria. Upon receipt, the oil bean seeds were visually inspected and defective seeds were discarded. The seeds were then transported to the laboratory and kept in airtight polyethylene containers in a dry and cool environment until ready for use. All the chemicals and reagents used in the study were of analytical grade

2.2 Preparation of samples

The African oil bean seeds were packaged in two polyethylene bags which were sealed for each treatment. Samples were then irradiated to absorbed doses of 5 and 10 kGy using a cobalt-60 gamma irradiation source (Model GS 1000, Category 4, Panorama Wet storage Source, Siemen, Germany) located at Shedan Science and Technology Complex (SHESTCO), Abuja, Nigeria. The samples were irradiated at room temperature. Following irradiation the 5 kGy irradiated sample was milled immediately while the 10 kGy irradiated sample was divided into two parts, a part was milled while the other part was subjected to hydrothermal treatment. Raw and 10kGy irradiated seeds were subjected to hydrothermal treatment for 6 hours at 100℃, dehulled, sun dried and milled into flour. The oil in each sample except the 5 kGy irradiated sample was extracted using the Sohxtlet apparatus in order to study the effect of combined treatment on the oil quality.

2.3 Determination of chemical composition

Quantitative composition was determined on each of the samples using the following analytical methods: Moisture content according to method 964.22 (AOAC, 1990); crude protein according to method 955.04 (AOAC, 1990); crude fat extracted overnight in a Soxhlet extractor with hexane and quantified gravimetrically; ash according to method 923.03 (AOAC, 1990); crude fibre determined after digesting a known weight of fat-free sample in refluxing 1.25% sulphuric acid and 1.25% sodium hydroxide; and carbohydrates determined by the difference method (subtracting the percent crude protein, crude fibre, crude fat, and ash from 100% dry matter). All analyses were carried out in triplicates. The energy contents of the samples were obtained by multiplying crude protein, crude fat and carbohydrate contents by factors of 4, 9 and 4, respectively (Enujiugha & Ayodele-Oni, 2003).

2.4 Mineral analysis

Analysis of sodium and potassium contents of the samples was carried out using flame photometry (Model PFP 7, Jenway, UK), while phosphorus was determined by the phosphovanado-molybdate (yellow) colorimetric method (AOAC, 1990). The other elemental concentrations were determined, after wet digestion of sample ash with a mixture of nitric and perchloric acids (1:1 v / v), using Atomic Absorption Spectrophotometer (AAS, Buck Model 20A, Buck Scientific, East Norwalk, CT06855, USA). All the determinations were carried out in triplicates.

2.5 Determination of anti-nutritional factors

The method of Wheeler and Ferrel (1971) as modified by Reddy *et al*. (1982) was used for phytic acid and phytate-phosphorus determinations. Phytic acid was extracted from each 3 g flour sample with 3% trichloroacetic acid by shaking at room temperature followed by high-speed centrifugation (30,000 x g for 5 min). The phytic acid in the supernatant was precipitated as ferric phytate, and iron in the sample was estimated. Phytate-phosphorus (phytate-P) was calculated from the iron results assuming a 4:6 iron: phosphorous molecular ratio according to method 970.39 (AOAC, 1990). The phytic acid was estimated by multiplying the amount of phytate-phosphorous by the factor 3.55 based on the empirical formula C6P6O24H18 (Enujiugha & Olagundoye, 2001).

Tannin contents were determined by the modified vanillin-HCl method (Burns, 1971; Price et al., 1978). A 2.00 g sample was extracted with 50 ml 99.9 % methanol for 20 min at room temperature with constant agitation. After centrifugation for 10 min at 653 x g, 5 ml of vanillin –HCl (2 % vanillin, 1 % HCl) reagent was added to 1 ml aliquots, and the colour developed after 20 min at room temperature was read at 500 nm. Correction for interference from natural pigments in the sample was achieved by subjecting the extract to the conditions of the reaction, but without vanillin reagent. A standard curve was prepared using catechin (Sigma Chemical, St. Louis, MO) after correcting for blank, and tannin concentration was expressed in mg / 100 g.

Determination of oxalate was by the AOAC (1990) method. One gram (1 g) of finely ground sample was dissolved in 75 ml of 1.5 N H_2SO_4. The solution was carefully stirred intermittently with a magnetic stirrer for about 1 h and filtered using Whatman no. 1 filter paper. A 25 ml sample of the filtrate (extract) was collected and titrated hot (80 - 90℃) against 0.1 N $KMnO_4$ solution to the point when a faint pink colour appeared that persisted for at least 30 seconds. The concentration of oxalate in each sample was got from the calculation: 1 ml 0.1 N permanganate = 0.006303 g oxalate. All procedures were carried out in triplicates.

2.6 Determination of functional properties

The determination of water and oil absorption capacities followed a modification of the method of Prinyawiwatkul *et al*. (1997). Each flour sample (5.0 g) was thoroughly mixed, without pH adjustment with 25 ml of deionized water or oil in 50-ml centrifuge tubes. Suspensions were stirred intermittently over a 30 min period at room temperature (25℃) and then centrifuged at 12,000 x g for 30 min at 25℃. The volume of decanted supernatant was measured, and the water and oil absorption capacities were then calculated. Triplicate samples were analyzed for each flour sample category.

The least gelation concentration was carried out as described by Enujiugha and Akanbi (2005). Triplicate suspensions of 1 - 20 % seed flour sample (dry w / v, at 1 % increment) were prepared in 10ml of deionized water and mixed thoroughly without pH adjustment. The slurries were heated in 125 x 20 mm screw- capped test tubes in a water bath at 95 ± 2℃. After 1h of heating, tubes were immediately cooled in tap water for 30 sec and then in ice water for 5 min to accelerate gel formation. All tubes were then held at 4℃ for 3 h. Least gelation concentration (percent) was determined as the concentration above which the sample remained in the bottom of the inverted tube.

The foaming properties of the samples were determined using the procedure of Coffmann and Garcia (1977). Exactly 2.0 g of sample was weighed into 60 ml distilled water in a 100 ml cylinder. Solid material was dispersed with spatula and the suspension was whipped for 5 min using ultra-Turax T25 mixer at a high speed. Volumes before and after whipping were noted and volume increase due to whipping was then calculated. The volume of foam in the standing cylinder was also recorded for foam stability studies at 1, 5, 10, 20, 30, 60, 90, 120 and 180 min after whipping. The results were expressed in percentages (g / g basis).

Emulsifying properties were determined using a modification of the method described by Ige*et al*. (1984). A known quantity (1.8 g) of sample was dispersed in 25 ml distilled water, and 25 ml vegetable oil (pure groundnut oil) was added. The 50 ml mixture was emulsified at high speed using ultra-Turax T25 mixer for 1 min. Emulsion was filled into centrifuge tubes and centrifuged for 5 min at 1,300 x 6 rpm. Percentage emulsion was then expressed as % Emulsion = 100(height of emulsified layer)/(height of whole solution in centrifuge tube). The results were expressed in percentages (g / g basis).

2.7 Determination of seed oil characteristics

The seed oils of the samples were extracted using Soxhlet apparatus and the rancidity indices (peroxide value, free fatty acids content and acid value) were determined using the methods of Pearson (1976). The peroxide values were expressed as milliequivalents of peroxide oxygen per kg of sample (mEq/kg) while the free fatty acids were expressed as g oleic acid per 100 g of sample (g/100g). The acid values were expressed as mg NaOH per g of sample (mg NaOH/g). Iodine value was determined by the AOAC (1990) method using Wij's solution.

2.8 Statistical analysis

Data collected from the study were subjected to analysis of variance (ANOVA) as described by Steel and Torrie, (1980). Differences among means were separated using Duncan's multiple range test; significances were accepted at 5 % level ($P \geq 0.05$). The statistical software used was SAS 9.0 (2008) for windows.

3. Results and Discussion

3.1 Nutritional evaluation of pentaclethra macrophylla

The quality or nutritional value of any consumable plant part including seeds depends on its basic constituents, including proteins, carbohydrates, minerals and vitamins. The results of the different treatment on the proximate composition per 100g dry weight and energy value in KJ of Pentaclethra macrophylla seeds are shown in Table 1.

The protein content decreased from 32.91 g/100g DM in raw Pentaclethra macrophylla to 31.31 g/100g DM in cooked Pentaclethra macrophylla. This could be attributed to the leaching of protein into processing water (Kingsley, 1995). Protein content reduced to 30.55g/100g DM when irradiation was combined with cooking. Irradiation treatment at 5 kGy and 10 kGy had no significant effect on protein, fat and carbohydrate content ($p > 0.05$). Similar findings were observed by Aziz and Mahrous (2004), who reported that there were no changes in protein, lipid or carbohydrate, contents of gamma-irradiated wheat and bean seeds at a dose level of 5 kGy. The results show that although irradiation in itself did not affect protein content, it aided the negative effects of cooking when in combination treatment.

Cooking reduced carbohydrate content from 14.54 g/kg DM to 12.96g/100g DM and there was no significant difference when cooking was combined with 10 kGy irradiation. During the cooking of legume seeds, two simultaneous processes occur inside and outside the cotyledon cells, the gelatinization of intracellular starch and denaturation of proteins are accompanied by softening of the seeds as a result of plasticization or partial solubilization of the middle lamella, which leads to separation of individual cotyledon cells. Legume seeds require a relatively long cooking time, ranging from 1 to 6 h and it is known that starch is mainly responsible for the textural quality of cereals and legumes and especially for changes during cooking (Osman, 1967). The amount of hydrolysis is intimately related to the extent of gelatinisation, which is itself a function of the temperature of processing and the amount of water present (Tester & Sommerville, 2000; R.F. Tester & M.D. Sommerville, 2000; Tester & Sommerville, 2000). Neither 0.5 kGy nor 10 kGy doses had significant effect on total available carbohydrates, total free sugars, total starches or dextrins or on the eight individual free sugars identified in pistachio kernels (Kashani & Valadon, 1984). Cooking increased fibre content of Pentaclethra macrophylla but the fibre content was significantly reduced when irradiation at 10 kGy was combined with cooking. Fibre levels may be reduced in direct proportion to the level of irradiation due to depolymerisation and delignification.

It appears that radiation results in random depolymerisation and decomposition of cellulose (Jerome*et al*.,1952) and seriously weakens the cellulosic fibre (Gilfillan & Linden, 1955).

The moisture content of the cooked seed and irradiated cooked seed increased significantly due to absorption of the cooking water. Gamma irradiation at 5 kGy and 10 kGy had no effect on the moisture content. Similar findings were o observed by Rady *et al*., (2002) who reported that gamma irradiation has no real effect on moisture content of oil seeds.

Irradiation is also acknowledged to cause fewer overall physical and chemical changes than cooking, freezing or canning (Josephson & Peterson, 1983; Josephson *et al*., 1978; Molins, 2001). Increased irradiation dose from 5 kGy to 10 kGy caused fewer overall chemical changes than cooking and combined treatment of 10 kGy and this conformed with the finding of El- Niely (1996) who found that irradiation of broad beans (Vicia faba) at levels of 2.5, 5, 10 and 20 kGy did not induce any significant change in their chemical composition. Farag, (1989) reported that soybeans irradiated at a dose level of 10 kGy retained their normal levels of moisture, crude protein, fat and ash. This dose level does not result in the denaturation of protein, and does not affect the nitrogen containing components of the food materials.

The fat content of Pentaclethra macrophylla increased from 47.30 g/100g DM to 50.39 g/100g DM during cooking this could be as a result of heating of the fat during cooking or the liquefaction of solid fats into oils (Carmordy & Wrangham, 2009).

Cooking and combined treatment of 10 kGy irradiation increased energy value as a result of the increase in fat content and calorific content. Irradiation produces so little chemical change in food that it is difficult to design a test to determine whether a food has been irradiated or not (Stevenson, 1994). However, the combination treatment was quite effective in bringing about changes in nutrient composition.

3.2 The effect of processing on the mineral composition of pentaclethra macrophylla

Various minerals have been shown to be essential for man. They are divided into major and trace elements. The major mineral elements are calcium, potassium, sulphur, sodium, chlorine, phosphorus and magnesium while the trace elements include iron, cobalt, manganese, copper, molybdenum, selenium, chromium, tin, zinc, nickel and fluorine. They are ingested as part of certain foods. Table 2 shows the mineral composition of the Penthaclethra macrophylla. The results of the mineral analysis indicate that the oil bean seeds are rich in potassium and calcium which are paramount for teeth and bone development. Cobalt and lead were not present. All treatment reduced the potassium content of Pentaclethra macrophylla. The decrease in potassium content in the cooked sample could be attributed to leaching during processing, especially cooking. Cooking of the oil bean seeds brought about a significant ($p < 0.05$) increase in calcium from 2.61 mg/100g to 3.31 mg/100g, zinc from 0.30mg/100g to 0.45 mg/100g phosphorus from 0.43 mg/100g to 0.48mg/100g and iron from 0.24mg/100g to 0.36mg/100g. Similar findings were reported for C. esculenta and some vegetables where cooking caused significant increases in the levels of some mineral elements (Mephba *et al.*, 2007). The increase in iron could be attributed to the quality of the cooking water as a result of the presence of dissolved minerals. Sodium content in Pentaclethra macrophylla was low (0.26mg/100g). Balogun and Fetuga (1980) linked the low sodium levels of some legume seeds to the subnormal concentrations of sodium in tropical crops, which are a reflection of the low sodium contents of the soils. A previous study observed an increase of calcium with processing (hydrothermal treatment and fermentation) of the Pentaclethra macrophylla seeds (Enujiugha & Olagundoye, 2001).

Sodium, zinc, iron and phosphorus were increased by 5 kGy irradiation but that of magnesium was not significant ($p > 0.05$). Increased dose of irradiation from 5kGy to 10kGy decreased all the minerals present. Combined treatment lead to the retention of sodium calcium, zinc and iron but the same process reduced magnesium and phosphorus significantly ($p < 0.05$).

The K / Na ratio of >1 in 5kGy irradiated, 10kGy irradiated and cooked 10kGy irradiated sample might be desirable since an average human diet is low in K and high in Na. Combination treatment increased the ratio slightly.

All the assayed micro-minerals (Zn, Fe) were generally low in the samples; iron had the lowest concentration. Underwood (2003) has suggested that food and nutrition insecurity which affects an estimated 815 million households (the majority in developing countries) is in large part due to micronutrient deficiencies.

3.3 Effect of processing on functional properties

Functionality as applied to food ingredients is defined as any property on which the utility of those foods depends. A functional food may be defined as a food having health promoting benefits and/or disease preventing properties over and above its usual nutritional value. The effects of cooking, irradiation and combined treatment on the functional properties of African oil bean seeds are presented in Table 3.

Water and fat retention are basic functional properties of proteins which determine the quality (juiciness, texture, binding of structure, appearance and mouth feel)of oil absorption involves the physical entrapment of oil by food components and the affinity of non-polar protein side chains for lipids (Kinsella, 1976). Irradiation at the dose of 5 kGy and 10 kGy increased oil and water absorption capacity ($p < 0.05$). The oil and water absorption capacity values obtain for cooked and irradiated (10kGy) cooked were not significantly different ($p > 0.05$) from that of the raw samples. The higher oil absorption capacity values may imply that non-polar amino acid residues predominate in Pentaclethra macrophylla. Increases in oil and water absorption up to 10 kGy may be due to unmasking of non-polar protein residues as a result of irradiation-induced denaturation (Urbain, 1986). Increased hydrophobicity due to irradiation exposure of previously buried non-polar protein sites has also been reported for red kidney bean proteins at 2 kGy (Dogbevi *et al*., 1999). This also conformed with the study of Abu *et al*., 2005 who reported that irradiation at 10 kGy significantly ($p<0.05$) increased oil absorption capacity values in both cowpea flours and paste.

Water absorption capacity is an index of the amount of water retained within protein matrix. Increased irradiation dose (5kGy and 10kGy) decreased water absorption capacity but the decrease was not significantly different from the value of the raw sample ($p > 0.05$). The results indicate that irradiation had no apparent effect on water absorption capacity. These findings were in accordance with those reported by Zayas (1997) and Azim *et al*. (2009) that the water holding capacity was not affected by gamma irradiation. Cooking and combined treatment reduced water absorption capacity from 0.84ml/g to 0.72ml/g and 0.73 ml/g respectively. The reduction of water absorption capacity by both treatments could be as a result hydrothermal treatment which blocked the tissue pores, thereby hindering water sippage and retention. Cooking has also been shown to reduce water absorption capacity in conophor nut (Enujiugha, 2003).

Irradiation treatment at 5kGy and 10kGy did not change the least gelation concentration of the Pentaclethra macrophylla. The gel forming ability is reported to be influenced by the nature of the protein, starch and gums in the sample as well as their interaction during heat treatment (Enujiugha *et al*., 2003). Cooking and combined treatment improved the binding properties of Pentaclethra macrophylla.

Foaming capacity is dependent on the protein, and the raw seed flour with the highest protein content demonstrated the highest foaming capacity. Foaming capacity was significantly reduced by cooking and combined treatment ($p<0.05$). This could be as a result of protein denaturation as well as dimunition protein solubility during heat treatment (Tagodoe & Nip, 1994). Increased irradiation dose to 10 kGy didn't affect foaming capacity and this did not conform to the findings of Abu *et al*. (2005) who reported that irradiation at 10 kGy decreased the foaming capacity of cowpea flours significantly ($p < 0.05$). It has been suggested that foaming properties are negatively related to protein denaturation in that native proteins have higher foaming abilities than denatured proteins (Yasumatsu *et al*., 1972). Although higher protein denaturation would have been expected if 10 kGy irradiation was done after Pentaclethra macrophylla seeds were cooked given the additional radiolytic effect of added water. The foaming capacity of combined treated sample (5%) didn't reduced more than the cooked samples (5%) ($p > 0.05$).

The foams from cooked seed and combined treated seed flours showed no stability after 30 min, while the raw seed flour, 5 and 10 kGy irradiated seed flour gave foam that was stable at 2 h, indicating that the native protein gives higher stability than denatured protein (Enujiugha & Akanbi, 2005). Similar observations had been reported for taro flour, tannia flour, cowpea flour and winged bean flour (Fagbemi & Olaofe, 2000; Narayana & Narasinga Rao, 1984; Bencini, 1986).

Emulsion capacity denotes the maximum amount of oil that can be emulsified by protein dispersion. The high emulsion capacity could be as a result of high content of free fatty acid which leads to increased oil absorption (Ihekoronye & Ngoddy, 1985). Cooking and combined treatment increased emulsion capacity of Pentaclthra macrophylla. Irradiation at 5 kGy and 10kGy decreased the emulsion capacity significantly ($p<0.05$). The decrease in emulsion property may be attributed to protein aggregation as well as surface hydrophobicity and change the characteristics, which affect emulsifying properties in different ways (Cheftel *et al*., 1985). Irradiation at 2, 10 and 50 kGy caused significant ($p<0.05$) decrease in emulsion capacity of cowpea flours and pastes when compared with the non-irradiated (control) samples. Also, it was reported that irradiation of cowpea at 2.0 KGy caused decrease in emulsion capacity (Abu *et al*., 2005).

3.4 Effect of processing on anti-nutritional factors

The levels of some anti-nutritional factors in the raw and processed seed flour samples are presented in Table 4. The anti-nutritional factors are generally reported to have the capacity of retarding growth and lowering digestibility and absorption of important dietary nutrients.

Each successive processing step significantly ($p< 0.05$) lowered the concentrations of oxalate and tannin in the Pentaclethra seeds. Oxalic acid and its salts can have deleterious effects on human nutrition and health, particularly by decreasing calcium absorption and aiding the formation of kidney stones (Noonan and Savage, 1999). High-oxalate diets can increase the risk of renal calcium oxalate formation in certain groups of people (Libert and Franceschi, 1987). The majority of urinary stones formed in humans are calcium oxalates stones (Hodgkinson, 1977). The oxalate content was recorded lowest (1.3mg/g) for the combined treated sample and highest (2.54 mg/g) for the raw seed. Cooking treatments were found effective measure to reduce the oxalate content in these wild tubers. Cooking may cause considerable skin rupture and facilitate the leakage of soluble oxalate into cooking water (Albihn and Savage, 2001), this may be the possible reason to observed high reduction in oxalate level upon cooking. The reduction in oxalate levels on cooking is expected to enhance the bioavailability of essential dietary minerals of the African oil bean, as well as reduce the risk of kidney stones occurring among consumers.

Phytic acid chelates mineral cations and proteins, forming insoluble complexes, which leads to reduced bioavailability of trace minerals and reduced digestibility of proteins (Reyden & Selvendran, 1993). According to Hurrell *et al*. (1992), phytic acid inhibits iron absorption, which invariably contributes to the high prevalence of iron deficiency in infants in developing countries. The reduction in phytate level could be interpreted as the main reason behind the observed increase in the concentrations of some of the minerals during cooking. It has been suggested that the traditional methods employed in processing the seeds, namely hydrothermal treatment, soaking and fermentation, could considerably reduce the levels of the anti-nutritional factors (Enujiugha & Ayodele-Oni, 2003). Also, the apparent decrease in phytate content during cooking may be partly due either to the formation of insoluble complexes between phytate and other components, such as phytate-protein and phytate-protein–mineral complexes or to the inositol hexaphosphate hydrolyzed to penta- and tetraphosphates (Siddhuraju and Becker, 2001). The observations are contrast to Wanasundera and Ravindran, (1992) who reported that phytate content in yam tubers were unaffected by the cooking. However, cooking has been reported to lower the phytate levels in several plant foodstuffs (Badifu, 2001; Reddy *et al*., 1982; Saikia *et al*., 1999; Vijayakumari *et al*., 1997).

Cooking and irradiation at 10 kGy also lead to a significant decrease in phytate level ($P<0.05$) but this was insignificant compared to 10 kGy irradiation ($p>0.05$) but the 10kGy treatment lead to a significant decrease from that of the raw oil bean seed which had a value of 2.27. Cooking and irradiation caused a significant decrease (40%) in sorghum porridge (Duodu *et al*., 1999). Similarly, treatment of soybean seeds with irradiation, alone or in combination with soaking, reduces the level of phytate compared to controls (Sattar *et al*., 1990). This reduction is probably due to chemical degradation of phytate to the lower inositol phosphates and inositol by the action of free radicals produced by the radiation (De Boland *et al*., 1975. View Record in ScopusCited By in Scopus (95) De Boland *et al*., 1975). Another possible mode of phytate loss during irradiation could have been through cleavage of the phytate ring itself.

3.5 Rancidity indices of the oil of pentaclethra macrophylla

The rancidity indices comprises of peroxide and iodine value, acid value as well as the free fatty acids content, as these are reflectives of oxidative (oxygen-enhanced) rancidity, total acidity and hydrolytic rancidity respectively. The products of rancidity are known to be hazardous to health since they are associated with aging, membrane damage, heart disease and cancer (Cosgrove *et al*., 1987). The rancidity indices of oil as affected by processing are presented in Table 5.

Free fatty acids content is an indicator of the level of hydrolytic rancidity of oil. The free fatty acid of Pentaclethra macrophylla oil sample reduced significantly upon cooking ($p<0.05$) from 5.22 to 4.43 (% FFA as oleic acid). This may be due to the loss of volatile free fatty acid leading to decreased free fatty acid content, alternatively the higher free fatty acid content in raw samples when compared to cooked samples could also be explained by the deactivation of enzymes, due to the heating process which would prevent the release of free fatty acids due to lipase activity in the cooked samples. These results are in agreement with those of Al-Saghir *et al*., (2004). View Record in Scopus Cited By in Scopus (26) Al-Saghir *et al*. (2004), who observed a decrease of free fatty acid in Salmon fillets, steamed or pan-fried, either with or without different types of oil. (Chantachum *et al*., 2000) also observed a lower free fatty acid content in oil prepared from tuna heads, by heating at 95 °C, when compared to the raw oil. All treatment significantly decreased ($p<0.05$) decreased free fatty acid content and this indicate that all the oil samples other than oil from raw seed sample thus processed oil samples have fairly low acidity, and thus are in a high state of purity according to Akpuaka and Nwankwor (2000).

In the tropics, where vegetable oils are the most common dietary lipid, it has been shown that it is desirable to ensure that the free fatty acid content of cooking oil lies within limits of 0.0–3.0% (Bassir, 1971). In the present study only the sample irradiated at 10 kGy had values within this range. The presence of high free fatty acid content leads to the formation of off flavour, depressed smoke point of cooking and frying fat, it leads to the loss of nutritive value, undesirable toughening, increased fat absorption and reduced water absorption (Ihekoronye & Ngoddy, 1985). Free fatty acid is usually removed during the refining process.

The acid values of the extracted oils indicate the total acidity as contributed by the fatty acids in the sample. Hydrolysis of glycerides to yield fatty acid occurs during storage.

The total acidity expressed as acid value was highest in the oil of raw sample (18.51 mg NaOH/g) followed by oil from the 10 kGy irradiated sample (15.70 mg NaOH/ g of oil) and was lowest in the oil from cooked sample (8.48 mg NaOH/g). The oil from the cooked sample would be most suitable for use in industrial manufacture of soap because it has the lowest acid value (Devine & Williams, 1961).

The peroxide value is an indicator of deterioration of fats. As oxidation takes place the double bonds in the unsaturated fatty acids are attacked forming peroxides. Fresh oils have been shown to have peroxide values lower than 10 mg/g oil and oils become rancid when the peroxide value ranges from 20.0 to 40.0 mg/g oil (Pearson, 1976). Ojeh (1981) reported that oils with high peroxide values are unstable and easily become rancid (having a disagreeable odour). The lower peroxide value recorded for oil from the raw sample (7.03mg/g oil) compared to the other samples is an indication of its relative stability to oxidative rancidity. There was a significant increase ($p<0.05$) in the value of oil from cooked and 10kGy irradiated sample and it was statistically different from the value of the oil from the cooked sample and the 10kGy irradiated sample which had 10.23mg/g oil and 8.75 mg/g oil respectively. The peroxide value of the oil from the cooked Pentaclethra macrophylla could be as a result of the heat treatment because high temperature accelerates oxidative rancidity (Ihekoronye & Ngoddy, 1985). Exited ions produced as a result of irradiation could have lead to the formation of free radicals which could have resulted in increased peroxide value in the oil of the 10kGy irradiated sample.

The oil from the raw seed and the 10kGy irradiated seed is stable but that from the raw sample is more stable. The increased that accompanied the combined treatment may be due to the combination of the effect of excited ions formed by irradiation and the heat treatment in form of cooking. However, the high peroxide value of oil from combined treatment this indicates an increase the susceptibility of the oil to lipid oxidation.

Iodine value is the measure of the proportion of unsaturated acids present. The principle of iodine value is due to the reactivity of double bonds with halogens. The oil with high iodine value has high degree of unsaturation. The level of unsaturation decreases with oxidation. The iodine value ranged from 18.74 mg/g oil in raw sample to 17.58 mg/g oil in 10kGy irradiated sample, to 13.31 mg/g oil in cooked sample to 8.86 mg/g oil in combined treated sample. All the values were statistically different at $p<0.05$. The reduction in the iodine value of the cooked sample could be as a result of thermal inhibition of lipase which occurs at 80℃ (Ihekoronye & Ngoddy, 1985). The oil from the raw oil bean had the highest iodine value and the oil obtained from the combined treated sample has the lowest.

Because there are a wide number of unsaturated acid which could be present in fats, iodine value alone does not provide a measure of specific unsaturated acid and a development of a more reliable method is required to determine fatty acid composition (Ihekoronye & Ngoddy, 1985).

4. Conclusion

The results of the present study have shown that neither 5kGy nor 10kGy doses had significant effect on protein, carbohydrate, fat, ash, fibre, moisture content and energy value of Pentaclethra macrophylla seed. Increasing the dose led to nutrient retention of the seed. Changes were observed in the combined γ-irradiated and cooked sample as a result of cooking and the sample had the highest energy value. Cooking and 5 kGy irradiation increased zinc, phosphorus and iron. Increased dose of irradiation from 5kGy to 10kGy decreased all the minerals present. Combined treatment led to the retention of sodium, calcium, zinc and iron but the same process reduced magnesium and phosphorus. The 10kGy irradiation had a negative effect on the mineral content of P. macrophylla seeds. Gamma-irradiation and cooking increased the ability of protein in P. macrophylla to form a structure matrix for holding water, sugar and flavour and other food ingredients, and its ability to form an emulsion. Combined treatment reduced the amount of water retained in the protein matrix and this reduced its tendency as a thickening agent and its ability to form stable foam. The reduction of all the antinutritional factors by combined γ-irradiation and cooking increased nutrient bioavailability and protein digestibility as these antinutritional factors are known to bind protein and other nutrients.

References

Abu, J. O., Klaus. M., Kwaku, G. D., Duodu, G., & Amanda, M. (2005). Functional properties of cowpea (Vigna unguiculata L. Walp) flours and pastes as affected by gamma-irradiation. *Journal of Food Chemistry, 93*, 103-111. http://dx.doi.org/10.1016/j.foodchem.2004.09.010

Achinewhu, S. C. (1982). Composition and food potential of African oil bean seed (Pentaclethra macrophylla) and velvet beans (Mucuna urines). *Journal of Food science, 47*, 1736. http://dx.doi.org/10.1111/j.1365-2621.1982.tb05025.x

Akpuaka, M. U., & Nwankwor, E. (2000). Extraction, analysis and utilization of a drying oil from Tetracarpidium conophorum. *Bioresource Technology, 73*, 195-196. http://dx.doi.org/10.1016/S0960-8524(99)00159-5

Albihn, P. B. E., & Savage, G. P. (2001). The effect of cooking on the location and concentration of oxalate in three cultivars of New Zealand-grown Oxalis tuberose. *Journal of the Science of Food and Agriculture, 81*, 1027–1033. http://dx.doi.org/10.1002/jsfa.890

Al-Saghir, S., Thurner, K., Wagner, K. H, Frisch., G., & Luf, W. (2004). Effects of different cooking procedures on lipid quality and cholesterol oxidation of farmed salmon fish (Salmo salar). *Journal of Agricultural and Food Chemistry, 52*, 5290–5296. http://dx.doi.org/10.1021/jf0495946

AOAC. (1990). Official Methods of Analysis, 15th Ed. Association of Official Analytical Chemists, Washington DC.

Azim, A. M., Nour, S. E., & Ahmed, G. O. (2009). Effect of Gamma Irradiation on Physico-chemical Characterstics of Groundnut (Arachis Hypogaea). *Australian Journal of Basic and Applied Science, 3*(3), 2856-2860.

Aziz, N. H., & Mahrous, S. R. (2004). Effects of gamma irradiation on aflatoxin B1 production by Aspergillus flavus and chemical composition of three crop seeds. *Nahrung, 48*, 234-238. http://dx.doi.org/10.1002/food.200300429

Badifu, G. I. O. (2001). Effect of processing on proximate composition, antinutritional and toxic contents of kernels from Cucurbitaceae species grown in Nigeria. *Journal of Food Composition and Analysis, 14*, 153-166. http://dx.doi.org/10.1006/jfca.2000.0964

Balogun, A. M., & Fetuga, B. L. (1980). Tannin, phytin and Oxalate content of some wild under-utilized crop-seeds in Nigeria. *Food Chemistry, 30*, 37-43. http://dx.doi.org/10.1016/0308-8146(88)90022-2

Bassir, O. (1971). *Handbook of practical biochemistry (2nd ed.)*. Ibadan, Nigeria: Ibadan University Press.

Bencini, M. C. (1986). Functional properties of drumdried chick pea flour. *Journal of Food Science,* 1521-1526.

Burns, R. (1971). Method for estimation of tannin in grain sorghum. *Agronomy Journal, 63*, 511-512. http://dx.doi.org/10.2134/agronj1971.00021962006300030050x

Cambell-Platt, G., & Grandson, A. S. (1990). Food irradiation and combination processes. *Int. J. Radiat. Appl. Instum, 35*, 237.

Carmody, R. N., & Wrangham, R. W. (2009). The energetic significance of cooking. *Journal of food chemistry, 57*(4), 379-391.

Chantachum, S., Benjakul, S., & Sriwirat, N. (2000). Separation and quality of fish oil from precooked and non-precooked tuna heads. *Food Chemistry, 69*, 289-294. http://dx.doi.org/10.1016/S0308-8146(99)00266-6

Cheftel, J. C., Cuq, J. L., & Lorient, D. (1985). Food Chemistry (2nded.), Marcel Dekker, New York.

Coffmann, C. N., & Garcia, V. A. (1977). Functional properties of the protein isolate from mung bean flour. *Journal of Food Science, 12*, 473-478.

Cosgrove, J. P., Church, D. F., & Pryor, W. A. (1987). The kinetics of the autoxidation of polyunsaturated fatty acids. *Lipids, 22*, 299-304. http://dx.doi.org/10.1007/BF02533996

De Boland, A. R., Garner, G. B., & O'Dell, B. L. (1975). Identification and properties of 'phytate' in cereal grains and oilseed products. *Journal of Agricultural and Food Chemistry, 23*, 1186-1189. http://dx.doi.org/10.1021/jf60202a038

Devine, J., & Williams, P. N. (1961). *The chemistry and technology of edible oils and fats (1st ed.)*. London: Pergamon Press.

Dogbevi, M. K., Vachon, C., & Lacroix, M. (1999). Physico-chemical properties of dry red kidney bean proteins and natural microflora as affected by gamma irradiation. *Journal of Food Science, 64*, 540-542. http://dx.doi.org/10.1111/j.1365-2621.1999.tb15080.x

Duodu, K. G., Minnaar, A., & Taylor, J. R. N. (1999). Effect of cooking and irradiation on the labile vitamins and antinutrient content of a traditional African sorghum porridge and spinach relish. *Food Chemistry, 66*, 21-27. http://dx.doi.org/10.1016/S0308-8146(98)00070-3

El-Niely, H. F. G. (1996). Chemical constituents and digestibility of broad beans subjected to irradiation treatment. MSc thesis, Women's College, Ain Shams University, Cairo, Egypt.

Enujiugha, V. N. (2003). Chemical and Functional Characteristics of Conophor Nut. *Pakistan Journal of Nutrition, 2*(6), 335-338. http://dx.doi.org/10.3923/pjn.2003.335.338

Enujiugha, V. N. (2008). Pentaclethra macrophylla African oil bean, pp. 398-400. In: The Encyclopedia of Fruit and Nuts (J. Janick and R. E. Paull, eds.), CAB International, Oxfordshire, UK.

Enujiugha, V. N., & Agbede, J. O. (2000). Nutritional and antinutritional characteristics of African oil beans (Pentaclethra macrophylla Benth) seeds. *Applied Tropical Agriculture, 5*, 11-14.

Enujiugha, V. N., & Akanbi, C. T. (2002). Adaptable techniques for the post harvest processing and preservation of African oil bean seeds proceedings, Regional Workshop On Promotion of Appropriate Agro-processing Technologies in West Africa, Ile-Ife, Nigeria (23rd -26thOctober, 2002), 164-169.

Enujiugha, V. N., & Akanbi, C. T. (2005). Compositional changes in African oil bean seeds during thermal processing. *Pakistan Journal of Nutrition, 4*(1), 27-31. http://dx.doi.org/10.3923/pjn.2005.27.31

Enujiugha, V. N., & Ayodele-Oni, O. (2003). Evaluation of nutrient and some antinutrient in lesser-known, underutilized oil seeds. *International Journal of Food Science and Technology, 38*, 525-528. http://dx.doi.org/10.1046/j.1365-2621.2003.00698.x

Enujiugha, V. N., Badejo, A. A., Iyiola, S. O., & Oluwamukomi, M. O. (2003). Effect of germination on the nutritional and functional properties of African oil bean (Pentaclethra macrophylla Benth) seed flour. *Journal of Food, Agriculture and Environment, 1*(3/4), 72-75.

Enujiugha, V. N., & Olagundoye, T. V. (2001). Comparative nutritional characteristics of raw, fermented and roasted African oil bean (Pentaclethra macrophylla Benth) seeds. *La Rivista Italiana delle Sostanze Grasse*, *78*(4), 247-250.

Fagbemi, T. N., & Olaofe, O. (2000). The chemical composition and functional properties of raw and precooked taro (Colocasia esculenta) flours. *Journal of Food Chemistry*.

Farag, M. E. (1989). Radiation deactivation of antinutritional factors: trypsin inhibitor and hemagglutinin in soybeans. *Egyptian Journal of Radiation Sciences and Applications, 6*, 207-215.

Farkas, J. (1990). Combination of irradiation with mild heat treatment. *Food control, 1*, 223-229. http://dx.doi.org/10.1016/0956-7135(90)90073-L

Gilfillan, E. S., & Linden, L. (1955). Effects of nuclear radiation on the strength of yearns. *Textile Research Journal, 25*, 773-777. http://dx.doi.org/10.1177/004051755502500905

Grant, I. R., & Patterson, M. F. (1995). Combined effect of gamma radiation and heating on the destruction of Listeria monocytogenesis and Salmonella typhirium in cook chill roast beef and gravy. *International Journal of Food Microbiology, 27*, 117-128. http://dx.doi.org/10.1016/0168-1605(94)00156-Z

Hodgkinson, A., (1977). *Oxalic acid in Biology and Medicine*. London, Academic Press.

Hurrel, R. F., Juillert, M. A., Reddy, M. B., Lynch, S. R., Dassenko, S. A., & Cook, J. D. (1992). Soy protein, phytate and iron absorption in humans. *American Journal of Clinical Nutrition, 56*, 573–578.

Ige, M. M., Ogunsua, A. O., & Oke, O. L. (1984). Functional Properties of the Proteins of Some Nigerian Oilseeds, Conophor Seeds and Three Varieties of Melon Seeds. *Journal of Agricultural and Food Chemistry, 32*, 822-825. http://dx.doi.org/10.1021/jf00124a031

Ihenkoronye, A. I., & Ngoddy, P. O. (1985). *Integrated Food Science and Techology for the Tropics*. Macmillian publishers, London, pp. 17-18, 45, 53, 64, 66.

Jerome, F. A., Millett, M. A., & Lawton, E. (1952). Effect of high energy cathode rays on cellulose. *Industrial and Engineering Chemistry, 44*, 2848-2852. http://dx.doi.org/10.1021/ie50516a027

Josephson, E. S., Thomas, M. H., & Calhoun, W. K. (1978). Nutritional aspects of food irradiation; an overview. *Journal of Food Processing and Preservation, 2*, 299-313. http://dx.doi.org/10.1111/j.1745-4549.1978.tb00564.x

Kashani, G. G. & Valadon, L. R. G. (1984). Effects of gamma irradiation on the lipids, carbohydrates and proteins of Iranian pistachio kernels. *Journal of Food Technology, 19*, 631–638. http://dx.doi.org/10.1111/j.1365-2621.1984.tb01880.x

Kingsley, M. O. (1995). Effect of processing some antinutritive and toxic component on the nutritional composition of African oil bean seed (Penthaclethra macrophylla Benth). *Journal of Food Science and Agriculture, 68*, 153-158. http://dx.doi.org/10.1002/jsfa.2740680204

Kinsella, J. E. (1976). Functional properties of proteins in foods: a survey. *Critical Reviews in Food Science and Nutrition, 1* (3), 219-280.

Libert, B., & Franceschi, V. R. (1987). Oxalate in crop plants. *Journal of Agricultural and Food Chemistry, 35*(6), 926-937. http://dx.doi.org/10.1021/jf00078a019

Mepba, H. D., Eboh, L., & Banigo, D. E. B. (2007). Effects of processingtreatments on the nutritive composition and consumer acceptance of some Nigerian edible leafy vegetables. *Afr. J. Food Agric. Nutr. Dev., 7*(1), 1-18.

Molins, M. (2001). Food Irradiation: Principles and Applications, Wiley Publication, USA.

Narayana, K., Narasinga Rao, M. S. (1984). Effect of partial proteolysis on the functional properties of winged bean (Psophocarpus tetragolobus) flour. *Journal of Food Science, 49*, 944-947. http://dx.doi.org/10.1111/j.1365-2621.1984.tb13247.x

Noonan, S. C., & Savage, G. P. (1999). Oxalic acid and its effects on humans. *Asia Pacific Journal of Clinical Nutrition, 8*, 64-74. http://dx.doi.org/10.1046/j.1440-6047.1999.00038.x

Ojeh, O. (1981). Effects of refining on the physical and chemical properties of cashew kernel oil. *Journal of Fats and Oils Technology, 16*, 513-517.

Osman, E. N. (1967). *Starch in food industry*. In: Whistler, R.L. and Paschall, E.F.,Editors, Starch: chemistry and technology (vol. 2), Academic Press, London.

Price, M. L., Scoyoc, S. V., & Butler, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry, 26*, 1214-1218. http://dx.doi.org/10.1021/jf60219a031

Prinyawiwatkul, W., Beuchat, L. R., McWatters, K. H., & Phillips, R. D. (1997). Functional properties of cowpea (Vigna unguiculata) flour as affected by soaking, boiling, and fungal fermentation. *Journal of Agricultural and Food Chemistry, 45*(2), 480-486. http://dx.doi.org/10.1021/jf9603691

Rady, A. H., Abdel Hady, S. M., Elnashabi F. M., Afifi, E. A., & Salam, E. M. (2002). Influence of Gamma rays and Microwave heating on the Quality of Olive fruits and their virgin oil. *Isotope and Rad. Res, 34*, 369-380.

Reddy, N. R., Sathe, S. K., & Salunkhe, D. K. (1982). Phytate in legumes and cereals. *Advances in Food Research, 28*, p. 1. http://dx.doi.org/10.1016/S0065-2628(08)60110-X

Reyden, P., & Selvendran, R. R. (1993). *Phytic acid: properties and determination*. In: R. Macrae, R. K. Robinson and M.J. Sadler, Editors, Encyclopedia of food science, food technology and nutrition, Academic Press, London, pp. 3582-3587.

Saikia, P., Sarkar, C. R., & Borua, I., (1999).Chemical composition, antinutritional factors and effect of cooking on nutritional quality of rice bean Virgna umbellate. *Journal of Food Chemsitry, 67*, 347–352. http://dx.doi.org/10.1016/S0308-8146(98)00206-4

Satter, A., & Neelofar Akhtar, M. A. (1990). Irradiation and germination effects on phytate, protein and amino acids of soybean. *Plant Foods for Human Nutrition, 40*, 185–195. http://dx.doi.org/10.1007/BF01104141

Siddhuraju, P., & Becker, K. (2001). Effect of various domestic processing methods on antinutrients and in vitro-protein and starch digestibility of two indigenous varieties of Indian pulses, Mucuna pruriens var utilis. *Journal of Agricultural and Food Chemistry, 49*(6), 3058–3067. http://dx.doi.org/10.1021/jf001453q

Steel, R. G. D., & Torrie, J. H. (1980). *Principles and procedures of statistics: A biometrical approach, 2nd ed.* McGraw-Hill Inc., New York

Stevenson, M. H. (1994). Identification of Irradiated foods. *Food Technology, 48*, 141-144.

Tagodoe, A., & Nip, N. W. (1994). Functional properties of raw and precooked taro flour. *International Journal of Food Science and Technology, 29*, 457-462. http://dx.doi.org/10.1111/j.1365-2621.1994.tb02087.x

Tester, R. F., & Sommerville, M. D. (2000). Swelling and enzymatic hydrolysis of starch in low water systems. *J. Cereal Sci., 33*, 193–203. http://dx.doi.org/10.1006/jcrs.2000.0350

Underwood, B. A. (2003). Scientific research: essential, but is it enough to combat world food insecurities? *Journal of Nutrition, 133*(Suppl. 1), 1434S-1437S.

Urbain, W. M. (1986). Food irradiation, Academic press, London.

Vijayakumari, K., Siddhuraju, P., & Janardhanan, K. (1997). Effect of domestic processing on the levels of certain antinutrients in Prosopis Chilensis (Molina) Stunz. Seeds. *Food Chemistry, 59* (3), 367-371. http://dx.doi.org/10.1016/S0308-8146(96)00249-X

Wanasundera, J. P. D., & Ravindran, G. (1992). Effects of cooking on the nutrient and antinutrient contents of yam tubers (Dioscorea alata and Dioscorea esculenta). *Food Chemistry, 45*, 247-250. http://dx.doi.org/10.1016/0308-8146(92)90155-U

Wheeler, E. L., & Ferrel, R. E. (1971). A method for phytic acid determination in wheat fractions. *Cereal Chemistry, 48*, 312-316.

Yasumatsu, K. K., Sawada, S., Moritaka, M., Misaki, J., Toda, T., Wada, T., & Ishii, K. (1972). Whipping and emulsifying properties of soybean products. *Journal of Agricultural and Biological Chemistry, 36*, 719. http://dx.doi.org/10.1271/bbb1961.36.719

Zayas, J. F. (1997). *Functionality of proteins in food.* New York:Springer-Verlag, pp. 1, 34–56, 144-146. http://dx.doi.org/10.1007/978-3-642-59116-7_1

Table 1. Proximatecomposition of raw and processed pentaclethra macrophylla seeds (%100g dry weight)

Parameters	Raw	Cooked	5kgy Irradiated	10kgy Irradiated	10kgy Irradiated And Cooked
Protein	32.91a±0.08	31.31b±0.31	32.50a±0.07	32.66a±0.16	30.55c±0.71
Fat	47.43c±0.10	50.39b±0.42	48.07c±0.31	47.42c±0.50	51.19a±0.30
Ash	2.91a±0.06	2.25b±0.47	2.79a±0.01	2.76a±0.25	2.28b±0.27
Fibre	2.22c±0.15	3.27a±0.01	2.41c±0.09	2.20c±0.17	2.73b±0.55
Nfe	14.54a±0.24	12.96b±0.10	14.23a±0.28	14.69a±0.23	12.71b±0.21
Mc	4.15c±0.72	9.36b±0.42	4.66c±0.33	4.75c±0.28	10.42a±0.62
E Value (Kj)	2583.81b±1.49	2643.60a±9.58	2595.37b±7.65	2579.61b±17.67	2655.27a±2.81

*All values are expressed as means of triplicate determinations (mean ± s.d.). Values along same row with the same following letters in superscript are not significantly different ($p > 0.05$).

Table 2. Mineral composition of raw and processed pentaclethra macrophylla seeds (mg/100g)

Parameters	Raw	cooked	5 kgy irradiated	10 kgy irradiated	10kgy irradiated and cooked
Pottasium	4.38a±0.51	1.85b±0.06	1.62c±0.09	0.92d±0.01	1.29dc±0.10
Sodium	0.26c±0.03	0.37c±0.02	1.56a±0.03	0.91b±0.00	0.30c±0.19
Calcium	2.61b±0.30	3.53a±0.10	3.59a±0.20	1.79c±0.58	2.45b±0.21
Zinc	0.30b±0.03	0.45a±0.04	0.47a±0.02	0.25c±0.01	0.33b±0.00
Iron	0.24bc±0.91	0.36a±0.08	0.28b±0.01	0.17c±0.03	0.23bc±0.09
Magnesium	0.52a±0.04	0.56a±0.03	0.59a±0.02	0.28c±0.01	0.47b±0.10
Phosphorus	0.43b±0.01	0.48a±0.20	0.48a±0.01	0.26d±0.22	0.35c±0.21

*All values are expressed as means of triplicate determinations (mean ± s.d.). Values along same row with the same following letters in superscript are not significantly different ($p > 0.05$). Cu and Pb were not detected in the samples.

Table 3. Functional properties of raw and processed penthaclethra macrophylla seeds

Parameters	Raw	Cooked	5kgy Irradiated	10kgy Irradiated	Parameters
Oac (ml/g)	1.27b±0.31	1.00b±0.00	1.39a±0.12	1.43a±0.31	Oac (ml/g)
Wac (ml/g)	0.84a±0.04	0.72c±0.00	0.80ab±0.04	0.76bc±0.00	Wac (ml/g)
Fc (%)	13.33a±2.89	5.00b±0.00	13.33a±2.89	11.67a±2.89	Fc (%)
Lgc (g/cm3)	10.33b±0.58	12.33a±0.58	10.67b±0.58	10.67b±0.58	Lgc (g/cm3)
Ec (%)	37.50b±2.00	42.00a±1.00	34.00c±2.00	35.33c±1.15	Ec (%)

All values are expressed as means of triplicate determinations (mean ± s.d.) Values along same row with the same following letters in superscript are not significantly different ($p > 0.05$).

Table 4. Antinutritional factors in pentaclethra macrophylla seeds

Parameters	Raw	Cooked	5kgy irradiated	10kgy irradiated	Irradiated and 10kgy cooked
Tannin (g/100g)	0.48a±0.00	0.34b±0.00	0.37b±0.00	0.32c±0.02	0.29d±0.01
Oxalate (mg/g)	2.54a±0.03	2.11bc±0.00	2.14b±0.01	1.94c±0.20	1.30d±0.10
Phytin-p (g/100g)	0.64a±0.00	0.63a±0.05	0.63a±0.01	0.55b±0.10	0.53b±0.04
Phytate(mg/100g)	2.27a±0.01	2.23a±0.18	2.23a±0.04	1.94b±0.35	1.87b±0.02

* All values are expressed as means of triplicate determinations (mean ± s.d.). Values along same row with the same following letters in superscript are not significantly different ($p > 0.05$).

Table 5. Physicochemical properties of the seed oil extract

Parameters	Raw	Cooked	10kGy Irradiated	Irradiated And10kGy Cooked
Peroxide value (mg/g oil)	7.03d±0.45	10.23b±0.38	8.75c±0.56	16.50a±0.53
Iodine value (mg/100g)	18.74a±0.15	13.13c±0.15	17.58b±0.31	8.86d±0.17
Acid value (mg NaoH/g oil)	18.51a±2.80	8.48d±0.80	15.70b±0.03	13.06c±0.28
Free fatty acid (%FFA as oleic)	5.22a±0.08	4.43b±0.11	0.24d±0.02	3.69c±0.08

*All values are expressed as means of triplicate determinations (mean ± s.d.). Values along same row with the same following letters in superscript are not significantly different ($p > 0.05$).

Osmotic Dehydration Process for Preservation of Fruits and Vegetables

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Abstract

Osmotic dehydration has received greater attention in recent years as an effective method for preservation of fruits and vegetables. Being a simple process, it facilitates processing of fruits and vegetables such as banana, sapota, fig, guava, pineapple, apple mango, grapes, carrots, pumpkins, etc. with retention of initial fruit characteristics viz., colour, aroma, texture and nutritional composition. It is less energy intensive than air or vacuum drying process because it can be conducted at low or ambient temperature. It has potential advantages for the processing industry to maintain the food quality and to preserve the wholesomeness of the food. It involves dehydration of fruit slices in two stages, removal of water using as an osmotic agent and subsequent dehydration in a dryer where moisture content is further reduced to make the product shelf stable.

Keywords: Osmotic dehydration, Preservation, Fruits, Vegetables, Organoleptic quality

1. Introduction

Fruits and vegetables contribute a crucial source of nutrients in daily human diet. The world fruit production is estimated to be 434.7 million metric tones and vegetables 90.0 million metric tones. India is the second largest fruits and vegetable producer and its annual production is 44 million metric tones from an area of 3, 949, 000 ha during 2000-2002 (Srivastava & Kumar, 2002). Fruits and vegetables losses in the developing countries are considerably high. In India, post harvest losses of fruits and vegetables are estimated as more than 25 percent.

Many processing techniques can be employed to preserve fruits and vegetables by drying and dehydration is one of the most important operations that are widely practiced because of considerable saving in packaging, storage etc.

Osmotic dehydration has received greater attention in recent years as an effective method for preservation of fruits and vegetables. Being a simple process, it facilitates processing of tropical fruits and vegetables such as banana, sapota, pineapple, mango, and leafy vegetables etc. with retention of initial fruit and vegetables characteristics viz., colour, aroma and nutritional compounds (Pokharkar & Prasad, 1998). It is less energy intensive than air or vacuum drying processes because it can be conducted at low or ambient temperature. It has potential advantages for the processing industry to maintain the food quality and to preserve the wholesomeness of the food. It involves dehydration of fruit slices in two stages, removal of water using as an osmotic agent (osmotic concentration) and subsequent dehydration in a dryer where moisture content is further reduced to make the product shelf stable (Ponting, 1973).

Osmotic concentration is the process of water removal from fruits and vegetables, because the cell membranes are semi-permeable and allow water to pass through them more rapidly than sugar. During osmosis small quantity of fruit acid is removed along with water. It is a dynamic process, in which water and acid are removed at first and then move slowly, while sugar penetration is very slight at first but increases with the time. Therefore,

the characteristics of the product can be varied by controlling temperature, sugar syrup concentration, concentration of osmosis solution, time of osmosis etc. to make osmotic concentration process faster.

2. Application of Osmosis in Food Processing

The osmotic dehydration process and influence of its process variables such as pretreatment, temperature of sugar solution and additives on the mass transfer in osmotic dehydration of various fruits was studied by Ponting *et al*. (1966) and reported that the apple slices reduced to 50 per cent of original weight by using 60 – 70 ℃ Brix sugar solution and superior quality. The study also indicated that there was no need of sulphur dioxide treatment to prevent loss of colour. The osmotic air-dried products were high in superior quality and reported that the osmosis process removed water from fruits and vegetables slices to the extent of 40 – 50 per cent of the weight, but not enough for storage. Therefore, to remove water up to safe levels further drying is needed. Bongirwar and Sreenivasan (1977) indicated that the high temperature above 60 ℃ modifies the tissue characteristics favouring impregnation phenomena and thus solid gain. Rahman and Lamb (1991) reported the rate of sucrose diffusion is a function of solute concentration and temperature. The diffusion coefficient decreased with the increase in solid content during the osmosis and increased with the drying air temperature.

3. Parameters Influencing the Osmotic Process

Variables like maturity, variety, pretreatments, temperature, nature and concentration of osmotic agent, agitation, geometry of the material, fruit pieces to osmotic solution ratio, physico-chemical properties, additives, structure and pressure affecting the osmotic dehydration process. The effect of the process variables on mass transfer and product quality have been reviewed by Ponting *et al*. (1966), Torreggiani (1993), Raout Wack (1994), Pokharkar (1994), Lewicki and Lenart (1995), Sudheer and Dash (1999) and more recently by Rastogi *et al*. (2002).

4. Raw Materials Characters for Osmotic Dehydration

4.1 Quality of raw material

The variety and maturity of fruits and vegetables mainly control water loss and solid gain in the osmosis process. Among different fruits variability is mainly related to the tissue compactness, initial insoluble and soluble solids content, intercellular spaces and enzymatic activity of the fruit. The kinetic rate of solid gain did not depend significantly on solute concentration or process temperature. Among different varieties of mango Dashehari and Totapuri at ripe stage were found suitable for osmotic dehydration (Tiwari & Jalali, 2004).

4.2 Shape, size and thickness of the fruit pieces

Water loss increases with increase in the surface area of fruit pieces. Panagiotou *et al*. (1998) observed that the size of fruit samples had a negative effect on water loss during osmotic treatment. Rahman (1992) observed that the distribution coefficient of water decreased with increasing temperature and surface area and it increased with the increase in syrup concentration and thickness of minimum geometric dimension. In general, a sample size of 3 mm to a maximum of 10 mm in rectangle, ring or cube shape was suggested for the use in osmotic dehydration process. The flow chart of general method of osmotic dehydration process is given Figure 1.

5. Osmotic Process Parameters

5.1 Pretreatments

Any pre treatment such as blanching or freezing prior to osmotic water removal was detrimental to the product quality. Dipping in 1 percent citric acid solution prior to drying or osmotic dehydration was used to prevent enzymatic browning of fruits. Immersion of product in alkaline or acid solutions of oleate esters prior to drying of fruits affected the prevention of discoloration (Hussain *et al*., 2004; Sunkja & Ragharan, 2004). Torreggiani, (1993) reported that pretreatment with chemicals (SO_2), or blanching prior to drying of fruits and vegetables effected the prevention of discolouration. Dipping the papaya and mango slices in 0.4 percent ascorbic acid solution or 0.4 per cent ascorbic acid + 0.1 percent KMS solution for 30 min prior to osmosis process effected to obtain a high acceptable product.

5.2 Immersion time

Keeping the concentration of the solution constant, the increase of the immersion time resulted in the increase of water loss, but the rate of increase was decreased. Studies on the optimization of duration of osmosis process indicated that mass exchange took place at the maximum rate within the first two hrs of the osmotic treatment. Tiwari and Jalali (2004) reported that during osmotic dehydration of mango and pineapple increase in osmotic duration resulted in increase in weight loss, but the rate of which occurs decreases. Gaspartero *et al*. (2003) and Mauro *et al*. (2004) reported that when banana and apple slices dipped in 70 and 50 ^{0}Brix respectively, osmotic solution temperature of 50 ^{0}C for 3 hrs immersion time gave optimum water loss and sugar gain. Osmotic

dehydration process followed by air-drying at 60^0C temperature with the air velocity of 2 m/s up to a constant weight.

5.3 Temperature of the osmotic solution

The temperature of osmotic solution markedly affected the rate of osmosis. Although the rate increased with temperature, it was limited up to 60 ^{0}C as higher temperature destroyed the cell membranes. Pokharkar and Prasad (1998) developed kinetic model for osmotic dehydration of banana slices and reported that the temperature of the osmotic solution affected the parameters like water and sugar gain of osmosis process.

5.4 Osmotic agents

Several studies were conducted to find out the effect of different osmotic agents on the osmotic dehydration process. The most commonly used osmotic agents were sucrose, glucose for fruits and NaCl for vegetables. Other osmotic agents include Calcium chloride, monohydroxy ethanol and polyhydroxy compounds such as lactose, malt dextrin, corn syrup and mixtures of these items. The effects of various osmotic agents are presented in Table 1.

5.5 Concentration of osmotic solution

Rahman and Lamb (1990) pointed out that water loss and sugar gain increased linearly with the increase of sugar concentration and temperature. The rate of sugar diffusion was a function of sugar concentration and temperature. Concentration of solution is a key factor in the osmotic dehydration process and many research workers have studied the effect of osmotic solution concentration on mass transfer of osmotic dehydration process. In general, syrup strength in the range of 60 to 70 ^{0}Brix has been found to be optimum (Chaudhary *et al*, 1993). It was also reported that higher concentration, faster is the rate of osmosis. Torreggiani (1993) suggested that, it was usually not worthwhile using higher concentration for osmosis process for more than 50 per cent of weight reduction because of decrease in osmotic rate with time.

5.6 Agitation / Circulation

When fruits are agitated in syrup, the rate of osmosis will be faster due to reduced mass transfer resistance at the surface by avoiding localized dilution process. But, it may cause damage to sample. Panagiotou *et al*, (1998) and Tiwari (2005) observed that the speed of agitation had a positive effect on water loss during osmotic treatment.

5.7 Fruit pieces to osmotic solution ratio

With an increase in solution to sample ratio, the rate of osmosis increases up to a certain extent. However, it is essential to use an optimum ratio since large ratios offer practical difficulties in handling the syrup fruit mixture for processing. A ratio of 1:2 or 1:3 is optimum for practical purposes (Tiwari, 2005).

6. Kinetic of Osmotic Dehydration

The kinetic of osmotic dehydration is determined by estimating the rate of water removal and solid gain. Generally higher rates of water removal take place within first hour of osmosis due the large driving force between the dilute fruit sap and osmotic solution (Sharma *et al*., 2004).

7. Mass transfer phenomena during osmotic dehydration

There are three major types of counter current mass transfer in osmotic concentration process (Karthiayani, 2004; Tiwari, 2005) (Figure 2).

1. Important water out flow from product to solution.
2. A solute transfer, from the solution to the product; it makes thus possible to introduce the desired amount of an active principle, a preservative agent, any solute or nutritional interest, or a sensory quality improvement of the product.
3. Leaching out of products own solutes (sugar, organic acids, minerals, vitamins etc.), which is quantitatively negligible when compares with the first two types of transfer, but essential with regard to the composition of final product.

The schematic diagram of mass transfer during osmosis process is given in Figure 2.

8. Drying Behaviour of Osmotically Concentrated Fruits

The high temperature short time drying process was possible for osmo-dried products as those having low moisture content. Generally osmotic concentration would not give low moisture content to be stored for long time. Osmo-dried products should be processed further (air drying, vacuum drying etc.) to obtain shelf-stable

products (Pointing, 1973). The osmo dried papaya and mango slices were dried in a cabinet dryer at 60 ^{0}C for 6 hrs to obtain 16 per cent moisture content (Gurumeenakshi *et al.*, 2005).

9. Packaging of Osmotically Dehydrated Products

In order to prevent absorption of moisture from atmosphere and to prevent spoilage due to contamination, good quality, food grade and airtight containers can be used to store osmotically dried foods. Aluminum foil, laminated polypropylene pouches are suggested as ideal packing materials (Sagar & Khurdiya, 1999). Ahemed and Choudhary (1995) used high-density polyethylene pouches for osmo-dried papaya. Dried products were kept at room temperature for six months and it was accepted with little changes.

10. Storage of Osmotically Dehydrated Products

The storage stability of osmotically dehydrated products varies from six months to one year. The papaya product obtained from osmotic dehydration process remains stable up to six months of storage at room temperature (Ahemed & Choudhary, 1995). Bongirwar and Sreenivasan (1977) reported that the osmotically dehydrated banana products can be preserved up to one year or more depending upon the storage conditions and packaging materials used. Storage studies on osmo-dehydrated mango slices showed that the keeping relative humidity between 64.8 to 75.5 per cent would be conducive for the retention of colour, flavour, texture and taste.

11. Microbial Studies of Osmo-dehydrated Products

Ramarjuna and Jayaraman (1980) studied the microbial quality of intermediate moisture banana stored at 0 ^{0}C and 37 ^{0}C and showed that at 0 ^{0}C the total plate count was 250 to 300 colonies/g but at room temperature and 37 ^{0}C, it was negligible and product was microbiologically safe for direct consumption. Khandekar *et al*, (2005) carried out research on standard plate count of fig toffee after six months of preservation. The toffees, which were treated with sodium benzoate, reported lower microbial count (11 x 10^3 /g) compared to untreated toffee (23 x 10^3 /g).

12. Advantages of Osmotic Dehydration

There are number of advantages of the osmotic dehydration process.

1. It minimizes the effect of temperature on food quality and preserves the wholeness of the food, as no high temperature/phase change is required in the process.
2. Mild heat treatment favours colour and flavour retention resulting in the product having superior organoleptic characteristics. It is more when sugar syrup is used as osmotic agent.
3. IT increases resistance to heat treatment
4. The process is quite simple, economical (energy requirement is 2-3 times less as compared to the conventional drying.
5. It prevents the enzymatic browning and inhibits activities of polyphenol oxidases.
6. It improves the texture and rehydration properties
7. The blanching process may be eliminated by this process, which reduces cost of processing
8. Acid removal and sugar uptake by fruits modifies the composition and improves the taste and acceptability which is called candying effect
9. The process could prove to be good for production of the ready to eat foods such as raisins etc.
10. The process reduces volume of the products thereby saving in the cost of processing, storage and transport
11. Constant immersion of product in osmotic agents avoids the O_2 exposure, the product retains better colour
12. It protects against the structural collapse of the product during subsequent drying. It helps to retain the shape of the dehydrated products

13. Conclusions

Osmotic dehydration process being a simple process, it facilitates processing of tropical fruits and vegetables such as banana, sapota, pineapple, mango, guava, carrot, pumpkin, papaya etc with retention of initial fruit characteristics viz., colour, aroma and nutritional compounds. In preservation of fruits and vegetables osmotic dehydration process add value to the finished product, which is wholesome, nutritious and available round the year.

References

Ahemed, J., & Choudhary, D. R. (1995). Osmotic dehydration of papaya. *Indian Food Pack, 49*, 5-11.

Biswal, R. N., & Maguer, Le. (1989). Mass transfers in plant material in contact with aqueous solution of ethanol and sodium chloride equilibrium data. *J. Food Process Eng., 11*, 159-176.

Bolin, H. R., Huxsoll, C. C., & Jackson, R. (1983). Effect of osmotic agents and concentration on fruit quality. *J. Food Sci. Technol, 48*, 202-205. http://dx.doi.org/10.1111/j.1745-4530.1989.tb00027.x

Bongirwar, D. R., & Sreenivasan, A. (1977). Osmotic dehydration of banana. *J. Food Sci. Technol, 14* (3), 104-112.

Chaudhari, A. P., Kumbhar, B. K., Singh, B. N. N., & Narain, M. (1993). Osmotic dehydration of fruits and vegetables. *Indian Food Industry, 12*, 20-27.

Farkas, D. F., & Lazor, M. E. (1969). Osmotic dehydration of apple pieces. Effect of temperature and syrup concentration. *J. Food Sci. Technol, 23*, 668-690.

Flink, J. M. (1975). Processing conditions for improved flavour quality of freeze-dried foods. *J. Agric. Food Chem, 23* (6), 1019-1026. http://dx.doi.org/10.1021/jf60202a018

Gaspartero, O. C. P., Silva, P. D. L., & Gertrudes, E. (2003). Study of conservation of banana by osmotic dehydration and drying in a conventional dryer. *J. Chemi. Eng, 3*, 25-29.

Gurumeenakshi, G., Manimegalai, G., Maragatham, S., & Jeberaj, S. (2005). Ascorbic acid and KMS as new food additives for osmo dried foods. *Beverage Food World, 32* (7), 50-51.

Hawkes, J., & Flink, J. M. (1978). Osmotic concentration of fruit slices prior to freeze dehydration. *J. Food Proc. And Preservation, 2*, 265-284. http://dx.doi.org/10.1111/j.1745-4549.1978.tb00562.x

Hussian, I., Iqbal, M., & Arub, N. (2004). Effect of sucrose and glucose mixture on the quality characteristics of osmotically dehydrated banana slices. *Pakistan J. Nutrition, 46*, 83-92.

Karthiayani, A. (2004). Osmotic dehydration of fruits and vegetables with special reference to vacuum treatment. *Food and Pack, 39*, 82-84.

Khandekar, S. V., Chavan, U. D., & Chavan, J. K. (2005). Preservation of pulp and preparation of toffee from fig fruit. *Beverage and Food World, 32*, 55-56.

Lenart, A., & Flink, J. M. (1984). Osmotic concentration of potato. Criteria for the end point of the osmotic process. *J. Food Sci. Technol, 19*, 45-63.

Lewicki, P. P., & Lenart, A. (1995). Osmotic dehydration of fruits and vegetables. In Handbook of Industrial Drying, Second edn. By Mujumdar, A. S., Marcel Dekker Inc. New York. pp. 691-713.

Mauro, A. M., Mounnrat, S. M., & Rodrogues, A. E. (2004). Vacuum drying of osmotic dehydrated apple slices. Drying, 2004, Proceedings of the 14th International drying Symposium, Brazil.

Panagiotou, N. M., Karathanos, V. T., & Maroulis, Z. B. (1998). Mass transfer modeling of the osmotic dehydration of some fruits. *Int. J. Food Sci. Technol, 33*, 267-284. http://dx.doi.org/10.1046/j.1365-2621.1998.00167.x

Pointing, J. D. (1973). Osmotic dehydration of fruits, recent modifications and applications. *J. Process Bio Technology, 12*(8), 8-20.

Pointing, J. D., Watterss, G. G., Forrey, R. R., Stangly, W. L., & Jackson, R. (1966). Osmotic dehydration of fruits. *J. Food Sci. Technol, 20* (10), 125-128.

Pokharkar, S. M. (1994). Studies on osmotic concentration and air-drying of pineapple slices. Ph.D. Thesis, Department of Agril. Engineering, Indian Institute of Technology, Kharagpur. Pp. 22-68.

Pokharkar, S. M., & Prasad, S. (1998). Mass trasfer during osmotic dehydration of banana slices. *J. Food Sci. Technol, 35* (4), 336-338.

Rahman, M. S. (1992). Osmotic dehydration kinetics of foods. *Indian Food Industry, 11* (5), 20-24. http://dx.doi.org/10.1111/j.1745-4530.1991.tb00088.x

Rahman, M. S., & Lamb, J. (1991). Air-drying behaviour of fresh and osmotically dehydrated pineapples. *J. Food Process Engi, 14*, 163-171.

Ramarjuna, M. N., & Jayaraman, K. S. (1980). Studies on the preparation and storage stability of intermediate banana. *J. Food Sci. Technol, 17*, 183.

Raout Wack, A. L. (1994). Recent advances in the osmotic dehydration of fruits. *J. Food Sci. Technol, 5*, 255-260. http://dx.doi.org/10.1016/0924-2244(94)90018-3

Rastogi, N. K., Ragavarao, K. S. M. S., Niranjan, K., & Knorr, D. (2002). Recent developments in osmotic dehydration: Methods and enhance mass transfer. *Trends in Food Sci. Technol, 13*, 48-59. http://dx.doi.org/10.1016/S0924-2244(02)00032-8

Sagar, V. S., & Khurdiya, D. S. (1999). Studies on dehydration of Dashehari mango slices. *Indian Food Pack, 53*(1), 5-9.

Sharma, K. D., Kunen, R., & Kaushal, B. B. L. (2004). Mass transfers Characteristics of yield and quality of five varieties of osmotically dehydrated apricot. *J. Food Sci. Technol, 41*, 264-275.

Srivastava, R. P., & Sanjeev Kumar. (2002). Fruit and Vegetable Preservation, Principles and Practices. Third edn. Army printing press Lucknow. India. Pp. 11-20.

Sudheer, K. P., & Dash, S. K. (1999). Osmo solar dehydation of ruits and vegetables. An overview. *Indian Food Pack, 53* (3), 28-39.

Sunkja, P. S., & Ragharan, G. S. V. (2004). Assessment of pre treatment methods and osmotic dehydration of Cranberries. *J. Canadian Bio systems, 46*, 52-56.

Tiwari, R. B. (2005). Application of osmo air dehydration for processing of tropical fruits in rural areas. *Indian Food Industry, 24*(6), 62-69.

Tiwari, R. B., & Jalali, S. (2004). Studies on osmtic dehydration of different varieties of mango. In proceeding of First Indian Horticulture congress-2004, New Delhi.

Torreggiani, D. (1993). Osmotic dehydration in fruit and vegetable processing. *Food Res. Intl, 26*, 59-68. http://dx.doi.org/10.1016/0963-9969(93)90106-S

Table 1. Different osmotic agents and their effects in osmotic dehydration process

Osmotic agent	**Remark**	**Reference**
Calcium Chloride	Increases the firmness of apple slices and preserves the texture during storage. Prevents browning because of synergistic effect with ascorbic acid or sulphur dioxide. Imparts better taste to the product if used above 0.5 per cent level.	Pointing, 1973
Ethanol	Decreases viscosity and freezing and freezing point of the osmotic solution in cooling and freezing processes.	Biswal and Le Maguer, 1989
Fructose	Increases the dry matter content by 50 per cent as compared to sucrose due to higher penetration rate. Water activity of the final product is also lower. However sucrose is preferred over fructose.	Bolin *et al.*, 1983
Invert sugar	Theoretically more effective than same concentration of sucrose because when completely inverted, it has twice as many molecules per unit volume. Practically little difference in the rate of osmotic dehydration of fruit by sucrose or invert syrups of the same concentration and temperature.	Pointing *et al.*, 1966
Lactose	It has much lower level of sweetness than sucrose. Low solubility in aqueous solution.	Hawkes and Flink, 1978
Malto Dextrin	It can be used as an osmosis solute at higher total solids concentration, or in mixed systems. Less effective than sucrose at the same concentration.	Hawkes and Flink, 1978
Sodium Chloride	NaCl: Mostly used for vegetables as it retards oxidative and non-enzymatic browning. Increases the driving force for the drying owing to the lowering capacity of the salt. Sometimes blanching effect on coloured products can be prevented using mixture of salt and sugar. Organoleptic should be 10-12 per cent. Hinders shrinkage at the surface layers.	Hawkes and Flink, 1978, Lenart and Flink, 1984
Sucrose / Sugar	Dry sugar is unsuitable because of oxidative browning during osmosis. Difficulty in disposing sugar syrup. Sugar solution is best as it reduces browning by preventing the entry of oxygen. Sweetness hinders its use in vegetable processing.	Pointing *et al*, 1966, Farkas and Lazer, 1969, Flink, 1975
Starch / Corn syrup	Favours similar final water content with minimal solid gain as that obtained with sucrose.	Flink, 1975

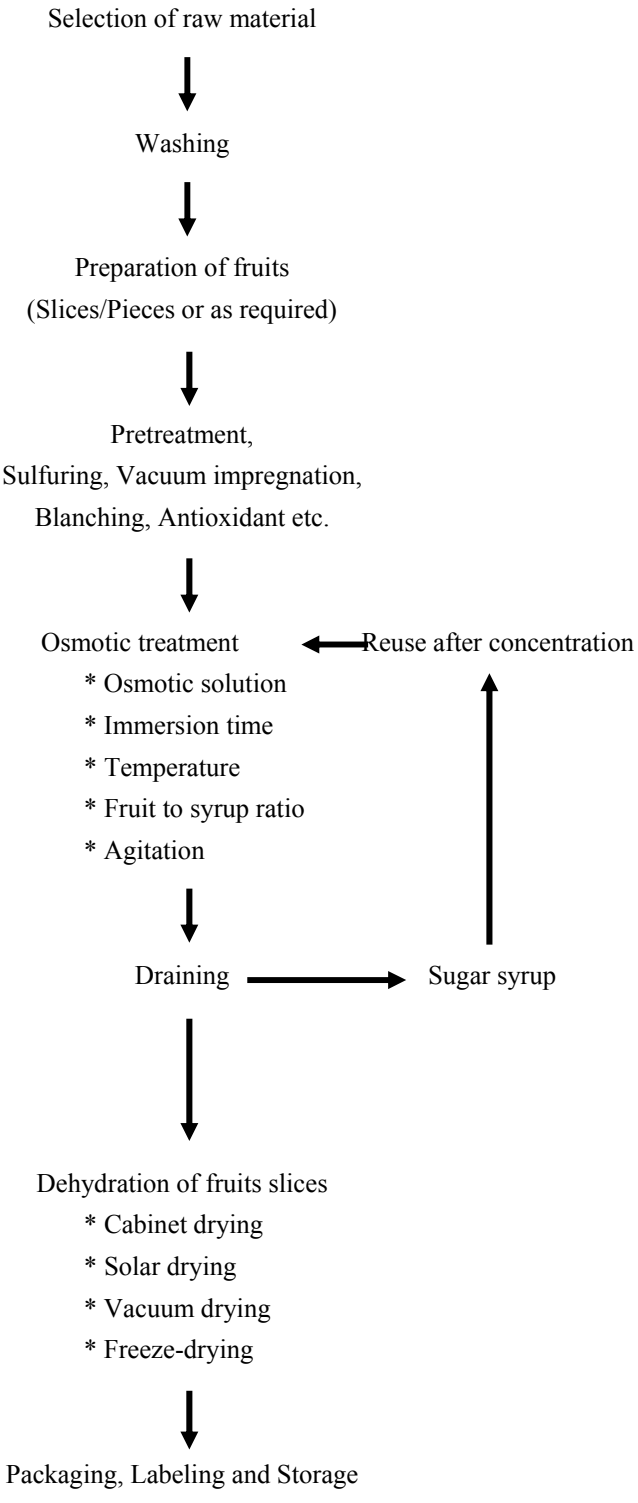

Figure 1. Flow chart of general method of osmotic dehydration process

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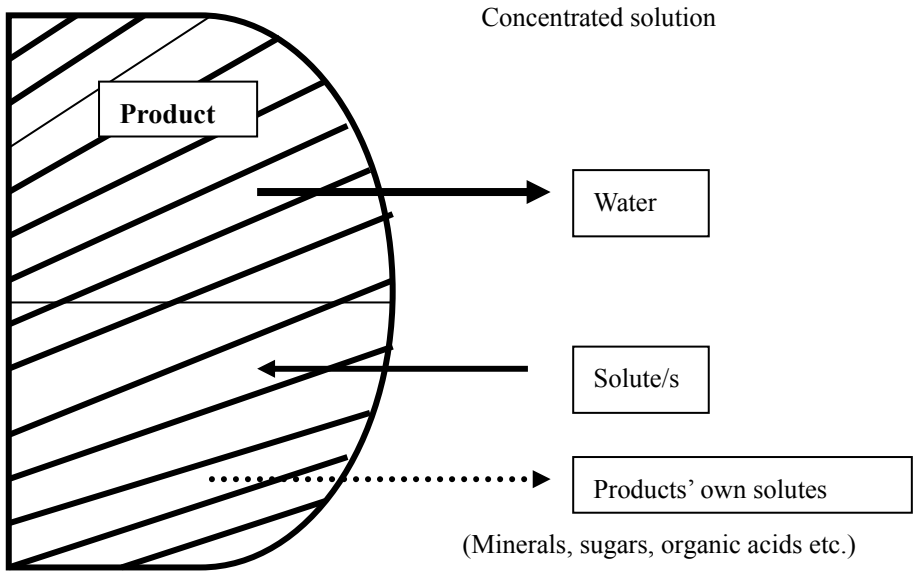

Figure 2. The schematic diagram of mass transfer during osmosis process (Raoult-Wack, 1994)

Predicting the Consumer Acceptability of Dried MD2 and Smooth Cayenne Pineapple Pulps from Chemical Composition

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Abstract

Drying of pineapple (*Ananas comosus*) pulp enhances shelf life while assuring all-year-round availability of pineapple in different forms. The difficulty in selecting suitable fruit pulps for producing acceptable dried pulp needs to be addressed in order to reduce variability in dried pineapple pulps and predict consumer acceptability. MD2 and Smooth cayenne fruits were used for the study. Relationships existing between chemical and sensory characteristics of oven-dried pineapple pulps were determined using standard procedures. While a strong positive correlation was found between total soluble solids (TSS) and taste (SC=0.98; MD2=0.99) a negative association was found between TSS and crispness (SC=-0.98; MD2=-0.99) as well as between total titratable acidity (TTA) and taste (SC=-0.99; MD2=-0.98). The study showed that TSS of the fresh fruit was the single most important predictor of overall acceptability of dehydrated pulps. TSS of fresh fruits could therefore be used to select fresh pulps for dehydration.

Keywords: Pineapple, Dehydration, Sensory evaluation, Smooth Cayenne, MD2

1. Introduction

Pineapple (*Ananas comosus*), is a member of the family Bromeliaceae. It is a good source of ascorbic acid (vitamin C), vitamin A, calcium, phosphorus, iron, potassium and thiamine (USDA, 2005). They are eaten as dessert fruits throughout the tropical and sub tropical areas of the world (Francis, 1982). Thailand, Philippines, Brazil and China are the main producers in the world supplying nearly 50 % of the total world output (Athey, 1995). Other important producers include India, Nigeria, Kenya, Indonesia, México and Costa Rica. According to Francis (1982), nearly 70% of the pineapple is consumed as fresh. The problem with fresh pineapple is how best the fruits can be stored at room temperature in order to minimize postharvest losses. This has resulted in the adoption of various processing techniques in extending the shelf life of the fresh fruits so as to ensure all year round availability of the fruit in different forms. Among the promising technologies is drying. Drying of fruits while extending shelf life could result in products with varying quality characteristics. The high variability in the eating quality of dehydrated pineapple pulp has been attributed to the use of fruits with different chemical characteristics, such as fruits at different stages of ripening (Barrett, Somogyi & Ramaswamy, 2005). In order to minimize the variability, ensure consumer acceptance and enhanced profitability dried pineapple fruit producers need a good criteria for selecting fresh fruits that would make good chips. This study therefore, sought to identify important easy-to-determine fresh pulp characteristics that could be used to predict quality and consumer acceptability dried pineapple pulps.

2. Materials and Methods

2.1 Location and location of experiment

Fully mature fresh Smooth Cayenne and MD2 fruits were harvested from Bomarts Farms Limited at Nsawam in the Eastern Region of Ghana and transported to the laboratory of the Department of Horticulture, Faculty of Agriculture, KNUST, Kumasi for analysis.

2.2 Sample preparation

Fresh Smooth Cayenne and MD2 pineapple at different stages of ripening (Unripe, half-ripe and fully ripe) fruits were washed, peeled and sliced into circular discs (slices) of dimension 1 cm thickness. The slices were then

spread evenly on trays lined with aluminum foil. Trays were then placed in a Wagtech oven (Model GP120SSE300HYD) at temperature of 67 ºC in an oven for 24 hours. The slices were periodically turned to ensure uniform drying. The dried slices were allowed to cool for about 30 minutes in a dessicator prior to analysis.

2.3 Quality assessment

Standard procedures were used for determination of moisture content, total titratable acidity (TTA), total soluble solids (TSS) and sensory characteristics.

2.3.1 Moisture content

Moisture content was determined according to the method of the AOAC (AOAC, 1990). Two (2 g) of pineapple pulp was placed in a crucible and dried in an oven for 24 hours at 60ºC. Moisture content was estimated using the formula: (Initial wt-dry wt/initial wt) ×100.

2.3.2 Total soluble solids (TSS)

The total soluble solids content was measured using a Lafayette hand refractometer. A 30g of the pulp tissue in 90ml of distilled water was blended with a laboratory blender for 2minutes and filtered. A single drop of the filtrate was placed on the prism of the refractometer. The percentage total soluble solid was read by pointing the refractometer towards a light source. The recorded value was then multiplied by three since the initial pulp sample was diluted three times with distilled water (Dadzie & Orchard, 1997).

2.3.3 Total titratable acidity (TTA)

Thirty grams (30g) of the pulp was weighed into a blender plus 90ml distilled water, blended for two minutes and filtered. 25ml of the filtrate was transferred into a 125ml conical flask. Another 25ml distilled water and four to five drops of phenolphthalein indicator were added. This solution was titrated against 0.1N sodium hydroxide until there was a sharp colour change from light yellow to pink. The titre volume of NaOH added was multiplied by the citric acid factor (0.07) to obtain the total titratable acidity (Dadzie & Orchard, 1997).

2.3.4 Sensory evaluation

Sensory evaluation was carried out on the chips using 30 untrained panelists. Sensory attributes assessed were appearance, taste, aroma, crispness, mouth feel and overall acceptability. A hedonic scale of 1-5 (1 - Dislike very much; 2 - Dislike slightly; 3 - Neither like nor dislike; 4 - Like slightly; 5 - Like very much) was used for the assessment.

2.4 Statistical analyses

Analysis of variance was carried out on collected triplicate data using GENSTAT (Discovery edition 3). Least significant difference (Lsd) test was used to determine differences between means.

3. Results and Discussion

3.1 Chemical composition of fresh pineapple fruits used to produce dried pineapple pulp

3.1.1 Moisture Content

During ripening the moisture content of Smooth Cayenne increased from 684.0 g kg^{-1} in unripe to 693.0 g kg^{-1} in full ripe representing a 1.3% increase with ripening (Table 1). On the other hand, MD2 increased from 798.0 g kg^{-1} in unripe to 812.0 g kg^{-1} in full ripe, representing an increase of 1.8% in the moisture of the fruits. This gradual increase in moisture content of the pineapple pulps during ripening could be attributed to loss of moisture from the peels to the pulp (Kays, 1991). The increase in moisture during ripening allows fruit solutes, usually sugars, to dissolve inducing sweetness.

(Table 1)

3.1.2 Changes in total soluble solids (TSS) Content

There was a general increase in total soluble solids in both varieties during ripening. Whereas TSS increased from 97.0 g kg^{-1} to 114.0 g kg^{-1} in Smooth Cayenne, MD2 increased from 117.0 g kg^{-1} to 126.0 g kg^{-1} representing increases of 17.5 % and 7.7 % respectively. Increase in TSS during ripening is a result of the transformation of fruit starch into soluble sugars under the action of the phosphorylase enzyme (Nakasone and Paull, 1998). The observed increase in sweetness during ripening could probably be attributed to increased levels of sucrose, glucose and fructose which are known to be responsible for sweetness in pineapple (Bates, Morris & Crandall, 2001).

(Table 2)

3.1.3 Changes in total titratable acid (TTA) content

The total soluble solids content of MD2 (1.8 g kg^{-1}) was significantly lower than Smooth Cayenne (4.5 g kg^{-1}). The total titratable acidity for Smooth Cayenne decreased from (4.9 g kg^{-1}) in unripe to (4.1 g kg^{-1}) in full ripe whiles that of MD2 decreased from (2.5 g kg^{-1}) in unripe to (1.4 g kg^{-1}) in full ripe. The two major organic acids in pineapple are citric and malic acids (Saradhuldhat & Paull, 2007). During ripening the acids are involved in protein synthesis resulting in the observed decrease in total titratable acidity (Lacey, Hancock & Ramsey, 2009).

(Table 3)

3.1.4 Changes in sugar: acid ratio

The sugar:acid ratio of the of both Smooth Cayenne and MD2 fruit pulps increased as ripening advanced. While for Smooth Cayenne the ratio increased from 19.8:1 to 27.8:1, MD2 increased from 46.8:1 to 90.0:1 with ripening (Table 4). During ripening fruit acids are degraded, the sugar content increases and the sugar:acid ratio increases (Shashirekha & Patwardhan, 1976) as was observed in this study. Sugar-acid ratio has been reported to contribute to the unique flavor of fruits (Colaric, Veberic, Stamjoar & Hudina, 2005). It is an indicator of commercial and organoleptic ripeness. Overripe fruits have very low levels of fruit acid and therefore lack characteristic flavor (OECD, 2011). The higher levels of sugar:acid ratio in MD2 probably contributed to MD2 having superior taste.

(Table 4)

3.2 Sensory evaluation

3.2.1 Taste

The characteristic taste of fruits is determined by the content of sugars and organic acids (Kader, 2008). According to the sensory panel, the taste of dehydrated MD2 pulp was significantly more acceptable than Smooth Cayenne irrespective of the stage of ripening (Table 5). Generally, the unripe dehydrated pulps were the least accepted among the 2 varieties. The full ripe pulps were the most agreeable to the sensory that scored them 4.9 and 4.5 for MD2 and Smooth Cayenne respectively. Whereas the differences between half-ripe and full ripe were significant for Smooth Cayenne, MD2 did not show any significant difference. This means that half-ripe MD2 could be used to produce chip without adverse effect on taste. The strong positive correlation between TSS and taste (MD2, r=0.98; Smooth Cayenne, r=0.99) as well as the strong negative correlation (MD2, r= -0.99; Smooth Cayenne, r= -0.98) between taste and TTA suggest that the taste of dehydrated MD2 and Smooth Cayenne pineapple pulps are influenced by TSS and TTA.

(Table 5)

3.2.2 Appearance

Appearance is one of the most important factor consumers take into consideration in purchasing a product. Dried pulp produced with Half ripe and full ripe fruits from both MD2 and Smooth Cayenne were perceived by the sensory panel to be similar in terms of their appearance. However, the differences between unripe and half ripe or fully ripe MD2 were found to be significant. According to Bartolome, Ruperez & Fuster (1996) the development of carotenoids provide ripe pineapples with an attractive colour. This probably contributed to the more acceptable appearance of the dehydrated pulp produced using fully ripe pulp. The results suggest that both Smooth Cayenne and MD2 could perform similarity in terms of the appearance of their pulps when their half ripe and fully ripe pulps are dehydrated.

(Table 6)

3.2.3 Aroma

Aroma is produced by the volatile synthesized during fruit ripening and may include aldehydes, alcohols, esters, lactones, terpenes and sulfur compounds (Kader, 2008). Volatiles responsible for aroma originate from proteins, carbohydrates, lipids and vitamins. Generally, the unripe pulps, irrespective of the variety were statistically lower than half ripe and full ripe which were judged to be similar. The aroma of MD2 (4.1) was reported to be statistically similar to Smooth Cayenne (3.8) as indicated in Table 7. In the study, it was observed that the full ripe dehydrated pulps were more accepted than the half-ripe and unripe, variety notwithstanding.

(Table 7)

3.2.4 Crispness

The crispness of the dried pulp from the unripe was most preferred to that of the half ripe and full ripe. The crispness of chips from full ripe pineapples were the least preferred with a mean sensory score of (2.5) in Smooth Cayenne and (1.5) in MD2 (Table 8). This implies that the crispness of Smooth Cayenne was more acceptable than MD2. This could be attributed to increased sugar levels thereby making the chips gummy instead of crispy in the case of MD2 variety (Food Resource, 2009; Kitts, 2011).

(Table 8)

3.2.5 Mouthfeel

Mouth feel gives an indication of the mouth sensation the chips give in the mouth. Table 9 shows the scoring for mouthfeel of dehydrated pineapple slices. There were statistical differences between the mouthfeel scores of unripe (3.7) and full ripe (4.5) Smooth Cayenne. On the other hand, there were differences between unripe MD2 (3.5) and half ripe (4.6) and full ripe (4.5) pulps as shown in Table 8. According to the sensory panel the mouth-feel of pulps at different stages of ripening were significantly different. The unripe stages were less acceptable than both half-ripe and ripe. The implication of this finding is that both half-ripe and fully ripe fruits of Smooth cayenne and MD2 could be used to produce dehydrated pulp of acceptably similar mouthfeel.

(Table 9)

3.2.6 Overall acceptability

While both unripe Smooth Cayenne and MD2 were generally, neither liked nor disliked, all their half ripe and full ripe dehydrated pulps were scored acceptable to the sensory panel. As regards the full ripe pulps, Smooth Cayenne was preferred. However, the most acceptable dehydrated pulp was produced by MD2 at the half ripe (4.7) as indicated in Table 10. The results of this study has shown that Smooth Cayenne (4.0) and MD2 (3.8) produced equally acceptable dehydrated pulp.

(Table 10)

3.3 Empirical relationships between chemical attributes and overall acceptability

There were strong positive correlations between taste and total soluble solids for Smooth Cayenne (r=0.98) and MD2 (r=0.99). However, the correlation between total titratable acidity and taste was an inverse one for both Smooth Cayenne (r=-0.99) and MD2 (0.98; Table 11). The results indicate that as TSS (sweetness) increases and TTA (acidity) decreases, the taste of the dried pulp improved (Kader, 2008). This explains why the dried pulp produced using ripe fruits was preferred to the unripe.

(Table 12)

Regression equations (Table 12) from the study suggested that the TSS content of the fresh pineapple fruits was important in explaining the variability in crispness (R^2=0.82), taste (R^2=0.78) and aroma (R^2=0.62) of the dried pulp. Whereas aroma and taste of the dried pulp improved with increasing TSS content of the fresh fruit pulp, crispness was adversely affected. On the other hand, mouthfeel of the dried pulp which was found to be a determinant of overall acceptability (R^2=0.80), was itself determined by taste (R^2=0.75) and crispness (R^2=0.64); which has been shown to be influenced by TSS content of the fresh pineapple fruit in this study. TSS of the fresh pineapple fruit pulp could therefore be used to predict the acceptability of sensory attributes of dehydrated pineapple pulp.

4. Conclusion

The most acceptable stage of ripening for MD2 for the production of dehydrated pulp was the 'half ripe stage'. On the other hand, Smooth Cayenne produced its best dehydrated pulp from the full ripe stage. Total Soluble Solids content of the pineapple fruits found to be the single most important predictor of the acceptability of the dried pulps. Producers of dried pineapple pulp could therefore predict the acceptability of their dried pulps by assessing the total soluble solid content of the fresh fruits.

References

AOAC. (1990). Official methods of analysis of the Association of Official Analytical Chemists, 15th ed, Washington D. C.

Arthey, D. (1995). Food Industries Manual. In: Ranken, M. D., Kill, R. C. and British Food Manufacturing Industries Research Association Eds. Fruit and Vegetable Product. London. Blackie Academic and Professional.

Barrett, D. M., Somogyi, L., & Ramaswamy, H. S. (Eds). (2005). *Processing fruits*. 2nd ed. CRC Press. London.

Bartolome, A., Ruperez, P., & Fuster, C. (1996). Freezing Rate and Frozen Storage Effects on Color and Sensory Characteristics of Pineapple Fruit Slices. *Journal of Food Science, 61*, 154–156. http://dx.doi.org/10.1111/j.1365-2621.1996.tb14747.x

Bates, R. P., Morris, J. R., & Crandall, P. G. (2001). Principles and practices of small and medium-scale fruit processing. FAO Agricultural Services Bulletin 146. [online] Available : http://www.fao.org/docrep/005/y2515e/y2515e00.htm [March 19, 2012]

Colaric, M., Veberic, R., Stampar, F., & Hudina, M. (2005). Evaluation of peach and nectarine fruit quality and correlation between sensory and chemical attributes. *Journal of the Science of Food and Agriculture, 85*, 2611-2616. http://dx.doi.org/10.1002/jsfa.2316

Dadzie, B. K., & Orchard, J. (1997). Routine Post Harvest Screening of Banana/Plantain Hybrids: Criteria and Methods. INIBAP Technical Guidelines 2. Wageningen, The Netherlands. [online] Available: http://bananas.bioversityinternational.org/files/files/pdf/publications/tg2_en.pdf [June 14, 2011]

Food Resource. (2009). Fruits-Vegetables. Oregon state University. [online] Available: http:/foodoregonstate.edu/learn/fruitveg.html [Accessed 26 April, 2011].

Francis, F. J. (1982). *Analysis of anthocyanins*. In: Markakus, P (Ed), Anthocyanins as food colour. New York: Academic Press.

Kader, A. A. (2008). Perspective: Flavor quality of fruits and vegetables. *Journal of the Science of Food and Agriculture, 88*, 1863-1868. http://dx.doi.org/10.1002/jsfa.3293

Kays, S. J. (1991). Postharvest Physiology of Perishable Plant Products. Van Nostrand Reinhold New York.

Kitts, D. D. (2011). The functional role of sugars in food. Canadian Sugar Institute. [online] Available: http:/www.sugar.ca/English/healthprofessionals/carboIssue4.cfm. [April 26, 2011]

Lacey, K., Hancock, N., & Ramsey, H. (2009). Measuring internal maturity of citrus. Western Australian Agriculture Authority. Farmnote no. 354. [online] Available: http://www.agric.wa.gov.au/objtwr/imported_assets/content/hort/fn/cp/citrusfruits/FN_testing_of_citrus.pdf. [April 25, 2011)

Nakasone, H. Y., & Paull, R. E. (1998). Tropical Fruits. CAB International,198 Madison Avenue, New York, U. S. A.

OECD. (2011). Guidance on objective tests to determine quality of fruits and vegetables and dry and dried produce. Available: http://www.oecd.org/dataoecd/32/47/19515719.pdf [April 25, 2011]

Saradhuldhat, P., & Paull, R. E. (2007). Pineapple organic acid metabolism and accumulation during fruit development. *Scientia Horticulturae, 112* (3), 297-303. http://dx.doi.org/10.1016/j.scienta.2006.12.031

Shashirekha, M. S., & Patwardhan, M. V. (1976). Changes in amino acids, sugars, and nonvolatile acids in a ripening mango fruit *(Mangifera indica,* 'Badami' variety). *Lebensmittel-Wissenschaft &Technologie, 9*, 369-370.

USDA (2011). National nutrient database for standard release 18. (2005). Available: http://usda.gov/ fnic/ food comp/ search/ [April 26, 2011]

Table 1. Changes in moisture content of Smooth Cayenne and MD2 pineapple fruit during ripening (g kg^{-1})

	Smooth Cayenne	MD2	Means
Unripe	684.0	798.0	741.0
Half ripe	685.0	808.0	747.0
Full ripe	693.0	812.0	752.0
Means	687.0	806.0	
Lsd at 0.05	0.10		

Table 2. Changes in total soluble solids during ripening (g kg^{-1})

	Smooth Cayenne	MD2	Means
Unripe	97.0	117.0	107.0
Half ripe	108.0	122.0	115.0
Full ripe	114.0	126.0	120.0
Means	106.0	121.0	
Lsd at 0.05	1.3		

Table 3. Changes in total titratable acidity during ripening (g kg^{-1})

	Smooth Cayenne	MD2	Means
Unripe	4.9	2.5	3.7
Half ripe	4.5	1.9	3.2
Full ripe	4.1	1.4	2.7
Means	4.5	1.8	
Lsd at 0.05	0.01		

Table 4. Changes in Sugar:Acid Ratio in pineapple fruits during ripening

	Smooth Cayenne	MD2	Means
Unripe	19.8	46.8	28.9
Half ripe	24.0	64.2	35.9
Full ripe	27.8	90.0	44.4
Means	23.6	67.2	
Lsd at 0.05	0.34		

Table 5. Sensory scoring for taste of chips

	Smooth Cayenne	MD2	Means
Unripe	3.2	3.8	3.5
Half ripe	4.0	4.6	4.3
Full ripe	4.5	4.9	4.7
Means	3.9	4.4	
Lsd at 0.05	0.37		

Table 6. Sensory scoring for appearance of chips

	Smooth Cayenne	MD2	Means
Unripe	3.9	3.9	3.9
Half ripe	4.1	4.4	4.2
Full ripe	4.2	4.1	4.2
Means	4.0	4.1	
Lsd at 0.05	0.45		

Table 7. Sensory scoring for aroma of chips

	Smooth Cayenne	MD2	Means
Unripe	3.1	3.5	3.3
Half ripe	4.0	4.3	4.1
Full ripe	4.2	4.4	4.3
Means	3.8	4.1	
Variety	Lsd (P=0.05) = 0.45		
Stage of ripening	Lsd (P=0.05) = 0.49		
Variety x Stage of ripening	Lsd (P=0.05) = 0.70		

Table 8. Sensory scoring for crispness of chips

	Smooth Cayenne	MD2	Means
Unripe	4.6	3.5	4.1
Half ripe	3.5	2.5	3.0
Full ripe	2.5	1.5	2.0
Means	3.5	2.5	

Lsd (p=0.05) = 0.27

Table 9. Sensory scoring for mouth-feel of chips

	Smooth Cayenne	MD2	Means
Unripe	3.7	3.5	3.6
Half ripe	4.1	4.6	4.3
Full ripe	4.5	4.5	4.5
Means	4.1	4.2	

Lsd (p=0.05) = 0.55

Figure 10. Sensory score for overall acceptability of chips

	Smooth Cayenne	MD2	Means
Unripe	3.3	3.5	3.4
Half ripe	4.1	4.7	4.4
Full ripe	4.5	3.3	3.9
Means	4.0	3.8	

Lsd (p=0.05) = 0.31

Table 11. Correlation between chemical and sensory parameters

Variety	Parameter	Taste	Crispness	Mouthfeel	Overall Acceptability
Smooth Cayenne	TSS	0.98	-0.98	0.97	0.99
	TTA	-0.99	0.99	-0.99	-0.98
MD2	TSS	0.99	-0.99	0.85	0.97
	TTA	-0.98	0.99	-0.82	-0.96

TSS – Total soluble Solids TTA – Total Titratable Acidity

Table 12. Empirical relationships existing between sensory and chemical properties of pineapple pulp

Equation No.	Linear Regression Equation	R2	P-value
1	Crispness = 13.7-0.09 Total Soluble Solids	0.82	0.014
2	Aroma = -0.50+0.04 Total Soluble Solids	0.62	0.041
3	Taste = -1.85+0.05 Total Soluble Solids	0.78	0.020
4	Mouthfeel = 1.45+0.65 Taste	0.75	0.025
5	Mouthfeel = 5.18+0.34 Crispness	0.64	0.057
6	Overall acceptability = 0.36+0.86 Appearance	0.92	0.002
7	Overall acceptability = 2.48+0.33 Mouthfeel	0.80	0.010
8	Overall acceptability = 3.07+0.19 Taste	0.50	0.020

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Natural Mineral Waters Enhance the Intestinal Health and Stimulate Anti-inflammatory Immune Response in Functional Cell Model of a Non-cancerogenic Human Gut

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Abstract

Background Although mineral waters have been used extensively in human nutrition and have widely recognized health related properties; the availability of data on the mechanisms of their actions is limited.

Methods and results We have therefore analyzed their ability to increase trans-epithelial resistance of small intestinal epithelia and cell renewal, the bioaccessibilityas well as evaluated their immunomodulatingpotentialin a 3D functional cell model of the gut. Results have showed that the mineral content is highly accessible to the human body. Moreover, carbonated natural mineral water increases the trans-epithelial resistance and epithelial proliferation. In the same time they enhance the anti-inflammatory response by inhibiting activation of TNF-α and stimulating the TGF-β cytokine in healthy epithelia as well as in macrophages.

Conclusions We can conclude that by this action the mineral waters have the potential to increase the integrity of the small intestinal wall and its immune status. In addition to providing scientific grounds for a health-beneficial use of natural mineral water, this manuscript provides a novel methodology for the assessment of food derived bioactives in the human gut.

Keywords: Bioaccessibility, TER, Mineral water, 3D cell model, Human gut

1. Introduction

Consumption of packaged water increases year after yeardue to growing awareness of pollution and its impact on the quality of drinking water as well as higher consumer preferences for a healthy diet. Natural mineral water is by definition water that originates in an underground water source which is highly protected against pollution and contains naturally dissolved minerals and gasses. Mineral substances in natural mineral water are in an already dissolved form and can be directly absorbed into the human body via the gut.

Studies have shown that calcium and magnesium in natural mineral water are absorbed to a greater extent than those in other foods, even in comparison with milk (Ekmekcioglu, 2000; Marktl, 2009).

It has been shown previously, in a study of 300 post-mastectomy breast cancer patients and 150 patients after gastric resection for cancer, that a course intake of mineral water improves immune status.In both groups an intake of mineral water significantly raised the levels of T- and B-lymphocytes and their functional activity, and reduced blood levels of IgG and CIC providing immunological enhancement of tumor antigens (Vladimirov *et al*., 2004).

Concerning immunomodulating properties of natural mineral water, it was shown that some mineral waters have the ability to enhance the lympho-proliferative response to mitogens.

IL-4 and IFN-gamma production also increased in stimulated culture supernatants. Conversely, natural mineral water containing cell medium induced a decrease in IL-4 production by normal peripheral blood lymphocytes. Furthermore, the water amended the clinical features as well as the immunological Th2 profile of atopic dermatitis (Portales *et al*., 2001).

Although natural mineral water has been consumed for centuries and has many observed positive effects on human physiology (Nocco, 2008; Perez-Granados *et al*., 2010), there have been, to our knowledge, no major mechanistic studies done to prove or to explain previously described effects on the human organism.

Therefore, in this work we determine the validity of natural mineral water consumption by assessing bioaccessibility of different minerals (Ca, Na, Mg, K, microelements) from natural mineral waters with or without CO_2, their potential to increase trans-epithelial resistance (TER) and cell proliferation, as well as the potential of natural mineral waters to activate the cytokine expression in healthy small intestinal tissue and in human monocytes.

Variousfavourable properties of different health promoting substances can be assessed *In vitro* by severalbiological tests, particularlyby using cell culture systems where direct effects of substances on intestinal and other cells can be monitored. While rapid growth in the field of genomics and proteomics with technological innovations in recent years provides new data, *In vitro* models are still essential tools in biological mechanistic and functional studies (Cencic & Langerholc, 2010; Botic *et al*., 2007; Nissen *et al*., 2009).

Unfortunately, so far mostly tumorigenic and transformed cell lines have been widely used for mechanistic studies of the gastro-intestinal tract, despite their phenotype very distinguished from that of the normal gut epithelia.

In addition, these cell lines are mostly cultivated as monolayers on plastic surfaces in experimental models, where the establishment of functional, epithelial character is not defined (Cencic *et al*., 2010)

For these purposes, we developed a novel 3D human intestinal cell model consisting of untransformed human small intestinal epithelial cell line (H4 cl. 1) and human monocytes/macrophages cell line (TLT) in a system that closely mimics the small intestinal environment.

2. Materials and Methods

2.1 Cells

Thefollowing cell lines were used: H4-1 (non-transformed human foetal small intestinal cells) and TLT (non-transformed human monocyte/ macrophages). H4-1 and TLT were derived from healthytissues and are available from BioNutriTech Ltd. (Lunel, France).

Cells were generallymaintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, L-glutamine 2 mmol/l, penicillin (100 U/ml, Sigma) and streptomycin (1 mg/ml) at 37 °C in 5% CO_2 atmosphere in tissue culture flasks.

2.2 Natural mineral water and cell media

To assay the biological effects of natural mineral water on different cell lines we incubated the cells during experimentation in cell culturemedia prepared with individual mineral water with or without CO_2 or distilled water as a control. Prior to each experiment we prepared fresh DMEM supplemented with 10% foetal calf serum, L-glutamine 2 mmol/l, penicillin (100 U/ml, Sigma) and streptomycin (1 mg/ml) by adding natural mineral water Radenska Classic (carbonated) or mineral water RadenskaNaturelle (uncarbonated) or distilled water,as a control, according to the producer's manual. In order to avoid influence of difference in pH we have adjusted the pH of all prepared media with natural mineral waters and distilled water to the same pH of 6,4 by using 1M HCl. The mineral water was donated from Radenskad.d. (Radenci, Slovenia). Their physical and chemical parameters relevant to this study are given in Table 1.

2.3 Trans-epithelial resistance (TER)

H4-1 cells were seeded on 12 well plates with microporous inserts (Milipore, USA) in a initial concentration of $1x10^5$ cells per well and incubated at 37 °C in atmosphere of 5% CO_2. As soon as a confluent layer was formed we have measured the TER with Milicell ERS-2 (Milipore, USA) in each well daily till the 7^{th} day when TER has reached in average a constant resistance of 667 Ohm as a sign for complete polarization and differentiation. At that point we have discarded the apical and basal supernatants and applied in each apical compartment 0,5 ml of media prepared with mineral waters and the control. In the basal compartments we have pipetted 1,5 ml of regular DMEM media. TER was measured right after application, after 6 hours and 24 hours.

After 24 hours of incubation of the cells with the mineral waters and the control we have collected the basal supernatants of each well for further incubation with TLT macrophages and analysis of ion concentration.

2.4 Bioaccessibility

We have determined the bioaccessibility of magnesium (Mg^{2+}), calcium (Ca^{2+}), sodium (Na^+), potassium (K^+), chlorine (Cl^-) and carbon dioxide (CO_2) by measuring.

the concentration of investigated ions in the collectedbasalsupernatants after a 24 hour incubation of the cells with mineral waters and the control with the Cobas c111 system (Roche, France). We expressed the bioaccessibility of single ionsas a percentage increase in concentration, compared to the control.

2.5 Cell proliferation assay

To perform cell proliferation assays, H4-1 cells were separately seeded in 96 well plates at a concentration of 10 viable cells per well. DMEM prepared with natural mineral waters and distilled water were added and incubated for 7 days at 37 °C in atmosphere of 5% CO_2. After incubation we stainedthecells with crystal-violet and counted the single formed colonies.

2.6 Immunomodulating activity

In order to determine the immunomodulating properties of natural mineral water we have cultivated TLT cells in 12 well plates at 37 °C in an atmosphere of 5% CO_2,until a confluent layer was formed. Later the supernatants have been exchanged with basal supernatants of the H4 3D model. After 24 hours of incubation the expressionof TNF-α, IL-10 and TGF-β in TLT supernatants and H4 basal supernatants has been determined by ELISA. The ELISA kits for TNF-α, IL-10 and TGF-β were purchased from Invitrogen (UK).The increase or decrease of cytokines of mineral water treated cells is expressed as a percentage increase or decrease, compared to the control (cells incubated with DMEM prepared with distilled water).

2.7 Statistical analysis

Analysis of variance followed by unpaired Student's t-test were used to determine statistical differences between multiple groups; p-values<0.05 were considered statistically significant. All findings are expressed by the mean ratios (%, ±SD) of results in treated wells as compared to those in control wells, of three individual experiments.

3. Results

3.1 TER

After initial application of carbonated, uncarbonated and control water on the H4-1 3D cell model a general decrease in trans-epithelial resistance was observed which started to increase in the course of the next 24 hours (Figure 1). The highest final TER was measured on cells incubated with carbonated mineral water (average TER of 737 Ohm) and the lowest on cells incubated with uncarbonated mineral water (average TER of 680 Ohm). Cells incubated with the control have shown a TER of 711 Ohms as shown in Figure 1.

3.2 Bioaccessibilityof minerals from natural mineral waters

Bioaccessibility of the minerals dissolved in the mineral waters with or without CO_2 in the novel human gut functional model was determined as described in Materials and Methods. The results clearly showed thatthe concentration of Mg^{2+} in the basal side of the H4-1 model incubated with carbonated mineral water increased by 100% compared to the control (DMEM prepared with distilled water only). Atthe same time we observed a high absorbance of Ca^{2+} via the small intestinal cell layer. The concentration of Ca^{2+} in the basal compartment of the H4-1 model incubated with carbonated mineral water has increased by 228.25% as compared to the control (DMEM prepared with distilled water only) (Figure 2).

The incubation of cells with uncarbonated mineral water resulted in a smaller increase in the concentration of Mg^{2+} and Ca^{2+} in the basal compartment of the functional cell model, as compared to carbonated mineral water. The concentration of Mg^{2+} has increased for 24% and that of Ca^{2+} for 109.71% (Figure 2).

The same pattern was observed in absorption of monovalent ions dissolvedin the tested mineral waters: a 7.83% increase of Na^+, a 12.09 % increase of K^+ and a 4.03% increase of Cl^- in the basal compartments of the cells incubated with carbonated mineral water (Figure 2).

A similar trend was observed in H4-1 cellsincubatedwith uncarbonated mineral water. Compared to the control, the concentrations of Na^+, K^+ and Cl^- increased for 3.26%, 6.19% and 2.77%, respectively.

The concentration of CO_2 roseby 41% in the basal compartments of the functional cell model when the cells werecultured in DMEMwith carbonated mineral water and by 16% when the cells were incubated in DMEM with uncarbonated mineral water, as shown in Figure 2.

3.3 Proliferative activity of natural mineral waters

Incubation of intestinal cells with carbonated and uncarbonated mineral water (Figure 3) resulted in an increase in the number of colonies formed by cells of normal gut epithelia (H4-1) compared to the control. Carbonated mineral water (average of 80.8 colonies) showed a stronger proliferative effectthan uncarbonated mineral water(73.3 colonies) whereas cells incubated with the control have formed in average 61,8 colonies.

3.4 Immunomodulatingproperties of natural mineral waters

By measuring the concentration of cytokines TNF-α, IL-10 and TGF-β in the supernatnats of TLT cells and basal supernatants of H4-1 cells a 5% down regulation of TNF-α and IL-10 was observed in supernatants of H4-1 cells incubated in DMEMwithcarbonated and uncarbonated mineral water,compared to that in the control (Figure 4). Atthe same time, an up regulation of 5 % was taking place for TGF-β forcellsincubated in DMEM with uncarboanted water and 27% for cells incubated in DMEM with carbonated mineral water.

During incubation of TLT macrophages with basal supernatants of H4-1 cells treated with mineral waters, a general down regulation of all cytokines could be observed when compared to the control (Figure 5).

4. Discussion

Despite many previously observed positive effects of natural mineral waters on human physiology (Nocco, 2008; Perez-Granados *et al*., 2010), there has been no evidence on potential mechanisms behind those effects.

In our novel human gut functional model we showed that minerals dissolvedincarbonated natural mineral waters are absorbed through the small intestinal epitheliumtoa much higher extent as compared to non-gaseous mineral waters (Figure 2) and that the minerals are highly available.

These findings are not surprising, as previously described by Missner et al. (Missner *et al.*, 2008), CO_2 increases the permeability of cellular membranes, resulting in a higher mineral intake.

In the same time carbonated mineral water is positively enhancing the trans-epithelial resistance of H4-1 cells (Figure 1). Intestinal epithelial cells are closely packed and form tight-junctions in order to regulate intestinal absorption and migration. TER is the parameter describing how well the cells are interlocked, differentiated and polarized (Langerholc *et al*., 2011). By enhancing TER, carbonated mineral water shows the potential to beneficially influence the integrity of the intestinal wall.

Regarding self-renewal, the intestine is one of the most proliferative organs of the human body. Frequent cellular turnover is necessary for its normal functioning therefore increased proliferation and renewal seems a promising attribute of carbonated mineral water.

The data obtained in testing the immunomodulatingproperties of natural mineral waters, especially of the mineral water with CO_2 indicated that natural mineral water has the ability to down regulate proinflammatorycytokine expression by small intestinal epithelial cells and underlying monocytes/macropages (Figure 4). The water has

significantly up-regulated the production of TGF-β in H4-1 cells and at the same time suppressed its activation in TLT macrophages (Figure 4). It was reported before that TGF-β appears to block the activation of lymphocytes and monocyte derived phagocytes (Leterrioand Roberts, 1998).

5. Conclusions

Modern day lifestyle, defined by a decrease in physical activity and an increase in unhealthy nutritional habits, gives rise to augmented incidence of intestinal inflammations, intestinal dysfunction and colon cancer (Labianca et al., 2010).

Our study suggests that regular intake of carbonated natural mineral water bears the potential to ease symptoms associated with above mentioned conditions. The findings demonstrate that natural mineral waters present a valuable source of macro- and microelements which are highly bioaccessible to the human organism, beneficially influence cellular turnover and contribute to the positive regulation of gut immunological responses. A clinical trial is currently running to support our claims.

In this manner, our report emphasizes also the importance of mechanistic studies to elucidate clinical evidence as well as the importance of utilizing appropriate models which mimic the *In vivo* environment as close as possible. In models that represent a healthy gut environment, untransformed and non-carcinogenic cell lines should be applied, such as in the model employed in this study.

References

Botić, T., Klingberg, T. D., Weingartl, H., & Cencic, A. (2007). A novel eukaryotic cell culture model to study antiviral activity of potential probiotic bacteria. *International Journal of Food Microbiology*, *115*, 227-34. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.10.044

Cencic, A., & Langerholc, T. (2010). Functional cell models of the gut and their applications in food microbiology - a review. *International Journal of Food Microbiology, 141*, 4-14. http://dx.doi.org/10.1016/j.ijfoodmicro.2010.03.026

Ekmekcioglu, C. (2000). Intestinal bioavailability of minerals and trace elements from milk and beverages in humans. *Nahrung*, *44*, 390-7. http://dx.doi.org/10.1002/1521-3803(20001201)44:6<390::AID-FOOD390>3.0.CO;2-Y

Kolesnikov, O. L., Selianina, G. A., Dolgushin, I. I., & Kolesnikova, A. A.(2002). The question of mechanisms of the immunotropic effect of mineral drinking waters. *Voprosy Kurortologii, Fizioterapiii Lechbnoi Fizicheskoi Kultury, 3*, 15-7.

Labianca, R., Beretta, G. D., Kildani, B., *et al*. (2010).Colon cancer. *Critical Reviews in Oncology/Hemathology, 74*, 106-133. http://dx.doi.org/10.1016/j.critrevonc.2010.01.010

Langerholc, T., Maragkoudakis, P. A., Wollgast, J., Gradisnik, L., & Cencic, A. (2011). Novel and established intestinal cell line models - An indispensable tool in food science and nutrition. *Trends in Food Science & Technology, 22*, 11-20. http://dx.doi.org/10.1016/j.tifs.2011.03.010

Letterio, J. J., & Roberts, A. B. (1998). Regulation of immune responses by TGF-β. *Annual Review of Immunology, 16*, 137-161. http://dx.doi.org/10.1146/annurev.immunol.16.1.137

Marktl, W. (2009). Gesundheitliche Bedeutung Natürlicher Mineralwässer. *Wiener KlinischeWochenschrift, 121*, 544–550. http://dx.doi.org/10.1007/s00508-009-1244-1

Missner, A., Kügler, P., Saparov, S. M., Sommer, K., Mathai, J. C., Zeidel, M. L., & Pohl, P. (2008). Carbon dioxide transport through membranes. *Journal of Biological Chemistry, 283*(37), 25340-25347. http://dx.doi.org/10.1074/jbc.M800096200

Nissen, L., Chingwaru, W., Sgorbati, B., Biavati, B., & Cencic, A. (2009). Gut health promoting activity of new putative probiotic/protective Lactobacillus spp. strains: a functional study in the small intestinal cell model. *International Journal of Food Microbiology*, *135*, 288-94. http://dx.doi.org/10.1016/j.ijfoodmicro.2009.08.027

Nocco, P. B. (2008). Mineral water as a cure. *Veroffentlichungen der Schweizerischen Gesellschaft fur Geschichte der Pharmazie, 29*, 13-402.

Pérez-Granados, A. M., Navas-Carretero, S., Schoppen, S., & Pilar-Vaquero, M. (2010). Reduction in cardiovascular risk by sodium-bicarbonated mineral water in moderately hypercholesterolemic young adults. *The Journal of Nutritional Biochemistry*, *21*, 948-953. http://dx.doi.org/10.1016/j.jnutbio.2009.07.010

Polushina, N. D., Vladimirov, V. I., & Frolkov, V. K. (2002). Effect of drinking mineral water on hormonal regulation of glucose homeostasis after mastectomy for breast cancer. *Voprosy Kurortologii, Fizioterapiii Lechbnoi Fizicheskoi Kultury, 6*, 19-21.

Portalès, P., Ariès, M. F., Licu, D., Pinton, J., Hernandez-Pion, C., Gall, Y., Dupuy, P., Charveron, M., & Clot, J. (2001). Immunomodulation induced by Avène spring water on Th1- and Th2-dependent cytokine production in healthy subjects and atopic dermatitis patients. *Skin Pharmacology and Applied Skin Physiology, 14*, 234-42. http://dx.doi.org/10.1159/000056352

Radenskad.d. (2011). Internal quality report. *Radenskad.d.*.

Vladimirov, V. I., Laktionova, A. I., & Polushina, N. D. (2004). Drinking mineral waters in rehabilitation of cancer patients. *Voprosy Kurortologii, Fizioterapiii Lechbnoi Fizicheskoi Kultury, 4*, 16-9.

Table 1. Physical and chemical parameters of the mineral waters

Parameter	Radenska Classic (Mineral water withCO_2)	Radenska Naturelle (Mineral water without CO_2)
pH	6,4	7,3
Mg^{2+}	92 mg/l	20 mg/l
Ca^{2+}	200 mg/l	47 mg/l
Na^{+}	440 mg/l	7,4 mg/l
K^{+}	80 mg/l	0,8 mg/l
Cl^{-}	44 mg/l	3,4 mg/l

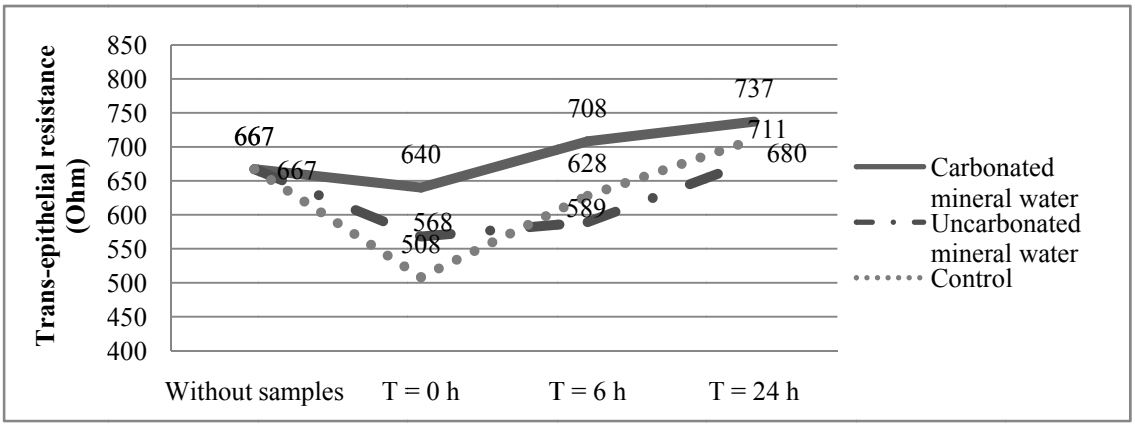

Figure 1. The initial TER of H4-1cells before samples were applied was 667 Ohm. After application of mineral waters a general decrease in TER has been observed. After 24 hours the TER of cells incubated with carbonated water was 737 Ohm, the one of cells incubated with uncarbonated water 680 TER and the control 711 Ohm. The values represent an average of 3 individual experiments

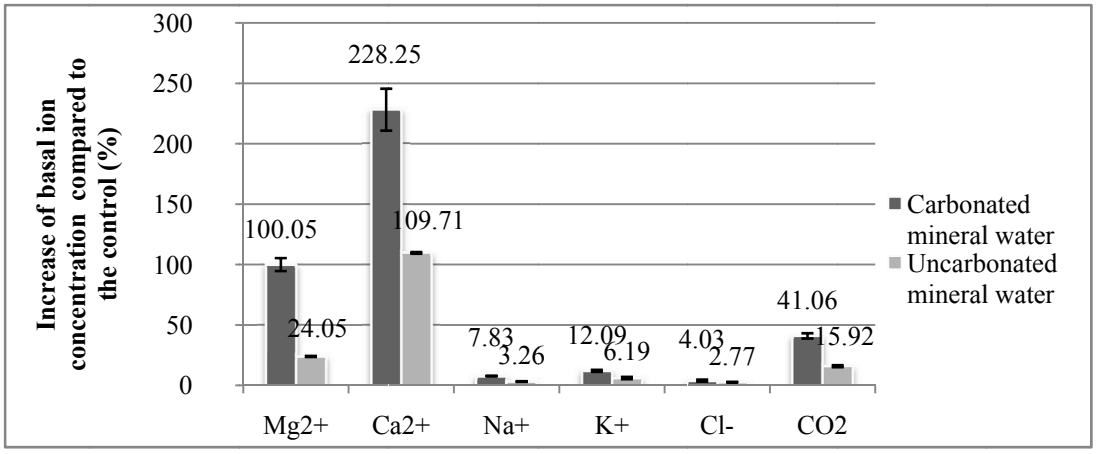

Figure 2. Increase of basal ion concentration. The increase of each ion is expressed as mean ±SD (n=3) of the average concentration as compared to the control

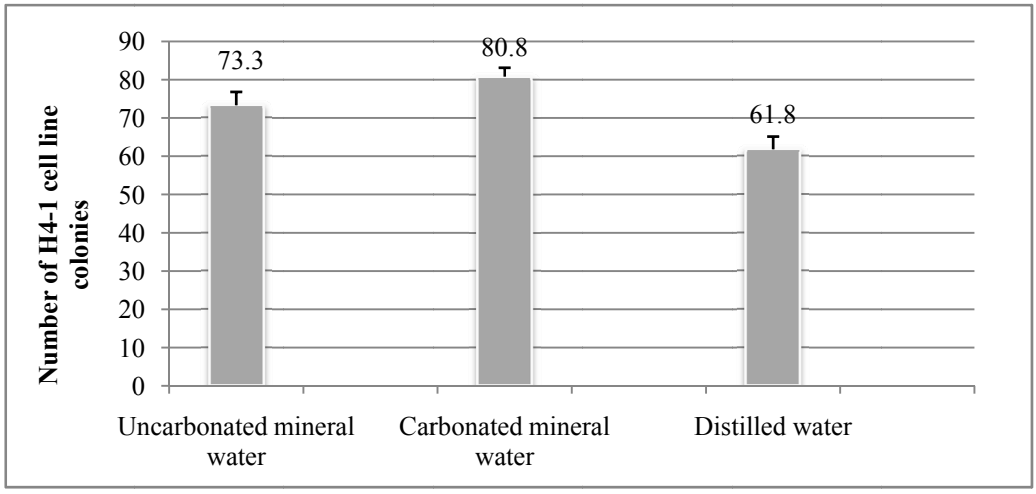

Figure 3. Number of colonies formed by H4-1 (untransformed intestinal epithelial cells) after incubation in DMEM with carbonated, uncarbonated and distilled water for 7 days at 37°C. The effect on the proliferation of each water is expressed as mean ±SD (n=3) of the number of colonies formed as compared to the control

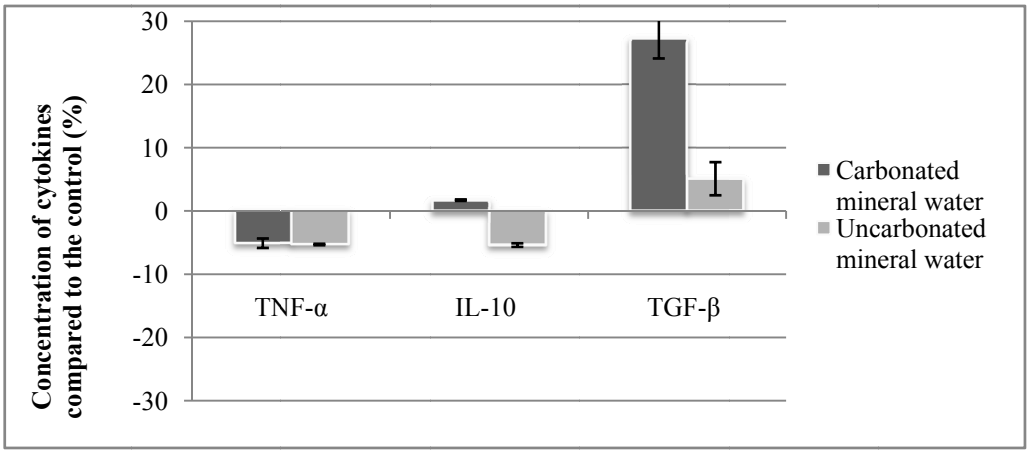

Figure 4. The difference of cytokine activation in H4-1 cells after incubation in DMEM with natural mineral waters and the control for 24 hours at 37°C expressed as percentage compared to the control (cells incubated in DMEM with distilled water). The percentage of difference is expressed as mean ±SD (n=3)

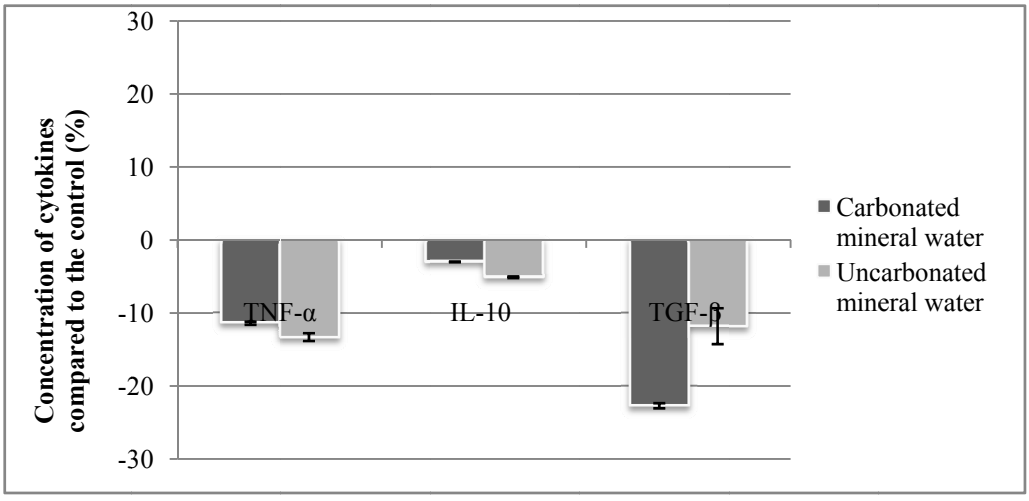

Figure 5. The difference of cytokine activation in TLT macrophage cells after incubation in DMEM with natural mineral waters and the control for 24 hours at 37°C expressed as percentage compared to the control (cells incubated in DMEM with distilled water). The percentage of difference is expressed as mean ±SD (n=3)

Mechanical and Structural Stability of an Extruded Starch-protein-polyol Food System

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Abstract

The objective of this work was to assess the structure stability of a starch-protein-polyol based food product manufactured by extrusion using texture analysis (TA), mechanical spectroscopy (DMTA), differential calorimetry (DSC) and X-ray diffraction. An accelerated storage trial showed an increase in the compression force from ~25N to ~82N after 120 days at 37 °C. The decrease in tan δ values from ~0.65 to ~0.40, from DMTA, suggested a reduction in the amorphous fraction of the starch present in the formulation. This data was supported by DSC, which showed an irreversible endothermic peak at ~58 ^{o}C with an associated melting enthalpy (ΔH) ~2.1 J/g. The kinetic was modeled by the Avrami equation as an empirical approximation giving the parameter *G* (h^{-1}) ~1.62E-02. This data indicated that the retrogradation of the starchy component is the main mechanism driving the changes in texture of the whole product, confirming the importance of its structuring functionality in this type of formulations.

Keywords: Starch, Polyol, Extrudate, Retrogradation

1. Introduction

Intermediate moisture food products provide important advantages over wet products in terms of palatability and shelf life. They show attractive textural attributes at water activities (Aw) lower than 0.7, minimizing spoilage

due to microorganisms (Fennema, 1996). This characteristic, commonly found in foods such as confectioneries, is also being applied to pet-care formulations such as flavored tooth-cleaner chews and meat-like treats, where mechanical properties are important quality attributes together with nutritional value. The composition of these formulations is commonly based on cereals as structure forming components. Other ingredients include proteins, lipids and low molecular weight components as plasticizers, which contribute to the rubber-like behavior at ambient temperature. Although these products are microbiologically stable during storage, an increase in the hardness has been reported (McMahon, Adams & McManus, 2009). This behavior would have an effect on the product's digestibility, increasing the risk of dental and digestive tract injuries (Torney, Unlu, Willcocks, Zubair & Bierer, 2009). Therefore, understanding the mechanisms driving these changes seems important in order to improve product formulation and shelf life. Due to their complexity, very little information is found in the literature on the fundamentals of the structural stability of real food products, limiting their study to empirical methods, which makes the understanding of ageing phenomena difficult and incomplete. Li, Szlachetka, Chen, Lin and Ruan (2008) studied the hardening of a high content protein bar by textural analysis and nuclear magnetic resonance (NMR), showing changes in molecular mobility during storage but with no clear explanation of its occurrence. More fundamental studies looking at mechanisms driving the structural stability and its kinetics are applied to simpler models systems.

Van Soest and Knooren (1997) studied the changes in mechanical properties of potato starch-gycerol based extruded sheet during storage, showing a correlation between an increase in elastic modulus and crystallinity dependent on water content, plasticizer concentration, and glass transition temperature (Tg). Farhat, Blanshard and Mitchell (2000) analyzed the retrogradation kinetics of waxy maize starch extrudates, observing an increase in crystallinity and a decrease in molecular mobility after 40 hours. They determined a strong dependence of retrogradation on water content and storage temperature. Kingcam, Devahastin and Chiewchan (2008) studied the effect of pre-treatments (blanching, freezing and thawing) on the degree of starch retrogradation of dried potato chips, showing that an increase in degree of crystallinity led to an increase in hardness and toughness produced by the formation of crystalline regions during the starch retrogradation.

In the case of the stability of intermediate and high moisture foods (>~10%), they can undergo changes in their structure which can be related to the reordering of the amorphous polymer chains towards a crystalline structure if stored at temperatures above their Tg (Farhat, Blanshard, Decamps, & Mitchell, 2000; Jouppila, Kansikas, & Roos, 1998; Mizuno, Mitzuiki, & Motoki, 1998). For example, it is generally accepted that starch retrogradation significantly contributes to bread staling, demonstrated by the formation of crystal-like structures using differential scanning calorimetry and X-ray diffraction (Gray & Bemiller, 2003; Jagannath, Jayaraman, Arya, & Somashekar, 1998; Karim, Norziah & Seow, 2000).

In terms of the prediction of the ageing phenomenon, several theories have been developed to describe the kinetics of crystallization of starch-based products, where the Avrami equation (Avrami, 1940) has been the one most widely used. Jouppila et al. (1998) studied the crystallization kinetics of amorphous corn starch at various temperatures and water content, and from an analysis of the parameters of the Avrami equation concluded that crystallization is faster at higher temperatures and $T - Tg > 0$ conditions. Takaya, Sano and Nishinari (2000) analyzed the retrogradation of the gelatinized corn starch system and from the slope analysis of the Avrami equation showed that starch gels, especially the amylose fraction, retrograde immediately on cooling, followed by rod-like growth of crystals. Kun, Ping, Li-Jun, Zhan-Hui and Li-Te (2007) studied the kinetics of staling of Man-tou (special kind of Chinese bread) using the Avrami equation and found that the hardening rate was higher than the crystallization rate, suggesting that starch crystallization is not the only factor that increases the hardness of this product.

The aim of this work was to identify and study the mechanism driving the changes in mechanical properties of an intermediate moisture starch-protein-poyol based product during storage using fundamental techniques including dynamic mechanical thermal analysis (DMTA), differential scanning calorimetry (DSC) and wide angle X-ray diffraction (WAXD). The overall change in hardness was monitored using a texture analyser, a well known empirical technique widely used in industry.

2. Materials and Methods

2.1 Sample composition and storage

A commercial extruded pet-care product, commonly known as kibble, was studied by standard analytical techniques to report its composition. Protein content was obtained by the Kjeldahl method (AOAC, 1995), fat content was obtained by standard Soxhlet extraction, and glycerol content was calculated with a colorimetric reaction kit (free glycerol determination kit, Sigma Aldrich, Missouri, USA) absorbing at 540nm.

Non-nitrogenous components, which were associated with the starch fraction, were obtained from the difference in weight fraction. The moisture of the samples was determined gravimetrically using a vacuum oven (Gallemkamp, UK) at 70 °C for 12 h.

A fresh product (24 h after manufacture), in its original package, was placed in sealed aluminum bags and stored at 37 °C, temperature commonly used in industry for accelerated stability tests (Mizrahi, 2000; Park, Rhee, Kim & Rhee, 1993; Varga, 2005), for ageing times of 15, 30, 45, 60, 75, 90, 105 and 120 days.

2.2 Mechanical properties

The mechanical properties during storage were assessed by measuring the force needed to vertically compress 30% of the product's height. A texture analyzer model TA.XT-PLUS (Stable Microsystems, UK) was used at a compression speed of 2 mm/s using a 70-mm diameter circular plate. The experiment was performed at ambient temperature (~25 °C). Five replicates of each sample were measured.

2.3 Dynamic mechanical thermal analyzer (DMTA)

Mechanical properties of the product under dynamic conditions were determined on a Rheometric Scientific Mk III DMTA (Rheometric Scientific, UK) instrument. Fresh and aged samples (30, 60 and 120 days of storage) were cut into flat ribbons of typical size ~1.5 cm length, ~0.7 cm width and ~0.2 cm thickness. The strips were covered with silicone oil (Dow Corning, USA) to avoid moisture loss during analysis. The instrument was set to single bending mode and a temperature scan from -100 °C to 120 °C at a heating rate of 3 °C/min. To assure the measurement on the stress-strain lineal zone, the set strain was x 4 (62 μm amplitude). The frequency tested was 10 Hz. Three replicates were measured for each sample.

2.4 Differential scanning calorimetry (DSC)

Thermal transitions of the product were evaluated using a DSC 7 differential scanning calorimeter (Perkin Elmer, UK) previously calibrated using indium ($T_{m,\ onset}$= 156.6 °C, ΔH = 28.6 J/g) and cyclohexane ($T_{m,\ onset}$= 7.0 °C). Before the analysis all samples were ground under cryogenic conditions using liquid nitrogen and mixed with distilled water at a 1:3 solid-to-water ratio, loaded into stainless steel DSC pans, and equilibrated overnight by continuous rotation at ambient temperature. The typical experimental conditions were: scanning temperature from 5 °C to 110 °C at a heating rate of 10 °C/minute, cooling to 5 °C at a rate of 30 °C/minute, and re-heating from 5 °C to 110 °C at a rate of 10 °C/minute. The measuring parameters considered were the endothermic peak temperature and melting enthalpy. The reported transition enthalpies were reported as J/g of dry sample. Three replicates were measured for each sample.

2.5 Wide angle X-ray diffraction (WAXD)

The samples were scanned using a Bruker D5005 X-Ray diffractometer (Bruker, UK) equipped with a copper source at operational settings of 40 kV and 30 mA (CuK wavelength → 0.154 nm). The experimental settings were an incident angle of 2θ = 4° to 38° at an angle step of 0.05° per 3 s. The rotational speed of the sample holder was set to 60 rpm. The obtained diffractogram were subtracted by the holder spectra and baseline corrected over the measurement scanning angles using the OPUS 3.0 software (Bruker, UK). The fresh and aged samples were scanned in duplicate.

2.6 Determination of ageing kinetics

The hardening kinetics was assessed empirically by testing the equation proposed by Avrami (1940) (Equation 1). This model has been successfully applied to various starch-based systems to predict variations in textural attributes relating this phenomenon to a molecular reordering process (Ottenhof, 2003):

$$Y_t = Y_\infty(Y_\infty - Y_o)e^{-k_r t^n} \quad (1)$$

where n represents the Avrami coefficient that is thought to depend on the type of crystal nucleation and the dimensions in which growth takes place, t is variable time, k_r is the crystal growth rate, Y_t is a physical parameter describing the crystallization dependency over time t, Y_o is the physical parameter at time 0, Y_∞ is the physical parameter at time equal to infinity (end plateau).

2.7 Statistical analysis

Statistical significance of the experimental data was evaluated by one-way Analysis of Variance (ANOVA) with a P value of 0.05 using the Data Analysis suite available in Excel Office 2003 (Microsoft Corp. USA).

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The fitting optimization of Equation 1 was done by independently adjusting Y_o, k and n. This procedure was performed by minimizing the sum of squares difference between the experimental and theoretical data using the Solver software suite available in Excel Office 2003 (Microsoft Corp. USA).

3. Results and Discussion

Proximal analysis of the product reported ~20.0 g of proteins, ~12.5 g of glycerol, ~2.0 g of lipids, 16.4 g of water and ~50.5 g of starchy components. All quantities are based on 100 g of dry product. The starch concentration present in the product agreed with the value reported by the manufacturer.

Gravimetric determination showed a small variation in moisture content between the fresh and aged product after 60 and 120 days (Table 1). The statistical analysis of this data did not indicate significant differences ($P > 0.05$). Therefore, it was concluded that the product did not dehydrate during storage.

The mechanical properties of the product measured by texture analysis showed a significant increase in stiffness during storage as indicated by greater compressive force. This behavior can be clearly seen in Figure 1. In the first 30 days of storage there was a slight increase ($P > 0.05$) of this parameter from ~25N to ~32N. After 30 days of storage a marked increase ($P < 0.05$) of the compression force was detected up to ~60 days of storage, with an increase from ~32 N to ~80 N. At this point the compression curve leveled off with a plateau value of ~82 N ($P < 0.05$) up to 120 days of storage. As mentioned previously, this increase in stiffness was not related to product dehydration, as the moisture content remained relatively constant during the experiment (Table 1), suggesting other mechanisms driving the hardening of the system. Pushpadass and Hanna (2009) showed that both tensile strengths and strains in extruded corn starch films plasticized with glycerol and stearic acid were strongly influenced by aging time (120 days, 20ºC), where tensile strengths increased during storage while tensile strain decreased with aging time, making films stiffer and less flexible. This behavior was explained by changes in the microstructure driven by increasing in crystalline content and free volume changes (Pushpadass & Hanna, 2009).

The experimental data obtained from DMTA show the typical profile for a polymeric structure (Figures 2A and B), which is interesting for such a complex composition. The elastic modulus (E') significantly decreased three-fold from *log E'*~9.3 Pa to *log E'*~6.0 Pa when the temperature increased from -100 °C to 40 °C. This reduction of three orders of magnitude was coupled to a maximum value of *tan* δ ~ 0.65 at ~ 20 °C. This behavior can be related to an α-like relaxation of the main polymer chains at the glass transition temperature. At this point, long range and cooperative motions of the polymeric chains seems to occur, increasing their structural mobility (Price, 2002; Roos, 2010). These data suggest that the physical state of the overall mixture, and particularly the starchy component present in the formulation, were in a rubber-like state under normal environmental conditions ($T \geq 25$ °C). These experimental data correlated well with the flexible nature of the food product at ambient temperature (data not shown). The *tan* δ vs. temperature graph also indicated a second transition at lower temperature (~ -40 °C) with an associated *tan* δ ~0.40 (Figure 2B). The origin of this transition is not clear, but it may represent a secondary or β-relaxation of the polymer structure (Kalichevsky, Jaroszkiewicz, Ablett, Blanshard, & Lillford, 1992; Price, 2002) or an α-relaxation from a separated phase or rich fraction of a secondary component. The experimental data suggest a rich region of the protein fraction present in the formulation. A similar behavior has been reported in the literature using mechanical spectroscopy to study a gelatin-starch model system (Mousia, Farhat, Blachot & Mitchell, 2000).

Similar *E'* values were found between the fresh and aged samples at temperatures lower than 20 °C. In this temperature range the amorphous fraction would be in the glassy state, behaving mechanically as a solid, therefore stiffness variations between the samples were not observed. At higher temperatures significant differences ($P < 0.05$) in *E'* values were obtained. Indeed, if the reference temperature is set at 25 °C (ambient temperature), clear differences in this parameter between the fresh product and that aged for 120 days were observed, with *log E'* ~6.3 Pa to *log E'* ~6.7 Pa, respectively (Figure 2A). These results correlate well with the experimental data from the compression tests performed at ~25 °C, where significant changes in compressive force were detected for the same storage time (Figure 1).

No variations were observed in the temperatures at which the marked drop in *E'* values and *tan* δ peaks occurred for the fresh and aged samples (Figures 2A and B). As moisture, an effective plasticizer, remained relatively constant throughout the storage evaluation, no change in these transition temperatures was observed.

Interestingly, a decrease in *tan* δ peak height was observed during storage (Figure 2B). This value decreased from *tan* δ ~0.65 for the fresh product to *tan* δ ~0.40 after 120 days of storage. This behavior may be associated with the reduction of the amorphous fraction present in the system, suggesting a possible reordering phenomenon occurring during storage (Price, 2002). Lionetto, Maffezzoli, Ottenhof, Farhat, and Mitchell (2005)

studied an extruded wheat starch with 37% moisture content (db) stored for 180 h, explaining the decrease in *tan δ* values from ~0.85 to ~0.25 by a re-crystallization phenomenon, which was in agreement with X-ray diffractograms (increase in relative intensity at around 2θ ~16º, ~18º and ~23.5º) and NMR relaxometry (decrease in relaxation time, T_2) during storage representing lower molecular mobility. Figure 2B also indicates a reduction in *tan δ* of the smaller peak occurring at – 40 °C, from ~0.39 to ~0.32, supporting the hypothesis that attributes the origin of this transition to the presence of a separated phase component changing its molecular configuration on ageing. Mousia *et al*. (2000) studied the mechanical transitions of amylopectin–gelatin mixtures by mechanical spectroscopy and attributed the presence of a transition at lower temperatures to the existence of a gelatin-rich phase present in the mixture.

Figures 3 A and B depict the 1st and 2nd DSC scans, respectively. The experimental data show a small reversible endotherm at ~25 °C with a melting enthalpy (ΔH_m) of ~0.3 J/g, which was associated with the melting of lipids present in the formulation. This was confirmed by a reduction of this enthalpy when the product was thoroughly washed by an organic solvent (data not shown). As expected, the ΔH_m values did not change significantly with storage time (Table 2). The DSC scans obtained for the fresh product and that stored for 30 days showed a small endotherm at ~58 °C with an associated ΔH_m ~0.10 J/g. This value would suggest some structural ordering of the starchy components present in the formulation. Major endotherm enthalpies were detected at the same temperature range for the product aged for 60 and 120 days, with ΔH_m ~1.03 J/g and ΔH_m ~2.10 J/g, respectively (Table 2), indicating further ordering of the structure. If the cereal (starch) weight fraction present in the formulation is considered, the melting temperatures and enthalpies obtained are equivalent to those found in the literature for the starch-water system. Indeed, Ottenhof, Hill and Farhat (2005) suggested that retrogradation kinetics of starch-water systems prepared by thermo-mechanical extrusion showed endothermic transitions at peak temperatures of 57.7 °C (ΔH_m ~11.06 J/g), 55.8 °C (ΔH_m ~9.0 J/g) and 53.0 °C (ΔH_m ~6.1 J/g) for potato, waxy maize and wheat starches, respectively.

X-ray diffractograms for the fresh and aged product in Figure 4 show a small peak for all the samples at a diffractive angle (2θ) ~32º, which was related to the presence of a salt in crystalline form. Enrione (2005) detected diffractive peaks at ~30° in a similar formulation which was related to the presence of potassium carbonate (K_2CO_3).

The curves for the fresh samples and those stored for 30 days were very similar, showing an amorphous-like pattern with not distinctive peak being detected. In the case of the product stored for 60 and 120 days, Figure 4 shows an indication of structural order taking place, represented by two peaks standing out from the background, one at a diffraction angle (2θ) ~20° and the other at 2θ ~23°. These peaks seem to be related to the formation of a rather ordered structure of the starchy component (Ottenhof *et al*., 2005). Bello-Perez, Ottenhof, Agama-Acevedo and Farhat (2005) detected an increase in peak intensity at the angles (2θ) ~17° and 23° when extruded banana starch with a moisture content of 30% (wb) was stored for 7 h at 25 °C. Similar results were reported by Pushpadass and Hanna (2009) working with extruded corn starch films plasticized with glycerol and stearic acid stored at ~20 °C for up to 120 days. Saiah, Sreekumar, Leblanc, Castandet and Saiter (2007) also reported an increase in crystallinity measured by X-ray diffraction in wheat extruded sheets plasticized with water and glycerol after storage of 12 months at room temperature.

The reordering process of the polymeric fraction, particularly the starchy component present in the formulation, suggests the relevance of the application of a mathematical representation of the experimental data in order to assess the kinetics of this phenomenon. Figure 1 shows the fitting of the Avrami equation (Equation 1) generating the parameters k ~6.4E-06, n ~2.90, Y_∞ ~84.0 N and Y_o ~27.0 N. If retrogradation is assumed to be the main mechanism driving the changes in mechanical properties of the product, the rate of retrogradation (G h^{-1}) was then calculated using the relation $G = k_r^{1/n}$ (Farhat *et al*., 2000), giving a value of ~1.6E-02 h^{-1}. This value was similar in order of magnitude to those indicated in the literature for extruded starch-based systems. Ottenhof et al. (2005) obtained a G value of ~5.0E-02 h^{-1} for extruded wheat starch systems in the presence of 34.1% (d.w.b) of moisture, and Enrione (2005) calculated a G value of ~6.5E-02 h^{-1} for wheat starch extrudates containing 16% moisture (wb) and 20% glycerol (db). Despite the usefulness of using this equation to assess the recrystallization kinetics of these systems, the interpretation of the Avrami coefficients should be treated with caution. Del Nobile, Martoriello, Mocci and La Notte (2003) commented that parameter n should be considered as a fitting variable rather than of the original interpretations of an integer number representing the mechanism of crystal growth. Farhat *et al*. (2000) used a least squares minimizing fitting routine to adjust parameters k, n, Y_∞ and Y_o instead of simple linear fitting because they considered that the model's assumptions would introduce distortions in the calculated kinetic parameters.

Therefore, more suitable mathematical models are necessary in order to improve the understanding of the retrogradation process and kinetics in complex food formulations.

4. Conclusions

The results indicate the relevance and importance of fundamental techniques to assess the structure and stability of complex food systems during storage. Particularly interesting in this work are the data obtained from mechanical spectroscopy showing a clear glass transition temperature properly describing the rubber-like structure of the product at ambient temperature. Transitions at lower temperatures indicated the presence of a protein-rich fraction present in the formulation. The hardening of the product during storage, indicated by an increase in the elastic modulus and compression force, was correlated to a reordering of amylopectin from the starchy component as indicated by melting endotherms detected by differential calorimetry. These data were supported by the reduction of the *tan* δ values at constant temperature reported for the stored product. This phenomenon was also observed by X-ray diffraction after 120 days of storage. The crystallization kinetics, calculated by the Avrami equation, was comparable to values reported in the literature for starch-based model systems, confirming the functionality of this biopolymer as structuring matrix in extruded foods products.

Abbreviations: Tg, glass transition temperature; DMTA, dynamic mechanical thermal analyser; DSC, differential scanning calorimetry; WAXD, wide angle X-ray diffraction; Aw, water activity; t, time; k_r, crystal growth rate; Y_t, physical parameter describing the crystallization dependency on time t; Y_o, value of physical parameter at time equal 0; Y_∞, value of physical parameter at time equal infinity (end plateau); E', elastic modulus; G, rate of retrogradation.

References

AOAC (1995). *Official Methods of Analysis of AOAC International*. (16th ed.) Washington DC: Association of Official Analytical Chemist.

Avrami, M. (1940). Kinetics of phase change. II Transformation-time relations fro random distribution of nuclei. *The Journal of Chemical Physics*, *8*, 212-224. http://dx.doi.org/10.1063/1.1750631

Bello-Perez, L. A., Ottenhof, M. A., Agama-Acevedo, E., & Farhat, I. A. (2005). Effect of Storage Time on the Retrogradation of Banana Starch Extrudate. *Journal of Agricultural and Food Chemistry*, *53*, 1081-1086. http://dx.doi.org/10.1021/jf048858I

Del Nobile, M. A., Martoriello, T., Mocci, G., & La Notte, E. (2003). Modeling the starch retrogradation kinetic of durum wheat bread. *Journal of Food Engineering*, *59*, 123-128. http://dx.doi.org/10.1016/S0260-8774(02)00441-7

Enrione, J. (2005). Mechanical Stability of Intermediate Moisture Starch-Glycerol Systems. PhD Thesis. Division of Food Sciences, The University of Nottingham.

Farhat, I. A., Blanshard, J. M. V., & Mitchell, J. R. (2000). The retrogradation of waxy maize starch starch-sugar extrudates. *Biopolymers, 53*, 411.

Farhat, I. A., Blanshard, J. M. V., Decamps, M., & Mitchell, J. R. (2000). Effect of Sugars on Retrogradation of Waxy Maize Starch-Sugar Extrudates. *Cereal Chemistry, 77*, 202-208. http://dx.doi.org/10.1094/CCHEM.2000.77.2.202

Fennema, O. R. (1996). *Food Chemistry*. (3rd ed.). New York: CRC Press.

Gray, J. A., & Bemiller, J. N. (2003). Bread Staling: Molecular Basis and Control. *Comprehensive Reviews in Food Science and Food Safety, 2*, 1-21. http://dx.doi.org/10.1111/j.1541-4337.2003.tb00011.x

Jagannath, J. H., Jayaraman, K. S., Arya, S. S., & Somashekar, R. (1998). Differential scanning calorimetry and wide-angle X-ray scattering studies of bread staling. *Journal of Applied Polymer Science, 67*, 1597-1603.

Jouppila, K., Kansikas, J., & Roos, Y. H. (1998). Factors affecting crystallization and crystallization kinetics in amorphous corn starch. *Carbohydrate Polymers, 36*, 143-149. http//dx.doi.org/10.1016/SO144-8617(98)00024-1

Kalichevsky, M., Jaroszkiewicz, E. M., Ablett, S., Blanshard, J. M. V., & Lillford, P. J. (1992). The Glass Transition of Amylopectine Measured by DSC, DMTA and NMR. *Carbohydrate Polymer, 18*, 77-88. http://dx.doi.org/10.1016/0144-8617(92)90129-E

Karim, A., Norziah, M. H., & Seow, C. C. (2000). Methods for the study of starch retrogradation. *Food Chemistry, 71*, 9-36. http://dx.doi.org/10.1016/S0308-8146(00)00130-8

Kingcam, R., Devahastin, S., & Chiewchan, N. (2008). Effect of starch retrogradation on texture of potato chips produced by low-pressure superheated steam drying. *Journal of Food Engineering, 89*, 72-79. http://dx.doi.org/10.1016/j.jfoodeng.2008.04.008

Kun, S., Ping, Q., Li-Jun, W., Zhan-hui, L., & Li-Te, L. (2007). Effect of Storage Time on the Physicochemical and Sensory Properties of Man-tou (Chinese Steamed Bread). *International Journal of Food Engineering, 3*, 1-19. http://dx.doi.org/10.2202/1556-3758.1217

Li, Y., Szlachetka, K., Chen, P., Lin, X., & Ruan, R. (2008). Ingredient Characterization and Hardening of High-Protein Food Bars: an NMR State Diagram Approach. *Cereal Chemistry, 85*, 780-786. http://dx.doi.org/10.1094/CCHEM-85-6-0780

McMahon, D. J., Adams, S. L., & McManus, W. R. (2009). Hardening of High-Protein Nutrition Bars and Sugar/Polyol–Protein Phase Separation. *Journal of Food Science*, 74, E312-E321. http://dx.doi.org/10.1111/j.1750-3841.2009.01225.x

Mizrahi, S. (2000). Accelerated shelf-life test. In D. Kilcast & P. Subramaniam (Eds.), *The stability and shelf-life of foods*. Florida: CRC/Woodhead.

Mizuno, A., Mitsuiki, M., & Motoki, M. (1998). Effect of Crystallinity on the Glass Transition Temperature of Starch. *Journal of Agricultural and Food Chemistry, 46*, 98-103. http://dx.doi.org/10.1021/jf970612b

Mousia, Z., Farhat, I. A., Blachot, J. F., & Mitchell, J. R. (2000). Effect of water partitioning on the glass-transition behaviour of phase separated amylopectin–gelatin mixtures. *Polymer, 41*, 1841-1848. http://dx.doi.org/10.1016/S0032-3861(99)00335-3

Ottenhof, M. A. (2003). A multi-technique study of the retrogradation of concentrated starch systems. PhD Thesis. Division of Food Sciences, The University of Nottingham.

Ottenhof, M.-A., Hill, S. E., & Farhat, I. A. (2005). Comparative Study of the Retrogradation of Intermediate Water Content Waxy Maize, Wheat, and Potato Starches. *Journal of Agricultural and Food Chemistry, 53*, 631-638. http://dx.doi.org/10.1021/jf048705y

Park, J., Rhee, K. S., Kim, B. K., & Rhee, K. C. (1993). High-Protein Texturized Products of Defatted Soy Flour, Corn Starch and Beef: Shelf-Life, Physical and Sensory Properties. *Journal of Food Science, 58*, 21-27. http://dx.doi.org/10.1111/j.1365-2621.1993.tb03202.x

Price, D. M. (2002). Thermomechanical, Dynamic Mechanical and Dielectric Methods. In P.J. Heines (Eds), *Principles of Thermal Analysis and Calorimetry*. Cambridge: The Royal Society of Chemistry. http://dx.doi.org/10.1039/9781847551764-00094

Pushpadass, H. A., & Hanna, M. A. (2009). Age-Induced Changes in the Microstructure and Selected Properties of Extruded Starch Films Plasticized with Glycerol and Stearic Acid. *Industrial & Engineering Chemistry Research, 48*, 8457-8463. http://dx.doi.org/10.1021/ie801922z

Roos, Y. H. (2010). Glass Transition Temperature and Its Relevance in Food Processing. *Annual Review of Food Science and Technology, 1*, 469-496. http://dx.doi.org/10.1146/annurev.food.102308.124139

Saiah, R., Sreekumar, P. A., Leblanc, N., Castandet, M., & Saiter, J. M. (2007). Study of Wheat-Flour-Based Agropolymers: Influence of Plasticizers on Structure and Aging Behavior. *Cereal Chemistry Journal*, *84*, 276-281. http://dx.doi.org/10.1094/CCHEM-84-30276

Takaya, T., Sano, C., & Nishinari, K. (2000). Thermal studies on the gelatinisation and retrogradation of heat–moisture treated starch. *Carbohydrate Polymers, 41*, 97-1000.

Torney, A. A, Unlu, E., Willcocks, N., Zubair, K., & Bierer, T. (2009). Method for designing an oral pet product using biometric analysis. United States Patent Application Publication, Pub. Nº: US 2008/0010080 A1.

Van Soest, & Knooren, N. (1997). Influence of glycerol and water content on the structure and properties of extruded starch plastic sheets during aging. *Journal of Applied Polymer Science, 64*, 1411-1422.

Varga, L. (2005). Use of a long-chain polyphosphate mixture for shelf-life extension of processed cheese spreads. *Acta Alimentaria, 34*, 493-498. http://dx.doi.org/10.1556/AAlim.34.2005.4.16

Table 1. Moisture content (MC) of fresh and aged commercial product

Storage time	MC (%, wet basis)
Fresh	16.2 (0.3)
60 days	16.2 (0.2)
120 days	15.2 (0.2)

Values in brackets are the standard deviation from 3 measurements.

Table 2. DSC endotherms and enthalpy of fresh and aged products stored for 30, 60 and 120 days at 37 °C.

	1st peak Temperature (ºC)	1st Peak Enthalpy (J/g)	2nd peak Temperature (ºC)	2nd Peak Enthalpy (J/g)
Fresh 1st scan	25.4 (1.2)	0.33 (0.08)	56.1 (1.7)	0.12 (0.03)
Fresh 2nd scan	25.2 (1.8)	0.31 (0.06)		
Aged 30 days 1st scan	24.1 (1.3)	0.28 (0.05)	57.4 (1.6)	0.11 (0.04)
Aged 30 days 2nd scan	24.9 (1.6)	0.25 (0.07)		
Aged 60 days 1st scan	25.5 (1.3)	0.26 (0.05)	57.1 (1.4)	1.03 (0.32)
Aged 60 days 2nd scan	25.1 (1.1)	0.25 (0.09)		
Aged 120 days 1st scan	25.3 (0.8)	0.28 (0.05)	58.6 (1.9)	2.10 (0.39)
Aged 120 days 2nd scan	25.4 (1.2)	0.31 (0.03)		

(*) values in brackets represent the standard deviation from 3 measurements.

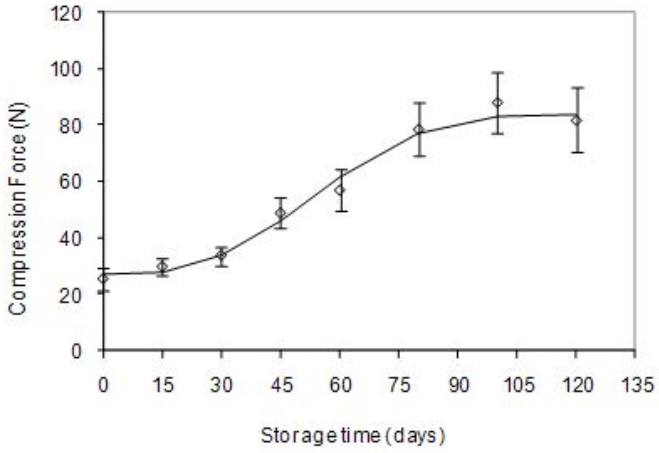

Figure 1. Changes in the compression force of the product versus storage time at 37 °C. Error bars represent the standard deviation from 5 measurements

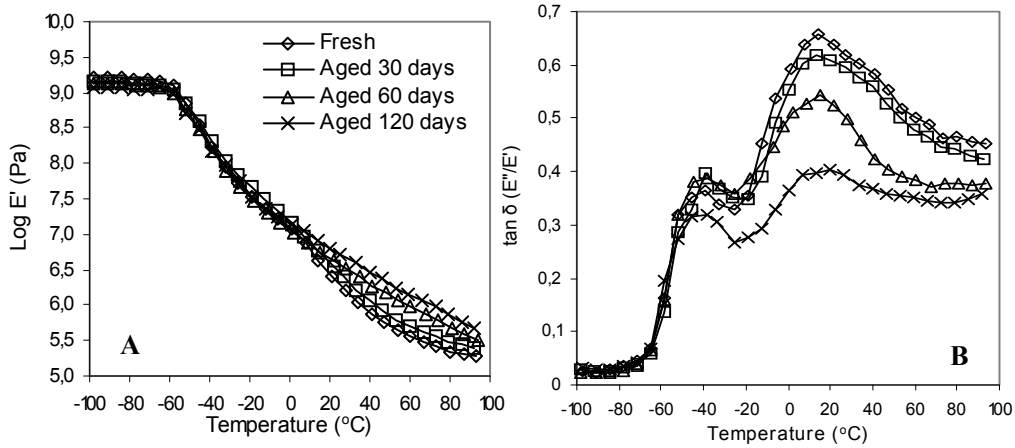

Figure 2. Elastic modulus *E'* (A) and *tan δ* (B) versus temperature for the commercial product stored for 0, 30, 60 and 120 days at 37 °C. Analysis performed at 10 Hz

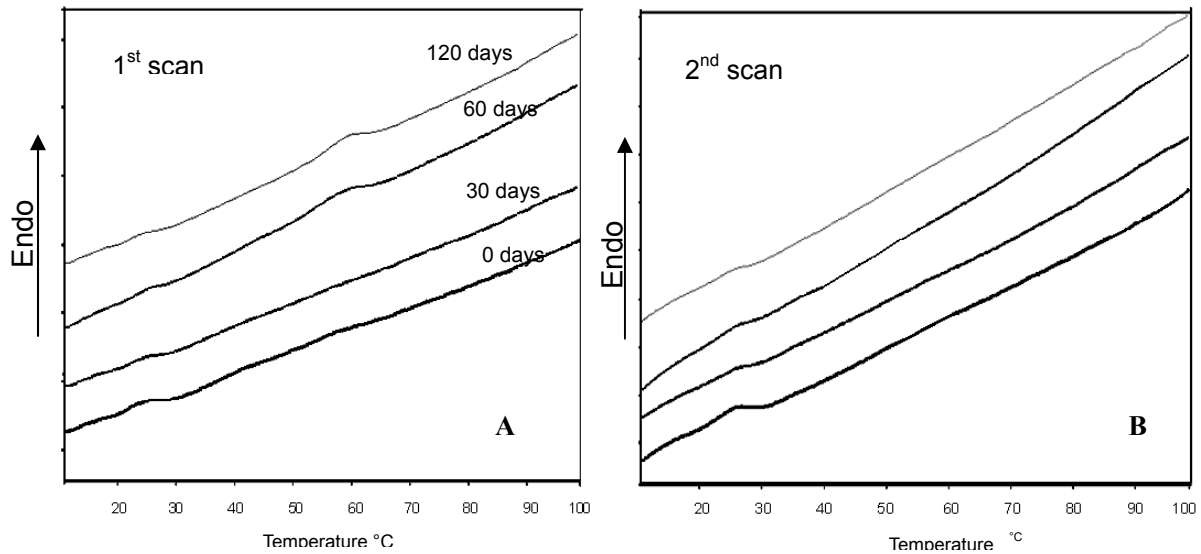

Figure 3. 1st and 2nd DSC scans for fresh and 30, 60 and 120 days stored product at 37 °C

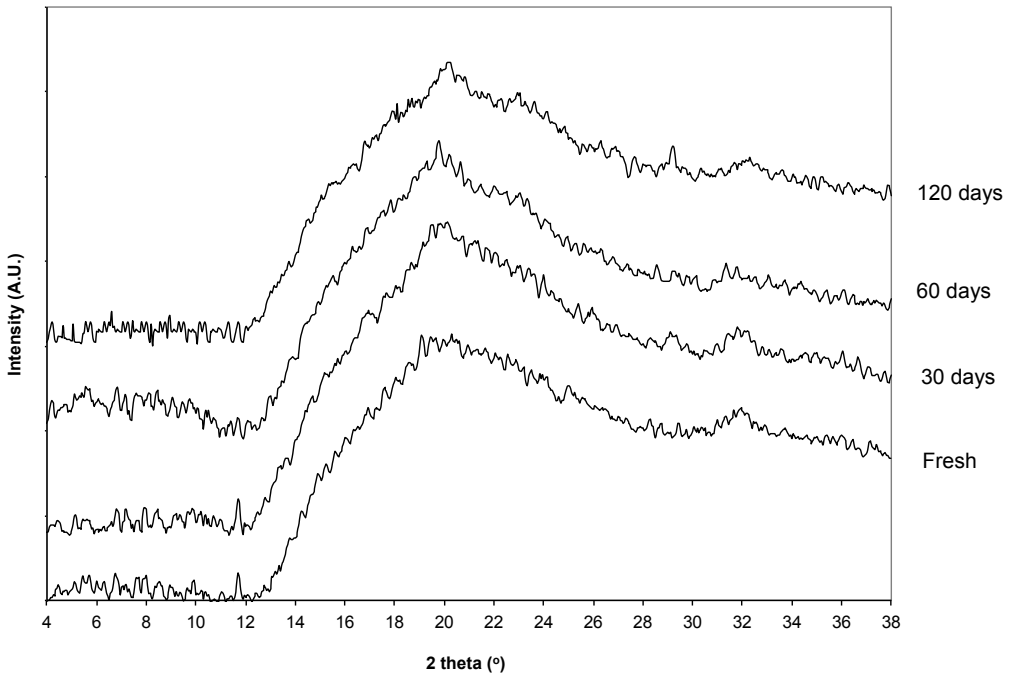

Figure 4. X-ray diffractograms for fresh product and product stored for 30, 60 and 120 days at 37 °C. (A. U.: Arbitrary Units)

A Comparison of Two Extraction Methods for Food Oxalate Assessment

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Abstract

Hyperoxaluria is a primary risk factor for the formation of calcium oxalate-containing kidney stones. Increased dietary oxalate intake and/or intestinal absorption may provide the critical quantity of additional oxalate that triggers the formation of kidney stones. The accurate determination of food oxalate is highly dependent on oxalate extraction, the first step in oxalate analysis. Potential problems include the possibility of elevated oxalate due to in vitro conversion from various oxalate precursors such as ascorbate and failure to dissolve all pre-existing calcium oxalate crystals. The primary objective was to compare the efficiency of the hot and cold extraction methods in extracting oxalate from 50 dry herb and 10 fresh fruit samples. Regardless of the method of extraction, leaves of Atriplex halimus and kiwifruit exhibited the highest concentrations of both total and soluble oxalate among the herbs and the fruits, respectively. The hot extraction method appeared to extract more total oxalate compared to the cold extraction method while there was no significant difference between the methods in efficiency of extracting soluble oxalate. The overall data suggested that the use of the hot acid method will yield a more accurate assessment of the total oxalate content of foods.

Keywords: Kidney stones, Oxalate extraction, Soluble oxalate, Total oxalate, Herbs, Fruits

1. Introduction

Oxalate is the conjugate base of oxalic acid which can bind to metal ions such as $Ca2^{+}$ and $Mg2^{+}$ to form precipitates in the body. Consumption of high oxalate-containing foods may result in hyperoxaluria and subsequent formation of insoluble calcium oxalate (CaOx) crystals, a primary component of kidney stones. Although urinary oxalate originates from two sources, endogenous synthesis from various precursors and exogenous intake from oxalate-containing foods, increased dietary oxalate intake and/or intestinal absorption may provide the critical quantity of additional oxalate that triggers the formation of CaOx kidney stones (Robertson et al. 1978; Robertson & Peacock, 1980).

Dietary oxalate is mainly derived from foods of plant origin. Efficiency of oxalate absorption depends on the amount present and form (soluble or insoluble), as well as other constituents of the diet such as calcium, magnesium and various fibers (Liebman & Al-Wahsh, 2011). Determination of oxalate content of foods is of special interest for kidney stone patients for whom decreasing urinary oxalate excretion by avoiding consumption of oxalate-rich foods may help prevent stone recurrence (Massey, 2007). However, data on the oxalate content of foods are incomplete and sometimes inaccurate. The accurate determination of food oxalate is highly dependent on oxalate extraction, the first step in oxalate analysis. Oxalate exists in plants in a crystalline form either as calcium oxalate or as a soluble anion (Holmes & Kennedy, 2000). Soluble oxalates, which consist of oxalic acid and soluble salts, can be released when foods are extracted with water; insoluble oxalates, presumed to be mainly calcium oxalate, freely dissolve in acid (Liebman & Al-Wahsh, 2011). Traditionally, dilute acid is used in extracting total oxalate including both the soluble and insoluble fractions.

Problems associated with oxalate extraction from food include the possibility of elevated oxalate due to in vitro conversion from various oxalate precursors such as ascorbate and failure to dissolve all pre-existing CaOx crystals (Hönow & Hesse, 2002). Oxalate extraction with hot acid has been used to ensure complete dissolution of CaOx. However, this method may result in in vitro oxalate generation with subsequent overestimation of oxalate content (Zarembski & Hodgkinson, 1962a). On the other hand, although cold acid extraction has been suggested to minimize in vitro oxalate synthesis, CaOx crystals may not completely solubilize which in turn may lead to underestimation of oxalate content (Hönow & Hesse, 2002). The primary objective of the present study was to compare the efficiency of the hot and cold extraction methods in extracting oxalate from dry and fresh food samples.

2. Materials and Methods

2.1 Samples

After short interviews with local herbalists to identify the most popular commercially available herbs, representative samples were purchased from local herbalist shops in Amman, Jordan. Fifty herbs were taxonomically identified at Hashemite University laboratories (Zarqa, Jordan) and then transported to the nutrition research laboratory at the University of Wyoming (Laramie, Wyoming). The herbs were ground into a fine powder using a coffee mill prior to soluble and total oxalate analyses.

The fruits used in this study were apples, strawberries, blueberries, grapes, kiwifruits, peaches, pears, oranges, bananas and cantaloupes. All fruits were purchased from local supermarkets in Laramie, Wyoming. Individual fruits were cut into small pieces and homogenized using a tissue homogenizer before oxalate extraction.

2.2 Hot extraction

0.5 g of finely ground herb or 4 g of homogenized fruit sample were weighed into 250 ml volumetric flasks and 50 ml of 2 N HCl (for total oxalate extraction) or 50 ml of distilled deionized water (for soluble oxalate extraction) were added. The flasks were placed in a shaking water bath at 80 ℃ for 30 min. The extracts were further diluted with 50 ml of distilled deionized water and then transferred into 15 ml centrifuge tubes and centrifuged at 4200 rpm for 10 min. The supernatants were filtered through Whatman #1 filter paper and kept frozen until the time of oxalate analysis. Each sample was extracted in duplicate.

2.3 Cold extraction

Total and soluble oxalates were extracted from the samples using the method of Ohkawa (Ohkawa, 1985) with some modifications. 0.15 g of the herb or 1.0 g of the fruit was weighed into a 15-ml centrifuge tube and 5 ml of 2 N HCl (for total oxalate extraction) or 5 ml of distilled deionized water (for soluble oxalate extraction) were added to the sample. The tube was tightly capped and vortexed for 5 min and then centrifuged at 4200 rpm for 10 min. The supernatant was transferred to a 25-ml volumetric flask and the remaining oxalate in the pellet was extracted two additional times. The final volume of the collected supernatant from the 3 successive extractions was diluted to 25 ml with distilled deionized water. The extracts were kept frozen until the time of oxalate analysis. Each sample was extracted in duplicate.

2.4 Quantification of total and soluble oxalate

The extracts were analyzed in duplicate for oxalate by using a commercially available enzymatic kit (Trinity Biotech, Berkeley Heights, New Jersey), which is based on measuring the amount of hydrogen peroxide liberated from the oxidation of oxalate by oxalate oxidase. Oxalate concentrations are expressed in mg/100 g of dry herbal sample weight and mg/100 g of fresh fruit weight (± standard deviation of two separate extractions).

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2.5 Statistical Analysis

The paired t test was used to test the null hypothesis that the average amount of extracted oxalate was the same between the two methods. To satisfy the assumption of normality, oxalate values were analyzed in transformed scale (loge(x)). In addition, simple linear regression was employed to describe the relation between the two extraction methods. Regression was conducted on the square root of the oxalate values to satisfy the assumption of normality. Statistical significance was declared at $\alpha = 0.05$ level. Statistical computations were made by using the Statistical Analysis System (SAS institute, version 9.2, Cary, North Carolina USA).

3. Results

Oxalate concentrations corresponding to the two extraction methods (hot and cold) for 50 herbs and 10 fruits are shown in Tables 1 and 2, respectively. Mean total oxalate concentrations using the hot extraction method were significantly higher than the values obtained from the cold extraction method (t_{49}=4.27, $p<0.01$ for herbs; t_9= 2.10, $p< 0.05$ for fruits). The mean difference (± standard error) between the two extraction methods (hot - cold) in total oxalate for the herb and fruit samples were 119.4 ± 32.5 and 1.6 ± 0.8 mg /100 g, respectively. However, with respect to extracting the soluble oxalate from both herb and fruit samples, there was no significant difference in mean oxalate concentrations between the two extraction methods. Regardless of the method of extraction, leaves of Atriplex halimus and kiwifruit exhibited the highest concentrations of both total and soluble oxalate among the herbs and the fruits, respectively.

Linear regression analysis of the total oxalate concentration values in herbs revealed a significant linear correlation ($r^2 = 0.97$, $p<0.0001$) between the two extraction methods (Figure 1). Back transformation indicated that for every 1 mg total oxalate/100 g increase using the cold extraction method, there was on average a 1.2 mg total oxalate/100 g increase using the hot extraction method. A similar correlation between the two methods occurred for the fruit samples ($r^2 = 0.98$, $p< 0.0001$), with an average 1.2 times more total oxalate obtained using the hot extraction method (Figure 2). Linear regression analysis of soluble oxalate in herbs also indicated a significant ($r^2 = 0.87$, $p<0.0001$) linear correlation between the two methods. In fruits, however, after excluding kiwifruit, which was considered to be an outlier because of its markedly higher oxalate concentration, the correlation between the two extraction methods was not significant ($r^2 = 0.25$, $p= 0.17$).

4. Discussion

The oxalate content of foods is of interest because consumption of a high oxalate diet may result in hyperoxaluria thereby increasing risk of kidney stones. Most very high oxalate-containing plants (more than 5% oxalate by dry weight) belong to the three families Amaranthaceae, Chenopodiaceae, and Polygonaceae (Siener et al. 2006). It is possible that a similar biosynthetic pathway and functional role for oxalate exists within one family or group of families that account for a specific range of oxalate levels (Libert & Franceschi, 1987). Among fifty herbs that were presently analyzed, Atriplex halimus contained the highest amount of both total and soluble oxalate, which exceeded 5% of dry weight when extracted by the hot extraction method. Atriplex halimus belongs to the Chenopodiaceae family; oxalate levels within this family vary from 1% to over 30% of the plant dry weight (Libert & Franceschi, 1987). Other important species of plants in the Chenopodiaceae family include beetroot, mangold, spinach, and quinoa. Eight tested herbs, Achillea millefolium, Artemisia abrotanum, Artemisia herba-alba, Calendula officinalis, Carthamus tinctorius, Chrysanthemum vulgare, Cichorium intybus and Matricaria chamomilla belong to the Asteraceae family, the largest family of flowering plants. In the present study, their total oxalate levels ranged from over 200 to 868 mg oxalate/100 g. The lowest oxalate levels were found in Trigonella foenum-graecum which belongs to the Fabaceae (Leguminosa) family and Citrullus colocynthis from the Cucurbitaceae family. One study reported that the total oxalate level of another plant, Trichosanthes cucumerina, of the Cucurbitaceae family, was 2.4–2.6 mg/100 g fresh weight (Adebooye & Oloyede, 2007). Because both Citrullus colocynthis and Trichosanthes cucumerina are fruits, it appears that fruit tissues from the Cucurbitaceae family of plants are relatively low in oxalate.

The distribution of oxalate within plants is uneven. Generally, oxalate contents are highest in the leaves, followed by the seeds; levels tend to be lowest in the stem (Noonan & Savage, 2002). Leaves are typically the part of the plant used to make herbs. In this study, most herbs that were high in oxalate (>1000 mg total oxalate/100 g) were made from dry leaf tissue including Atriplex halimus which had the highest total oxalate level among all the herbs tested.

In addition, high levels of oxalates are commonly observed in tropical plants like taro and sesame seeds (Savage & Mårtensson, 2010; Ishi & Takiyama, 1994). According to the present results, species of Atriplex halimus, Laurus nobilis and Sarothamnus scoparius were rich in total oxalate; these species grow in tropical or low latitude areas.

In contrast to the dietary intake of most plant products, the herbs analyzed in the present study are always prepared raw and consumed in the form of an infusion or tea. Soluble oxalates would be expected to reach out and dissolve in the boiling water. Thus, with the use of traditional herbs in this manner, it may be important to consider oxalate levels, particularly in individuals predisposed to CaOx stone formation.

Previous work suggested that most fruits contain only small quantities of oxalate (Zarembski & Hodgkinson, 1962b), while some such as kiwifruit and star fruit (Carambola) were reported to be moderately high (Wang et al., 2006; Rassam & Laing, 2005). Kiwi (Actinida chinesis) was reported to contain a range of 18-45 mg total oxalate/100 g fresh weight in different genotypes (Rassam & Laing, 2005). The present study yielded 37.4 mg total oxalate and 6.6 mg soluble oxalate per 100 g in kiwifruits when using the hot extraction method, while the cold extraction method yielded 30.6 mg total oxalate and 6.5 mg soluble oxalate per 100 g.

Oranges, bananas, peaches and pears have been previously reported to contain within the range of 2-10 mg total oxalate/100 g fresh weight (Hönow & Hesse, 2002) which are consistent with the presently reported values. Apples and and grapes were previously reported to be low oxalate fruits with total oxalate levels between 0-2 mg/100 g (Holmes & Kennedy, 2000; Hönow & Hesse, 2002). In the present study, the two extraction methods yielded a range of 1.3-2.6 mg total oxalate/100 g for apple and 1.5-2.8 mg total oxalate/100 g for grape.

Earlier studies are inconsistent with regard to the reported oxalate level of strawberries with total oxalate levels ranging from 2.9 mg/100g (Hönow & Hesse, 2002) to 23.4 mg/100g (Ogawa, Takahashi & Kitagawa, 1984). In the present study, strawberries were reported to contain 6.0 mg and 2.5 mg of total and soluble oxalate/100g, respectively. The variation in oxalate values in different sources of plants can be affected by factors such as soil quality, climate or different state of fruit ripeness (Libert & Franceschi, 1987). In addition, discrepancies could also be due to differences in preparation of the samples and analytical techniques.

Controversy remains with respect to the ideal temperature for oxalate extraction. Room temperature could cause erroneously low values due to an incomplete extraction while high temperature could lead to high values due to the possible in vitro generation of oxalate from various food constituents such as ascorbate and other precursors. Therefore, although hot acid and water methods ensure a complete extraction of oxalate, there is a possibility that certain compounds contained in food could be converted to oxalate.

Ascorbate can be converted to oxalate non-enzymatically. Therefore, the presence of ascorbate is one of the major factors that can affect the measurement of oxalate. Chalmers, Cowley & McWhinney (1985) reported that, in urine samples, ascorbate is most stable at about pH 4.5-5.0, while there was a considerable conversion of ascorbate to oxalate at pH 7.0. In addition to pH, heating temperature and time may also be important factors.

In the present study, comparison of the two extraction methods indicated that the hot acid extraction yielded significantly more total oxalate than cold acid extraction for both herb and fruit samples. There are two possible reasons for the elevation: a greater efficiency of hot acid in extracting oxalate and oxalate generation from precursors upon heating. Because ascorbate has been reported to be stable at pH < 5 and because the dried herbs and most of the analyzed fruits were low in ascorbate, it is unlikely that ascorbate conversion to oxalate would account for the elevated oxalate values. In addition, the fruit samples highest in ascorbate (orange, strawberries, and kiwifruit) were not characterized by a relatively greater elevation in both total and soluble oxalate levels when comparing the hot and cold extraction methods, which should have occurred if there had been significant in vitro conversion from ascorbate.

The possibility that oxalate was generated from other precursors when using the hot acid and water extraction method should also be acknowledged. However, the consistently observed increase in total oxalate with the hot acid method (i.e., an average of 1.2 times more oxalate for both herb and fruit samples) suggested that the discrepancy between the two methods is most likely due to a higher efficiency of oxalate extraction with heating. This assertion is further supported by the finding of greater total oxalate levels with the use of hot compared to cold acid in conjunction with no difference in soluble oxalate levels between the hot and cold water extractions, which would be predicted if there was a more efficient dissolution of insoluble oxalate crystals, such as calcium oxalate, with hot acid.

In conclusion, the overall data suggested that the use of the hot acid method will yield a more accurate assessment of the total oxalate content of foods while either method can be used for the assessment of soluble oxalate.

References

Adebooye, O. C., & Oloyede, F. M. (2007). Effect of phosphorus on the fruit yield and food value of two landraces of Trichosanthes cucumerina L. – Cucurbitaceae. *Food Chemistry, 100*(3), 1259-1264.

http://dx.doi.org/10.1016/j.foodchem.2005.10.002

Chalmers, A. H., Cowley, D. M., & McWhinney, B. C. (1985). Stability of ascorbate in urine: relevance to analyses for ascorbate and oxalate. *Clinical Chemistry, 31*(10), 1703-1705.

Holmes, R. P., & Kennedy, M. (2000). Estimation of the oxalate content of foods and daily oxalate intake. *Kidney International, 57*, 1662-1667. http://dx.doi.org/10.1046/j.1523-1755.2000.00010.x

Hönow, R., & Hesse, A. (2002). Comparison of extraction methods for the determination of soluble and total oxalate in foods by HPLC-enzyme-reactor. *Food Chemistry, 78*(4), 511-521. http://dx.doi.org/10.1016/S0308-8146(02)00212-1

Ishi, Y., & Takiyama, K. (1994). Extraction of calcium, oxalate and calcium oxalate crystals from sesame seeds (in Japanese with English abstract). *Bunseki Kagaku, 43*(2), 151-155.

Libert, B., & Franceschi, V. R. (1987). Oxalate in crop plants. *Journal of Agricultural and Food Chemistry, 35*(6), 926–938. http://dx.doi.org/10.1021/jf00078a019

Liebman, M., & Al-Wahsh, I. A. (2011). Probiotics and other key determinants of dietary oxalate absorption. *Advances in Nutrition, 2*, 254-260. http://dx.doi.org/10.3945/an.111.000414

Massey, L. K. (2007). Food oxalate: Factors affecting measurement, biological variation, and bioavailability. *Journal of the American Dietetic Association, 107*(7), 1191-1194. http://dx.doi.org/10.1016/j.jada.2007.04.007

Noonan, S. C., & Savage, G. P. (2002). Oxalate content of foods and its effect on humans. *Asia Pacific Journal of Clinical Nutrition, 8*(1), 64-74. http://dx.doi.org/10.1046/j.1440-6047.1999.00038.x

Ohkawa, H. (1985). Gas chromatographic determination of oxalic acid in foods. *Journal of the Association of Official Analytical Chemists, 68*(1), 108-111.

Ogawa, Y., Takahashi, S., & Kitagawa, R. (1984). Oxalate content in common Japanese foods. *Acta Urologica Japonica, 30*, 305–310.

Rassam, M., & Laing, W. (2005). Variation in ascorbic acid and oxalate levels in the fruit of Actinidia Chinensis tissues and genotypes. *Journal of Agricultural and Food Chemistry, 53*(6), 2322-2326. http://dx.doi.org/10.1021/jf048197s

Robertson, W. G., & Peacock, M. (1980). The cause of idiopathic calcium stone disease: Hypercalciuria or hyperoxaluria? *Nephron, 26*(3), 105-110. http://dx.doi.org/10.1159/000181963

Robertson, W. G., Peacock, M., Heyburn, P. J., Marshall, D. H., & Clark, P. B. (1978). Risk factors in calcium stone disease of the urinary tract. *British Journal of Urology, 50*(7), 449-454. http://dx.doi.org/10.1111/j.1464-410X.1978.tb06189.x

Savage, G. P., & Mårtensson, L. (2010). Comparison of the estimates of the oxalate content of taro leaves and corms and a selection of Indian vegetables following hot water, hot acid and in vitro extraction methods. *Journal of Food Composition and Analysis, 23*(1), 113–117. http://dx.doi.org/10.1016/j.jfca.2009.07.001

Siener, R., Hönow, R., Seidler, A., Voss, R., & Hesse, A. (2006). Oxalate contents of species of the Polygonaceae, Amaranthaceae and Chenopodiaceae families. *Food Chemistry, 98*(2), 220-224. http://dx.doi.org/10.1016/j.foodchem.2005.05.059

Wang, Y. C. L., Liu, B. M., Supernaw, R. B., Lu, Y. H., & Lee, P. Y. (2006). Management of star fruit–induced neurotoxicity and seizures in a patient with chronic renal failure. *Pharmacotherapy, 26*(1), 143-146. http://dx.doi.org/10.1592/phco.2006.26.1.143Refine or Alter Search

Zarembski, P. M., & Hodgkinson, A. (1962a). The determination of oxalic acid in food. *Analyst, 87*, 698-702. http://dx.doi.org/10.1039/AN9628700698

Zarembski, P. M., & Hodgkinson, A. (1962b). The oxalic acid content of English diets. *British Journal of Nutrition, 16*(1), 627-634. http://dx.doi.org/10.1079/BJN19620061

Table 1. Mean (± standard error) of total and soluble oxalate concentrations in 50 herbs (mg/100 g dry weight) using two extraction methods (means of n = 2)

Herb	Family	Parts analyzed	Total oxalate		Soluble oxalate	
			Hot extraction	Cold extraction	Hot extraction	Cold extraction
Achillea millefolium	Asteraceae	Flowers and stems	533 ± 13	466 ± 4	129 ± 8	101 ± 8
Alchemilla vulgaris	Rosaceae	Leaves	1104 ± 1	1050 ± 13	100 ± 3	72 ± 0
Althaea rosea	Malvaceae	Leaves and flowers	1798 ± 20	1653 ± 60	112 ± 6	114 ± 7
Angelica officinalis	Apiaceae	Aerial parts	535 ± 12	485 ± 18	140 ± 10	102 ± 10
Arctostaphylos uva-ursi	Ericaceae	Leaves	2269 ± 11	2162 ± 6	60 ± 2	55 ± 5
Artemisia abrotanum	Asteraceae	Aerial parts	530 ± 18	488 ± 30	309 ± 32	18 ± 2
Artemisia herba-alba	Asteraceae	Aerial parts	868 ± 15	700 ± 40	107 ± 4	127 ± 4
Atriplex halimus	Chenopodiacea	Leaves	5311 ± 50	4452 ± 177	2293± 48	2298 ± 219
Calendula officinalis	Asteraceae	Flowers	502 ± 4	408 ± 9	131 ± 1	144 ± 1
Capparis spinosa	Capparaceae	Leaves	190 ± 16	209 ± 8	91 ± 5	97 ± 2
Capsella bursa pastoris	Brassicaceae	Aerial parts	353 ± 3	291 ± 2	131 ± 1	124 ± 1
Carthamus tinctorius	Asteraceae	Flowers	392 ± 1	370 ± 29	63 ± 1	112 ± 3
Cassia senna	Caesalpiniaceae	Leaves	1898 ± 29	1727 ± 9	120 ± 9	123 ± 1
Cetraria islandica	Parmeliaceae	Leaves	584 ± 15	470 ± 45	83 ± 1	215 ± 23
Chrysanthemum vulgare	Asteraceae	Flowers	562 ± 1	495 ± 9	81 ± 5	81 ± 8
Cichorium intybus	Asteraceae	Leaves	298 ± 7	236 ± 11	109 ± 1	73 ± 4
Citrullus colocynthis	Cucurbitaceae	Fruits and seeds	48 ± 1	37 ± 1	32 ± 1	34 ± 1
Crataegus oxyacantha	Rosaceae	Leaves and fruits	1843 ± 77	1939 ± 151	114 ± 9	267 ± 26
Cuminum cyminum	Apiaceae	Seeds	714 ± 19	639 ± 21	140 ± 4	120 ± 11
Cuscuta epithymum Mur	Cuscutaceae	Aerial parts	273 ± 25	240 ± 6	130 ± 12	136 ± 6
Erythraea centaurium	Gentianaceae	Aerial parts	193 ± 14	111 ± 4	78 ± 1	5 ± 1
Foeniculum vulgare	Apiaceae	Seeds	1086 ± 63	935 ± 24	194 ± 5	263 ± 4
Galium aparine	Rubiaceae	Aerial parts	1031 ± 25	825 ± 9	461 ± 9	322 ± 11
Humulus lupulus	Cannabaceae	Leaves	2292 ± 47	2568 ± 20	110 ± 2	256 ± 10
Hypericum perforatum	Clusiaceae	Flowers	187 ± 4	188 ± 6	82 ± 6	62 ± 3
Hyssopus officinalis	Lamiaceae	Aerial parts	201 ± 20	279 ± 2	104 ± 5	144 ± 4
Juniperus communis	Cupressaceae	Aerial parts	4493 ± 74	3592 ± 191	63 ± 5	195 ± 2
Laurus nobilis	Lauraceae	Leaves	1972 ± 15	1710 ± 11	168 ± 1	154 ± 6
Lavandula officinalis	Lamiaceae	Flowers	1078 ± 2	971 ± 5	310 ± 0	241 ± 19
Matricaria chamomilla	Asteraceae	Aerial parts	202 ± 4	152 ± 2	85 ± 1	76 ± 1
Melilotus officinalis	Fabaceae	Aerial parts	514 ± 29	795 ± 10	107 ± 2	93 ± 0
Melissa officinalis	Lamiaceae	Leaves	692 ± 27	676 ± 11	132 ± 7	101 ± 9
Myrtus communis	Myrtaceae	Leaves	1574 ± 13	1440 ± 35	176 ± 11	136 ± 4
Origanum vulgare	Lamiaceae	Leaves	442 ± 2	307 ± 28	137 ± 5	126 ± 12
Ortie urticadiocia	Urticaceae	Leaves	545 ± 19	864 ± 5	176 ± 1	33 ± 2
Paronychia argentea	Caryophyllaceae	Aerial parts	2664 ± 122	1858 ± 49	367 ± 16	278 ± 20
Peganum harmala	Zygophyllaceae	Seeds	1921 ± 39	1547 ± 7	1452 ± 12	1510 ± 83
Pimpinella anisum	Apiaceae	Seeds	1011 ± 7	920 ± 16	273 ± 11	505 ± 36
Plantago lanceolata	Plantaginaceae	Aerial parts	367 ± 20	446 ± 17	100 ± 0	117 ± 6
Primevere officinalis	Primulaceae	Leaves	2254 ± 54	2135 ± 18	209 ± 4	188 ± 16
Rosa canina	Rosaceae	Flowers	1100 ± 33	820 ± 23	115 ± 6	99 ± 5
Rosmarinus officinalis	Lamiaceae	Aerial part	403 ± 11	291 ± 4	101 ± 4	82 ± 4
Ruta graveolens	Rutaceae	Leaves	1908 ± 1	1661 ± 45	78 ± 3	127 ± 6
Salvia triloba	Lamiaceae	Leaves	798 ± 16	694 ± 8	163 ± 8	150 ± 6
Sarothamnus scoparius	Fabaceae	Aerial parts	1627 ± 16	1351 ± 55	72 ± 6	57 ± 5
Teucrium polium	Lamiaceae	Aerial part	1132 ± 10	1068 ± 6	93 ± 2	183 ± 18
Thymus serpyllum	Lamiaceae	Leaves	394 ± 27	327 ± 14	162 ± 11	147 ± 13
Tilia playtphyllos	Tiliaceae	Leaves	1783 ± 21	1512 ± 14	510 ± 46	440 ± 37
Trigonella foenum-graecum	Fabaceae	Seeds	42 ± 2	36 ± 3	18 ± 2	27 ± 1
Viscum album	Viscaceae	Aerial part	355 ± 11	302 ± 16	45 ± 4	54 ± 4

Table 2. Mean (± standard error) of the total and soluble oxalate contents in 10 fruits (mg/100 g fresh weight) using two extraction methods (means of n = 2)

Fruit	Brand	Total oxalate		Soluble oxalate	
		Hot extraction	Cold extraction	Hot extraction	Cold extraction
Strawberry	Dole Strawberry Fraises	6.0 ± 1.8	6.1 ± 0.0	2.5 ± 0.1	2.5 ± 0.1
Pears	Asia Pear	3.3 ± 0.4	3.5 ± 0.4	2.3 ± 1.0	3.3 ± 0.6
Banana	Del Monte	6.1 ± 1.5	2.8 ± 0.1	2.5 ± 0.3	1.4 ± 0.6
Apple	Red Delicious	2.6 ± 0.3	1.3 ± 0.2	2.2 ± 0.2	1.1 ± 0.0
Grape	PLU Raisins Rouge Sans Pepius	1.5 ± 0.1	2.8 ± 0.1	1.1 ± 0.4	1.7 ± 0.5
Kiwifruit	Zespri green	37.4 ± 2.5	30.6 ± 0.3	6.6 ± 0.2	6.5 ± 1.3
Peach	Tree Ripe Chile	1.8 ± 0.5	2.0 ± 0.4	0.3 ± 0.3	0.1 ± 0.1
Blueberry	Gourmet Trading Company Argentina	5.4 ± 0.6	4.4 ± 0.3	3.7 ± 0.5	1.4 ± 0.1
Orange	Sunkist Satsuma Mandarins	10.2 ± 0.2	6.0 ± 0.6	2.9 ± 0.3	3.2 ± 0.0
Cantaloupe	Unknown	5.4 ± 1.0	3.8 ± 0.1	3.5 ± 0.2	2.3 ± 0.0

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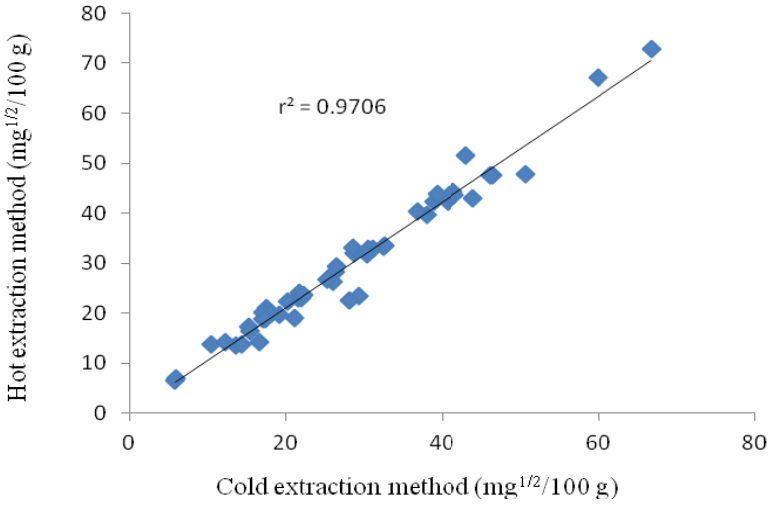

Figure 1. Regression analysis of the total oxalate values of herbs by using hot extraction against cold extraction after square root transformation ($\sqrt{x}$)

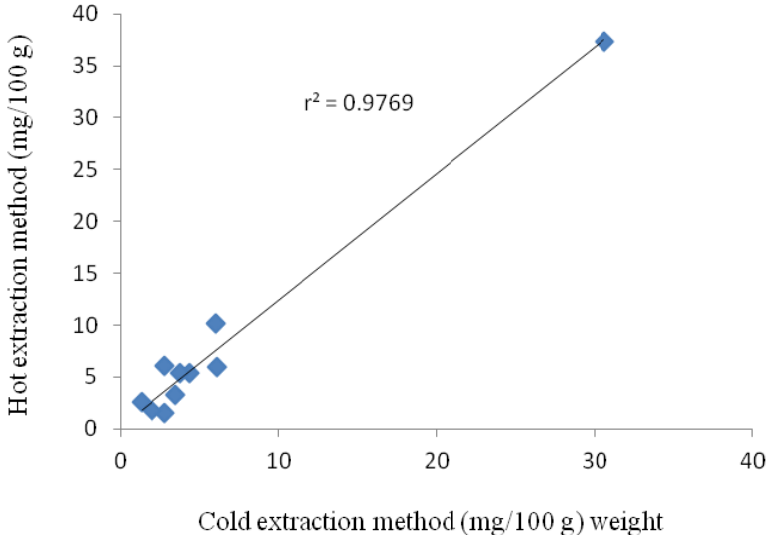

Figure 2. Regression analysis of the total oxalate values of fruits by using hot extraction against cold extraction

Changing Agriculture and Vegetable Supply in China and Analysis the Drivers for Change

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Abstract

Chinese food is famous throughout the world. The China vegetable industry is a dynamic, growing horticultural sector whose products appeal to consumers. The fruit and vegetable market is the largest sector of the retail food market in China, yet barely affected by the development of new retail formats. Fruit and vegetable sales tend to be dominated by markets where local farmers supply produce direct from the field to the end consumer. A major driver for continual change in agric food business, to secure greater efficiencies, profitability and service, is the aim for much better integration in the food chain.

The aim of 'supply chain management' is to better integrate business planning, and to effectively balance supply and demand across the entire chain. It tries to bring suppliers and customers more effectively together in one coherent and integrated business process (Alexander, 2003).

In this paper, the opportunity is taken to examine some of the key drivers, in particular the importance of changing consumer lifestyles and behaviour, and their influences on food purchase. The significance of a better-integrated food chain is explored, as are proposals for successful future development of agric food business, in China. In this dissertation, the author used face-to-face interview and group interviews to get primary information.

Keywords: Changing agriculture, Vegetable supply chain, Drivers for change

1. Changing Agriculture

Chinese transition from planned economy to market economy began with the rural reform 26 years ago, which practiced the household contract responsibility system that replaced the collective production team system. From 1978 to 1984, the annual increase in agricultural output was up to 7.7%, and farmers' income obtained a rapid annual increase of 13.4%. These achievements provided confidence for the second-period market-oriented reform of urban industrial enterprises. According to statistics from National Bureau of Statistics and World Bank, in the past 26 years, Chinese economic growth rate was 9.3%, and in terms of comparable price, Chinese GDP in 2002 was 8.5 times higher than that in 1978 when the reform and opening just began. China is a country with the most rapid economic growth and a rare case of lasting rapid economic growth in the world. However, in the recent years, Chinese three-dimensional rural issues concerning agriculture, countryside and farmers gradually became more conspicuous, which aroused the full recognition in and outside the country. The 16th National Congress of the Communist Party of China held last year proposed the goal to roundly construct a well off society by the time of 2020. The consensus among people of all walks of life is that there is no national well off society.

At the beginning of this year, the Central Committee of the Communist Party of China held a Central Conference on Rural Work and mentioned that rural work is part and parcel of the Party's task. Rural issues are the toughest unsolved problem in present China.

In general discussions, agricultural issue is mentioned in the same breath with issues of countryside and farmers, however it is not so important. In the past 26 years, the growth rate in agricultural production is 6% per year. Even from the end of 1990s to the beginning of 21 century (1997-2002), at the time that people considered the three-dimensional issues (agriculture, countryside and farmers) were grave; the growth rate in Chinese agricultural production per year was still up to 5.7% averagely. Generally speaking, it is a great achievement that the growth in agricultural production is one percent higher than population growth. Such a growth rate is quite admirable in both domestic and foreign agricultural development history. The issue of countryside implies

farmers' burdens and the aggravation of urban and rural income gap, which is due to the slowness in farmers' income rise (Beers, Beulens & van Dalan, 2000). Therefore, as to three-dimensional rural issues, especially a series of problems occurring since the 1990s, the key issue is the slowness in farmers' income rise. The solution of three-dimensional rural issues will be focused on sustained rise in farmers' income in the long term.

Before China entering WTO (World Trade Organisation), there were a lot of discussions in and outside China on how WTO will influence Chinese agriculture and farmers (Lin Yufu, 2003). Some viewpoints were quite pessimistic, considering that WTO would greatly impact Chinese farmers' income rise and even cause a tragedy. Because at the time that China passed the bilateral agreement with the U.S., China asked the U.S. for granting permanent normal trade relations to China supplementary. When President Clinton submitted the relevant bill to the Congress, for this supplementary term needed the permission from the American Congress, he declared that in the Sino-American negotiation on agriculture, the U.S. obtained a great success. In terms of cold war thinking, the great success of one side of the negotiation means the deadly failure of the other. Hence, the U.S. propaganda deepened the pessimistic thoughts on Chinese agricultural condition after entering WTO. The argument of this opinion is as follows: a farm in China is only as large as one acre, but in the USA, Canada and Australia, it is generally 200-300 acre. Therefore, Chinese farmers can hardly compete against farmers in the USA, Canada or Australia. Apart from that, the whole development level of Chinese agriculture is very low and the technologies and degree of mechanization fall far behind developed countries. Those scholars accordingly considered that after entering WTO Chinese agriculture will be badly impacted, and there will be a lot of bankrupt farmers in countryside. The estimation seems to be proved by political propaganda. For instance, agricultural issue was one of the toughest questions when China negotiated with other WTO member countries for the WTO agreement (Cheng, 2000).

Nevertheless, there was optimism in the discussions before entering WTO. Especially those scholars expert on agricultural issues are relatively optimistic at large. Vegetable and processed production belongs to labor-intensive agricultural products.

China lacks comparative advantages in land-intensive agricultural products. If there are no trade barriers, with the increase of Chinese citizens' income, China will import more and more land-intensive food and other agricultural products. But after entering WTO, the trade relation is relatively normal, the export channel will be relatively smooth, and therefore, the export volume of Chinese labor-intensive agricultural products, like vegetable and fruit, will consequently increase, which will be good for the structural adjustment on Chinese agriculture and the increase of farmers' income.

China has become a WTO member. The conditions reflecting in these years basically conform to optimists' estimates.

The rapidly increasing agricultural exports are mainly the labor-intensive products with Chinese comparative advantages, such as vegetable, fruit and so on. Normally, after entering WTO, since China permits food import, the import volume should consequently increase. However, first, Chinese food production is overproduced in these several years so the import desire is weak. Second, the volume of global food production decreases on account of climate factors, and hence the international food price is higher than that in China, so China will not import food. Besides, the volume of corn exports increases.

Since China has formally been a WTO member, the policies to solve Chinese three-dimensional rural issues should be concerned under the WTO frame. As it mentioned above, the core of three-dimensional rural issues is the slowness in farmers' income rise. As for how to increase farmers' income, at present, the internal academic circle and the decision-making circle achieve consensus at large that the key to increasing farmers' income is as follows: agricultural population, 72.6% of total national population, is transferred from agriculture to non-agriculture, and from countryside to city; a part of rural labor is transferred into non-agricultural urban industries, while the remains need to set about adjusting the structure of agricultural products, from land-intensive food production with low added value to labor-intensive production of vegetable and fruit with high added value; the technologies of agricultural production continuously improve and the infrastructures constantly advance (Li & Yu, 2001).

What chances will we meet, or what conditions do we need, to implement those three aspects of Chinese rural development after entering WTO? First, after entering WTO, Chinese domestic market will further integrates with international market. Various tariff and non-tariff walls will be reduced or eliminated. Foreign products will more easily enter Chinese market, and in turn, other WTO member countries will eliminate some tariff and non-tariff walls against China. It means that Chinese economy will be well developed according to Chinese comparative advantages. In the present development period, Chinese advantages are the abundance, high quality

and low salary of labor. The labor-intensive products are Chinese comparative advantages, for which WTO provides a great chance to develop. The development of labor-intensive industries will offer plenty of employment. The surplus labor in rural areas will be quickly transferred from agriculture to non-agricultural industries. In this regard, WTO will be of great help to Chinese farmers' income rise and the solution of three-dimensional rural issues.

WTO is also helpful to improve agricultural technology. After entering WTO, various tangible and intangible technologies including seed and equipments more easily enter China. Chinese scientific research on food is internationally outstanding, but those agricultural products with comparative advantages in vegetable, fruit, livestock products and so on fall far behind the international level in food safety and organic food production. WTO will let Chinese farmers make good use of good international technologies, increase the competitive capacity of Chinese labor-intensive agricultural products, and raise farmers' income.

How does WTO influence the structural adjustment on Chinese agriculture? Chinese comparative advantages in agriculture are labor-intensive agricultural products, which will have a great development chance if there are no trade barriers after entering WTO. China has no comparative advantages in labor-intensive food production. If Chinese economy has an average growth rate of 7-8% per year in the next twenty or thirty years, China will have less and less comparative advantages in food production and need more and more food import. The challenge China confronts is how to smoothly export the agricultural products with comparative advantages and import more non-comparative advantage food with the economic growth in the future.

As to the above challenge, just entering WTO won't solve the problem, because developed countries create various policy barriers to agricultural product trade. China hopes to export more labor-intensive agricultural products, while the developed countries, such as the U.S., Canada, Australia and etc, hope to export land-intensive food to China (Wen Tiejun, 2003). However, the main barrier at present is the concern for food safety of all the countries. From the economic angle, as a rapid developing country, importing more non-comparative advantage food is good for the improvement of resource configuration, economic growth and even farmers' income rise. However, some large exporting countries often take food as their weapon to achieve their political goals, so all countries do not wish their food safety is controlled by other countries. For its survival, China cannot and is unwilling to import a lot of food. However, it will be a good time for the structural adjustment on Chinese agricultural industry and the rise in farmers' agricultural income after entering the WTO. (Source: Huasheng Bao website at http://www.hsm.com.cn/)

2. Drivers for Change

2.1 Major 'drivers for change' in the agric food sector

-Consumer tastes and behaviour

-Competition and production efficiency

-Advances in technology

-Institutional pressures and regulatory requirements

-Environmental considerations

-International and globalisation influences

-Policy influences

(Source: W.P.Davies Drivers for change in modern food supply)

2.2 These influences can themselves be multi-dimensional

On the demand side for example, 'consumer change' involves considerations of demographics and population structures, cultural and religious issues, economic status and disposable income, aspirations and lifestyle. In the same way the supply side will be influenced in many ways by advancing technology and new innovations in different parts of the food chain (David, 2000).

These factors can also interact in complex ways.

For example, consumer reactions to new technologies, environment issues and animal welfare concerns in the food chain can exert political pressures for changes in legislation, regulation and international trade (Baines& Davies 1998, Davies 1999).

3. Consumer Concerns

Consumer concerns in China have prompted higher quality standards, control procedures and information

requirements. Food products with positive health attributes are likely to find increasing markets with particular opportunities for new functional foods and so-called 'nutraceuticals' (Enrique's & Goldberg2000).

Retailers, acting as the gateway, are the funnel points for food supply, and they are consolidating and declining in numbers. This concentration of power, in dominating consumer relationships, as the 'food chain captains', seems likely to continue (Foreign Economic Bureau, 2000). The question remains of whether this concentration of power is good news for consumers and, as importantly, whether this high degree of domination is desirable for other contributors to the food chain for future production and processing enterprises.

Consumers seem to associate these issues with healthier lifestyles and longevity, including increasing public interest in, and access for recreation. Increasing restrictions on input-use, such as pesticides and fertilizers, and increasing requirements for environmental quality, in particular of water and soils, are changing approaches to fanning. There are significant limitations.

This new food culture based on increasing consumer consciousness, and concerns, which focus on health, nutrition, environmental concerns and more traditional approaches to farming, has boosted the market for organic and more 'natural' products in China.

4. Understanding the Changing Consumer

4.1 'Understanding the consumer' and 'working with the consumer'

'Understanding the consumer' and 'working with the consumer' have become essential elements of food chain studies. The key to more successful food supply hinges on gaining a much better understanding of consumer behavior, and what influences and informs choices about food.

Do we know what consumers want and how capable are we of providing the right food products?

Agricultural production is increasingly market led and needs to focus more and more on the needs of the marketplace to maximize profitability (Hutton, 2003). In developed markets the links and understanding between farmer producing foods and consumers have been historically.

Much better integration in the food chain is not only essential for better relationships with the end consumer ('the market') but also for seizing 'added value' opportunities. Shifting farm commodity production of low value to more value added products is dependent on a much better understanding of the marketplace, and an increased capability to better manage the entire food chain business.

Retailers are making increasing attempts, in particular, to understand the consumer and segment the market. What consumers want, their motivations, their changing lifestyles and individual circumstances are all considered very significant. How well are these changing requirements understood, however, in primary agricultural production? (W. P. Davies, 2003)

4.2 The changing lifestyles and circumstances in China

A number of changing lifestyles and circumstances are evident in social trends in China. In particular:

- An increasing proportion of working women
- More couples without children
- More single parents
- Increasing numbers of active/retired and elderly persons
- More men shopping
- More adventurous 'eating-out'
- Increasing numbers of microwaves and freezers
- Shorter food preparation times
- Increasing snacking and grazing habits, with fewer meal occasions at home
- Increasing interest in personal health and food safety
- Increasing interest in a more diverse diet and exotic foods
- Greater concerns for the environment and animal welfare issues
- Much more concern about good health and diet habit

4.3 What consumers consider in food supply

Food and eating are an integral part of over 4,000 years of Chinese culture. In a nation that has frequently suffered from starvation, a common greeting is "Have you eaten yet?" Even now, meals still hold a central place in Chinese society (Huang, 2003). Today's consumer exhibits a number of characteristics that need to be considered in relation to food supply.

- More demanding
- More fresh
- More convenient
- More healthy
- More safety
- More nutrition
- Better product quality

All of which need to be accommodated in food supply to meet diverse consumer needs (Malcolm 2002).

4.4 To meet changing market requirements

As a result a number of changes in food product development have taken place to meet changing market requirements in China. Including:

- A greater range of convenience foods
- More fully, or partly -prepared, food products
- More 'health-giving' products
- More sophistication in the marketplace
- More products for different sectors
- A greater range of product sizes and packaging types
- More value added products
- More label information

4.5 The lifestyle shopping requirements

In South-East Asia, the importance of the 20-39 year age group is being increasingly recognised in food retailing, and of the expanding middle classes with an increasing level of disposable household income (Glover 1999). Consumers are seeking many of the lifestyle shopping requirements such as:

- A need for much greater convenience
- More 'one stop shopping'
- More convenience foods (refrigerated and packaged)
- A wider range of foods and more choice
- More branded products
- A more healthy, hygienic and cleaner shopping environment
- Longer opening hours
- Easier access and more car parking.

It has to be recognized, increasingly. Some of these needs may seem far removed from the production process on farm, and even in some cases from later downstream processing and distribution, but the issues of transparency in the food chain and accountability now underpin quality assurance schemes.

References

Alexander, Repple. (2003). *The Market Mix* (August 03, 2003).

Beers, G., Beulens, A., & van Dalan, J. (2000). *Emerging Supply Chain Management Systems: Part 1.*

Cheng, G. Q. (2000). *China agriculture under WTO frame* in world agriculture.

Li, D., & Yu, W. J. (2001). *marketing* China Ren Min University publishing Company (chapter 8).

David, Drakakis-Smith. (2000), *Third World Cities* (2nd Ed.) (chapter 6).

Foreign Economic Bureau. (2000). *Supermarket Development in China: Implications for US-China Trade in Food Products.*

Hutton, D. (2003). "Food safety and the consumer in the EU". Jornada Internacional de Seguridad Alimentaria del Productor al Consumidor. Madrid.

Huang, J. K. (2003). *China Agriculture in the past and future*, the agricultural policy study center of China academy of sciences.

Chemical and Protein Quality of Soybean (*Glycine max)* and Tigernut (*Cyperus esculentus)* Based Weaning Food

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Abstract

Malnutrition and poverty poses a major challenge to low-income families in developing nations and the twin issues are very critical for a growing infant. Commercially processed weaning foods are expensive for these categories of families; hence the objective was to formulate and evaluate the functional, antinutritional and protein quality of composite weaning food based on soybean and tigernut flour. Soybean and Tigernut seeds were processed into flour and three weaning diets: STF_1 (Tigernut; 75%, Soybean 15%); STF_2 (Tigernut; 65%, soybean 25%) and STF_3 (Tigernut; 55%, Soybean 35%) with 10% full cream milk powder (FCM) addition were produced according to FAO/WHO/UNU recommendations. Commercial weaning food (CB) was used as control. Effect of tigernut flour addition on the functional, antinutritional and protein quality of the formulated blends were evaluated using standard methods. The protein quality was evaluated using rat assay. The functional properties of the samples were significantly ($P<0.15$) different from the commercial sample. STF_3 sample had significantly ($P<0.05$) lower swelling index (SI), Packed bulk density, (PBD), loose bulk density (LBD) and water absorption capacity (WAC) with values of 3.63±0.10, 0.53±0.01 g/cm, 0.32±0.03 g/cm and 187.00 ± 2.10 ml/100g compared to values of 6.14±0.22, 0.55 ± 0.01g/cm, 0.42±0.01g/cm and 374.00 ± 3.40 for commercial sample, respectively. Total oxalic, soluble oxalic acid, phytic acid and tannins values of the diets were significantly ($P<0.05$) higher than CB, and the lowest values were for STF_S. Protein quality indices of the samples showed significant ($P<0.05$) difference. The NPU, PER, NPR, TD and BV of STF_3 compared favorably with CB. STF_3 sample supported good growth for the growing rats. The results suggested that STF3 is nutritionally balanced & possessed good growth promoting quality for a growing infant and could be adopted at the house hold-level to curb infant malnutrition and death.

Keywords: Protein quality, Tigernut, Soybean, Weaning food, Functional properties, Antinutritional

1. Introduction

The weaning period is a crucial event in an infant's life due to the inability of the mother's milk to adequately meet nutrient needs (Cameron & Hofvander, 1983; WHO, 2003). Appropriate weaning food is imperative to partially replace the mother's milk during weaning. Nutritional status in children is most critical during the weaning stages when both macro and micronutrients are required in sufficient amount to maintain growth and development. Due to high level of poverty in developing nations like Nigeria, many families cannot afford commercial brands resulting in children being weaned on high starchy gruels, low in proteins and other nutrients. Protein energy malnutrition (PEM), and infant mortality and morbidity are consequently on the increase in these rural households due to inadequacy of nutrients for the infants' nourishment and growth. Consequently, the development of low cost high protein weaning food from underutilized readily available raw materials is a constant challenge for developing nation. Hence, there is growing awareness and concerns about the development of cheap balanced weaning foods from available underutilized crops. Tigernut (*Cyperus esculentus)* is an underutilized crop in the family of cyperaceae, which produce rhizomes from the base and tubers that are somewhat spherical. Tigernut tubers contain about 8% protein and has 20-30% tigernut oil, which is nourishing to the epidermis (Alobo & Ogbogo, 2007). The necessary essential minerals, calcium, magnesium and iron required for bones, tissue repairs, muscles and blood stream, as well as vitamin B, that assist in balancing the central nervous system is contained in tigernut tubers (Oladele & Aina, 2007). Chevallier (1996) also reported that tigernut tubers could be used for the treatment of flatulence, indigestion, diarrhea, dysentery and excessive thirst and contain higher essential amino acids than those proposed in the protein standard by the FAO/WHO

(1985) for satisfying adult needs (Bosch *et al*., 2005). The use of such readily available underexploited crop to complement with legumes such as soybeans in developing a simple household low cost weaning food hold promise for PEM and infant mortality alleviation. The aim of this study was to evaluate the chemical and protein quality of soybean seed and tigernut tubers composite based weaning food.

2. Materials and Method

Mature dried tigernut (Cyperus esculentus), soybean (Glycine max), and full cream milk powder (FCM) were bought from a local market in Calabar, Nigeria. Corn Starch, Sucrose, and casein were bought from a reputable chemical supplier in Calabar. A commercial weaning food brand used as control was purchased from a local superstore.

All the chemical reagent were of anaylical grade purchased from Aldrich (Germany).

2.1 Preparation of soybean and tigernut flour

Soybean seeds were sorted to remove extraneous materials and soaked overnight in portable tap water, drained and then cooked for 30 min with portable tap water twice its volume. The cooked beans were dehulled, washed thoroughly and then dried in an oven (Gallenkamp Plus 11) at 60 $^{\circ}$C to a constant weight. The sample was milled using a hammer mill (Christy & Norris Ltd., Chelmsford, England) to pass through a 60 μm sieve to obtain soybean flour (SF).

The method of Ade-Omowaye *et al*., (2008) was used in the production of tigernut flour. Tigernut tubers were sorted to remove damaged and other extraneous materials, then dried in a cabinet dryer at 60 $^{\circ}$C for 24 h, and milled to pass through 60 μm sieve to obtain tigernut flour (TF). The flours were packed and sealed separately in a high density polyethylene pouches and stored at -4 $^{\circ}$C until analyzed.

2.2 Diet formulation

Three diet formulations: STF_1, STF_2 and STF_3 in duplicates were prepared by mixing varying proportions of the flours with 10 percent full cream powered milk (FCM) as shown in Table 1.

2.3 Analysis

2.3.1 Functional property

The method of Leach *et al*. (1959) was applied to determine Swelling Index (SI). One gram of sample was washed into a beaker and rewashed (W_1). The sample was dispersed in 50 mL of distilled deonized water (ddw) using magnetic stirrer and then heated at temperatures of 40, 50, 60, 70, 80 and 90 ^{o}C for 10 min in a thermostate water bath (Technical and Technical, Texas, USA). The slurry was cooled to room temperature (25 ^{o}C) and then centrifuged at 2200 rpm for 15 min. The residue obtained after centrifugation with the water it retained was rewashed (w_2) and the SI calculated.

Water absorption capacity (WAC) was determined using Solsulki *et al*. (1976) method. Ten milliliter of ddw was added to 2 g sample contained in a 25 ml centrifuge tube and stirred for 30 seconds with a glass rod .The suspension was given a 10min rest interval while the particles adhering to the sides of the tubes were scrubbed down with a glass rod. The sample was stirred seven additional times with each period lasting for 20sec and 10 min rest period between each stirring. The tube was centrifuged (Bench top centrifuge, model: MSE England) at 5100 rpm for 25 min, after which the water was decanted. The percentage of absorbed water was calculated.

The method of Akpapunam & Markakis (1981) was used for the determination of pack bulk density (PBD) and loose bulk density (LBD). Sample was weighed into a 5 mL measuring cylinder (W_1). For PBD, it was gently taped to eliminate air spaces between the flour sample in the cylinder and the volume was noted as the volume of the sample used (W_2). The mass of the sample and the cylinder was recorded. PBD expressed in gram per cm was calculated as the differential in weight. For LBD, space was not eliminated by tapping but the sample contained in the cylinder was left to stand for 10 min before weighing.

2.3.2 Antinutritional factors

Hoff & Singleton (1977) method was used for Tannin determination. Briefly, 0.05 g ground defatted sample was suspended in 5 mL of EtOH in a test tube for 15 min, shaken for 2 min (Gallenkemp Flask Shaker) and centrifuged in a benchtop centrifuge for 5 min at 1500 rpm. Sample extract (1mL) was pipetted into a 100 mL volumetric flask containing 75ddw, then 5 mL of folin-Denis reagent and 10 mL sodium carbonate solution were added. The solution was made up to 100 mL with ddw, shaken properly and allowed to stand for 30 min, after which the absorbance was read in a spectrophotometer (Perking Elmer Lambda 3 B) at 760 nm. Tannin content was read from a standard curve of tannic acid. The combined methods of Makower (1970) and Wheeler & Ferrel

(1971) were used for phytic acid estimation. Briefly, the sample (0.2 g) was extracted four times for 40 min with 3% Trichloracetic acid (TCA) and then centrifuged for 30 min at 5000 rpm. Aliquot (10 mL) of the supernatant was precipitated with 4 mL $FeCl_3$ solution containing 0.2% $FeCl_3$ in 3% TCA. The solution was heated for 45 min in a water bath (Aston VII) at 100 °C and centrifuged at 5000 rpm for 15 min. The $Fe(OH)_3$ obtained was dissolved in 40 mL of hot 3.2 N $HN0_3$ and the Iron determined calorimetrically (Corning Calorimeter, 253). The absorbance of the solution was read in a spectrophotometer (Perking Elmer Lambda 3B) at 480 nm against a reagent blank for each set of sample. The Iron content was calculated from a standard curve. Phytate phosphorus was calculated from determination assuming 4:6 Iron: Phosphorus molecular ratio, while phytic acid contains 28:20% phosphorus.

The method described by Dye (1956) was used for the oxalate determination. Briefly, sample (10 g) was digested in a water bath (Aston VII) for 4 h at 50 °C with 190 mL ddw and 10 mL of 6 N HCl. The digest was centrifuged for 30 min and the filtrate diluted to 250 mL with ddw. Aliquot of 50 mL was taken and evaporated to 25 mL, and the brown precipitate filtered off and washed with hot distilled ammonia until the pink color of methyl red indicator changed to faint yellow. The solution was heated in a water bath to 90 °C, precipitated with 10 mL of 5% $CaCl_2$ solution, allowed to stand overnight and then centrifuged (Bench top centrifuge, MSE, England). The precipitate was washed with hot 25% H_2SO_4 diluted to 125 mL and warmed to 90 °C, then filtered with 0.05 N $KMnO_4$ and the total oxalate calculated. The same procedure was used for soluble oxalate except that 10 g sample was digested in a water bath with 200 mL ddw at 90 °C for 4 h.

2.3.3 Protein quality evaluation

The randomised design was used in the study. Thirty five wistar strain albino rats aged 28-35 days with average initial weight of 33-60 g obtained from the pharmacology Department, University of Calabar, Nigeria were divided into A, B, C, D, E and F. The rats were housed in individual metabolic wire mesh bottomed cages in a room at 25 ± 2 °C with facilities for fecal and spilled food collection. Each group of five replicates was fed a different experimental diet for 28 days and acclimatization for 3 days. Drinking water provided with nipple drinkers and the test diets supplied in troughs were fed to the rats ad libitum. The feed intake was measured individually twice daily and body weight at 3 days intervals. The fecal droppings of the rats were collected daily, dried at 85 °C to a constant weight and then ground into powder for fecal nitrogen determination. Urine samples were collected in sample bottles containing 0.1 N HCl to prevent loss of ammonia and stored in a freezer until analyzed for urinary nitrogen. Data on feed consumption and spilled food were collected by recording the feed measured out for each rat at the beginning and the quantity remaining after feeding. Gain or Loss in weights of the rats was also recorded. At the end of the feeding period, the rats were sacrificed with chloroform and dissected. The carcasses and alimentary tract contents were dried at 85 °C to a constant weight, ground and then stored for carcass nitrogen determination. Faecal and carcass nitrogen of the rats were determined by the Kjedhal method (AOAC, 1990). Protein Efficiency Ratio (PER) was obtained by the method of Bender & Doell (1957), while Net Protein Utilization (NPU), Biological Value (BV), True Digestibility (TD), Net Protein Retention (NPR) were calculated based on the method of Miller & Bender (1955).

2.4 Data analysis

Data generated were analyzed using SPSS Software Program for Analysis of Variance (ANOVA) at 5 percent confidence interval. Mean separation was done by LSD.

3. Results and Discussion

3.1 Functional properties

Table 2 shows the results of the functional properties of the formulated weaning Diets (FWD) and the control. There was a significant difference ($P<0.05$) in the Swelling Index (SI) values, which ranged from 3.65 ± 0.10 to 6.14 ± 0.22. The control (CB) sample had significantly higher ($P<0.05$) SI value of 6.14 ± 0.22, while the lowest value was 3.63 ± 0.10 was for STF_3. SI refers to the expansion accompanying spontaneous uptake of solvent. Increase in soybean addition produced a reduction in SI values of the FWD, while SI value decreased as the tigernut flour addition decreased. The effect could probably be due to lose association of amylase and amylopectin in the native granules of tigernut starch and weaker association forces maintaining the granules structures (Adebayo-Oyetoro *et al*., 2011). Among the FWD, STF_1 had the highest SI (4.77 ± 0.30), while the least was STF_3 (3.63 ± 0.10). Swelling causes changes in hydrodynamic properties of the food, thus impacting characteristics such as body, thickening and increase in viscosity to foods. This suggests that among these FWDs, STF_1 with the highest SI will produce a thick viscous gruel compared to STF_3 and STF_1, which may be due to higher carbohydrate content arising from increased level of tigernut flour, than the others with higher soybean substitution level. The SI is an important parameter since it determines the consistency of the diet. Flours with

high SI value indicates high water absorption capacity and will therefore hold large volume of water during cooking into gruels, to yield voluminous low energy and nutrient food (Cameroon & Hafvander, 1983). According to WHO (2003), appropriate complementary diet is one which produce a gruel or porridge that is neither too thick for the infant to consume nor so thin that energy and nutrient density are reduced. The FWD with lower SI which compared favorably with CB would therefore deliver more energy and nutrient to the infant. Thus, STF_3 with the lowest value (3.63 ± 0.10) may be more desirable for a complementary diet. Similar trend have been reported by earlier workers (Adebayo-Oyetora *et al*., 2011; Ikpeme -Emmanuel et al., 2009; Sanni *et al*., 1999; Omueti *et al*., 2009).

The water absorption capacity WAC of the samples were significantly different ($P<0.05$). The CB sample had the highest value of 374.00 ± 3.40 mL/100 g. The WAC values ranged from 187.00 ± 2.10 mL/100 g to 374.00 ± 3.40 mL/100 g. WAC is the ability of a product to associate with water under a condition where water is limiting (Omueti *et al.,* 2009). There was a progressive reduction in WAC values as the level of tigernut flour decreased. The ability of flour to absorb water was reported to have a significant correlation with its starch content (Mbofung *et al*., 2006). Among the FWD, STF_1 had the highest ($P<0.05$) WAC value of (220.00±2.00 mL/100 g), which may be due to exposure of more damaged starch, which are sites for water binding (Milan-Camilo *et al*., 2000). This could also be that STF_1 contained higher fiber and starch content, (which have been found to cause water binding) because of higher amount of tigernut flour (Ade-Omowage *et al.,* 2008) The significantly higher ($P<0.05$) WAC values of STF_1 and STF_2 compared to STF_3 may be due to higher tigernut flour content (75%) and 65%), respectively, which resulted in the presence of many exposed hydroxyl groups on the molecules, which were available for water binding (Nelson & Cox, 2000). Our result is in agreement with the reports of earlier workers (Adebayo Oyetora *et al*., 2011; Sanni *et al*., 1999; Ikpeme-Emmanuel *et al*., 2009; Omueti *et al*., 2009). Among the FWDs, STF_3 had the lowest WAC value (187.00 ± 2.10 mL/100 g) which is significant because lower WAC is desirable for making thinner gruels with high caloric density per unit volume. However, the highest value of WAC for CB (374.00 ± 3.40) could be attributable to higher protein content, as proteins are hydrophilic in nature and so absorb and bind more water (Otegbayo *et al*., 2000). The decreasing levels of both WAC and SI with decrease in tigernut flour and increase in soybean flour are a reflection of the level of damaged starch and hemicelluloses contained in these samples (Pomeranz & Moore, 1975). The Bulk Density (BD) is a reflection of the load the sample can carry if allowed to rest directly on one another. The lower the bulk density value, the higher the amount of flour particles that can stay together thereby increases the energy content derivable from such diets (Onimawo & Egbekun, 1998). The Pack Bulk Density (PBD) of the samples were significant ($P<0.05$) and the values ranged from 0.53 ± 0.01g/cm to 0.59 ± 0.01g/cm. STF_1 had the highest value of 0.59 ± 0.04 g/cm, while the STF_3 had the lowest value of 0.53 ± 0.02 g/cm. Among the FWDs, there was a decrease in the PBD with increase in soybean flour. Similar trend was also reported by Edima *et al*., (2005). The PBD value for STF_1 and STF_2 were significantly higher ($P < 0.05$) than CB, while the PBD value for STF_3 was lower than the other FWDs as well as CB, thereby making the blend suitable for the formulation of high nutrient dense weaning food (Desikachar, 1979). This is because high bulk limits the caloric and nutrient intake per feed per child and infants are sometimes unable to consume enough to satisfy their energy and nutrient requirements (Omueti *et al*., 2009). Apart from dietary bulk of the gruel or porridge made from the complementary diets, the bulk density is also important in the packaging requirement and material handling of the complimentary diet (Karuna *et al*., 1996). Earlier workers have reported bulk density values of 0.48-0.66 g/mL for raw and malted wheat flour (Magnesia & Wafflemix, 2007) and 0.57 to 0.64 g/mL for tigernut and wheat flour, which are comparable to the values obtained in this study. The Loose Bulk Density (LBD) represents the lowest attainable energy without compression. The LBD values ranged from 0.32 ± 0.03 g/cm to 0.42 ± 0.01 g/cm. The CB had the highest LBD value (0.42 ± 0.01 g/cm), while STF_3 had the least. The LBD is related to the bulk density, the higher the LBD, the higher the bulk density. This is because the LBD indicates the free space between the food when packed. According to Omueti *et al*., (2009), a large free space is undesirable in packaging of foods because it constitute a large oxygen reservoir. The difference between the loose and the bulk densities of FWDs was slight, indicating that the volume of the FWD in a package will not decrease excessively during storage or distribution.

3.2 Antinutritional factors

Table 3 shows the results of the antinutritional factors. The result showed that total and soluble oxalates values obtained for the FWD were significantly higher ($P<0.05$) than the CB. The total and soluble oxalate levels ranged from 18.75 ± 0.02 mg/100 g to 45.34 ± 0.20 mg/100 g and from 9.57 ± 0.20 mg/100 g to 22.27 ± 0.10 mg/100 g, respectively. The STF_3 had the lowest value for both total and soluble oxalates. There was a progressive reduction in the level of total and soluble oxalate of the FWD with increase in soybean flour

substitution and reduction in tigernut flour substitution level. The values were lower than a total oxalate of 366.6 mg/100 g and a soluble oxalate of 250 mg/100 g for a multimix diet and a total oxalate of 128.3 mg /100 g and soluble oxalate of 110 mg/100 g for the commercial brand reported by Okoro (1986). Oxalates in large amounts bind with calcium forming calcium oxalate, which is insoluble and not absorbed by the body. They are therefore considered poisonous at high concentration, but harmless when present in small amounts (Fox & Cameron, 1986). High oxalate level in food has been implicated as the cause of kidney stones because high level of oxalates correlates with increase in calcium absorption in the kidney (Chai & Liebman, 2004).

Tannin has the ability to form insoluble complexes with proteins thereby reducing digestibility of food proteins. The tannin values ranged from 0.06 ± 0.07 mg/100 g to 0.14 ± 0.11 mg/100 g. There was no significant difference ($P<0.05$) between STF_1 and STF_2 and between STF_3 and CB, though all the values were low. This suggests that the processing technique used in this study (soaking, dehulling, and drying) were effective in reducing the tannin contents of the samples. Our values were higher than the tannin values (0.40 to 0.70 mg/100 g) reported by Akaninwor & Okechukwu (2004) for sweet potato and soybean weaning food. Toxicity of tannins absorbed from the gut and interference with the absorption of Iron and a possible carcinogenic effect has been reported (Buttler, 1989). Tannins also inhibit the activities of trypsin, chymotrypsin, amylase and lipase (Griffiths & Mosley, 1980; Delumen & Salamat, 1980). Reduction of tannins during production improve nutritional value of food by increasing protein digestibility (Bassey, 2004) and hence the formulated diet with low tannins levels would have good digestibility.

The phytic acid level was significantly higher ($P<0.05$) in the formulated diets compared to the commercial brands. The levels ranged from 0.99 ± 0.30 mg/100 g to 1.96 ± 0.03 mg/100 g. STF_1 had the highest phytic acid level of 1.96 ± 0.03 mg/100 g, while the least value of 1.79 ± 0.04 mg/100 g was for STF_3 among the FWDs. Phytic acids are insoluble and cannot be absorbed in the human intestine. However the level of phytic acid found in the FWD was very low and would not be injurious to health. The values for all the antinutritional factors in the FWD were low and as such would not cause any harm to an infant.

3.3 Protein quality

Table 4 shows result of the protein quality of the samples. The rats fed with CB gained significantly ($P<0.05$) more weight than others fed on the FWD. Among the FWDs, STF_3 had significantly ($P<0.05$) higher weight gain (31.93 ± 6.45 g) compared to values of 20.57 ± 8.47 g and 25.93 ± 3.97 g, respectively for STF_1 and STF_2. Rats fed on the basal diet lost weight and their body weights was significantly lower ($P<0.05$) than rats fed on other diets. Ayalogu *et al*., (2003) reported similar findings. There was a progressive weight increase from STF_1 fed rats to STF_3 fed rats, which corresponded to increase in protein ingested from STF_3. This implies that STF_3 diet promoted growth better than other FWD. The observed high weight gain of rats fed on CB may be attributable to the addition of sucrose and vanillin to the product by the manufactures, which resulted in better palatability. Rats fed on casein diet had the lowest fecal nitrogen of 0.15 ± 0.01g ($P<0.05$). The FWDs had significantly lower fecal nitrogen than the CB diet. Fecal nitrogen affects digestibility consequently, rats fed on STF_3 had higher nitrogen intake, since it had lower fecal nitrogen value of 0.27 ± 0.02 g. The highest carcass nitrogen content was observed for rats fed on CB ($P<0.05$), followed by rats on STF_3, while the least value was for STF_1 ($P < 0.05$). This might be due to the relative proportion of protein in these diets. The FWD had lower urinary nitrogen ($P<0.05$) compared to the CB diet, which could be due to nutrient-nutrient interaction between soybean and tigernut, resulting in better digestibility with lower urinary nitrogen. NPU is the percentage of nitrogen intake retained as body nitrogen. The values ranged from 50.00 ± 0.10% to 77.25 ± 0.06%. The casein diet had the highest NPU ($P<0.05$), followed by CB diet. Among the FWDs, STF_3 had the highest value ($P<0.05$) of 60.44 ± 0.03%. The NPU values for STF_3 and CB were comparable. PER (Protein Efficiency Ratio) is the weight gained per gram protein consumed. The PER values ranged from 1.36 ± 0.02 to 2.83 ± 0.10. PER of CB diet was higher ($P<0.05$) compared to other samples. This could be that the higher percentage of milk protein was incorporated into the formulation of the CB diet by the manufactures. The FWD had significantly lower ($P<0.05$) PER values, though the STF_3 value (2.26 ± 0.04) was above the 2.1 minimum PER value recommended for such flours by Protein Advisory Group. NPR (Net Protein Ratio) provides information on the ability of proteins to support both maintenance and growth. The values ranged from 1.83 ± 0.03 to 3.47 ± 0.30. NPR value of rats fed on CB diet was significantly higher ($P<0.05$) than for rats fed on other diets. This was followed by NPR value for casein diet which compared favorably with NPR value of rats fed on STF_3 diet. The true disgestibility (TD) values gives information on the percentage of nitrogen intake absorbed by the body. The values ranged from 86.10 ± 0.50% to 98.80 ± 0.02%. TD values of rats fed on casein diet was significantly higher ($P<0.05$), followed by the TD value for the CB diet. The TD values of the FWDs were comparable; however, STF_3 diet had the highest ($P<0.05$) value. The gradual decrease in TD values of the rats fed on the FWDs could be due to higher amounts

of antinutrients from STF_3 to STF_1, which are known to reduce protein digestibility and consequently bioavailability. However, TD values of the FWDs were higher than the (85%) values recommended for children (PAG, 1971). Biological value (BV) gives information on how much of the absorbed nitrogen is actually retained or utilized by the body. BV values ranged from 67.29 ± 0.06% to 92.77 ± 0.01% ($P<0.05$). The BV of rats fed on casein diet was higher ($P<0.05$) than for rats fed on other diets. The BV value of rats fed on STF_3 diet was higher ($P<0.05$) than the other FWDs, while BV of CB was highest (90.06 ± 0.03%). This indicates that rats fed on CB had higher nitrogen retention than those on other diets (as was also reflected by high NPR value). This also suggested that the essential amino acids in the product were present in sufficient quantity to meet the needs for growth (Young & Bier, 1987). According to Codex Alimentarius standards, the nutritional composition of the foods indicates their suitability for young children. Our results are in agreements with reports of earlier workers (Ijarotimi & Ayantokun, 2006; Ijarotimi & Olopade, 2009; Gahlawat, 1992; Onweluzo & Nwabugwu, 2009; Essien *et al*., 2010).

4. Conclusion

The study revealed that STF_3 containing 55% tigernut, soybean 35% and milk 10% had good growth promoting quality and therefore would be suitable as a weaning diet because it supported growth in rat models. This would consequently add value to the under-utilized nutrient dense tigernut tubers and so would encourage biodiversification of food crops.

References

Adebayo-Oyetora, A. C., Olatidoye, O. P., Ogundipe, O. O., Balogun, O. I., & Faboya, A. O. (2011). Quality evaluation of weaning food produced from blend of Ofada rice (Orzae Sativa) & Bambara groundnut (Voandzeia subterranean). *Journal of Environmental Agriculture & Food Chemistry, 10*(6), 2322-2330.

Ade-Omowage, B. I. O., Akinwande, B. A., Bolorinwa, I. F., & Adebuyi, A. O. (2008). Evaluation of tigernut (cyperus esculentus)–wheat composite flour and bread. *African Journal of Food Science, 2*, 87-91.

Akaninwor, J. O., & Okechukwu, P. N. (2004). Comparative nutrient and anti-nutrient levels in commercial and formulated weaning mixtures: BIOKEMISTRY, *16*(1), 15-21.

Alobo, A. P., & Ogbogo, P. O. (2007). Selected properties of tigernut starch as affected by physical and chemical modifications. Proceeding of the 31st annual NIFST Conference, Abuja, Nigeria 22nd -25th October 2007.

Anderson, W., Smith, B. M., & Gustafon, N. J. (1994). Health benefits and practical aspects of high fibre diets. *American J. Clinical Nutri., 59*, 12425-12475.

AOAC. (1990). Official methods of Analysis. Association of Official Analytical Chemistry. Washington D. C., USA.

Bender, A. E., & Doell, B. H (1957). Biological Evaluation of proteins: A new Aspect. *British Journal of Nutrition, 11*,140-148. http://dx.doi.org/10.1079/BJN 19570029

Buttler, L. G. (1989). *Effects of Condensed tannin on animal nutrition: In Chemistry and significance of condensed Tannins*. R. W. Hemingway and J. J. Karchesy (Ed). New York: Planum Press. http://dx.doi.org/10.1007/978-1-4684-7511-1_24

Cameron, M., & Hofvander, Y. (1983). *Manual on feeding infants and youngs children*. Oxford: Oxford University Press.

Chai, W., & Liebman, M. (2004). Assessment of oxalate absorption from almonds and black beans with and without the use of extrinsic label. *J. Urol, 172*, 953-957. http://dx.doi.org/10.1097/01.ju.0000135918.00761.8a

Chevallier, A. (1996). *The Encyclopedia of Medicinal plants*. Dorling Kindersley. London.

Codex Alimentarius Commissions, Joint FAO/WHO Standard Program: (1976). Recommended International Standards for foods for infants & children Rome: FAO.

Delumen, B., & Salament, L. A. (1980). Trypsin inhibitor activity in winged bean (Psophocarpus tetragonolobu) and possible role of tannin. *Journal of Agric & Food Chemistry, 28*, 533-536. Htt://dx.doi.org/10.1021/jf60229a042

Desikachar, H. S. R. (1979). Development of weaning food of high caloric density and low hot paste viscosity using traditional technologies. *Food Nutrition Bulletin, 4* (9), 21-33.

Dye, W. B. (1956). Chemical Studies of Halogeton glumeratus. *Journal of Weeds, 4*, 55-60. http://dx.doi.org/10.2307/4040009

Edema, M. O., Sanni, L. O., & Sanni, A. I. (2005). Evaluation of maize-soybean flour blends for sour maize bread production in Nigeria. *African Journal of Biotechnology, 4* (9), 911-917.

Essien, E. B., Abbey, B. W., & Akaninwor, J. O. (2010). Nutritional evaluation of some traditional weaning foods from Akwa Ibom State, Nigeria. *Nigerian J. of Biochemistry & Molecular Biology, 25* (1), 65 – 72.

FAO/WHO. (1985). Energy and Protein requirement, Geneva Report of a joint FAO/WHO/UNI expert consultation. WHO Technical report series, No. 724.

Fox, B. A., & Cameron, A. G. (1986). *A Chemical approach to food and nutrition*. London, University of London Press Ltd, p. 102.

Gahlawat, P. (1992). Formulation and Nutritional value of home made weaning foods. *Nutritional Research, 12* (10), 1171-1180. http://dx.doi.org/10.1016/S0271-5317(05)80774-2

Griffiths, D. W., & Mosley, G. (1980). The effect of diets containing field beans of or low polyphenolic content on the activity of digestive enzymes in the intestine of rats. *Journal of Sciences Food and Agri., 31*, 225-259. http://dx.doi.org/10.1002/jsfa.2740310307

Hoff, J. E., & Singleton, F. (1977). A method for the determination of tannin. *Journal of Food Science, 42*, 1566. http://dx.doi.org/10.1111/j.1365-2621.1977.tb08427.x

Ijarotimi, O. S., & Ayantokun, O. A. (2006). Nutritional composition, sensory and biological evaluation of a potential weaning diet from low cost food materials (*Sorghum bi Colour* and *Cajanus Cajan*). *J. Food Tech, 4* (3),178-184.

Ijarotimi, O. S., & Olopade, A. J. (2009). Determination of amino acid content and protein quality of complementary food produced from locally available food materials in Ondo State, Nigeria. *Mal. J. Nutr, 15* (1), 87-95.

Ikpeme-Emmanuel, C. A., Okoi, J., & Osuchukwu, N. C. (2009). Functional, antinutritional and sensory acceptability of taro and soybean based weaning food. *African Journal of Food Sci, 3* (11), 372-377.

Karuna, D., Noel, G., & Dilip, K. (1996). Production and use of raw potato flour in Mauritanian traditional foods. *Food & Nutritional bulletin, 17* (2), 12-14.

Magnesia, M., & Wafflemix, W. S. (2007). Engineer resources Bulk density and specific gravity chart. Camber southeast. Inc (www.powderandbulk.com/resources/material _bulk_density_charts.htm) Accessed 16 Aug. 2011.

Makower, R. N. (1970). Extraction and determination of Phytic acid in bean (P. Vulgaria). *Cereal Chemistry, 47*, 288-295.

Mbofung, C. M. F., Abubakar, Y.N., Nyintang, A., Abduo, B., & Balaam, F. (2006). Physicochemical and functional properties of six varieties of taro (calocascia esculenta L. Schott) flour. *J. Food Technol, 4* (2), 135-14.

Milan-Camilo, J., Reyes-moreno, C., Armienta-Rodelo, A., Carabez-trejo, & Mora-Escobel, R. (2000). Physicochemical and nutritional characteristics of extruded flours from fresh and hardened chick peas. *Lebensm-wiss, 33*, 117-123. http:/dx.doi.org/10.1006/fstl.1999.0620

Murphy, M. G., Skonberg, D. J., & Camire, M. E. (2003). Chemical Composition and physical properties of extruded snacks containing crab-processing by-product and winged bean flour for food. *Food Science, 47*, 1534-1538.

Nelson, D. I., & Cox, M. M. (2000). *Lehninger – Principles of Biochemistry*. Replika press- PVT Ltd. India, pp. 117-124.

Okoro, E. U. (1986). Chemical and Biological Evaluation of the Nutritional value of a multimix weaning food formulated using local foodstuffs. M.Sc. Thesis, University of Calabar.

Oladele, A. K., & Aina, J. O. (2007). Chemical composition and functional properties of flour from two varieties of tigernut (Cyperus esculentus). *Afri J. Biotechnol, 6* (21), 2473-2476.

Omueti, O., Otegbayo, B., Jaiyeola, O., & Afolabi, O. (2009). Functional properties of complementary diets developed from soybean (Glycine max), groundnut (Arachis hypogeal) and crayfish (macrobrachium spp). *Journal of Environmental Agricultural & Food Chemistry, 8* (8), 563-573.

Onimawo, I. A., & Egbekun, M. k. (1998). *Comprehensive Food Science and Nutrition*. Benin City: Ambik Publishers. Nigeria.

Onwelugo, J. C., & Nwabugwu, C. C. (2009). Development and Evaluation of weaning foods from pigeon pea and millet. *Pakistan J of Nutri, 8* (6), 725 -730. http://dx.doi.org/10.3923/pjn.2009.725.730

Otegbayo, B. O., Sobande, F. O., & Aina, J. O. (2000). Effects of Soy-substitution on physicochemical qualities of extruded plantain Snack. *Tropical Oil Seeds Journal, 5*, 69-78.

Pomeranz, Y., & Moore, R. B. (1975). Reliability of several methods for protein determination in wheat. *Bakers Digest, 58*, 45-48.

Protein Advisory Group (PAG) Guideline. (1972). Guideline on Protein-Rich Mixture for use as weaning foods. New York: United Nations.

Sanni, A. I., Onilude, A. A., & Ibidapo, O. T., (1999). Physicochemical characteristics of weaning food formulated form different blends of cereal and soybean. *European Food Research & Technol, 208* (3), 221 – 224.

Sosulki, S., Garret, M. D., Slunkard, A. E. (1976). Functional Properties of ten legume flours. *Institute of Food Science and Technol J, 9*, 66-69.

Wheeler, E. L., & Ferrel, R. E. (1971). A method for phytic acid determination in wheat and wheat flour. *Cereal chemistry, 48*, 313-314.

WHO, (2003). Feeding and Nutrition of infants and young children. Guidelines for the WHO European region with emphasis on the former soviet union. *WHO Regional Publication European series. 87*, 1-296. .http://www.crossref.org/simpleTextQuery

Young, V. R., & Bier, D. M. (1987). Amino Acid Requirements in the Adult Human: How well Do we know them? *Journal of Nutrition, 117*, 1484-1488

Table 1. Percentage composition for formulation of tigernut and soybean weaning food

COMPOSITE	PERCENT DRY WEIGHT BASIS (% dwb)		
	TF	SF	FCM
STF_1	75	15	10
STF_2	65	25	10
STF_3	55	35	10

SF = Soybean Flour; Tigernut Flour = TF.

Table 2. Functional properties of soybean and tigernut based weaning food

PROPERTY	DIETS			
	STF_1	STF_2	STF_3	CB
Swelling Index (SI)	4.77 ± 0.30^{c}	4.03 ± 0.04^{b}	3.65 ± 0.10^{d}	6.14 ± 0.22^{a}
Packed Bulk Density (PBD) (g/cm)	0.59 ± 0.04^{d}	0.58 ± 0.01^{a}	0.53 ± 0.02^{c}	0.55 ± 0.01^{b}
Loose Bulk Density (LBD) (g/cm)	0.37 ± 0.02^{b}	0.34 ± 0.02^{c}	0.32 ± 0.03^{d}	0.42 ± 0.01^{a}
Water absorption Capacity (WAC) (ml/100g)	220.00 ± 2.00^{b}	200.00 ± 1.89^{c}	187.00 ± 2.10^{d}	374.00 ± 3.40^{a}

*Means of three determination. Values not followed by the same letter in the same row are significantly different ($P<0.05$). STF_1 (Tigernut 75%; Soybean 15%), STF_2: (Tigernut 65%, soybean: 25%), STF_3: (Tigernut 55%, Soybean 35%); CB: Commercial Brand (control).

Table 3. Antinutritional contents of soybean and tigernut based weaning food (mg/100 g)

Diets	Oxalic acid		Phytic acid	Tannins
	Total Oxalic acid	Soluble oxalic acid		
STF_1	45.34 ± 0.20[a]	22.13 ± 0.03[a]	1.96 ± 0.03[a]	0.14 ±0.11[a]
STF_2	43.25 ± 0.30[b]	15.25 ± 0.10[b]	1.88 ±0.02[b]	0.11 ± 0.10[b]
STF_3	37.25 ± 0.01[c]	11.73 ± 0.03[c]	1.79 ±0.04[c]	0.08 ±0.01[a]
CB	28.75 ± 0.02[d]	9.75 ± 0.20[d]	0.99 ±0.30[d]	0.06 ±0.02[a]

*Means of three determination; Values not followed by the same letter in the same row are significantly different ($P<0.05$). STF_1 (Tigernut 75%, Soybean 15%), STF_2: (Tigernut 65%, soybean 25%); STF_3: (Tigernut 55%, Soybean 35%); CB: Commercial Brand (control).

Table 4. Protein quality of soybean and tigernut based weaning foods

Parameter	DIETS					
	STF_1	STF_2	STF_3	CB	CD	BD
Weight gain (g)	20.57±8.47[d]	25.93±3.97[c]	31.93±6.45[b]	43.87±7.51a	30.87±7.51[b]	9.85±0.05[e]
Carcass Nitrogen (g)	1.42 ± 0.26[e]	1.53 ± 0.05[d]	1.89 ± 0.21[b]	2.30 ± 0.04[a]	1.76 ± 0.21[c]	0.21± 0.02[f]
Carcass Protein (g)	8.85 ± 0.26[e]	9.69 ± 0.31[d]	11.81±1.30[b]	14.40± 0.28[a]	11.00± 0.08[c]	1.33± 0.14[f]
Fecal Nitrogen (g)	0.47 ± 0.31[d]	0.38 ± 0.02[c]	0.27 ± 0.02[b]	0.53 ± 0.03[a]	0.15 ± 0.01[e]	0.01 ± 0[f]
Urinary Nitrogen (g)	0.08 ± 0.02[e]	0.18 ± 0.02[c]	0.23 ± 0.02[b]	0.40 ± 0.03[a]	0.04 ±0.10[f]	0.11±0.01[d]
Protien Intake (g)	14.07±0.23[d]	14.09±0.11[c]	22.87±0.01[a]	22.19 ± 09[b]	17.03±.47[d]	0.93±0.19*
Nitrogen Intake (g)	2.25 ± 0.03[d]	3.05 ± 0.02[c]	3.66 ± 0.02[a]	3.55 ± 0.02[b]	2.20 ± 0.07[d]	0.15± 0.03[e]
NPU (%)	50.00±0.10[d]	48.85±0.03[e]	60.44±0.03[c]	63.09± 0.10[b]	77.27± 0.06[a]	-
PER	1.40± 0.01[C]	1.36 ± 0.02[d]	2.26 ± 0.04[c]	2.83 ± 0.10[a]	2.46 ± 0.03[b]	-
NPR	1.83 ± 0.03[d]	1.87 ± 0.01[d]	2.16 ± 0.03[c]	2.85 ± 0.01[b]	3.47 ± 0.30[a]	-
TD (%)	86.10±0.50[d]	87.67±0.02[c]	88.14±0.30[c]	90.36± 0.01[b]	98.80± 0.02[a]	-
BV (%)	67.29±.006[d]	65.72±0.04[e]	80.54±0.05[c]	90.06± 0.03[b]	92.77± 0.01[a]	-

*Means of three determinations, values not followed by the same letter in the same row are significantly different ($P<0.05$) STF_1 (tigernut 75; soybean 15%); STF_2 (tigernut flour 65%; soybean 25%), STF_3 (tigernut 55%; soybean 35%), CB: Commercial brand; BD: Basal diet (non-protein diet), CD:Casein diet.

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Fiber intake Inconsistently Alters Gut Hormone Levels in Humans Following Acute or Chronic Intake

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Abstract

Diet composition affects the release of gut hormones involved in the regulation of appetite and energy intake. While some research suggests high fiber foods cause greater satiety than low fiber foods, few studies have measured gut hormone levels as a mechanism by which fiber may influence appetite. A review of the literature was conducted to better understand the effect of fiber on gut hormone concentrations in humans, which specific focus on peptide YY, glucagon-like peptide-1, cholecystokinin, and ghrelin. Considerable variation was found in study design, population, fiber type and level. Few studies reported a significant effect of fiber on gut hormone levels, and data suggest caloric load may have a more significant influence on gut hormone release. While it is possible that circulating gut hormones are not the mechanism by which fibers influence satiety, it is also possible that variability in study design prevents definitive conclusions around this relationship.

Keywords: Dietary fiber, Peptides, Gut hormones, Cholecystokinin, Ghrelin, Glucagon-like peptide-1, Peptide YY

1. Introduction

A variety of peptides are released from the gastrointestinal (GI) tract in response to the nutritional state. These gut hormones are considered to be important factors in the control of appetite and satiety. The strength and timing of postprandial gut hormone release is clearly influenced by macronutrient distribution and total meal composition. Certain macronutrients are thought to be more satiating due to their ability to influence gut hormones; however, the impact of fiber on this relationship is not clearly understood. While some research suggests high fiber foods result in greater satiety than low fiber foods, few studies have measured circulating gut hormone response after fiber intake in humans. Therefore, a review of the literature was conducted to better understand fibers' impact on gut hormone concentrations in the blood. Although many peptides and hormones are released from cells in the GI tract and may influence satiety (e.g. oxyntomodulin, pancreatic polypeptide, glucose-dependent insulinotropic polypeptide, leptin, adiponectin, enterostatin, glucagon, insulin, amylin), only

four – peptide YY (PYY), glucagon-like peptide-1 (GLP-1), cholecystokinin (CCK), and ghrelin – were chosen for this review due to their relatively well established effects on appetite.

1.1 Peptide YY

PYY is a 36-amino-acid polypeptide synthesized and secreted from the L-cells of the terminal ileum (Batterham *et al*., 2007). Upon release, the molecule undergoes cleavage by the enzyme dipeptidyl peptidase IV (DPP-IV) to yield a truncated peptide, PYY3-36, which is the predominant circulating form in the fed and fasted state(Sloth, Davidsen, Holst, Flint, & Astrup, 2007). PYY3-36 binds with high affinity to the Y2 receptor, located throughout the central nervous system (CNS) and vagal afferents (Neary *et al*., 2005). PYY is thought to inhibit appetite by acting centrally on homeostatic centers in the hypothalamus to reduce expression of neuropeptide Y (NPY), an orexigenic peptide. Neural reflexes are also important, since PYY concentrations increase before nutrients reach the site of PYY release and vagotomy abolishes the appetite suppressing effect of PYY (Abbott *et al*., 2005). PYY also activates the ileal brake, which slows gastric emptying and nutrient absorption, and may extend feelings of satiety (Maljaars, Peters, Mela & Masclee, 2008).

Plasma PYY levels rise within 15 minutes of a meal, and peak approximately an hour after nutrient ingestion (Adrian *et al.*, 1985). The magnitude of PYY release depends on both the caloric load and macronutrient content of the meal. When balanced for total energy, meals high in fat and protein appear to increase PYY more than carbohydrate-rich meals (Batterham *et al*., 2006; Essah, Levy, Sistrun, Kelly & Nestler, 2007; MacIntosh *et al*., 1999). Intravenous infusion of PYY has been shown to significantly increase satiety and reduce energy intake in humans (Batterham *et al*., 2002; Batterham *et al*., 2003; Batterham *et al*., 2007; Degen *et al*., 2005). However, many studies use pharmacological doses which can lead to side effects such as nausea and vomiting, which may interfere with appetite ratings. Researchers have reported lower fasting and postprandial PYY concentrations in obese participants compared to lean individuals, and this is reversed following bariatric surgery (Batterham *et al*., 2003; le Roux *et al*., 2006). This suggests PYY may play an important role in energy homeostasis, and has led to interest in PYY as a potential antiobesity therapeutic agent.

1.2 Glucagon-like peptide-1

GLP-1 is formed from the cleavage of proglucagon, and is released primarily from the L-cells of the distal small intestine. Further N-terminal truncation is required to produce the biologically active form, GLP-17-36 (Chaudhri, Small & Bloom, 2006). GLP-1 undergoes rapid degradation by DPP-IV and only 10-15% reaches the systemic circulation intact (Holst, 2007). GLP-1 receptors are expressed in the gut, brainstem, hypothalamus, and vagal afferent nerves. It is thought that GLP-1 may access the CNS directly via the area postrema, which lacks a blood-brain barrier, but the significance of this pathway is unknown (Dhillo & Bloom, 2004). GLP-1 exerts several physiological effects that may influence appetite. As an incretin hormone, GLP-1 amplifies the insulin response to glucose ingestion and inhibits the release of glycogen from the liver (Baggio & Drucker, 2007; Cummings & Overduin, 2007; Huda, Wilding & Pinkney, 2006). In the GI tract, GLP-1 inhibits gastric and pancreatic exocrine secretion, as well as gastric emptying, which may enhance satiety (Cummings & Overduin, 2007; Huda *et al*., 2006; Naslund, Bogefors *et al*., 1999).

Upon eating, plasma GLP-1 levels increase within 10-15 minutes and peak by 40 minutes (Orskov, Wettergren & Holst, 1996). This initial increase occurs prior to nutrients reaching the small intestine, and is likely mediated by neural inputs. GLP-1 release is proportionate to the number of calories consumed. Additionally, when matched for energy, meals high in carbohydrates and protein seem to be more potent stimulators of GLP-1 secretion than high fat meals (Bowen, Noakes, Trenerry & Clifton, 2006; Raben, Agerholm-Larsen, Flint, Holst & Astrup, 2003). In humans, GLP-1 infusion has been shown to increase satiety and decrease food intake in healthy normal weight and obese participants, as well as individuals with type 2 diabetes (Flint, Raben, Astrup & Holst, 1998; Gutniak *et al*., 1997; Naslund *et al*., 1999; Verdich *et al*., 2001). A meta-analysis by Verdich *et al*. concluded that infusion with physiological doses of GLP-1 reduced energy intake by an average of 12% (Verdich *et al*., 2001).

1.3 Cholecystokinin

CCK is released primarily from I-cells in the duodenum and proximal jejunum, but small amounts are also produced by neurons in the GI tract and nervous system (Liddle, 1997; Rehfeld, 2004). CCK is formed by selective processing of its precursor, proCCK, which results in multiple bioactive forms ranging in size from 8 to 58 amino acids (Reeve, Eysselein, Walsh, Ben-Avram & Shively, 1986; Rehfeld & Hansen, 1986). All isoforms show affinity for the CCK receptor, located on the gallbladder, pancreas, and stomach, as well as in the hindbrain and hypothalamus (Rehfeld, Sun, Christensen & Hillingso, 2001). CCK-induced satiation appears to be mediated neuronally via activation of vagal afferents in the stomach and duodenum (Kopin *et al*., 1999). In

addition, CCK slows gastric emptying, which may increase stomach distension and causes greater satiety (Liddle, Morita, Conrad & Williams, 1986).

Plasma CCK typically increases within 15 minutes of a meal, and the duration of elevation depends both on caloric load and macronutrient content. When matched for energy, fat and protein appear to be stronger stimuli for CCK release than carbohydrates (Liddle, 1997). In humans, infusion with CCK reduces meal size and duration, and has been estimated to suppress energy intake by an average of 22.5% (de Graaf, Blom, Smeets, Stafleu & Hendriks, 2004). There also appear to be gender differences in the CCK response, with women experiencing greater CCK elevation than men; however, it is not clear if this corresponds to differences in appetite sensations between genders (Nolan, Guss, Liddle, Pi-Sunyer & Kissileff, 2003; Schneeman, Burton-Freeman & Davis, 2003).

1.4 Ghrelin

Ghrelin is a 28-amino-acid peptide hormone originating primarily from the stomach, with lesser amounts formed in the small intestine and other organs (Kojima *et al*., 1999). Circulating ghrelin is present in both an acylated and non-acylated form, but only the acylated form binds the ghrelin receptor and is considered biologically active (van der Lely, Tschop, Heiman & Ghigo, 2004). Ghrelin receptors are widely distributed throughout the body in tissues such as brain, stomach, intestine, pancreas, and heart (Cummings, 2006). Ghrelin is thought to interact with NPY and agouti-related peptide (AgRP)-expressing neurons of the arcuate nucleus of the hypothalamus through vagal afferents or more directly via the bloodstream (Huda *et al*., 2006). NPY and AgRP are orexigenic peptides and promote food intake (Huda *et al*., 2006).

Ghrelin is unique in that it is the only peripheral hormone known to be a powerful stimulant of appetite and food intake (Cummings & Overduin, 2007). Plasma ghrelin levels increase markedly prior to a meal, suggesting a role in meal initiation (Cummings *et al*., 2001). In general, nutrient intake suppresses plasma ghrelin levels. While caloric load is the most important determinant of the magnitude and duration of ghrelin suppression, macronutrient composition of the meal also plays a role. When matched for total energy, lipids appear to be less effective than carbohydrates or protein at suppressing ghrelin (Al Awar, Obeid, Hwalla & Azar, 2005; Monteleone, Bencivenga, Longobardi, Serritella & Maj, 2003; Tannous dit El Khoury, Obeid, Azar & Hwalla, 2006). Peripheral infusion with ghrelin increases energy intake and hunger in humans (Wren *et al*., 2001). In addition, there is evidence that obese individuals may be more sensitive to the effects of ghrelin (Druce *et al*., 2005). Ghrelin may also play a role in long-term weight regulation as levels increase with weight loss and decrease with weight gain (Cummings *et al*., 2002).

In summary, the presence or absence of food in the GI tract causes the release of a number of peptides that act to optimize the digestive process and regulate appetite and energy expenditure. Levels of these hormones are influenced by meal composition, caloric load, body weight, gender, and other factors. This study aimed to determine the effect of fiber intake on circulating gut hormone levels in humans.

2. Methods

PubMed/Medline was used to identify original research and review articles on September 27, 2010. The following key words and search terms were used: (dietary fiber OR fiber OR fibre OR whole grain OR complex carbohydrate) AND (gut hormones OR ghrelin OR peptide YY OR peptide tyrosine tyrosine OR PYY OR glucagon-like peptide-1 OR glucagon like peptide OR GLP OR GLP-1 OR cholecystokinin OR CCK). All searches were limited to human studies, English language, and peer-reviewed publications. References from original research and review articles were scanned to identify other potentially relevant studies. The following inclusion criteria were used: Adults (19+ years); healthy individuals of any body weight; clinical trials; measurable fiber level and type; outcome data for PYY, GLP-1, CCK, or ghrelin; attrition rates ≤20%; and studies completed between 1990 and the present. Exclusion criteria included infants; children; people <19 years of age; people with diabetes, hyperlipidemia, hypertension, hypercholesterolemia, or any other health disorders. Studies that used descriptive (retrospective or prospective) study design only, or studies without a measurable fiber intervention were excluded, as were studies with an attrition rate >20%. Studies that met the inclusion criteria were further examined for relevance, validity, and quality by evaluating sample population and size, study design (crossover vs. parallel), randomization, blinding, choice of control, and appropriateness of statistical analyses. These characteristics were organized into tables. Studies that lacked a control were excluded at this level of evaluation.

3. Results

3.1 Effect of fiber on peptide YY concentrations

The PubMed search generated a list of 27 publications, including 22 original research articles and 5 review articles (Figure 1). Eleven primary research articles were relevant to the research question, of which 9 were obtained from the PubMed search and 2 were discovered by examining the reference lists from the review articles. Nine of the 11 relevant publications met the quality criteria and were included in the final analysis. More than 11 types of fibers were studied and doses ranged from 3.8 to 27 g. Fiber was primarily supplied via a supplemented grain product (e.g. bread, muffins, or cereal) which was consumed alone or as part of a mixed meal. Alternatively, fiber was provided as a powder added to a beverage. General study characteristics and outcomes are summarized in Table 1.

Three studies examined the effects of β-glucan fibers on PYY response. Two studies used randomized, crossover designs and controls matched for calories and macronutrients. The first measured 3 g barley β-glucan in 14 normal weight volunteers (Vitaglione, Lumaga, Stanzione, Scalfi & Fogliano, 2009). Area under the curve (AUC) measured over 3 hours was significantly higher following β-glucan intake compared to control. In a similar study, 3 doses (2.2-5.5 g) of oat β-glucan were tested in 14 overweight men and women (Beck, Tapsell, Batterham, Tosh & Huang, 2009). PYY levels were compared to control at individual time points (AUC was not compared). The highest dose of β-glucan resulted in significantly higher PYY levels after 4 hours compared to control. A dose response effect was observed for increasing levels of β-glucan. Although these studies are limited by small sample size, they suggest a dose of 3-6 g β–glucan may raise postprandial PYY levels.

One study examined the effects of chronic β-glucan supplementation on PYY levels. In a parallel design, overweight women (n=66) consumed a low calorie diet supplemented with 0, 5-6 or 8-9 g β-glucan for 3 months (Beck, Tapsell, Batterham, Tosh & Huang, 2010). Total fasting PYY decreased in all groups compared to baseline, but the decrease was significantly less for the high dose compared to control. However, it is not possible to distinguish the effects of fiber supplementation from the effects of caloric restriction and weight loss on gut hormone levels.

Two randomized, crossover studies examined the effect of wheat and/or oat fiber on PYY response. Juvonen *et al*. tested 10 g wheat or oat bran alone, 5 g of each in combination, and a control and found no differences in PYY response among treatments (Juvonen *et al*., 2010). Similarly, Weickert *et al*. tested 10.5 g of added wheat or oat fiber in 14 women and found that postprandial PYY AUC0-300 was blunted following wheat fiber, while PYY levels after oat fiber did not differ from control (Weickert *et al*., 2006).

Other fiber sources were tested in single studies, but at varying doses. In a randomized, crossover design, subjects (n=20) consumed 0, 4, 8, or 12 g of a mixed fiber (pectin, barley β-glucan, guar gum, pea fiber, and citrus fiber) (Willis *et al*., 2010). PYY3-36 AUC0-60 did not differ among treatments; however, many samples fell below the assay detection level. In another randomized crossover study, subjects (n=16) consumed 4 isoenergetic meals with varying amounts of psyllium (6.2-27 g) and soy protein or a bread control (Karhunen *et al*., 2010). The high fiber meals caused a longer elevation of PYY levels compared to control, but this was only significant at 90 min and 120 minutes after the meal; AUC0-120 did not differ.

Two studies using parallel designs examined chronic consumption of a fiber source. Subjects (n=10) consumed 16 g/d of an inulin/oligofructose blend or control for 2 weeks, at which point postprandial PYY was measured in response to a free choice buffet breakfast (Cani *et al*., 2009). Mean total PYY levels were compared at individual time points (AUC was not measured). Plasma PYY was significantly increased 10 minutes after breakfast in subjects who had been consuming the inulin treatment compared to control. Another study examined the effect of increasing doses (5 to 10 g) of a functional fiber blend consumed for 3 weeks (Reimer *et al*., 2010). Following intervention, fasting PYY was significantly higher in the supplemented group compared to control, but only in a subset of individuals with BMI <23. In addition, PYY levels at week 3 were not different from baseline.

Overall, the available evidence does not show a clear effect of fiber on PYY levels. Acute feeding studies reported that small amounts of β-glucan or large amounts of psyllium increased postprandial PYY, while wheat and oat brans and a mixed fiber blend did not increase PYY compared to control meals. Chronic, daily consumption of β-glucan combined with energy restriction was shown to decrease fasting levels of PYY, while a mix of inulin and oligofructose or a functional fiber blend had little effect on fasting PYY levels. In general, the available studies are limited by sample size and study design. The wide variety of fiber types and doses used make it is difficult to discern an overall relationship between fiber and PYY response.

3.2 Effect of fiber on glucagon-like peptide-1 concentrations

The PubMed search generated a list of 53 publications, including 37 original research articles and 16 review articles, meta-analyses, or letters to the editor (Figure 1). Nineteen primary research articles were relevant to the research question, of which 17 were obtained from the PubMed search and 2 were discovered by examining the reference lists from the review articles. Of the 19 relevant publications, 16 met the quality criteria and were included in the final analysis. Many types of fiber were evaluated, with doses ranging from 1.7 g to 29 g fiber. In 11 studies, the fiber was provided as a supplemented grain product (most commonly bread), while in the other 5 studies, a powdered fiber supplement was mixed with a beverage or other test product. General study characteristics and outcomes are summarized in Table 2.

Several studies tested multiple types and amount of fiber, but only one combination showed a positive impact on GLP-1 levels. In a randomized, crossover design, subjects (n=15) consumed 7 test meals with varying levels (9.9-81 g) of dietary fiber plus resistant starch (RS) from various forms of barley, oats, and modified corn starch or a low fiber control (Nilsson, Ostman, Holst & Bjorck, 2008). Test meals were consumed in the evening, and GLP-1 was measured the next morning following a standard breakfast. The total GLP-1 AUC0-120 was significantly higher than control following consumption of the test meal containing 20.2 g fiber + RS from ordinary barley. There were no other differences among treatments. This suggests the source of fiber may be more important than the dose, since other treatments with similar amounts of fiber + RS had no effect. Another study that compared 5.5 g whole wheat barley to control found no differences in GLP-1 AUC0-300 following the test meal (Najjar *et al*., 2009).

Additional studies have evaluated the effect of fiber from other whole grain sources on GLP-1 response. Two crossover studies compared various types and doses (6.1-29 g) of rye bread to a low-fiber white bread matched for available carbohydrates. In both studies, GLP-1 AUC0-180 did not differ among treatments (Juntunen *et al*., 2002; Juntunen, Laaksonen, Poutanen, Niskanen & Mykkanen, 2003). A high fiber rye bread (whole meal rye bread enriched with rye bran) providing 29 g fiber caused significantly greater GLP-1 values compared to control at 150 and 180 minutes postprandially (Juntunen *et al*., 2003). However, this product was also higher in energy, fat, and protein, so it is unclear if fiber was responsible for the observed effects. A rye bread enriched with β-glucan (17.1 g fiber, including 5.4 g β-glucan) also increased GLP-1 compared to control later in the postprandial period (120 and 150 minutes) (Juntunen *et al*., 2002). In a randomized, crossover design, Weickert *et al*. examined the effect of 10.5 g wheat or oat fiber compared to control and found no differences among treatments in GLP-1 measured as AUC0-300 or individual time points (Weickert *et al*., 2005). Similarly, ancient wheat Einkorn (4-6 g) did not alter GLP-1 AUC0-180 compared to a modern wheat bread (Bakhoj, Flint, Holst & Tetens, 2003). However, fiber differences between control and treatment were minor and the sample size was small (n=11).

Psyllium was tested in two randomized, crossover trials. In the first, subjects (n=10) consumed a meal with added psyllium (1.7 g) and/or fat or an unsupplemented meal matched for available carbohydrates (Frost, Brynes, Dhillo, Bloom & McBurney, 2003). GLP-1 AUC0-240 was significantly higher than control in the meal with added psyllium and fat, but this effect was likely due to caloric differences between meals. AUC was not different between control and the low fat psyllium treatment, which was matched for calories (Frost *et al*., 2003). In a later study, isoenergetic meals with varying levels of psyllium (6.2-27 g) and protein were compared to an unsupplemented control (Karhunen *et al*., 2010). GLP-1 AUC0-120 did not differ among treatments, but GLP-1 concentrations decreased below baseline following consumption of the high fiber, high protein treatment, indicating a negative effect of fiber and/or protein on GLP-1 levels.

Two studies tested pea fiber, either alone or as part of a mixed fiber blend. In a study by Raben *et al*., subjects (n=10), consumed a meal supplemented with 25.5 g pea fiber or low fiber control matched for energy and macronutrients (Raben, Christensen, Madsen, Holst & Astrup, 1994). There were no differences in GLP-1 between treatments when measured as AUC0-240 or at individual time points. Willis *et al*. examined the effect of muffins supplemented with 0, 4, 8, or 12 g of a mixed fiber (pectin, barley β-glucan, guar gum, pea fiber, and citrus fiber) and found that GLP-1 AUC0-60 was significantly higher for the 0 g dose than the 4 and 12 g doses, which again suggests a potential suppressive effect of fiber on GLP-1 (Willis *et al*., 2010).

Three randomized, crossover trials measured GLP-1 response to fiber dissolved in a test beverage. Two studied the effect of a preload of guar gum (2.5 g) + galactose or water (control), followed by a test meal (Adam & Westerterp-Plantenga, 2005a; Adam & Westerterp-Plantenga, 2005b). In both studies, GLP-1 levels were increased compared to control between 30 and 60 minutes postprandially. However, this is not a useful comparison since GLP-1 is known to increase as a result of caloric load. The fiber treatment contained 200 kcal

and was compared to a non-caloric control. In another study, there were no differences in GLP-1 AUC0-360 between a beverage containing 24 g inulin + 56 g high fructose corn syrup (HFCS) and beverages with 80 g or 56 g of HFCS alone (Tarini & Wolever, 2010).

Three studies with parallel design have evaluated the effect of chronic fiber supplementation on GLP-1 levels. Consumption of oat β-glucan (5-6 or 8-9 g/d) as part of a reduced calorie diet led to a reduction in fasting GLP-1 levels after 3 months (Beck *et al*., 2010). Values were not different from a control group on the same low calorie diet, suggesting that weight loss has a greater effect on gut hormone levels than fiber. In another study, subjects consumed increasing doses (5 to 10 g) of a novel functional fiber or control for 3 weeks (Reimer *et al*., 2010). There were no differences in fasting GLP-1 levels at the end of the treatment period. Similarly, fasting GLP-1 levels were not different in subjects receiving 16 g/d of an inulin/oligofructose blend or control for 2 weeks (Cani *et al*., 2009). However, GLP-1 was elevated compared to control at 10 minutes following a standard meal in subjects who had consumed fiber; AUC was not evaluated. These studies suggest chronic fiber intake independent of weight changes does not impact GLP-1 levels. In addition, due to the parallel design, these studies must be interpreted with caution, given the inter-individual variability in gut hormone levels.

The available research suggests that fiber does not increase GLP-1 levels compared to control. Most studies were limited by sample size or design. Only one study reported an increase in GLP-1 AUC following fiber intervention (20.2 g ordinary barley), and other studies with similar types or doses of fiber found no effect. High doses of fiber (17-29 g) from rye bread significantly increased GLP-1 between 2 and 3 hours after a test meal, but at no other time points. Other fiber interventions showed no effect on GLP-1 concentrations when matched for calorie content.

3.3 Effect of fiber on cholecystokinin concentrations

The PubMed search generated a list of 64 publications, including 47 original research articles and 17 review articles, meta-analyses, or letters to the editor (Figure 1). No additional articles were discovered from examination of review article reference lists. Eleven primary research articles were relevant to the research question, of which 9 met the quality criteria and were included in the final analysis. Fiber came from a variety of sources, but β-glucan sources were the most common; fiber doses ranged from 3.7 to 35.5 g fiber. While most studies provided fiber as part of a mixed meal, one used a fiber-supplemented liquid formula. Control meals were generally well matched to the treatment meals in terms of energy and macronutrients. General study characteristics and outcomes are summarized in Table 3.

Several studies evaluated CCK response to supplementation with fibers containing β-glucans. Test cereals containing varying amounts of oat β-glucan (2.16-5.65 g) were compared to a low fiber cereal in a randomized, crossover design (Beck *et al*., 2009). There was a significant dose response for women (n=7), but the combined sex analyses showed no differences in CCK AUC0-240. A similar gender effect was observed in subjects consuming mixed meals containing 7 g (control) or 20 g fiber (added fiber in the high fiber meal was primarily from oats) (B. Burton-Freeman, Davis & Schneeman, 2002). In women, the high fiber meal elicited a significantly higher mean CCK response compared to control, while the CCK response between meals did not differ in men. In another study, male volunteers (n=11) consumed pasta made from barley with high β-glucan content (15.7 g fiber; 5 g β-glucan) or control (Bourdon *et al*., 1999). CCK AUC0-360 did not differ, but the pattern of CCK response was different. While CCK concentrations returned to baseline by 3 hours after the low fiber meal, CCK levels did not return to baseline until 6 hours following the high fiber meal.

In a chronic study using a parallel design, consumption of oat β-glucan (5-6 or 8-9 g/d) as part of a reduced calorie diet for 3 months did not alter fasting CCK levels compared to control in women (Beck *et al*., 2010). Another chronic study evaluated addition of 20 g partially hydrolyzed guar gum (PHGG) to a very low calorie formula diet in obese women (n=25) (Heini *et al*., 1998). Women received PHGG during either week 3 or 5 of the diet. Following a meal challenge using the formula diet, average CCK concentrations did not differ between treatment and control.

Additional randomized, crossover trials have evaluated different types of fiber or types of carbohydrate. In a small study, men (n=10) consumed a test meal with 12 g fiber from bean flakes or a low fiber meal matched for energy and macronutrients (Bourdon *et al*., 2001). The bean flake meal produced almost twice the CCK AUC0-360 response, which was statistically significant. Pasman *et al*. compared the effect of isoenergetic meals containing complex or simple carbohydrates in 26 male volunteers (Pasman, Blokdijk, Bertina, Hopman & Hendriks, 2003). The complex carbohydrate meal contained 6.7 g of fiber, provided primarily by rye bread. There was no difference in CCK response between the meals when measured as AUC0-240 or at individual time points.

Two studies compared meals that differed in glycemic index. The first found that consumption of a low fiber (2.4 g), high glycemic index meal resulted in a significantly greater CCK AUC0-480 compared to a high fiber (35.5 g), low glycemic index meal in female volunteers (n=22) (B. M. Burton-Freeman & Keim, 2008). In contrast, when matched for fiber content (29-30 g), consumption of a low glycemic index meal resulted in significantly greater CCK AUC0-420 in men (n=12) (Reynolds, Stockmann, Atkinson, Denyer, & Brand-Miller, 2009).

The available evidence indicates that fiber does not have a consistent effect on CCK levels. In a small, but well designed study, fiber from bean flakes caused a clear increase in CCK compared to control, but the results are applicable only to men. Acute consumption of fiber from oats may increase CCK in women only, while chronic intake of fiber has no effect. In addition, meals varying in type of carbohydrate yielded inconsistent effects on CCK. Most studies were limited by small sample size, and may not be representative of the general population since they were conducted in certain genders, BMI ranges, or individuals on a reduced calorie diet.

3.4 Effect of fiber on ghrelin concentrations

The PubMed search generated a list of 51 publications, including 40 original research articles and 11 review articles, meta-analyses, or letters to the editor (Figure 1). Twenty-three primary research articles were relevant to the research question, of which 19 were obtained from the PubMed search and 4 were discovered by examining the reference lists from the review articles. Of the 23 relevant publications, 19 met the quality criteria and were included in the final analysis. A variety of individual fibers and fiber blends were studied, with doses ranging from 2 to 52 g fiber. Twelve studies provided fiber as a supplemented grain product or as part of a mixed meal, 5 added powdered fiber to a liquid or semi-solid product, and 2 added fiber to water. General study characteristics and outcomes are summarized in Table 4.

Several studies measured ghrelin response to β-glucan supplementation. In a randomized, crossover design, subjects (n=14) consumed isoenergetic breads enriched with 3 g barley β-glucan or control (Vitaglione *et al*., 2009). Ghrelin AUC60-180 was significantly lower following the fiber treatment. In contrast, there were no differences in ghrelin AUC0-240 among subjects (n=14) consuming cereal supplemented with varying doses of oat β-glucan (2.16-5.65 g) or control matched for available carbohydrate and protein (Beck *et al*., 2009). Similarly, in a 3 month parallel trial, supplementation with oat β-glucan (5-6 or 8-9 g) had no effect on fasting ghrelin levels in women on a reduced calorie diet (Beck *et al*., 2010). However, it possible that any effect of fiber would have been overshadowed by the influence of weight change on gut hormone levels. Additional randomized, crossover trials using 10-10.5 g fiber from oats or wheat did not show a suppressive effect of fiber on ghrelin levels compared to an isoenergetic control (Juvonen *et al*., 2010; Weickert *et al*., 2006). In fact, one study found that 10.5 g wheat fiber resulted in significantly higher ghrelin AUC0-180 compared to control (Weickert *et al*., 2006).

A series of crossover studies examined the effects of carob fiber on postprandial ghrelin levels. In the first study, subjects (n=20) consumed a liquid meal alone or enriched with 5, 10, or 20 g carob fiber (Gruendel *et al*., 2006). Acylated (but not total) ghrelin was significantly lower 60 minutes after the test meal for all doses of fiber compared to control. There were no other differences over the 5 hour postprandial period, and AUC was not analyzed. In contrast, the same doses of carob fiber added to glucose water had no effect on acylated ghrelin, but the 10 g dose decreased total ghrelin compared to control (Gruendel, Otto *et al*., 2007). In a third study, volunteers consumed calorie and nutrient matched meals with or without 50 g carob fiber, followed by an overnight fast (Gruendel *et al*., 2007). Ghrelin was measured the next morning following ingestion of a standardized white bread. Fasting acylated (but not total) ghrelin was significantly higher following consumption of the meal enriched with carob fiber; there were no differences in postprandial ghrelin levels.

There were 9 additional acute, crossover studies with fiber and ghrelin, each testing different types of fiber. In a study by Karhunen *et al*., subjects (n=16) consumed isoenergetic meals with varying levels of psyllium (7.6-27 g) and protein or a low fiber, low protein control in randomized order(Karhunen *et al.*, 2010). The declines in total ghrelin, measured as AUC0-120 after the high fiber meals were blunted and differed significantly from the low fiber meals. Similarly, in subjects (n=11) consuming a meal with 6 g arabinoxylan or control matched for energy and macronutrients, ghrelin suppression was greater following control(Mohlig *et al*., 2005). In a study by Willis *et al*., subjects (n=20) consumed muffins with 0, 4, 8, or 12 g of a mixed fiber in random order (Willis *et al*., 2010). There were no differences in AUC0-90 between treatments and control, but the highest dose led to significantly higher values than the lower doses. Consumption of rye products with varying levels of fiber (6.5-12.3 g) did not alter ghrelin AUC0-180 compared to low fiber control matched for available carbohydrates

(Rosen *et al*., 2009). These studies suggest that fiber does not have a suppressive effect on ghrelin, and that certain fibers may actually blunt the decline in postprandial ghrelin levels.

In contrast, several studies have reported greater declines in ghrelin levels following fiber compared to control. Consumption of bread enriched with lupin kernel flour (15 g fiber) resulted in significantly lower plasma ghrelin values than a calorie-matched white bread over a 3 hour postprandial period (Lee *et al*., 2006). However, the enriched bread also contained twice the protein as control, so it is unclear if the effects are due to fiber, protein, or the combination. Consumption of 6 g fiber from plums produced significantly lower ghrelin values compared to white bread, but only at 15 and 30 minutes after the meal; there were no differences in ghrelin AUC0-120 (Furchner-Evanson, Petrisko, Howarth, Nemoseck & Kern, 2010). Addition of 24 g inulin to a HFCS beverage caused a significant decrease in ghrelin levels compared to control, but not until 4 hours later, after a standard test lunch was consumed (Tarini & Wolever, 2010). This suggests that fiber may produce a 2nd meal effect on ghrelin levels. Although these studies suggest a suppressive effect of fiber on ghrelin, any effects appear to be short lived.

Two studies tested the influence of different types of carbohydrate on ghrelin levels. Ghrelin response was not different when subjects consumed a high glycemic index meal or a low glycemic index meal with similar fiber content (Reynolds *et al*., 2009). In another study, subjects consumed meals containing simple or complex carbohydrates at varying calorie levels, but with similar fiber content (Blom *et al*., 2005). The decrease in ghrelin AUC0-240 was greater for the high calorie, simple carbohydrate meal than for the high calorie, complex carbohydrate meal, which suggests carbohydrate structure may affect ghrelin levels, regardless of fiber content.

Two additional studies examined the effect of chronic fiber supplementation on fasting ghrelin levels. In a randomized, crossover design, subjects consumed 12 g/d pullulan, RS, soluble fiber dextrin, soluble corn fiber or control for 2 weeks each. There were no differences in fasting ghrelin among treatments (Stewart, Nikhanj, Timm, Thomas & Slavin, 2010). Similarly, consumption of a novel functional fiber for 3 weeks did not alter fasting ghrelin levels compared to a control diet (Reimer *et al*., 2010). However, this study was limited by parallel design.

The available evidence suggests fiber does not positively influence postprandial ghrelin levels. The majority of studies found that fiber had no effect or a negative effect on ghrelin (higher levels compared to control) over a range of fiber sources and doses. In the few studies showing a suppressive effect of fiber, lower ghrelin values were only observed at limited time points throughout the postprandial period. However, many of these studies were limited by small sample size, lack of crossover design, or use of a control that differed in variables other than fiber content.

4. Discussion

The available literature on fiber and gut hormones is limited in both quality and quantity. Few studies with strong design (randomized, controlled, double-blind, crossover trials) measure gut hormone levels following acute fiber intake. Therefore, to provide a more complete assessment of the literature, studies with parallel design and those that measured fasting hormone levels after chronic fiber intake were also included in this review. Gut hormone levels can be highly variable from individual to individual, so the reliability of results from those studies is unknown. There is also little consistency in the types of fibers and doses used across studies, and a wide variety of isolated fibers, synthetic fibers, and high-fiber whole foods were used. Furthermore, control treatments differed greatly among studies and were not always appropriate for examining the effect of fiber supplementation. Since the primary outcome was gut hormone levels compared to control, the use of inappropriate control treatments could significantly alter the results. These variations make it difficult to discern the true effect of fiber on gut hormone levels.

Few studies have been conducted investigating the effect of fiber on PYY release. Only nine publications met the inclusion criteria for the current review, resulting in 20 fiber-control comparisons based on many different fiber types and levels. Of those comparisons, the influence of fiber on circulating PYY levels was seen with acute feeding of test meals containing 3-6 g barley or oat β-glucan or greater than 25 g psyllium. Generally, fat and protein, as well as calorie load of a meal, have a greater influence on release of PYY into circulation than carbohydrates (Batterham *et al*., 2006; Essah *et al*., 2007; MacIntosh *et al*., 1999). Fiber, as a member of the carbohydrate family of macronutrients, might not be expected to influence PYY to a great extent beyond the provision of calories to a meal.

Sixteen publications investigating the effect of fiber on GLP-1 release met the inclusion criteria for the current review, resulting in 34 fiber-control comparisons based on many different fiber types and levels. Of those comparisons, influence of fiber meals on circulating GLP-1 levels were seen primarily when differences in

calorie content of the products were reported. For instance, in a study of psyllium, an increase in circulating GLP-1 was found when fat, and therefore calories, was added to the test meal, but not when the meals were matched for energy (Frost *et al*., 2003). Circulating GLP-1 levels are known to be influenced by calories consumed, however when calorie content of a meal is held constant, carbohydrates and proteins are potent stimulators of GLP-1 release (Bowen *et al*., 2006; Raben *et al*., 2003). The results of this review suggest that calories are a more potent stimulator of GLP-1 release into the bloodstream than fiber. Any effect of fiber on appetite through GLP-1 action may be mediated directly via the vagal nerve and not as a result of circulating GLP-1.

Nine publications investigating the effect of fiber on circulating levels of CCK met the inclusion criteria for the current review, resulting in only 14 fiber-control comparisons based on many different fiber types and levels. In general, the results would suggest that fibers are not efficacious in promoting higher levels of circulating CCK. These results should not be surprising as carbohydrates have not been found to be as robust in their influence on circulating CCK levels as either protein or fat. Based on this review, two areas of interest for further investigation are the influence of beans and glycemia on CCK release (Bourdon *et al*., 2001; Reynolds *et al*., 2009). Although only 1 study has been published examining bean flakes, the results were quite promising with twice the response, based on AUC, when compared to a control meal. The efficacious component of the bean may be the protein and/or phytonutrient co-passengers in the formulation. Glycemic index of a meal was examined by Reynolds and coworkers (2009) with a report that, when controlled for fiber content of the meal, glycemic index significantly influenced the CCK response to the meal (Reynolds *et al*., 2009). Preliminary research has suggested that glycemia may influence appetite and satiety and this is the first report that suggests that one mechanism may be related to CCK release. More research is needed in both of these areas to confirm these early findings.

Ghrelin is known to be influenced by consumption of food. The increase in ghrelin levels between meals is generally reversed once food is consumed. Some data suggest that protein and carbohydrates are more effective than lipids at attenuating the rise in ghrelin, however the presence of food in the gut may be the primary precipitating factor. Nineteen publications investigating the effect of fiber on attenuating the rise in circulating levels of ghrelin met the inclusion criteria for the current review, resulting in 44 fiber-control comparisons. Although several studies examining specific time points following the meal suggest that the influence of fiber on ghrelin may be time-specific, other data suggest that inclusion of fiber in a meal may actually blunt the postprandial decrease in ghrelin. In general, data reported as AUC did not support the hypothesis that fiber suppresses ghrelin levels.

Other issues complicating gut hormone research are related to the technological aspects and limitations involved in the measurement of gut hormones. Most studies rely on more affordable, but less sophisticated techniques, such as enzyme immunoassay or radio immunoassay, for gut hormone analysis (Delzenne *et al.*, 2010). These often measure the total amount of the peptide, rather than a specific form. In many cases, only certain forms of a hormone may be bioactive, so measuring the total concentration may not be entirely informative. In addition, some studies have shown changes in one form of a peptide, but not another (e.g. acylated ghrelin vs. total ghrelin), suggesting that measuring total peptide amounts is providing an incomplete picture (Gruendel *et al*., 2006). Furthermore, degradation of some peptides (e.g. GLP-1 by DPP-IV) both in the blood and in stored samples could lead to inaccurate measurements and interpretations. In addition, since many gut hormones bind their receptors and exert actions in the gut, measurement of these peptides in venous blood may not be meaningful in terms of their physiological effects.

The primary reason for measuring gut hormones following fiber intervention is to identify potential mechanisms by which fiber may influence appetite. However, it is important to consider the fact that individual gut hormones are not released in isolation following a meal. Instead, they are released in concert with other hormones and peptides which act together to control the digestion and absorption process and signal energy needs. Nevertheless, most studies focus on individual hormones as independent contributors to the primary outcome of appetite. Specific combinations of gut hormones have been shown to have additive effects on outcomes such as inhibition of food intake, and other synergistic relationships may exist (Neary *et al*., 2005). By studying each hormone in isolation, we may be missing the bigger picture.

The available research does not support a consistent effect of fiber on modifying circulating gut hormone levels. While it is possible that fiber does not influence appetite via gut hormone pathways, it is also possible that the lack of consistent study design merely prevents us from forming conclusions around this relationship. Current research uses a wide variety of fiber sources with different physical and chemical properties which may influence gut hormone response. Different fiber types may influence gut hormone levels based on their

physicochemical properties, but additional research is required to examine this relationship. The relationship between fiber intake and appetite may also work by mechanisms not detectable with the measurement of circulating gut hormone levels.

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References

Abbott, C. R., Monteiro, M., Small, C. J., Sajedi, A., Smith, K. L., Parkinson, J. R., *et al*. (2005). The inhibitory effects of peripheral administration of peptide YY(3-36) and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal-brainstem-hypothalamic pathway. *Brain Research, 1044*(1), 127-131. http://dx.doi.org/10.1016/j.brainres.2005.03.011

Adam, T. C., & Westerterp-Plantenga, M. S. (2005a). Glucagon-like peptide-1 release and satiety after a nutrient challenge in normal-weight and obese subjects. *The British Journal of Nutrition, 93*(6), 845-851. http://dx.doi.org/10.1079/BJN20041335

Adam, T. C., & Westerterp-Plantenga, M. S. (2005b). Nutrient-stimulated GLP-1 release in normal-weight men and women. Hormone and Metabolic Research.Hormon- Und Stoffwechselforschung.Hormones Et *Metabolisme, 37*(2), 111-117. http://dx.doi.org/10.1055/s-2005-861160

Adrian, T. E., Ferri, G. L., Bacarese-Hamilton, A. J., Fuessl, H. S., Polak, J. M., & Bloom, S. R. (1985). Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology, 89*(5), 1070-1077.

Al Awar, R., Obeid, O., Hwalla, N., & Azar, S. (2005). Postprandial acylated ghrelin status following fat and protein manipulation of meals in healthy young women. *Clinical Science (London, England : 1979), 109*(4), 405-411.

Baggio, L. L., & Drucker, D. J. (2007). Biology of incretins: GLP-1 and GIP. *Gastroenterology, 132*(6), 2131-2157. http://dx.doi.org/10.1053/j.gastro.2007.03.054

Bakhoj, S., Flint, A., Holst, J. J., & Tetens, I. (2003). Lower glucose-dependent insulinotropic polypeptide (GIP) response but similar glucagon-like peptide 1 (GLP-1), glycaemic, and insulinaemic response to ancient wheat compared to modern wheat depends on processing. *European Journal of Clinical Nutrition, 57*(10), 1254-1261. http://dx.doi.org/10.1038/sj.ejcn.1601680

Batterham, R. L., Cohen, M. A., Ellis, S. M., Le Roux, C. W., Withers, D. J., Frost, G. S., *et al*. (2003). Inhibition of food intake in obese subjects by peptide YY3-36. *The New England Journal of Medicine, 349*(10), 941-948. http://dx.doi.org/10.1056/NEJMoa030204

Batterham, R. L., Cowley, M. A., Small, C. J., Herzog, H., Cohen, M. A., Dakin, C. L., *et al*. (2002). Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature, 418*(6898), 650-654. http://dx.doi.org/10.1038/nature00887

Batterham, R. L., ffytche, D. H., Rosenthal, J. M., Zelaya, F. O., Barker, G. J., Withers, D. J., *et al*. (2007). PYY modulation of cortical and hypothalamic brain areas predicts feeding behaviour in humans. *Nature, 450*(7166), 106-109. http://dx.doi.org/10.1038/nature06212

Batterham, R. L., Heffron, H., Kapoor, S., Chivers, J. E., Chandarana, K., Herzog, H., *et al*. (2006). Critical role for peptide YY in protein-mediated satiation and body-weight regulation. *Cell Metabolism, 4*(3), 223-233. http://dx.doi.org/10.1016/j.cmet.2006.08.001

Beck, E. J., Tapsell, L. C., Batterham, M. J., Tosh, S. M., & Huang, X. F. (2009). Increases in peptide Y-Y levels following oat beta-glucan ingestion are dose-dependent in overweight adults. *Nutrition Research, 29*(10), 705-709. http://dx.doi.org/10.1016/j.nutres.2009.09.012

Beck, E. J., Tapsell, L. C., Batterham, M. J., Tosh, S. M., & Huang, X. F. (2010). Oat beta-glucan supplementation does not enhance the effectiveness of an energy-restricted diet in overweight women. *The British Journal of Nutrition, 103*(8), 1212-1222. http://dx.doi.org/10.1017/S0007114509992856

Blom, W. A., Stafleu, A., de Graaf, C., Kok, F. J., Schaafsma, G., & Hendriks, H. F. (2005). Ghrelin response to carbohydrate-enriched breakfast is related to insulin. *The American Journal of Clinical Nutrition, 81*(2), 367-375.

Bourdon, I., Olson, B., Backus, R., Richter, B. D., Davis, P. A., & Schneeman, B. O. (2001). Beans, as a source of dietary fiber, increase cholecystokinin and apolipoprotein b48 response to test meals in men. *The Journal of Nutrition, 131*(5), 1485-1490.

Bourdon, I., Yokoyama, W., Davis, P., Hudson, C., Backus, R., Richter, D., *et al*. (1999). Postprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with beta-glucan. *The American Journal of Clinical Nutrition, 69*(1), 55-63.

Bowen, J., Noakes, M., Trenerry, C., & Clifton, P. M. (2006). Energy intake, ghrelin, and cholecystokinin after different carbohydrate and protein preloads in overweight men. *The Journal of Clinical Endocrinology and Metabolism, 91*(4), 1477-1483. http://dx.doi.org/10.1210/jc.2005-1856

Burton-Freeman, B., Davis, P. A., & Schneeman, B. O. (2002). Plasma cholecystokinin is associated with subjective measures of satiety in women. *The American Journal of Clinical Nutrition, 76*(3), 659-667.

Burton-Freeman, B. M., & Keim, N. L. (2008). Glycemic index, cholecystokinin, satiety and disinhibition: Is there an unappreciated paradox for overweight women? *International Journal of Obesity, 32(*11), 1647-1654.

Cani, P. D., Lecourt, E., Dewulf, E. M., Sohet, F. M., Pachikian, B. D., Naslain, D., *et al*. (2009). Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. *The American Journal of Clinical Nutrition, 90*(5), 1236-1243. http://dx.doi.org/10.3945/ajcn.2009.28095

Chaudhri, O., Small, C., & Bloom, S. (2006). Gastrointestinal hormones regulating appetite. Philosophical Transactions of the Royal Society of London. *Series B, Biological Sciences, 361*(1471), 1187-1209. http://dx.doi.org/10.1098/rstb.2006.1856

Cummings, D. E. (2006). Ghrelin and the short- and long-term regulation of appetite and body weight. *Physiology & Behavior, 89*(1), 71-84. http://dx.doi.org/10.1016/j.physbeh.2006.05.022

Cummings, D. E., & Overduin, J. (2007). Gastrointestinal regulation of food intake. *The Journal of Clinical Investigation, 117*(1), 13-23. http://dx.doi.org/10.1172/JCI30227

Cummings, D. E., Purnell, J. Q., Frayo, R. S., Schmidova, K., Wisse, B. E., & Weigle, D. S. (2001). A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes, 50*(8), 1714-1719. http://dx.doi.org/10.2337/diabetes.50.8.1714

Cummings, D. E., Weigle, D. S., Frayo, R. S., Breen, P. A., Ma, M. K., Dellinger, E. P., *et al*. (2002). Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *The New England Journal of Medicine, 346*(21), 1623-1630. http://dx.doi.org/10.1056/NEJMoa012908

de Graaf, C., Blom, W. A., Smeets, P. A., Stafleu, A., & Hendriks, H. F. (2004). Biomarkers of satiation and satiety. *The American Journal of Clinical Nutrition, 79*(6), 946-961.

Degen, L., Oesch, S., Casanova, M., Graf, S., Ketterer, S., Drewe, J., *et al*. (2005). Effect of peptide YY3-36 on food intake in humans. *Gastroenterology, 129*(5), 1430-1436. http://dx.doi.org/10.1053/j.gastro.2005.09.001

Delzenne, N., Blundell, J., Brouns, F., Cunningham, K., De Graaf, K., Erkner, A., *et al*. (2010). Gastrointestinal targets of appetite regulation in humans. *Obesity Reviews : An Official Journal of the International Association for the Study of Obesity, 11*(3), 234-250. http://dx.doi.org/10.1111/j.1467-789X.2009.00707.x

Dhillo, W. S., & Bloom, S. R. (2004). Gastrointestinal hormones and regulation of food intake. *Hormone and Metabolic Research = Hormon- Und Stoffwechselforschung = Hormones Et Metabolisme, 36*(11-12), 846-851.

Druce, M. R., Wren, A. M., Park, A. J., Milton, J. E., Patterson, M., Frost, G., *et al*. (2005). Ghrelin increases food intake in obese as well as lean subjects. *International Journal of Obesity, 29*(9), 1130-1136. http://dx.doi.org/10.1038/sj.ijo.0803001

Essah, P. A., Levy, J. R., Sistrun, S. N., Kelly, S. M., & Nestler, J. E. (2007). Effect of macronutrient composition on postprandial peptide YY levels. *The Journal of Clinical Endocrinology and Metabolism, 92*(10), 4052-4055. http://dx.doi.org/10.1210/jc.2006-2273

Flint, A., Raben, A., Astrup, A., & Holst, J. J. (1998). Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *The Journal of Clinical Investigation, 101*(3), 515-520. http://dx.doi.org/10.1172/JCI990

Frost, G. S., Brynes, A. E., Dhillo, W. S., Bloom, S. R., & McBurney, M. I. (2003). The effects of fiber enrichment of pasta and fat content on gastric emptying, GLP-1, glucose, and insulin responses to a meal. *European Journal of Clinical Nutrition, 57*(2), 293-298. http://dx.doi.org/10.1038/sj.ejcn.1601520

Furchner-Evanson, A., Petrisko, Y., Howarth, L., Nemoseck, T., & Kern, M. (2010). Type of snack influences satiety responses in adult women. *Appetite, 54*(3), 564-569. http://dx.doi.org/10.1016/j.appet.2010.02.015

Gruendel, S., Garcia, A. L., Otto, B., Mueller, C., Steiniger, J., Weickert, M. O., *et al*. (2006). Carob pulp preparation rich in insoluble dietary fiber and polyphenols enhances lipid oxidation and lowers postprandial acylated ghrelin in humans. *The Journal of Nutrition, 136*(6), 1533-1538. http://dx.doi.org/10.1017/S0007114507777127

Gruendel, S., Garcia, A. L., Otto, B., Wagner, K., Bidlingmaier, M., Burget, L., *et al*. (2007). Increased acylated plasma ghrelin, but improved lipid profiles 24-h after consumption of carob pulp preparation rich in dietary fibre and polyphenols. *The British Journal of Nutrition, 98*(6), 1170-1177. http://dx.doi.org/10.1017/S0007114507701642

Gruendel, S., Otto, B., Garcia, A. L., Wagner, K., Mueller, C., Weickert, M. O., *et al*. (2007). Carob pulp preparation rich in insoluble dietary fibre and polyphenols increases plasma glucose and serum insulin responses in combination with a glucose load in humans. *The British Journal of Nutrition, 98*(1), 101-105. http://dx.doi.org/10.2337/diacare.20.12.1874

Gutniak, M. K., Larsson, H., Sanders, S. W., Juneskans, O., Holst, J. J., & Ahren, B. (1997). GLP-1 tablet in type 2 diabetes in fasting and postprandial conditions. *Diabetes Care, 20*(12), 1874-1879.

Heini, A. F., Lara-Castro, C., Schneider, H., Kirk, K. A., Considine, R. V., & Weinsier, R. L. (1998). Effect of hydrolyzed guar fiber on fasting and postprandial satiety and satiety hormones: A double-blind, placebo-controlled trial during controlled weight loss. *International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity, 22*(9), 906-909. http://dx.doi.org/10.1038/sj.ijo.0800680

Holst, J. J. (2007). The physiology of glucagon-like peptide 1. Physiological Reviews, 87(4), 1409-1439. http://dx.doi.org/10.1152/physrev.00034.2006

Huda, M. S., Wilding, J. P., & Pinkney, J. H. (2006). Gut peptides and the regulation of appetite. *Journal of the International Association for the Study of Obesity, 7*(2), 163-182. http://dx.doi.org/10.1111/j.1467-789X.2006.00245.x

Juntunen, K. S., Laaksonen, D. E., Poutanen, K. S., Niskanen, L. K., & Mykkanen, H. M. (2003). High-fiber rye bread and insulin secretion and sensitivity in healthy postmenopausal women. *The American Journal of Clinical Nutrition, 77*(2), 385-391.

Juntunen, K. S., Niskanen, L. K., Liukkonen, K. H., Poutanen, K. S., Holst, J. J., & Mykkanen, H. M. (2002). Postprandial glucose, insulin, and incretin responses to grain products in healthy subjects. *The American Journal of Clinical Nutrition, 75*(2), 254-262.

Juvonen, K., Salmenkallio-Marttila, M., Lyly, M., Liukkonen, K. H., Lahteenmaki, L., Laaksonen, D., *et al*. (2010). Semisolid meal enriched in oat bran decreases plasma glucose and insulin levels, but does not change gastrointestinal peptide responses or short-term appetite in healthy subjects. Nutrition, Metabolism, and Cardiovascular Diseases : NMCD. http://dx.doi.org/10.1016/j.numecd.2010.02.002

Karhunen, L. J., Juvonen, K. R., Flander, S. M., Liukkonen, K. H., Lahteenmaki, L., Siloaho, M., *et al*. (2010). A psyllium fiber-enriched meal strongly attenuates postprandial gastrointestinal peptide release in healthy young adults. *The Journal of Nutrition, 140*(4), 737-744. http://dx.doi.org/10.3945/jn.109.115436

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., & Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature, 402*(6762), 656-660. http://dx.doi.org/10.1038/45230

Kopin, A. S., Mathes, W. F., McBride, E. W., Nguyen, M., Al-Haider, W., Schmitz, F., *et al*. (1999). The cholecystokinin-A receptor mediates inhibition of food intake yet is not essential for the maintenance of body weight. *The Journal of Clinical Investigation, 103*(3), 383-391. http://dx.doi.org/10.1172/JCI4901

le Roux, C. W., Batterham, R. L., Aylwin, S. J., Patterson, M., Borg, C. M., Wynne, K. J., *et al*. (2006). Attenuated peptide YY release in obese subjects is associated with reduced satiety. *Endocrinology, 147*(1), 3-8. http://dx.doi.org/10.1210/en.2005-0972

Lee, Y. P., Mori, T. A., Sipsas, S., Barden, A., Puddey, I. B., Burke, V., *et al*. (2006). Lupin-enriched bread increases satiety and reduces energy intake acutely. *The American Journal of Clinical Nutrition, 84*(5), 975-980.

Liddle, R. A. (1997). Cholecystokinin cells. *Annual Review of Physiology, 59*, 221-242. http://dx.doi.org/10.1146/annurev.physiol.59.1.221

Liddle, R. A., Morita, E. T., Conrad, C. K., & Williams, J. A. (1986). Regulation of gastric emptying in humans by cholecystokinin. *The Journal of Clinical Investigation, 77*(3), 992-996. http://dx.doi.org/10.1172/JCI112401

MacIntosh, C. G., Andrews, J. M., Jones, K. L., Wishart, J. M., Morris, H. A., Jansen, J. B., *et al*. (1999). Effects of age on concentrations of plasma cholecystokinin, glucagon-like peptide 1, and peptide YY and their relation to appetite and pyloric motility. *The American Journal of Clinical Nutrition, 69*(5), 999-1006.

Maljaars, P. W., Peters, H. P., Mela, D. J., & Masclee, A. A. (2008). Ileal brake: A sensible food target for appetite control. A review. *Physiology & Behavior, 95*(3), 271-281. http://dx.doi.org/10.1016/j.physbeh.2008.07.018

Mohlig, M., Koebnick, C., Weickert, M. O., Lueder, W., Otto, B., Steiniger, J., *et al*. (2005). Arabinoxylan-enriched meal increases serum ghrelin levels in healthy humans. Hormone and Metabolic Research.Hormon- Und Stoffwechselforschung. *Hormones Et Metabolisme, 37*(5), 303-308. http://dx.doi.org/10.1055/s-2005-861474

Monteleone, P., Bencivenga, R., Longobardi, N., Serritella, C., & Maj, M. (2003). Differential responses of circulating ghrelin to high-fat or high-carbohydrate meal in healthy women. *The Journal of Clinical Endocrinology and Metabolism, 88*(11), 5510-5514. http://dx.doi.org/10.1210/jc.2003-030797

Najjar, A. M., Parsons, P. M., Duncan, A. M., Robinson, L. E., Yada, R. Y., & Graham, T. E. (2009). The acute impact of ingestion of breads of varying composition on blood glucose, insulin and incretins following first and second meals. *The British Journal of Nutrition, 101*(3), 391-398. http://dx.doi.org/10.1017/S0007114508003085

Naslund, E., Barkeling, B., King, N., Gutniak, M., Blundell, J. E., Holst, J. J., *et al*. (1999). Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men. *Journal of the International Association for the Study of Obesity, 23*(3), 304-311. http://dx.doi.org/10.1038/sj.ijo.0800818

Naslund, E., Bogefors, J., Skogar, S., Gryback, P., Jacobsson, H., Holst, J. J., *et al*. (1999). GLP-1 slows solid gastric emptying and inhibits insulin, glucagon, and PYY release in humans. *The American Journal of Physiology, 277*(3 Pt 2), R910-6.

Neary, N. M., Small, C. J., Druce, M. R., Park, A. J., Ellis, S. M., Semjonous, N. M., *et al*. (2005). Peptide YY3-36 and glucagon-like peptide-17-36 inhibit food intake additively. *Endocrinology, 146*(12), 5120-5127. http://dx.doi.org/10.1210/en.2005-0237

Nilsson, A. C., Ostman, E. M., Holst, J. J., & Bjorck, I. M. (2008). Including indigestible carbohydrates in the evening meal of healthy subjects improves glucose tolerance, lowers inflammatory markers, and increases satiety after a subsequent standardized breakfast. *The Journal of Nutrition, 138*(4), 732-739.

Nolan, L. J., Guss, J. L., Liddle, R. A., Pi-Sunyer, F. X., & Kissileff, H. R. (2003). Elevated plasma cholecystokinin and appetitive ratings after consumption of a liquid meal in humans. *Nutrition (Burbank, Los Angeles County, Calif.), 19*(6), 553-557. http://dx.doi.org/10.1016/S0899-9007(03)00039-X

Orskov, C., Wettergren, A., & Holst, J. J. (1996). Secretion of the incretin hormones glucagon-like peptide-1 and gastric inhibitory polypeptide correlates with insulin secretion in normal man throughout the day. *Scandinavian Journal of Gastroenterology, 31*(7), 665-670. http://dx.doi.org/10.3109/00365529609009147

Pasman, W. J., Blokdijk, V. M., Bertina, F. M., Hopman, W. P., & Hendriks, H. F. (2003). Effect of two breakfasts, different in carbohydrate composition, on hunger and satiety and mood in healthy men. *Journal of the International Association for the Study of Obesity, 27*(6), 663-668. http://dx.doi.org/10.1038/sj.ijo.0802284

Raben, A., Agerholm-Larsen, L., Flint, A., Holst, J. J., & Astrup, A. (2003). Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake. *The American Journal of Clinical Nutrition, 77*(1), 91-100.

Raben, A., Christensen, N., Madsen, J., Holst, J., & Astrup, A. (1994). Decreased postprandial thermogenesis and fat oxidation but increased fullness after a high-fiber meal compared with a low-fiber meal. *American Journal of Clinical Nutrition, 59*(6), 1386-1394.

Reeve, J. R.,Jr, Eysselein, V., Walsh, J. H., Ben-Avram, C. M., & Shively, J. E. (1986). New molecular forms of cholecystokinin. microsequence analysis of forms previously characterized by chromatographic methods. *The Journal of Biological Chemistry, 261*(35), 16392-16397.

Rehfeld, J. F. (2004). Clinical endocrinology and metabolism. cholecystokinin. Best Practice & Research. *Clinical Endocrinology & Metabolism, 18*(4), 569-586. http://dx.doi.org/10.1016/j.beem.2004.07.002

Rehfeld, J. F., & Hansen, H. F. (1986). Characterization of preprocholecystokinin products in the porcine cerebral cortex. evidence of different processing pathways. *The Journal of Biological Chemistry, 261*(13), 5832-5840.

Rehfeld, J. F., Sun, G., Christensen, T., & Hillingso, J. G. (2001). The predominant cholecystokinin in human plasma and intestine is cholecystokinin-33. *The Journal of Clinical Endocrinology and Metabolism, 86*(1), 251-258. http://dx.doi.org/10.1210/jc.86.1.251

Reimer, R. A., Pelletier, X., Carabin, I. G., Lyon, M., Gahler, R., Parnell, J. A., *et al*. (2010). Increased plasma PYY levels following supplementation with the functional fiber PolyGlycopleX in healthy adults. *European Journal of Clinical Nutrition, 64*(10), 1186-1191. http://dx.doi.org/10.1038/ejcn.2010.141

Reynolds, R. C., Stockmann, K. S., Atkinson, F. S., Denyer, G. S., & Brand-Miller, J. C. (2009). Effect of the glycemic index of carbohydrates on day-long (10 h) profiles of plasma glucose, insulin, cholecystokinin and ghrelin. *European Journal of Clinical Nutrition, 63*(7), 872-878. http://dx.doi.org/10.1038/ejcn.2008.52

Rosen, L. A., Silva, L. O., Andersson, U. K., Holm, C., Ostman, E. M., & Bjorck, I. M. (2009). Endosperm and whole grain rye breads are characterized by low post-prandial insulin response and a beneficial blood glucose profile. *Nutrition Journal, 8*, 42. http://dx.doi.org/10.1186/1475-2891-8-42

Schneeman, B. O., Burton-Freeman, B., & Davis, P. (2003). Incorporating dairy foods into low and high fat diets increases the postprandial cholecystokinin response in men and women. *The Journal of Nutrition, 133*(12), 4124-4128.

Sloth, B., Davidsen, L., Holst, J. J., Flint, A., & Astrup, A. (2007). Effect of subcutaneous injections of PYY1-36 and PYY3-36 on appetite, ad libitum energy intake, and plasma free fatty acid concentration in obese males. American Journal of Physiology. *Endocrinology and Metabolism, 293*(2), E604-9. http://dx.doi.org/10.1152/ajpendo.00153.2007

Stewart, M. L., Nikhanj, S. D., Timm, D. A., Thomas, W., & Slavin, J. L. (2010). Evaluation of the effect of four fibers on laxation, gastrointestinal tolerance and serum markers in healthy humans. *Annals of Nutrition & Metabolism, 56*(2), 91-98. http://dx.doi.org/10.1159/000275962

Tannous dit El Khoury, D., Obeid, O., Azar, S. T., & Hwalla, N. (2006). Variations in postprandial ghrelin status following ingestion of high-carbohydrate, high-fat, and high-protein meals in males. *Annals of Nutrition & Metabolism, 50*(3), 260-269.

Tarini, J., & Wolever, T. M. (2010). The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. *Nutrition Et Metabolisme, 35*(1), 9-16.

van der Lely, A. J., Tschop, M., Heiman, M. L., & Ghigo, E. (2004). Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocrine Reviews, 25*(3), 426-457. http://dx.doi.org/10.1210/er.2002-0029

Verdich, C., Flint, A., Gutzwiller, J. P., Naslund, E., Beglinger, C., Hellstrom, P. M., *et al*. (2001). A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans. *The Journal of Clinical Endocrinology and Metabolism, 86*(9), 4382-4389. http://dx.doi.org/10.1210/jc.86.9.4382

Vitaglione, P., Lumaga, R. B., Stanzione, A., Scalfi, L., & Fogliano, V. (2009). Beta-glucan-enriched bread reduces energy intake and modifies plasma ghrelin and peptide YY concentrations in the short term. *Appetite, 53*(3), 338-344. http://dx.doi.org/10.1016/j.appet.2009.07.013

Weickert, M. O., Mohlig, M., Koebnick, C., Holst, J. J., Namsolleck, P., Ristow, M., *et al*. (2005). Impact of cereal fibre on glucose-regulating factors. *Diabetologia, 48*(11), 2343-2353. http://dx.doi.org/10.1007/s00125-005-1941-x

Weickert, M. O., Spranger, J., Holst, J. J., Otto, B., Koebnick, C., Mohlig, M., *et al*. (2006). Wheat-fibre-induced changes of postprandial peptide YY and ghrelin responses are not associated with acute alterations of satiety. *The British Journal of Nutrition, 96*(5), 795-798. http://dx.doi.org/10.1017/BJN20061902

Willis, H. J., Thomas, W., Eldridge, A. L., Harkness, L., Green, H., & Slavin, J. L. (2010). Increasing doses of fiber do not influence short-term satiety or food intake and are inconsistently linked to gut hormone levels. *Food & Nutrition Research,* 54.

Wren, A. M., Seal, L. J., Cohen, M. A., Brynes, A. E., Frost, G. S., Murphy, K. G., *et al*. (2001). Ghrelin enhances appetite and increases food intake in humans. *The Journal of Clinical Endocrinology and Metabolism, 86*(12), 5992. http://dx.doi.org/10.1210/jc.86.12.5992

Table 1. Studies measuring effect of fiber on PYY

Ref	N	X/P	C/A	Fiber Type	Fiber Dose	PYY Increase vs. Control
Vitaglione 2009	14	X	A	barley β-glucan concentrate	3 g	Yes
Beck 2009	14	X	A	oat β-glucan	2.2 g	No
				oat β-glucan	3.8 g	No
				oat β-glucan	5.5 g	Yes (2-4 h after test meal)
Beck 2010	66	P	C	β-glucan	5-6 g/d x 3 months	No (fasting values)
				β-glucan	8-9 g/d x 3 months	No (fasting values)
Juvonen 2010	20	X	A	wheat bran	10 g	No
				oat bran	10 g	No
				wheat bran + oat bran	5 g each	No
Weickert 2006	14	X	A	wheat fiber	10.5 g	No
				oat fiber	10.6 g	No
Willis 2010	20	X	A	mixed fiber	4 g	No
				mixed fiber	8 g	No
				mixed fiber	12 g	No
Karhunen 2010	16	X	A	psyllium + low protein	7.6 g	No
				psyllium + low protein	27 g	Yes
				psyllium + high protein	6.2 g	No
				psyllium + high protein	25.8	Yes
Cani 2009	10	X	C/A	inulin/oligofructose blend	16 g x 2 wks	Yes (but only at 10 min after standardized non-fiber meal on day 14)
Reimer 2010	54	P	C	Functional fiber blend	5 g/d x 1 wk, then 10 g/d x 2 wks	Yes (in BMI <23; values not different from baseline)

A, acute intake; C, chronic intake; C/A, chronic intake, acute meal challenge; P, parallel design; X, crossover design

Table 2. Studies measuring effects of fiber on GLP-1

Ref	N	X/P	A/C	Fiber Type	Fiber Dose	GLP-1 Increase vs. Control
Beck 2010	66	P	C	β-glucan	5-6 g/d x 3 months	No (fasting values)
				β-glucan	8-9 g/d x 3 months	No (fasting values)
Willis 2010	20	X	A	mixed fiber	4 g	No
				mixed fiber	8 g	No
				mixed fiber	12 g	No
Karhunen 2010	16	X	A	psyllium + low protein	7.6 g	No
				psyllium + low protein	27 g	No
				psyllium + high protein	6.2 g	No
				psyllium + high protein	25.8	No
Cani 2009	10	X	C/A	inulin/oligofructose blend	16 g x 2 wks	Yes (but only at 10 min after standardized non-fiber meal on day 14)
Reimer 2010	54	P	C	functional fiber blend	5 g/d x 1 wk, then 10 g/d x 2 wks	No (fasting values)
Nilsson 2008	15	X	A	ordinary barley	20.2 g	Yes
				cut ordinary barley	19.4 g	No
				ordinary barley	9.9 g	No
				high amylose barley	38.1 g	No
				high b-glucan barley	81 g	No
				resistant starch	11.5	No
Najjar 2009	10	X	A	whole wheat bread	6.3 g	Yes
				whole wheat barley bread	5.5 g	No
Juntunen 2002	20	X	A	whole kernel rye	12.8 g	No
				whole meal rye with oat b-glucan concentrate	17.1 g	Yes (but only at 120 and 150 min after meal)
				dark durum wheat pasta	5.6 g	No
Juntunen 2003	19	X	A	endosperm rye	6.1 g	No
				whole-meal rye	15.2 g	No
				whole-meal rye enriched with rye bran	29 g	Yes (but only at 150 and 180 min after meal)
Weickert 2005	14	X	A	wheat fiber	10.5 g	No
				oat fiber	10.6 g	No
Bakhoj 2003	11	X	A	ancient wheat Einkorn	4-6 g	No
Frost 2003	10	X	A	psyllium	1.7 g	No
				psyllium + fat	1.7g	Yes
Raben 1994	10	X	A	pea fiber	25.5 g	No
Adam 2005a	58	X	A	guar gum (+galactose)	2.5 g	Yes (but vs. water; important kcal difference)
Adam 2005b	30	X	A	guar gum (+galactose)	2.5 g	Yes (but vs. water; important kcal difference)
Tarini 2010	12	X	A	inulin (+HFCS)	24 g	No

A, acute intake; C, chronic intake; C/A, chronic intake, acute meal challenge; HFCS, high fructose corn syrup; P, parallel design; X, crossover design

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Table 3. Studies measuring effect of fiber on CCK

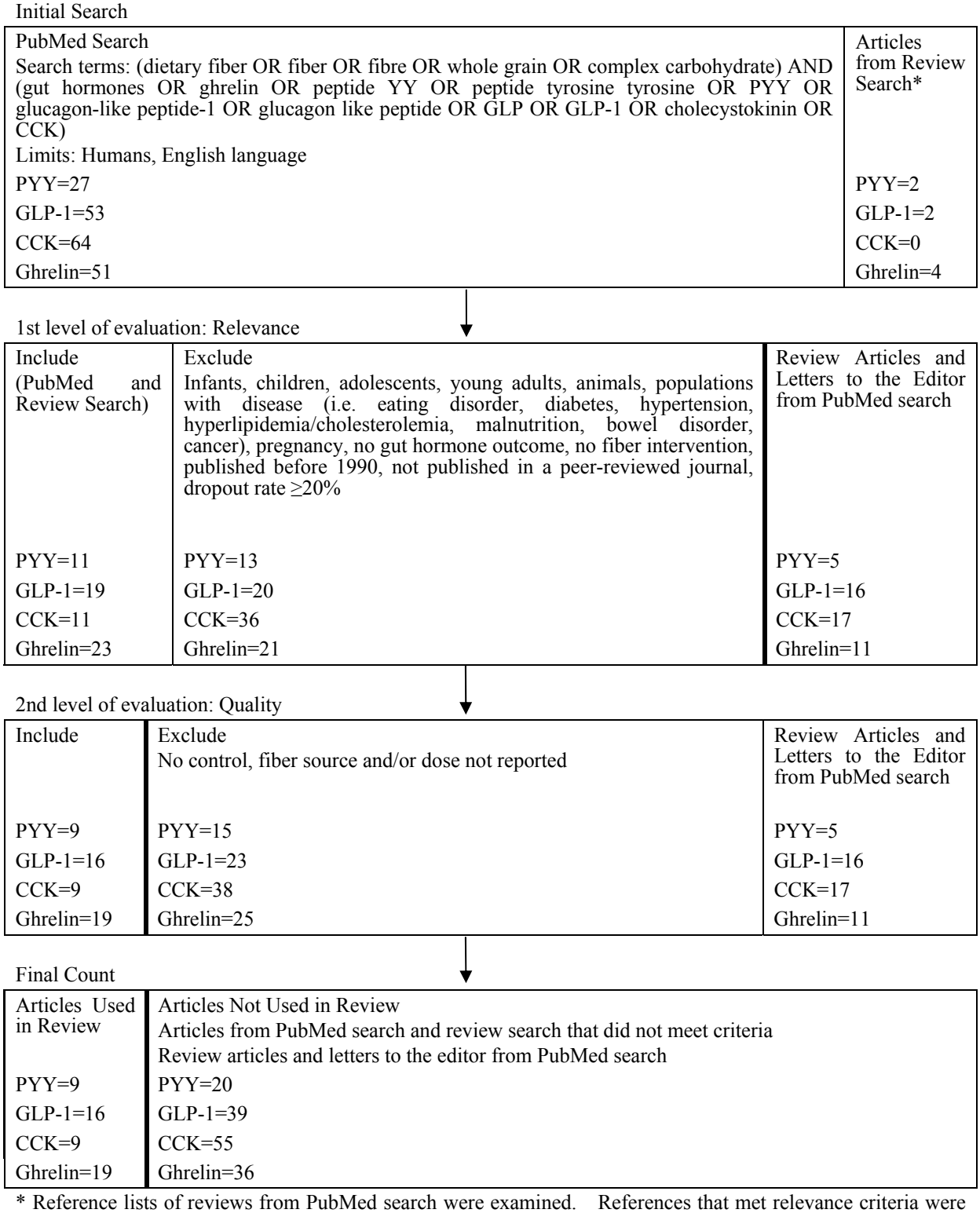
Ref	N	X/P	C/A	Fiber Type	Fiber Dose	CCK Increase vs. Control
Beck 2009	14	X	A	β-glucan	2.16 g	No
				β-glucan	3.82 g	No
				β-glucan	5.45 g	No
				β-glucan + oat β-glucan concentrate	5.65 g	No (Dose response in women)
Beck 2010	66	P	C	β-glucan	5-6 g/d x 3 months	No (fasting values)
				β-glucan	8-9 g/d x 3 months	No (fasting values)
Burton-Freeman 2008	16	X	A	oat bran	20 g	Yes (women only)
Bourdon 1999	11	X	A	β-glucan enriched fraction of barley flour	15.7 g, including 5g β-glucan	No (elevated above baseline for 6 hrs vs. 3 hrs in ctl.)
				barley flour naturally high in β-glucan	15.7 g, including 5g β-glucan	No
Heini 1998	25	X	C	partially hydrolyzed guar gum	20 g	No
Bourdon 2001	10	X	A	bean flakes	12 g	Yes
Pasman 2003	26	X	A	complex carbohydrate	6.7 g	No (vs. low fiber, simple carbohydrate meal)
Burton-Freeman 2008	22	X	A	low glycemic index meal	35.5 g	No
Reynolds 2009	12	X	A	low glycemic index meal	30 g	Yes (vs. high glycemic meal with equal fiber)

A, acute intake; C, chronic intake; C/A, chronic intake, acute meal challenge; P, parallel design; X, crossover design

Table 4. Studies examining effects of fiber on ghrelin

Ref	N	X/P	C/A	Fiber Type	Fiber Dose	Ghrelin Decrease vs. Control
Vitaglione 2009	14	X	A	barley β-glucan concentrate	3 g	Yes (AUC60-180)
Beck 2009	14	X	A	β-glucan	2.16 g	No
				β-glucan	3.82 g	No
				β-glucan	5.45 g	No
				β-glucan + oat β-glucan	5.65 g	No
Beck 2010	66	P	C	β-glucan	5-6 g/d x 3 months	No (fasting values)
				β-glucan	8-9 g/d x 3 months	No (fasting values)
Juvonen 2010	20	X	A	wheat bran	10 g	No
				oat bran	10 g	No
				wheat bran + oat bran	5 g each	No
Weickert 2006	14	X	A	wheat fiber	10.5 g	No
				oat fiber	10.6 g	No
Willis 2010	20	X	A	mixed fiber	4 g	No
				mixed fiber	8 g	No
				mixed fiber	12 g	No
Karhunen 2010	16	X	A	psyllium + low protein	7.6 g	No
				psyllium + low protein	27 g	No
				psyllium + high protein	6.2 g	No
				psyllium + high protein	25.8	No
Reimer 2010	54	P	C	functional fiber blend	5 g/d x 1 wk, then 10 g/d x 2 wks	No
Tarini 2010	12	X	A	inulin (+HFCS)	24 g	Yes (after a lunch 4-6 hours after the test meal)
Reynolds 2009	12	X	A	low glycemic index meal	30 g	No
Gruendel 2006	20	X	A	carob fiber (in mixed meal)	5 g	Yes (acylated only)
				carob fiber (in mixed meal)	10 g	Yes (acylated only)
				carob fiber (in mixed meal)	20 g	Yes (acylated only)
Gruendel 2007; 98(1)	20	X	A	carob fiber (in glucose water)	5 g	No
				carob fiber (in glucose water)	10 g	No
				carob fiber (in glucose water)	20 g	No
Gruendel 2007; 98(6)	19	X	A	carob fiber	45 g	No
Mohlig 2005	11	X	A	Arabinoxylan	6 g	No
Rosen 2009	12	X	A	Endosperm rye bread	6.7 g	No
				Whole grain rye bread	9.6 g	No
				Rye bran bread	12.3g	No
				Endosperm rye porridge	6.5 g	No
				Whole grain rye porridge	10.1 g	No
Lee 2006	17	X	A	lupin kernel	15 g	Yes
Furchner-Evanson 2010	19	X	A	fiber from dried plums	6 g	Yes (but only at 15 and 30 min after meal)
Blom 2005	20	X	A	low kcal meal (fiber from fruit)	14 g	No
				high kcal, simple carbohydrate	12 g	No
				high kcal, complex carbohydrate	12 g	No
Stewart 2010	20	X	C	pullulan	12 g/d x 2 wks	No (fasting values)
				resistant starch	12 g/d x 2 wks	No (fasting values)
				soluble fiber dextrin	12 g/d x 2 wks	No (fasting values)
				soluble corn fiber	12 g/d x 2 wks	No (fasting values)

A, acute intake; C, chronic intake; C/A, chronic intake, acute meal challenge; HFCS, high fructose corn syrup; P, parallel design; X, crossover design



* Reference lists of reviews from PubMed search were examined. References that met relevance criteria were included and later examined for quality

Figure 1. Search process and selection criteria diagram

Behavior of *Brucella abortus* and *Brucella melitensis* in Raw Meatball (Cig Kofte)

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Abstract

In the present study, we aimed to determine the survival and reproducibility of the said pathogenic microorganisms during the storage period (24 h) of raw meatball samples contaminated with reference strains of *Brucella abortus* and *Brucella melitensis* at levels of 10^4 and 10^6 cfu/g. *Brucella abortus* (NCTC 11363) and *Brucella melitensis* (NCTC 10094) strains were incubated in sterile full-cream milk with 12 % fat at 37°C for 48 hours under a 6% CO_2 aerobic atmosphere. Subsequent to the incubation, culture counts were performed on Brucella Agar Base using tryptose soy broth. As a result during our analysis at the 12th hour of the storage, it was noted that while pH remained 5.2, aw 0.96 and moisture %52.16, population of *B. abortus* in-group A2 rose from 10^4 cfu/gr to 10^5 cfu/gr ($P<0.05$). At hour 24, pH was found 5.1, aw 0.92 and moisture % 49.07 whereas population of *B. abortus* decreased to 10^5 cfu/gr in-group A1 and to 10^4 cfu/gr in-group A2 ($P<0.05$). In-group B1, there was no change in the number of *B. melitensis*. It was also observed at hour 24 population of *B. melitensis* in-group B2 increased to 10^5 cfu/gr ($P<0.05$).

Keywords: *Brucella abortus*, *Brucella melitensis*, Meat ball, Cig kofte, Traditional meat product

1. Introduction

Çiğ köfte (raw meatball) is a traditional meat dish/delicacy that is usually made and served as an appetizer in Turkey, particularly in Southeastern Anatolia, on special occasions and at communal events (Yıldırım *et al*, 2005). Since no standards exist for the preparation of çiğ köfte, the ingredients and amount of content vary depending on preference. It is a meat product that doesn't undergo any thermal treatment and is consumed raw. It is produced by kneading finely ground lean mutton or beef, with a mixture of bulgur (fine wheat groats), onion, garlic, paste, parsley, and spices (powdered isot, red pepper, black pepper, cinnamon, clove, cumin, mint) with water or ice (Uzunlu & Yıldırım, 2003; Yıldırım *et al*, 2005).

Studies conducted in Turkey on raw meatballs (Sagun *et al.*, 1997a; Kuplulu & Sarımehmetoglu, 2003; Cetin *et al.*, 2008), have revealed that microbiological quality of the food appears hardly acceptable for human consumption and that it poses a significant threat to public health.

Brucellosis is a zoonotic infection, which has spread across many countries and remains endemic in developed ones, too (WHO, 2006). It is reported by the World Health Organisation (WHO) that an estimated 500,000 new human cases occur annually worldwide (Pappas *et al.*, 2006). Brucellosis is endemic almost in all regions of the world, including the Mediterranean countries such as Spain, Portugal, Southern France, Italy, Greece, Turkey; North Africa, the Near East, India, Mexico, and Central and South America (Yüce & Çavuş, 2006). For humans, the contraction of the disease arises from intake of raw or unpasteurized infected milk or dairy products. However inhalation of contaminated dust, and contact with infected carcasses, and uterine contents and discharges (Chahota *et al*., 2003), and via consumption of meat and meat products (Robinson *et al.*, 2000).

In the present study, we aimed to determine the survival and reproducibility of the said pathogenic microorganisms during the storage period (24 h) of raw meatball samples contaminated with reference strains of *Brucella abortus* (NCTC 11363) and *Brucella melitensis* (NCTC 10094) at levels of 10^4 and 10^6 CFU/g, and to discover whether they pose a threat to public health or not.

2. Material and Methods

2.1 Preparation of raw meatballs

Raw Meatballs were made by mixing and kneading ground beef and bulghur, each ingredient in equal amounts of 2 kgs, powdered cumin, red pepper, black pepper, garlic, onion, parsley, tomato paste, and salt, based on the method as prescribed by Durmaz *et al*. (2007). The mixture was hand shaped into small balls each weighting approximately 25 g and stored at 4 ^oC for 24 hours. All çiğ köfte samples were analyzed in duplicate after storage for 0, 6, 12 and 24 h.

2.2 Microbiological analysis

2.2.1 Preparation and Inoculation of Test Strains

Brucella abortus (NCTC 11363) and *Brucella melitensis* (NCTC 10094) strains were obtained from Refik Saydam National Publich Health Agency and incubated in sterile full-cream milk with 12 % fat at 37°C for 48 hours under a 6% CO_2 aerobic atmosphere. Subsequent to the incubation, culture counts were performed on Farrell's Agar plates (Oxoid CM 169; Brucella Selective Supplement Oxoid SR 83) using tryptose soy broth as diluent in the range up to $10^{-9.}$ The amount derived from the initial solutions inoculated showed that it could contaminate 1 g of raw meatball at levels of 10^4 cfu/g and 10^6 cfu/g. After the samples were inspected for any presence of *Brucella abortus* and *Brucella melitensis,* they were contaminated with strains of *B. abortus* and *B. melitensis* at levels of 10^4 cfu/g and 10^6 cfu/g (Estrada *et al*., 2005). The prepared samples (A1: 10^6 cfu/g *B. abortus*; A2: 10^4 cfu/g *B. abortus*; B1: 10^6 cfu/g *B. melitensis*; B2: 10^4 cfu/g *B. melitensis*) were then stored at +4^oC for 24 hours.

2.2.2 Sampling and Preparation of Dilutions

Throughout the storage period, samples were taken from all raw meatballs at hours 0, 6, 12, and 24. 10 g specimens were weighed out into sterile Stomacher bags, and 90 ml of sterile peptone saline (% 0,85 NaCl + % 0,1 peptone) was added to each container. The samples were immediately homogenized in the stomacher (Interscience, UK) for 2 minutes and were diluted ten-fold. Following the homogenization, serial decimal dilutions were performed until reaching 10^{-7} (Pichhardt, 1993).

2.2.3 Enumeration of Total Mesophilic Aerobic Bacteria and Lactic Acid Bacteria

The dilutions prepared were planted in Plate Count Agar (PCA, Oxoid, CM325 – at 32^oC for 48 h) to count the population of mesophilic aerobic bacteria, and in Man Rogasa Sharpe Agar (MRS, Oxoid, CM 361 – pH:5.7- at 35^oC for 48 h, anaerobic) to count lactic acid bacteria (Pichhardt, 1993).

2.2.4 Enumeration of *Brucella abortus* ve *Brucella melitensis*

25 g samples were weighed out from each raw meatball specimen under aseptic conditions and placed into sterile stomacher bags. 225 ml of BPW (Buffered Peptone Water, Oxoid CM0509) was added and homogenized. After being blended in the stomacher, the decimal dilutions obtained from homogenized samples were diluted ten-fold in BPW, being prepared until reaching $10^{-9.}$ The samples were plated onto Farrell's agar (Oxoid CM 169; Brucella Selective Supplement Oxoid SR 83) by means of spread plate technique. The petri dishes planted were subjected to incubation at 37°C for 48 hours under a % 6 CO_2 environment. At the end of the incubation,

colonies with typical *Brucella* were enumerated and justified through agglutination tests (*B. abortus* antisera Difco 2871-47-7, *B. melitensis* antisera Difco 2889-47-7) (Estrada *et al*., 2005).

2.3 Chemical analysis

pH values of the sampled raw meatballs were measured by means of pH metre (InoLab pH 720 model, Germany), based on method as prescribed by Troller and Scott (1992). Determination of moisture was achieved by AOAC (1990), and water activity (aw) values were analyzed according to the method by Rodel *et al.* (1975).

2.4 Statistics analysis

The data obtained from two replications were analysed by ANOVA using the SPSS statistical package program and differences among the means were compared using Duncan's Multiple Range test.

3. Result

Survival and reproducibility of the pathogenic microorganisms in raw meatball samples (A1: 10^6 cfu/g *B. abortus*, A2: 10^4 cfu/g *B. abortus*, B1: 10^6 cfu/g *B. melitensis*, B2: 10^4 cfu/g *B. melitensis*) contaminated with reference strains of *Brucella abortus* (NCTC 11363) and *Brucella melitensis* (NCTC 10094) at levels of 10^4 and 10^6 CFU/g can be seen in Table 1. During our analysis at the 6th hour of the storage, no change was recorded in the values of pH 5.3, aw 0.98 and moisture 64.10% as well as in numbers of *B. abortus* and *B. Melitensis* in all four groups. However, at the 12th hour of the storage, it was noted that while pH remained 5.2, aw 0.96 and moisture 52.16%, population of *B. abortus* in-group A2 rose from 10^4 cfu/gr to 10^5 cfu/gr ($P<0,05$). At hour 24, pH was found 5.1, aw 0.92 and moisture 49.07% whereas population of B. *abortus* decreased to 10^5 cfu/gr in group A1 and to 10^4 cfu/gr in group A2. In group B1, there was no change in the number of *B. melitensis*. It was also observed at hour 24 that although values for pH (5.1), aw (0.92) and moisture (%49.07) dropped, population of *B. melitensis* in group B2 increased to 10^5 cfu/gr ($P<0,05$).

4. Discussion

In the present study, we aimed to determine the survival and reproducibility of the said pathogenic microorganisms during the storage period (24 h) of çiğ köfte samples contaminated with reference strains of *Brucella abortus* (NCTC 11363) and *Brucella melitensis* (NCTC 10094) at levels of 10^4 and 10^6 CFU/g, and to discover whether they pose a threat to public health or not.

Studies carried out have showed that 54.7% of total food poisoning cases are caused by the consumption of meat and meat products (Farber *et al*., 1989). A major epidemiological factor in contracting the disease lies in the fact that in certain societies people are in the habit of consuming raw meat, eg raw liver or other offal with spices. (Syrjamaki *et al.*, 1984). Following slaughter, micro-organisms, which cause foodborne infection and intoxication in humans, are transmitted to the end product as a result of crosss-contamination during carcass boning/cutting, processing, packing, and storage, thereby posing a threat to consumer health. Researchers have reported that infected lymph nodes are particularly noted for their being the most likely source for endogenic contamination of ground meat (Erol, 1999a). Due to its texture and processing techniques, ground meat turns out to be the leading meat product suitable for microbial contamination (Sinell, 1992).

Under the present study, in our analysis on the sample raw meatballs at the 6th hour of the storage, the original values of pH 5.3, aw 0.98 and moisture % 64,10 showed no change. Also, numbers of *B. abortus* and *B. Melitensis* didn't alter in any of the four groups: A1, A2, B1 and B2. At hour 12 of the storage, however, it was noted that while pH remained 5.2, aw 0.96 and moisture 52.16%, population of *B. abortus* in group A2 rose from 10^4 cfu/gr to 10^5 cfu/gr. Our measurement at the 24th hour revealed pH 5.1, aw 0.92 and moisture % 49.07, whereas population of *B. abortus* decreased to 10^5 cfu/gr in group A1 and to 10^4 cfu/gr in that of A2. In group B1, no change was noted in the number of *B. melitensis*. It was also observed that although values for pH (5.1), aw (0.92) and moisture (49.07%) dropped, population of *B. melitensis* in group B2 increased to 10^5 cfu/gr.

Optimum pH value for the reproduction of *Brucella* spp. is 6,6-7,4, with maximum 8.7 and minimum 5.8 (Frobisher, 1968). However, various studies have yielded results which exhibit that Brucella spp can somehow survive even at lower pH values in different foods. Robinson *et al.* (2000), reported that *Brucella abortus* managed to survive for 34 days in the milk set at pH 5.0-5.8 by means of lactic acid, and for 2 days at pH 3.9. In the study carried out by Estrada *et al.* (2005), sterile skim milk was inoculated with *Brucella abortus* at 10^5 cfu/g with a yoghurt starter culture of lactic acid bacteria, and was incubated at $+4^oC$. Their results demonstrated that after 10 days of storage at 4 degrees C, B. abortus was recovered in fermented milk at a level of 10^5 cfu/g , despite the low pH value below 4.0. Ozturk and Nazli (1996), have reported that they experimentally contaminated sheep cheese and cow's cheese stuffed into sheepskin bags with *Brucella melitensis* at 10^9 cfu/g , and that the pathogen decreased to 10^2 cfu/g in both cheese varieties on the 21st day of the maturation. However,

Brucella melitensis couldn't be isolated at pH 5.0 in either samples on the 30th day of the maturation. Researchers have also documented that following contamination, *Brucella* spp. managed to survive for 14 days in chilled meat (Taşkın, 2007), and for several years in frozen tissues and organs (Altekruse *et al.*, 1998).

This experimental study demonstrated that while levels of reference strains *B. abortus* and *B. melitensis* in raw meatball samples stored for 24 hours at +4 ^{o}C did not change significantly, the total population of mesophilic aerobic bacteria (10^7 cfu/g) increased (Table 1). When the literature was screened, no similar experimental study related to meat and meat products occurred. As to experimental studies on milk and dairy products, while Ozturk and Nazlı (1996) observed that *brucellae* in cheese were inhibited at pH 5.0, Robinson *et al.* (2000) and Estrada *et al.* (2005), found that they were not inhibited even at lower pH levels (3.9 and 4.0). In the present study, at 24-hour storage period, pH ranged between 5.3 - 5.1, and *B. abortus* and *B. melitensis* were not inhibited at pH 5.1. The fact that *B. abortus* and *B. melitensis* survive for different time periods or is inhibited at different pH levels may depend on products' composition; amount of moisture, protein and fat content; their texture; and storage period and conditions.

Çiğ köfte is a traditional and popular meat dish/delicacy throughout Turkey; however, since it is consumed raw, it gives rise to the spread of various pathogenic bacteria as well as *Brucella* outbreaks and poses a major threat to public health. Indeed, it has been documented by different investigators in Turkey that sanitary quality of ground meat, which is the basic ingredient for making çiğ , köfte (Tekinşen *et al.*, 1980; Yetim, 1985; Akın & Kaya, 1988; Sancak *et al.*, 1993; Ciftcioglu & Ugur, 1992; Guven *et al.*, 1997; Erol, 1999b; Sireli & Erol, 1999), as well as the spices used (Sagun *et al*., 1997b; Erol *et al*., 1999; Vural, 2004) is hazardous for public health.

Antimicrobial effect of spices added to çiğ köfte is not powerful enough to eliminate the risk of pathogenic microorganisms. Because the antibacterial effect of spices such as isot and black pepper used in the preparation of çiğ köfte is limited, population of microorganisms doesn't undergo any significant change under different temperatures and periods (Uzunlu & Yıldırım, 2003). Experimental studies have demonstrated that many pathogenic bacteria can sustain their survival and are not inhibited in çiğ köfte environments (Sagun *et al*., 2003; Uzunlu & Yıldırım, 2003; Sireli *et al*., 2008). Therefore, the folk belief that added spices will thoroughly kill the pathogenic microorganisms in raw meatballs remains only a myth.

Brucellosis remains a major public health issue in Turkey. Varying rates of Brucella prevalance among humans have been noted by researchers in our country (Durmaz *et al*., 1997; Altındiş, 2001; Sumer *et al*., 2003; Atmaca *et al*., 2004; Cetinkaya *et al*., 2005; Demirturk *et al*., 2008). In Turkey, brucellosis is a particularly common problem in dairy cattle (Gokcen and Eskiizmirliler, 1998; Iyisan *et al*., 2000; Solmaz *et al*., 2002; Ceylan *et al*., 2003) and in sheep (Gokcen & Eskiizmirliler, 1998; Muz *et al*., 1999; Iyisan *et al*., 2000; Ongor *et al*., 2001; Ceylan *et al*., 2003). It is unfortunate that sale and slaughtering of animals suffering from brucella still continues in abattoirs due to negligence and lack of proper inspection at livestock markets and slaughterhouses. Meat and ground meat obtained from such animals may end up at points of sale, posing a risk for public health. Fearing possible health risks, some consumers have, in recent years, turned to çiğ köfte completely made with bulghur as a replacement for ground meat. We suggest that that a more pro-active and pre-emptive policy should be adopted to eradicate brucella infections, which is especially widespread in dairy cattle and sheep in Turkey; that procedures and practices like HACCP and GMP should be rigorously implemented during the production phase of meat and meat products by following the "from farm to table" continuum; that all the community working in the field of animal husbandry should be trained; that alternative methods should be taken up for making this traditional meat product, such as preparing çiğ köfte without adding any ground meat, or exposing it to thermal treatment in a way that will not spoil its characteristics; that different methods, such as food irradiation techniques should be developed to enhance the microbiological quality of çiğ köfte; and that training courses and seminars should be organized for çiğ köfte producers so that awareness could be raised among them.

References

Akın, A., & Kaya, B. (1988). Ankara'da Satılan Etlerin Mikrobiyolojik Kalite Kontrolü, 1-Kıymaların Mikrobiyolojik Kalite Kontrolü. *Doğa Tıp ve Ecz. Derg, 12*, 183-189.

Altekruse, S. F., Timbo, B. B., & Mawbroyi, J. C. (1998). Cheese-associated outbreaks of human Ilness in the United States. *Journal of Food Protection, 61*(10), 1405-1407.

Altındiş, M. (2001). Afyon Bölgesi Besicilerinde, Kasaplarda, Süt Ürünleri Toplayıcısı, imalathanelerinde Çalışanlarda Bruselloz Seropozitifliği. *İnfeksiyon Dergisi, 15*, 11-15.

AOAC. (1990). Method 926.08, 925.09. Association of Official Analytical Chemists. Official Methods of Analysis. (15th ed). AOAC, Arlington: USA.

Arslan, A., Guven, A., Saltan, S., & Patir, B. (1992). Microbiologic quality of raw meat balls in Elazig. Firat University, *Health Science Journal*, 6, 13-18.

Atmaca, S., Özekinci, T., Akpolat, N., Elçi, S., Suay, A., & Asıkan, E. (2004). Brucellosis Seroprevalance in Southeast Turkey (Diyarbakır). *Turkish Journal of Medical Sciences*, *34*, 251-255.

Cetin, O., Bingol, E. B., & Akkaya, H. (2008). The Microbiological, Serological and Parasitological Quality of Cig Kofte (Raw Meatball) and Its Lettuce Marketed in Istanbul Polish. *Journal of Environmental Studies, 17*, 701-706.

Çetinkaya, Z., Aktepe, O. C., Çiftci, I. H., & Demirel, R. (2005). Seroprevalence of Human Brucellosis in a Rural Area of Western Anatolis, Turkey. *Journal of Health Population and Nutrition, 23*, 137-141.

Ceylan, E., Irmak, H., Buzğan, T., Karahocagil, M. K., Evirgen, Ö., Sakarya, N., Akdeniz, H., & Demiröz, A. P. (2003). Van İline Bağlı Bazı Köylerde İn san ve Hayvan Populasyonunda Bruselloz Seroprevalansı. *Van Tıp Dergisi, 10*, 1-5.

Chahota, R., Sharma, M., Katoch, R. C., Verma, S., Singh, M. M., Kapoor, V., & Asrani, R. K. (2003). Brucellosis outbreak in an organized dairy farm involving cows and in contact human beings, in Himachal Pradesh, India. *Veterinarski Arhiv, 73*, 95-102.

Çiftçioğlu, G., & Uğur, M. (1992). Kıyma, sucuk ve tavuk etlerinde *Listeria monocytogenes* kontaminasyonu, *İstanbul Üniversitesi Veteriner Fakültesi Dergisi, 18*, 33-44.

Demirturk, N. T., Demirdal, N., Erben, S., Demir, Z., & Asci, T. P. (2008). Brucellosis: A retrospective evaluation of cases and review of Brucellosis treatment. *Tropical Doctor, 38*, 59-62. http://dx.doi.org/10.1258/td.2006.006266.

Durmaz, H., Sagun, E., Sancak, H., & Sagdic, O. (2007). The fate of two *Listeria monocytogenes* serotypes in ‘‘cig kofte’’ at different storage temperatures. *Meat Science, 76*, 123-127. http://dx.doi.org/10.1016/j.meatsci.2006.10.020.

Durmaz, R., Durmaz, B., Rafiq, M., & Sönmez, E. (1997). Seropositivity of Brucellosis in Malatya, Turkey. *Turkish Journal of Medical Sciences, 27*,125-128.

Erol, İ. (1999a). Hayvansal gıdalardan kaynaklanan Salmonella İnfeksiyonları. *İnfeksiyon Dergisi, 13*, 123-127.

Erol, İ. (1999b). Ankara’da tüketime sunulan kıymalarda Salmonellaların varlığı ve serotip dağılımı. *Turkish Journal of Veterinary and Animal Sciences, 23*, 321-325.

Erol, İ., Küplülü, Ö., & Karagöz, S. (1999). Ankara’da tüketime sunulan bazı baharatın mikrobiyolojik kalitesi. *Ankara Veteriner Fakültesi Dergisi, 46*, 115-125.

Estrada, A. Z., De La Garza, L. M., Mendoza, M. S., Lopez, E. M. S., Kerstupp, S. F., & Merino, A. L. (2005). Survival of Brucella abortus in milk fermented with a yoghurt starter culture. *Revista Latinoamericana de Microbiologia, 47*, 88-91.

Farber, J. M., Sanders, G. W., & Johnston, M. A. (1989). A survey of various foods for the presence of Listeria species. *Journal of Food Protection, 52*, 456-458.

Frobisher, M. (1968). *Fundamentals of Microbiology (*8th ed). Press of W.B. Saunders company, Philadelphia, London: Toronto. 740.

Gökcen, S., & Eskiismirliler, S. (1998). Bornova Veteriner Kontrol Ve Araştırma Enstitüsü’ne 1988-1997 Yılları Arasında Ege Bölgesi İllerinden Gönderilen Sığır, Koyun Ve Keçi Kan Serumlarında *Brucella* Pozitiflik Oranı. *BornovaVeteriner Kontrol ve Araştırma Enstitüsü Müdürlüğü Dergisi, 23*, 1-10.

Güven, A., Gülmez, M., & Kamber, U. (1997). Kars ilinde tüketime sunulan kıymalarda bazı patojen mikroorganizmaların araştırılması ve kıymaların mikrobiyolojik kalitesinin belirlenmesi. *Kafkas Universitesi Veteriner Fakultesi Dergisi, 3*, 57-65.

İyisan, A. S., Akmaz, O., Duzgun, S. G., Ersoy, Y., Eskiizmirliler, S., & Guler, L. (2000). Sero-epidemiology of Brucellosis on cattle and sheep in Turkey. *Journal of Pendik Veterinary Microbiology, 31*, 21-75.

Küplülü, Ö., & Sarımehmetoğlu, B. (2003). The Microbiological Quality of Çiğ Köfte Sold in Ankara. *Turkish Journal of Veterinary and Science, 27*, 325-329.

Muz, A., Ertaş, H. B., Öngör, H., Gülcü, H. B., Özer, H., Eröksüz, H., Dabak, M., Başbuğ, O., & Kalender H. (1999). Elazığ ve Çevresinde Koyun ve Keçilerde Abortus Olgularının Bakteriyolojik, Serolojik ve Patolojik Olarak İncelenmesi. *Journal of Veterinary and Animal Sciences, 23*, 177-188.

Nielsen, K. H., Kelly, L., Gall, D., Balsevicius, S., Basse, J., Nicoletti, P., & Kelly, W. (1996). Comparison of enzyme immunoassays for the diagnosis of bovine Brucellosis. *Preventive Veterinary Medicine, 26*, 17-32.

Ocal, H. M. (1997). *Ozellikleri ve Guzellikleriyle Cig Koftemiz*. Ozlem Kitabevi. Sanliurfa. Turkey.

Öngör, H., Muz, A., & Çetinkaya, B. (2001). Atık yapmış koyunlarda Brucellozis'in teşhisinde ELISA ile diğer serolojik testlerin karşılaştırılması. *Turkish Journal of Veterinary Animal Sciences, 25*, 21-26.

Öztürk, G. Y. & Nazlı, B. (1996). Deneysel olarak enfekte edilen sütte yapılan tulum peynirlerinde *Brucella melitensis*'in mevcudiyeti üzerine araştırmalar. *Pendik Veteriner Mikrobiyolojisi Dergisi, 27*, 123-142.

Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L., & Tsianos, E. V. (2006). The new global mab of human Brucellosis. *Lancet Infectious Diseases, 6*, 91-99. http://dx.doi.org/10.1016/S1473-3099(06)70382-6

Pichhardt, K. (1993). *Lebensmittel-Mikrobiologie*. Springer Verlag: Berlin.

Robinson, R. K., Batt, C. A., & Patel, P. D. (2000). *Encycclopedia of food Microbiology*. In Brucella. (ed) Academic pres., London, *1*, 319-327.

Rödel, W., Panert, H., & Leistner, L. (1975). Verbessertes aw-Wert-Messer zur Bestimmung der Wasseraktivitat von Fleisch und Fleisch Waren. *Fleischwirtschaft, 4*, 557-558.

Sagun, E., Alişarlı, M., & Durmaz, H. (2003). Farklı Sıcaklıklarda Muhafazanın Çiğ Köftede *Staphylococcus aureus*'un Gelişimi ve Enterotoksin üretimi üzerine Etkisi. *Turkish Journal of Veterinary Animal Sciences, 27*, 839-845.

Sagun, E., Sancak, Y. C., Durmaz, H., & Akkaya, L. (1997a). A study on hygienic quality of raw meat balls consumed in Van. *Yuzuncu Yil University Veterinary Faculty Journal, 3*, 64-67.

Sagun, E., Sancak, Y. C., Durmaz, H., & Ekici, K. (1997b). Van'da tüketime sunulan baharatların mikrobiyolojik kalitesi, *Yuzuncu Yil University Veterinary Faculty Journal, 8*, 1-5.

Sancak, Y. C., Boynukara, B., & Ağaoğlu, S. (1993). Van'da Tüketime Sunulan Kıymaların Mikrobiyolojik Kalitesi. *Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi, 4*, 73-86.

Sinell, H. J. (1992). Faktoren, die mikrobiellen Verderb bestimmen. In: Einführung in die Lebensmittelhygiene, Paul Prey Verlag, Hamburg: Berlin, 85-105.

Şireli, U. T., & Erol, İ. (1999). Hazır kıymalarda Listeria türlerinin araştırılması. *Turkish Journal of Veterinary Animal Sciences, 23*, 373-380.

Şireli, U. T., Göncüoğlu, M., & Pehlivanlar, S. (2008). Growth of *Listeria monocytogenes* in çiğ köfte (raw meat ball). *Ankara Üniversitesi Veteriner Fakultesi Dergisi, 55*, 83-87. http://dx.doi.org10.1501/Vetfak_0000000304.

Solmaz, H., Tütüncü, M., Gülhan, T., Ekin, İ. H., & Talaş, İ. (2002). Van Yöresi Süt Sığırlarında Brucellozis'in İnsidensi Üzerine İnceleme. *Yuzuncu Yil University Veterinary Faculty Journal, 13*, 54-56.

Sümer, H., Sümer, Z., Alim, A., Nur, N. & Özdemir, L. (2003). Seroprevalence of Brucella in an Elderly Population in Mid Anatolia, Turkey. *Journal of Health Population and Nutrition, 21*, 158-161.

Syrjamaki, C., Migliazzo, A., Yarbzough, J., & Le, M. (1984). Brucella abortus Endocarditis Following Ingestion of Cow's Blood. *Nebraska Medical Journal, 69*, 141-142.

Taşkın, N. (2007). Ankara ilinde tüketime sunulan sokak sütlerinde ve beyaz peynirlerde Brucella varlığının ELISA yöntemi ile araştırılması ve Brucella bakterisinin kefirde yaşam süresinin tayini. Gazi Üniversitesi Fen Bilimleri Enstitüsü. Yüksek Lisans Tezi. Ankara.

Tekinşen, O. C., Yurtyeri, A., & Mutluer, B. (1980). Ankara'da Satılan Hazır Kıymalarının Bakteriyolojik Kalitesi. *Ankara Üniversitesi. Veteriner Fakültesi Dergisi, 27*, 45-63. http://dx.doi.org/10.1501/Vetfak_0000000885.

Troller, J. A., & Scott, V. N. (1992). Measurement of water activity (Aw) and acidity. In: Compendium of Methods for the Microbiological Examination of Foods (edited by C. Vanderzant and D.F. Splittstoesser), (3rd ed.). American Public Health Association. Washington, DC.

Uzunlu, S., & Yıldırım, I. (2003). Microbiological quality of raw meat ball and investigation of its microbial variation at the different storage time and temperature. *Gıda, 28*, 553-558.

Vural, A. (2004). Investigation on the microbiological quality of nonpacking some spices in Diyarbakır province. *Kafkas Universitesi Veteriner Fakültesi Dergisi, 10*, 13-18.

WHO. (2006). Brucellosis in humans and animals. WHO Press, World Health Organization. Geneva: Switzerland.

Yetim, H. (1985). Erzurum piyasasında tüketime sunulan sığır kıymalarının bazı saprofit ve bir kısım patojen bakteriler yönünden incelenmesi, Atatürk Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi. Erzurum.

Yıldırım, İ., Uzunlu, S., & Topuz, A. (2005). Effect of gamma irradiation on some principle microbiological and chemical quality parameters of raw Turkish meat ball. *Food Control, 16*, 363-367. http://dx.doi.org/10.1016/j.foodcont.2004.04.001.

Yüce, A., & Çavuş, S.A. (2006). Türkiye'de Bruselloz: Genel Bakış. *Klinik Dergisi, 19*, 87-97.

Table 1. Behavior of *Brucella abortus* and *Brucella melitensis* in Raw Meatball

Groups	Holding time (h)	*Brucella (cfu/g)*	TAMB *(cfu/g)*	LAB *(cfu/g)*	pH	a_w	Humidity (%)
	0	$6,20^{b}$	$4,00^{d}$	$3,04^{d}$	$5,30^{a}$	$0,98^{a}$	$64,23^{a}$
A1	6	$6,45^{a}$	$4,66^{c}$	$4,08^{c}$	$5,30^{a}$	$0,97^{a}$	$64,11^{b}$
	12	$6,08^{b}$	$5,82^{b}$	$4,34^{b}$	$5,20^{b}$	$0,95^{b}$	$52,16^{c}$
	24	$5,56^{c}$	$7,72^{a}$	$4,41^{a}$	$5,10^{c}$	$0,91^{c}$	$49,07^{d}$
	0	$4,70^{b}$	$4,32^{d}$	$3,26^{d}$	$5,30^{a}$	$0,98^{a}$	$64,23^{a}$
A2	6	$4,56^{b}$	$4,65^{c}$	$4,36^{c}$	$5,30^{a}$	$0,97^{a}$	$64,12^{a}$
	12	$5,08^{a}$	$5,81^{b}$	$4,52^{a}$	$5,20^{b}$	$0,95^{b}$	$52,16^{b}$
	24	$3,30^{c}$	$7,75^{a}$	$4,40^{b}$	$5,10^{c}$	$0,91^{c}$	$49,07^{c}$
	0	$5,60^{c}$	$4,34^{d}$	$3,15^{d}$	$5,30^{a}$	$0,98^{a}$	$64,23^{a}$
B1	6	$6,72^{a}$	$4,66^{c}$	$4,38^{c}$	$5,30^{a}$	$0,97^{a}$	$64,11^{b}$
	12	$6,20^{b}$	$5,88^{b}$	$4,61^{a}$	$5,20^{b}$	$0,95^{b}$	$52,16^{c}$
	24	$5,48^{c}$	$7,77^{a}$	$4,45^{b}$	$5,09^{c}$	$0,91^{c}$	$49,07^{d}$
	0	$4,30^{c}$	$4,51^{d}$	$3,11^{d}$	$5,30^{a}$	$0,98^{a}$	$64,23^{a}$
B2	6	$4,56^{b}$	$4,64^{c}$	$4,04^{c}$	$5,29^{a}$	$0,97^{a}$	$64,12^{b}$
	12	$4,11^{d}$	$5,86^{b}$	$4,52^{a}$	$5,20^{b}$	$0,95^{b}$	$52,15^{c}$
	24	$5,11^{a}$	$7,70^{a}$	$4,38^{b}$	$5,10^{c}$	$0,91^{c}$	$49,07^{d}$

A1: 10^6 cfu/gr *B. abortus*; A2: 10^4 cfu /gr *B. abortus*; B1: 10^6 cfu /gr *B. melitensis*; B2: 10^4 cfu /gr *B. melitensis*;

TAMB: Total Aerobic Mesophilic Bacteria; LAB: Lactic Acid Bacteria

a-d Means in a same column with different letters are significantly different ($p<0,05$).

Bioavailability of Tea Components

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Abstract

This review discusses the bioavailability of active components of green and black teas--in hot brewed tea, cold tea, and dietary supplements containing tea extracts, based on literature published in 1995-2011. Many publications demonstrate that consumption of tea increases the antioxidant status of a person (between 3.5-76%) and reduces the concentration of oxidative stress biomarkers in biological fluids. In 1-2 hours after tea intake, epigallocatechin gallate (EGCG), epicatechin (EC), and epicatechin gallate (ECG) at a level of 5-150 ng/ml were detected in plasma by HPLC. The results of pharmacokinetics and metabolism of biologically active tea components analyzed within 24 hours in plasma, urine, and feces by HPLC-MS and GC-MS are presented. Dozens of metabolites were identified in urine and plasma—these are methylated, sulfated, and glucuronide conjugates of catechins. Some metabolites were shown to have high antioxidant activity. The role of the small intestine and colon in absorption of catechins was also identified.

Keywords: Tea, Bioavailability, Polyphenols, Tea catechins, Antioxidants

1. Introduction

The biological activity of green tea and other teas is directly related to their bioavailability, therefore the bioavailability of tea is a primary parameter.

Bioavailability is typically estimated by measuring that portion of a drug (as a percentage) which reaches the systemic blood flow after its non-systemic administration.

In order to use tea components as effective antioxidant therapy, one needs first to know the content of polyphenol antioxidants in different varieties of tea, and, secondly, their bioavailability. It is well known that not all polyphenols are absorbed with equal efficiency. Antioxidants are extensively metabolized by liver enzymes.

Knowledge of polyphenol metabolic processes and bioavailability is needed to evaluate their biological activity in tissues. Knowledge about the bioavailability of antioxidants is also essential for understanding their effect of on human health.

Flavonoid aglycones (without sugar residues) can be absorbed in the small intestine. However, most flavonoids are present in foods as glycosides, esters, or polymers and they often cannot be absorbed in these forms. Before

being absorbed, these flavonoids must be hydrolyzed by intestinal enzymes or gut flora. During absorption, polyphenols are conjugated first in the intestine and later in the liver. These reactions mainly include methylation, sulfation, and glucuronidation. Polyphenols are able to penetrate tissues (Holst & Williamson, 2004).

Polyphenols and their derivatives are eliminated from the body in urine and bile.

The bioavailability of tea components, in particular catechins, may be determined by several methods:

– by the increase of the antioxidant activity (capacity) of plasma or human serum after consumption of tea or its individual components;

– by direct determination of catechins in biological fluids and tissues one or two hours after consuming a certain quantity of tea or individual catechins;

– by determining the effect of consumed tea and its components on reduction of oxidative stress markers.

Table 1 presents data related to increased antioxidant activity (AA) of human plasma after consumption of different quantities of tea.

Tea consumption reduces concentrations of oxidative stress biomarkers in biological fluids. The most well-known oxidative stress biomarkers are: 8-hydroxydeoxyguanosine (8-OHdG), tyrosine derivatives, malondialdehyde, F_2-isoprostane, and phosphatidylcholine hydroperoxide (PCOOH) – marker of oxidative injury of plasma lipoproteins (Miyazawa, 2000).

The consumption of 900 ml of green tea per day for 7 days was shown to reduce 8-OHdG in human urine by 40% and malondialdehyde by 80%, while there were wide individual variations noted (Klaunig *et al*., 1999). Consumption of green tea reduced PCOOH concentrations in human plasma by 60% (Nokagawa *et al*., 1999)

Increased consumption of green tea reduced the concentration of F2-isoprostane in humans and animals (Morrow *et al*., 1999).

An anticarcinogenic effect of green tea and its catechins has been observed in many studies (Yang *et al*., 2000). Especially strong results were obtained in animals.

Prolonged exposure to UV radiation promotes the formation of cyclobutane pyrimidine dimer (CPD) which plays an important role in skin carcinogenesis (Katiyar *et al*., 2001). If skin is treated with an EGCG solution (1-4 mg/cm^2) 20 min before UV radiation, the formation of CPD is significantly reduced (Katiyar *et al*., 2000).

2. Investigation of the Bioavailability of Tea Catechins

Bioavailability and metabolism of some individual catechins such as epigallocatechin gallate (EGCG), epicatechin (EC), epigallocatechin (EGC) and epicatechin gallate (ECG) as well as total catechins of green and black tea in humans have been studied rather frequently (Stalmach *et al*., 2009; Daniele Del Rio *et al*., 2010; Sun *et al*., 2009; Henning *et al*., 2004; Chow *et al*., 2001; Higdon *et al*., 2003; Chow *et al*., 2005; Nakagawa *et al*., 2009; Crozier *et al*., 2009; Li *et al*., 2000; Meng *et al*., 2001; Auger *et al*., 2008; Stalmach *et al*., 2009; Spencer *et al*., 2003; Aura, 2008; Selma *et al*., 2009; Feng, 2006; Mulder *et al*., 2005; Dalluge & Nelson, 2000; Lee *et al*., 2011; Lee *et al*., 2011; Ku *et al*., 2010; Lee *et al*., 2010; Roowi *et al*., 2010).

Bioavailability and metabolism processes of black tea theaflavins and thearubigins were studied significantly less (Mulder *et al*., 2001; Wiseman *et al*., 2001). High dose (700 mg) of mixed theaflavins was given two healthy volunteers, one male and one female. Only theaflavin was detected because enzyme treatment also removed ester gallate. Maximum theaflavin concentration detected in the plasma of the female and the male were 1.0 and 0.5 μg/L, respectively, and maximum concentrations in urine were 0.6 and 4.2 μg/L, respectively, all at 2 hours (Mulder *et al*., 2001).

The beneficial health effects of tea catechins are fundamentally related to their bioavailability, absorption, distribution in various organs, metabolism, and excretion from the body.

The bioavailability is estimated by the concentration of a particular catechin or its metabolites in a certain organ. It is impossible to accurately determine these parameters in vivo in humans due to the difficulty of accessing the organs; therefore, such studies are conducted on animals. However, the bioavailability and metabolism of catechins in humans and animals may vary. The absolute bioavailability is often estimated by the number of active compounds into the blood.

As already indicated, bioavailability consists of several interrelated processes: liberation, absorption, distribution, metabolism, and excretion (Holst & Williamson, 2004).

The absorption is hard to assess precisely. Sometimes it is judged by excretions in the urine. However, the total absorption cannot be assessed in this way because some catechins could be metabolized by the liver and conjugate in the intestines (Warden *et al*., 2001). Only about 1.7% of consumed catechins are found in plasma, urine, and feces of a person who drinks black tea. Bioavailability of gallocatechins is lower than that of catechins without galloyl groups (Warden *et al*., 2001).

Catechins (EC, ECG, EGC, and EGCG) are relatively high molecular weight compounds (300-450) comprised of more than 5 hydroxyl groups. They have low bioavailability due to their large size (Lipinsi *et al*., 2001). The level of catechins and other flavonoids in plasma does not exceed 1 μM when consumed in typical amounts (1-2 cups, 100-200 mg of catechins).

The total concentration of catechins (both free and conjugated) is about 2-3 μM or less (Yang *et al*., 1998). These plasma concentrations are much lower than those used in studies in vitro.

Various measurements, including HPLC, showed that the concentration of catechins in human plasma increases in 1-2 hours after tea consumption. But the bioavailability of tea catechins is relatively low—only 0.2–2% of the consumed amount of catechins gets into plasma of healthy humans (Wiseman *et al*., 2001). When green tea is consumed in high doses, the overall level of catechins in human plasma is about 0.6-1.8 μM (Holst & Williamson, 2004).

In one study, the bioavailability of catechins (flavan-3-ols) after consumption of cold tea in sealed packages was investigated in 20 volunteers (Del Rio *et al*., 2010). After consumption, the urine of volunteers was analyzed over a 24 hour period by HPLC–electrospray MS/MS. Eight metabolites of flavan-3-ols and unmetabolized gallic acid were identified in urine. Urine contained 7.2% of flavan-3-ols and 4.5% of gallic acid in relation to their total amounts contained in the consumed tea. The maximum level of 5 metabolites (epicatechin sulfate, methyl-epicatechin sulfate, epicatechin glucuronide, epigallocatechin glucuronide, and methyl-epigallocatechin glucuronide) were observed in the urine 4 hours after consumption of cold tea; the maximum level of 2 metabolites (methyl-epigallocatechin and methyl-epigallocatechin sulfate) were present in the urine only after 10 hours; whereas the content of epigallocatechin in the urine was seen as constantly increasing over a period of 24 hours. Bioavailability observed in this study (Del Rio *et al*., 2010) was consistent with a previous study (Stalmach *et al*., 2008).

The bioavailability of pure EGC, ECG, and EGCG in healthy people was investigated. After consumption of 1.5 mM of EGC, ECG, and EGCG alone, the average plasma concentration was 5 μM, 3.1 μM, and 1.3 μM respectively (Van Hof, 1998). One large review (Sun *et al*., 2009) summarizes pharmacogenetic parameters of tea metabolites in human body after consumption. After oral consumption of 100 to 1600 mg of pure epigallocatechin gallate, 0.26 to 6.35 μm of epigallocatechin gallate was detected in plasma in 2-3 hours; some conjugated compounds of epigallocatechin gallate in the amount of 0.28 to 7.40 μm were also found in 1.9 to 4.6 hours.

These data show that the bioavailability of various catechins varies greatly. EGCG is the least bioavailable. During absorption, catechins undergo intensive bioconversions, including methylation, sulfonation, etc (Meng *et al*, 2002). It is assumed that about 80% of tea catechins in plasma and urine are in the form of their conjugates. Some conjugates have intact hydroxyl substituents, which can capture superoxide free radicals, and their antioxidant efficiency remains quite high (Kuhnie *et al*, 2000, Voidyanathon & Walle, 2002, Li *et al*., 2001, Li *et al*., 2000). Even having low bioavailability, tea catechins have a salutary effect in many diseases.

Table 2 provides a list of studies which determined the catechin content in different biological fluids and tissues of humans and animals. Table 3 shows specific examples of bioavailability of green tea, black tea, and tea catechins separately.

The evidence for antioxidant effects of tea demonstrated in clinical trials is summarized in one review (Manach *et al*., 2004). The authors have come to the unequivocal conclusion that flavonoids-tea antioxidants-are absorbed in the intestine, which leads to a significant increase in antioxidant capacity of human plasma an hour after tea consumption.

In one study, it is assumed that part of the catechins is present in a metabolized form (Natsume *et al*., 2003). In particular, dimerization of EGCG in an alkaline medium results in new compounds (theasinensins A and D) which antioxidant activity is 2-3 times greater.

In another study, an improved HPLC method with a coulometric detector was proposed for the polyphenols of green and black tea in different biomatrixes (plasma, urine, saliva, and tissues) (Pietta *et al*., 1998).

Polyphenols were extracted with ethyl acetate. Major catechins, theaflavins, and catechin metabolites were identified by HPLC.

The catechins were determined in plasma, saliva, and urine. Tissue samples for the determination of catechins in tissues were taken from rats.

Approximately 180 ng/ml of EGCG, 156-158 ng/ml of EGC, 68-70 ng/ml of EC, and 85-87 ng/ml of ECG were detected in plasma (ng = - $1 \cdot 10^{-9}$ g). The detection limit was at 5-10 ng/ml.

The proposed method gives reproducible and reliable results in determining the eight major polyphenols at the same time.

The bioavailability of green tea and its individual catechins was investigated in several studies (Table 3) (Nokagawa *et al*., 1999, Clifford *et al*., 2000, Manach *et al*., 2004).

In example number 4, 6 mg of EGCG was detected in the entire blood by HPLC upon consumption of 82 mg, which is less than 7% of the consumed amount.

3. Metabolism and Pharmacokinetics of the Biologically Active Tea Components.

Catechin metabolites (e.g., EC, ECG, EGC, and EGCG) were determined in humans after oral intake of green tea (Pietta *et al*., 1998a, Pietta *et al*., 1998b, Mudler *et al*., 2005, Yoshino *et al*., 1999, Stalmach *et al*., 2009).

In human plasma, the portion of the methylated EGC is higher than that of ECG and EGCG (Ho et al., 1995).

In human plasma, a greater amount of EGCG is present in the free form than EGC and EC which are mostly in the conjugated form (Meng *et al*., 2002, Umegaki *et al*., 2001, Kotani *et al*., 2003). In one hour after drinking tea, 77% of EGCG was in the free form in human plasma, whereas the result was 31% and 21% of EGC and EC respectively (Meng *et al*., 2002). After consuming a tea supplement and pure catechin, over 80% of catechin found in plasma was in the bound form. EGC has not been detected at all, whereas 57-71% of ECG was present in the glucuronidated form, 23-36% in the sulfonated form, and 3-13% in the free form. In urine, over 90% of EGC was in the sulfonated form (Unno *et al*., 2005).

The catechins which are not absorbed in the small intestine, reach the large intestine which contains trillions of microorganisms. Bacterial enzymes degrade polyphenols to simpler molecules, in particular to oxyaromatic acids. Then these products of microbial metabolism are absorbed. Some of these metabolites were identified (Li *et al*., 2000).

Absorption, metabolism, and excretion in urine of green tea flavonols were studied by HPLC-MS in 10 healthy volunteers who were taking 500 ml of Choladi green tea (Choladi, India), containing 648 μmol of flavan-3-ols (Stalmach *et al*., 2009). After consumption of green tea, plasma and urine were analyzed over a 24 hour period. Ten metabolites of O-methylated, sulfated, and glucuronide conjugates of epicatechin and epigallocatechin at a concentration of 29-126 nM were found to occur 1.6-2.3 hours after ingestion. This proves that absorption occurs in the small intestine. Plasma also contained unmetabolised epigallocatechin gallate and epicatechin gallate at concentrations of 55 and 25 nM, respectively. Fifteen metabolites of epicatechin and epigallocatechin were identified in urine. No metabolites of epigallocatechin gallate and epicatechin gallate were detected. Overall flavanol excretion was estimated at 8.1% of intake but excretion of certain flavanols varied from 11.4% to 28.5% of intake. These studies have shown that epicatechins are more bioavailable than other catechins in green tea.

Hippuric acid is the main metabolite when black tea is consumed (Tsuchiya *et al*., 1997, Yang *et al*., 2000). Hippuric acid was also detected in urine when green tea was consumed (Tsuchiya *et al*., 1997). After consumption of black tea, 1,3-dihydroxyphenyl-2-0-sulfate was identified as a metabolite (Yang, 2000). Extremely low levels of theaflavins were detected in human plasma and urine after consumption of black tea (Clifford *et al*., 2000).

One study claims that about 43% of tea polyphenols is metabolized to hippuric acid (Masukawa *et al*., 2006). It has been suggested that thearubigins also are degraded to hippuric acid (Tsuchiya *et al*., 1997). But hippuric acid can also be derived from aromatic amino acids. That is why 3-hydroxyhippuric acid is considered as the best biomarker for tea consumption (Rechner *et al*., 2001).

In one study (Lee *et al*., 2010), catabolites in urine and feces were investigated by GC-MS in patients with a functioning colon and in patients without a colon. In the first group of patients, the following components were detected in the urine within 24 hours: pyrocatechin, pyrogallol, 4-hydroxybenzoic acid, 4-hydroxyphenylacetic acid, 3-methoxy-4-hydroxy-phenylacetic acid, and hippuric acid. In patients without a colon, only hippuric acid and 4-hydroxyphenylacetic acid were found in small amounts. The above acids were detected in feces 24 hours later.

Only very few studies related to the determination of the tea components in human tissues were published. In one study, the bioavailability of both green and black tea polyphenols was investigated by their content in human prostate tissue samples (Kim *et al*., 2000). The content of both free and conjugated EGC and EGCG in these tissues was significantly higher in people who consumed tea than in people who consumed an aqueous solution of caffeine.

Tea metabolites are primarily excreted through the urine, and highly conjugated tea components through the bile (Manach *et al*., 2004). The half-life of tea catechins in the body is approximately 2-3 hours. EGCG is excreted more slowly (Manach *et al*., 2004).

In one study it was shown that the bioavailability of tea polyphenols in tea dietary supplement capsules is much higher than after consumption of green and black tea (Serafini *et al*., 2000). This fact requires further research. The effect of food matrix on the bioavailability has not yet been investigated.

It is crucial to evaluate the bioactivity the glucuronidated, sulfated, and methylated derivatives of catechins (metabolites) to establish a link between tea consumption and cancer risk (Nokagawa *et al*., 1999).

Once absorbed, the tea catechins are metabolized by glucuronidation, methylation, and sulfonation converting the catechins into more hydrophilic compounds (Yang *et al*., 1998 & Van Hof, 1998). Metabolic reactions are catalyzed by enzymes.

Only a very small amount of conjugated catechins are present in plasma. Prior to determining the catechin metabolites in plasma and urine samples by HPLC, they must be pre-treated with β-glucuronidase and sulphatase in order to remove the respective functional groups.

The main metabolite which was identified in plasma is 4'-methyl-EGC. In 2 hours after consumption of green tea, the amount of 4'-methyl-EGC present in human plasma is 4-6 times greater than EGC (Yang *et al*., 1998). Approximately 90% of 4'-0-methyl-EGC is excreted via the urine within 8 hours.

The EGCG metabolites, 4', 4"-di-0-methyl-EGCG, were identified in human plasma and urine after tea consumption using LC/MS/MS (Meng *et al*., 2002).

The enzymes catalyze many reactions in small and large intestines, including decomposition of the flavonoids down to oxyaromatic acids. These compounds are also antioxidants and have a beneficial physiological effect. Unfortunately, very few studies were conducted to these metabolites.

In one study, the bioavailability and pharmacokinetics of catechins in the blood and urine after consumption of green and black tea, as well as green tea dietary supplements, was investigated in 30 volunteers. The content of catechins (EC, ECG, EGC, and EGCG) in the original beverages was determined by HPLC with coulometric detector.

The total amount of EC, ECG, EGC, and EGCG in the green and black tea drinks and in tea dietary supplements was 679.0±9.4, 496.5±3.3 and 386±3.2 mg respectively. Indian Darjeeling tea which contains a large amount of EGCG (on a level with green tea), was used for black tea. After catechin-containing drink consumption, total catechin content in plasma was measured in 30 healthy volunteers within eight hours. The highest content in plasma was 1-2 hours after the drinks were taken, and then the concentration of catechins slowly decreased to the initial zero value.

The greatest value of the total content of catechins in plasma, 1.2 μM/L, was reached after consumption of green tea. The maximum plasma concentration after consumption of black tea and dietary supplements was approximately 0.5 μM/l.

As was found previously, tea catechins are largely metabolized or broken down into simpler phenolic acids, such as 4-hydroxybenzoic, 3,4-dihydroxybenzoic, and 3-methoxy-4-hydroxyhippuric acids[48]. These acids also showed in vitro antioxidant activity. As noted above, EGCG is dimerized in an alkaline medium resulting in new compounds, theasinensins A and D, which have higher antioxidant activity than EGCG.

Influence of joint consumption of epigallocatechin gallate and caffeine on their absorption and metabolism in humans was also explored (Kiyotaka *et al*., 2009). Caffeine reduces sulfonation and glucuronidation of epigallocatechin gallate. A metabolomic analysis of green tea was carried out by nuclear magnetic resonance (NMR) followed by chemometrics (Mulder *et al*., 2005).

4. Conclusion

It can be concluded that when green tea is consumed orally, the tea catechins reach human plasma at the micromolar level (less than 1-2%). The metabolites of tea components are present in plasma to a greater extent, and they also show antioxidant activity and can protect people from various diseases.

However, the bioavailability of tea catechins was not sufficiently investigated. In order to assess the biological activity of tea components in vivo, various tea metabolites must be thoroughly studied and identified, and their antioxidant and other properties must be assessed.

Detailed study of the tea components' pharmacokinetic processes, such as absorption process, duration of their presence in the body, and excretion time, is also necessary.

It is absolutely necessary in order to be able to reasonably determine the optimal frequency of tea consumption in order to maintain a constant concentration of its biologically active components in human plasma during antioxidant therapy aimed at combating various diseases.

References

Auger, C., Hara, Y., & Crozier, A. (2008). Bioavailability of polyphenon E flavan-3-ols in humans with an ileostomy. *Journal of Nutrition*, *138*, 1535S-1542S.

Aura, A. M. (2008). Microbial metabolism of dietary phenolic compounds in the colon. *Phytochemistry Reviews*, *7*(3), 407-429. http://dx.doi.org/10.1007/s11101-008-9095-3

Benzie, I. F., Szeto, Y. T., Strain, J. J., & Tomlinson, B. (1999). Consumption of green tea causes rapid increase in plasma antioxidant power in humans. *Nutrition and Cancer, 34*, 83-87. http://dx.doi.org/10.1207/S15327914NC340112

Chow, H. H, Hakim, I. A, Vining, D. R, Crowell, J., Ranger-Moore, J., Chew, W., Celaya, C., Rodney, S., Hara, Y., & Alberts, D. (2005). Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of polyphenon E in healthy individuals. *Clinical Cancer Research, 11*(12), 4627–4633. http://dx.doi.org/10.1158/1078-0432.CCR-04-2549.

Chow, H. H., Cai, Y., Alberts, D., Hakim, I., Dorr, R., Shahi, F., Crowell, J. A., Yang, C. S., & Hara, Y. (2001). Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. *Cancer Epidemiology, Biomarkers & Prevention*, *10*(1), 53-58

Clifford, M. N., Coleland, E. L., Bloxsidge, J. P., & Mitchell, L. A. (2000). Hippuric acid as a major excretion Product Associated with Black Tea Consumption. *Xenobiotica, 30*(3), 317-326.

Crozier, A., Jaganath, I. B., & Clifford, M. N. (2009). Dietary phenolics: chemistry, bioavailability and effects on health. *Natural Product Reports*, *26*, 1001-1043. http://dx.doi.org/10.1039/B802662A.

Dalluge, J., & Nelson, B. (2000). Determination of tea catechins. *Journal of Chromatography A, 881(1-2)*, 411-424. http://dx.doi.org/10.1016/S0021-9673(00)00062-5

Del Rio, D., Stalmach, A., Calani, L., & Crozier, A. (2010). Bioavailability of Coffee Chlorogenic Acids and Green Tea Flavan-3-ols. *Nutrients, 2*(8), 820-833. http://dx.doi.org/10.3390/nu2080820

El-Hady, D., & El-Maali, N. (2008). Determination of catechin isomers in human plasma subsequent to green tea ingestion using capillary electrophoresis with a high-sensitivity cell. *Talanta, 76*(1), 138-145. http://dx.doi.org/10.1016/j.talanta.2008.02.026

Feng, W. Y. (2006). Metabolism of green tea catechins: an overview. *Current Drug Metabolism, 7*(7), 755-809.

Henning, S. A., Aronson, W., Niu, Y., *et al*. (2006). Tea Polyphenols and theaflavins are present in prostate tissue of humans and mice after green and black tea consumption. *Journal of Nutrition, 136*, 1839-1843.

Henning, S., Niu, Y., Lee, N., Thames, G., Minutti, R., Wang, H., Go, V., & Heber, D. (2004). Bioavailability and antioxidant activity of tea flavanols after consumption of green tea, black tea, or a green tea extract supplement. *The American Journal of Clinical Nutrition, 80*(6), 1558-1564.

Higdon, J. V., & Frei, B. (2003). Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. *Critical Reviews in Food Science and Nutrition, 43*(1), 89-143. http://dx.doi.org/10.1080/10408690390826464

Ho Y., Lee Y. L., & Hsu K. Y. (1995). Determination of (+)-catechins in plasma by high-performance liquid chromatography using fluorescence detection. *Journal of Chromatography B, 383*(2), 383-389, http://dx.doi.org/10.1016/0378-4347(94)00535-D

Holst, B., & Williamson, G. (2004). *Methods to Study Bioavailability of Phytochemicals. Phytochemicals in Health and Disease. Ed.* R. Fenwick, New York: Marcel Dekker. http://dx.doi.org/10.2174/138920006778520552

Katiyar, S. K., & Elmets, C. A. (2001). Green tea polyphenolic antioxidants and skin photoprotection (review). *International Journal of Oncology, 18*, 1307-1313.

Katiyar, S. K., Perez, A., & Mukhtar, H. (2000). Green tea polyphenol treatment to human skin prevents formation of ultraviolet light B-induced pyrimidine dimmers in DNA. *Clinical Cancer Research, 6*, 3864-3869

Kim, S., Lee, M. J., Hong, J., Li, C., Smith, T., Yang, G., Seril, D., & Yang, C. (2000). Plasma and tissue levels of tea catechins in rats and mice during chronic consumption of green tea polyphenols. *Nutrition and Cancer, 37*, 41-48. http://dx.doi.org/10.1207/S15327914NC3701_5

Kivits, G. A., van der Sman, F. J. P., & Tijburg, L. B. M. (1997). Analysis of catechins from green and black tea in humans. *International Journal of Food Science and Nutrition, 48*(6), 387-392.

Klaunig, J. E., Xu, Y., Han, C., Kamendulis, L., Chen, J., Heiser, C., Gordon, M., & Mohler III, E. (1999). The effect of tea consumption on oxidative stress in smokers and nonsmokers. *Proceedings of the Society for Experimental Biology and Medicine, 220*(4), 249-254. http://dx.doi.org/10.3181/00379727-220-44375

Kotani, A., Miyashita, N., & Kusu, F. (2003). Determination of catechins in human plasma after commercial canned green tea ingestion by high-performance liquid chromatography with electrochemical detection using a microbore column. *Journal of Chromatography B*, *25*(2), 269-275. http://dx.doi.org/10.1016/S1570-0232(02)01036-X

Ku, K. M., Choi, J. N., Kim, J., Kim, J. K., Yoo, L. G., Lee, S. J., Hong, Y. S., & Lee, C. H. (2010). Metabolomics analysis reveals the compositional differences of shade grown tea (*Camellia sinensis L.*). *Journal of Agriculture and Food Chemistry, 58*(1), 418–426. http://dx.doi.org/10.1021/jf902929h

Kuhnie, G., Spencer, J., Schroeter, H., Shenoy, B., Debman, E., Srai, S., Rice-Evans, C., & Hahn, U. (2000). Epicatechin and catechin are O-methylated and glucuronidated in the small intestine. *Biochemical and Biophysical Research Communications, 277*(2), 507-512. http://dx.doi.org/10.1006/bbrc.2000.3701

Lee, H. C., Jenner, A. M., Low, C. S., & Lee, Y. K. (2006). Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microflora. *Research in Microbiology, 157*(9), 876-884. http://dx.doi.org/10.1016/j.resmic.2006.07.004.

Lee, J. E., Lee, B. J., Chung, J. O., Hwang, J. A., Lee, S. J., Lee, C. H., & Hong, Y. S. (2010). Geographical and climatic dependencies of green tea (*Camellia sinesis*) metabolites: A 1H NMR-based metabolomics study. *Journal of Agriculture and Food Chemistry, 58*(19), 10582-10589. http://dx.doi.org/10.1021/jf102415m.

Lee, J. E., Lee, B., Chung, J. O., Shin, H. J., Lee, S. J., Lee, C. H., & Hong, Y. S. (2011). 1H NMR-based metabolomics characterization of green tea (Camellia sinensis) during fermentation. *Food Research International, 44*, 597-604. http://dx.doi.org/10.1016/j.foodres.2010.12.004.

Lee, J. E., Lee, B. J., Hwang, J., Ko, K. S., Chung, J. O., Kim, E. H., Lee, S. J., & Hong, Y. S. (2011). Metabolic Dependence of Green Tea on Plucking Positions Revisited: A Metabolomic Study. *Journal of Agriculture and Food Chemistry, 59*(19), 10579-10585. http://dx.doi.org/10.1021/jf202304z

Li, C., Lee, M. J., Sheng, S. M., Meng, X., Prabhu, S., Winnik, B., Huang, B., Chung, J., Yan, S., Ho, C-T., & Yang, C. (2000). Structural identification of two metabolites of catechins and their kinetics in human urine and blood after tea ingestion. *Chemical Research in Toxicology, 13*(3), 177-184. http://dx.doi.org/10.1021/tx9901837

Li, C., Meng, X., Winnik, B., Lee, M-J., Lu, H., Sheng, S., Buckley, B., & Yang, C. (2001). Analysis of urinary metabolites of tea catechins by liquid chromatography/electrospray ionization mass spectrometry. *Chemical Research in Toxicology, 14*(6), 702-707. http://dx.doi.org/10.1021/tx0002536

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (1997). Experimental and computational approaches to estimate solubility and permeability of drug discovery and development settings. *Advanced Drug Delivery Reviews, 23*, p. 3-25. http://dx.doi.org/10.1016/S0169-409X(96)00423-1

Longley-Evans, S. C. (2000). Consumption of black tea elicits an increase in plasma antioxidant potential in humans. *International Journal of Food Sciences and Nutrition, 51*(5), 309-315. http://dx.doi.org/10.1080/096374800426902

Manach, C., Sealbert, A., Morand, C., Reemesy, C., & Jimenez, L. (2004). Polyphenols: food sources and bioavailability. *The American Journal of Clinical Nutrition, 79*(5), 727-747.

Manach, C., Williamson, G., Morand, C., Scalbert, A., & Remesy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *The American Journal of Clinical Nutrition, 81*(1), 2305-2425

Masukawa, Y., Matsui, Y., Shimizu, N., Kondou N., Endou, H., Kuzukawa, M., & Hase, T. (2006). Determination of green tea catechins in human plasma using chromatography-electrospray ionization mass spectrometry. *Journal of Chromatography B, 834(1-2)*, 26-34. http://dx.doi.org/10.1016/j.jchromb.2006.02.008

Meng, X., Lee, M. J., Li, C., Sheng, S., Zhu, N. Q., Sang, S. N., Ho, C. T., & Yang, C. S. (2001). Formation and identification of 4'-O-methyl-(-)-epigallocatechin in humans. *Drug Metabolism & Disposition, 29*(6), 789–793

Meng, X., Sang, S., Zhu, N., Lu, H., Sheng, S., Lee, M-J., Ho, C-T., & Yang, C. (2002). Identification and characterization of methylated and ring-fission metabolites of tea catechins formed in humans, mice, and rats. *Chemical Research in Toxicology, 15*(8), 1042-1050. http://dx.doi.org/10.1021/tx010184a

Miyazawa, T. (2000). Absorption, metabolism and antioxidative effects of tea catechin in humans. *BioFactors, 13*, 55-59

Morrow, J. D., Chen, Y., Brame, C. J., Yang, J., Sanchez, S., Xu, J., Zackert, W., Awad, J., & Roberts, L. J. (1999). The Isoprostanes: unique prostaglandin-like products of free-radical-initiated lipid peroxidation. *Drug Metabolism Reviews, 31*(1), 117-139. http://dx.doi.org/10.1081/DMR-100101910

Mulder, T. P., Rietveld, A., & van Amelsvoort, J. (2005). Consumption of both black tea and green tea results in an increase in the excretion of hippuric acid into urine. *The American Journal of Clinical Nutrition, 81*(1), 256S-260S.

Mulder, T. P., van Platerink, C., Wijnand Schuyl, P., & van Amelsvoort, J. (2001). Analysis of theaflavins in biological fluids using liquid chromatography-electrospray mass spectrometry. *Journal of Chromatography B: Biomedical Sciences and Applications, 760*(2), 271-279. http://dx.doi.org/10.1016/S0378-4347(01)00285-7

Nakagawa, K., Nakayama, K., Nakamura, M., Sookwong, P., Tsuduki, T., Nuno, H., Kimura, F., & Miyazawa, T. (2009). Effects of co-administration of tea epigallocatechin-3-gallate (EGCG) and caffeine on absorption and metabolism of EGCG in humans. *Bioscience, Biotechnology, and Biochemistry, 73*(9), 2014-2017. http://dx.doi.org/10.1271/bbb.90195

Natsume, M., Osakabe, N., Oyama, M., Sakasi, M., Baba, S., Nakamura, Y., Osawa, T., & Terao, J. (2003). Structures of (-)-epicatechin glucuronide identified from plasma and urine after oral ingestion of (-)-epicatechin: differences between human and rat. *Free Radical Biology and Medicine, 34*(7), 840-849. http://dx.doi.org/10.1016/S0891-5849(02)01434-X

Nokagawa, K., Ninomiya, M., Okubo, T., Aoi, N., Juneja, L., Kim, M., Yamanaka, K., & Miyazama, T. (1999). Tea catechin supplementation increases antioxidant capacity and prevents phospholipids hydroperoxidation in plasma of humans. *Journal of Agricalture and Food Chemistry, 47*(10), 3967-3973. http://dx.doi.org/10.1021/jf9811951

Pietta, P. G., Simonetti, P., Gardana, C., Brusamolino, A., Morazzoni, P., & Bombardelli, E. (1998a). Catechin metabolites after ingestion of green tea infusions. *BioFactors, 8*(1-2), 111-118. http://dx.doi.org/10.1002/biof.5520080119

Pietta, P. G., Simonetti, P., Gardana, C., Brusamolino, A., Morazzoni, P., & Bombardelli, E. (1998b). Relationship between rate and extent of catechin absorption and plasma antioxidant status. *International Union of Biochemistry and Molecular Biology, 46*(5), 895-903. http://dx.doi.org/10.1080/15216549800204442

Rechner A. R., Spencer, J., & Kuhnle, G. (2001). Novel biomarkers of the metabolism of caffeic acid derivatives in vivo. *Free Radical Biology and Medicine, 30*(11), 1213-1222. http://dx.doi.org/10.1016/S0891-5849(01)00506-8

Roowi, S., Stalmach, A., Mullen. W., Lean, M., Edwards, C., & Crozier, A. (2010). Green tea flavan-3-ols: colonic degradation and urinary excretion of catabolites by humans. *Journal of Agriculture and Food Chemistry, 58*(2), 1296–1304. http://dx.doi.org/10.1021/jf9032975.

Selma, M. V., Espin, J. C., & Tomas-Barber, F. (2009). Interaction between phenolics and gut microbiota: role in human health. *Journal of Agriculture and Food Chemistry, 57*(15), 6485–6501. http://dx.doi.org/10.1021/jf902107d

Serafini, M., Ghiselli, A., & Ferro-Luzzo, A. (1996). In vivo antioxidant effect of green and black tea in man. *European Journal of Clinical Nutrition, 50*(1), 28-32.

Serafini M., Laranjinha, J., Almeida, L., & Maiani, G. (2000). Inhibition of human LDL lipid peroxidation by phenol-rich beverages and their impact on plasma total antioxidant capacity in humans. *The Journal of Nutrition Biochemistry, 11*(11-12), 585-590. http://dx.doi.org/10.1016/S0955-2863(00)00124-8

Shahrzad, S., & Bitsch, I. (1998). Determination of gallic acid and its metabolites in human plasma and urine by high-performance liquid chromatography. *Journal of Chromatography B: Biomedical Sciences and Applications, 705*(1), 87-95. http://dx.doi.org/10.1016/S0378-4347(97)00487-8

Spencer, J. P. E. Metabolism of tea flavonoids in the gastrointestinal tract. (2003). *Journal of Nutrition, 133*, 3255S–3261S

Stalmach, A., Mullen, W., Steiling, H., Williamson, G., & Crozier, A. (2010). Absorption, metabolism, and excretion of green tea flavan-3-ols in humans with an ileostomy. *Molecular Nutrition & Food Research, 54*(3), 323-334. http://dx.doi.org/10.1002/mnfr.200900194

Stalmach, A., Troufflard, S., Serafini, M., & Crozier, A. (2009). Absorption, metabolism and excretion of Choladi green tea flavan-3-ols by humans. *Molecular Nutrition & Food Research*, 53(1), S44–S53. http://dx.doi.org/10.1002/mnfr.200800169.

Sun T., Ho C.-T., & Shahidi F. (2009). Bioavailability and metabolism of tea catechins in human subjects. In: Tea and Tea Products. Chemistry and Health – Promoting Properties. Ed. by Ho C.-T., Lin J.- K., Shahidi F. CRC Press. Taylor and Francis Group, Boca Raton, London, New York, 111-129.

Sung, H., Nah, J., Chun, S., Park, H., Yang, S., & Min, W. (2000). In vivo antioxidant effect of green tea. *European Journal of Clinical Nutrition, 54*(7), 527-529. http://dx.doi.org/10.1038/sj.ejcn.1600994

Takino, M., Daishima, S., Yamaguchi, K., & Nakahara, T. (2003). Quantitative liquid chromatography-mass spectrometry determination of catechins in human plasma by automated on-line extraction using turbulent flow chromatography. *Analyst, 128*, 46-50. http://dx.doi.org/10.1039/B209675G

Tsuchiya, H., Sato, M., Kato, H., Okubo, T., Juneja, L. R., & Kim, M. (1997). Simultaneous determination of catechins in human saliva by high-performance liquid chromatography. *Journal of Chromatography B: Biomedical Sciences and Applications, 703*(1-2), p. 253-258. http://dx.doi.org/10.1016/S0378-4347(97)00412-X

Umegaki, K., Sugisaws, A., Yamada, K., & Higuchi, M. (2001). Analytical method of measuring tea catechins in human plasma by solid-phase extraction and HPLC with electrochemical detection. *Journal of Nutritional Science and Vitaminology, 47*(6), 402-408.

Unno, T., Sagesaka, Y. M., & Kakuda, T. (2005). Analysis of tea catechins in human plasma by high performance liquid chromatography with solid-phase extraction. *Journal of Agriculture and Food Chemistry, 53*(26), 9885-9889. http://dx.doi.org/10.1021/jf0522199

Vaidyanathan, J. B., & Walle, T. (2002). Glucuronidation and sulfation of tea flavonoid (-)-epicatechin by the human and rat enzymes. *Drug Metabolism and Disposition, 30*(8), 897-903. http://dx.doi.org/10.1124/dmd.30.8.897

Van het Hof, K. H., de Boer, H., Wiseman, S., Lien, N., Westrate, J., & Tijburg, L. (1997). Consumption of green and black tea does not increase resistance of low-density lipoprotein to oxidation in humans. *American Journal of Clinical Nutrition, 66*, 1125-1132.

Van het Hof, K. H., Kivits, G., Weststrate, J., & Tijburg, L. (1998). Bioavailability of catechins from tea: the effect of milk. *European Journal of Clinical Nutrition, 52*(5), 356-359.

Warden, B. A., Smith, L. S., Beecher, G. R., Balentine, D. A., & Clevidence, B. A. (2001). Catechins are bioavailable in men and women drinking black tea throughout the day. *Journal of Nutrition, 131*(6), 1731-1737.

Wiseman, S., Mulder, T., & Rietveld, A. (2001). Tea Flavonoids: Bioavailability *in Vivo* and effects on cell signaling pathways *in vitro*. *Antioxidants and Redox Signaling, 3*(6), 1009-1021. http://dx.doi.org/10.1089/152308601317203549

Yang, B., Arai, K., & Kusu, F. (2000). Determination of catechins in human urine subsequent to tea ingestion by high-performance liquid chromatography with electrochemical detection. *Analytical Biochemistry, 283*(1), 77-82. http://dx.doi.org/10.1006/abio.2000.4624

Yang, C. S., Chung, J. Y., Yang, G., Chhabra, S., & Lee, M-J. (2000). Tea and tea polyphenols in Cancer Prevention. *Journal of Nutrition, 130*, 472S-478S.

Yang, C., Chen, L., Lee, M., Balentine, D., Kuo, M., & Schantz, S. (1998). Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. *Cancer Epidemiology, Biomarkers & Prevention, 7*, 351-354.

Yoshino, K., Suzuki, M., Sasaki, K., Miyase, T., & Sano, M. (1999). Formation of antioxidants from (-)-epigallocatechin gallate in mild alkaline fluids, such as authentic intestinal juice and mouse plasma. *The Journal of Nutritional Biochemistry, 10*(4), 223-229. http://dx.doi.org/10.1016/S0955-2863(98)00103-X

Table 1. Antioxidant Activity (AA) of Human Plasma After Consumption of Different Quantities of Tea

No.	Quantity of Consumed Tea	Increase of AA in %	Reference
1	300 ml of green tea 300 ml of black tea	40	Serafini et al., 1996
2	900 ml of green tea per day for 4 weeks	3.5	Van het Hof et al., 1997
3	300 ml of tea	15	Pietta et al., 1998a
4	400 ml of tea catechins	16	Pietta et al., 1998b
5	300-400 ml of green tea	Increase in relation to water consumption	Benzie et al., 1999
6	Green tea 150 ml 300 ml 45 ml	 0 7 12	Sung et al., 2000
7	300 ml of green tea 300 ml of black tea	40 52	Serafini et al., 2000
8	1200 ml of black tea (200 ml every hour for 6 hours)	65-76	Longley-Evans, 2000

Table 2. Determination of Catechins in Biological Fluids and Tissues of Humans and Animals

No.	Type of Biological Fluid or Tissue	Reference
1.	Plasma	Ho et al., 1995; Kivits et al., 1997, Shahrzad and Bitsch, 1998; Umegaki et al., 2001 ; Takino et al., 2003 ; Kotani et al., 2003 ; Unno et al., 2005 ; Masukawa et al., 2006 ; El-Hady et al., 2008 ; Stalmach et al., 2009
2.	Serum	Kivits et al., 1997 ; El-Hady et al., 2008
3.	Saliva	Tsuchiya et al., 1997
4.	Urine	Yang et al., 2000; Clifford et al, 2000; Manach et al., 2005 ; Mulder et al., 2005 ; Auger et al., 2008 ; Stalmach et al., 2009
5.	Feces	Lee et al., 2006
6.	Prostate cells	Henning et al., 2006
7.	Brain	Rechner et al., 2001
8.	Cancer cells	Kim et al., 2000

Table 3. Bioavailability of Tea Catechins

No.	Type of Tea or Catechin	Amount of Consumed Catechins	Amount of Catechins Found in Blood Plasma	References
1.	Green Tea	90-150 mg	0.1-0.7 μM/g	Clifford et al., 2000
2.	Green Tea	679 mg	1.3 μM/g	Nokagawa et al., 1999
3.	Black Tea	496 mg	0.5 μM/g	Manach et al., 2004
4.	EGCG	82 mg	$1.22 \cdot 10^{-3}$ mg/ml	Clifford et al., 2000

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In Vitro Bioavailability of Mineral Nutrients in Breakfast Cereals

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Abstract

The bioavailability of both micro- and macroelements was investigated under conditions simulating the digestion processes in the human alimentary system. A one-step enzymatic extraction was applied using buffered solutions containing pepsin, trypsin, α-amylase or pancreatin, which are enzymes that hydrolyse different nutritional food components such as peptides, carbohydrates and lipids, as the extractant. Corn flakes and multigrain breakfast cereals containing taste additives from a local market in the Wroclaw agglomeration were selected for study as an important kind of ready-to-eat meal. The most popular brands (corn flakes from Nestlé, Mlekołaki and Hanne as well as wheat-based breakfast cereals from Chocapic, Nesquik and Fitness) were analysed. Microwave digestion was employed for sample preparation, and the total concentrations of both micronutrients (Al, Ca, Cu, Fe, Mg, Mn, P, Sr and Zn) and metals released during enzymatic *in vitro* digestion were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES). Analysis of a Standard Reference Material was performed to validate the applied analytical procedure. Fractionation of the metals bound to the peptides, lipids and carbohydrates was evaluated and discussed.

Keywords: Micronutrients bioavailability, Enzymatic extraction, Fractionation, Breakfast cereals, Micro- and macroelements

1. Introduction

Oral ingestion is the only natural path to supply humans with trace elements and other essential nutrients, which are indispensable for growth, normal physiological functioning and the maintenance of life because the body cannot synthesise them. The best way to provide a sufficient amount of nutrients is to ensure the consumption of an adequately balanced diet. Therefore, quality control of the identity, purity and concentration of characterizing compounds ensures the quality, safety and efficacy of food products. It is widely known that the toxicity of metals and metalloids depends on their concentration and bioavailability. Consequently, the identification and quantitative determination of the elemental chemical forms should be assessed in addition to the total metal content. One of the possibilities for the verification of environmental risks to humans from metals present in food is to measure their bioavailability (bioaccessibility). Bioaccessibility is the maximum amount of the compound released from the matrix during gastrointestinal digestion that becomes available for intestinal absorption (Oomen *et al.*, 2002).

For that purpose, *in vitro* digestion procedures were developed based on human physiology.

Digestion using simulated gastric and intestinal fluids provides valuable information on mineral fractionation and allows the estimation of their bioavailability (Elless, Blaylock, Huang & Gussman, 2000). Simulation of stomach conditions consists mainly of reconstructing the essential constituents of gastric juices. Pepsin, which occurs in gastric juices, is a proteolytic enzyme that digests proteins in the highly acidic (pH between 2 and 3) environment of the stomach. This enzyme begins digestion by splitting proteins into smaller pieces. The simulated gastric juice, i.e., a solution containing pepsin, sodium chloride and hydrochloric acid, was used to release metals from food and dietary supplements (Ponce de Leon, Sutton, Caruso & Uden, 2000; Silva *et al.*, 2001; Reyes *et al.*, 2006; Bermúdez-Soto, Tomás-Barberán & Garciá-Conesa, 2007; Kulkarni, Acharya, Rajurkar

& Reddy, 2007). Intestinal digestion involves the activity of trypsin, amylase, pancreatin, bile salts and bicarbonates and takes place in the small intestine. At this phase in digestion, proteins, polysaccharides and fats are hydrolysed into products that can be absorbed, i.e., surpass the intestinal membrane. Enzymatic digestion procedures replicating intestinal digestion consist of the application of both single enzymes, such as trypsin (Pardo-Martínez, Viñas, Fisher & Hill, 2001; Peña-Farfal *et al.*, 2005), α-amylase (Caruso, Heitkemper & Hymer, 2001; Peña-Farfal *et al.*, 2004), lipase (Peña-Farfal *et al.*, 2004) and pronase (Dernovics, Stefánka & Fodor, 2002), or their natural combination, pancreatin (Miller, Schricker, Rasmussen & Van Campen, 1981; Pardo-Martínez, Viñas, Fisher & Hill, 2001; Peña-Farfal *et al.*, 2004; Kulkarni, Acharya, Rajurkar & Reddy, 2007) and synthetic mixtures, such as pancreatin with α-amylase (Azenha & Vasconcelos, 2000; Reyes *et al.*, 2006) or pronase with amylase (Casiot, Szpunar, Łobiński, Potin-Gautier, 1999).

All the enzymatic extraction procedures were conducted at 37°C, which is similar to the normal body temperature. To simulate the gastrointestinal movement and mixture of food during enzymatic hydrolysis, the samples were shaken (Peña-Farfal *et al.*, 2005; Reyes *et al.*, 2006), stirred, either mechanically or magnetically (Ponce de Leon, Sutton, Caruso & Uden, 2000; Caruso, Heitkemper & Hymer, 2001; Kulkarni, Acharya, Rajurkar & Reddy, 2007), or swirled (B'Hymer & Caruso, 2000) for a set period of time primarily in a water bath. Usually, procedures simulating gastric digestion last from 1 to 6 hours (Peña-Farfal *et al.*, 2005; Peña-Farfal *et al.*, 2005; Kulkarni, Acharya, Rajurkar & Reddy, 2007), whereas the samples are incubated in the intestinal juices for 1 to 24 hours (Pardo-Martínez, Viñas, Fisher & Hill, 2001; Dernovics, Stefánka & Fodor, 2002; Peña-Farfal *et al.*, 2004). A distinct reduction of the length of the enzymatic hydrolysis procedure to 30 minutes was achieved using ultrasonic energy (Peña-Farfal *et al.*, 2005).

Enzymatic extraction procedures were employed to assess the bioaccessibility of metals in various food and environmental samples, such as mixtures of food (meals) (Miller, Schricker, Rasmussen & Van Campen, 1981), baby foods (Pardo-Martínez, Viñas, Fisher & Hill, 2001), fish (swordfish, sardine and tuna) (B'Hymer & Caruso, 2000), mussel soft tissues (Peña-Farfal *et al.*, 2004), edible seaweeds (Peña-Farfal *et al.*, 2005), bovine milk (Silva *et al.*, 2001), freeze-dried apples (Caruso, Heitkemper & Hymer, 2001), chokeberries (Bermúdez-Soto, Tomás-Barberán & Garciá-Conesa, 2007), wheatgrass (Kulkarni, Acharya, Rajurkar & Reddy, 2007), mushrooms (Dernovics, Stefánka & Fodor, 2002), yeast and yeast-based food supplements (Casiot, Szpunar, Łobiński & Potin-Gautier, 1999; B'Hymer & Caruso, 2000; Reyes, Encinar, Marchante-Gayón, Alonso & Sanz-Medel, 2006; Bermúdez-Soto, Tomás-Barberán & Garciá-Conesa, 2007), wine (Azenha & Vasconcelos, 2000) and soil (Oomen *et al.*, 2002).

The majority of the undertaken research focused on the determination of only one or a few different elements. Essential micronutrients, such as Ca (Silva *et al.*, 2001), Cu (Miller, Schricker, Rasmussen & Van Campen, 1981), Fe (Miller, Schricker, Rasmussen & Van Campen, 1981; Silva *et al.*, 2001), Mg (Silva *et al.*, 2001), Se (Ponce de Leon, Sutton & Caruso, Uden, 2000) and Zn (Silva, Lopes, Nóbrega, Souza & Nogueira, 2001), or toxic elements, such as As (Pardo-Martínez, Viñas & Fisher, Hill, 2001; Peña-Farfal *et al.*, 2004), Hg (Cabañero, Madrid & Cámara, 2004) and Pb (Azenha & Vasconcelos, 2000), were investigated. Only a few studies were performed using enzymatic extraction procedures for multi-elemental analysis, which can lead to measurements based on the quantification of more than eight different elements (Oomen *et al.*, 2002; Peña-Farfal *et al.*, 2004; Peña-Farfal *et al.*, 2005). The concentration of both the major and trace elements examined was mainly assessed using spectroscopic methods, in particular FAAS (Azenha & Vasconcelos, 2000), HG-AAS (Pardo-Martínez, Viñas, Fisher & Hill, 2001), AFS (Dernovics, Stefánka & Fodor, 2002; Cabañero, Madrid & Cámara, 2004), ICP-OES (Dernovics, Stefánka & Fodor, 2002; Peña-Farfal *et al.*, 2005, ICP-MS (Casiot, Szpunar, Łobiński & Potin-Gautier, 1999; Cabañero, Madrid & Cámara, 2004; Reyes *et al.*, 2006), INNA (Kulkarni, Acharya, Rajurkar & Reddy, 2007).

The aim of the present work, continuation of of our recent study (Leśniewicz, Kretowicz, Wierzbicka, Żyrnicki, 2009), was to determine the mineral composition, nutritive value and the (*in vitro*) bioavailability of minerals and trace elements in ready-to-eat breakfast cereals by applying enzymatic digestion procedures. Two types of products, corn and multigrain breakfast cereals, were analysed. The enzymes in the gastric and pancreatic juices were used to investigate metal fractionation. The extraction effectiveness was investigated to evaluate the liberation of metals through the enzymatic hydrolysis of macronutrients, such as peptides, lipids and carbohydrates, present in the corn flakes and flavoured breakfast cereals examined.

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2. Materials and Methods

2.1 Samples

Popular brands of breakfast cereals – ready-to-eat food grain products playing important role in children and adolescent diet - purchased from the local market in the Wrocław commercial area were studied. The packages containing the corn and multigrain breakfast cereals were randomly selected for analysis, and a detailed description of the analysed products is given in Table 1. The Standard Reference Material, Corn Flour INCT-CF-3, was used to assess the accuracy and precision of the applied procedures.

2.2 Reagents, glassware and plastics

All chemicals used in this study were analytical grade and tested for possible contamination. For sample digestion, concentrated HNO_3 (Merck KGaA, Germany) and 30% (m/v) H_2O_2 (Polish Chemical Reagents, Poland) were used. Pepsin from a hog's stomach, trypsin from a porcine pancreas, α-amylase from a hog's pancreas and pancreatin from a porcine pancreas (BioChemika, Fluka) were used to prepare the extractants. Aqueous standard solutions were prepared by diluting the ICP multi-element standards (Merck KGaA, Germany). All dissolutions and dilutions were performed using 18.3 MΩ cm^{-1} water (EASYpure™ system, Barnstead, Thermolyne Corporation, USA).

Glass and plastic test tubes and bottles were washed with distilled water, cleaned with diluted nitric acid in an ultrasonic bath and rinsed several times with deionized water.

2.3 Extraction method - enzymatic hydrolysis

A conventional, one-step, solid-liquid leaching was performed at 37 ºC in a water bath shaker (elpan-Laboratory Instruments, type 357). The following extractants were used:

1. a solution containing pepsin, hydrochloric acid and sodium chloride (1.6 g pepsin, 1,0 g of NaCl and 3.5 mL of 37 % HCl made up to the 500 mL with deionized water (Ponce de Leon, Sutton, Caruso, Uden, 2000)) with a composition similar to gastric juices;
2. an α-amylase buffered solution (1,4286 g α–amylase dissolved in phosphate buffer solution, pH = 7.1 and made up to 500 mL with the same buffer solution (Harper, Rodwell, Mayer, 1983));
3. a buffered solution containing trypsin (1,4286 g trypsin dissolved and filled up to the 500 mL with phosphate buffer solution, pH = 7.5 (Peña-Farfal *et al.*, 2005));
4. a solution containing pancreatin (1,4286 g pancreatin dissolved in phosphate buffer solution, pH = 7.5 and made up to 500 mL with the same solution (Peña-Farfal *et al.*, 2004; Intawongse & Dean, 2006)).

In a plastic test-tube, 20.0 mL of the extractant solution was added to 0.5 g of the dry material. The closed tube was shaken for either 2 h (for the pepsin solution) or 6 h (for trypsin, α-amylase and pancreatin solutions) at 37˚C on a mechanical shaker at a speed of 200 r. p. m.. The supernatant was separated from the solid residue by centrifuging for 15 minutes at 9000 r.p.m. (High Speed Brushless Centrifuge – MPW 350). All of the extracts investigated were stored in clean polyethylene bottles at 4 ˚C before analysis.

Five parallel analyses were performed for each examined sample. For each set of five replicates of the digested or extracted samples, a blank was simultaneously subjected to the complete procedure, analysed and used to correct the analytical signals. All of the extraction procedures were applied to the examined samples twice.

2.4 Measurement of element contents

A standard microwave digestion procedure was used prior to the total concentration measurements. The concentrations of elements (i.e., Al, Ca, Cu, Fe, Mg, Mn, P, Sr and Zn) in the digests and extracts were measured using inductively coupled argon plasma-optical emission spectrometry (ICP-OES). A Jobin-Yvon 38S spectrometer was equipped with a cross-flow nebuliser and Scott-type spray chamber for the digest measurements and a V-groove nebuliser and cyclonic chamber for the extracted samples. The instrument operating parameters and analytical line wavelengths used are shown in Table 2.

3. Results and Discussion

Many types of breakfast cereals are produced using a variety of taste additives. As a general rule, the mineral content is different in corn flakes and multi-grain breakfast cereals enriched with additives. Therefore, for studies of mineral bioavailability, the three products based on wheat, rice and corn grains as well as three based only on corn flour (see Table 3) with the highest element content were selected based on our previous study (Leśniewicz, Kretowicz, Wierzbicka & Żyrnicki, 2009) .

Enzymatic extraction experiments were performed to study the availability of micronutrients in these breakfast cereals simulating the processes of the digestion in the human gastrointestinal system.

The enzymes responsible for gastric and intestinal digestion were used for these studies (Harper, Rodwell & Mayer, 1983).

Pepsin, an enzyme present in gastric juice, as well as amylase, trypsin and pancreatin, which exist in pancreatic juice, were applied during this study. Pepsin and trypsin break down dietary proteins into their component parts, i.e., peptides and amino acids, which can then be readily absorbed by the intestinal lining. Amylase degrades starches into sugars. Pancreatin, a mixture of trypsin, amylase and lipase, hydrolyses proteins into oligopeptides, starches into oligosaccharides and maltose and triglycerides into fatty acids and glycerols (Harper, Rodwell & Mayer, 1983).

The enzymatic hydrolysis efficiency was calculated as a ratio of the metal concentration in the extract to the element concentration in the samples after complete decomposition in a microwave system (Leśniewicz, Kretowicz, Wierzbicka & Żyrnicki, 2009)

The results of the liberation of metals from both the examined corn flakes and multi-grain, flavoured breakfast cereals by the enzymatic activity are shown in Figures 1-4.

The activity of pepsin in an HCl solution with pH = 2 resulted in a high mineral extraction effectiveness from breakfast cereals made of wheat, rice and corn. Over 80 % of the Zn, P, Mn, Mg, Ca and Sr content was removed from the organic matrix of the Chocapic, Nesquik and Fitness cereals. For these products, extraction efficiencies equal to or greater than 50 % were observed for Fe and Cu. Al was the only exception with at most 20 % leaching into acidic pepsin solution. A similar tendency was observed for the examined corn flakes brands, albeit at a lower extraction efficiency. For those breakfast cereals, extraction using a solution of pepsin released more than 80 % of the total Zn, Mn, Ca and Sr and 50 % of the total P, Mg and Cu. An effectiveness of less than 50 % was observed for Fe for all examined corn flakes samples. Less than 20 % of the total metal concentration determined by the pepsin solution was observed only in the case of Al. It is clear that ready-to-eat breakfast cereals can be a significant source of minerals, and micronutrients in such products are easily removable from the organic matrix by extraction under conditions similar to gastric digestion. Mineral susceptibility to digestion by acidic pepsin solutions is due to the metal that binds to the peptide or its specific connection to the organic matrix.

Moreover enzymes present in the pancreatic juice and, therefore, active during intestinal digestion were also studied. For evaluation of pancreatic enzymes influence on the examined samples, buffered solutions containing individual enzymes, i.e. trypsin, amylase and pancreatin were applied as extractants. As a general rule, higher enzymatic efficiencies were observed for corn flakes than for breakfast cereals made of wheat, rice and corn flour mixtures. The mineral leaching effectiveness of solutions containing trypsin, amylase and pancreatin for breakfast cereals made from corn is at least twice that for multigrain and flavoured breakfast cereals.

The effectiveness of the trypsin solutions was generally lower than those observed for extractants prepared from pepsin. In the case of multi-grain flakes, the efficiency of trypsin solution was at most 40 %. The extraction efficiencies for Mn, Mg, Cu and Sr from Chocapic, Nesquik and Fitness cereals were close to 30 %, and the concentrations of Zn and Ca in the extractant were the lowest, with only 10 % of the total content being removable. For breakfast cereals made from corn, a high quantity, 30 to 100 %, of Zn, Mn, Mg, Ca and Cu was removable from the organic matrix during extraction with the trypsin solution. Only in the case of Al and Sr fraction bound to proteins was low (approximately 10 % was the leachable) for the trypsin solution).

Amylase, an enzyme that breaks down carbohydrates, was most effective at removing Mn, Mg and Cu from multigrain cereals and Zn, Mn, Mg, Ca, Cu and Al from corn flakes. Extraction efficiencies were between 40 and 60 % for multigrain cereals with taste additives and between 50 and 100 % for corn flakes. Under these conditions, less than 20 % of the total Zn and Sr and less than 5 % of the total Fe, Ca and Al was removable from the breakfast cereals made from wheat, rice and corn. By contrast, the lowest extraction efficiency observed for Fe and Sr in corn flakes was close to or higher than 20 %.

Application of the pancreatin solution removed up to 60 % of the minerals from multigrain cereals and up to 100 % from the corn-based products. This solution removed the largest percentage of Mn, Mg, Cu and Al from Chocapic, Nesquick and Fitness cereal brands. In the case of corn flakes, the highest extraction efficiency was observed for Zn, Mn, Mg, Cu and Al. The lowest extraction efficiencies for the pancreatin solution were observed for Zn, Fe and Ca in the wheat, rice and corn flour flakes as well as for Fe and Ca in the corn-based breakfast cereals, with average values of 20 and 30 %, respectively.

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A comparison of the extraction effectiveness obtained for all of the pancreatic enzymes to the efficiency of the pepsin and hydrochloric acid mixture indicates that the enzymes active during intestinal digestion are less effective. Undoubtedly, the efficiency of the leaching process by trypsin, amylase and pancreatin solutions is higher for corn flakes than breakfast products based on wholemeal wheat, rice and corn. At the same time, the extraction efficiencies obtained for Zn, Mn, Mg, Cu, Al and Sr by amylase and pancreatin solutions are definitely higher than those obtained using the trypsin extractant for both kinds of products analysed. These results suggest a connection between the elements and the carbohydrate fraction hydrolysed by amylase or elements and the lipids degradable by the lipase in pancreatin (Harper, Rodwell & Mayer, 1983). Only in the case of one element - calcium - in multigrain breakfast cereals highest amount of was released by buffered solution containing trypsin, proteolitic enzyme.

Taking the origin and composition of the products into account with regards to their reaction to the extraction conditions, a few relationships could be pointed out. First of all, a proportional leaching of the elements from Chocapic, Nesquik and Fitness brand cereals by the amylase and pancreatin solutions was observed. For Mn, Mg and Sr, the highest extraction efficiency was from Nesquik and the lowest was from Fitness breakfast cereals. According to the manufacturer's data, Nesquik is made of wholemeal wheat, corn and rice flours as well as cocoa, whereas Fitness is composed of just wheat and rice grains. The maximum amount of Cu leachable by pancreatin extractants is obtained from Fitness, while the minimum is obtained from Nesquik. Metals are easily removable from Chocapic brand cereals, which are made of wholemeal wheat, wheat and rice flour with the addition of white chocolate and cocoa; however, the enzymatic extraction efficiency is not the greatest among the cereals studied, which is probably due to the presence of taste additives. The extraction efficiencies of the various kinds of corn flakes differ obviously, and the highest susceptibility of the matrix components to enzymatic hydrolysis were observed for Nestlé brand corn flakes, which have a higher corn content. At the same time, the lowest extraction efficiency was observed for Sr for all enzymes, both Cu and Fe for all pancreatic enzymes and Al for the pancreatin solution. The least amount of Zn was liberated from Hanne corn flakes for all extractants used. Additionally, the extraction efficiencies obtained for P, Mn, Fe, Mg, Ca and Cu from Hanne corn flakes were the least for the pepsin and HCl mixture. A constant proportion of Fe was leached by the pancreatic enzyme extractants.

Investigation of the Certified Reference Material, Corn Flour INCT-CF-3, served as an evaluation of the accuracy of the element concentration measurements, which was very high for most of the determined metals. For Zn, P, Mg and Ca, the recovery was in the range 91-99,6 %, while Mn and Sr recovery was satisfactory if the uncertainties from the standard deviation were considered. Significant disagreement between the experimentally measured and certified element content was only observed for Cu and Al.

Generally speaking, the precision of the measurements, expressed as RSD, was found to be less than 5 % for the digests and 15 % for the extracts.

4. Conclusions

A higher enzymatic extraction efficiency was observed for the acidic pepsin solution than for all of the examined pancreatic enzymes, which is due to the minerals either being present in an acid-soluble form or linked to the pepsin-degradable peptides.

For all elements, the effectiveness of metal liberation by pepsin in a 0.1 M HCl solution was considerably higher for wheat-based cereals than for corn flakes, and these efficiencies were comparable only for Fe. In the case of the multigrain cereal flakes, the extraction efficiencies for individual elements were close to 100% for the brands Chocapic, Nesquik and Fitness. In the case of corn flakes, various enzymatic extraction efficiencies were observed for each element from the different brands Mlekołaki, Nestlé and Hanne.

In contrast to the pepsin and HCl mixture, the extraction efficiencies of the trypsin solution were higher for the corn cereals than for the wheat-based products. The opposite tendency was observed only for Sr. The quantified liberation of individual elements by trypsin varied for all of the analysed products and was significantly lower than for the amylase and pancreatin solutions. The only exception was the response of Ca to the peptide-hydrolysing enzyme, which indicates that Ca is connected to the protein fraction.

Similarly to the behaviour of trypsin, buffered amylase and pancreatin solutions more efficiently liberated metals from corn flakes than from wheat cereals.

The various distributions of the different metals bound to the peptides, carbohydrates and lipids found here in ready-to-eat breakfast cereals were expected; however, the substantial differences between similar products supplied by different manufacturers was both unexpected and evident. In the case of wheat products, these

differences can be explained by assuming the presence of various additives. However, the results achieved for corn cereals (e.g., the differences in the extraction efficiencies of Zn, Mn, Mg, Al and Sr for amylase and Zn and Sr for trypsin or pancreatin) clearly indicate that the explanation is more complicated.

The relatively high liberation of Sr, which is a toxic element - causing problems with bone growth, especially for children - by these enzymes seems to be an important fact worthy of attention. References

References

Azenha, M. A. G. O., & Vasconcelos, M. T. S. D. (2000). Assessment of the Pb and Cu *in vitro* availability in wines by means of speciation procedures. *Food Chem Toxicol, 38*, 899-912. http://dx.doi.org/10.1016/S0278-6915(00)00082-X

B'Hymer, C., & Caruso, J. A. (2000). Evaluation of yeas-based selenium food supplements using high-performance liquid chromatography and inductively coupled plasma mass spectrometry. *J Anal Atom Spectrom, 12*, 1531-1539. http://dx.doi.org/10.1039/B006437H

Bermúdez-Soto, M.-J., Tomás-Barberán, F.-A., & Garciá-Conesa, M.-T. (2007). Stability of polyphenols in chokeberry (*Aronia melanocarpa*) subjected to *in vitro* gastric and pancreatic digestion. *Food Chem., 102*, 865-874. http://dx.doi.org/10.1016/j.foodchem.2006.06.025

Cabañero, A. I., Madrid, Y., & Cámara, C. (2004). Selenium and mercury bioaccessibility in fish samples: an in vitro digestion method. *Anal Chim Acta, 526*(1), 51-61**.** http://dx.doi.org/10.1016/j.aca.2004.09.039

Caruso, J. A., Heitkemper, D. T., & Hymer, C. B. (2001). An evaluation of extraction techniques for arsenic species from freeze-dried apple samples. *Analyst, 126*, 136-140. http://dx.doi.org/10.1039/b009825f

Casiot, C., Szpunar, J., Łobiński, R., & Potin-Gautier, M. (1999). Sample preparation and HPLC separation approaches to seciation analysis of selenium in yeast by ICP-MS. *J Anal Atom Spectrom, 14*, 645-650. http://dx.doi.org/10.1039/A809027K

Dernovics, M., Stefánka, Zs., & Fodor, P. (2002). Improving selenium extraction by sequential enzymatic processes for Se-speciation of selenium-enriched *Agaricus bisporus*. *Anal Bioanal Chem, 372*, 473-480. http://dx.doi.org/10.1007/s00216-001-1215-5

Elless, M. P., Blaylock, M. J., Huang, J. W., & Gussman, Ch. D. (2000). Plants as a natural source of concentrated mineral nutritional supplements. *Food Chem., 71*, 181-188. PII: S0308-8146(00)00142-4

Harper, H. A., Rodwell, V. W., & Mayer, P. A.(1983). Zarys chemii fizjologicznej, Państwowy Zakład Wydawnictw Lekarskich, Warszawa – in polish.

Intawongse, M., & Dean, J. R. (2006). In-vitro testing for assessing oral bioaccessibility of trace metals in soil and food samples. *Trends Anal Chem., 25* 9), 876-886. http://dx.doi.org/10.1016/j.trac.2006.03.010

Kulkarni, S. D., Acharya, R., Rajurkar, N. S., & Reddy, A. V. R. (2007). Evaluation of bioaccessibility of some essential elements from wheatgrass (*Triticum aestivum* L.) by *in vitro* digestion method. *Food Chem, 103*, 681-688. http://dx.doi.org/10.1016/j.foodchem.2006.07.057

Leśniewicz, A., Kretowicz, M, Wierzbicka, K., & Żyrnicki, W. (2009). Inorganic micronutrients in food products of plant origin used for breakfast in Poland. *Int J Environ An Ch, 89*(8-12), 621-634. http://dx.doi.org/10.1080/03067310902962494

Miller, D. D., Schricker, B. R., Rasmussen, R. R., & Van Campen, D. (1981). An in vitro method for estimation of iron availability from meals. *Am J Clinl Nutr, 34*, 2248-2256.

Oomen, A. G., Hack, A., Minekus, M., Zeijdner, E., Cornelis, C., Schoeters, G., Verstraete, W., Van de Wiele, T., Wragg, J., Rompelberg, C. J. M., Sips, A. J. A. M., & Van Wijnen J. H. (2002). Comparison of Five In Vitro Digestion Models To Study the Bioaccessibility of Soil Contaminants. *Environ Sci Technol., 36* (15), 3326-3334. http://dx.doi.org/10.1021/es010204v

Pardo-Martínez, M., Viñas, P., Fisher, A., & Hill, S. J. (2001). Comparison of enzymatic extraction procedures for use with directly coupled high performance liquid chromatography-inductively coupled plasma mass spectrometry for the speciation of arsenic in baby foods. *Anal Chim Acta, 441*, 29-36. PII: S0003-670(01)01070-4

Peña-Farfal, C., Moreda-Piñeiro, A., Bermejo-Barrera, A., Bermejo-Barrera, P., Pinochet-Cancino, H., & de Gregori-Henríquez, I. (2004). Use of enzymatic hydrolysis for the multi-element determination in mussel soft tissue by inductively coupled plasma-atomic emission spectrometry. *Talanta, 64*, 671-681. http://dx.doi.org/10.1016/j.talanta.2004.03.042

Peña-Farfal, C., Moreda-Piñeiro, A., Bermejo-Barrera, A., Bermejo-Barrera, P., Pinochet-Cancino, H., & de Gregori-Henríquez, I. (2005). Speeding up enzymatic hydrolysis procedures for the multi-element determination in edible seaweed. *Anal Chim Acta, 548*, 183-191. http://dx.doi.org/10.1016/j.aca.2005.06.004

Ponce de Leon, C. A., Sutton, K. L., Caruso, J. A., & Uden, P. C. (2000). Chiral speciation of selenoamino acids and selenium enriched samples using HPLC coupled to ICP-MS. *J Anal Atom Spectrom, 15*, 1103-1107. http://dx.doi.org/10.1039/B001069N

Reyes, L. H., Encinar, J. R., Marchante-Gayón, J. M., Alonso, J. I. G., & Sanz-Medel, A. (2006). Selenium bioaccessibility assessment in selenized yeast after "in vitro" gastrointestinal digestion using two-dimensional chromatography and mass spectrometry. *J Chromatogr A, 1110*, 108-116. http://dx.doi.org/10.1016/j.chroma.2006.01.088

Silva, F. V., Lopes, G. S., Nóbrega, J. A., Souza, G. B., & Nogueira, A. R. A. (2001). Study of the protein-bound fraction of calcium, iron, magnesium and zinc in bovine milk. *Spectrochim Acta B, 56*, 1909-1916. http://dx.doi.org/10.1016/S0584-8547(01)00313-5

Wragg, J., Rompelberg, C. J. M., Sips, A. J. A. M., & Van Wijnen J. H. (2002). Comparison of Five In Vitro Digestion Models To Study the Bioaccessibility of Soil Contaminants. *Environ Sci Technol, 36* (15), 3326-3334. http://dx.doi.org/10.1021/es010204v

Table 1. Composition of the analysed breakfast cereals

Brand name	Producer	Composition (according to producer data):	
		main compounds	nutrients content [g/100 g]
Corn flakes			
Corn flakes MLEKOŁAKI	Lubella S.A. ul. Wronkowska 1 20–469 Lubin	corn flour (91.7 %), barley malt, fructose, glucose, salt, emulsifier	protein: 6.6 carbohydrates: 84.9 fat: 2.3
Corn flakes Nestlé	Cereal Partners Poland Toruń–Pacific ul. Szosa Lubicka 38 /58 87 – 100 Toruń	corn (97 %), sugar, salt, glucose, barley malt, emulsifier	protein: 7.3 carbohydrates: 83.3 fat: 1.5
Corn flakes Hanne	C. Halne Mühlenwerke GmbH & Co KG Postfach 10 0551 D–32505 Bad Oeynhaus	corn (91 %), sugar, salt, barley malt	protein: 7.3 carbohydrates: 82.4 fat: 1.2
Flavoured and multigrain flakes			
Fitness	**Brands manufactured for NESTLÉ** Cereals Partners Poland, Toruń-Pacific Sp. z o.o, ul. Szosa Lubicka 38/58, 87-100 Toruń.	fitness flakes (97 %): cereal grains – wheat (39.1 %), rice, sugar, brown sugar syrup, emulsifier, acidity regulator, antioxidant	protein: 8.0 carbohydrates: 79.8 fat: 1.3
Chocapic Duo		flour (52.9 %): wholemeal wheat, wheat, rice, white chocolate (10.7 %), cocoa (6.4 %), glucose, barley malt, palm oil, emulsifier	protein: 8.3 carbohydrates: 74.4 fat: 7.5
Nesquik		flours (62.5 %): wholemeal wheat, corn, rice, sugar, cocoa (5.6 %), glucose, palm oil, salt, low-fat cocoa, acidity regulator	protein: 7.3 carbohydrates: 79.1 fat: 3.8

Table 2. Instrumental and operating conditions for ICP-AES

Discharge parameters:	
Forward power	1000 W [(1)]/ 1200 W [(2)]
Frequency	27.3 MHz
Plasma gas flow rate	13 L min^{-1} [(1)] / 14 L min^{-1} [(2)]
Sheath gas flow rate	0.2 L min^{-1}
Nebulizer gas flow rate	0.3 L min^{-1}
Sample uptake	1.0 mL min^{-1} [(1)] / 1.33 mL min^{-1} [(2)]
Monochromator:	1m Czerny-Turner type: HR 1000
Gratings	4320 and 2400 grooves mm^{-1}
Slit width (entrance/exit)	20 µm / 50 µm
Photomultiplier:	R 955
Plasma observation zone:	radial, 12 mm above load coil
Analytical lines (wavelengths in nm):	

Al	396.152	Mn	259.373
Ca	317.933	P	213.618
Cu	324.754	P	214.914
Fe	259.940	Sr	407.771
Mg	280.270	Zn	202.548
Mg	285.213		

[(1)] – applied for digested samples

[(2)] – applied for extracted samples

Table 3. Content of the macro- and microelements in the analysed corn and multi grain flakes – mean value ± standard deviation [µg/g, - dry weight]

Element \ Brand	Corn flakes Mlekołaki	Corn Flakes Nestlé	Corn flakes Hanne	Nestlé Fitness	Chocapic Duo	Nesquik
Al	1.46 ± 0.04	1.00 ± 0.55	5.82 ± 0.3	2.01 ± 0.33	14.6 ± 0.90	9.66 ± 0.44
Ca	1061 ± 33	30.5 ± 2.7	54.7 ± 3.1	4604 ± 91	2657 ± 122	2731 ± 50
Cu	0.20 ± 0.06	0.30 ± 0.06	0.32 ± 0.10	1.86 ± 0.06	3.67 ± 0.14	3.39 ± 0.09
Fe	41.0 ± 8.5	165 ± 2	10.9 ± 0.8	117 ± 4.8	167 ± 4.4	123 ± 5
Mg	466 ± 12	73.9 ± 3.9	363 ± 12	547 ± 36	818 ± 29	664 ± 22
Mn	1.87 ± 0.06	0.36 ± 0.07	1.57 ± 0.08	15.4 ± 0.16	15.8 ± 0.37	12.5 ± 0.40
P	1419 ± 26	341 ± 17	1161 ± 62	1700 ± 133	1700 ± 116	1330 ± 75
Sr	0.70 ± 0.01	1.72 ± 0.10	0.13 ± 0.03	1.36 ± 0.05	1.94 ± 0.08	1.63 ± 0.01
Zn	6.95 ± 0.78	0.44 ± 0.26	9.26 ± 0.72	14.8 ± 1.4	12.9 ± 0.16	10.7 ± 0.44

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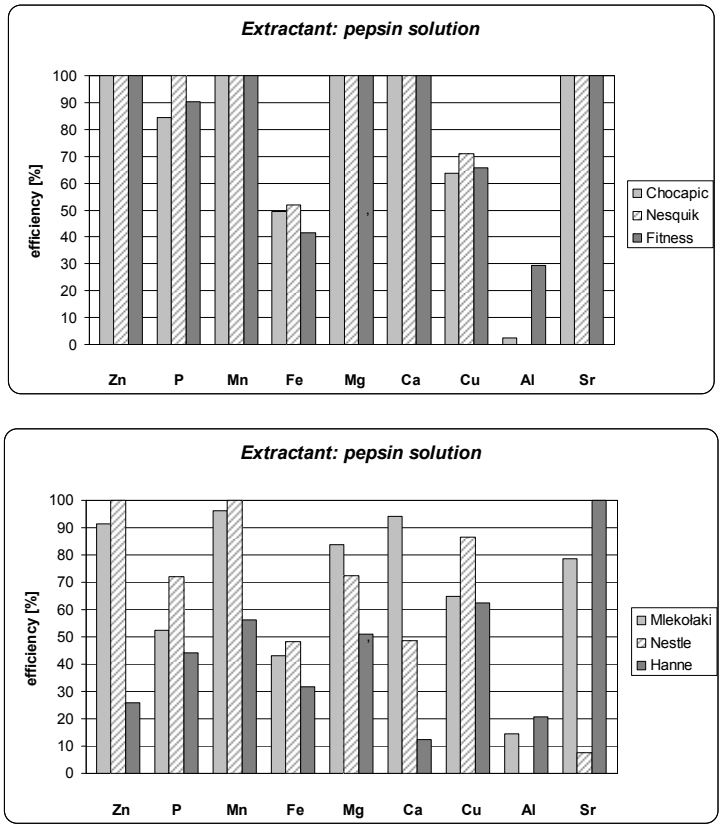


Figure 1. Enzymatic extraction efficiency for pepsin solution

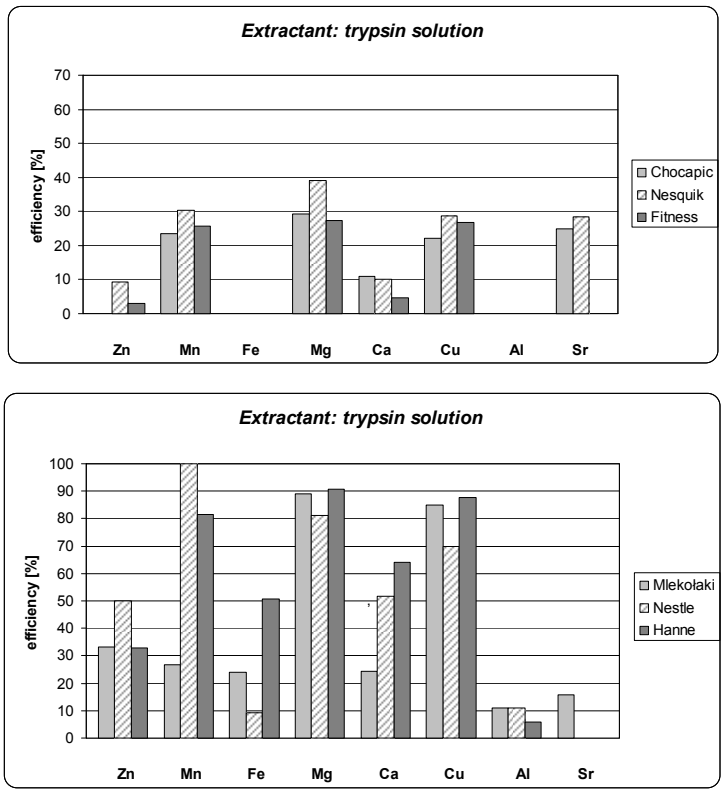
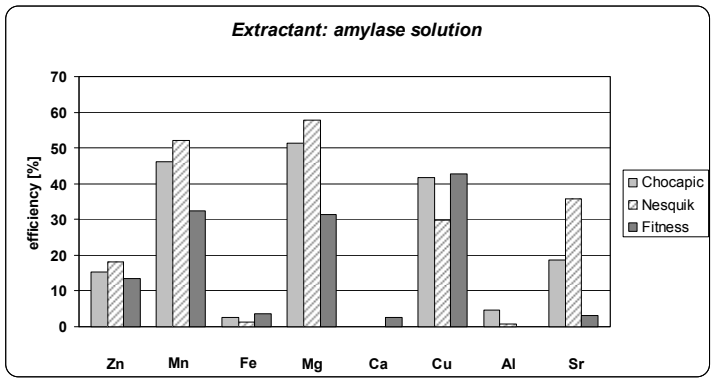


Figure 2. Enzymatic extraction efficiency for trypsin solution



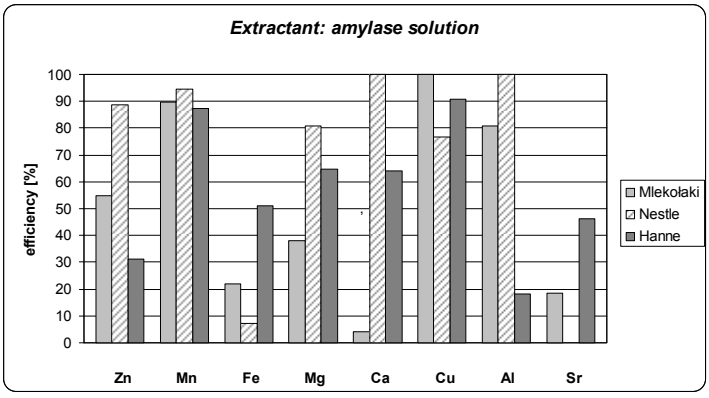
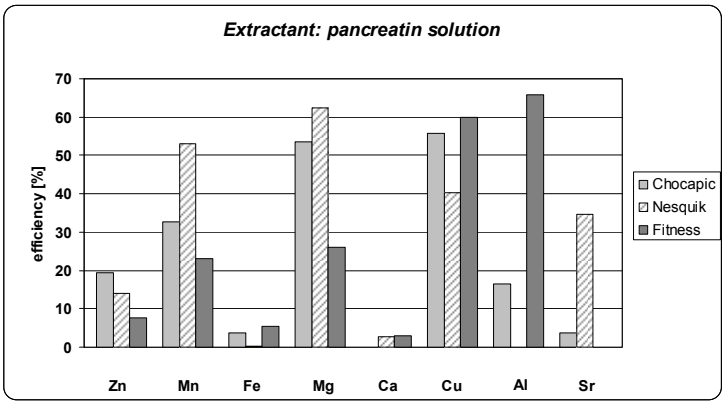


Figure 3. Enzymatic extraction efficiency for amylase solution



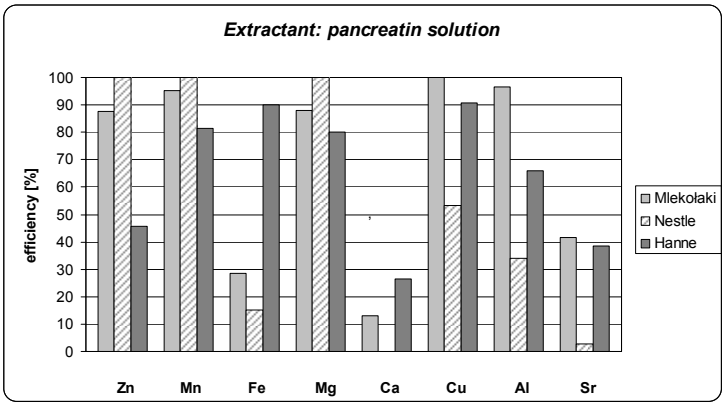


Figure 4. Enzymatic extraction efficiency for pancreatin solution

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