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Potency of *Lactobacillus plantarum* Dad-13 and Sweet Potato (*Ipomoea batatas*) Fiber as Immunomodulator in Rats Infected With *Salmonella* Typhimurium

Nurliyani[1], Madarina Julia[2], Eni Harmayani[3], Muthi Ikawati[4] & Endang Baliarti[1]

[1] Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia

[2] Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia

[3] Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia

[4] Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia

Correspondence: Nurliyani, Faculty of Animal Science, Universitas Gadjah Mada, Jl. Fauna 3 Kampus UGM, Bulaksumur, Yogyakarta 55281, Indonesia. E-mail: nurliyani@yahoo.com

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Abstract

Lactobacillus plantarum Dad-13 that isolated from "dadih" (traditional Indonesian fermented milk) has been known as probiotic, while sweet potato fiber has been proven as an effective prebiotic. The objective of this study was to evaluate the potency of *Lactobacillus plantarum* Dad-13 and sweet potato fiber as immunomodulators in terms of intestinal secretory immunoglobulin A (sIgA) and splenocyte gamma-interferon (IFN-γ). Sixty male Sprague Dawley rats (uninfected and infected) were divided into five groups: AIN-93, Indonesian children diet (ICD), Sweet potato fiber (SPF), SPF + *Lactobacillus plantarum* Dad-13 (SPFL), and fructooligosaccharides + Lacto-B (FOSL). After diet intervention, the rats were killed and sampled including intestinal fluid, spleen and caecal digesta. The results showed that soluble fiber such as sweet potato fiber could not increase the number of lactobacilli in infected rats, but could play a role in mucosal immune response through the increasing of sIgA. While, *Lactobacillus plantarum* Dad-13 contained in the combination with sweet potato fiber may has potency in systemic immune stimulation, because of the tendency to increase level of splenocyte IFN-γ in infected rats.

Keywords: *Lactobacillus plantarum* Dad-13, sweet potato fiber, immunomodullator, lactobacilli, *Salmonella* Typhimurium

1. Introduction

The isolate of *Lactobacillus plantarum* Dad-13 from "dadih" was identified using *recA* gene-based multiplex primers (Rahayu, Yogeswara, Tami, & Suparmo, 2011). Traditionally, dadih made from buffalo milk are fermented in a bamboo tube and covered with banana leaves, and then incubated at room temperature (27-33 ºC) for 2 d (Sughita, 1985).

The gut microbiota or microflora has a crucial role in human health and disease. The gastrointestinal tract (GIT) is comprised of the entire digestive system from the stomach to the anus. The colon or the large intestine is the organ which is the preferred site for bacterial colonization. Particular changes in the intestinal ecosystem might contribute to the development of certain illness, such as inflammatory bowel disease, antibiotic-associated diarrhea, colon cancer, hypercholesterolemia, and others (Vyas & Ranganathan, 2012).

The human gut is dominated by several bacterial phyla including Bacteroidetes, Firmicutes, and Actinobacteria (Vyas & Ranganathan, 2012). The caecal microbiota was more complex than the jejunal and ileal microbiota. Although facultative anaerobes were also the predominant species in caecal microbiota, obligate aerobes belonging to the Bacteroides group, the *C. coccoides* group and the *C. leptum* subgroup were also present (Hayashi, Takahashi, Nishi, Sakamoto, & Benno, 2005).

The gut immune system is influenced by many factors, including dietary components and commensal bacteria. The composition of commensal bacteria can be influenced by various factors, including host genetics, nutrition, antibiotic treatment, infection, and sequential microbial colonization in the neonatal period. Therefore, prebiotics

and/or probiotics are a powerful strategy for manipulating the microbial composition and immune responses of the host (Vieira, Teixeira, & Martin, 2013).

Gastric juice and bile salt resistant, as well as antagonisms toward pathogenic bacteria were used to screen the probiotic candidates. Based on these criteria, *Lactobacillus plantarum* Dad-13 was included as probiotic. *L. plantarum* Dad-13 was able to inhibit the growth of pathogenic bacteria including *Shigella dysenteriae* dky-4, two strains of pathogenic *Escherichia coli* dky-1 and dky-2, and *Salmonella* Typhimurium dky-3. This strain showed resistance to bile salt (up to 3%), and resistance to gastric juice at pH 2.0-3.0 for 6 h (Rahayu, Yogeswara, Tami, & Suparmo, 2011).

In vivo studies showed that *Lactobacillus plantarum* Dad-13 increased total lactobacilli by 1.2 log cycle, but did not reduce the count of *E. coli* and coliforms. Infection of *E. coli* and addition of *L. plantarum* Dad-13 changed the ratio among fecal microbiota of rats (Sumaryati, Utami, & Suparmo, 2009). *L. plantarum* competes with *E. coli* for intestinal colonization in the presence of the mannose-dependent adherence mechanism. This mechanism was partly responsible for the competition with *E. coli* early after colonization, and also influences intestinal and systemic immunity (Herias et al., 1999). The species *Lactobacillus plantarum* 283 is a colonizer of the small human intestine, and *Lactobacillus plantarum* 299 is colonizer in human colon (Johansson et al., 1993). In another study, *Lactobacillus plantarum* Mut7 that had been isolated from fermented cassava possess probiotic criteria such as survival under acidic condition and bile acid, and antagonism against pathogenic bacteria such as *Salmonella choleraesuis* and *Shigella flexneri* (Lestari, Rahayu, & Aman, 2008). Heat-killed *L. plantarum* Mut7 has immunomodulatory effect in human HB4C5 cell line and the capability for increasing the production of IgM was the highest among other indigenous probiotic (Lestari, Harmayani, & Sugahara, 2010). There is limited information on *in vivo* study of immunomodulatory properties of *L. plantarum* Dad-13.

Diarrheal infections caused by *Salmonella*, are one of the major causes of childhood morbidity and mortality in developing countries. *Salmonella* causes various diseases that range from mild gastroenteritis to enteric fever, depending on the serovar involved, infective dose, species, age and immune status of the host (Castillo, Perdigón, & de LeBlanc, 2011). In the previous study, sweet potato fiber extract could increase the lactobacilli population and prevent the *Salmonella* Typhimurium diarrhea. This sweet potato (Bestak variety) containing 11.36% total fiber consisted of 9.23% insoluble fiber and 2.13% soluble fiber (Astuti, 2005). In addition, sweet potato fiber extracts containing fructooligosaccharides (FOS), inulin and raffinose, and showed potency as prebiotic source. This score of prebiotic activity of SPF was similar to FOS, but was higher than inulin (Lestari, Soesatyo Iravati, & Harmayani, 2013). Therefore, the SPF can be developed as functional foods.

Lactobacilli are able to utilise the most commonly used prebiotic oligosaccharides, and the best growth was observed on inulin, followed by lactulose and raffinose (Kunová, Rada, Lisová, Ročková, & Vlková, 2011). According to Gibson and Fuller (2000), preferred target organisms for prebiotics are not only lactobacilli, but also species belonging to the *Bifidobacterium* genera. The most efficient prebiotics may also reduce or suppress numbers and activities of organisms seen as pathogenic. However, contradicting results on prevention of pathogenic *Salmonella* infections with prebiotics have been published. Study by Bruggencate, Bovee-Oudenhoven, Lettink-Wissink, Katan, and Van der Meer (2004), inulin and FOS showed increased salmonella numbers in caecal contents six days after infection. Both inulin and FOS significantly stimulated intestinal colonisation and translocation of salmonella to extraintestinal sites. Additionally, supplementing a cornstarch-based rodent diet with 10% FOS or xyclooligosaccharides (XOS) was found to increase the translocation of *S.* Typhimurium SL1344 to internal organs in mice (Petersen et al., 2009).

Secretory IgA (sIgA) serves as the first line of defense in protecting the intestinal epithelium from enteric toxins and pathogenic microorganisms, through a process known as immune exclusion. This sIgA promotes the clearance of antigens and pathogenic microorganisms from the intestinal lumen by blocking their access to epithelial receptors, entrapping them in mucus, and facilitating their removal by peristaltic and mucociliary activities (Mantis, Rol, & Corthésy, 2011). While, IFN-γ and tumor necrosis factor alpha (TNF-α) cytokines, and also NOS_2 are key elements of cellular immunity (cell-mediated defense) against intracellular pathogens (Parent et al., 2006) such as *Salmonella* Typhimurium (Leung & Finlay, 1991).

The purpose of this study was to evaluate the effect of supplementation of sweet potato fiber and combination of *Lactobacillus plantarum* Dad-13 and sweet potato fiber on total number of caecal lactobacilli and its immunomodulatory properties in rats infected with *Salmonella* Typhimurium.

2. Materials and Methods

2.1 Bacterial Isolates, Sweet Potato Tuber, Commercial Prebiotic, and Probiotics

Bacterial isolate derived from "dadih" was provided by Food and Nutrition Culture Collection (FNCC) Center for Food and Nutrition Studies, Universitas Gadjah Mada. The sweet potato tuber that was used in this study was Bestak variety from Central Java, Indonesia. The characteristic this tuber were brownish white color in their skin and yellowish white in the interior tuber, spherical and tapered at both ends with diameter was around 8.0 cm. Fructooligosaccharides (FOS) as a commercial prebiotic was provided by Sari Husada Milk Industry (Yogyakarta, Indonesia), whereas the Lacto-B as a commercial probiotic was produced by Novell Pharmaceutical Laboratories (Jakarta, Indonesia). The Lacto-B powder contains three kinds of bacteria (*Streptococcus thermophilus, Lactobacillus acidophilus* and *Bifidobacterium longum*) and is known as anti-diarrhea in infant and children.

2.2 Preparation of Ethanol-Insoluble Residues From Sweet Potato

Fibers from sweet potato tuber (*Ipomoea batatas*) were prepared according to Mongeau & Brassard (1990) with slight modifications. Whole sweet potato was peeled (2 × 2 × 2 cm) and diced prior to extraction, and then steamed for 30 min. The steamed tuber was extracted by using 80% ethanol at 60 ºC for 20 min in Waring blender. The warm solutions were filtered by filter paper (Whatman 41), and then the ethanol-insoluble solid was washed with acetone (1:2) for 30 min to produce fluffy white materials. The materials were dried in oven at 50 ºC for 10 h and blended.

2.3 Experimental Design

Materials in this study consisted of sixty male Sprague Dawley rats three weeks old (were assumed as 1-2 years old children) around 50 g weighed from Laboratory of Integrated Research and Assessment, Universitas Gadjah Mada. Before the treatment of diets, the rats were aclimated for 7 d with standard diet AIN-93G (Reeves, Neilsen, & Fahey, 1993), and given drinking water adlibitum. The rats were divided into five groups: 1) AIN-93, 2) Indonesian children diet (ICD), 3) Sweet potato fiber (SPF), 4) *Lactobacillus plantarum* Dad-13+ SPF (SPFL), and 5) Lacto-B + fructooligosaccharides (FOSL). Each group consisted of 12 rats and was treated with diet for 14 d. Six rats in each group were infected orally with *Salmonella* Typhimurium (10^5 CFU/ml) at day 15 (total 30 rats), and continued to recieve the treated diets for 7 d and then killed. The uninfected rats were killed after recieving the diet for 14 d. The caecal, intestinal fluid and spleens of rats were sampled for evaluation of total lactobacilli, sIgA and IFN-γ, respectively. The fecal consistency was observed visually in uninfected and infected rats 7 d before and after infection. The lactobacilli were counted by standard plate count (SPC) method. While the intestinal sIgA and IFN-γ in spleen lympocyte culture supernatant, were analyzed by enzyme-linked immunosorbent assay (ELISA) technique. All procedures related to animal experiment were conducted following the recommendation of Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine Universitas Gadjah Mada, Indonesia (number of Ethics Committee Approval: KE/FK/374/EC).

2.4 Diets Preparation

Five diets were prepared: 1) Standard diet AIN-93G (Reeves, Neilsen, & Fahey, 1993) as negative control. 2) Indonesian children diet (ICD). The diet composition resembled Indonesian children' food and was made under the recommended dietary allowance for children aged 1-2 years. 3) Sweet potato fiber (SPF) diet. This sweet potato fiber was added (fortified) to standard diet AIN-93. 4) Sweet potato fiber + *Lactobacillus plantarum* Dad-13 diet (SPFL). This sweet potato fiber was added to standard diet AIN-93, whereas the *Lactobacillus plantarum* Dad-13 (10^8 CFU/ml) was administered by oral force feeding. 5) FOS + Lacto-B (FOSL) diet as positive control. FOS was added to the standard diet AIN-93 and Lacto-B (10^8 CFU/ml) was administered by oral force feeding. The composition for five diets is given in Table 1.

Table 1. Composition of standard and intervention diet

	g/kg				
Composition	AIN-93	ICD	SPF	SPFL	FOSL
Casein	200.0	59.3	200.0	200.0	200.0
L-Cystine	3.0	-	3.0	3.0	3.0
Sucrose	100.0	-	100.0	100.0	100.0
Corn starch	529.5	-	529.5	529.5	529.5
Fiber (CMC)	50.0	-	50.0	50.0	50.0
Soy oil	70.0	-	70.0	70.0	70.0
Choline bitartat	2.5	-	2.5	2.5	2.5
AIN-93 Min-Mix	35.0	35.0	35.0	35.0	35.0
AIN-93 Vit-Mix	10.0	10.0	10.0	10.0	10.0
Sweet potato fiber	-	-	32.0	32.0	-
Fructooligosaccharides	-	-	-	-	32.0
L. plantarum Dad-13	-	-	-	10^8 CFU/ml	-
Lacto-B	-	-	-	-	10^8 CFU/ml
Rice	-	763.3	-	-	-
Palm oil	-	132.4	-	-	-

2.5 Fecal Consistency

Fecal consistency was observed visually in uninfected and infected rats during experiment, by the same individual each morning according to Correa-Matos et al. (2003). The fecal consistency was graded using the following scale: 1 = solid; 2 = semisolid; 3 = loose; 4 = watery. The fecal consistency was observed everyday before and after infection for 7 d.

2.6 Total Lactobacilli

Total number of caecal lactobacilli was counted by using standard plate count method on selective medium Rogosa Agar (Jakesevic et al., 2011). Sample of caecal digesta was weighed and serial dilutions were made with physiological salt, and then certain dilutions were inoculated on sterile agar plates. Inoculated agar plate was incubated for 48-72 h at 37 ºC, the number of bacterial colony was counted and expressed in log CFU/g digesta.

2.7 Intestinal Fluid Collection

Rat intestinal fluid was taken by flushing with 4 ml of cold phosphate-buffered saline (PBS) pH 7 containing 2 mM phenylmethyl sulfonyl fluoride (PMSF) (Sigma), 10 mg tosylphenylalanine chloromethyl ketone (TPCK) (Sigma), 0.02% NaN_3, and 5 mM ethylene diamine tetra acetic acid (EDTA) (Sigma). Flushing fluid was collected in a sterile of 15 ml conical tubes, then were centrifuged at 926 × g (Sorval, Biofuge primo R), the supernatant was taken and stored at -20 ºC until analyzed (Yun, Lillehoj, Shu, & Min, 2000).

2.8 Analysis of Intestinal sIgA

Intestinal fluid sIgA was analyzed according to the instructions on the Rat sIgA ELISA Kit (Uscn Life Science Inc., Wuhan, China) as follows: 100 µl standard solution, samples and blank were pippeted into a well on the plate, then covered with a plate sealer and incubated at 37 ºC for 2 h. The solution was discarded and then added 100 µl solution of detection reagent A (biotin-conjugated polyclonal antibody specific for sIgA) into each well and incubated for 1 h at 37 ºC. The solution was removed and then the plate was washed three times with washing solution 1×. Then added 100 µl solution of detection reagent B (Avidin conjugated to Horseradish Peroxidase / HRP) and incubated for 30 min at a temperature of 37 ºC. Plate was washed five times, then added 90 µl of substrate solution (tetramethyl benzidine) into each well and incubated for 15-25 min at a temperature of 37 ºC in the dark space (fluid will be blue color). Stop solution (sulfuric acid) of 50 µl was added into each well (the liquid will change color to yellow), then read on a microplate reader (Model 680 XR, Bio-RAD) wavelength of 450 nm.

2.9 Lymphocyte Culture Supernatant Collection

Isolation and collection procedures of spleen lymphocyte were performed according to Klein, Witonsky, Ahmed, Holladay, and Gogal (2006) with slight modifications. Briefly, spleen was excised aseptically and placed in 10 ml of Rosewell Park Memorial Institute (RPMI)-1640 medium (Sigma) containing 10% FBS (fetal bovine serum: Gibco) and 2% penicillin-streptomycin (Gibco). The spleen was ripped with syringe tip, and also by pipetting up and down and spraying by RPMI medium a few times by using disposable syringe for releasing the lymphocytes. After releasing the lymphocytes from the spleen, the suspension was allowed to separate from cell debris. The supernatant was removed into conical tubes, and then the cells were counted by haemacytometer. The cell concentrations to be cultured were 5×10^5 /ml. The lymphocytes were cultured in plate with 96 wells in RPMI medium, and 5 µg/ml of phytohaemagglutinine (PHA) mitogen (Murex) was added to in each well. The volume of the lymphocyte culture was 100 µl in each well. The plate was placed into 5% CO_2 incubator for 72 h at 37 ºC. The lymphocyte culture supernatants were removed and analyzed for cytokine IFN-γ production.

2.10 Analysis of IFN-γ in Spleen Lymphocyte Culture Supernatant

The amount of IFN-**γ** contained in rat spleen lymphocyte culture supernatant was measured with specific rat IFN -**γ** ELISA kit (Bender MedSystem, Vienna, Austria). Briefly, microwell plate was washed twice with wash buffer. The standard was reconstituted with 750 µl aquabidest for Standard of IFN-**γ**, and the prepared standard was made into a 1:2 dilution in a small tube. For IFN-γ: 225 µl of reconstituted standard IFN-**γ** (concentration = 4000.0 pg/ml) was pipetted into S1 tube (standard 1) containing 225 µl sample diluent (concentration= 2000.0 pg/ml). This procedure was done for the next tubes until the concentration of the final tube (S7) was 31.3 pg/ml. Each standard (100 µl) was pipetted into well for standard, and 100 µl of sample diluent was pipetted into well for blank. Each well for sample was filled with 50 µl of sample diluent and added 50 µl sample. Biotin conjugate solution was added into microwell plate, and covered with adhesive film. The plate was incubated at room temperature (18-25 ºC) for 2 h. Adhesive film was removed, and microwell plate was washed 3 times with 400 µl wash buffer for each well. Streptavidin –HRP solution (100 µl) was added into all well, and covered with adhesive film. The plate was incubated at room temperature for 1 h. Micro plate was washed 3 times and added with 100 µl TMB (tetramethyl-benzidine) substrate to all well. The plate was incubated at room temperature for 10 min, avoiding exposure from direct light. The enzyme reaction was stopped by pipetting 100 µl of stop solution into each well. The absorbance of each microwell was read at a wave length of 405 nm.

2.11 Statistical Analysis

The data of total caecal lactobacilli, sIgA and IFN-**γ**, were analyzed by ANOVA using SPSS 12.0 (2003), Chicago, USA.

3. Results and Discussion

3.1 Fecal Consistency

The effect of infection with *Salmonella* Typhimurium in fecal consistency in rats that received various diet interventions is shown in Table 2.

Table 2. Fecal consistency of uninfected and rats infected with *Salmonella* Typhimurium

Diets	Uninfected				Infected			
	Solid (1)	Semi solid (2)	Loose (3)	Wattery (4)	Solid (1)	Semi solid (2)	Loose (3)	Wattery (4)
AIN-93	-	-	6	-	-	1	5	-
ICD	6	-	-	-	6	-	-	-
SPF	-	6	-	-	-	3	3	-
SPFL	-	6	-	-	-	1	5	-
FOSL	-	6	-	-	-	4	2	-
Total (n=30)	6	18	6	-	6	9	15	-

Consistency of feces in rats that received Indonesian children diets (ICD) tended to be solid in both in uninfected

and infected rats. The fecal consistency in rats fed sweet potato fiber (SPF), sweet potato fiber + *Lactobacillus plantarum* Dad 13 (SPFL) and fructooligosaccharides + Lacto-B (FOSL) were semi solid in uninfected rats, whereas this consistency in infected rats largely became loose. In the negative control rats (AIN-93), the fecal consistency tended to loose both in uninfected and infected rats. There was no watery consistency in rat fecal of all diets in uninfected or infected rats.

Although the diarrhea prevalence tend to more frequent after salmonella infection, but there was no clear pattern. The rats fed Indonesian chidren's diet (ICD) had more solid in fecal consistency than the rats fed other diets, and there was no change the fecal consistency in infected rats. This may be due the ICD containing more rice which has less fiber, so that the moisture content was lower. According to Li, Andrews, and Pehrsson (2002), cooked white rice containing 0 soluble fibers, 0.34% insolubel fiber and 0.34% total dietary fiber (TDF).

Salmonella Typhimurium is bacteria which causes diarrhea in infant and children, but the effect on rats is not as consistent as in humans. In this study, the uninfected rats fed SPF, SPFL and FOSL had semi solid in fecal consistency, whereas in infected rats, the fecal matter partially became loose in consistency. In the previous study, the rats fed 10% sweet potato fiber showed solid in fecal consistency after infection with salmonella, showing no diarrheal symptoms (Astuti, 2005). This is in accordance with Tauxe and Pavia (1998) which stated that clinically important infections due to *Salmonella* Typhimurium occur only in humans, and humans are the only known reservoir for this organism. Similar to the National Research Council (1991), that *S. enteritidis* serotype Typhimurium is the most common serotype infecting laboratory rodents, although the prevalence of asymptomatic carriers is unknown but probably low. Reports of natural outbreaks of disease are rare in the literature, probably because most infections are asymptomatic in normal hosts. Diarrhea is an uncommon finding. Many host, pathogen, and environmental factors determine the pathologic findings and severity of infection, including host age and genotype; make up of the intestinal flora; nutritional state; immune status; presence of concurrent infections; bacterial serotype; and environmental stressors such as food and water deprivation, temperature, iron deficiency, and experimental manipulations.

3.2 Caecal Lactobacilli

There were no significant differences in total lactobacilli in rats that received AIN-93, ICD and FOSL both in uninfected and infected rats. However, the total lactobacilli in infected rats fed SPF and SPFL decreased significantly ($p<0.01$) compared with uninfected rats. Lactobacilli in rats fed FOSL also decrease in infected rats, even though not significant (Figure 1).

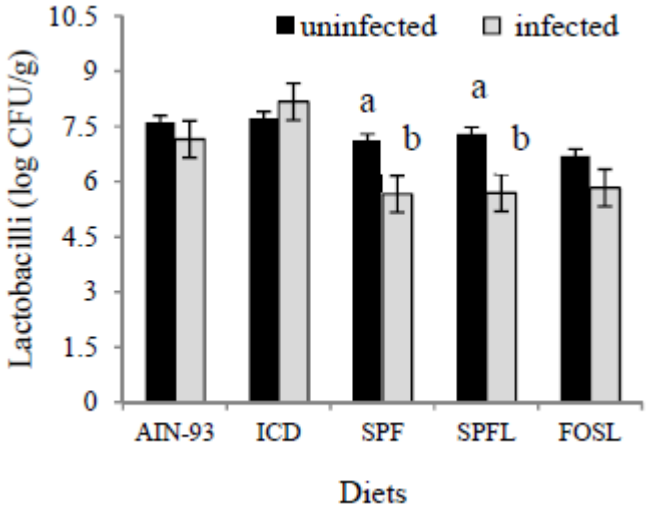

Figure 1. The average of total caecal lactobacilli in uninfected and infected rats fed different diets (AIN-93, ICD, SPF, SPFL, FOSL). Different letters (a, b) indicate significant difference ($p<0.01$) between uninfected and infected rats in each diet intervention, whereas no letters on top indicate no significant difference

The lower count of lactobacilli in three kinds of diets in this study (SPF, SPFL, FOSL) (Figure 1) indicates these dietary fiber could stimulate the growth of *Salmonella* Typhimurium and then compete with lactobacilli. This effect is likely due to the rapid fermentation of sweet potato fiber and fruktooligosaccharides to production of

metabolites such as SCFA. Inulin and oligofructose are rapidly and completely fermented by the colonic microflora with the production of acetate and other short chain fatty acid (SCFA) (Jenkins, Kendall, & Vuksan, 1999). Rapid production of metabolites results in subsequent irritation and impairment of the mucosal barrier. Total caecal SCFA pools were higher while pH was lower from ingesting oligosaccharide-containing diets compared with control or cellulose diets.

Dietary incorporation of fermentable, indigestible oligosaccharides, by providing SCFA, lowering pH, and increasing bifidobacteria, may be beneficial in improving gastrointestinal health (Campbell, Fahey, & Wolf, 1997). Salmonella can use the SCFA conditions of the mammalian intestinal tract as a signal for invasion. Low total SCFAs (30 mM) with a predominance of acetate induce invasion, whereas high total SCFAs (200 mM) with greater concentrations of propionate and butyrate suppress it (Lawhon, Maurer, Suyemoto, & Altier, 2002). The epithelial cell injury caused by rapid SPF, SPFL and FOSL fermentation in this study cause salmonellae crosses in the distal gut by a paracellular and transcellular route (Kops, Lowe, Bement, & West, 1996). The ability of *Salmonella* Typhimurium to invade the intestinal mucosal cells is an important step in pathogenesis (Durant, Corrier, & Ricke, 2000). *Salmonella* Typhimurium causes enteric and systemic disease by invading the intestinal epithelium of the distal ileum, a process requiring the invasion genes of Salmonella pathogenicity island 1 (SPI-1). The concentration and composition of SCFAs in the distal ileum provide a signal for productive infection by Salmonella, whereas those of the large intestine conditions inhibit invasion (Lawhon, Maurer, Suyemoto, & Altier, 2002). In addition, SCFA may serve as an environmental signal that triggers the expression of invasion genes in the gastrointestinal tract (Durant, Corrier, & Ricke, 2000).

3.3 Intestinal sIgA

Demonstrated on Figure 2, only sIgA in infected rats treated with SPF increased significantly ($p<0.05$) compared with uninfected rats, whereas there were no significant differences in sIgA between uninfected and infected rats in other diets.

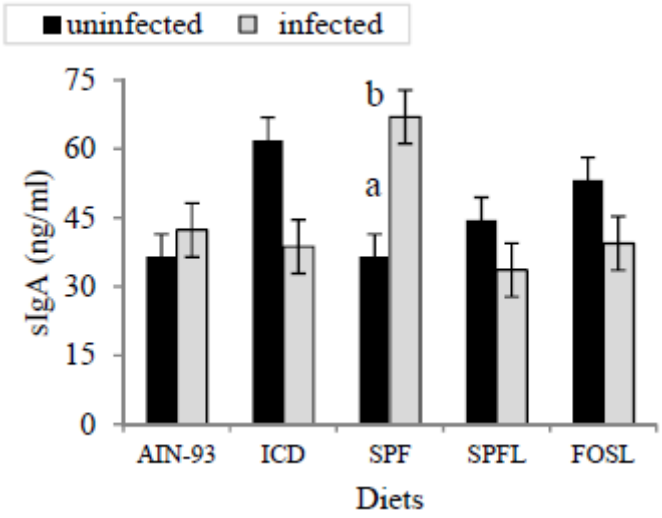

Figure 2. The average of intestinal sIgA in uninfected and infected rats fed different diets (AIN-93, ICD, SPF, SPFL, FOSL). Different letters (a, b) indicate significant difference ($p<0.01$) between uninfected and infected rats in each diet intervention, whereas no letters on top indicate no significant difference

The increase in level of sIgA in infected compared to uninfected rats fed sweet potato fiber may be due the SPF containing more than one prebiotic namely FOS, inulin and raffinose (Lestari, Soesatyo, Iravati, & Harmayani, 2013). This prebiotics combination may have a better effect on the growth of probiotic and their SCFA production in intestine. In addition, the dietary fiber could act as a more effective prebiotic by inducing major shifts in gut microbial composition and directly affecting the mucosal immune system, resulting in an improvement in enteric inflammatory disorders and the systemic immune response (Vieira, Teixeira, & Martins, 2013). These results are similar to those reported by Bakker-Zierikzee et al. (2006), that formula-fed infants may benefit from infant formulas containing a prebiotic mixture of GOS and FOS because of the observed clear tendency to increase faecal sIgA secretion. Adding viable *Bifidobacterium animalis* strain Bb-12 (6.0×10^9 CFU

/100 ml formula) did not reveal any sign for such a trend. Infants fed on the probiotic formula showed a highly variable faecal sIgA concentration with no statistically significant differences compared with the standard formula group. In this study, the infected rats fed SPFL or FOSL tended to decrease intestinal sIgA compared to uninfected rats, although not statistically different (Figure 2). This indicates that sIgA level is more influenced by prebiotic combination rather than combination of single prebiotic and probiotic.

Contrary to a study by Biedrzycka et al. (2003), young rats receiving more than 10^9 Bifidobacterium cells daily for 14 d, have higher bacterial antigen-specific IgA in serum blood compared to the control rats, both in non-infected and Salmonella-challenged animals. This result study by Biedrzycka et al. (2003), similar with the result in other study, that levels of sIgA and lymphocyte proliferation rate were also significantly increased in probiotic dahi-fed mice and infected with *Salmonella enteridis* compared with mice fed milk and control dahi. (Jain, Yadav, & Sinha, 2009). This different results from this study may be due to differences in the probiotic bacterial strain and dose, and also experimental animal used.

Most dietary fibers that have fermentable carbohydrates could be considered as prebiotics as well. There is a hypothesis that any type of dietary or food supplement that could promote the growth of beneficial bacteria and consequently promote homeostasis in the gut and good health could be considered as a prebiotic, even though the supplement may not meet the required criteria. Prebiotics and/or probiotics are a powerful strategy for manipulating the microbial composition and immune responses of the host (Vieira, Teixeira, & Martins, 2013).

SCFA production, particularly butyrate, in the colon may reduce the requirement of epithelial cells for glutamine, thereby sparing it for other cells, such as those of the immune system. This is possibly as a result of it's ability to spare glutamine as a substrate for the colonic mucosa by provision of increased SCFA. Because glutamine is a preferred substrate for lymphatic tissue, it is possible that this may improve immune function under some circumstances. Such an effect may also be relevant to inulin and oligofructose if SCFA production is increased in the proximal, even if not in the distal colon (Jenkins & Kendall, 1999). This hypothesis is supported by the observation that lactulose administration can increase serum glutamine levels (Jenkins, & Kendall, 1997). Glutamine is an important energy source for immune lymphocytes, because glutamine at near-physiological concentration can be readily utilized by these cells (Wu, Field, & Marliss, 1991) for rapidly dividing cells such as lymphocytes and epithelial cells of intestinal mucosa (Windmueller, 1982). It is well established that the fermentation of inulin and oligofructose increases the production of SCFA, primarily acetate, butyrate and propionate in the gut (Gibson & Roberfroid, 1995). Nevertheless, a number of studies support that SCFA improve components of non specific immune responses (Pratt, Tappenden, McBurney, & Field, 1996). Modulation of T-cell responses by n-butyrate could also result from altered antigen-presenting cells (APC) function, possibly as a consequence of downregulating distinct adhesion and/or costimulatory receptors as well as of inducing apoptosis. A potential clinical relevance of SCFA for reducing T-cell-mediated immune reactions via modulating APC function is speculated (Bohmig et al., 1997).

3.4 IFN-γ in Spleen Lymphocyte Culture

The level of IFN-γ in infected rats fed SPF was lower ($p<0.05$) than uninfected rats, same as in the ICD diet. In the AIN-93 diet, the IFN tended to decrease in infected rats, even though the decrease was not significant. However, the level of IFN-γ in infected rats fed SPFL and FOSL diets increased compared with uninfected rats, even though the increase was not significantly different (Figure 3).

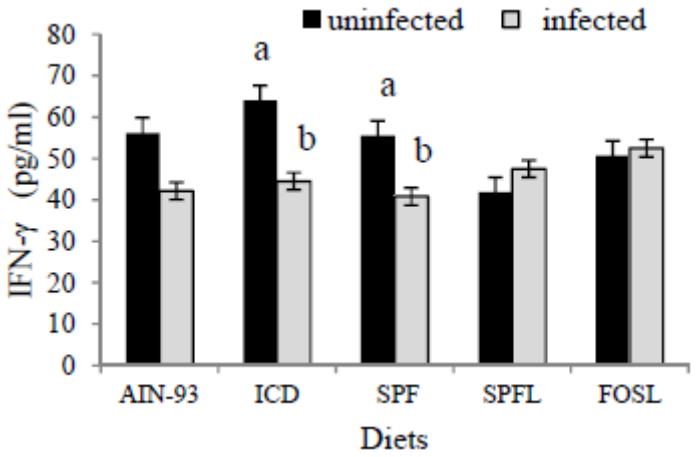

Figure 3. The average of IFN-γ in spleen lymphocyte culture in uninfected and infected rats fed different diets (AIN-93, ICD, SPF, SPFL, FOSL). Different letters (a, b) indicate significant difference ($p<0.05$) between uninfected and infected rats in each diet intervention, whereas no letters on top indicate no significant difference

Even though not significant, the increasing IFN-γ in infected rats fed SPFL and FOSL indicate that stimulation for this cytokine needs probiotic, because in infected rats fed SPF the cytokine decreased significantly (Figure 3). No significant increase in IFN-γ, may be caused of the low doses of probiotic *Lactobacillus plantarum* Dad-13 in SPFL diet and also Lacto-B in FOSL diet. In the previous study by Yuan, Wen, Liu, and Li (2013), the dose effects of *Lactobacillus acidophilus* on IFN-γ producing T cell was similar between the intestinal and systemic lymphoid tissues. These findings have significant implications in the use of probiotic lactobacilli as immunostimulatory versus immunoregulatory agents. Therefore, that probiotics can be ineffective or even detrimental if not used at the optimal dosage for the appropriate purposes, highlighting the importance of not only strain but also dose selection in probiotic studies. According to Vinderola, Matar, and Perdigón (2005), that probiotic-bacteria-intestinal epithelial cell (IEC) interaction, which releases signals from the IEC, could play a major role in the innate immune response induced by lactic acid bacteria (LAB), depending on the dose administered (Galdeano, de LeBlanc, Bonet, & Perdigon, 2007). As the induce cytokine profiles and intrinsic adjuvanicity properties by LAB was also strain- dependent (Maassen et al., 2000).

Since the increasing of splenocyte IFN-γ in infected rats fed SPFL diet was higher than FOSL diet (commercial probiotic), this means *Lactobacillus plantarum* Dad-13 which is the local isolate, has potency as immunomodulator in systemic immune response. In the previous study, probiotic dahi augmented lymphocyte proliferation and enhanced T-cell response towards Th1 by stimulating the production of IL-2, IL-6 and IFN-γ. This twist in immune response may help to eradicate *S. enteritidis* infection (Jain, Yadav, Sinha, Naito, & Marotta, 2008).

After interaction between probiotic bacteria and the immune cells in the Peyer's patches (PP), the probiotic bacteria or their fragments are internalized by M cells or in a paracellular way through follicle-associated epithelial cells of the PP. After that, the bacteria or their particles interact with the macrophages and dendritic cells, which are activated to produce cytokines. As consequence of the bacterial stimulation to the immune cells in this inductor site of the immune response, cytokine production is enhanced. The cytokines released by probiotic stimulation in PP are the biological messengers of the complex network of signals that activate the systemic immune response (Galdeano, de LeBlanc, Bonet, & Perdigon, 2007).

The functions of IFN-γ have classically focused on the interactions of macrophages and $CD4^+$ T cells. The interaction of a T-cell receptor with an antigen bound to a major histocompatibility complex (MHC) molecule triggers production of IFN-γ by T cells. This IFN-γ then acts to activate macrophages, up-regulating a number of gene products and rendering macrophages additionally cytotoxic by increasing oxidative burst and the production of other oxidants such as nitric oxide. Recently, IFN-γ was shown to be produced by a number of other immune cell types, including natural killer (NK) cells and macrophages; and to regulate the functions of many of these cell types (Ellis & Beaman, 2004).

4. Conclusions

To induce a systemic immune response associated IFN-γ production in rat infected with *Salmonella* Typhimurium, probiotics such as *Lactobacillus plantarum* Dad-13 influenced more than prebiotic contained in sweet potato fiber. Nevertheless, fiber components contained in the sweet potato has a significant role in the mucosal immune system through the increasing of sIgA in rat rat with *Salmonella* Typhimurium, although there was no increase in caecal lactobacilli. This research strengthens the evidence of previous studies, which reported that rapid fermented fiber increased salmonella translocation, thus decreased lactobacilli population in intestine. Further research is required to study the effect of various doses of *L. plantarum* Dad-13, and various combinations of local prebiotic sources with different levels of solubility on stimulation of mucosal and systemic immune responses in rats infected. The microbial diversity of rat caecum is an interesting subject for further study and need to be analyzed using molecular technique.

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References

Astuti, I. B. (2005). *Dietary Fiber of Sweet Potato [Ipomoea batatas (L) Lam] and their Potency as Prebiotic to Prevent Pathogenic Diarrhea*. Thesis. Food Science and Technology, Universitas Gadjah Mada.

Bakker-Zierikzee, A. M., Tol, E. A., Kroes, H., Alles, M. S., Kok, F. J., & Bindels, J. G. (2006). Faecal SIgA secretion in infants fed on pre-or probiotic infant formula. *Pediatric Allergy Immunology, 17*(2), 134-40. http://dx. doi.org/10.1111/j.1399-3038.2005.00370.x

Biedrzycka, E., Bielecka, M., Wróblewska, B., Jêdrychowski, L., Zduñczyk, Z., & Haros, C. M. (2003). Immunostimulative activity of probiotic bifidobacterium strains determined *in vivo* using elisa method. *Polish Journal of Food and Nutrition Sciences, 12* (suppl 1), 20-23.

Bohmig, G. A., Krieger, P-M, Saemann, M. D., Wenhardt, C., Pohanka, E., & Zlabinger, G. J. (1997). n-Butyrate downregulates the stimulatory function of peripheral blood-derived antigen-presenting cells: A potential mechanism for modulating T-cell responses by short-chain fatty acids. *Immunology, 92*(2), 234-243. http://dx.doi.org/10.1046/j.1365-2567.1997.00337.x

Bruggencate, S. J. M. T., Bovee-Oudenhoven, I. M. J., Lettink-Wissink, M. L. G., Katan, M. B., & Van der Meer, R. (2004). Dietary fructo-oligosaccharides and inulin decrease resistance of rats to salmonella: protective role of calcium. *Gut, 53*(4), 530-535. http://dx. doi.org/10.1136/gut.2003.023499

Campbell, J. M., Fahey, G. C. Jr., & Wolf, B. W. (1997). Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. *Journal of Nutrition, 127*(1), 130-136.

Castillo, N. A., Perdigón, G., & de LeBlanc, A. de M. (2011). Oral administration of a probiotic Lactobacillus modulates cytokine production and TLR expression improving the immune response against *Salmonella enterica* serovar Typhimurium infection in mice. *BMC Microbiology, 11*, 1-12. http://dx.doi.org/10.1186/1471-2180-11-177

Correa-Matos, N. J., Donovan, S. M., Isaacson, R. E., Gaskins, H. R., White, B. A., & Tappenden, K. A. (2003). Fermentable fiber reduces recovery time and improves intestinal function in piglets following *Salmonella* Typhimurium infection. *Journal of Nutrition, 133*(6), 1845-1852.

Durant, J. A., Corrier, D. E., & Ricke, S. C. (2000). Short-chain volatile fatty acids modulate the expression of the hilA and invF genes of *Salmonella* Typhimurium. *Journal of Food Protection, 63*(5), 573-578.

Ellis, T. N., & Beaman, B. L. (2004). Interferon-γ activation of polymorphonuclear neutrophil function. *Immunology, 112*(1), 2-12. http://dx. doi.org/10.1111/j.1365-2567.2004.01849.x

Galdeano, C. M., de LeBlanc A. de M., Bonet M. E. B., & Perdigon, G. (2007). Proposed model: Mechanisms of immunomodulation induced by probiotic bacteria. *Clinical and Vaccine Immunology, 14*(5), 485-492. http://dx.doi.org/10.1128/CVI.00406-06

Gibson, G. R., & Fuller, R. (2000). Aspects of *in vitro* and *in vivo* research approaches directed toward identifying probiotics and prebiotics for human use. *Journal of Nutrition, 130*(2), 391S-395S.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonie microbiota: introducing the concept of prebiotics. *Journal of Nutrition, 125*(6), 1401-1412.

Hayashi, H., Takahashi, R., Nishi, T., Sakamoto, M., & Benno, Y. (2005). Molecular analysis of jejunal, ileal, caecal and rectosigmoidal human colonic microbiota using 16S rRNA gene libraries and terminal restriction fragment length polymorphism. *Journal of Medical Microbiology, 54*, 1093-1101. http://dx.doi.org/10.1099/jmm.0.45935-0

Herías, M. V., Hessle, C., Telemo, E, Midtvedt, T., Hanson, L. A., & Wold, A. E. (1999). Immunomodulatory effects of *Lactobacillus plantarum* colonizing the intestine of gnotobiotic rats. *Clinical Experimental Immunology, 116*(2), 283-90. http://dx. doi.org/10.1046/j.1365-2249.1999.00891.x

Jain, S., Yadav, H., Sinha, P. R., Naito, Y., & Marotta, F. (2008). Dahi containing probiotic Lactobacillus acidophilus and Lactobacillus casei has a protective effect againt Salmonella enteridis infection in mice. *International Journal of Immunopathology and Pharmacology, 21*(4), 1025-1033.

Jain, S., Yadav, H., & Sinha, P. R. (2009). Probiotic dahi containing *Lactobacillus casei* protects against *Salmonella enteritidis* infection and modulates immune response in mice. *Journal of Medicinal Food, 12*(3), 576-583. http://dx. doi.org/10.1089/jmf.2008.0246

Jakesevic, M., Aaby, K., Borge, G-I. A., Jeppsson, B., Ahrné, S., & Molin, G. (2011). Antioxidative protection of dietary bilberry, chokeberry and *Lactobacillus plantarum* HEAL19 in mice subjected to intestinal oxidative stress by ischemia-reperfusion. *BMC Complementary & Alternative Medicine, 11*(8), 1-12. http://dx.doi.org/10.1186/1472-6882-1

Jenkins, D. J. A., Kendall, C. W. C., & Vuksan V. (1999). Inulin, oligofructose and intestinal function. *Jounal of Nutrition, 129*(7), 1431S-1433S.

Johansson, M. L., Molin, G., Jeppsson, B., Nobaek, S., Ahrné, S., & Bengmark, S. (1993). Administration of different Lactobacillus strains in fermented oat meal soup: *in vivo* colonization of human intestinal mucosa and effect on the indigenous flora. *Applied Environmental Microbiology, 59*, 15-20.

Klein, A. B., Witonsky, S. G., Ahmed, S. A., Holladay, S. D., & Gogal, R. M., Jr . (2006). Impact of different cell isolation techniques on lymphocyte viability and function. *Journal of Immunoassay & Immunochemistry, 27*(1), 61-76. http://dx.doi.org/10.1080/15321810500403755

Kops, S. K., Lowe, D. K., Bement, W. M., & West, A. B. (1996). Migration of *Salmonella* Typhi through intestinal epithelial monolayers: an *in vitro* study. *Microbiology and Immunology, 40*(11), 799-811. http://dx.doi.org/10.1111/j.1348-0421.1996.tb01145.x

Kunová, G., Rada, V., Lisová, I., Ročková, S., & Vlková, E. (2011). *In vitro* fermentability of prebiotic oligosaccharides by lactobacilli. *Czech Journal Food Science, 29* (Special Issue), S49-S54.

Lawhon, S. D., Maurer, R., Suyemoto, M., & Altier, C. (2002). Intestinal short-chain fatty acids alter *Salmonella* Typhimurium invasion gene expression and virulence through BarA/SirA. *Molecular Microbiology, 46*(5), 1451-1464. http://dx. doi.org/10.1046/j.1365-2958.2002.03268.x

Lestari, L. A., Harmayani, E., & Sugahara, T. (2010). Immunomodulatory effect of heat-killed indigenous probiotic in HB4C5 cell-line and Balb/c Mice. In *Proceeding of the 2nd International Symposium on Probiotic and Prebiotic*, 4-5 August, Jakarta.

Lestari, L. A, Rahayu, E. S., & Aman, A. T. (2008). Antagonism properties of indigenous probiotic isolated from traditional fermented foods against diarrhoeal pathogens. In *Proceeding of the International Seminar on Probiotic and Prebiotic*, 19 August, Jakarta.

Lestari, L. A., Soesatyo, M. H. N. E., Iravati, S., & Harmayani, E. (2013). Characterization of Bestak sweet potato (*Ipomoea batatas*) variety from Indonesian origin as prebiotic. *International Food Research Journal, 20*(5), 2241-2245.

Leung, K. Y., & Finlay, B. B. (1991). Intracellular replication is essential for the virulence of *Salmonella* Typhimurium. *Proceedings of the National Academy of Sciences, 88*, 11470-11474. http://dx.doi.org/10.1073/pnas.88.24.11470

Li, B. W., Andrews, K. W., & Pehrsson, P. R. (2002). Individual sugars, soluble, and insoluble dietary fiber contents of 70 high consumption foods. *Journal of Food Composition and Analysis, 15*(6), 715-723. http://dx.doi.org/10.1006/jfca.2002.1096

Maassen, C. B., van Holten-Neelen, C., Balk, F., den Bak-Glashouwer, M. J., Leer, R. J., Laman, J. D., Boersma, W. J., & Claassen, E. (2000). Strain-dependent induction of cytokine profiles in the gut by orally administered Lactobacillus strains. *Vaccine, 18*(23), 2613-2623.

http://dx.doi.org/10.1016/S0264-410X(99)00378-3

Mangeau, R., & Brassard, R. (1990). Determination of insoluble, soluble, and total dietary fiber: Collaborative study of a rapid gravimetric method. *Cereal Foods World, 35*, 319-322.

Mantis, N. J., Rol, N., & Corthésy, B. (2011). Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. *Mucosal Immunology, 4*, 603-611. http://dx. doi.org/10.1038/mi.2011.41

National Research Council. (1991). *Infectious diseases of mice and rats: a report of the Institute of Laboratory Animal Resources Committee on Infectious Diseases of Mice and Rats.* National Academy Press, Washington, D.C.

Parent, M. A., Wilhelm, L. B., Kummer, L. W., Szaba, F. M., Mullarky, I. K., & Smiley, S. T. (2006). Gamma interferon, Tumor necrosis factor alpha, and nitric oxide synthase 2, key elements of cellular immunity, perform critical protective functions during humoral defense against lethal pulmonary Yersinia pestis infection. *Infection and Immunity, 74*(6), 3381-3386. http://dx. doi.org/10.1128/IAI.00185-06

Petersen, A., Heegaard, P. M. H., Pedersen, A. L., Andersen, J. B., Sørensen, R. B., Lahtinen, H. F. S. J., … Licht, T. R. (2009). Some putative prebiotics increase the severity of *Salmonella enterica* serovar Typhimurium infection in mice. *BioMed Central Microbiology, 9*(245), 1-11. http://dx. doi.org/10.1186/1471-2180-9-245

Pratt, V. C., Tappenden, K. A., McBurney, M. I., & Field, C. J. (1996). Short-chain fatty acid-supplemented total parenteral nutrition improves nonspecific immunity after intestinal resection in rats. *Journal of Parenteral and Enteral Nutrition, 20*(4), 264-271. http://dx.doi.org/10.1177/0148607196020004264

Rahayu, E. S., Yogeswara, A., Utami, T., & Suparmo. (2011). Indigenous Probiotic Strains of Indonesia and their application for fermented food. The 12thAsean Food Conference.BITEC Bangna, Bangkok, Thailand 16-18, June, 2011.

Reeves, P. G., Neilsen, F. H., & Fahey, G. C, Jr. (1993). AIN-93 purified diets for laboratory rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Formulation of the AIN-76A Rodent Diet. *Journal of Nutrition, 123*(11), 1939-1951.

SPSS 12.0. (2003). Brief Guide. SPSS Inc., 233 South Wacker Drive 11th Floor Chicago, United States of America.

Sughita, I. M. (1985). Dadih: Minang traditional buffalo milk product, benefits, constraints, and prospects in the era of industrialization West Sumatra. Seminar on Application of Technology. Faculty of Animal Science, Andalas University, Indonesia.

Sumaryati, B. T., Utami, T., & Suparmo. (2009). The effects of infection of Escherichia coli and addition of *Lactobacillus plantarum* Dad 13 to Wistar rats fecal microbiota. *Agritech, 29*, 165-170.

Tauxe, R. V., & Pavia, A. T. (1998). *Salmonellosis: nontyphoidal*. In A. S. Evans, P. S. Brachman (Eds.), *Bacterial infections of humans: epidemiology and control*. (3rd ed, pp. 613- 628), New York: Plenum Publishers. http://dx.doi.org/10.1007/978-1-4615-5327-4_32

Vieira, A. T., Teixeira, M. M., & Martins, F. S. (2013). The role of probiotics and prebiotics in inducing gut immunity. *Frontiers in Immunology, 4*(445), 1-12. http://dx. doi.org/10.3389/fimmu.2013.00445

Vinderola, C. G., Matar, C., & Perdigón, G. (2005). Role of the epithelial cells in the immune effects mediated by gram-positive probiotic bacteria. Involvement of Toll-like receptors. *Clinical and Diagnostic Laboratory Immunology, 12*(9), 1075-1084. http://dx. doi.org/10.1128/CDLI.12.9.1075-1084

Vyas, U., & Ranganathan, N. (2012). Probiotics, Prebiotics, and Synbiotics: Gut and Beyond. Gastroenterology Research and Practice, 1-16. http://dx.doi.org/10.1155/2012/872716

Windmueller, H. G. (1982). Glutamine utilization by the small intestine. *Advances in Enzymology, 53*, 201-237. http://dx. doi.org/10.1002/9780470122983.ch6

Wu, G., Field, C. J., & Marliss, E. B. (1991). Glutamine and glucose metabolism in rat splenocytes and mesenteric lymph node lymphocytes. *American Journal of Physiology, 260*(1), E141- E147.

Yuan, L., Wen, K., Liu, F., & Li, G. (2013). Dose effects of LAB on modulation of rotavirus vaccine induced immune response. In *Lactic Acid Bacteria - R & D for Food, Health and Livestock Purposes* (pp. 263-280), InTech. http://dx.doi.org/10.5772/50379

Yun, C. H., Lillehoj, H. S., Shu, J., & Min, W. (2000). Kinetics differences in intestinal and systemic interferon- and antigenic-specific antibodies in chickens experimentally infected with Eimeria maxima. *Avian Diseases, 44*(2), 305-312. http://dx.doi.org/10.2307/1592544

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Influence of Functional Sweet White Lupin Biscuits on Lipid Profile and Food Efficiency of Induced Hyperlipidemia Rats

Rasha Mousa Ahmed Mousa[1]

[1] Home Economic Department, Faculty of Specific Education, Assiut University, Assiut, Egypt

Correspondence: Rasha Mousa Ahmed Mousa, Home Economic Department, Faculty of Specific Education, Assiut University, Assiut, Egypt. E-mail: rashamousa@aun.edu.eg; rshmousa73@gmail.com

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Abstract

In recent years, functional foods have attracted much interest to prevent nutrition-related diseases such as hyperlipidemia and weight gain. In this regard, this study was designed to examine the effect of use sweet white lupin (SWL) oil and flour with/without germination as a source of active healthy components to prepare functional biscuits for lowering blood lipids and growth. Functional biscuits were formulated by replacing wheat flour and butter in biscuit formulae by SWL extracted flour and SWL oil in the range of 20-30% (w/w) and 30-40% (v/w), respectively. Results indicated that the feed of hyperlipidemic rats on diets supplemented with different functional SWL biscuits for 6 weeks significantly ($P < 0.05$) reduced serum total cholesterol, triglycerides, low density lipoproteins, very low density lipoproteins, ratio of total cholesterol/high density lipoproteins cholesterol, ratio of low density lipoproteins/high density lipoproteins cholesterol and atherogenic index. Furthermore, the feed of functional SWL biscuits significantly reduced the body weight gain of rats and their food efficiency compared to that of rats fed on hyperlipidemic diet. On the other hand, there was an increase in the value of high density lipoproteins cholesterol and its ratio with total cholesterol. All these findings supported that the addition of 25% germinated SWL flour and 35% or 40% germinated SWL oil in biscuits gave interested results compared to the common wheat biscuits. Therefore, the proposed functional SWL biscuits could be able to regulate the blood cholesterol and the body growth levels of individuals and patients.

Keywords: biscuits, cholesterol, food efficiency, germination, lipoprotein, lupins, triglycerides

1. Introduction

Hyperlipidemia is a general term for the high concentrations of any or all types of the lipids in serum (lipid profile) that include cholesterol low density lipoproteins (LDL-cholesterol; LDL-c), cholesterol very low density lipoproteins (VLDL-cholesterol; VLDL-c), cholesterol high density lipoproteins (HDL-cholesterol; HDL-c), total cholesterol (TC) and triglycerides (TG) (National Cholesterol Education Program (NCEP), 2002). Cholesterol and triglycerides levels are measured in milligrams (mg) of cholesterol per deciliter (dL) of blood in the United States and some other countries while Canada and most European countries measure cholesterol in millimoles (mmol) per liter (L) of blood (Integrated Guidelines, 2011); Consider these general guidelines, the recommended normal ranges of LDL-c, HDL-c, TG and TC are < 100 mg/dL (2.5 mmoL/L), > 45 mg/dL (1.6 mmol/L), < 150 mg/dL (1.7 mmol/L) and < 200 mg/dL (5.1 mmol/L), respectively. The ratios of TC/HDL-c, LDL-c/HDL-c (risk ratio; RR), 100.HDL-c/TC (HTR%) and atherogenic index (AI) are predictors of coronary risk (National cholesterol education program, 1994). Meanwhile, the changes in eating habits including high fat and low fiber diets increased also the risk of obesity and overweight. Risk of these diseases increases steadily with increasing the values of body mass index (BMI) or food efficiency ratio (FER).

Legumes played an important role for improving the lipid profile in humans since they are rich sources of protein, fiber, certain minerals and vitamins (ELMaki et al., 2007). Among the legumes, soybean and its protein fractions appeared a significant reduction in TC and LDL-c through the modulation of genes related to the lipid metabolism (Anderson, Johnstone, & Cook-Newell, 1995). However, the presence of isoflavones that have potentially harmful effects on humans (Sirtori, 2001) reduced the impact of soybeans. Other seeds such as peas, lentils, chickpeas, beans and lupin are also investigated due to their chemical composition and great potential in the prevention of lipid disorders (Duranti, 2006; Sirtori et al., 2004; Smith et al., 2006). Lupin is the only another protein-rich grain legume and contains low levels of isoflavons. More than 300 lupin species were described, but

only five species are cultivated. One of the most important species is the sweet white lupin (SWL); It is most commonly found in Mediterranean countries especially in Egypt, Portugal, Greece, and Italy (Hassanein, El-Shami, & El-Mallah, 2011).

The chemical composition of SWL confirmed that it is a good source of nutrients, not only proteins but also dietary fibers, minerals, vitamins and healthy oils. The amount of SWL oil was found to be in the range of 5-20% (v/w) of total lupin grain. SWL Oil was characterized by the low percent of saturated fatty acids (≤ 10%), absence of trans fatty acids, absence of cholesterol and high percent of healthy unsaturated fatty acids (≥ 90%) including 30-50% oleic acid, 15-45% linoleic acid and 3-11% linolenic acid (Gravelle, Barbut, & Marangoni, 2012; Stortz, Zetzl, Barbut, Cattaruzza, & Marangoni, 2012; Tarancón, Fiszman, Salvador, & Tárrega, 2013). Lupin flour has always been a rich of proteins and dietary fibers with the range of 36-52% and 30-40% (w/w), respectively (Mohamed & Rayas-Duarte, 1995). The SWL protein has two major fractions, albumins and globulins in a ratio of 1:9, respectively. In turn, the globulins consisted of the two classical major storage proteins called conglutins β and two minor distinct protein types, conglutins γ and ξ (Blagrove & Gillespie, 1975). Conglutin γ is an oligomeric lupin seed glycoprotein comprising 5% of the total lupin proteins (Duranti, Restani, Poniatowska, & Cerletti, 1981). However, the presence of anti-nutrients reduced the broad impact of lupin grains for improvement of the public health.

Germination of legumes is a simple, low-cost and effective process for decreasing the levels of anti-nutrients and for achieving desirable changes in nutritional and sensory characteristics through a variety of reactions including synthesis, degradation and transformation of biomolecules during transformation of seeds into plants. To improve the quality of SWL seeds, germination was used to change protein, lipid and carbohydrate levels (Dueñas, Hernández, Estrella, & Fernández, 2009). Analysis of the oil extract after germination revealed that the concentrations of phytosterols and total polyphenols were increased (Rumiyati & James, 2013). In addition, the concentration of anti-nutritional factors such as phytate, trypsin inhibition, α-galactoside and alkaloids in lupin were significantly diminished by germination (Cuadra et al., 1994). However, no study was undertaken to demonstrate the anti-hyperlipidemic activity of the germinated sweet white lupin (Lupinus albus; SWL) formulated in food products such as biscuits. Biscuits are the most common food product consumed by the upper, middle and lower income countries (Monteiro, Moubarac, Cannon, Ng, & Popkin, 2013).

According to most definitions, “functional foods” are foods that provide a health benefit beyond basic nutrition (Hassan, Rasmy, Foda, & Bahgaat, 2012). Fortified foods, along with other enhanced or enriched foods, are considered functional foods. The individual and/or simultaneous substituting with SWL extracted flour and SWL oil to prepare functional SWL biscuits was previously studied in the range of 20-30% (w/w) and 30-40% (v/w), respectively by Mousa (2014). It was found that the simultaneous substitution with germinated SWL flour and oil raised significantly the values of protein, fiber and total phenolic compounds (TPCs). However, the proposed substitution showed marked reductions in the values of fat, carbohydrate, calorie and Na/K ratio. The sensory acceptability of functional SWL biscuits showed acceptable attributes of taste, appearance, texture and aroma with an observable improvement in the color of biscuits. Therefore, the present investigation was designed to evaluate the hypolipidemic activity (Youssef, Youssef, & Mousa, 2014) and food efficiency of functional SWL biscuits on hyperlipidemia albino rats. Functional SWL biscuits were prepared by simultaneous substitution of butter and wheat flour with sweet white lupin (SWL) oil and extracted flour with/without germination.

2. Materials and Methods

2.1 Materials

Sweet white lupin grains (Lupinus albus, Termis), refined wheat flour (Triticum Aestivum, 72% extraction hard red winter), butter (Cow's milk ≥ 80% fat), sugar powder (100% pure cane sugar), sodium bicarbonate, ammonium bicarbonate, skim milk powder (Defatted Cow's milk powder), salt mixture, orange (*Citrus sinensis* (L.)) peel powder, corn starch, corn oil and animal fats were procured from local market. Methanol, n-hexane, ether, nitric acid and perchloric acid were obtained from Fluka company Co. (Germany). Casein, 85% pure cholesterol, vitamin mixture, choline chloride, cellulose and cholic acid were obtained from Sigma Chemical Co (Germany). Kits of total cholesterol, HDL-cholesterol, and triglycerides were obtained from Stanbio Laboratory, Texas, USA. Double distilled water was used for the chemical analysis.

2.2 Germination of Sweet White Lupin (SWL) Grains

Prior to germination, clean lupin grains (300.0 g) were soaked in 2 L of distilled water at room temperature in the dark for 18 h. Following this the soaking water was decanted and the grains were twice rinsed with water. Then, grains were spread on dishes lined and covered with moistened paper towels. These dishes were placed on trays in an incubator set at 25 ºC and relative humidity (RH) 90-95%. The seeds in dishes were germinated for 3

days (Mousa, 2014). During the germination period, grains were washed twice per day with distilled water. At the end, the grains were dried under the sun. The above process was done in three replications to confirm the precision of the results. Finally, the germinated SWL grains or ungerminated SWL grains were ground and then sieved thrice through 10 mesh to flour. Grinding was done in Ushamixer Grinder. The obtained flours were stored in the dark at 4 °C prior to use.

2.3 Simultaneous Procuration of SWL Oil and Extracted Flour

A portion of the obtained germinated/ungerminated SWL flour (200.0 g) was exposed for oil extraction. The oil was extracted by n-hexane using a Buchi E-816 Soxhlet extraction unit (Switzerland). The weighed samples were placed into a thimble and put on pre-weighed extraction cups that placed on the extraction chamber in the soxhlet (Rumiyati & James, 2013). Consequently, the oil was extracted by n-hexane for 60 min. The extracted oil samples were kept in well stoppered dark containers under 4 °C (to prevent their autoxidation) until use as a fat replacer in the formulated biscuits. Simultaneously, the remained flour amount from oil extraction was dried over anhydrous sodium sulphate and the solvent was removed off by rotatory evaporator under vacuum. The dried extracted flour was cooled, weighed and used for the partial substitution of wheat flour in the formulated biscuits.

2.4 Preparation of Functional SWL Biscuits

The functional SWL biscuits were prepared as cited in Table 1 and were previously described by Mousa (2014). For the preparation of control/reference wheat biscuit (F1), butter (10.0 g) and sugar powder (20.0 g) were creamed together for 3 min in a mixer at 60 rpm. Then, 2.0 g of skim milk powder, 1.0 g of salt, 0.4 g of sodium bicarbonate, 1.5 g of ammonium carbonate and 5.0 g of orange peel powder were added in water (20.0 mL) and mixed together for 8 min at 125 rpm. The wheat flour (100.0 g) was added to the above mixture and mixed again for 3 min at 60 rpm. The dough was sheeted to a 3.5-mm thickness and cut with a biscuit cutter (50-mm diameter). The biscuits were baked on an aluminium tray in an electric oven at 200 ºC for 5 min. They were cooled for 30 min at room temperature and stored in low density polyethylene bags until further use.

For the preparation of functional wheat-SWL biscuits, six formulations (F2-F7) were prepared by the replacement of wheat flour with SWL in the presence of other ingredients (e.g. skim milk powder, orange peel powder, etc.) as described above. In these formulations, F2 and F3 samples were prepared by the simultaneous variation of wheat flour and butter with 20% SWL extracted flour and 30% SWL oil without germination and after germination, respectively. After that, the other remained samples (F4-F7) were formulated by the simultaneous variation with germinated SWL extracted flour in the range of 20-30% (w/w) and germinated SWL oil in the range of 30-40% (v/w).

Table 1. Variations in the component of formulated biscuits

Sample*	**Wheat Flour (g)**	**SWL Flour (g)**	**Butter (g)**	**SWL oil (mL)**	**Water (mL)**
F1 (control biscuit)	100.0	---	10.0	---	20.0
F2 (without germination)	80.0	20.0	7.0	3.0	24.0
F3 (with germination)	80.0	20.0	7.0	3.0	24.0
F4	75.0	25.0	7.0	3.0	26.0
F5	70.0	30.0	7.0	3.0	27.0
F6	75.0	25.0	6.5	3.5	26.0
F7	75.0	25.0	6.0	4.0	26.0

* Other ingredients of the formulations (F2-F6) are the same as described in the text for F1.

2.5 Rats Experimental Design

Ninety adult male white albino rats (Sprague) weighing between 100 and 120 grams were obtained from the animal house of King Fahad Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. The animals were housed as groups in wire cages under standard laboratory conditions (20-25 ºC, 40-60% humidity,

10-12 hours light/dark cycle). The animals were given free access to diet and water during the feeding period. Diet and water were given *ad- libitum* for six weeks. Before starting the experiment, the rats were fed for a week as adaptation period for acclimatization. Body weight and food intake were daily weighed through the experimental feeding period.

The rats were randomly divided into 9 groups of 10 rats each. Each rat was ranked on the tail to differentiate between animals. Daily administration was continued for 6 weeks. Group (1) involved 10 rats that were fed by the basal diet only (a negative control). The basal diet used is outlined in Tables 2 and 3 (Youssef et al., 2014). Group (2) received hyperlipidemic diet (Table 4) and served as a positive control. Group (3) received hyperlipidemic diet contained 10% (w/w) wheat flour biscuits (sample F1). Group (4) received hyperlipidemic diet contained 10% (w/w) of wheat-ungerminated SWL biscuits (sample F2). Groups from (5) to (9) received hyperlipidemic diet contained 10% (w/w) of wheat-germinated SWL formulae from (F3) to (F7).

Table 2. Constituents of the basal diet for 100 gm diet

Item	**%**
Corn starch	67.8
Casein	12.5
Corn oil	10.0
Vitamin mixture	1.0
Salt mixture	3.5
Cellulose	5.0
Choline chloride	0.2

Table 3. Constituents of vitamins mixtures used in the basal diet

Item	**Amount (g)**
Vitamin A palmitate 500.000 IU/gm	0.8
Vitamin D3 100.00 IU/gm	1
Vitamin E acetate 500 IU/gm	10
Menadione sodium bisulfite 62.,5% menadione	0.08
Biotin 1.0%	2
Cyano cobalaming 0.01%	1
Folic acid	0.2
Nicotinic acid	3
Calcium pantothenate	1.6
Pyridoxine HCl	0.7
Riboflavin	0.6
Thiamin-HCl	0.6
Sucrose	978.42

Table 4. Constituents of the hyperlipidemic diet for 100 g diet

Item	%
Corn starch	66.3
Casein	12.5
Animal fat	10
Cholesterol	1
Cholic acid	0.5
Vitamins mixture	1
Salt mixture	3.5
Cellulose	5
Choline chloride	0.2

2.6 Biochemical Measurements

2.6.1 Blood Sampling

At the end of the experiment, rats were fasted overnight and anesthetized (Youssef et al., 2014). Blood samples were collected from all animals from the retro-orbital plexus of each group into clean, dry and labeled tube. The tubes contained heparin (10.01 U/ml) as anticoagulant. Blood was centrifuged (3500 rpm for 15 min) to separate serum which was tightly kept in sealed aliquot tubes at -20 ºC until biochemical assay was carried out.

2.6.2 Determination of Total Triglycerides

Fully enzymatic determination of total triglycerides (TG) in serum was estimated spectrophotometrically at 546 nm according to the method of Wahlefeld (1974) of the enzymatic hydrolysis of triglycerides using kits followed by colorimetry determination of liberated glycerol.

2.6.3 Determination of Serum Total Cholesterol, LDL-c, VLDL-c and HDL-c

Enzymatic determination of total cholesterol (TC) in serum was carried out according to the method of Allian, Poon, Chan and Richmond (1974) using Stanbio kits (Texas, USA).

The determination of High Density Lipoprotein (HDL-c) was carried out according to Warnick, Benderson and Albers (1983) by the kits that were provided from Stanbio (Texas, USA). Low Density Lipoprotein (LDL-c) is precipitated from serum by magnesium chloride/dextrin sulfate reagent. HDL-c is then determined in supernatant using cholesterol reagent. LDL-c and VLDL-c were determined by the equations published by Friedewald, Levy and Fredrickson (1972).

2.7 Statistical Analysis

The values of measurements were expressed as means±standard deviation (SD). One-way analysis of variance (ANOVA) and Tukey's *post hoc* test were performed to test the significance of differences ($P < 0.05$) between groups (Gustavo, José, Robison, Paulo, & José, 2012). The statistical analyses were carried out using Minitab software version 14.13 (USA).

3. Results and Discussion

3.1 Body Weight Gain, Food Intake and Food Efficiency Ratio

The results given in Table (5) revealed that the daily body weight gain (DBWG) and daily food intake (DFI) were positive in all studied groups (1-9) for the experimental rats. The rats of group fed on the hyperlipidemic diet (positive control) showed an increase in the DBWG compared to the rats of group fed on basal diet (negative control). Feeding on the diets enriched with biscuits composed of wheat flour and ungerminated/germinated sweet white lupin (SWL) oil and SWL flour decreased significantly ($P < 0.05$) the DBWG in the range from 1.28±0.30 g to 2.19±0.31 g compared to the positive control. The lowest value of DBWG was obtained with the rats of group (4) fed on hyperlipidemic diet contained 10% of ungerminated SWL biscuits formula F2. While, the highest value of DBWG was achieved within the rats of group (8) fed on hyperlipidemic diet contained 10% of germinated SWL formula F6 (25% oil and 35% flour). The value of DBWG in group 8 was very close to that of negative control (2.17±0.21). These results are in agreement with that of Atiat, Eman, Mona, and Ibrahim (1999), and Dadai, Walker, Sambrook, Welch and Owen (1996) who found a reduction of gain in body weight of

rats fed on legumes compared with hypercholesterolemic control. The decrease in final body weight may be due to the increase of legume protein intake and high fiber content that may cause weight loss (Anderson & Major, 2002). On the other hand, the DFI value (14.32±0.59 g) of positive control was markedly decreased ($P < 0.05$) compared with the negative control (15.92±0.55 g). This could be attributed to the presence of high amounts of fat (10%) and cholesterol (1%) in the hyperlipidemic diet. By enrichment of hyperlipidemic diet with 10% wheat biscuits and SWL biscuits (groups 3-9), the DFI amounts were increased more than that of the positive control. The highest value of DFI (15.42±0.5 g) was observed in group 9 of rats fed on hyperlipidemic diet contained 10% of germinated SWL formula F7 (25% oil and 40% flour) followed by group 8 (15.12±0.35 g) of rats fed on hyperlipidemic diet contained 10% of germinated SWL formula F6 (25% oil and 35% flour). The values of DFI in groups 8 and 9 are more in match with that of negative control (15.92±0.55) than others. This could be due to that germination could achieve desirable changes in nutritional characteristics of the SWL grains (Mousa, 2014). Furthermore, germination decreased the levels of anti-nutrients to improve the quality of SWL seeds. Food efficiency ratio (FER) was calculated by dividing DBWG on DFI (Sulieman & El-Newary, 2014). No significant difference ($P > 0.05$) of FER was noticed among groups, except groups 2 and 4 fed on hyperlipidemic diet and hyperlipidemic diet enriched with 10% ungerminated SWL biscuits. All these findings reflected the benefits of using germinated SWL oil and flour on the DBWG and DFI of induced hyperlipidemic rats.

Table 5. Effect of the studied groups on the body weight (g), food intake (g) and food efficiency of the experimental rats (induced hyperlipidemia)

Parameter	Starting weight (g)	DBWG (g)	DFI (g)	FER
Group 1 (BD)	109.53	2.17±0.21^{c}	15.92±0.55^{e}	0.14
Group 2 (HLD)	108.91	2.74±0.36^{d}	14.32±0.59^{a}	0.19
Group 3 (HLD+F1)	101.73	2.03±0.26^{c}	14.82±0.42^{b}	0.14
Group 4 (HLD+F2)	112.87	1.28±0.30^{a}	14.92±0.30^{b}	0.09
Group 5 (HLD+F3)	115.38	1.84±0.27^{b}	14.97±0.42^{b}	0.12
Group 6 (HLD+F4)	119.39	1.89±0.36^{b}	15.02±0.30^{b}	0.13
Group 7 (HLD+F5)	109.54	2.05±0.18^{c}	15.05±0.42^{b}	0.14
Group 8 (HLD+F6)	108.98	2.19±0.31^{c}	15.12±0.35^{c}	0.14
Group 9 (HLD+F7)	109.21	2.16±0.22^{c}	15.42±0.50^{d}	0.14

DBWG: Daily body weight gain; DFI: Daily food intake.

FER: Food efficiency ratio = DBWG/DFI (Sulieman & El-Newary, 2014).

BD: Basal diet; HD: Hyperlipidemic diet.

F1: Hyperlipidemic diet contained 10% (w/w) of wheat biscuits

F2: Hyperlipidemic diet contained 10% (w/w) of ungerminated SWL biscuits.

F3: Hyperlipidemic diet contained 10% (w/w) of germinated SWL (20% oil and 30% flour).

F4: Hyperlipidemic diet contained 10% (w/w) of germinated SWL (25% oil and 30% flour).

F5: Hyperlipidemic diet contained 10% (w/w) of germinated SWL (30% oil and 30% flour).

F6: Hyperlipidemic diet contained 10% (w/w) of germinated SWL (25% oil and 35% flour).

F7: Hyperlipidemic diet contained 10% (w/w) of germinated SWL (25% oil and 40% flour).

Values are expressed as mean±SD.

Values in each column which have different letters are significantly different at ($P < 0.05$).

3.2 Serum Lipid Profile

In the present study, the effect of short-term (6 weeks) consumption of hyperlipidemic diet supplemented with 10% ungerminated/germinated wheat-SWL biscuits and biscuits prepared from 100% refined wheat flour on lipid profile was studied and the results were presented in Tables 6 and 7.

The effects of feeding fat and cholesterol- enriched diet supplemented with different biscuit samples to the experimental rats for 6 weeks on the total cholesterol (TC) and triglycerides (TG) values are presented in Table 6. The results indicated a significant elevation ($P < 0.05$) in both TC and TG in hyperlipidemic diet group (positive control) as compared to negative control group (basal diet). Through the studied groups, data revealed that the reduction in TC and TG levels was obvious by the feeding of diets contained SWL biscuits (groups 4-9) compared to wheat biscuits (group 3). Furthermore, the reduction in TC and TG values was higher in formulated biscuits by germinated SWL oil and flour (group 5-9) than that of ungerminated SWL grains (group 4). The range of reduction in TC and TG levels was significantly ($P < 0.05$) changed by increasing the amounts of simultaneous substitution with germinated SWL oil and extracted flour in the formulated biscuits compared to control samples and wheat biscuits. The highest reductions of TC levels were observed in the groups 8 and 9 of rats fed on hyperlipidemic diet contained 10% of germinated SWL formula F6 (25% oil and 35% flour) and 10% of germinated SWL formula F7 (25% oil and 40% flour) as cited in Table 6. The values of TC in the groups of 8 and 9 were 169.80±0.34 and 168.10±0.16 mg/dL, respectively compared to 145.21±0.25 mg/dL of negative control and 405.16±0.32 of positive control. While, the lowest reductions in TC levels were achieved in the group of rats fed with wheat biscuits (208.10±0.15 mg/dL) and ungerminated SWL biscuits (198.80±0.18 mg/dL). The same findings were observed in the case of TG levels where the highest reductions were observed in the groups 8 and 9 of rats fed with germinated SWL formula F6 (91.23±0.35 mg/dL) and germinated SWL formula F7 (89.87±0.17 mg/dL) as compared with other examined groups. As well, the percent of decrements in groups 8 and 9 were 22.93% and 24.08%, respectively compared to the negative control. While, the lowest reductions were observed in the case of wheat biscuits (139.72±0.32 mg/dL) and ungerminated SWL formulae F2 (112.94±0.03 mg/dL). Therefore, the TC and TG values of the groups 8 and 9 were the best and all of them were cited in the normal ranges of TC (< 200 mg/dL) and TG (< 150 mg/dL). This substantial reduction in the values of TC and TG with SWL biscuits simultaneously substituted with germinated SWL oil and extracted flour after germination could be attributed to the combined effect of unsaturated fatty acids, dietary fiber and plant protein present in SWL seeds after germination. These results are in agreement with Jose et al. (2005) who reported that plasma total cholesterol concentration was decreased when rats were fed on lupin containing diet compared with control. Also, Fatima, Ann, Ian, Vernon and Robert (1996) and Mahfouz Elaby and Hassouna (2012) found that the mean plasma triacylglycerol level of positive control (hypercholesterolemic rats) was greater than that of rats fed on the legumes diets. The reduction of TG in the liver of rats using lupin protein has already been reported by Sirtori et al. (2004). It was attributed to the expression of the genes of the enzyme SREBP-1c, which is responsible for regulating the synthesis of fatty acids and triglycerides in the liver.

Table 6. Effect of the formulated biscuits on total cholesterol (TC) and triglycerides (TG) of the experimental rats (induced hyperlipidemia)

Groups	Total cholesterol (mg/dL) (Normal range < 200)	Triglycerides (mg/dL) (Normal range < 150)
Group 1 (-ve control)	145.21±0.25^{a}	118.37±0.08^{c}
Group 2 (+ve control)	405.16±0.32^{e}	183.72±0.21^{e}
Group 3	208.10±0.15^{d}	139.72±0.32^{d}
Group 4	198.80±0.18^{c}	112.94±0.03^{b}
Group 5	191.40±0.09^{c}	109.24±0.29^{b}
Group 6	190.50±0.11^{c}	110.83±0.14^{b}
Group 7	183.20±0.29^{c}	103.77±0.04^{b}
Group 8	169.80±0.34^{b}	91.23±0.35^{a}
Group 9	168.10±0.16^{b}	89.87±0.17^{a}

Values are expressed as mean±SD.

Values in each column which have different letters are significantly different at ($P < 0.05$).

The effect of feeding hyperlipidemic diet supplemented with different biscuits samples to the experimental rats for 6 weeks on low density lipoproteins cholesterol (LDL-c), very low density lipoproteins cholesterol (VLDL-c)

and high density lipoproteins cholesterol (HDL-c) are given in Table (7). The obtained results revealed significant decrease (P< 0.05) in HDL-c and significant increase (P < 0.05) in LDL-c and VLDL-c in hyperlipidemic diet group (positive control), as compared with negative control group. It could also be seen from the values cited in Table 7 that serum HDL-c concentration increased significantly (p < 0.05) in the groups of rats fed on hyperlipidemic diet contained 10% of germinated SWL formula F6 (25% oil and 35% flour) and 10% of germinated SWL formula F7 (25% oil and 40% flour). The values of HDL-c in the groups 8 and 9 were 70.43±0.11 and 71.51±0.19 mg/dL, respectively with high increments compared to the positive control (34.52±0.07 mg/dL) and very close to the negative control (72.21±0.13 mg/dL). While, the lower increments of HDL-c values were recorded with the rats fed with wheat biscuits (42.47±0.28 mg/dL) and ungerminated SWL biscuits F2 (49.73±0.03 mg/dL). On the other hand, the effect of studied groups on LDL-c and VLDL-c contents of the experimental rats (induced hyperlipidemia) was also studied. The LDL-c and VLDL-c values were calculated from the equations LDL-c = TC - (TG/5) – HDL and VLDL-c = TG/5 (Friedewald et al., 1972). The data revealed that all studied groups reduced LDL-c levels of the experimental rats in the range from 137.69±0.19 mg/dL (group 3) to 78.62±0.15 mg/dL (group 9). However, groups 8 and 9 recorded the highest decrement in LDL-c levels accounting to 81.12±0.09 and 78.62±0.15 mg/dL, respectively compared with the positive control (333.90±0.14 mg/dL). As well, the values of VLDL-c showed the highest significant decrements (P< 5) in the groups 8 and 9 of rats fed on hyperlipidemic- enriched diet with 10% of germinated SWL formula F6 (25% oil and 35% flour) and 10% of germinated SWL formula F7 (25% oil and 40% flour). Both of them recorded percent decrements VLDL-c of 22.90% in group 8 and 24.08% in group 9 compared to the negative control. In general, the values of HDL-c, LDL-c and VLDL-c in groups 8 and 9 were cited in the normal international ranges of > 45, < 100 and < 150 mg/dL, respectively.

Table 7. Effect of the formulated biscuits on serum lipoproteins of the experimental rats (induced hyperlipidemia)

Groups	HDL-c (mg/dL) (Normal range > 45)	LDL-c* (mg/dL) (Normal range < 100)	VLDL-c** (mg/dL) (Normal range < 150)
Group 1 (-ve control)	72.21±0.13^{d}	49.33±0.22^{a}	23.67±0.10^{d}
Group 2 (+ve control)	34.52±0.07^{a}	333.90±0.14^{f}	36.74±0.18^{f}
Group 3	42.47±0.28^{b}	137.69±0.19^{e}	27.94±0.05^{e}
Group 4	49.73±0.03^{b}	126.48±0.21^{e}	22.59±0.14^{c}
Group 5	60.44±0.13^{c}	109.12±0.25^{d}	21.84±0.26^{c}
Group 6	65.31±0.12^{c}	103.02±0.07^{d}	22.17±0.20^{c}
Group 7	69.32±0.23^{c}	93.13±0.26^{c}	20.75±0.19^{b}
Group 8	70.43±0.11^{d}	81.12±0.09^{b}	18.25±0.27^{a}
Group 9	71.51±0.19^{d}	78.62±0.15^{b}	17.97±0.14^{a}

* LDL-c = TC - (TG/5) – HDL ** VLDL-c = TG/5 (Friedewald et al., 1972).

Values are expressed as mean±SD.

Values in each column which have different letters are significantly different at (P < 0.05).

Therefore, the best values of all lipoprotein parameters TC, TG, HDL-c, LDL-c and VLDL-c were recorded with the SWL biscuits simultaneously substituted with germinated SWL oil (25%) and extracted flour (35% and 40%) after germination. It was appeared a significant improvement (P < 5) in all lipoprotein parameters by decreasing the values of LDL-c and VLDL-c as well as increasing the value of HDL-c compared to the positive control. The values of HDL-c, LDL-c and VLDL-c tended to match the control values in rat group fed on basal diet. The reason could be due to the effect of soluble dietary fiber, protein combined by unsaturated fatty acids that are improved after the germination process of SWL seeds. Huff and Telford (1985), Kingman (1991), and Andersson and Major (2002) reported that the LDL-c reduction observed by feeding legumes or their fractions to hypercholesterolemic subjects could result from reduced LDL synthesis and/or increased LDL metabolism. On the other hand, Sirtori et al. (2004) clearly indicated that protein from a naturally isoflavone-poor legume such as while lupin can effectively reduce cholesterolemia and, most likely, up regulate LDL receptor activity, a widely

accepted mechanism of cholesterol reduction associated with the intake of proteins. Moreover, El-Malky and Gouda (2007) indicated that germinated lupin seeds reduced the increase of serum glucose, TC, TG, LDL-c, VLDL-c and enhanced the increase of HDL-c. As well, Osman, Mahmoud, Romeilah and Fayed (2011) observed the supplementation of a hypercholesterolemia-induced diet with sweet lupin seeds significantly lowered the plasma levels of TC, TG and LDL-C. ALT, AST and LDH activities slightly decreased in treated groups compared with the hypercholesterolemic group (positive control).

3.3 Predictors of Coronary Risk

Total cholesterol/ High density lipoproteins cholesterol (TC/HDL-c), Low density lipoproteins cholesterol/High density lipoproteins-cholesterol (LDL/HDL-c; risk ratio; RR) and High density lipoproteins-cholesterol/Total cholesterol ratios (HTR%) are predictors of coronary risk (National cholesterol education program, 1994). The results presented in Table (8) indicated that the groups of rats fed on hyperlipidemic diet (positive control) showed significant increments ($P < 0.05$) in the values of RR and TC/HDL-c concurrent with a marked decrement in the value of HTR% compared to the rats fed with basal diet (negative control). It was obvious that the feeding with SWL biscuits supplemented with germinated SWL oil and extracted flour had significant lower ratios of TC/HDL-c and RR compared to the hyperlipidemic diet (positive control), wheat biscuits (group 3) and ungerminated SWL biscuits (group 4). The groups 8 and 9 of rats fed on hyperlipidemic- enriched diet with 10% of germinated SWL formula F6 (25% oil and 35% flour) and 10% of germinated SWL formula F7 (25% oil and 40% flour) showed TC/HDL-c ratios that characterized with low coronary risk (2.41±0.23 and 2.35±0.18) (Hassan et al., 2012). Meanwhile, feeding of wheat biscuits to rats resulted in a TC/HDL-c ratio which approached to the ratio that characterized with middle coronary risk (4.90±0.22). In the case of RR values, the feeding of SWL biscuits formulae F6 and F7 in the rats groups 8 and 9 recorded the lowest risk ratios of 1.15±0.10 and 1.10±0.17 with significant decrements ($P < 0.05$) compared with the induced hyperlipidemic rats (positive control) (9.67±0.11 RR). It could be concluded that, the effect of studied biscuits on TC/HDL-c and RR in descending order was wheat biscuits (group 3) > ungerminated SWL biscuits group (4) > germinated SWL biscuits (20% oil and 30% flour) > germinated SWL biscuits (25% oil and 30% flour) > germinated SWL biscuits (30% oil and 30% flour) > germinated SWL biscuits (25% oil and 35% flour) > germinated SWL biscuits (25% oil and 40% flour). The TC/HDL-c and RR of groups 8 and 9 were tended to match that of the negative control. Furthermore, the group 9 of rats fed on biscuits formula F7 exhibited maximum increase in HTR% (42.54%) followed by the group 8 of rats fed on biscuits formula F7 (41.48%) as could be seen in Table 8. Whereas, the groups 3 and 4 of rats fed on wheat biscuits and ungerminated SWL biscuits showed the lowest increase in the HTR (20.41% and 25.02%). It could be seen from the obtained results that adding germinated SWL oil and extracted flour enriched biscuits to the diet could produce significant improvement for the beneficial lipoprotein ratios to reduce the risk of heart disease in hyperlipidemic individuals. Gustavo, José, Robison, Paulo and José (2012) demonstrated that protein isolate from *Lupinus albus* has a metabolic effect on endogenous cholesterol metabolism and a protector effect on the development of hepatic steatosis. Whereas, El-Malky and Gouda (2007) indicated that germinated lupin seeds reduced the hepatic enzymes ALT and AST and avoided the damage in liver tissues resulted from hyperlipidemic diet effect compared to the positive control.

Atherogenic index (AI) indicates the deposition of foam cells or plaque or fatty infiltration or lipids in heart, coronaries, aorta, liver and kidney (Hassan et al., 2012). The higher the AI, the higher is the risk of above organs for oxidative damage (Basu et al., 2007). Atherogenic lipoprotein profile of plasma is an important risk factor for coronary artery disease. The results cited in Table 8 revealed that the rats of group 9 that fed on hyperlipidemic-enriched diet with 10% of germinated SWL formula F7 (25% oil and 40% flour) showed a maximum lower AI value (1.35) followed by the group 8 of rats fed on hyperlipidemic- enriched diet with 10% of germinated SWL formula F6 (25% oil and 35% flour) with 1.41 as compared to the positive control (10.74). These results are in accordance with those obtained by Marchesi et al. (2008) who studied the hypolipidemic and anti-atherogenic effect of lupin protein isolates in rabbits and reported a significant reduction of cholesterol and a reduction of the risk of developing atherosclerosis. Furthermore, Mahfouz et al. (2012) recommended to utilize white lupin to prepare healthy diets to protect against hypercholesterolemia.

Table 8. Predictors for coronary risk

Groups	RR	TC/HDL-c	HTR%	AI
Group 1 (-ve control)	0.68±0.18	2.01±0.19	49.73	1.01
Group 2 (+ve control)	9.67±0.11	11.74±0.20	8.52	10.74
Group 3	3.24±0.24	4.90±0.22	20.41	3.90
Group 4	2.54±0.12	3.99±0.10	25.02	2.99
Group 5	1.81±0.19	3.17±0.11	31.58	2.17
Group 6	1.58±0.09	2.92±0.12	34.28	1.92
Group 7	1.34±0.25	2.64±0.25	37.84	1.64
Group 8	1.15±0.10	2.41±0.23	41.48	1.41
Group 9	1.10±0.17	2.35±0.18	42.54	1.35

RR: risk ratio= LDL-c/HDL-c.

HTR: High density lipoproteins-cholesterol/ Total cholesterol ratio = (HDL-c / TC) × 100.

AI: Atherogenic index = (TC - HDL-c) / HDL-c.

4. Conclusion

In the present study, it was observed that feeding of high cholesterol and fat diets supplemented with functional sweet white lupin (SWL) biscuits to the hyperlipidemic rats resulted in a significant decrease ($P < 0.05$) in the values of TC, TG, LDL-c and VLDL-c concurrent with a significant increase ($P < 0.05$) in the value of HDL-c compared with wheat biscuits. In addition, the feeding of hyperlipidemic diet contained 10% of functional SWL biscuits reduced markedly the predictors for the coronary heart risk such as RR, AI and TC/HDL-c compared to the group of rats fed on hyperlipidemic diet (positive control). Furthermore, it was found that the enrichment of hyperlipidemic diets with germinated SWL oil and extracted flour improved markedly the depression of serum cholesterol and food efficiency compared to the ungerminated ones. The acceptable results of these biological parameters were achieved by the simultaneous substitution with 25% germinated SWL flour and 35% or 40% germinated SWL oil. Therefore, it could be suggested that the proposed functional SWL biscuits were able to regulate the blood cholesterol and the body growth levels of individuals and patients. The modification in the lipid profile and food efficiency of induced hyperlipidemia rats caused by the feeding of functional SWL biscuits may have some physiological importance and may be easily extrapolated to humans.

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References

Allian, C. C., Poon, L. S., Chan, C. S. G., & Richmond, W. (1974). Enzymatic colourimetric method of the determination of plasma total cholesterol. *Clin. Chem, 20*, 470.

Anderson, J. W., & Major, W. (2002). Pulses and lipaemia, short and long-term effect: potential in the prevention of cardiovascular disease. *Br. J. Nutr., 88*(3), 263-271. http://dx.doi.org/10.1079/BJN2002716

Anderson, J. W., Johnstone, B. M., & Cook-Newell, M. E. (1995). Metaanalysis of the effects of soybean protein intake on serum lipids. *N. Engl. J. Med, 333*, 276-282. http://dx.doi.org/10.1056/NEJM199508033330502

Atiat, M. E., Eman, M. S., Mona, S. H., & Ibrahim, S. S. (1999). Influence of two of dietary fiber on the development of experimental atherosclerosis in rats. *Home Econ. J, 15*, 1-18.

Basu, M., Prasad, R., Jayamurthy, P., Pal, K., Arumughan, C., & Sawhney, R. C. (2007). Anti-atherogenic effects of seabuckthorn (*Hippophaea rhamnoides*) seed oil. *Phytomedicine, 14*, 770-777. http://dx.doi.org/10.1016/j.phymed.2007.03.018

Blagrove R. J., & Gillespie J. M. (1975). Isolation, purification and characterization of the seed globulins of *Lupinus albus*. *Aust. J. Plant Physiol, 2*, 13-27. http://dx.doi.org/10.1071/PP9750013

Cuadra, C., Muzquiz, M., Burbano, C., Ayet, G., Calvo, R., Osagie, A., & Cuadrado C. (1994). Alkaloid, β-galactoside and phytic acid changes in germinating lupin seeds. *J. Sci. Food Agric, 66*(3), 357-364. http://dx.doi.org/10.1002/jsfa.2740660313

Dadai, F. D., Walker, A. F., Sambrook, I. E., Welch, V. A., & Owen, R. W. (1996). Comparative effects on blood lipids and faecal Steroids of five legume species incorporated into a semi-purified, hypercholesterolamic rats dite. *Br. J. Nutr., 75*, 557-571. http://dx.doi.org/10.1079/BJN19960159

Dueñas, M., Hernández, T., Estrella, I., & Fernández, D. (2009). Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (*Lupinus angustifolius* L.). *Food Chem, 117*(4), 599-607. http://dx.doi.org/10.1016/j.foodchem.2009.04.051

Duranti, M. (2006). Grain legume proteins and nutraceutical properties. *Fitoterapia, 77*, 67-82. http://dx.doi.org/10.1016/j.fitote.2005.11.008

Duranti, M., Restani, P., Poniatowska, M., & Cerletti, P. (1981). The seed globulins of *Lupinus albus. Phytochemistry, 20*, 2071-2075. http://dx.doi.org/10.1016/0031-9422(81)80087-8

ELMaki, H. B., Abdel Rahaman, S. M., Idris, W. H., Hassan, A. B., Babiker, E. E., & ELTinay, A. H. (2007). Content of antinutritional factors and HCl extractabitity of minerals from white bean (*Phoseolus vulgaris*) cultivars: Influence of soaking and/or cooking. *Food Chem, 100*, 362-368. http://dx.doi.org/10.1016/j.foodchem.2005.09.060

El-Malky, W. A., & Gouda, H. A. (2007). Effect Of Green Leaves And Germination And Boiling Treatments Of Fenugreek And Lupin Seeds On Chemical Composition, Serum Glucose, Lipid Profile And Hepatic Enzymes Of Rats. *Eg. J. Biomed. Sci, 23*(1), 39-59.

Fatima, D. D., Ann, F. W., Ian, E. S., Vernon, A. W., & Robert, W. O. (1996). Comperative effects on blood lipids and faecal steroids of five legume species incorporated into a semipurified, hypercholesterolaemic rat diet. *Br. J. Nutr, 75*, 557-571. http://dx.doi.org/10.1079/BJN19960159

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Determination of high density lipoprotein cholesterol by selective precipitation. *Clin. Chem, 18*, 499-502.

Gravelle, A. J., Barbut, S., & Marangoni, A. G. (2012). Ethylcelluloseoleogels: Manufacturing considerations and effects of oil oxidation. *Food Res. Internat, 48*, 578-583. http://dx.doi.org/10.1016/j.foodres.2012.05.020

Gustavo, G. F., José, P. B., Robison, J. C., Paulo, H. N. S., & José, A. G. A. (2012). Cholesterol-lowering effect of whole lupin (*Lupinus albus*) seed and its protein isolate. *Food Chem, 132*(3), 1521-1526. http://dx.doi.org/10.1016/j.foodchem.2011.11.145

Hassan, A. A., Rasmy, N. M., Foda, M. I., & Bahgaat, W. K. (2012). Production of Functional Biscuits for Lowering Blood Lipids. *World J. Dairy & Food Sci, 7*(1), 1-20.

Hassanein, M. M. M., El-Shami, S. M., & El-Mallah, M. H. (2011). Investigation of lipids profiles of Nigella, Lupin and Artichoke seed oils to be used as healthy oils. *J. Oleo Sci, 60*(3), 99-107. http://dx.doi.org/10.5650/jos.60.99

Huff, M. W., & Telford, D. E. (1985). Direct synthesis of low-density lipoprotein apoprotein B in the miniature pig. *Metabolism, 34*, 36-42. http://dx.doi.org/10.1016/0026-0495(85)90057-5

Integrated guidelines for cardiovascular health and risk reduction in children and adolescents expert panel. (2011). Summary Report. *Pediatrics, 128*, S213-S256.

Jose, M. M., Michel, R., Manuel, C. A., & Ana, M. (2005). Cholesterol-lowering effects of dietary blue Lupin (*Lupines angustifolius* L.) in intact and ilearectal anastomosed pigs. *J. Lipid Res, 4*6, 1539-1547. http://dx.doi.org/10.1194/jlr.M500129-JLR200

Kingman, S. M. (1991). The influence of legume seeds on human plasma lipid concentration. *Nutr. Res. Rev, 4*, 97-123. http://dx.doi.org/10.1079/NRR19910010

Mahfouz, S. A., Elaby, S. M., & Hassouna, H. Z. (2012). Effects of some legumes on hypercholesterolemia in rats. *J. Am. Sci, 8*(12), 1453-1460.

Marchesi, M., Parolini, C., Diani, E., Rigamonti, E., Cornelli, L., & Arnoldi, A. (2008). Hypolipidaemic and antiatherosclerotic effects of lupin proteins in a rabbit model. *Br. J. Nutr, 4*, 1-4. http://dx.doi.org/10.1017/S000711450894215X

Mohamed, A. A., & Rayas-Duarte, P. (1995). Composition of *Lupinus albus. Cereal Chem, 72*(6), 643-647.

Monteiro, C. A., Moubarac, J. C., Cannon, G., Ng, S. W., & Popkin, B. (2013). Ultra-processed products are becoming dominant in the global food system. *Obes. Rev, 2*, 21-28. http://dx.doi.org/10.1111/obr.12107

Mousa R. M. A. (2014). Nutritional assessment of biscuits formulated by simultaneous substitution with sweet white lupin oil and extracted flour after germination. *Am. J. Food Nutr, 2*, 108-116. http://dx.doi.org/10.12691/ajfn-2-6-3

National cholesterol education program (NCEP). (2002). Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult treatment panel III, final report). *Circulation, 106*, 3143-3421.

National cholesterol education program. (1994). Second report of the expert panel on detection, evaluation and treatment of high blood cholesterol in adults. *Circulation, 89*(3), 1333-1445.

Osman, M., Mahmoud, G. I., Romeilah, R. M., & Fayed, S. A. (2011). Lupin seeds lower plasma lipid concentrations and normalize antioxidant parameters in rats. *Grasas y aceites, 62*(2), 162-170. http://dx.doi.org/10.3989/gya.056310

Rumiyati, J. V., & James, A. P. (2013). Total phenolic and phytosterol compounds and the radical scavenging activity of germinated australian sweet lupin flour. *Plant Foods Hum. Nutr, 68*, 352-357. http://dx.doi.org/10.1007/s11130-013-0377-6

Sirtori, C. R. (2001). Risks and benefits of soy phytoestrogens in cardiovascular diseases, cancer, climacteric symptoms and osteoporosis. *Drug Safety, 24*, 665-682. http://dx.doi.org/10.2165/00002018-200124090-00003

Sirtori, C. R., Lovati, M. R., Manzoni, C., Castiglioni, S., Duranti, M., Magni, C., … Arnoldi, A. (2004). Proteins of white lupin seed, a naturally isoflavone-poor legume, reduce cholesterolemia in rats and increase LDL-receptor activity in hepG2 cells. *J. Nutr, 134*, 18-23.

Smith, S. C., Choy, R., Johnson, S. K., Hall, R. S., Wildeboer-Veloo, A. C. M., & Welling, G. W. (2006). Lupin kernel fibre consumption modifies faecal microbiota in healthy men as determined by rRNA gene fluorescent in situ hybridisation. *Eur. J. Nutr, 45*, 335-341. http://dx.doi.org/10.1007/s00394-006-0603-1

Stortz, T. A., Zetzl, A. K., Barbut, S., Cattaruzza, A., & Marangoni, A. G. (2012). Edible oleogels in food products to help maximize health benefits and improve nutritional profiles. *Lipid Technol, 24*, 151-154. http://dx.doi.org/10.1002/lite.201200205

Sulieman, A. M., & El-Newary, S. A. (2014). Hypolipidemic effect of *Cordia dichotoma* forst. Pulp in high-fat diet-fed rats. *World J. Dairy & Food Sci, 9*(2), 260-271.

Tarancón, P., Fiszman, S. M., Salvador, A., & Tárrega, A. (2013). Formulating biscuits with healthier fats. Consumer profiling of textural and flavour sensations during consumption. *Food Res. Internat, 53*, 134-140. http://dx.doi.org/10.1016/j.foodres.2013.03.053

Wahlefeld, A. W. (1974). In Methods of Enzymatic Analysis. HU Bergmeyer, Ed Academic Press, New York, 5, 1831-1835. http://dx.doi.org/10.1016/B978-0-12-091304-6.50036-7

Warnick, G. R., Benderson, V., & Albers, N. (1983). Selected Methods. *Clin. Chem, 10*, 91-99.

WHO. (2011). Global status report on non-communicable diseases. Retrieved from www.who.int/countries/egy/

Youssef, M. K. E., Youssef, H. M. K. E., & Mousa, R. M. A. (2014). Evaluation of Antihyperlipidemic Activity of Citrus Peels Powders Fortified Biscuits in Albino Induced Hyperlipidemia. *Food Public Health, 4*(1), 1-9. http://dx.doi.org/10.5923/j.fph.20140401.01

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The Effect of Buffalo Meat on Composition, Instrumental and Sensory Characteristics of Traditional Greek Sausages

D. Petridis[1], A. Zotos[1], B. Skapetas[2] & V. A. Bampidis[2]

[1] Department of Food Technology, School of Agricultural Technology, Food Technology and Nutrition, Alexander Technological Educational Institute of Thessaloniki, 57400 Thessaloniki, Greece

[2] Department of Agricultural Technology, School of Agricultural Technology, Food Technology and Nutrition, Alexander Technological Educational Institute, 57400 Thessaloniki, Greece

Correspondence: D. Petridis, Department of Food Technology, School of Agricultural Technology, Food Technology and Nutrition, Alexander Technological Educational Institute of Thessaloniki, 57400 Thessaloniki, Greece. Tel: 30-231-001-3917; Fax: 30-231-079-1375. E-mail: petridis@food.teithe.gr

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Abstract

Five (5) mixtures of buffalo/pork meat (70/0, 52.5/17.5, 35/35, 17.5/52.5 and 0/70), maintaining stable the amount of pork backfat, were prepared and analyzed for their chemical composition, fatty acids profile, instrumental parameters and sensory attributes. The results of the study showed that the addition of buffalo meat produced sausages with higher protein and less fat content. A slight decrease in ω6/ω3 ratio was observed and an increase in CLA fatty acids. Principal Component Analysis revealed that the lower fat content in the sausages the higher the levels of CLA18:10trans 12cis and CLA18:9cis 11trans, whereas, SFA is abundant at the highest fat levels. Redness and hardness instrumentally increased in the sausages with the addition of buffalo meat, while juiciness decreased, probably due to the decreased lipid content. The mixture with the ratio of 52.5/17.5 buffalo/pork positively maximized sensorial consistency, elasticity and cohesiveness of the traditional sausages, while the addition of 70.0/0.0 mixture, hardness and redness.

Keywords: Greek buffalo meat, traditional Greek sausages, FAME, instrumental, sensory

1. Introduction

Essential fatty acids, fat soluble vitamins and energy are the three basic physiological functions contributed to the fat content of any food (Muguerza, Fista, Ansorena, Astiasaran, & Bloukas, 2002). In meat products fat is a crucial parameter since it contributes to flavor, texture, mouth feel, juiciness and overall sensation of lubricity of the product. Therefore, any fat reduction in meat products can affect their acceptability by the consumers (Giese, 1996). However, it has been worldwide scientifically announced that lowering the amount of fat, particularly saturated fatty acids as well as linoleic acid and increased ratio of ω–6/ω–3 fatty acids is a major risk factor for western type cancers, thrombotic diseases, apoplexy, allergic hyperreactivity and diseases for which anti-inflammatory drugs are effective (AHA, 1986; Zotos & Vouzanidou, 2012).

Greeks are quite keen on traditional sausages which are very popular all over the country. According to Greek Food Legislation (1987) they are characterized as fresh (non-cooked) sausages which should not contain more than 35% fat although Papadima, Arvanitoyannis, Bloukas, and Fournitzis (1999) have found that the fat content ranges from 15.4 to 56.8%. Neverthereless, fat acts as a reservoir for flavor compounds and contributes to product texture and juiciness (Papadima & Bloukas, 1999). The granulated fat in non-cooked sausages helps the sausage mixture to be loosened and consequently it attributes to the release of moisture from the inner layers of the product (Wirth, 1988). Therefore, reducing the fat content in sausages may alter product quality.

Most Greek agricultural families produce traditional sausages. They are mainly consisted by pork meat and fat which are chopped and thoroughly mixed with salt, leek and seasonings. The sausage mixtures were stuffed in natural casings obtaining from the cleaned small intestine of pigs. The sausages were pierced on all sides to allow ensnared air to get away and stored for some days in a warm room in order the expected dryness to be achieved. Sausages which are going to be consumed within a few weeks stored at low temperature rooms (Papadima & Bloukas, 1999).

The Food and Agriculture Organization of the United Nations refers to the Greek buffalo (*Bubalus bubalis*) population as a separate breed named "Ellinikos vouvalos (Greek buffalo)", and characterizes this population as endangered-maintained (FAO, 2007) species. The Greek buffalo is part of biodiversity in many Greek wetlands enriching ecosystems with its aesthetic value. Moreover, the Greek buffalo, as a food producing animal, provides valuable products (milk, meat; Zotos & Bampidis, 2014), which constitute exquisite dishes, contributing to economic growth in the surrounding areas of farming thus there is an increasing interest nowadays for its breeding in Greece, with its population being approximately 4,000 (GBBC, 2014). While Greek buffalo meat gaining popularity in Greece, there are opportunities for the development of the buffalo meat industry to cater for the needs of the Greek market. The advantages of buffalo meat are the high protein, low fat and low cholesterol contents as well as the attribution of lower calories than beef meat (Vasanthi, Venkataramanujam, & Dushyanthan, 2007). Thus, the aim of this work was to study the effect of Greek buffalo (*Bubalus bubalis*) meat on the proximate composition, fatty acids profile, instrumental and objective sensory characteristics of traditional Greek sausages, maintaining at the same time a consistent addition of pork backfat.

2. Materials and Methods

2.1 Preparation of Samples

The raw materials used for the preparation of traditional sausages such Greek buffalo (thigh) and pork (thigh) meat, pork backfat and natural cases of porcine small intestine were provided by Kerkini market, North Greece (41°14' N, 23°06' E) and stored at 4 °C. The used additives such as oregano, pepper, salt and cumin were purchased from the local market. The quantities were buffalo meat 10.5 kg, pork meat 10.5 kg and pork backfat 9 kg. The mixtures of the above raw materials are shown in Table 1.

Raw materials were sliced into small pieces and mixed thoroughly with the above mentioned additives. They were then minced using a Seydelmann mince machine with internal mesh of 8 mm and external 5 mm.

The batter was encased in natural cases of porcine small intestine 30-32 mm using a TalsaH26PA filler machine. The cases were densely pierced on all sides to allow entrapped air to escape. Then sausages were mounted on trolleys and placed in the chamber at 4 °C for 72 hours in order a natural drying process to be achieved. The drying process followed by a grilling process for approximately 20 min and the samples were presented to panelists after being cooled at approximately 30 °C. Samples for further analysis were refrigerated at 2±1 °C.

Table 1. The five mixtures of the raw materials used to produce traditional sausages

Buffalo meat %	Pork meat %	Buffalo meat (Kg)	Pork meat (Kg)	30% Pork backfat (Kg)	2% Salt (Kg)	0.2% Pepper (Kg)	0.3% Cumin (Kg)	0.3% Oregano (Kg)	Sausages (Kg)
52.5	17.5	3.15	1.05	1.8	0.12	0.012	0.018	0.018	6.168
17.5	52.5	1.05	3.15	1.8	0.12	0.012	0.018	0.018	6.168
70	0	4.20	0	1.8	0.12	0.012	0.018	0.018	6.168
0	70	0	4.20	1.8	0.12	0.012	0.018	0.018	6.168
35	35	2.10	2.10	1.8	0.12	0.012	0.018	0.018	6.168
Sum (Kg)		10.50	10.50	9.0	0.60	0.060	0.090	0.090	30.840

2.2 Proximate Analysis

The recommended method by the CEC (Commission of European Communities) ISO 1442-1973 was used to determine moisture content (CEC, 1979). The method of Hanson and Olley (1963) was applied to determine lipid content as well as to extract lipids. Total protein (crude protein, N × 6.25) content of the samples was determined using the Kjeldahl method according to standard AOAC method (AOAC, 2002a). The ash content was determined by mineralization at 550^{o}C according to standard AOAC method (AOAC, 2002b).

2.3 Fatty Acid Analysis

The extracted lipids were further prepared for fatty acid analysis according to the procedure of Zotos, Hole and Smith (1995). Fatty acids methyl esters were analyzed using a Focus GC (Thermo Finnigan, USA) with an FID detector, equipped with auto sampler and a capillary column AT AquaWax 60 m × 0.25 mm ID, thickness 0.25

μm (Alltech, USA). The oven temperature set at 150 °C for 1 min and programmed to 220 °C at 3 °C min^{-1} and held there for 30 min, the total analysis time was 54 min. The volume of the sample was 1 μL, the injection temperature 220 °C at splitless mode with 0.80 s splitless time and the detection temperature 250 °C. The carrier gas was high purity helium with a linear flow rate 1.2 mL min^{-1}. The various fatty acids were identified by comparison with standard fatty acids methyl esters (C14:0, C16:0, C16:1ω-7, C18:0, C18:1ω-9, C18:1ω-7, C18:2ω-6, CLA (C18 9cis 11trans, C18 9cis 11cis, C18 9trans 11trans, C18 10trans 12cis), C18:3ω-3, C20:1ω-9, C20:4ω-6, C20:5ω-3 & C22:6ω-3, Larodan Fine Chemicals, Sweden).

2.4 Instrumental Analysis (TPA)

Sausages were instrumentally analyzed by the Texture Profile Analysis (Friedman, Whitney & Szczesniak 1963; Bourne, 1978), a well known method for texture characterization (Chen, 2009) which gives good correlation relationships between mechanical and sensory attributes for meat products such as rib steaks (Caine, Aalhus, Best, Dugan, & Jeremiah, 2003) and frankfurter-type sausages (Yang, Keeton, Beilken & Trout, 2001; Ritzoulis, Petridis, Derlikis, Fytianos, & Asteriou, 2010). The principle of the method is the application of two successive compressions to a test sample using a instrumental testing machine in imitation of a chewing process. The obtained force–displacement/time curves can be used for an approximate quantification of a number of kinesthetic parameters such as hardness, cohesiveness, viscosity, elasticity, adhesiveness, springiness, brittleness, chewiness, and gumminess. Hardness, cohesiveness, chewiness, springiness and gumminess were measured in this work. Experiments were performed using a TA-XT texture analyzer (TA instruments, New Castle, DE), as described before by Ritzoulis et al. (2010). The analyzer was equipped with a 50-mm-diameter aluminum cylinder, operating with a compression rate of 5 mm/sec. Samples, 20 mm in length, were cut using a dedicated template ring, and axially compressed to 40% of their original height. The capacity of the load cell used was 30 g. All tests were performed at least six times. The OriginPro 8.0 (OriginLab Corporation, Northampton, MA) computer program was chosen to obtain graphic displays of the texture analyzer data and perform the necessary calculations.

2.5 Sensory Analysis

Sensory evaluation was performed using a balanced incomplete block design with the following features (plain 11.1a, Cochran & Cox, 1957): t=5 treatments (samples), k=3 treatments assessed by each panelist, b=10 panelists, r=6 replicates per treatment και λ=3 similar pairs of treatments in the design. Each slice was cut into 3-cm cylinders. Samples were assessed at room temperature and were presented to panelists hosted in special booths in white plastic dishes. The order of assessment was randomized within each session. The samples were taken out from refrigeration (4 °C) 30 min prior to their testing and left for equilibration at room temperature and were immersed in boiling water for 3 min immediately prior to testing. Panelists were asked to evaluate five sensory attributes in terms of perception intensity:

- Hardness as the force required to penetrate sample with molar teeth.
- Consistency as the evaluation of the amount of deformation before rupture.
- Elasticity as the degree of bouncing between two consecutive bites.
- Juiciness as juice feeling in the mouth and gum.
- Red color intensity as the result of comparison of the degree of redness between slices of the various samples under white light.

A panel of 8 members of the School's staff plus two research students were chosen to participate in the sensory evaluation. They were previously allowed to gain experience by frequently consuming meat products available in the lab and were particularly trained on how to test various sausage formulations deliberately differing in sensory characteristics such as hardness, cohesiveness etc. Ten booths installed in the laboratory of sensory studies of the department were used to host the panelists who were asked to draw a vertical line on a 15 cm long unstructured scale (Muñoz & Civille, 1998) corresponding to a particular perceptive intensity of an attribute. The left end (0 cm) of the line was marked as not at all hard to bite, elastic, fatty, texturally consistent, and reddish. The right end (15 cm) was marked for the texture as very hard to bite and so forth. The experiment was conducted three times and adjusted means of attributes from each run were deduced forming eventually three replicates per sample.

2.6 Statistical Analysis

Descriptive statistics were employed for the chemical composition of data. Instrumental and sensory attributes were tested statistically using one way analysis of variance (fixed effect-sausage samples with five treatments)

and those found significant at 0.05 probability level were checked for differences between buffalo treatments by plotting the 95% confidence intervals of means. Statistically significant pair-wise differences of means assume non-overlap intervals. Principal Component Analysis (PCA) was furthermore conducted to detect potential relationships between fatty acids and fat level of samples and also between the texture and sensory profile of sausages and the buffalo addition in samples. Three replicates per treatment were taken for chemical analysis, six to eight for instrumental analysis and three for sensory evaluation.

3. Results and Discussion

3.1 Proximate Composition of Raw Material, Unprocessed and Grilled Sausages

The proximate composition of the raw materials used in this work as well as of the unprocessed and grilled sausages are shown in Table 2. As can be observed the buffalo meat has higher protein content than pork meat and less fat content. These differences affected the proximate composition of either unprocessed or grilled sausages. Thus, in the mixture 70.0/0.0 (buffalo/pork) the higher protein content was detected (approximately 42% in the unprocessed and 44% in the grilled sausages) on the contrary in mixture 0.0/70.0 (buffalo/pork) protein content shown the lowest value (approximately 36.5% in the unprocessed and 39% in the grilled sausages). Similar results were also obtained for fat content (Table 2). Proportional variations between moisture, protein and fat in buffalo sausages according to fat content were also reported by Krishnan & Sharma (1990). Thus, they were reported that moisture was 66.19, 63.07 and 60.59%, protein 17.01, 16.39 and 15.44% and fat 13.85, 17.45 and 21.06% in sausages with 85/15, 80/20 and 75/25 meat and pork fat respectively.

Table 2. Proximate composition of raw meat materials on wet weight basis (mean ± StDev) and of unprocessed and grilled sausages on dry weight basis

Raw material	Moisture %	Ash %	Fat %	Protein %
Pork meat	73.11±6.6	1.07±0.18	5.18±0.40	19.74±1.37
Buffalo meat	74.88±0.22	1.11±0.05	2.14±0.05	23.74±2.06
Pork backfat	10.99±0.57	0.20±0.02	87.49±0.48	2.93±0.01
Unprocessed sausages				
Buffalo/Pork meat		Ash %	Fat %	Protein %
52.5 / 17.5		6.15±0,04	56.10±0.73	38.74±0.20
17.5 / 52.5		5.68±0.01	58.33±0.69	38.58±1.87
70.0 / 0.0		6.68±0.20	52.36±0.16	41.95±4.40
0.0 / 70.0		5.78±0.01	56.10±1.36	36.64±0.01
35.0 / 35.0		6.71±0.10	52.54±0.31	40.88±1.03
Grilled sausages				
Buffalo/Pork meat		Ash %	Fat %	Protein %
52.5 / 17.5		5.16±0.04	53.62±0.62	42.38±0.51
17.5 / 52.5		5.52±0.07	53.88±0.12	41.06±1.78
70.0 / 0.0		5.73±0.21	50.68±0.44	44.04±3.23
0.0 / 70.0		5.28±0.12	56.25±0.43	39.18±0.64
35.0 / 35.0		5.71±0.02	52.88±0.36	41.65±1.41

Data are means of triplicate determinations ± standard deviation.

3.2 Fatty Acids Profile and Relationships

The fatty acid profiles of the five mixtures (buffalo/pork meat) are shown in Table 3. As it can be observed the main fatty acids are the oleic (C18:1ω-9), palmitic (C16:0), stearic (C18:0) and linoleic (C18:2ω-6). It can also

be observed that the contribution of the buffalo meat is not significant since the fatty acid profiles remained almost similar. However, a slight decrease of ω6 fatty acids was detected due to addition of buffalo meat having an effect on the proportional slight decrease of the ω6/ω3 ratio. The CLA fatty acids were also presented in higher concentrations in the sausages where buffalo meat was added (Table 3). The aforementioned fatty acids with the same order were also reported as the main fatty acids in dry fermented sausages (Muguerza, Gimenoa, Ansorena, Bloukas, & Astiasarán, 2001).

Table 3. Fatty acid profiles of the 5 above mentioned sausage mixture (buffalo/pork)

Fatty acids %	52.5/17.5	17.5/52.5	70.0/00.0	0.0/70.0	35.0/35.0
C14:0	1.40±0.01	1.46±0.01	1.37±0.01	1.53±0.13	1.43±0.16
C15:0	0.69±0.01	0.30±0.01	0.24±0.01	0.07±0.01	0.42±0.08
C16:0	26.75±0.06	26.46±0.52	26.07±0.22	26.88±0.80	26.09±1.29
C16:1ω7	2.65±0.01	2.45±0.01	2.49±0.01	2.90±0.11	2.72±0.33
C17:0	0.91±0.01	0.44±0.01	0.53±0.04	0.35±0.01	0.46±0.05
C18:0	12.55±0.10	12.94±0.10	12.86±0.06	12.57±0.28	12.53±0.16
C18:1ω-9	37.00±0.29	38.28±0.28	41.64±0.01	40.99±0.99	38.79±0.30
C18:2ω-6	11.54±0.07	11.24±0.08	10.36±0.03	11.25±0.06	11.11±0.05
CLA C18:9cis 11trans	0.92±0.04	0.77±0.01	1.09±0.04	0.63±0.01	0.87±0.02
CLA C18:10trans 12cis	0.22±0.01	0.18±0.01	0.27±0.01	0.19±0.01	0.20±0.01
C18:3ω-3	0.89±0.01	0.84±0.01	0.89±0.01	0.77±0.02	0.90±0.01
C18:3ω-6	0.23±0.03	0.37±0.04	0.18±0.02	0.38±0.01	0.23±0.01
C20:1ω-9	0.69±0.02	0.67±0.04	0.55±0.01	0.39±0.01	0.50±0.03
C20:2ω-6	0.11±0.01	0.09±0.01	0.14±0.05	0.07±0.01	0.15±0.03
C20:4ω-6	0.20±0.05	0.20±0.01	0.20±0.01	0.28±0.01	0.21±0.01
Saturated (SFA)	42.30±0.19	41.60±0.60	41.06±0.11	41.38±0.66	40.91±1.16
Monounsaturated (MUFA)	40.33±0.26	41.40±0.23	44.68±0.01	44.27±0.89	42.00±0.06
Polyunsaturated (PUFA)	14.10±0.08	13.67±0.08	13.11±0.13	13.56±0.02	13.65±0.06
Σ ω3	0.89±0.01	0.84±0.01	0.89±0.01	0.77±0.02	0.90±0.01
Σ ω6	12.29±0.03	12.06±0.09	11.14±0.09	12.16±0.04	11.89±0.04
ω6/ω3	13.81±0.19	14.35±0.11	12.58±0.20	15.79±0.42	13.21±0.04

Data are means of triplicate determinations ± standard deviation.

The effect of fat content on fatty acid content is shown in Figure 1 and the correlation coefficients between fatty acids and the two major components of PCA are shown in Table 4. Longer arrows in Figure 1 indicate attributes with higher effects, oblique and obtuse angles positive and negative correlations. The lower (oblique) or higher (obtuse) aperture between two lines the higher the correlation coefficient. Vertical lines indicate angle with zero correlation. Samples located near to an arrow exhibit strong effect on that attribute. PCA reveals an interesting relationship between fatty acids and fat content, bearing in mind that the initial preparation of sausages included steadily pork backfat concentration 30%. Fatty acids such as C20:4ω-6, MUFA, PUFA, C18:3ω-3 and C18:2ω-6 are the most responsible for the formation of major axis 1 (Table 4) explaining 55% of the total variation and SFA and CLA18:10trans 12cis for the formation of axis 2 explaining another 25.6% summing up to 80.64% variation. On the other hand, CLA18:9cis 11trans shares nearly equal correlations with both axes acting therefore equivalently. The fat content of the sausages correlates strongly and negatively only with axis 2 (r=-0.913) and that is clearly exemplified in Figure 1. The lowest fat levels appear in the top of axis 2 (25.76%) coinciding with

high % levels of CLA18:10trans 12cis and CLA18:9cis 11trans. On the contrary, SFA is abundant at the highest fat levels (29.15 and 29.73%) at the bottom of axis 2, whereas PUFA, C18:3ω-6 and C20:4ω-6, are distributed, the latter in an inverse direction, at medium fat levels (27.90%) close to axis 1. MUFA correlates inversely (negatively) and strongly with PUFA, C18:2ω-6 and SFA.

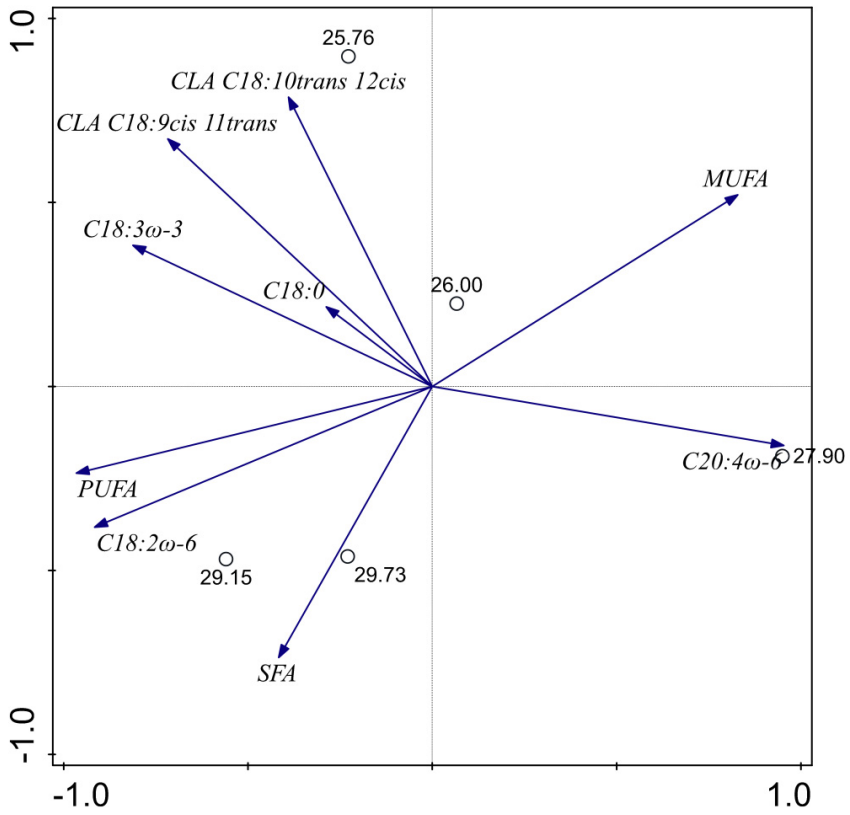

Figure 1. PCA biplot showing the correlations among fatty acids and the % distribution of fat in sausage mixtures. Lipid content on wet weight basis

Table 4. Correlation coefficients between fatty acids and the two major components. Values greater than |0.700| are shown in boldface. Asterisk denotes the correlation of fat concentration in the sausages, entered as a supplementary variable in the PCA, with the two axes

Fatty acids	Factor 1	Factor 2
C20:4ω-6	**-0.954**	-0.161
MUFA	**-0.829**	0.521
C18:0	0.288	0.216
CLA C18:10trans 12cis	0.390	**0.786**
SFA	0.417	**-0.737**
CLA C18:9cis 11trans	0.717	0.673
C18:3ω-3	**0.812**	0.383
C18:2ω-6	**0.916**	-0.382
PUFA	**0.966**	-0.236
*Fat %	0.183	**-0.913**

3.3 Instrumental and Sensory Properties of the Traditional Sausages

Three instrumental attributes, hardness, chewiness and gumminess, were found statistically significant and all appeared to increase proportionally to the buffalo content increase in the samples, more specifically at levels greater than 35% concentration (Figure 2). This should be expected since these sausages have higher protein and less lipid content. It is worth noted that any fat reduction can affect the acceptability of the products (Muguerza et al., 2002). Three also sensory attributes differentiate their intensity among buffalo levels (Figure 2). Redness elevated significantly to the buffalo content increase and distinctly between levels starting from as low as 5cm (low redness) and ending up to 11.5 cm (fair redness) proving to become an excellent indicator of buffalo amount in the sausages. An increase of sensory hardness is obvious with buffalo supplementation, actually distinct between 17.5 and 70% (confidence intervals do not overlap). Finally, juiciness declines with buffalo enrichment down to 7 cm (moderate intensity), presumably due to gradual pork content increase in the samples.

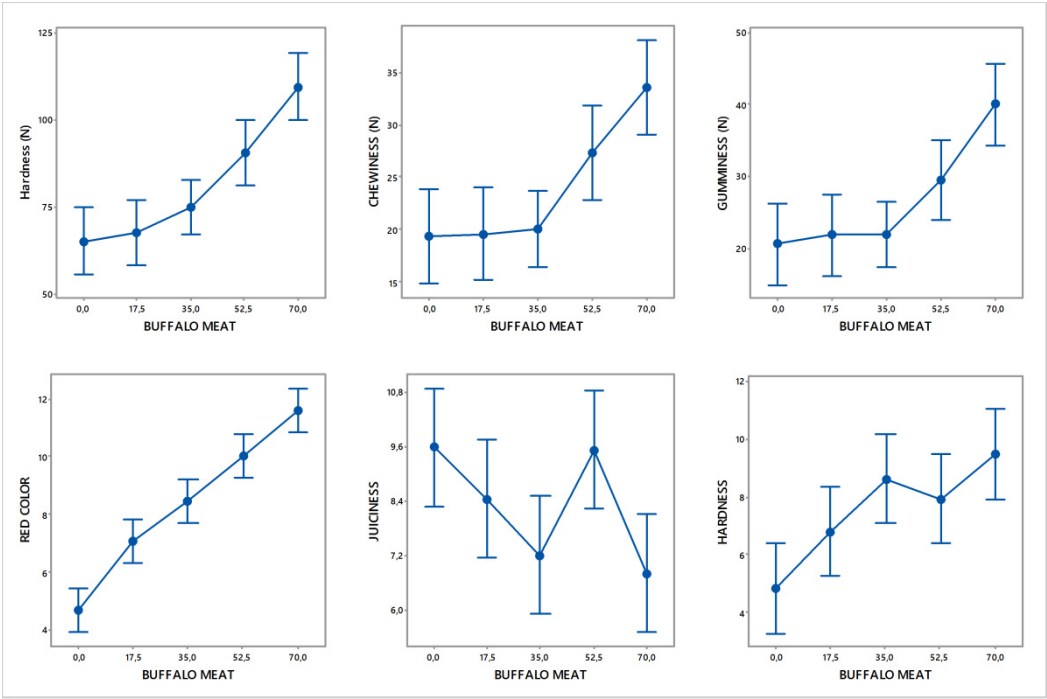

Figure 2. Mean change of instrumental and sensory attributes along the buffalo percentage increase. Vertical bars represent the 95% confidence intervals of means based on the mean square of ANOVA

The composition, instrumental and sensory profile of traditional sausages is explicitly described by the two major components in Table 5 and Figure 3. Sensory redness, juiciness and hardness, and instrumental hardness, gumminess, chewiness and springiness in joint with protein content best explain axis 1 (60.1% of the total variation) and ash and fat content axis 2 (21.3% totaling 81.5%). The relationships are further transitioned in Figure 3, in which three bundles of common attributes prevail. Between 0% and 17.5% buffalo amount in the samples, three variables of different origin correlate strongly: springiness, juiciness and fat content. Around 52.5% buffalo content, consistency, elasticity and cohesiveness maximize positively their performance. When buffalo occurs exclusively in the sausages (70% and pork 0%), then the protein content reaches high levels accompanied by high intensity of sensory hardness and redness, and all of them are adequately justified by high effects of instrumental hardness, gumminess and chewiness. Similar results regarding hardness were also reported by Rey, Martinez and Urrea (2011). They found an average hardness of 28.89 N and 19.63 N for sausages from buffalo and beef meat respectively. It was further reported that reducing the fat content of the sausages significantly increased ($p<0.05$) instrumental hardness and firmness (Muguerza et al., 2002).

Table 5. Correlation coefficients between each attribute and the two major components. Values greater than |0.700| are boldfaced. Asterisk denotes the correlation of buffalo concentration in sausages, entered as a supplementary variable in the PCA, with the two axes. Upper cases denote sensory and chemical variables and lower cases mechanical

Attribute	Factor 1	Factor 2
COLOR	**-0.959**	-0.167
Hardness	**-0.953**	-0.125
HARDNESS	**-0.924**	0.076
Gumminess	**-0.921**	-0.187
Chewiness	**-0.902**	-0.222
PROTEIN	**-0.875**	-0.101
CONSISTENCY	-0.668	-0.479
ASH	-0.595	**0.803**
ELASTICITY	-0.534	-0.574
Cohesiveness	-0.356	-0.685
FAT	0.578	**-0.802**
JUICINESS	**0.713**	-0.429
Springiness	**0.808**	-0.363
*Buffalo meat	**-0.966**	-0.129

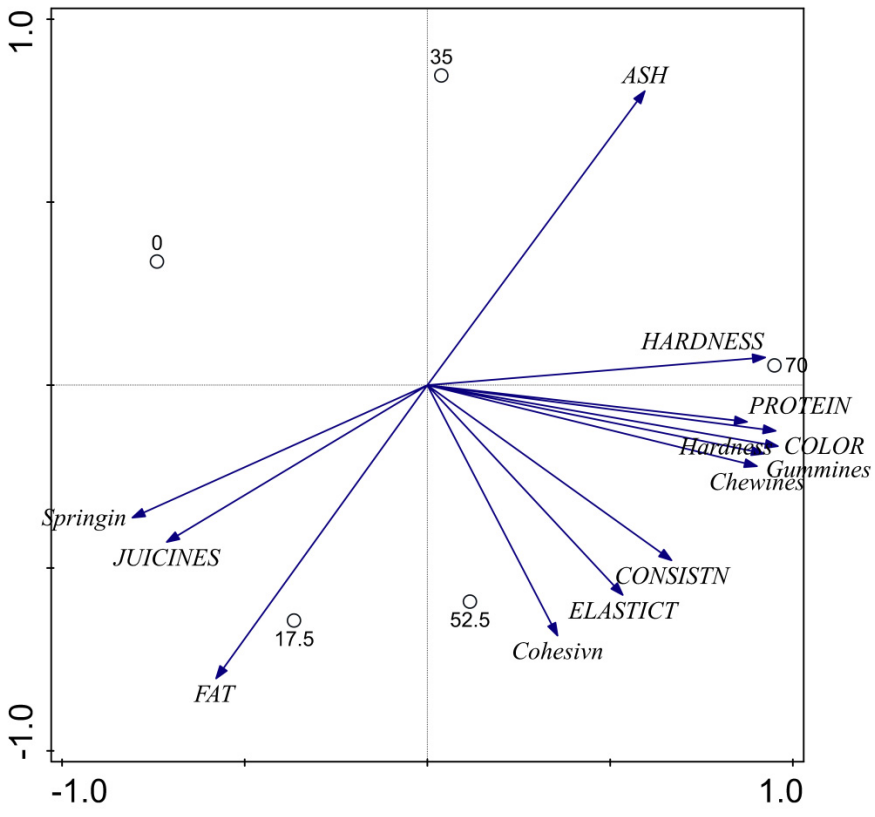

Figure 3. PCA biplot showing the correlations among attributes and the % distribution of buffalo meat samples. Upper cases denote sensory and chemical variables and lower cases instrumental

4. Conclusions

The addition of buffalo meat in traditional Greek sausages slightly increased protein and decreased fat content. A slight decrease of ω6/ω3 ratio and an increase of C18:10trans 12cis and C18:9cis 11trans fatty acids were also observed. Both C18:10trans 12cis and C18:9cis 11trans fatty acids are related to the fat content, thus the less fat content the higher the levels of the CLA fatty acids, while on the contrary, SFA is abundant at the highest fat levels. Instrumental redness and hardness increased with the addition of buffalo meat while juiciness decreased. The addition of 52.5/17.5 buffalo/pork positively maximized sensorial consistency, elasticity and cohesiveness while the addition of 70.0/0.0 mixture hardness and redness.

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References

AHA. (1986). Dietary guidelines for healthy adult Americans. *Americans Heart Association, 74*, 1465-1475.

AOAC. (2002a). *Official Methods of Analysis of AOAC International. 33.3.10, Method 920.109*. Gaithersburg, MD: AOAC.

AOAC. (2002b). *Ash of seafood's, method 938.08*. In W. Horwitz (Ed.) Official Method of Analysis of AOAC International. Maryland: AOAC International.

Bourne, M. C. (1978). Texture profile analysis. *Food Technology, 32*, 62-72.

Caine, W. R., Aalhus, J. L., Best, D. R., Dugan, M. E. R., & Jeremiah, L. E. (2003). Relationship of texture profile analysis and Warner Bratzler shear force with sensory characteristics of beef rib steaks. *Meat Science, 64*, 333-339. http://dx.doi.org/10.1016/S0309-1740(02)00110-9

CEC. (1979). *Commission of European Communities*. Method-ISO 1442-1973.

Chen, J. (2009). Food Oral Processing–A review. *Food Hydrocolloids, 23*, 1-25. http://dx.doi.org/10.1016/j.foodhyd.2007.11.013

Cochran, W. G., & Cox, G. M. (1957). *Experimental Designs*. New York: Wiley.

Food and Agriculture Organization (FAO). (2007). Breeds currently recorded in the Global Databank for Animal Genetic Resources. Rome, Italy, p. 155.

Friedman, H. H., Whitney, J. E., & Szczesniak, A. S. (1963). The texturometer–A new instrument for objective texture measurement. *Journal of Food Science, 28*, 390-396. http://dx.doi.org/10.1111/j.1365-2621.1963.tb00216.x

Giese, J. (1996). Fats, oils and fat replacers. *Food Technology, 50*, 78-83.

Greek Buffalo Breeders Cooperation (GBBC). (2014). Data concerning the Greek buffalo population. Vironia, Serres, Greece. Personal communication.

Hanson, S. W. F., & Olley, J. (1963). Application of the Bligh and Dyer method of lipid extraction to tissue homogenates. *Biochemical Journal, 89*, 101-102.

Krishnan, K. R. & Sharma, N. (1990). Studies on Emulsion-type Buffalo Meat Sausages Incorporating Skeletal and Offal Meat with Different Levels of Pork Fat. *Meat Science, 28*, 51-60. http://dx.doi.org/10.1016/0309-1740(90)90019-3

Muguerza, E., Gimeno, O., Ansorena, D., Bloukas, J. G., & Astiasarán, I. (2001). Effect of replacing pork backfat with pre-emulsified olive oil on lipid fraction and sensory quality of Chorizo de Pamplona - a traditional Spanish fermented sausage. *Meat Science, 59*, 251-258. http://dx.doi.org/10.1016/S0309-1740(01)00075-4

Muguerza, E., Fista, G., Ansorena, D., Astiasaran, I., & Bloukas J. G. (2002). Effect of fat level and partial replacement of pork backfat with olive oil on processing and quality characteristics of fermented sausages. *Meat Science, 61*, 397-404. http://dx.doi.org/10.1016/S0309-1740(01)00210-8

Muñoz, A. M., & Civille, C. V. (1998). Universal, product and attribute specific scaling and the development of common lexicons in descriptive analysis. *Journal of Sensory Studie*s, 13, 57-75. http://dx.doi.org/10.1111/j.1745-459X.1998.tb00075.x

Papadima, S. N., Arvanitoyannis, I., Bloukas, I. G., & Fournitzis, G. C. (1999). Chemometric model for describing Greek traditional sausages. *Meat Science, 51*, 271-277. http://dx.doi.org/10.1016/S0309-1740(98)00137-5

Papadima, S. N., & Bloukas, J. G. (1999). Effect of fat level and storage conditions on quality characteristics of traditional Greek sausages. *Meat Science, 51*, 103-113. http://dx.doi.org/10.1016/S0309-1740(98)00103-X

Rey, J. F., Martinez, C. L., & Urrea, A. (2011). Evaluation of sensory characteristics and texture of an economic Buffalo meat (*Bubalus bubalis*) sausage and an economic beef (*Bos incidus*) sausage with addition of bovine hemoglobin powder. *11th International Congress of Engineering and Food (ICEF11), Procedia Food Science, 1*, 545-548.

Ritzoulis, C., Petridis, D., Derlikis, E., Fytianos, K., & Asteriou, P. (2010). Utilization of inverse water–in–oil emulsions as fat replacers in Frankfurter model sausages: Influence of fat – emulsion content on the organoleptic and mechanical properties. *Journal of Texture Studies, 41*, 62-74. http://dx.doi.org/10.1111/j.1745-4603.2009.00213.x

Vasanthi, C., Venkataramanujam, V., & Dushyanthan, K. (2007). Effect of cooking temperature and time on the physico-chemical, histological and sensory properties of female carabeef (buffalo) meat. *Meat Science, 76*, 274-280. http://dx.doi.org/10.1016/j.meatsci.2006.11.018

Wirth, F. (1988). Technologies for making fat-reduce meat products. *Fleischwirtschaft, 68*, 115-1156.

Yang, A., Keeton, J. T., Beilken, S. L., & Trout, G. R. (2001). Evaluation of some binders and fat substitutes in low-fat Frankfurters. *Journal of Food Science, 66*, 1039-1046. http://dx.doi.org/10.1111/j.1365-2621.2001.tb08232.x

Zotos, A., & Bampidis, V. A. (2014). Milk fat quality of Greek buffalo (*Bubalus bubalis*). *Journal of Food Composition and Analysis, 33*, 181-186. http://dx.doi.org/10.1016/j.jfca.2013.12.004

Zotos, A., Hole, M., & Smith, G. (1995). The effect of frozen storage of Mackerel (*Scomber scombrus*) on its quality when hot smoked. *Journal of the Science of Food and Agriculture, 67*, 43-48. http://dx.doi.org/10.1002/jsfa.2740670108

Zotos, A., & Vouzanidou, M. (2012). Seasonal changes in composition, fatty acid, cholesterol and mineral content of six highly commercial fish species of Greece. *Food Science and Technology International, 18*, 139-149. http://dx.doi.org/10.1177/1082013211414785

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Physicochemical and Antioxidant Properties of *Cymbopogon citratus* Essential Oil

María del Carmen Vázquez-Briones[1], Luis Ricardo Hernández[2] & José Ángel Guerrero-Beltrán[1]

[1] Departamento de Ingeniería Química, Alimentos y Ambiental, Universidad de las Américas Puebla, Cholula, Puebla, México

[2] Departamento de Ciencias Químico Biológicas, Universidad de las Américas Puebla, Cholula, Puebla, México

Correspondence: José Ángel Guerrero-Beltrán, Departamento de Ingeniería Química, Alimentos y Ambiental, Universidad de las Américas Puebla, Cholula, Puebla 72810, México. Tel: 52-222-229-2126. E-mail: angel.guerrero@udlap.mx; joseangel150@hotmail.com

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Abstract

In this study, the essential oil (EO) of lemongrass (*Cymbopogon citratus*) or "lemon tea" leaves was studied. The EO was obtained by the steaming (0.75 ± 0.05%) and distillation (1.5 ± 0.07%), assisted by microwaves, methods. The EO had a refractive index of 1.483 ± 0.001 (20 °C) and a density of 0.873 ± 0.005 g/mL (27 °C). Color parameters of the oil corroborate the yellow hue observed by the naked eye. The Gas chromatography-Mass spectrometry (GC-MS), Fourier Transform Infrared (FT-IR) Spectrometry, and Nuclear Magnetic Resonance (NMR) techniques permitted to characterize the EO and revealed the chemical structure of the major component: citral (neral and geranial). The phenolic compounds content was 149.2 ± 6.0 mg Gallic acid equivalents (GAE) per 100 mL of oil and the antioxidant activity was 44.06 ± 0.20 mg Trolox (T) per 100 mL of essential oil.

Keywords: essential oil, *Cymbopogon citratus*, distillation, antioxidant activity, NMR, GC-MS, FT-IR

1. Introduction

Essential oils are defined as volatile substances of a complex mixture of chemical components (terpenes, monoterpenes, terpenoids, alcohols, aldehydes, and ketones) which evaporate when contact with air and are biosynthesized by plants (Parikh & Desai, 2011; Acevedo, Navarro, & Monroy, 2013). They can be obtained from different parts of plants and are generally recognized as safe (GRAS). Attention is now given to natural antimicrobial substances of plant origin since they could be a rich source of bioactive compounds (Burt, 2004; Silva, Gutierrez, Weisheimer, & Schapoval, 2008; García et al., 2008; Bakkali et al., 2008) and they might replace synthetic additives. The essential oils of basil (Zivanovic, Chi, & Draughon, 2005), garlic, cinnamon, lemongrass (Pranoto, Salokhe, & Rakshit, 2005; Xing et al., 2011; Maqbool et al., 2011; Azarakhsh et al., 2013), oregano (Dos Santos et al., 2012; Vatavali et al., 2013) and rosemary (Ponce, Roura, Del Valle, & Moreira, 2008) have been added as active chemical compounds in edible coatings.

The scientific name of lemongrass is *Cymbopogon citratus*. The *Cymbopogon* word derives from the Greek words "kymbe" (boat) and "pogon" (beard), referring to the arrangement of the spike of the flower. The word *citratus* derives from the old Latin, meaning lemon-scented leaves (Shah et al., 2011). The common name of *Cymbopogon citratus* in Mexico is "zacate limón" (lemongrass) or "té limón". It is a perennial tropical grass; is resistant to different temperatures and can grow in warm, semi-warm and temperate climates. It is from 60 to 120 centimeters high, its leaves are green, long and slats and have pleasant aroma and taste. This grass is native to India (Parikh & Desai, 2011). Because of its pleasant flavor, in Mexico is consumed as infusion in water or milk just because the herbs intake is a custom in the Mexican population (Juárez-Rosete et al., 2013). The essential oil of *Cymbopogon citratus* has shown to have anti-inflammatory, analgesic and antipyretic properties, (Gbenou et al., 2013) besides having antimicrobial effects (Hammer, Carson, & Riley, 1999; Pranoto, Salokhe, & Rakshit, 2005; Adukwu, Allen, & Phillips, 2012). Tzortzakis & Economakis (2007) reported that the essential oil of lemongrass inhibited the growth of *Botrytis cinerea*. Later Raybaudi-Massilia, Rojas-Graü, Mosqueda-Melgar, and Martín-Belloso (2008) reported that the oil of lemongrass could suppress the growth of mesophiles and psychrophiles in fresh-cut apples.

The aim of this study was to obtain and characterize the essential oil of *Cymbopogon citratus*.

2. Materials and Methods

2.1 Plant Material

Fresh *Cymbopogon citratus* leaves were acquired in the Puebla City Central Market, Puebla, Mexico.

2.2 Essential Oil Extraction

Fresh *Cymbopogon citratus* leaves were dried at room temperature for one week; leaves were extended on trays, turning them three times daily for ventilation, accelerate drying, and preventing the growth of microorganisms. The essential oil of *Cymbopogon citratus* was obtained by two methods. *Hydrodistillation*: 50 g of ground plant material was extracted for about 60 minutes (Baizabal, 2010) using a Clevenger apparatus. *Microwave assisted extraction*: 50 g of ground leaves and 200 mL of distilled water were placed in a 500 mL spherical flask; the distillation was performed for about 30 min. The distillation apparatus was placed inside of a conventional Daewoo DC (model KOR 6LYB) microwave oven. A power of 600 W was used. The yield of oil was calculated by the following equation:

$$\text{Yield (\%)} = (\text{Oil (mL)})/(\text{Plant (g)}) \times 100$$

2.3 Physicochemical Properties

2.3.1 Refraction Index

To measure the refractive index of oil, an Atago ND R5000 refractometer (Osaka, Japan) was used according to the 921.08 AOAC (2000) method.

2.3.2 Density

It was assessed according to the 985.19 AOAC (2000) method using a 2 mL pycnometer.

2.3.3 Color

The CIELAB color parameters were measured using a Colorgard 05 colorimeter (Gardner Laboratory, USA) in the transmittance mode. The equipment calibration was performed with the black mosaic and the calibration parameters: $L^* = 100$, $a^* = 0$, and $b^* = 0$. A cell of quartz and a volume of 3 mL of oil was used.

2.4 Gas Chromatography-Mass Spectroscopy

It was performed using an Agilent Technologies 6850 gas chromatograph (Santa Clara, CA, USA) coupled to an Agilent Technologies 5975 mass selective detector (Santa Clara, CA, USA). An Agilent capillary column (HP-5 ms nonpolar 5 % phenyl methyl polysiloxane) of 30 m, 0.25 mm in diameter and 0.25 microns in thickness was used. Helium, at a flow rate of 1.1 mL/min, was used as the carrier gas. The oven temperature of the gas chromatograph was maintained at 300 °C. The temperature in the column was started at 60 °C and maintained for 2 min, then increased, until reaching 250 °C, at a rate of 10 °C/min. Therefore, the final temperature was maintained for 10 min. One microliter of oil was injected into the column using a split injection of 10:1. The injector temperature was 250 °C (Conde-Hernández & Guerrero-Beltrán, 2014). The components were identified by their fragmentation patterns of mass spectra compared with data stored in the National Institute of Standards and Technology Mass Spectral database (NIST-MS, 2010).

2.5 Fourier Transform Infrared (FT-IR) Spectrometry

Spectrometry of the EO of *Cymbopogon citratus* was carried out with a Spectrum One FT-IR Spectrometer, (PerkinElmer®, Waltham, Massachusetts, USA). An EO sample was placed directly on the surface of the ATR top plate at room temperature; measurements were performed in the IR region at 4000-650 cm^{-1}. Four scans were performed at a resolution of 4.00 cm^{-1} at a scan speed of 0.20 cm/s. The crystal used was diamond SSNS. An air spectrum was used as reference (Wany et al., 2014).

2.6 Nuclear Magnetic Resonance (NMR)

NMR spectroscopic measurements were performed using a Varian Gemini 200 spectrometer (Varian Associates Inc., Palo Alto, CA, USA) (operating at 200 MHz for hydrogen) using deuterated chloroform solution containing tetramethyl silane (TMS) as internal standard (Fortuna et al., 2011).

2.7 Phenolic Compounds

The Folin & Ciocalteu method (Singleton, & Rossi, 1965) was used for determining phenolic compounds with some modifications. Fifty microliters of EO were diluted with 950 µL of ethanol. From this dilution, 500 µL were taken and added with 3 mL of distilled water and 250 µL of Folin-Ciocalteu reagent. The reaction was

stopped adding 750 µL of 20 % Na_2CO_3. Finally, the solution was brought to 5 mL with distilled water. The reaction was left in the dark for 2 hours. Samples were filtered through Whatman paper No. 4 before recording absorbances. A blank was prepared with 4 mL of distilled water, 250 µL of Folin-Ciocalteu reagent, and 750 µL of 20% Na_2CO_3. The absorbance was recorded at 765 nm using a UNICO® 2800H UV–visible spectrophotometer (Shanghai, China). Phenolic compounds were expressed in mg equivalent of Gallic acid per 100 mL of oil. The measurements were performed in triplicate. The phenolics concentration was calculated from a standard curve of Gallic acid (0-0.0672 mg): Abs = (15.03/mg)*[mg]+0.0886 (R^2 = 0.973). From a standard solution of Gallic acid (0.186 mg/mL), nine tubes were prepared to obtain concentrations of 0 to 400 µL.

2.8 Antioxidant Capacity (AC)

The method used to determine antioxidant capacity of the essential oil was adapted from Brand-Williams, Cuvelier and Berset (1995) with some modifications. *Radical solution*: it was prepared dissolving 3.94 mg of DPPH (2,2-Diphenyl-1-picrylhydrazyl) (Sigma-Aldrich, Toluca, Edomex, Mexico) in 100 mL of ethanol. *AC:* Fifty µL of EO was mixed with 950 µL of ethanol, 2 mL of DPPH radical solution, made up to 4 mL with ethanol, and thoroughly mixed. The mixture was left to react in the dark for 30 minutes. The absorbance of samples (A_s) was recorded at 517 nm in a UNICO® 2800H UV–visible spectrophotometer (Shanghai, China). A blank (A_b) was prepared using the same procedure substituting only the sample for ethanol. The percentage of inhibition (I) was calculated as follow:

$I = ((A_b\text{-}A_s)/A_b)*100$

The antioxidant capacity (mg T/100 mL of EO) was calculated from a standard curve of Trolox (0-0.028 mg): I = (2699.2/mg)*[mg]-4.3749 (R^2 = 0.989). To prepare the calibration curve a standard solution of Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, 97 %) was prepared (0.35 mg/mL) in ethanol. Eight tubes were used to obtain concentrations in the range 0 to 0.028 mg of Trolox.

2.9 Statistic Analysis

An analysis of variance and Tukey test were performed to state differences between means using the Minitab 17 software (LEAD Technologies Inc., NJ). A P value of 0.05 was considered statistically significant.

3. Results and Discussion

3.1 Essential Oil Yield

Yields of 0.752 ± 0.05 and 1.5 ± 0.07% (V/W) of EO were obtained by the steam and microwave assisted distillation methods, respectively. Differences ($p < 0.05$) within means of yields were observed. Very probably the core of ground plant placed in the spherical flask was efficiently heated due to microwaves. As observed in other works, when using a simple distillation procedure, sometimes the steam do not reach the center of the ground plant; therefore, the yield of the oil extraction is not optimized. The moisture content of the dried *C. citratus* was 9.14 ± 0.70%.

3.2 Physical Properties

The values of refractive index, color, and density are shown in Table 1. These values are close to those reported by Tovar et al. (2011) (ρ = 0.885 g/mL; IR = 1.482), Paviani, Pergher, and Dariva (2006) (ρ = 0.848 g/mL), Essien, Essien, Ita, & Ebong (2008) (ρ = 0.888 g/mL; IR = 1.477), Ibrahim, Ibo and Adejare (2010) (ρ = 0.866 g/mL; IR = 1.472), and Monteiro et al. (2011) (ρ = 0.949 g/mL; IR = 1.332) who reported similar refractive index and specific gravity values for essential oils of lemongrass, lemongrass, citron (*Citrus medica* L.), ginger, and allspice (*Pimenta dioica*), respectively, obtained by hydrodistillation. Color parameters indicate a yellow hue (68.81 ± 0.36) which was observed in the EO. Tovar et al. (2011) reported high values for L^* (98.59 to 101.63) but similar values for a^* (-2.45), and b^* (6.28) color parameters for the *Cymbopogon citratus* EO.

Table 1. Refractive index, density, and color parameters of dried *C. citratus* EO obtained by distillation assisted by microwaves

Characteristic		Value
Refraction index (20 °C)		1.483 ± 0.001
Density (g/mL) (27 °C)		0.873 ± 0.005
Color parameters	*L**	97.04 ± 0.25
	*a**	-2.44 ± 0.04
	*b**	6.29 ± 0.12

3.3 Chemical Compounds by GC-MS

Tables 2 and 3 list the main components of the EO of *C. citratus* revealed by the GC-MS technique.

Table 2. Chemical compounds of essential oil of dried *C. citratus* obtained by steam distillation

Chemical	Area (%)
Cis-linalool oxide	0.7
Linalool	0.64
2-hydroxy-1, 1, 10-trimethyl-6,9-decalin epidioxi	8.09
Oxirane methanol, 3-methil-3-(4-methyl-3-pentenyl)	28.4
Dihydronopol	2.52
Neral	19.35
Geranial	15.97
Geranic acid	5.76
Neric acid	9.15
2-tridecanone	1.21
7-Methyl-z-tetradecen-1-ol- acetate	2.37
1-allyl-2-hydroxy-6-methyl-cyclohexanecarboxylic acid	1.1
9-methyl-z10-tetradecen-1-ol-acetate	4
Cholestan-3-ol, 2-methylene (3β, 5α)	0.3
Ethyl-linoleate	0.43

Table 3. Chemical compounds of essential oil of dried *C. citratus* obtained by distillation assisted by microwaves

Chemical	Area (%)
3-Methyl-2-butenal	0.16
Nerol	1
Limonene	0.46
Citronellal	0.21
2-cyclohexen-1-one	0.33
Cis-linalool oxide	0.4
Linalool	0.86
Neral	29
Geranial	22.63
Methyl acetate	2.56
Oxirane carboxaldehyde, 3-methyl-3-(4-methyl-3-pentenyl)	25.29
Cis-pulegone oxide	3.25
Neric acid	9.19
Carotol	0.88
7-methyl-z-tetradecen-1-ol-acetate	0.25

The predominant component of the essential oil of *C. citratus* obtained by the two distillation methods of extraction was citral (mixture of geranial and neral aldehydes) (Figure 1). These results agree with those reported by Mahanta et al. (2007) and Negrelle and Gomes (2007). Adukwu, Allen, and Phillips (2012) and Andrade et al. (2012) informed that citral was the main component in *C. flexuosusu* and *C. nardus*, respectively. The highest percentage (51.63%) of citral in the EO obtained by the microwave assisted distillation method is close to that reported by León-Anzueto et al. (2011) (59.7 to 64.0%) in *C. citratus* (DC.) Stapf. Chanthai, Prachakoll, Ruangviriyachai, and Luthria (2012) reported that the plant has a content of citral of 65 to 80%. Mohamed Hanaa, Sallam, El-Leithy, and Aly (2012) reported concentrations of geranial (31.53%, 39.86% and 37.24%), neral (30.08%, 34.52% and 31.28%) and myrcene (16.61%, 14.49% and 15.42%) in essential oils extracted from leaves of *Cymbopogon citratus*, dried in the sun, in the shade, and in oven, respectively. The differences in type of components reported by other researchers is probably due to the climatic conditions where the plant was grown. The quality of *Cymbopogon citratus* is determined by the content of citral. It has been observed that citral has an inhibitory effect on bacteria and fungi (Adinarayana et al., 2012; Marques et al., 2013).

Geranial, α-citral Neral, β-citral

Figure 1. Chemical structures of geranial and neral

3.4 Infrared Spectrum

As mentioned above, the main component of the *C. citratus* EO is citral. In the IR spectrum (Figure 2) of EO of *C. citratus*, the functional groups of citral were observed. In the vibrations at 2968 cm^{-1}, a predominant asymmetric stretching of $-CH_3$ is observed corresponding to an alkyl saturated aliphatic group and at 2915 and 2857 cm^{-1}, symmetric and asymmetric stretching of $-CH_2$ are observed. The intense band observed at 1671 cm^{-1} is due to vibrations of C=C (cis and trans), confirming the presence of conjugated double bonds (C = C-CHO) in citral which are common in acyclic monoterpenes. The peak at 1632 cm^{-1} indicates stretching of C=O of the aldehyde group. At the 1442 cm^{-1} peak, bending of the $-CH_2$ group is observed. At 1377 cm^{-1} bending of $-CH_3$ group is observed. From 1194 to 1120 cm^{-1}, stretching of -C-O and vibrations of the -CH skeleton are observed. At 841 cm^{-1}, substitution in 1,3 and 1,4 are observed. Similar peaks were reported by Wany et al. (2014) for geranial from other species of *Cymbopogon winterianus* (citronella grass).

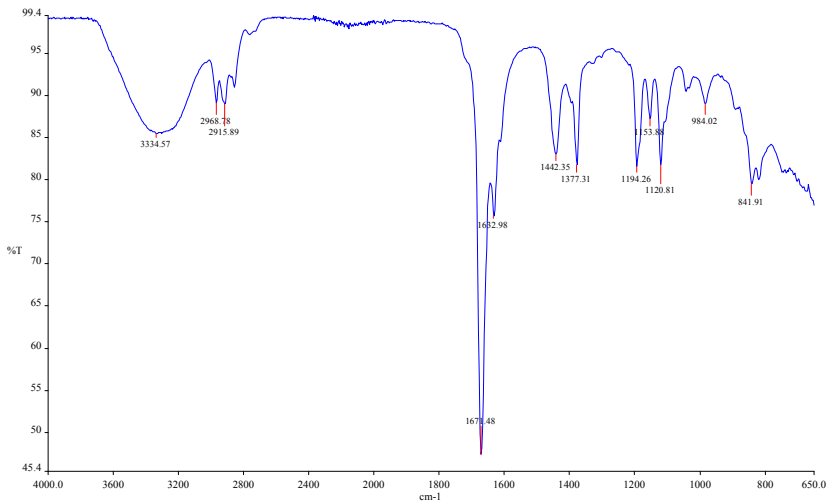


Figure 2. Infrared spectrum of *Cymbopogon citratus* essential oil

The IR spectrum of *C. citratus* EO is consistent with data obtained from gas chromatography coupled to mass spectroscopy, IR shows principally the citral spectrum (Mahanta et al., 2007; Marques et al., 2013) which is the main component in the mixture of chemicals in this oil. In addition, the IR spectrum shows absorption at 3334 cm^{-1}, characteristic of the -OH group which is also in agreement with results of the GC-MS analysis where the presence of nerol and other alcohols in small proportions were observed.

3.5 Nuclear Magnetic Resonance (NMR)

The NMR analysis allowed the characterization and structural elucidation of the main component in the essential oil of *C. citratus*. The NMR spectra of hydrogen (1 H) (Figure 3) and carbon-13 (C 13) (Figure 4) clearly show that the EO of *C. citratus* is composed of citral (Restrepo, Vinasco, Jaramillo, & Colmenares, 2009), where the two typical signals of the group aldehyde correspond to geranial and neral in proportions of 61 and 39%, respectively.

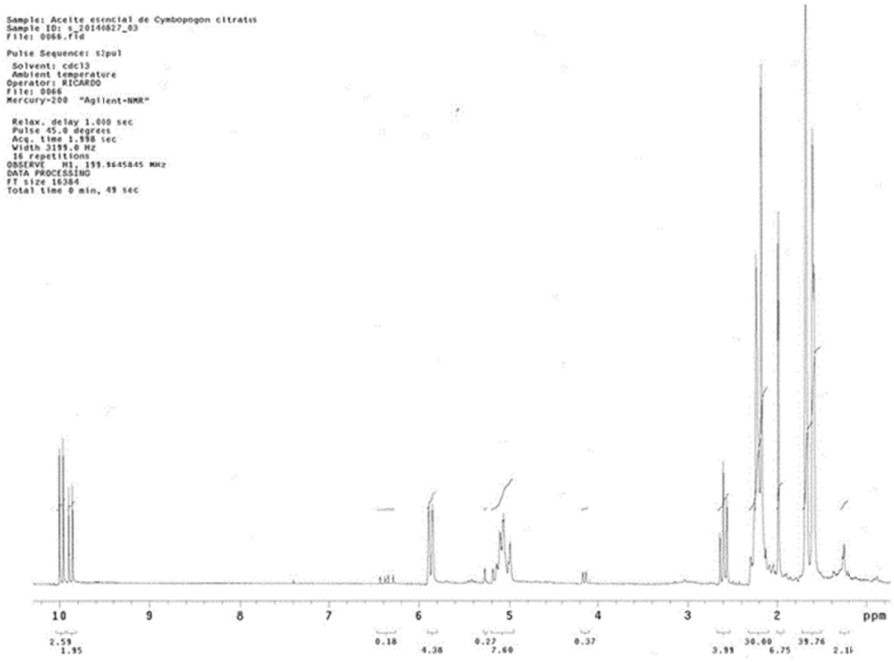
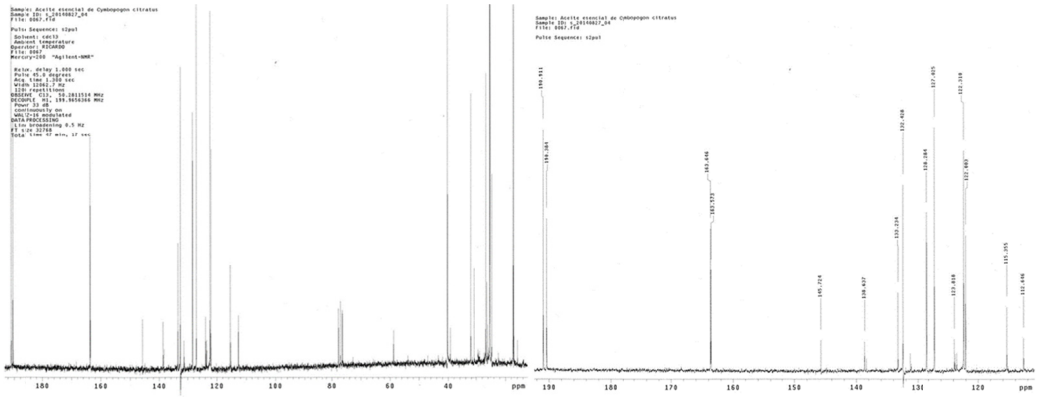
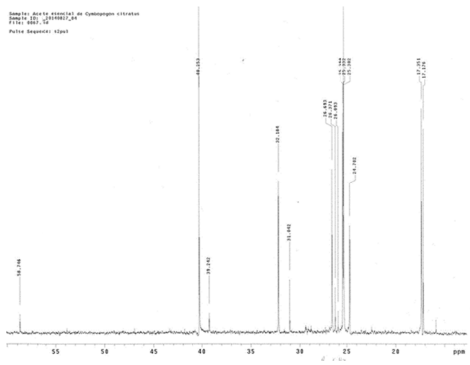


Figure 3. 1 H NMR spectrum of *Cymbopogon citratus* essential oil



A B



C

Figure 4. 13 C NMR spectrum of *Cymbopogon citratus* essential oil

3.6 Phenolic Compounds

Fruits and vegetables containing phenolic compounds may have high antioxidant activity, which means that they could have positive effects on the preservation of foods quality and human health (Becker, Nissen, & Skibsted, 2004). Some studies have shown that phenolic compounds having antioxidant activity may retard aging, prevent degenerative diseases such as cancer, cardiovascular disorders, and brain dysfunctions (Ames, Shigenaga, & Hagen, 1993). In this study, a phenolic content of 149.2 ± 6.0 mg GAE per 100 mL of oil (obtained by distillation assisted by microwaves) was obtained. Koh, Mohd, Mokhtar, & Iqbal (2012) reported a phenolic content of 30.74 ± 1.13 mg GAE per g of sample in ethanol extracts of *Cymbopogon citratus*. Mirghani, Liyana, and Parveen (2012) reported 184.69 mg of GAE per 100 mL of EO of *Cymbopogon citratus* lives. Several factors can affect the content of phenolic compounds in plants: preparation (drying time, temperature, among others) and growing conditions of the plant as well as the extracting method and the technique of analysis (Moraes-de-Souza et al., 2008).

3.7 Antioxidant Capacity

The essential oil of *C. citratus* had an antioxidant capacity of 44.06 ± 0.20 mg Trolox per 100 mL of essential oil (obtained by distillation assisted by microwaves), equivalent to 55.57% of inhibition. Selim (2011) reported values of 40.63 to 63.75% inhibition for *Cymbopogon proximus* (Stapf). Mirghani, Liyana, and Parveen (2012) reported higher values of inhibition (78.89%) when diluting the EO at 1:2 oil:methanol proportion. The differences in the antioxidant capacity values, reported by different researchers, can be attributed to factors such as climate, soil composition, and season as well as part, age and stage of growing of the plant (Angioni et al., 2006) in addition to the EO or extract concentration (Mirghani, Liyana, & Parveen, 2012). The antioxidant activity of the EO of *C. citratus* could be of great interest in the food industry to be used as a natural additive for flavoring.

4. Conclusions

The distillation, assisted by microwaves, was the best method for extracting the EO. The physicochemical properties of the EO of *C. citratus* were determined. The EO of *C. citratus* had high citral (a mixture of geranial and neral aldehydes) concentration which was corroborated by different instrumental techniques (GC-MS, FT-IR, and NMR). The EO showed high phenolics content (149.20 mg GAE per 100 mL) and antioxidant capacity (44.06 mg Trolox per 100 mL of essential oil).

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References

AOAC. (2000). *Official Methods of Analysis of the AOAC International*. (2000). 7th Edition, Volume II, Gaithersburg, Maryland, USA. Chapter 41, pp. 2-4.

Acevedo, D., Navarro, M., & Monroy, L. (2013). Composición química del aceite esencial de hojas de orégano (*Origanum vulgare*). *Información Tecnológica, 24*(4), 43-48. http://dx.doi.org/10.4067/S0718-07642013000400005

Adinarayana, G., Rahul, G., Kiran, R. S., Syamsundar, K. V., & Rajeswara, B. R. (2012). Evaluation of antimicrobial potential of field distilled and water-soluble essential oils of *Cymbopogon flexuosus*. *Journal of Pharmacognosy, 3*(2), 142-146.

Adukwu, E. C., Allen, S. C. H., & Phillips, C. A. (2012). The anti-biofilm activity of lemongrass (*Cymbopogon flexuosus*) and grapefruit (*Citrus paradisi*) essential oils against five strains of *Staphylococcus aureus*. *Journal of Applied Microbiology, 113*, 1217-1227. http://dx.doi.org/10.1111/j.1365-2672.2012.05418.x

Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences of the United States of America, 90*, 7915-7922. http://dx.doi.org/10.1073/pnas.90.17.7915

Andrade, M. A., Cardoso, M. G., Batista, L. R., Mallet, A. C. T., & Machado, S. M. F. (2012). Óleos essenciais de *Cymbopogon nardus*, *Cinnamomum zeylanicum* e *Zingiber officinale*: composição, actividades antioxidante e antibacteriana. *Revista Ciência Agronômica, 43*(2), 399-408. http://dx.doi.org/10.1590/S1806-66902012000200025

Angioni, A., Barra, A., Coroneo, V., Dessi, S., & Cabras, P. (2006). Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. *stoechas* essential oils from stem/leaves and flowers. *Journal of Agricultural and Food Chemistry, 54*, 4364-4370. http://dx.doi.org/10.1021/jf0603329

Azarakhsh, N., Osman, A., Ghazali, H. M., Tan, C. P., & Mohd Adzahan, N. (2013). Lemon grass essential oil incorporated into alginate-based edible coating for shelf-life extension and quality retention of fresh-cut pineapple. *Postharvest Biology and Technology, 88*, 1-7. http://dx.doi.org/10.1016/j.postharvbio.2013.09.004

Baizabal, C. R. H. (2010). *Evaluación de la capacidad antioxidante y antimicrobiana del aceite esencial y del polvo de romero* (*Rosmarinus officinalis* L.) *en queso fresco de vaca*. Bachelor Thesis. Universidad de las Américas Puebla. Puebla, Mexico.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils – a review. *Food and Chemical Toxicology, 46*, 446-475. http://dx.doi.org/10.1016/j.fct.2007.09.106

Becker, E. M., Nissen, L. R., & Skibsted, L. H. (2004). Antioxidant evaluation protocols: food quality or health effects. *European Food Research and Technology, 219*, 561-571. http://dx.doi.org/10.1007/s00217-004-1012-4

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology, 28*(1), 25-30. http://dx.doi.org/10.1016/S0023-6438(95)80008-5

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods a review. *International Journal of Food Microbiology, 94*, 223-253. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.03.022

Chanthai, S., Prachakoll, S., Ruangviriyachai, C., & Luthria, D. L. (2012). Influence of extraction methodologies on the analysis of five major volatile aromatic compounds of citronella grass (*Cymbopogon nardus*) and lemongrass (*Cymbopogon citratus*) grown in Thailand. *Journal of AOAC International, 95*(3), 763-772. http://dx.doi.org/10.5740/jaoacint.11-335

Conde-Hernández, L. A., & Guerrero-Beltrán, J. Á. (2014). Total phenolics and antioxidant activity of *Piper auritum* and *Porophyllum ruderale*. *Food Chemistry 142*, 455-460. http://dx.doi.org/10.1016/j.foodchem.2013.07.078

Dos Santos, N. S. T., Athayde, A. J. A., De Oliveira, C. E. V., Veríssimo, C., De Melo, S., Sousa, R., ... De Souza, E. L. (2012). Efficacy of the application of a coating composed of chitosan and *Origanum vulgare* L. essential oil to control *Rhizopus stolonifer* and *Aspergillus niger* in grapes (*Vitis labrusca* L.). *Food Microbiology, 32*, 345-353. http://dx.doi.org/10.1016/j.fm.2012.07.014

Essien, E., Essien, J., Ita, B., & Ebong, G. (2008). Physicochemical properties and fungitoxicity of the essential oil of *Citrus medical* L. against groundnut storage fungi. *Turkish Journal of Botany, 32*(2), 161-164.

Fortuna, A. M., Juarez, Z. N., Bach, H., Nematallah, A., Av-Gay, Y., Sánchez-Arreola, E., ... Hernández, L. R. (2011). Antimicrobial activities of sesquiterpene lactones and inositol derivatives from *Hymenoxys robusta*. *Phytochemestry, 72*, 2413-2418. http://dx.doi.org/10.1016/j.phytochem.2011.09.001

García, R., Alves, E. S. S., Santos, M. P., Aquije, G. M. F. V., Fernández, A. A. R., Santos, R. B., ... Fernández, P.

M. V. (2008). Antimicrobial activity and potential use of monoterpenes as tropical fruits preservatives. *Brazilian Journal of Microbiology, 39*, 163-168. http://dx.doi.org/10.1590/S1517-83822008000100032

Hammer, K. A., Carson, C. F., & Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology, 86*, 985-990. http://dx.doi.org/10.1046/j.1365-2672.1999.00780.x

Ibrahim, T. A., Ibo, D., & Adejare, A. R. (2010). Comparative phytochemical properties of crude ethanolic extracts and physicochemical characteristics of essential oils of *Myristical fragrans* (nutmeg) seeds and *Zingiber officinate* (ginger) roots. *Electronic Journal of Environmental, Agricultural and Food Chemistry, 9*(6), 1110-1116.

Juárez-Rosete, C. R., Aguilar-Castillo, J. A., Juárez-Rosete, M, E., Bugarín-Montoya, R., Juárez-López, P., & Cruz, C. E. (2013). Hierbas aromáticas y medicinales en México: tradición e innovación. *Revista Bio Ciencias, 2*(3), 119-129.

Koh, P. H., Mohd, R. A., Mokhtar, & Iqbal. M. (2012). Antioxidant potential of *Cymbopogon citratus* extract: alleviation of carbon tetrachloride-induced hepatic oxidative stress and toxicity. *Human and Experimental Toxicology, 31*(1) 81-91. http://dx.doi.org/10.1177/0960327111407226

León-Anzueto, E., Abud-Archila, M., Dendooven, L., Ventura-Canseco, L. M. C., & Gutiérrez-Miceli, F. A. (2011). Effect of vermicompost, worm-bed leachate and arbuscular mycorrizal fungi on lemongrass (*Cymbopogon citratus* (DC) Stapf.) growth and composition of its essential oil. *Electronic Journal of Biotechnology, 14*(6), 1-11.

Mahanta, J. J., Chutia, M. Bordoloi, M., Pathak, M. G. Adhikary, R. K., & Sarma, T. C. (2007). *Cymbopogon citratus* L. essential oil as a potential antifungal agent against key weed moulds of *Pleurotus* spp. Spawns. *Flavour and Fragrance Journal, 22*, 525-530. http://dx.doi.org/10.1002/ffj.1835

Maqbool, M., Ali, A., Alderson, P. G., Mohamed, M. T. M., Siddiqui, Y., & Zahid, N. (2011). Postharvest application of gum Arabic and essential oils for controlling anthracnose and quality of banana and papaya during cold storage. *Postharvest Biology and Technology, 62*, 71-76. http://dx.doi.org/10.1016/j.postharvbio.2011.04.002

Marques. A. M., Lima, C. H. P., Alviano, D. S., Alviano, C. S., Esteves, R. L., & Kaplan, M. A. C. (2013). Traditional use, chemical composition and antimicrobial activity of *Pectis brevipedunculata* essential oil: A correlated lemongrass species in Brazil. *Journal of Food and Agriculture, 25*(10), 798-808.

Mirghani, M. E. S., Liyana, Y., & Parveen, J. (2012). Bioactivity analysis of lemongrass (*Cymbopogan citratus*) essential oil. *International Food Research Journal, 19*(2), 569-575.

Mohamed Hanaa, A. R. Sallam, Y. I., El-Leithy, A. S., & Aly, S. E. (2012). Lemongrass (*Cymbopogon citratus*) essential oil as affected by drying methods. *Annals of Agricultural Science, 57*(2), 113-116. http://dx.doi.org/10.1016/j.aoas.2012.08.004

Monteiro, O. O., Souza, A. A., Soledade, L. L., Queiroz, N. N., Mouchrek Filho, V. V., & Vasconcelos, A. A. (2011). Chemical evaluation and thermal analysis of the essential oil from the fruits of the vegetable species *Pimenta dioica* Lindl. *Journal of Thermal Analysis & Calorimetry, 106*(2), 595-600. http://dx.doi.org/10.1007/s10973-011-1438-4

Moraes-de-Souza, R. A., Oldoni, T. L. C., Regitano-d'Arce, M. A. B., & Alencar, S. M. (2008). Actividad antioxidante y compuestos fenólicos en infusiones herbarias consumidas en Brasil. *Ciencia y Tecnología Alimentaria (Brasil), 6*(1), 41-47. http://dx.doi.org/10.1080/11358120809487626

National Institute of Standards and Technology (NIST). (2010). *Automated mass spectral library with search program* (Data version: NIST11, Version 2.0).

Negrelle, R. R. B., & Gomes, E. C. (2007). *Cymbopogon citratus* (DC.) Stapf: chemical composition and biological activities. *Revista Brasileira de Plantas Medicinais Botucatu (Brasil), 9*(1), 80-92.

Parikh, J. K., & Desai, M. A. (2011). Hydrodistillation of essential oil from *Cymbopogon flexuosus*. *International Journal of Food Engineering, 7*(1), 1-9. http://dx.doi.org/10.2202/1556-3758.2067

Paviani, L., Pergher, S. B. C., & Dariva, C. (2006). Application of molecular sieves in the fractionation of lemongrass oil from high-pressure carbon dioxide extraction. *Brazilian Journal of Chemical Engineering, 23*, 219-225. http://dx.doi.org/10.1590/S0104-66322006000200009

Ponce, A. G., Roura, S. I., Del Valle, C. E., & Moreira, M. R. (2008). Antimicrobial and antioxidant activities of

edible coating enriched with natural plant extracts: in vitro and in vivo studies. *Postharvest and Biology Technology, 49*, 294-300. http://dx.doi.org/10.1016/j.postharvbio.2008.02.013

Pranoto, Y., Salokhe, V. M., & Rakshit, S. K. (2005). Physical and antimicrobial properties of alginate-based edible film incorporated with garlic oil. *Food Research International, 38*, 267-272. http://dx.doi.org/10.1016/j.foodres.2004.04.009

Raybaudi-Massilia, R. M., Rojas-Graü, M. A., Mosqueda-Melgar, J., & Martín-Belloso, O. (2008). Comparative study on essential oils incorporated into an alginate-based edible coating to assure the safety and quality of fresh-cut Fuji apples. *Journal of Food Protection, 71*, 1150-1161.

Restrepo, J., Vinasco, L. E., Jaramillo, L. P., & Colmenares, A. J. (2009). Encapsulamiento de los aceites esenciales de citral (*Cymbopogon citratus*) en β-ciclodextrinas usando CO_2 supercrítico. *Ingeniería y Competitividad, 11*(2), 9-19.

Selim, S. A. (2011). Chemical composition, antioxidant and antimicrobial activity of the essential oil and methanol extract of the Egyptian lemongrass *Cymbopogon proximus* Stapf. *Grasas y aceites, 62*(1), 55-61. http://dx.doi.org/10.3989/gya.033810

Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., & Mann, A. S. (2011). Scientific basis for the therapeutic use of *Cymbopogon citratus*, Stapf (Lemon grass). *Journal of Advanced Pharmaceutical Technology and Research, 2*, 3-8. http://dx.doi.org/10.4103/2231-4040.79796

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*(3), 144-158.

Silva, C. B., Gutierrez, S. S., Weisheimer, V., & Schapoval, E. S. S. (2008). Antifungal activity of the lemongrass oil and citral against Candida spp. *Brazilian Journal of Infectious Diseases, 2*(1), 63-66.

Tovar, L. P., Pinto, G. M. F., Wolf-Maciel, M. R., Batistella, C. B., & Maciel-Filho, R. (2011). Short-path-distillation process of lemongrass essential oil: physicochemical characterization and assessment quality of the distillate and the residue products. *Industrial & Engineering Chemistry Research, 50*(13), 8185-8194. http://dx.doi.org/10.1021/ie101503n

Tzortzakis, N. G., & Economakis, C. D. (2007). Antifungal activity of lemongrass (*Cymbopogon citratus* L.) essential oil against key postharvest pathogens. *Innovative Food Science and Emerging Technology, 8*, 253-258. http://dx.doi.org/10.1016/j.ifset.2007.01.002

Vatavali, K., Karakosta, L., Nathanailides, C., Georgantelis, D., & Kontominas, M. G. (2013). Combined effect of chitosan and oregano essential oil dip on the microbiological, chemical, and sensory attributes of red porgy (*Pagrus pagrus*) stored in ice. *Food and Bioprocess Technology, 6*, 3510-352. http://dx.doi.org/10.1007/s11947-012-1034-z

Wany, A., Kumar, A., Nallapeta, S., Jha, S., Nigam, V. K., & Pandey, D. M. (2014). Extraction and characterization of essential oil components based on geraniol and citronellol from Java citronella (*Cymbopogon winterianus* Jowitt). *Journal of Plant Growth Regulation, 73*, 133-145. http://dx.doi.org/10.1007/s10725-013-9875-7

Xing, Y., Li, X., Xu, Q., Yun, J., Lu, Y., & Tang, Y. (2011). Effects of chitosan coating enriched with cinnamon oil on qualitative properties of sweet pepper (*Capsicum annuum* L.). *Food Chemistry, 124*, 1443-1450. http://dx.doi.org/10.1016/j.foodchem.2010.07.105

Zivanovic, S., Chi, S., & Draughon, A. F. (2005). Antimicrobial activity of chitosan films enriched with essential oils. *Journal of Food Science, 70*, M45-M51. http://dx.doi.org/10.1111/j.1365-2621.2005.tb09045.x

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Valuing Preferences for Environmental Sustainability in Fruit Production by United Kingdom and Japanese Consumers

Peter R. Tait[1], Caroline Saunders[1] & Meike Guenther[1]

[1] Agribusiness and Economics Research Unit, Lincoln University, Canterbury, New Zealand

Correspondence: Peter R. Tait, Agribusiness and Economics Research Unit, Lincoln University, PO Box 85084, Lincoln 7647, Canterbury, New Zealand. Tel: 64-3-423-0384. E-mail: peter.tait@lincoln.ac.nz

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Abstract

Reduction of carbon intensity of high volume grocery products is potentially a major contributor in meeting climate targets. In a choice experiment concerning fruit purchase decisions in the United Kingdom and Japan, this study estimates consumer willingness to pay for sustainability attributes of production alongside vitamin content, including water use efficiency, waste and packaging, and carbon emissions. Results indicate that sustainability attributes significantly influence consumers' fruit purchase decisions. Preferences are found to be very similar between countries, with reduction of carbon emissions the most valued sustainability attribute by both UK and Japanese consumers and increased vitamin content the least. This study's findings provide implications for carbon emission labeling development in the context of international food supply chains, and primary sector strategy encouraging initiatives to improve environmental performance domestically.

Keywords: carbon labelling, food sustainability, choice experiment, willingness to pay, cross-country comparison

1. Introduction

Changes in consumer demands in many primary sector markets are constantly driving changes in the value chains that primary industries participate in. There is an increasing expectation that products have environmental sustainability credentials in production (Guenther, Saunders, & Tait, 2012) such as information about climate change impacts (Rousseau & Vranken, 2013). Groceries account for about a third of total environmental impact and emissions arising from European Union countries, making reduction of carbon intensity of high volume grocery products a potential major contributor in meeting climate targets (Upham, Dendlar, & Bleda, 2011). Understanding the importance of carbon labelling in consumer choices and how it relates to other environmental attributes is crucial to determining potential efficacy of contributing to reductions. The potential for carbon labelling of food products to change consumer behaviour has been recognised (Vanclay et al., 2011; Cohen & Vandenbergh, 2012) and the practice of carbon labelling is likely to grow in importance (Roos & Tjarnemo, 2011). While some attention has been focused on estimating monetary values of consumer preferences for changes in carbon emissions levels of food products (Caputo Nayga & Scarpa, 2013) including fruit (Aoki & Akai, 2013; Onozaka & McFadden, 2011) meat products (Koistinen et al., 2013) and non-food products including flowers (Michaud, Llerena, & Joly, 2013; and air travel (Mackerron, Egerton, Gaskell, Parpia, & Mourato, 2009) the literature is scarce relative to that for other credence attributes such as organics or food safety. This study is motivated by a need to improve understanding of the relative importance of multiple environmental sustainability attributes of primary sector fruit production including carbon emissions reductions. The primary objective is to analyse the potential role of carbon labelling of fruit in consumers purchase behaviour as a tool in the formation of effective climate change strategies. Identifying consumer demand could contribute to incentivising adoption of carbon reduction strategies. Current strategies include adaptation of traditional processes (van Rikxoort, Schroth, Läderach, & Rodríguez-Sánchez, 2014) organic systems (Aguilera, Guzmán, & Alonso, 2014) localisation of consumption (Cleveland et al., 2011) and reduction in waste (Svanes & Aronsson, 2013).

This study estimates consumer values for carbon labelling of fruit in both the United Kingdom (UK) and Japan, where carbon labelling schemes are currently applied to food products. While carbon labelling schemes have been launched in several countries (Guenther et al., 2012) we chose the UK and Japan as representing early

adopters of carbon labelling schemes, having culturally diverse populations, and with significant demand for fruit products. Consumer demand for carbon labelling in the UK has been found to be relatively strong (Gadema & Oglethorpe, 2012). The UK Carbon Trust introduced one of the world's first carbon labels, the Carbon Reduction Label, in 2006 with the proviso that products bearing the label have to reduce emissions associated with producing the product by 20% over two years following certification otherwise they risk losing the right of use the label. In Japan, consumer demand for carbon labelling has also been recognised (Aoki & Akai, 2013). Japan introduced a carbon labelling scheme in 2008 with retailers voluntarily attaching a Carbon Footprint Label to their products providing detailed breakdowns of each products carbon footprint (Ministry of Economy Trade and Industry [METI], 2010). There is a recognition that consumers from different countries may respond differently to the same environmental attribute with willingness to pay (WTP), especially for socially responsible and origin-based food products, dependent in part on the culture and traditions of countries' consumers (McCluskey & Loureiro, 2003). Considering the possible implications for collaborative climate mitigation policy, and specialization of export strategies, there is surprisingly scant literature providing direct cross-country comparisons of consumer preferences for sustainability attributes of food products (Basu & Hicks, 2008; Tonsor, Schroeder, Pennings, & Mintert, 2009).

The economic valuation literature contains many applications of symbol and icon type eco-labelled products that signal attainment of a certain level of environmental performance, such as the often cited exemplar of the Nordic Swan (Bjorner, Hansen & Russell, 2004). Typical symbol and icon-type labels are defined by a generally simple presentation format intended to communicate single criteria (Jaffry, Pickering, Ghulam, Whitmarsh, & Wattage, 2004; McCluskey & Loureiro, 2003). Despite their popularity, there is growing recognition of the limitations of symbol and icon type eco-label formats. Due to their inherent simplicity they could be considered inadequate in addressing the complex information requirements of emerging consumer preferences (Czamezki, 2011). Problems can emerge concerning the divergence of simplicity in presentation with the complexity of preferences. A significant dispute concerns the risk that overly simplistic labelling schemes may lead to potentially misleading environmental evaluations by consumers, in a consequence known as the halo effect (Andrews, Burton, & Kees, 2011). This means that consumers may generalise that the product is more favourable on other environmental elements not explicitly identified. The implication for environmental sustainability labelling suggests the use of a multiattribute labelling format rather than an overly simplistic symbol or icon type format. Multiattribute labels would allow consumers to identify and express preferences over individual environmental outcomes previously aggregated into a single metric. Moreover, valuation studies employing icon-based label designs to estimate consumer WTP are not able to determine the value of preferences for changes in individual component measures of an eco-label standard.

The paper has two main objectives. The first is to advance understanding of the relative importance to individual consumers of minimising carbon emissions compared with other environmental sustainability attributes, water use efficiency, and reduction of waste and packaging. The second is to provide a direct cross-country comparison extending understanding of similarities and differences in consumer preferences for credence attributes in culturally diverse countries. By employing a choice experiment approach comprising of multiple environmental sustainability measures of food presented simultaneously, this study is able to provide more detailed information estimating consumer WTP and trade-offs over a range of environmental measures. There is some evidence suggesting that consumers' food purchase decisions may be primarily driven by private benefits such as enhanced health outcomes, rather than from public benefits typically associated with environmental goods (Rousseau & Vranken, 2013). With this in mind we include vitamin content as a fruit attribute in the choice experiment. This allows for investigation of the relative importance placed by consumers on private versus public benefits.

The paper proceeds by presenting next the choice experiment method employed to estimate consumers WTP. Section 3 then details survey development and implementation. Model estimation results are presented and WTP is calculated and discussed in Section 4. The paper concludes with implications.

2. Choice Modelling Method

Consumer markets that would allow for identification of relative importance between environmental attributes such as carbon emissions and water use efficiency are absent. This analysis employs the stated preference method of survey based choice experiments to collect information on consumers' fruit preferences. There is an established literature of application to food in Japan (Aizaki, Nanseki, & Zhou, 2013; Hu, Chen, & Yoshida, 2006; Iwamoto, Yamamoto, Sato & Sawada, 2003; Managi, Tamamoto, Iwamoto, & Masuda, 2008) and the UK (Bitzios, Fraser, & Haddock-Fraser, 2011; Balcombe, Fraser, & Di Falco, 2010; Jaffrey et al., 2004). In this study, alternative fruit options are described by the environmental impacts of production, vitamin content and price.

Consumers are asked to indicate their preferred alternative in each scenario. The observed choice and associated attribute levels of each alternative are modelled in a probabilistic econometric framework using Random Utility Models (RUM) underpinned by the theory of choice behaviour known as Random Utility Theory (McFadden, 1974; Ben-Akiva & Lerman, 1985). In this way, choice experiments provide a utility theoretic measure of preferences over various product characteristics.

The RUM can be made operational by formulising the relationship of an individual's utility function as follows:

$$U_{ni} = \beta_{0,n} + \sum \beta_k x_{ni} + \varepsilon_{ni} \quad (1)$$

Where, U_{ni} is the measure of utility from alternative i for individual n and it is a function of constant variable β_0, the sum of the utilities for each k attribute where β_k is the utility weight to be estimated and x is a vector of observed parameters, and ε_{ni} is an unobserved error term which is randomly distributed. The random component allows analysts to express consumer choice in probabilistic terms that enables the underlying preferences for attributes to be extracted.

$$P_{(ni|A)} = \Pr(U_{ni} > U_{nj}) \qquad i, j \in A \text{ and } i \neq j \quad (2)$$

Where the probability of choosing alternative i in choice set A ($P_{(ni|A)}$) is commensurate with the probability that the utility U_{ni} is greater than the utility of the other alternatives U_{nj} in A. Assuming that the error term is distributed independently and identically (IID) with extreme value type I, results in the multinomial logit (MNL) model (McFadden, 1974). A more flexible alternative is the Random Parameter Logit (RPL) model which represents a full relaxation of the IID assumption, accommodates correlations among panel observations and accounts for uncontrolled heterogeneity in tastes across respondents (Train, 2009). Preference heterogeneity is introduced in the individual specific random parameters for attributes (Greene & Hensher, 2007; Train, 2009). The parameter vector can now be expressed as the population mean β and the individual specific deviation η_n from a specified continuous distribution (Train, 2009). Hence the utility function can be rewritten as:

$$U_n = \beta X_n + \eta_n X_n + \varepsilon_n \quad (3)$$

The stochastic part of utility may now be correlated among alternatives and across the sequence of choices via the common influence of η_n (Hensher & Greene, 2003). The choice probability resulting from this specification does not have a closed form solution and requires estimation by simulated Maximum Likelihood (ML). The ML algorithm searches for a solution by simulating draws from distributions with given means and standard deviations. Probabilities can then be calculated by integrating the joint simulated distribution (the mixture distribution of the IID distribution of ε_n and the specified distribution for η_n). The preferred model specifications used here assume all randomly specified parameters are normally distributed allowing for both positive and negative preferences. WTP of fruit attribute j by consumer i is calculated as the ratio of the estimated model parameters accommodating the influence of the random component (Cicia, Cembalo, Del Giudice, & Scarpa, 2013) as:

$$WTP_i^j = -\left(\frac{\beta_j + \varepsilon_{ij}}{\beta_{price} + \varepsilon_{ip}} \right) \quad (4)$$

3. Survey Development

In order to explore possible attributes to be valued in the choice experiment, literature review was accompanied by focus groups with the general public, and interviews with key fruit industry stakeholders. Two focus groups consisting of 12 participants each were recruited by a professional marketing research company. Focus group participants were chosen based on their prominent role in household shopping and were selected from middle and upper income levels, semi-professionals, and as individuals who stated they were concerned about health and environmental issues. The latter views were collected from their response to screening questions. The first group were primarily single and a mixture of gender up to 30 years old. The second group were older, with or without children, but otherwise shared the same demographic characteristics. Each participant was remunerated $60 for their ninety minutes participation. The focus groups were an important method in trying to understand consumer views and attitudes towards environmental sustainability and how they relate to agricultural production, and particularly of carbon footprint labelling. Both focus group meetings followed a similar format including discussion of individual products and awareness and perceptions of environmental sustainability.

Overall, the awareness of sustainability issues was similar across both groups, and it was made clear by participants that sustainability is important, even though it may not be the primary driver of their purchase decisions.

To stimulate discussion of carbon labelling, participants were presented with three types of carbon labels to assess their preferences and user interpretation. The first label referred to the absolute level of carbon dioxide contained in a product, the second showed that an emissions standard had been met, while the third indicated that a percentage reduction of emissions had been achieved relative to a regular product. Although both groups understood the intent of the labels, there was no clear distinction in which label overcame all concerns expressed by the majority of participants. Participants were concerned about how a standard was set and how it would be measured, suggesting that significant effort would be required to gain enough information from secondary sources so as to gauge the strength of the standard. A weakness perceived in using a percentage reduction was the bass level of emissions was unknown, but participants agreed that if all products displayed such labels it would be useful for food product comparison. Participants were also concerned about how an absolute carbon measure was set, and were missing reference point and background information that made interpretation of an absolute value difficult. This finding is consistent with criticism of the absolute carbon measure approach as being cognitively difficult for respondents to ascertain meaning from as significant knowledge is required to be able to use information on absolute quantities, and that consumers are more likely to be able to comprehend relative changes (Upham et al., 2011). Taken as a whole, these considerations informed the decision to use a relative measure of carbon emissions change expressed as a percentage change from current levels.

Table 1. Choice experiment attribute descriptions and levels

Price	This attribute compares the price for the fruit in the survey to the price you currently pay for the fruit you normally buy. The fruit in the survey may cost more or may cost less than you currently pay.			
	- 10%	No change	+ 10%	+ 20%
Carbon emissions reduction	This attribute concerns the amount of carbon dioxide (CO_2) equivalents emitted during production and distribution of the fruit. For many of the options in the survey, emissions have been reduced. Most scientists believe that greenhouse gas emissions, often expressed as CO_2-equivalents, are causing global climate change or global warming.			
	- 30%	- 20%	- 10%	No change
Water efficiency	This attribute focuses on the use of water in production and distribution. Greater efficiency means that less water is used to grow the fruit and get it to the consumer.			
	+ 60%	+ 40%	+ 20%	No change
Vitamins	Fruit is a good source of vitamins. There are natural ways to grow and distribute fruit that is high in vitamins, such as selecting varieties that have higher levels of vitamins or reducing vitamin loss during storage. These changes are reflected in the higher vitamin content of some of the options in the survey.			
	+ 100%	+ 66%	+ 33%	No change
Waste/ packaging reduction	This attribute indicates that the product is produced and distributed in ways that reduce waste and packaging. Reducing waste and packaging means less use of natural resources.			
	- 60%	- 40%	- 20%	No change

Alongside carbon emissions, interviews with industry stakeholders revealed a strong indication that participants were predominantly concerned about ongoing issues around water scarcity and quality. To reflect this concern an attribute measuring the degree of water efficiency was developed and included in the model. The next sustainability attribute to be incorporated into the model was reductions in product waste/packaging. This is a theme that has significant policy traction in Japan, but less so in the UK, and a comparison of WTP could therefore aid in indicating the impact of differing policy environments on consumer preferences. Fruit is considered a healthy food option and increased consumption is often proffered on this basis, and so changes in vitamin levels were also included as an attribute important to consumers. The inclusion of vitamins also helps to

indicate the relative preferences for attributes with private benefits versus those with public good benefits such as carbon reduction. The above discussion determined the final attributes selected for the choice experiment in this study and are described in Table 1, which also shows the information presented to respondents in the survey. The chosen levels reflect possible achievable changes in the attributes that were identified with consultation from major primary industry stakeholders including food scientists.

The final questionnaire included twelve choice sets each made up of a paired comparison of two alternatives employing a D-efficient fractional factorial experimental design, and included the ability of respondents to opt-out of making a choice. Surveys of fruit consumers were implemented online in each country recruited from an online panel database maintained by the research company Research Now™ during October 2011. To improve reliability respondents had to have bought fruit in the previous month. The sampling process employed a pre-stratification approach to enhance representativeness of each countries age and household income population distributions.

4. Results and Discussion

Respondent demographics are given in Table 2. Statistical analysis was conducted employing econometric software Limdep v.9™. Alternative model specifications including an attribute non-attendance model yielded no qualitative improvement over parameter estimates presented (Table 3).

Table 2. Sample demographics

		United Kingdom (%)	Japan (%)
Male		45	50
Age	19-29	6	19
	30-49	21	30
	50-59	17	18
	60+	56	33
Location	Urban	23	48
	Suburban	46	44
	Rural	31	7
Education	High School	44	32
	Junior College	1	19
	Tertiary qualification	44	43
	Postgraduate qualification	7	4
Income (GB£)	< 15 000	19	12
	15 001 – 40 000	42	42
	40 001 – 60 000	17	22
	> 60 001	11	18

Consistent across countries, all attribute parameters are highly statistically significant and of the expected signs. Consumers are more likely to select a fruit option with higher levels of carbon emissions reduction, water use efficiency, vitamin content, or waste / packaging reductions; and are less likely to choose fruit options with a higher price. Significant taste heterogeneity is observed around the means of all random parameters in both the UK and Japan models, with reductions in carbon emissions subject to the greatest respondent taste variation in both countries.

Table 3. Random Parameter Model estimates

	United Kingdom	Japan
Random parameters in utility functions		
Carbon	5.89 (0.88)***	3.11 (0.49)***
Water	2.58 (0.38)***	1.45 (0.24)***
Waste	3.75 (0.57)***	0.68 (0.24)***
Vitamins	0.94 (0.28)***	0.59 (0.14)***
Price	-16.74 (1.68)***	-9.72 (0.85)***
Nonrandom parameters in utility functions		
ASC	-0.19 (0.11)*	0.18 (0.07)**
Distributions of standard deviations of random parameters		
Carbon	8.94 (2.76)***	3.44 (1.12)***
Water	3.34 (1.25)***	2.11 (0.56)***
Waste	4.82 (1.33)***	2.06 (0.41)*
Vitamins	3.95 (0.79)***	1.12 (0.22)***
Price	-16.74 (1.68)***	-9.72 (2.21)***
McFadden Pseudo-R^2	0.39	0.25
Number of observations	2 280	2 448

Notes. ***, **, * denotes statistical significance at 1%, 5%, and 10% level respectively. Standard Errors in brackets.

We simulate the unconditional distributions of WTP for fruit attributes and report the medians, lower and upper quartiles (Table 4.) As both the price and non-price attribute levels are defined as percentage changes the interpretation of WTP is the percentage change in the price of fruit for a percentage change in the level of an attribute (Snowball & Willis, 2006). Ranking the WTP estimates reveals an important finding that both countries value carbon emissions reductions the highest, at over twice the value of the next ranked attribute. While increased vitamin content is valued the least in both countries by a substantial margin, with the lower quartile of UK respondents indicating negative preferences for increases in vitamin content. Strikingly, the estimates are very similar across countries. The single statistically significant difference is in preferences for reductions in waste and packaging, valued by UK respondents at over twice that of Japanese (Poe, Giraud, & Loomis, 2005).

Table 4. Consumer Willingness to pay estimates

	United Kingdom	Japan
Carbon	22% (17%, 26%)	23% (18%, 26%)
Water	10% (8%, 12%)	11% (8%, 12%)
Waste	11% (7%, 15%)	5% (4%, 6%)
Vitamins	2% (-1%, 3%)	4% (3%, 5%)

Note. Median of unconditional simulated distribution (lower quartile, upper quartile).

Our results are consistent with Michaud et al. (2013) who found lowering the carbon footprint of roses was valued more than applying a composite standard that comprised of energy efficiency, waste management, fertiliser use and social requirements. Conversely, Onozaka et al. (2011) found that United States consumers valued reductions in the carbon footprint of tomatoes less than organic certification, and placed no value on carbon footprint reductions for apples. Similarly, Koistinen et al. (2013) found lowering carbon footprint in mincemeat production was not an attribute that consumers were willing to pay much for, and was the least valued of all attributes considered. This difference may in part be explained by the lack of a stand-alone carbon

labelling scheme in these studies locations. Whereas Japans' carbon labelling system has been in place since 2008 covering over 460 products and services, and the UK system was launched in 2008 covering around 4,000 products and services (Guenther et al., 2012). This disparity may result in differing levels of consumers' awareness of the connection between food products and carbon emissions, resulting in less developed preferences for this attribute. There is also the possibility that the divergent values observed may be specific to the differing food products considered.

Comparing WTP estimated here within a Japanese context, the experiment economics approach used by Aoki and Akai (2013) estimated that high environmentally conscious Japanese consumers are willing to pay ¥0.851 more per Satsuma mandarin per one gram reduction in carbon. The authors employ a range of observed market prices in their laboratory experiment and likewise a range of observed carbon levels. Using the averages of these series, ¥35 cost for a mandarin and 30 grams of carbon per mandarin, facilitates a comparison with values estimated in the present paper. Therefore, for a 10% reduction in carbon emissions, high environmentally conscious Japanese consumers are willing to pay about 7% more for a mandarin. This is significantly lower than the 23% value estimated here, even when considering the lower end of the WTP distribution. There are several possible reasons for this including the hypothetical nature of the choice experiment. Another possibility relates to the sample composition obtained by Aoki and Akai. Their sample recruitment approach resulted in over 40% students from the authors' university, and the remaining located adjacent to the university. It may be reasonable to expect the sample on average to be younger and have lower incomes relative to the general population and therefore a lower relative ability to pay, leading to a lower WTP estimate. The ability to pay is in part ameliorated by the design of the experiment that initially endows participants with money with which to purchase the mandarins. However the tighter relative budget constraint facing the typical student could reasonably be expected to incentivise the amount spent to be minimised, as any monies remaining are retained by participants. This would lead to lower WTP estimates.

The assertion that preferences over food attributes are likely to be differentiated by culturally diverse consumer groups across international borders (McCluskey & Loureiro, 2003) has been supported within the limited number of direct cross-country comparisons of preference valuation available. A study on consumer preferences for environmentally-friendly seafood in the US and Norway found differences in consumer preferences across countries (Johnson, Wessells, Donath, & Asche, 2001). Lusk, Roosen and Fox (2003) found differences across France, Germany, the UK, and the United States for preferences over beef fed GM corn. Some studies have found totally opposing preferences, for example Chinese consumers were found to be willing to pay a premium for GM soybean and rice, while Japanese consumers required a price discount (Li, Curtis, McCluskey, & Wah, 2003). Another GM valuation study (Tonsor, Schroeder, Fox, & Biere, 2005) found that French and German consumers have a higher WTP to avoid genetically modified feed than British consumers. While German and British consumers would pay more for growth hormone-free beef and French and German consumers are willing to pay for farm-specific source verification. Other studies finding disparities include preferences for food safety in beef steaks across Canada, Japan, Mexico and the US (Tonsor et al., 2009); meat traceability in the US, Canada, the UK and Japan (Dickinson & Bailey, 2005); Fair Trade coffee in Germany and the US (Basu & Hicks, 2008); Farm animal welfare in France, Germany, Spain, Italy and the UK (Nocella, Hubbard, & Scarpa, 2010).

When looking at which differences in consumer WTP exist between Japan and the UK, this study finds that WTP for reductions in waste and packaging is the only statistically significant difference. This suggests that preferences for environmentally sustainable production outcomes focused on carbon reduction and water use efficiency are relatively consistent across UK and Japanese consumers, at least for fruit. This implies that primary sector environmental policies applied domestically may be more readily aligned internationally than previous cross-country comparisons would indicate. UK consumers value reductions in production waste and packaging over two times greater than their Japanese counterparts. This finding may be a reflection of the already relatively high level of recycling activity in Japan compared to the UK. Only 16 per cent of total municipal waste is landfilled in Japan compared to 49% in the UK (European Environmental Agency [EEA], 2013). This cultural difference may lead Japanese consumers to not consider recycling a distinct product attribute as it is already strongly embedded within product design and part of expected behaviour in line with established social norms.

5. Conclusions

This study was motivated by a need to improve understanding of the relative importance of multiple environmental sustainability attributes of primary sector fruit production. An important finding is that improved carbon emissions reduction is valued most of all attributes, across both countries and by over twice as much as the next valued environmental attribute. The implication for the primary sectors of both countries is that

consumers foremost prefer environmental policy focused at climate targets, and are WTP for products that deliver this benefit. The finding of consistent preferences across cultural and country borders has implications for international supply chains. This result suggests that development of carbon labelling schemes within a domestic context may be more readily transferable to international supply chains than typically assumed. The ability to maintain uniformity in labelling scheme designs for products consumed domestically, as well as being exported, could be achieved without significant loss of generality of the effect on consumer behaviour. This is a useful finding considering that international coordination is required for effective climate mitigation policy. Facilitating this coordination is made simpler if consumers react to labels in a similar manner.

Future research extending the range of products and countries analysed is needed to establish whether the findings of relatively consistent preferences for environmentally sustainable production across countries may be more widespread than suggested here. In addition, the development of comparable revealed preference studies to form external validity remains as ground for future research.

References

Andrews, J. C., Burton, S., & Kees, J. (2011). Is simpler always better? Consumer evaluations of front of package nutrition symbols. *Journal of Public Policy and Marketing, 30*(2), 175-190. http://www.jstor.org/stable/23209273

Aizaki, H., Nanseki, T., & Zhou, H. (2013). Japanese consumer preferences for milk certified as good agricultural practice. *Animal Science Journal, 84*, 82-89. http://dx.doi.org/10.1111/j.1740-0929.2012.01043.x

Aoki, K., & Akai, K. (2013). Do consumers select food products based on Carbon dioxide emissions? In C. Emmanouilidis, M. Taisch, & D. Kiritsis (Eds.), *Advances in Production Management Systems: Competitive Manufacturing for Innovative Products and Services* (pp. 345-352). International Federation for Information Processing Advances in Information and Communication Technology. http://dx.doi.org/10.1007/978-3-642-40361-3_44

Aguilera, E., Guzmán, G., & Alonso, A. (2014). Greenhouse gas emissions from conventional and organic cropping systems in Spain. II. Fruit tree orchards. *Agronomy for Sustainable Development*. http://dx.doi.org/10.1007/s1359-014-0265-y

Basu, A. K., & Hicks, R. L. (2008). Label performance and the willingness to pay for Fair Trade coffee: A cross-national perspective. *International Journal of Consumer Studies, 32*, 470-478. http://dx.doi.org/10.1111/j.1470-6431.2008.00715.x

Balcombe, K., Fraser, I., & Di Falco, S. (2010). Traffic lights and food choice: A choice experiment examining the relationship between nutritional food labels and price. *Food Policy, 35*, 211-220. http://dx.doi.org/10.1016/j.foodpol.2009.12.005

Ben-Akiva, M., & Lerman, S. (1985). *Discrete choice analysis: Theory and application to predict travel demand*. MIT Press, Cambridge.

Bitzios, M., Fraser, I., & Haddock-Fraser, J. (2011). Functional ingredients and food choice: results from a dual-mode study employing means-end-chain analysis and a choice experiment. *Food Policy, 36*, 715-725. http://dx.doi.org/10.1016/j.foodpol.2011.06.004

Bjorner, T. B., Hansen, L. G., & Russell, C. S. (2004). Environmental labelling and consumers' choice-an empirical analysis of the effect of the Nordic Swan. *Journal of Environmental Economics and Management, 47*, 411-434. http://dx.doi.org/10.1016/j.jeem.2003.06.002

Caputo, V., Nayga, J. R. M., & Scarpa, R. (2013). Food miles or carbon emissions? Exploring labelling preference for food transport footprint with a stated choice study. A*ustralian Journal of Agricultural and Resource Economics, 57*(4), 465-482. http://dx.doi.org/10.1111/1467-8489.12014

Cicia, G., Cembalo, L., Del, Giudice, T., & Scarpa, R. (2013). Country-of-origin effects on Russian wine consumers. *Journal of Food Products Marketing, 19*(4), 247-260. http://dx.doi.org/10.1080/10454446.2013.724369

Cleveland, D. A., Radka, N. C., Müller, N. M., Watson, T. D., Rekstein, N. J., Wright, H. V. M., & Hollingshead, S. E. (2011). Effect of localizing fruit and vegetable consumption on greenhouse gas emissions and nutrition, Santa Barbara County. *Environmental Science and Technology, 45*, 4555-4562. http://dx.doi.org/10.1021/es1040317

Cohen, M. A., & Vandenbergh, M. P. (2012). The potential role of carbon labelling in a green economy. *Energy Economics, 34*, S53-S63. http://dx.doi.org/10.1016/j.eneco.2012.08.032

Czamezki, J. J. (2011). The future of food eco-labelling: organic, carbon footprint and environmental life-cycle analysis. *Stanford Environmental Law Journal, 30*, 1-49. http://digitalcommons.pace.edu/lawfaculty/914

Dickinson, D. L., & Bailey, D. (2005). Experimental evidence on willingness to pay for red meat traceability in the United States, Canada, the United Kingdom and Japan. *Journal of Agricultural and Applied Economics, 37*(3), 537-548.

Gadema, Z., & Oglethorpe, D. (2012). The use and usefulness of carbon labelling food: A policy perspective from a survey of UK supermarket shoppers. *Food Policy, 36*, 815-822. http://dx.doi.org/10.1016/j.foodpol.2011.08.001

Greene, W. H., & Hensher, D. A. (2007). Heteroscedastic control for random coefficients and error components in mixed logit. *Transportation Research Part E: Logistics and Transportation Review, 43*, 610-623. http://dx.doi.org/10.1016/j.tre.2006.02.001

Guenther, M., Saunders, C. M., & Tait, P. R. (2012). Carbon labeling and consumer attitudes. *Carbon Management, 3*(5), 445-455. http://dx.doi.org/10.4155/cmt.12.50

Hensher, D. A., & Greene, W. H. (2003). The mixed logit model: the state of practice. *Transportation, 30*(2), 133-176. http://dx.doi.org/10.1023/A:1022558715350

Hu, W., Chen, K., & Yoshida, K. (2006). Japanese consumers' perceptions on and willingness to pay for credence attributes associated with Canola oil. *Journal of Agricultural and Applied Economics, 38*(1), 91-103.

Iwamoto, H., Yamamoto, Y., Sato, K., & Sawada, M. (2003 February). *Effects of HACCP and Eco Labels on Japanese consumers' choice of milk.* Paper presented at the forty seventh Annual Conference of the Australian Agricultural and Resource Economics Society, Fremantle, Australia. http://purl.umn.edu/57896

Jaffry, S., Pickering, H., Ghulam, Y., Whitmarsh, D., & Wattage, P. (2004). Consumer choices for quality and sustainability labelled seafood products in the UK. *Food Policy, 29*, 215-228. http://dx.doi.org/10.1016/j.foodpol.2004.04.001

Johnson, R. J., Wessells, C. R., Donath, H., & Asche, F. (2001). Measuring Consumer Preferences for Ecolabeled Seafood: An International Comparison. *Journal of Agricultural and Resource Economics, 26*(1), 20-39.

Koistinen, L., Pouta, E., Heikkilä, J., Forsman-Hugg, S., Kotro, J., Mäkelä, J., & Niva, M. (2013). The impact of fat content, production methods and carbon footprint information on consumer preferences for minced meat. *Food Quality and Preference, 29*(2), 126-136. http://dx.doi.org/10.1016/j.foodqual.2013.03.007

Li, Q., Curtis, K. R., McCluskey, J. J., & Wah, T. I., (2003). Consumer Attitudes toward Genetically Modified Foods in Beijing, China. *AgBioForum, 5*(4), 145-152.

Lusk, J. L., Roosen, J., & Fox, J. A. (2003). Demand for Beef from cattle administered Growth hormones or Fed genetically modified corn: a comparison of consumers in France, Germany, The United Kingdom and the United States. *American Journal of Agricultural Economics, 85*(1), 16-29. http://www.jstor.org/stable/1244924

Mackerron, G. J., Egerton, C., Gaskell, C., Parpia, A., & Mourato, S. (2009). Willingness to pay for carbon offset certification and co-benefits among (high-) flying young adults in the UK. *Energy Policy, 37*(4), 1372-1381. http://dx.doi.org/10.1016/j.enpol.2008.11.023

Managi, S., Tamamoto, Y., Iwamoto, H., Masuda, K., (2008). Valuing the influence of underlying attitudes and the demand for organic milk in Japan. *Agricultural Economics, 39*(3), 339-348. http://dx.doi.org/10.1111/j.1574-0862.2008.00337.x

McCluskey, J. J., & Loureiro, M. L. (2003). Consumer Preferences and Willingness to Pay for Food Labeling: A Discussion of Empirical Studies. *Journal of Food Distribution Research, 34*(3), 96-102. http://purl.umn.edu/27051

McFadden, D. (1974). Conditional Logit analysis of qualitative choice behaviour. In P. Zarembka (Ed.), *Frontiers in econometrics* (pp. 105-42). New York: Academic Press.

Ministry of Economy, Trade and Industry. (2010). *Commencement of in-store sales of products carrying the Carbon Footprint Label*. METI Japan. Retrieved from

http://www.meti.go.jp/english/press/data/20100114_01.html

Michaud, C., Llerena, D., & Joly, I. (2013). Willingness to pay for environmental attributes of non-food agricultural products: a real choice experiment. *European Review of Agricultural Economics, 40*(2), 313-329. http://dx.doi.org/10.1093/erae/jbs025

Nocella, G., Hubbard, L., & Scarpa, R. (2010). Farm animal welfare, consumer willingness to pay and trust: Results of a cross-national survey. *Applied Economic Perspectives and Policy, 32*(2), 275-297. http://dx.doi.org/10.1093/aepp/ppp009

Onozaka, Y., & McFadden, D. T. (2011). Does local labeling complement or compete with other sustainable labels? A conjoint analysis of direct and joint values for fresh produce claims. *American Journal of Agricultural Economics, 93(3)*, 693-706. http://www.jstor.org/stable/41240352

Poe, G. L., Giraud, K. L., & Loomis, J. B. (2005). Computational Methods for Measuring the Difference of Empirical Distributions. *American Journal of Agricultural Economics, 87*(2), 353-365. http://dx.doi.org/10.1111/j.1467-8276.2005.00727.x

Roos, E., & Tjarnemo, H. (2011). Challenges of carbon labelling of food products: A consumer research perspective. *British Food Journal, 113*(8), 982-996. http://dx.doi.org/10.1108/00070701111153742

Rousseau, S., & Vranken, L. (2013). Green market expansion by reducing information asymmetries: Evidence for labeled organic food products. *Food Policy, 40*, 31-43. http://dx.doi.org/10.1016/j.foodpol.2013.01.006

Snowball, J. D., & Willis, K. G. (2006). Estimating the marginal utility of different sections of an arts festival: the case of visitors to the South African National Arts Festival. *Leisure Studies, 25*(1), 43-56. http://dx.doi.org/10.1080/0261436052000330410

Svanes, E. & Aronsson, A. K. S. (2013). Carbon footprint of a Cavendish banana supply chain. *International Journal of Life Cycle Assessment, 18*, 1450-1464. http://dx.doi.org/10.1007/s11367-013-0602-4

Tonsor, G. T., Schroeder, T. C., Fox, J. A., & Biere, A. (2005). European preferences for beef steak attributes. *Journal of Agricultural and Resource Economics, 30*(2), 367-380.

Tonsor, G. T., Schroeder, T. C., Pennings, J. M. E., & Mintert, J. (2009). Consumer valuations of beef steak food safety enhancement in Canada, Japan, Mexico, and the United States. *Canadian Journal of Agricultural Economics, 57*(3), 395-416. http://dx.doi.org/10.1111/j.1744-7976.2009.01158.x

Train, K. (2009). *Discrete choice methods with simulation*. Cambridge, UK: Cambridge University Press. http://dx.doi.org/10.1017/CBO9780511805271

Upham, P., Dendler, L., & Bleda, M. (2011). Carbon labelling of grocery products: public perceptions and potential emissions reductions. *Journal of Cleaner Production, 19*(4), 348-355. http://dx.doi.org/10.1016/j.jclepro.2010.05.014

Vanclay, J. K., Shortiss, J., Aulsebrook, S., Gillespie, A. M., Howell, B. C., Johanni, R., … Yates, J. (2011). Customer response to Carbon labelling of groceries. *Journal of Consumer Policy, 34*(1), 153-160. http://dx.doi.org/10.1007/s10603-010-9140-7

Van Rikxoort, H., Schroth, G., Läderach, P., & Rodríguez-Sánchez, B. (2014). Carbon footprints and carbon stocks reveal climate-friendly coffee production. *Agronomy for Sustainable Development,* 34, 887-897. http://dx.doi.org/10.1007/s1359-014-0223-8

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Determination of the Chemical Composition of Tea by Chromatographic Methods: A Review

Alexandr Ya Yashin[1], Boris V. Nemzer[2,3], Emilie Combet[4] & Yakov I. Yashin[1]

[1] Inter Lab Research Center, 129226 Moscow, Russia

[2] VDF FutureCeuticals, Inc., 2692 North State Rt. 1-17, Momence, IL 60954, USA

[3] Department of Food Science and Human Nutrition, University Illinois at Urbana Champaign, Urbana, IL, USA

[4] College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, University Avenue G12 8QQ, UK

Correspondence: Boris V. Nemzer, FutureCeuticals, Inc., 2692 N. State Rt. 1-17, Momence, IL, 60954, USA. Tel: 1-812-507-1427. E-mail: bnemzer@futureceuticals.com

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Abstract

Despite the fact that mankind has been drinking tea for more than 5000 years, its chemical composition has been studied only in recent decades. These studies are primarily carried out using chromatographic methods. This review summarizes the latest information regarding the chemical composition of different tea grades by different chromatographic methods, which has not previously been reviewed in the same scope. Over the last 40 years, the qualitative and quantitative analyses of high volatile compounds were determined by GC and GC/MS. The main components responsible for aroma of green and black tea were revealed, and the low volatile compounds basically were determined by HPLC and LC/MS methods. Most studies focusing on the determination of catechins and caffeine in various teas (green, oolong, black and pu-erh) involved HPLC analysis.

Knowledge of tea chemical composition helps in assessing its quality on the one hand, and helps to monitor and manage its growing, processing, and storage conditions on the other. In particular, this knowledge has enabled to establish the relationships between the chemical composition of tea and its properties by identifying the tea constituents which determine its aroma and taste. Therefore, assessment of tea quality does not only rely on subjective organoleptic evaluation, but also on objective physical and chemical methods, with extra determination of tea components most beneficial to human health. With this knowledge, the nutritional value of tea may be increased, and tea quality improved by providing via optimization of the growing, processing, and storage conditions.

Keywords: tea, chemical composition, catechins, HPLC, GC, theaflavins

1. Introduction

Tea extracts have gained popularity as ingredients in dietary supplements and functional foods. Interest in tea chemical composition analysis has increased dramatically in the last decade due to an abundance of scientific data regarding the positive effect of tea on human health. Epidemiological and animal studies suggest that tea is protective against certain cancers, cardiovascular diseases, and neurodegenerative diseases (Yang & Koo, 2000; Mandel & Youdim, 2004; Butt et al., 2015). Tea has a complex chemical composition, containing over 2000 components. Green tea, oolong tea, and black tea differ in their phytochemical contents. Chromatographic methods are keys for the analysis of such complex multi-component mixtures in tea and widely used for identification, tea quality estimation and quantitative analysis of tea bioactive compounds. Overview of the different chromatographic methods used for tea analysis is provided here.

2. Primary Compounds Present in Tea and Their Structural Formulas

The structural formulas of the most biologically active components of tea will be discussed in this section. Unfortunately, the published literature, especially popular science, contains many inaccuracies and errors regarding the nature of the compounds conferring its biological activity to tea. A list of the main chemical compounds present in tea is provided in Table 1.

Table 1. Primary chemical compounds present in tea (analyzed by Chromatographic Methods)

No.	Compound name	Notes: main representative elements
1.	Catechins	Flavanols: 12 catechins are indentified, including 8 occurring in significant quantity, i.e., (+)-catechin, (-)- epicatechin, (-)-gallocatechin, (-)-epigallocatechin, (-)-catechin gallate, (-)-epicatechin gallate, (-)-gallocatechin gallate, (-)-epigallocatechin gallate
2.	Oxyaromatic acids	Gallic, caffeic, quinine, chlorogenic, n-coumaric acids
3.	Flavonols	Quercetin, kaempferol, myricetin
4.	Theaflavins	Theaflavin, theaflavin-3-O-gallate, theaflavin-3'-O-gallate, theaflavin-3-3'-O-gallate
5.	Teagallins	Teagallin
6.	Thearubigins	High-molecular weight polymers of catechin gallates with molecular weight from 1000 to 40000 Da
7.	Pigments	Carotenoids and chlorophyll
8.	Alkaloids	Caffeine, theophylline, theobromine
9.	Sugars	Glucose, fructose, saccharose
10.	Amino acids	Isoleucine, leucine, methionine, threonine, phenylalanine, glutamine, asparagine, alanine, serine, proline, histidine, glutamic acid, aspartic acid, theanine
11.	Vitamins	C, α-, β-, γ-, δ-tocopherols, riboflavin
12.	Dibasic acids	Succinic, malic, tartaric, citric, quinic, aspartic, glutamic, oxalic acids
13.	Cations	K^+, Na^+,Ca^{2+}, Mg^{2+}, NH_4^+, Al^{3+}
14.	Metals	Fe, Zn, Cu, Ni, Al
15.	Lignans and triterpenoid saponins	Mixture of many compounds

In particular it regards to catechins, the most useful components of tea and especially green tea. There are twelve catechins in total identified by chromatography, although only eight catechins are present in tea in significant quantity (Zeeb et al., 2000). However, most published studies focus specifically on a limited subset of catechins, with the most abundant catechins, epigallocatechin gallate and epigallocatechin, which constitute over 70% of the total amount of all catechins. The catechins ratios found in different tea grades will be specified in later sections. The structural formulas of the eight most widely known catechins are shown in Figure 1. The stereoisomers are catechin and epicatechin, gallocatechin and epigallocatechin, catechin gallate and epicatechin gallate.

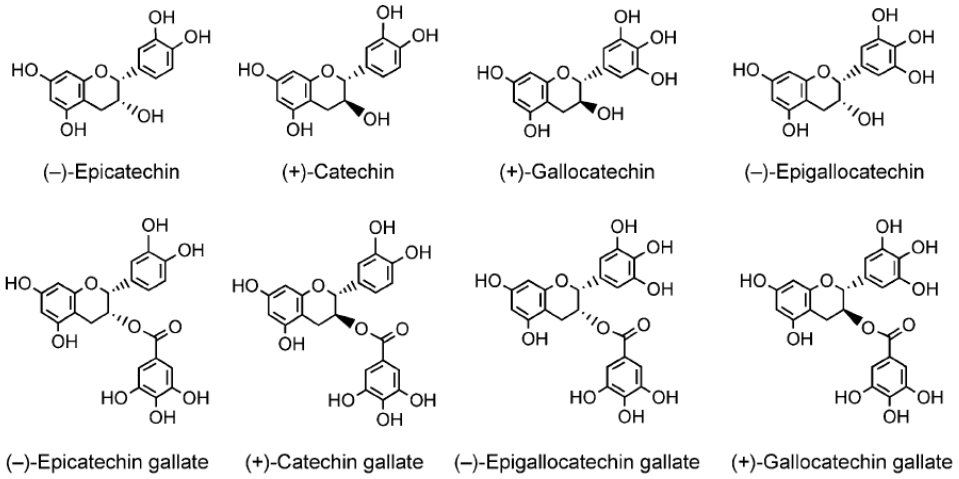


Figure 1. Eight major catechins found in tea

Black tea contains large amounts of theaflavins and thearubigins, which are condensation and polymerization products of catechins. Precursors of theaflavin are EC+EGC, precursors of theaflavin-3-gallate are EC+EGCG, precursors of theaflavin-3'-gallate are ECG+EGC, and precursors of theaflavin-3,3'-digallate are ECG+EGCG.

Structural formulas of theaflavins and other chemicals are shown in Figure 2. Theasinensin A was detected in Oolong tea. Oligomeric catechins presented in tea are thearubigins – 4"-O-methyl-EGCG and 4',4"-dimethyl-EGCG (Kartsova & Alekseeva, 2008).

Tea contains also significant amounts of gallic acid and caffeine, as well as small amounts of theobromine and theophylline (Figure 4) (Rio et al., 2004). Among amino acids, theanine, and gamma-aminobutyric (GABA) acid (Figure 5) are worth noting (Syu et al., 2008).

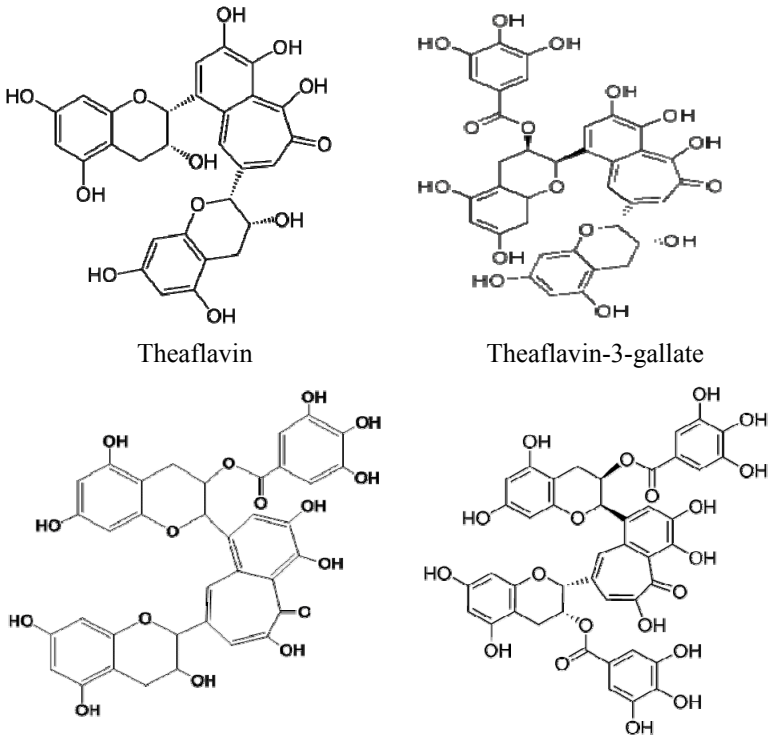


Figure 2. Structure formula of tea theoflavins

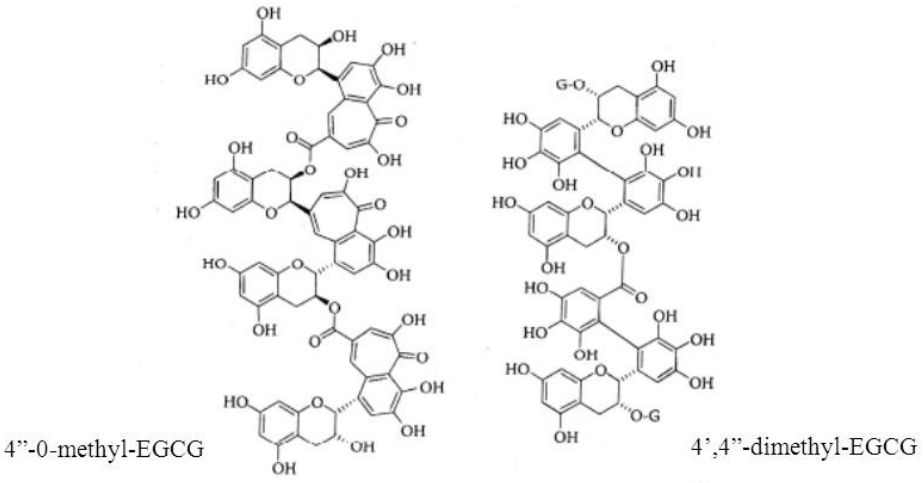


Figure 3. Structure of oligomeric catechins

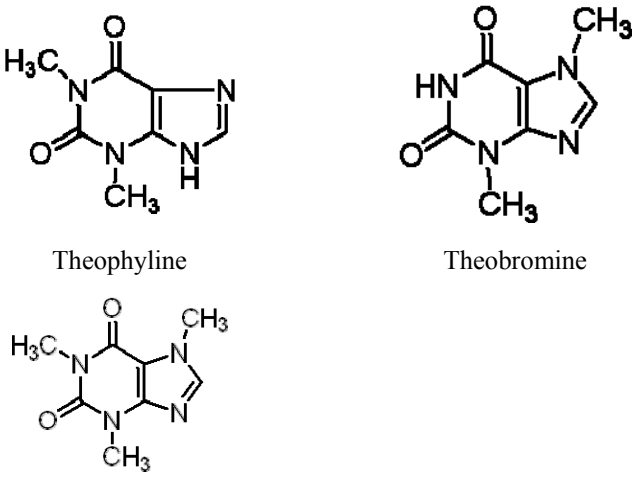


Figure 4. Structure of tea methylxanthins

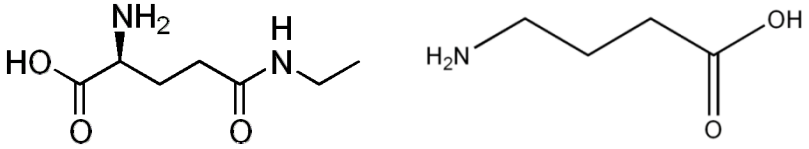


Figure 5. Structure formulas of tea Theanine and γ-Aminobutyric acid (GABA)

3. Chromatographic Methods Used to Determine the Chemical Composition of Tea

Chromatographic methods are keys for tea analysis, relying on the separation of individual components on dedicated chromatographic columns, with the separation based on the interaction of each component with the stationary phase of the column and the mobile phase. Once separated, each component is quantified at the column outlet with special detectors (including absorbance detector, diode array, electrochemical detection and mass spectrometer).

Chromatographic methods have been applied to identify and quantify a broad range of sample types, from gases to high-molecular weight compounds, as well as cation and anion mixtures. Unsurprisingly, chromatography and capillary electrophoresis are the primary methods used to analyze the components of tea (see Table 2).

In order to identify the separated tea compounds (i.e., to conduct qualitative analysis) gas chromatography with mass spectrometry and liquid chromatography with mass spectrometry have recently come into wide use (Table 1).

The low and non-volatile compounds of tea components are best analyzed using liquid chromatography with amperometric detector (AD) or liquid chromatograph with AD and absorbance detectors. Typical chromatogram of green tea is shown in Figure 6.

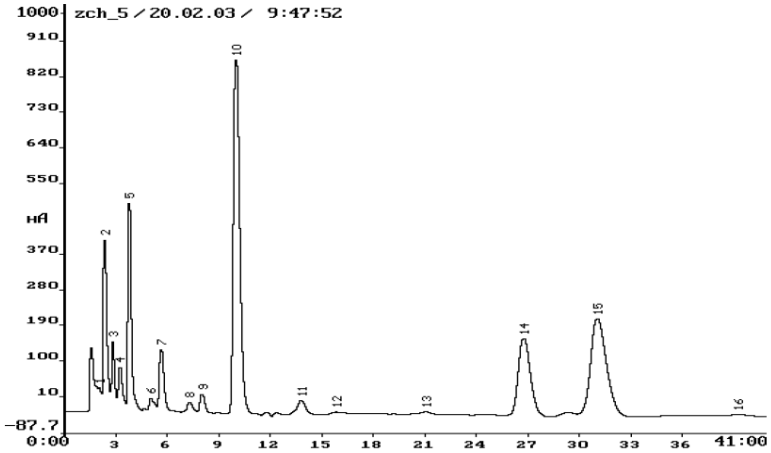


Figure 6. Chromatogram of green tea (brewing time 5 min) on a 150x4.6mm column with C18 Luna (5μ) eluted with acetonitrile and water; 2 – (-)- gallocatechin (GC), 5 – (-)- epigallocatechin (EGC), 10 – (-)- epicatechin (EC), 14 – (-)- epigallocatechin gallate (EGCG), 15 – (-)- epicatechin gallate (ECG) (Yashin et al., 2005-1

Table 2. Chromatographic methods used for the chemical analysis of tea

No.	Methods	Notes: Specific Characteristics and Application	Reference
1	Gas chromatography (GC)	Volatile components which determine the aroma of tea. Low volatile components in the form of derivatives, particularly in the form of trimethylsilyl derivatives.	Bondarivich et al., 1967; Pierce et al., 1969; Guth and Grosch, 1993.
	Headspace GC		Page and Charbonneau, 1984: Baptista, Tavares et al., 1998; Hu, Ding et al., 2007
	SPME-GC	Vitamin K, volatile flavour compounds,	Baptista, Tavares et al., 1998; Baptista, da P. Tavares et al., 1999; Reto, Figueira et al., 2007; Wang, Lee et al., 2008
	GC-ECD	Pesticides, organochlorides, aroma precursors	Gu, Yao et al. , 2011; Li, Li et al., 2004; Huang and Huang, 2006; Mo, Zheng et al., 2009
	GC-FID	Metabolic fingerprinting	

	Gas chromatography with mass spectrometry (GC-MS)	Used for identification of a range of compounds, including	Donovan et al., 1999; Kanrar et al. , 2010; Steiniger et al. , 2010; Xu et al., 2010; Tsumura et al. 1994; Li et al. 2004; Huang and Huang 2006; Cho et al., 2008; Schurek et al., 2008; Mo et al., 2009; Moinfar and Hosseini, 2009;
		Pesticides, Essential oils, volatile compounds, metabolic fingerprinting, acrylamides	Hu, Liang et al., 2010; Clark and Bunch, 1997; Bilia, Flamini et al., 2002; Mizukami, Kohata et al., 2006; Pongsuwan, Fukusaki et al., 2007; Rawat, Gulati et al., 2007; Pongsuwan, Bamba et al., 2008; Ciecierska and Obiedzinski 2009; del Mar Caja, Preston et al., 2009
2	Paper chromatography	Two-way chromatography with visual spectrophotometry.	Singh et al., 1999
3	Thin layer chromatography	Separation of catechins on cellulose plates; chemically modified phases spread on plates.	Vovk et al., 2005; Fecka et al., 2001
		Methyl-xanthine, catechols, flavonoids	Cimpoiu, Hosu et al., 2010; Tirimann. As, 1973; Ligor, Kornysova et al., 2008; Kartsova and Alekseeva, 2009
4	High performance liquid chromatography (HPLC):		
	HPLC-UV	Determination of polyphenols, monosaccharides, flavonoids, caffeine	Rechner et al., 2002; Guillarme, Casetta et al. , 2010; Bramati et al., 2002; Pelillo et al., 2002; Bramati et al., 2003; Pelillo et al., 2004; Yamauchi et al., 2008; Cordero et al., 2009; Lv et al., 2009
	HPLC-ECD	Selective determination of polyphenols, including catechins	Sano et al., 2001; Novak et al., 2010
	HPLC-FD	Determination of (+)-catechin in tea	Ho et al., 1995
		Polycyclic hydrocarbons	Kayali-Sayadi et al., 1998
	CL-HPLC	Determination of catechins in tea.	Nokagawa and Miyazawa, 1997
		Oxalic acid	Wu, He et al., 1998
	HPLC-MS	Identification of theaflavins, etc.	Chen et al., 1998
		Pesticides	Kanrar, Mandal et al., 2010; Huang, Zhang et al., 2009

	HPLC-MS	Cathechins, Flavonoids, polyphenols	Kuhnert, Clifford et al., 2010; Kiehne and Engelhardt, 1996; Kiehne, Lakenbrink et al., 1997; Umashankar, 2001; Pelillo, Biguzzi et al., 2002; Clifford, Stoupi et al., 2007; Venzie, Castro et al., 2007; Cordero, Canale et al. 2009
	Ultra HPLC	catechins	Jiang, Zhang, 2014
	Nano HPLC	polyphenols, methylxantines in tea	Fanali, 2013
	Two dimensional UHPLC	analysis of green and black teas	Scopano, 2013
	Simulated moving bed chromatography	epigallocatechin gallate	Wong, 2013
6	High-speed countercurrent chromatography	Separation of black tea theaflavins. Catechins, theflavins	Köhler et al., 2004 Wedzicha, Lo et al., 1990; Degenhardt, Engelhardt et al., 2000; Baumann, Adler et al., 2001; Du, Jiang et al., 2001; Cao, Lewis et al., 2004
7	Preparative HPLC and countercurrent chromatography	Isolation of pure epigallocatechin gallate from green tea. Polyphenols, alkaloids	Kim et al., 2002 Degenhardt, Engelhardt et al., 2000; Yuan, Chen et al., 2004
8	Capillary electrophoresis	Separation green tea components. Amino acids, inorganic cations, trace elements, polyphenols and alkaloids	Horie et al., 1997 Li, Huang et al., 2010; Ning, Gong et al., 2010; Horie, Mukai et al., 1997; Arce, Rios et al., 1998; Horie, Yamauchi et al., 1998; Barroso and van de Werken, 1999; Carducci, Dabas et al., 2000; Horie and Kohata, 2000; Lee and Ong, 2000; Wright, Aucamp et al., 2001; Feng, Wang et al., 2003; Kartsova and Ganzha, 2006; Hsieh and Chen 2007; El-Hady and El-Maali 2008; Kodama, Ito et al., 2008; Lee, Jeon et al., 2008; Chi, Li et al., 2009; Li, Zhou et al., 2009
9	Chiral chromatography	Separation of catechin optical isomers.	Kofink et al., 2007; Koenig, Evers et al., 1989; Stalcup, Ekborg et al., 1993; Kodama, Yamamoto et al., 2004; Gotti, Furlanetto et al., 2009
10	Ion chromatography	Separation of cations and anions of dibasic acids.	Ding et al., 1997

	Ion chromatography	Anionic minerals, organic acid, inorganic ions	Kuang, Miao et al., 2010; Ding, Chen et al., 1997; Kralj, Krizaj et al., 2005; Michalski, 2006; Kumar, Narayan et al., 2008
11	Size-exclusion (molecular-sieve) chromatography	Separation of high-molecular weight compounds.	Flaten and Lund, 1997; Odegard and Lund, 1997; Odegard and Lund, 1997; Matsuura, Hokura et al., 2001; Kasai and Nakatsubo, 2006

Abbreviations: UV-ultraviolet detector, ECD-electrochemical detector, FD-fluorometric detector, CL-chemiluminescence, MS-mass spectrometry.

The first publications which presented chromatographic analysis of tea appeared over 30 years ago (Bondarovich et al., 1967; Pierce et al., 1969; Yashin et al., 2009; Hoeffer & Coggon, 1976). Large volumes of chromatographic data on various types of tea have been published, including numerous reviews and books (Bokuchava & Skobeleva, 1969; Bokuchava et al., 1980; Wickremasinghe, 1978; Finger et al., 1992; Balentine et al., 1997; Dalluge & Nelson, 2000; Yashin et al., 2005a; Yashin Ya. & Yashin A., 2004; Yashin et al., 2005b; Harbowy & Balentine, 1997; Hara et al., 1995). Tea constituents of the foremost importance have been determined, as listed in Table 1.

For analysis of tea the following standard methods have been released (OSHA Tea Analysis; ISO Standards; British Standard). The sensory method is used for tea quality determination (Adman, 2013). The quantitative analysis of green tea extracts can also be provided by ESI-MS without chromatography (Savic, 2014).

The following primary goals for tea chemical composition analysis can be identified:

– Determination of how the quantitative content of tea active components, particularly catechins, depends on the tea growing conditions;

– Determination of how tea quality depends upon its chemical composition, in order to optimize production technology;

– Objective assessment of tea quality based on the content of certain compounds or compound groups (despite of great achievements attained by physicochemical methods used for compositional analysis of tea, tea quality is primarily assessed organoleptically by tea tasters)

– Determination of health benefits of certain constituents found in tea—this knowledge can be used as part of strategies for disease prevention;

– Determination of bioavailability and bioaccessibility of the most beneficial components present in tea;

– Exploring the potential of tea and tea products as part of antioxidant treatment of oxidative stress.

Some of these objectives can be achieved by chromatographic methods. In some areas, satisfactory results have already obtained, whereas in others, the work is still at the initial stage.

3. Determination of Volatile Impurities in Tea by Gas Chromatography

Several volatile compounds contribute to the aroma of tea beverages, and are identified by GC-MS in conjunction with head-space analysis or solid-phase microextraction (SPME). The list of compound classes and the number of compounds indentified in the aroma of black tea by GC-MS is provided in Table 3 (Bokuchava & Skobeleva, 1986)

Table 3. Classes of compounds contained in aroma of black tea

No.	Compound class	Number of identified compounds
1.	Acids	71
2.	Ketones	57
3.	Aldehydes	55
4.	Esters	55
5.	Alcohols	46
6.	Hydrocarbons	37
7.	Pyridines	23
8.	Pyrazines	22
9.	Phenols	19
10.	Amines and nitrogen-containing compounds	18
11.	Lactones	16
12.	Pyrroles	10
13.	Furans	9
14.	Thiazoles	7
15.	Sulfides and sulfur-containing compounds	5
16.	Oxazoles	2
17.	Thiophenes	1
18.	Other compounds	14

Total: 467 compounds.

Approximately 500 volatile compounds total have been identified in tea extracts. Alcohols are considered as forming in tea leaves through biosynthesis. Most of the remaining volatile constituents of black tea are formed from their precursors (i.e., carotenoids, lipids, or amino acids) during the fermentation process.

Some of the most important compounds which affect tea aroma are listed below, and include monoterpene hydrocarbons, β-myrcene, limonene, n-cumene, terpinolene, sesquiterpene hydrocarbons, methylnaphthalenes, monoterpene alcohols (linalol, nerol, geraniol, citronellol, α-terpineol, 4-terpineol, menthol, borneol, and farnesol), sesquiterpene alcohols (α- and δ-cadinols, cedrol, etc.), terpenoid aldehydes (neral, geranial, citronellal, β-cyclocitral, safranal, etc.), aromatic aldehydes, ketones (2-heptanone, 5-isopropyl-2-heptanone, 3,5-octadiene-2-one, etc.), isoprenoid ketones (geranylacetone, α-ionone, β-ionone, 5,6-epoxy-β-ionone, 4-oxo-β-ionone, etc.), phenol derivatives (guaiacol, n-ethylguaiacol, vinylguaiacol, isoeugenol), thiophene, oxazoles (4,5-dimethyloxazole, and 2,4,5-trimethyloxazole), sulfur compounds (methanethiol, dimethylsulfide), and many others (Bokuchava & Skobeleva, 1986).

The volatile constituents in different grades of green tea are compared in article (Shimoda et al., 1995). Tea tasters use various definitions to assess tea aroma, for example, fresh, sharp, strong, aromatic, fragrant, vivid, deep, medium, delicate, fruity, berry-like, sweetish, burnt, wood-like, rustic, satiated, sultry, smooth, and many others.

The following compounds determine a typical aroma of green tea: D-nerolidol, 6-methyl-α-ionone, coumarone, indole and coumarin (Shimoda et al., 1995). 3-Hexene acid and methyl jasmonate along with aliphatic alcohols and aldehydes create the freshness of the green tea aroma. 3,5,5-Trimethyl-2(5H)-furanone and 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone impart the depth to aroma. Linalool, 2,6-dimethyl-1,3,7-octatriene-6-ol, benzeneacetaldehyde, and 3-hexanyl hexanoate enhance the floral and fruity tones of the aroma. 1-Ethyl-1H-pyrrole-2-carboxaldehyde, 3-ethyl-4-methyl-1H-pyrrole-2,5-dione, 3-ethyl-3-methyl-2,5-pyrrolidinedione, coumarone, and coumarin contribute to a burnt and slightly sweet aroma. For identification of the volatile constituents, capillary column gas chromatography in temperature-programming

mode with flame ionization or mass spectrometry detection is primarily used (Heins et al., 1966).

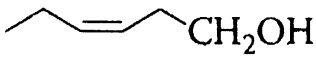
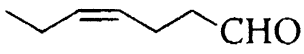
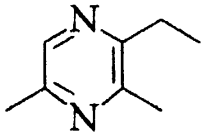
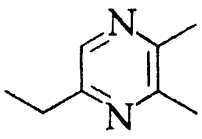
Structural formulas for the compounds which impart various aromas to green and black teas are provided in Tables 4 (Ho et al., 2009). Volatile components of dry tea leaves were mostly determined using static head-space analysis and GC-MS (Heins et al., 1966; Reymond et al., 1966). In particular, the aroma of Ceylon black tea was examined by means of GC-MS and head-space analysis with charcoal adsorption (Wickremasingle et al., 1973). Other analytical methods involved for aroma analysis included static headspace analysis (Witzthum & Werkhoff, 1978), as well as extraction with diethyl ether, steam distillation followed by extraction with isopentane and diethyl ether (Yamanishi et al., 1965), liquid-liquid extraction with pentane and dichloromethane, vacuum distillation, and separation by column liquid chromatography on silica gel columns (Yamanishi et al., 1972). Meanwhile, 57 compounds were identified in extracts by means of GC-MS and IR spectroscopy.

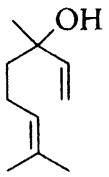
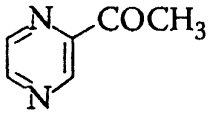
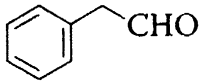
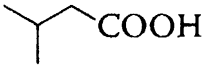
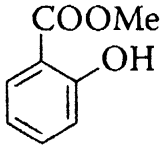
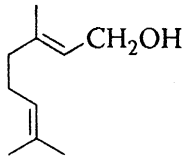
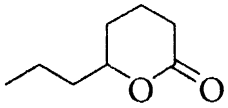
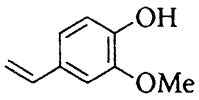
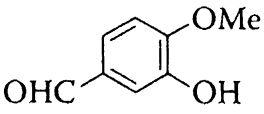
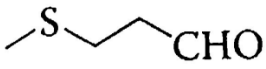
In some publications the teas are classified by aromaticity indices (Owuor et al., 1991). Aromaticity index is a ratio between the peak areas of two compound groups. The first group consists of twelve compounds with undesirable smell, including hexanal, 2,4-heptadienal, 3-hexenol, 2-hexenal, n-pentanol, n-hexanol, while the second group consists of twelve compounds which impart a pleasant aroma to tea, including linalool and its oxides, benzaldehyde, geraniol, α-terpineol, methyl salicylate, benzyl alcohol, β-ionone. These indices have been shown to be connected to the conditions of gathering tea leaves, the height of tea bushes, and types of nitrogen fertilizers. Moreover, Japanese researchers differentiate different tea tree clones by terpene indices (i.e., content of terpene compounds in tea aroma) (Owuor et al., 1987).

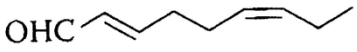
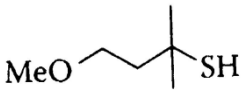
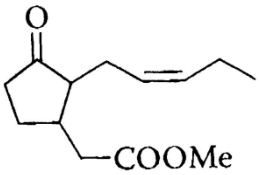
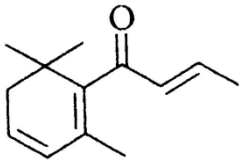
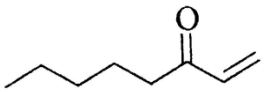
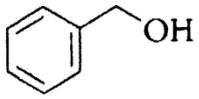
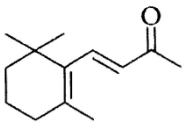
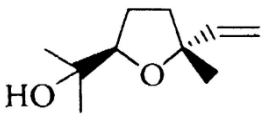
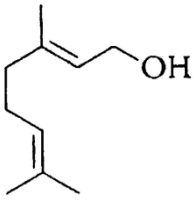
GC-MS was initially used for determining the difference in aromas of different tea grades (Mick and Shreier, 1984). However, during such analysis, decomposition of some unstable tea components was noted, in particular, hexenal, which may distort the true composition of tea aroma (Kinugasa & Takaeo, 1990). Analysis of the enantiomers (optical isomers) of some tea compounds is an entirely new approach to analyzing tea aroma (Wekhoff et al., 1991).

Volatile compounds of green, black, oolong and white teas by dispersive liquid-liquid microextraction coupled with GC have been reported (Sereshti, 2013). For ranking of Japanese green tea (sen-cha) by volatile profiling is used GC-MS and multivariate analysis (Jumtee et al., 2013). The aroma of Pu-erth tea characterized using headspace – solid phase microextraction, combined with GC-MS and GC-olfactometry (Lu et al., 2012).

Table 4. Main volatile components determined in tea samples

No.	Compound name	Structural formula	Type of aroma
		Green Tea	
1	(Z)-3-hexenol		Greenery
2	(Z)-4-heptenal		Hay-like
3	2-ethyl-3,5-dimethylpyrazine		Nut-flavored
4	5-ethyl-2,3-dimethylpyrazine		Nut-flavored

5	Linalool		Floral
6	2-acetylpyrazine		Roasted
7	Phenylacetaldehyde		Sweetish, honey-like
8	3-methylbutanoic acid		Oily
9	Methyl salicylate		Minty
10	Geraniol		Floral
11	5-octanolide		Sweetish
12	2-methoxy-4-vinylphenol		Spicy
13	Vanillin		Vanillin
	Balck Tea		
1	Methional		Potato-like

2	(E, Z) – 2,6 - nonadienal		Cucumber-like
3	4-methoxy-2-methyl-2-butanethiol		Meaty
4	(Z)-methyl jasmonate		Floral
5	β-damascenone		Honey like
6	1-octen-3-one		Smell of mushrooms
7	Benzyl alcohol		Scorched, weakly aromatic
8	β-ionone		Floral, wood-like
9	Menalool oxide		Sweetish, floral, creamy
10	Geraniol		Sweetish, honey-like

4. Determination of Amino Acids and Sugars in Teas by Anion Exchange Chromatography

Direct determination of amino acids and sugars in teas by means of anion exchange chromatography using a pulsed amperometric detector with a gold electrode has been described in (Ding et al., 2002). A 250 × 0.2 mm separation column with AminoPAC PA10 at 30 °C was employed. The gradient elution was performed with a

mixture of deionized water, 0.25 μM sodium hydroxide, and 1.0 μM sodium acetate as a mobile phase (eluent). The mixture composition changed over time according to a definite principle. The flow rate was 0.25 ml/min. The root mean square deviation (RMSD) was 4.6%. The detection limit was within the range of 0.12-4.9 pmol, linear range was three orders of magnitude with a correlation coefficient of 0.99.

The samples were prepared as follows: 0.5 g of tea leaves were placed in a flask and filled with hot water (80 °C), brewed for 30 min, then filtered through a membrane filter with a pore size of 0.45 μm. The solution without suspensions was diluted 25 times and analyzed by chromatography.

The results of sugar analysis in Longjing tea and Bi Luo Chun tea are provided in Table 5. The total content of sugars is approximately 12 mg/g of dry tea leaves; saccharose (sucrose) was found in the largest amount among all sugars.

Theanine (non-protein amino acid) was found in a relatively large amount, about 11 mg/g; the aggregate amount of all 14 remaining amino acids was also about 11 mg/g. Five of 14 amino acids are indispensable (Table 6). Theanine deserves separate consideration. Theanine (γ-glutamylethylamide) structure is shown in Figure 6. The content of theanine in different grades of tea varied within the range of 1.5-3% of dry weight. Tea primarily contains L-theanine and small amounts of D-theanine. Theanine easily dissolves in water but does not dissolve well in ethanol or esters. L-theanine is a weak amino acid, stable in acidic medium at pH=3. Theanine significantly contributes to the taste of green tea. Green tea is typically characterized by four tastes: bitterish, astringent, sweetish, and piquant. The sweetish, piquant tastes arise via the presence of theanine. That is why the content of theanine is one of the most important indicators of the tea quality in world markets. The best Chinese grades of green tea and Japanese Gyokuro tea contain up to 2% theanine.

Table 5. Content of Sugars in Green Tea Determined by HPLC with Amperometric Detector (Ding et al., 2002)

No.	Name of sugars	Content in dry tea, mg/g	
		Longjing	Bi Luo Chun
1.	Saccharose	7.8	9.9
2.	Glucose	1.3	0.9
3.	Fructose	0.8	0.9

Table 6. Content of amino acids in green tea determined by HPLC with pulsed amperometric detector (Ding et al., 2002)

No.	Amino acids	Content, mg/g	
		Longjing	Biluochun
1.	Isoleucine	0.10	—
2.	Leucine	0.32	0.21
3.	Methionine	0.10	—
4.	Threonine	0.27	0.23
5.	Phenylalanine	0.26	0.13
6.	Glutamine	2.24	3.11
7.	Asparagine	0.3	0.25
8.	Alanine	0.33	0.51
9.	Serine	0.69	0.55
10.	Proline	0.20	0.11
11.	Histidine	0.30	0.35
12.	Glutamic acid	3.05	3.13
13.	Aspartic acid	2.21	2.67
14.	Tyrosine	0.36	0.30
15.	Theanine	10.73	9.91

5. Application of High Performance Liquid Chromatography (HPLC) for the Determination of non-Volatile Compounds in Tea

5.1 Analytical Methods:

HPLC is the most frequently used methods to determine catechins, alkaloids, theaflavins, and thearubigins in teas. Thearubigins are high-molecular weight compounds with molecular weight varying from 1000 to 40,000 (Haslam, 2003). HPLC is also used to determine phenolic acids (such as gallic and caffeic acids, etc.), flavonols (such as quercetin, kaempferol, and myracetin), lignans, triterpenoid saponins, pigments (chlorophyll and carotenoids) in tea. Among various HPLC methods, reversed-phase chromatography on C_{18} bonded phase columns is used most often; while C_8 bonded phase columns are used less often.

Comparative selectivity separation and yield sequence was studied on 150 ×4.6 mm columns filled with various bonded phases, such as C_{18} perfluorophenyl and nitrile phases (Blythe & Rereira, 2006). Yield sequences of catechins of the standard mixture were as follows:

C_{18} – C, EGCG, EC, GCG, ECG, and CG;

Perfluorophenyl phase – C, EC, EGCG, GCG, ECG, and CG;

Phase with a CN group – C, EC, EGCG, ECG, GCG, and CG.

In particular, switching from C18 to perfluorophenyl phase changed the order of peaks 2 and 3; while switching from C18 to CN-rich phase changed the order of peaks 2 and 3, 4 and 5.

In various research papers, the 100, 150, or 250 mm columns with an inner diameter of 3, 3.9, 4, 4.6, or 5mm were used to determine catechins and other constituents in tea. C18 reversed-phase sorbents of the following companies were employed: Hypersil ODS, Zorbax, Cosmosil, Nucleosil, Water, TSKgel, Lichrosorb, Tosoh, and Wakosil. Selected mobile phases (eluent) were: water-acetonitrile, water-methanol, and water-isopropanol. Apart from isocratic elution, the gradient elution is often used. A broad range of detection systems are utilized with HPLC, including UV detectors, diode array spectrophotometers, electrochemical detectors (ECD), such as amperometric and coulometric detectors, mass spectrometry detectors, and fluorometric detectors. Absorbance, SPD and ECD detectors are used most often. Absorption regions at 210, 230, 254, and 370-280nm are used to determine catechins, with evidence showing that when determining five catechins with electrochemical detectors, the sensitivity is 1000 times compared to using a UV detector (Umegaki et al., 1996), while others have shown ECD to be 300 times more sensitive than UV detectors, particularly, for determining catechins (Kumamoto et al., 2000). Meanwhile, amperometric detectors register catechins at potentials of 400mV and 900mV, with maximum signal observed at 750-800 mV (Long et al., 2001). However, in some cases, it is recommended to use 500-600 mV in order to eliminate concurrent oxidation compounds (Umegaki et al., 1996).

5.2 Analyte Extraction:

Aqueous extracts at 80-100°C are used most frequently, followed by other solvents and their mixtures, such as ethanol, methanol, and esters. Black tea extracted for 2, 8, and 16 hours with different solvents, namely, absolute acetone, N,N-dimethylformamide (DMF), ethanol, methanol, and their 50% aqueous solutions (Turkman et al., 2007) – with varying recovery depending on the solvent used (Table 7). The total polyphenol content and antioxidants, as well as antibacterial activities, were determined in extracts. Polyphenol content varied within the range of 0.44-114 mg/g of dry tea, with regard to gallic acid, depending on the solvent and extraction time (Table 7).

Table 7. Extraction of polyphenols from tea using different solvents (mg/g)

Extraction time, min	Methanol		Ethanol		Acetone		Dimethylformamide,	
	50%	> 99%	50%	> 99%	50%	> 99%	50%	> 99%
2	58.4	8.6	74.4	0.5	114.0	0.44	95.6	18.6
8	62.9	22.8	85.4	1.9	92.8	1.4	105.0	44.1
18	68.7	23.1	86.3	2.1	98.2	1.6	109.4	49.1

Aqueous acetone and DMF have shown the best results, whereas pure acetone was least effective. When antibacterial activity was studied, staphylococcus was sensitive to all tea extracts except the methanolic extract.

The impact of temperature on the catechin extraction at 100, 80, and 60 °C for 5 min was studied, as well as

extraction duration (USDA Database for Flavonoid Content, Release 2.1, 2007). Catechin content increases by 30-40% if the extraction was carried out for 10 min. EGCG extraction is the most dependant on temperature – with 60% of EGCG extracted at 80 °C and only 50% at 60 °C. Meanwhile, extraction of catechin was least dependant on temperature – with 98% and 85% extracted at 80 °C and 60 °C respectively.

The conditions for the extraction of catechins from green tea using hot water have been optimized in (Vuong et al., 2012). Method GuEhERS has been applied for extraction pesticides in teas (Lozano et al., 2013; Zhai, 2014) and planar solid phase extraction for analysis pesticides residue in tea by LC-MS (Oellig & Schwack, 2013)

5.3 Determination of Non- Volatile Compounds

Averaged catechins content (USDA Database, 2007; Arts et al., 2000; Bronner & Beecher, 1998; Ding et al., 1992; Hertog et al., 1993; Khokhar & Magnusdottir, 2002; Lee & Ong, 2000; Price et al., 1998; Price & Spitzer, 1993; Sakakibara et al., 2003; Steinhaus & Engelhardt, 1989; Wang & Helliwell, 2001) were provided in Table 8; there were 68 different grades of green tea, 83 grades of black tea, and 7 oolong teas involved in analyses. Mean value of all catechins in 68 grades of green tea was 12% of dry weight, 3.3% in 83 grades of black tea, and 5.2% in oolong. Among the 68 grades of green tea, the minimum and maximum values for some catechins were different by an order of magnitude, i.e., 10 times. Maximum content of catechins in green tea was 33.1% of dry weight.

Table 8. Content of flavonoids (% dry weight) in the leaves of green tea, oolong tea, and black tea (mg/g) (USDA Database, 2007; Arts et al., 2000; Bronner & Beecher, 1998; Ding et al., 1992; Hertog et al., 1993; Khokhar & Magnusdottir, 2002; Lee & Ong, 2000; Price et al., 1998; Price & Spitzer, 1993; Sakakibara et al., 2003; Steinhaus & Engelhardt, 1989; Wang & Helliwell, 2001)

No.	Type of flavonoids	Type of tea		
		Green	Oolong	Black
Flavan-3-ols				
1	(-)-Epicatechin	8.1	2.5	2.6
2	(-)-Epicatechin-3-gallate	14.9	6.3	6.9
3	(-)-Epigallocatechin	20.6	7.5	9.6
4	(-)-Epigallocatechin-3-gallate	71.1	34.1	11.2
5	(+)-Catechin	0.6	0.3	1.4
6	(+)-Gallocatechin	0.0	0.0	0.9
7	Theaflavin	0.04	0.1	1.6
8	Theaflavin-3,3ʹ-digallate	0.0	0.2	1.7
9	Theaflavin-3'-gallate	0.04	0.0	1.6
10	Theaflavin -3-gallate	0.01	0.0	1.3
11	Thearubigins	1.31	0.0	59.2
Flavonols				
12	Kaempferol	1.5	0.02	1.3
13	Myricetin	1.1	0.0	0.4
14	Quercetin	2.6	0.02	2.0

A study focusing on the content of six catechins, purine alkaloids, and gallic acid was determined by HPLC in 45 tea grades from China, Japan, and Taiwan (Lin et al, 1998). Separation was performed on a 250 × 4.6 mm column with Cosmosil C18-MS, with particle size of 5μ eluted with methanol, redistilled water, and formic acid (19.5:80.2:0.3), the dose of 20 μ. The results were registered by UV detector at a wavelength of 280 nm. RMS deviation varied in the range of 4-10%. The averaged results of measurements taken for 15 grades of green tea from China, 13 grades of green tea from Japan, 9 grades of oolong tea, and 7 grades of Pu-erh tea are provided in Table 9.

Table 9. Content of catechins, gallic acid, caffeine, theophylline, and theobromine in green tea, oolong tea, black tea, and pu-erh tea, in mg/g (Lin et.al., 1998)

No.	Tea grade	Catechins						Total amount of catechins	Alkaloids			Gallic acid
		EGCG	EGC	ECG	EC	C	GCG		Caffeine	Theo-phylline	Theo-bromine	
1.	Green tea from China (mean value for 15 teas)	134	4.4	29	5.5	0.2	2.6	180 (130-220)	77.3	0.8	6.0	5.2
2.	Green tea from Japan (mean value for 13 teas)	131.4	8.8	19.5	8.7	0.2	3.8	178 (90-240)	76.8	0.6	3.1	2.3
3.	Oolong tea (averaged value for 9 teas from China and Taiwan)	49.7	3.5	8.4	2.7	0.1	0.9	65.1	63.8	0.4	2.8	4.5
4.	Black tea (85% fermentation)	3.0	1.9	—		0.4	—	5.2	160.3	—	2.6	18.3
5.	Pu-erh tea (Mean value for 7 teas)	1.5	1.9	0.3	0.9	—	0.1	4.9	77.0	0.3	6.3	14.9

Of particular interest, the aggregate values of the six catechins were very close for Chinese and Japanese teas, as well as the caffeine content. For some components, the average level of EGCG, C, and gallic acid were also similar. The content of EGC, EC, and GCG catechins was higher in Japanese green teas, whereas content of ECG, gallic acid, theophylline, and theobromine was higher in Chinese green teas. Oolong tea contains less catechins than green teas, and the Pu-erh tea contains only a small amount of catechins. The amount of gallic acid significantly increases during fermentation process due to its liberation from catechin gallates.

The total content of catechins significantly decreases during the fermentation process. This issue was studied with TTE tea, a tea variety cultivated in Taiwan which is highly resistant to various insect infestations. This tea variety was used to make green tea, paochong tea, oolong tea, and black tea with a known percent of fermentation. The content of catechins changes depending on the fermentation degree in ten times.

One simple and quick method to determine the four primary catechins, caffeine, and gallic acid in tea using HPLC with a diode array detector (Khokhar & Magnusdottir, 2002). Extraction was carried out thrice, with a 30% methanol solution. The separation was performed in a gradient mode on a 4.5 mm × 25 cm column with C_{18} Alltech adsorbosil, 5 μ, for 20 min. Methanol, acetate with an aqueous buffer were used as an eluent. UV detection was performed mainly at 280 and 360 nm wavelength (Table 10). The yield sequence of the compounds was as follows: gallic acid (GA), epigallocatechin (EGC), epigallocatechin gallate (EGCG), epicatechin (EC), epicatechin gallate (ECG) and caffeine.

The aggregate content of catechins stayed within the range of 100-123 mg/g (10-12.3%) in green tea; 40-63 mg/g (4-6%) in oolong tea; 15.3 mg/g (1.53%) in black tea, and 12.8 mg/g (1.28%) in Pu-erh tea. As fermentation degree increases, the content of gallic acid increases from 0.4 to 5.5 mg/g, the content of caffeine varies within the range of 19-26 mg/g (1.9-2.6%) of dry weight (except for Fujion oolong tea).

Five catechins and caffeine were determined by HPLC-MS (with chemical ionization) with preparative concentration by means of solid-phase microextraction on capillaries coated with polypyrrole. Catechins were determined in both positive and negative ionization modes (Wu et al., 2000). Detection limits of catechins were under 0.5 ng/ml and caffeine about 0.01 ng/ml. When concentration is employed, the detection limits are lower by an order of magnitude.

The determination results for five primary catechins and caffeine in green tea, oolong tea, and black tea, both pure and aromatized with fruits, flowers, or spices are provided in Table 11.

The table shows the results of measurements taken after brewing tea for 5 and 10 min. In all cases, the content of catechins at brewing time of 10 min is higher approximately by 30% compared to the brewing time of 5 min. The aggregate content of catechins varies within the range of 68.5 to 101 mg/g of dry weight in green teas (i.e., approximately 7-10%), approximately 50 mg/g (5%) in oolong teas, and approximately 10-12 mg/g (1-1.2%) in aromatized black teas.

The content of caffeine is about 30-40 mg/g (3-4%) in green teas, 26 mg/g (2.6%) in oolong teas, and 15-33 mg/g (1.5-3.3%) in black teas.

Table 10. Content of catechins, gallic acid, and caffeine in various tea grades (Zuo et al., 2002)

	Tea grade	Content, mg/g of dry tea						
		EGC	EGCG	EC	ECG	Total amount of catechins	Gallic acid	Caffeine
1.	Meifoo green tea	27.7	52.7	10.3	21.8	112.5	0.74	26.8
2.	Shanghai green tea	30.8	51.1	7.25	11.3	100.45	0.37	23.0
3.	Hongzhou Lung Ching green tea	37.6	62.4	6.6	16.3	122.9	1.84	28.5
4.	Jasmine green tea	27.6	54.2	6.9	15.8	104.6	1.13	29.6
5.	Fujian oolong tea	10	22.2	2.63	6.06	40.89	1.42	7.44
6.	Jiangxi oolong tea	15.9	28.2	2.96	6.45	63.51	1.67	18.7
7.	Fujian black tea	5.71	3.79	1.36	4.45	15.31	2.06	21.6
8.	Pu-erh tea	6.23	1.99	3.24	1.32	12.78	5.53	22.4

Table 11. Content of catechins and caffeine in various teas determined by HPLC-MS (Wu et al., 2000)

	Tea grade	Brewing time, min	Content in mg/g of dry tea						
			EGC	C	EC	EGCG	ECG	Total amount of catechins	Caffeine
1.	Chinese green tea	10	23.3	1.0	10.1	52.2	14.3	100.9	39.5
		5	15.2	0.7	8.7	43.1	9.0	76.6	30.4
2.	Lung Ching green tea	10	13.6	0.9	8.0	37.0	9.8	69.3	29.4
		5	10.2	0.6	6.3	31.0	7.8	55.9	22.9
3.	Jasmine green tea	10	9.2	2.2	11.5	31.5	14.1	68.5	29.4
		5	6.9	1.6	8.5	22.5	10.4	49.9	20.7
4.	Tie Guan Yin oolong tea	10	8.8	0.7	5.5	27.8	6.3	49.1	26.5
		5	6.2	0.5	3.5	20.0	5.9	36.0	20.5
5.	Black tea	10	5.2	1.0	8.7	18.8	8.4	42.1	33.8
6.	Black tea (Krasno-rozovy), aromatized	10	0.7	0.4	1.3	6.0	3.7	12.1	38.9
7.	Orange Pekoe black tea (Tetley), aromatized	10	0.8	0.3	0.9	4.2	3.4	9.5	14.9

A method for simultaneous determination of all polyphenols in vegetables and teas is proposed in work (Sakakibara et al., 2003). 100 polyphenols which occur most often in significant quantities in these products were selected; these include benzoic and cinnamic acids, flavonoids (flavones, flavonols, flavonone, isoflavones, catechins, theaflavins, chalcones, anthocyanins, anthraquinones, etc.). The retention parameters were determined on a 250x4.6mm column with C_{18} Capcell pek (5 μ) at 35 °C in gradient mode (eluted with methanol–sodium phosphate–water), and optimal wavelengths for diode array spectrophotometer, slope of the calibration curves, and detection limits were selected. Using the data obtained above, polyphenols content was determined in 63 vegetables, fruits, and teas. The measurement data for some polyphenols found in black teas from Japan, oolong tea from China, and black tea from Kenya as well as total content of catechins, total content of all polyphenols, and the portion of catechins in all polyphenols is provided in Table 12.

Table 12. Content of polyphenols in green teas from Japan, oolong tea from China, and black tea from Kenya, in mg/g (Karori et al., 2007)

No.	Polyphenols	Green tea		Oolong tea	Black tea
		Gyokuro	Sencha		
1.	Catechin	8.72	2.78	2.07	1.58
2.	Gallocatechin	2.27	14.60	9.99	–
3.	Catechin gallate	0.32	–	0.45	1.15
4.	Gallocatechin gallate	4.47	3.75	2.97	2.71
5.	Epicatechin	23.60	58.00	6.65	20.10
6.	Epigallocatechin	80.60	179.00	49.10	9.19
7.	Epicatechin gallate	14.00	23.50	8.94	8.23
8.	Epigallocatechin gallate	91.70	149.00	53.80	10.20
9.	Theaflavin	–	–	0.27	3.10
10.	Theaflavin-3-gallate	–	–	0.26	4.30
11.	Theaflavin-3'-gallate and theaflavin-3,3'-gallate	–	–	0.70	9.60
12.	Kaempferol-3-O-glucoside	2.68	1.82	0.83	3.05
13.	Kaempferol-3-O-rutinoside	0.12	–	0.60	2.53
14.	Kaempferol glycoside	2.50	2.59	0.43	1.15
15.	Quercetin-3-O-rhamnoside	0.30	1.16	1.39	6.33
16.	Quercetin glycoside	1.50	7.69	1.98	–
17.	Myricetin-3-O-rutinoside	0.98	5.17	2.08	2.08
18.	Gallic acid	1.54	2.54	13.30	17.90
	Total content of all polyphenols	235.30	451.60	153.81	101.20
	Total content of catechins	225.68	430.63	133.97	53.16
	The portion of catechins in Total amount of polyphenols	96%	95%	87%	52%

Five primary catechins and caffeine were determined in 31 commercial tea samples from China, Taiwan, and Japan by means of HPLC in boiling water extracts and 75% ethanol extracts (Table 13) (Lin et al., 2003). According to their total content of catechins and epigallocatechin gallate (EGCG), various teas were classified in the following order: green tea > green tea (fresh leaves)> oolong tea > black tea > Pu-erh tea. Extracts obtained using 75% ethanol solution showed a higher level of EGCG and total content of all catechins. Meanwhile, when ranked according to their caffeine content, the teas produced from the same tea bush but fermented to different degrees were arranged in the following sequence: Black tea > oolong > Green tea > fresh tea leaves.

The level of flavonols and flavones intake by a healthy population in the USA is 20-22 mg per day (Sampson et

al., 2002). In food products and beverages, the flavonols are primarily quercetin, kaempferol, and myricetin, and flavones (apigenin and luteolin). Tea and onions are primary sources of these flavonols and flavones both for men and women. Black teas (Lipton, Tetley, and Salada) were found to contain 26.0 mg/kg of quercetin, approx. 4.0 mg/kg of kaempferol, and 19-21 mg/kg of myricetin. New method (RP-HPLC) developed for determination of quercetin in green tea has recently been published (Savic et al., 2013). The proportion of tea with regard to total flavonol and flavone intake is 26-35%.

In Great Britain, black tea is the primary source of polyphenols among regular tea drinkers (Khokhar and Magnusdottir, 2002). Six grades of black tea purchased in Great Britain were analyzed specifically for this purpose. The overall antioxidant activity of the selected teas was determined by means of TEAC, ORAC and FRAPS methods; individual polyphenols were identified by HPLC. Epigallocatechin gallate, epicatechin gallate, teagallin, four theaflavins, quercetin-3-rutinoside, and 4-caffeoylquinic acid were identified. Thearubigins made 75-82% of all polyphenols. On average, one cup of brewed tea (one tea bag) was found to contain 262 mg of GAE, 65 mg of which were individual polyphenols. The antioxidant potential of individual tea phenolics from Kenyan green and black teas were provided using an on-line high-performance liquid chromatography (Stewart et al., 2005).

Table 13. Content of epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), catechin (C), epicatechin (EC), and total amount of all catechins in various grades of tea from China, Taiwan, and Japan, in mg/g of dry tea (W – hot water extracts, A – alcoholic extract, 75%)

No.	Type of tea, country	Tea name	EGCG		EGC		ECG		C		EC		Total catechins	
			W	A	W	A	W	A	W	A	W	A	W	A
1.	Green, China	Xian-Zhe-Zhu-Jian	24.47	29.38	26.29	20.88	2.54	9.98	1.00	0.50	2.54	1.94	59.65	62.68
2.	Green, China	E-Mei-Shan - Snow Bud	14.74	14.90	37.20	28.06	3.14	5.51	1.10	0.49	3.70	2.49	59.88	51.45
3.	Green, China	Lu-Shan Ckoud (D)	32.08	47.34	83.83	77.58	7.80	11.68	1.39	2.41	6.37	6.59	136.47	145.60
4.	Green, China	Lu-Shan Ckoud (K)	30.64	43.48	40.11	18.81	8.68	11.95	1.70	1.82	3.93	2.91	85.06	78.97
5.	Green, Taivan	Longjing	31.17	48.18	67.31	70.98	5.18	9.37	0.98	0.81	4.14	4.29	108.78	133.63
6.	Green, Japan	Decoct Tea	21.19	35.76	83.62	83.07	3.45	6.79	0.64	–	5.69	5.93	114.59	131.55
7.	Green, Japan	Decoct Tea	21.30	36.95	82.44	94.72	3.64	6.43	0.96	–	6.05	6.54	114.39	144.64
8.	Оолонг, China	Xiao-Hong-Pao	7.85	30.68	56.28	87.67	0.96	5.10	2.47	3.03	3.44	5.03	71.00	131.51
9.	Oolong, China	Rock Tea	3.69	14.67	47.02	43.83	0.49	2.90	1.78	2.08	3.15	2.95	56.13	66.43
10.	Oolong, China	Xiao-Hong-Pao	13.15	28.10	38.39	72.74	3.16	9.22	1.41	2.58	5.12	5.53	61.23	118.17
11.	Oolong, Taiwan	Ping-Lin	10.49	27.74	78.54	99.64	1.19	4.23	0.48	0.61	4.01	5.25	94.71	137.47
12.	Oolong, Taiwan	Shi-Zhuo-Zheng Lu	7.76	29.23	73.43	115.00	1.06	4.75	0.50	0.84	3.89	6.23	86.64	156.05
13.	Black, Taiwan	Lipton black	2.42	4.14	10.83	61.78	1.93	3.77	–	–	1.17	1.06	16.35	70.75
14.	Pu-erh, China	Xiao-Huang-Yin	5.96	4.79	13.82	5.78	9.56	10.08	1.42	0.72	4.87	2.58	35.63	23.95

An intake level of catechins, caffeine, and total polyphenols via tea was determined in Great Britain (GB). Tea was purchased in typical supermarkets both as tea bags and in bulk. Tea was prepared with boiling water and brewed for 5 minutes. The measurement results are provided in Table 14 (Khokhar & Magnusdottir, 2002).

Table 14. Total polyphenols, total catechins and caffeine content in tea (Khokhar et al., 2002)

No.	Type of tea	Content, mg/g of dry tea		
		Total amount of polyphenols	Total amount of catechins	Caffeine
1.	Green tea	87-106.2	51.5-84.3	11-20
2.	Black tea	80.5-134.9	5.6-47.5	22-28
3.	Fruit tea	—	8.5-13.9	—

A daily intake of three cups of tea a day (200 ml each brewed with 1% of tea leaves by weight), equates to 405.5 mg, 92.7 mg, and 61.5 mg of catechins from green, black, and fruit tea respectively. Meanwhile, this equates to 92-146 mg of caffeine daily with the same amount of tea.

Average content of catechins in different types of teas is provided. Range of catechin content in tea: green tea – 16-30, oolong – 8-20, black – 3-10 (in % of dry weight).

The published literature provides some conflicting information regarding the total content of polyphenols in green and black teas, with some researchers reporting 67.7 mg/g of polyphenols in black tea and 62.3 mg/g in green tea (Hoff & Singletion, 1977); with others reporting a higher content of polyphenols in green tea compared to black tea, namely, 95.4 mg/g and 80.1 mg/g respectively (Manzocco et al., 1998). This difference may be related to many factors, such as type of tea, storage conditions, extraction conditions, etc.

The primary phenolic components of black tea are theaflavins, thearubigins, and theabrownins. These compounds were measured in 56 grades of tea purchased in Australian surpermarkets (Yao et al., 2006). The content of theaflavins varied within the range of 0.29-1.25%. Low content of theaflavins suggests incomplete fermentation and/or long storage period. Solubility of thearubigins and theabrownins in tea bags was between 82 and 92%. This indicates differing permeability of tea bags.

Theaflavins are the first oxidation products of catechins and catechin gallates during the fermentation process. Theaflavins can be further oxidized forming more highly polymerized molecules, thearubigins; yet further oxidation creates condensed theabrownins, which presumably are thearubigins bonded with proteins.

Thearubigins in general are characterized as fractions insoluble in ethyl acetate which are separated by butanol into two fractions, both soluble and insoluble (Roberts et al., 1956). Theaflavins impart an astringent taste to black tea, and a bright golden color to brewed black tea. Meanwhile, thearubigins contribute a reddish color and full rich taste, theabrownins impart a dark brown color and negatively affect the quality of tea. Theaflavins and thearubigins should not be present in pure green teas, or should be present only in very small amounts.

However, in some green teas purchased in Australian supermarkets theaflavins and thearubigins were detected in quantities close to those contained in black teas. This fact may indicate that some production and/or storage protocols for these green teas were breached. Therefore, the analysis of tea polyphenols contained in the final product may be used to control shortcomings of production processes and violation of storage conditions.

For assessment of black tea quality, we suggest consideration of the proportion of theaflavins concentration to the thearubigin concentration. Fresh good black tea must contain over 1% of theaflavins and approximately 10% of thearubigins, and their ratio must be greater than 0.1. These indicators were measured for the most popular grades of black tea (Hara et al., 1995). Tea tasters confirm the high quality of those teas in which the ratio of theaflavins to thearubigins is greater than 0.1. Therefore, the above ratio could be used for assessment of tea quality as well as for ensuring the right production technology and storage conditions.

As early as 1972, it was determined that black tea contains 5% unoxidized polyphenols and 25% oxidized (Sanderson, 1972). Brewed black tea contains only 4.5% simple polyphenols and 15% oxidized polyphenols by total weight of dry tea, i.e., the total content of polyphenols is 19.5%.

It was determined that the total content of polyphenols in Kenyan black tea is 20% (5% simple unoxidized polyphenols, 2% theaflavins, and 13% thearubigins) (Obanda et al., 1997). Many Indian teas were found to contain an average of 20% polyphenols (Dev Choudhury & Goswami, 1983).

The total content of polyphenols in green teas ranged between 21-33% (average 25%).

The difference in the content of polyphenols, both in black and green teas, is connected with the place where they were grown: tea from India and Sri Lanka usually has higher total polyphenols content than teas from China. An overall aggregate content of polyphenols in two grades of green tea from Sri Lanka was determined as 26% and 34%, whereas Chinese teas had only 21% and 23% (Owuor et al., 1987).

Therefore, the total content of polyphenols, in addition to the aromaticity index, can be used to assess the origin and quality of tea (Owuor et al., 1987) the content of amino acids (Hara et al., 1995) and caffeine (Owuor and Chavanji, 1986). Low total polyphenols content in some teas, both black and green, may indicate that these teas were stored for too long. In the case of black teas it can also suggest their incomplete fermentation.

6. Conclusion

As follows from this article, the composition of the same type of tea may vary significantly depending on the place where it was grown (i.e., soil, climate, height, precipitation, etc.), production technology, storage conditions, etc.

The composition of green and black teas accounting for possible variations is provided in Table 15 (Robb and Brown, 2001; Peterson et al., 2005)

Table 15. The variation in composition of green and black teas

No.	Compound	Content	
		Green tea	Black tea
1.	Catechins	10-30%	3-10%
2.	Theaflavins	0	2-6%
3.	Thearubigins	0	10-20%
4.	Phenolic acids	2%	1%
5.	Flavonols	2%	1%
6.	Other polyphenols	3-6%	3-10%
7.	Caffeine, theobromine, and theophylline	3-6%	3-6%
8.	Amino acids	~10 mg/g	~5 mg/g
9.	Theanine	2%	–
10.	Peptides, proteins	6%	6%
11.	Organic acids	2%	2%
12.	Mono- and disaccharides	11 mg/g	11 mg/g
13.	Mineral substances	10-13%	10-13%

The total content of polyphenols can serve as an indicator of tea quality.

The presence of theaflavin and thearubigin in green tea indicates breach of production technology and storage conditions (these components should not be present in true green tea).

For assessment of black tea quality, we suggest consideration of the proportion of theaflavins concentration to the thearubigin concentration. High-quality black tea must contain over 1% of theaflavins and approximately 10% of thearubigins, and their ratio must be greater than 0.1. Tea tasters confirm the high quality of black tea only at such ratio of theaflavins to thearubigins.

Therefore, the analysis data on aggregate polyphenols as well as their individual constituents may be used for the overall assessment of tea quality, production technology, and storage conditions. Many analytical methods have been developed for the analysis of bioactive compounds in tea, including catechins, theaflavins, thearubigins, and methyl xanthines. HPLC methods are by far the most common procedures employed due to its separation power and its capabilities of coupling to different highly sensitive detectors such as ESD and MS. LS-MS is most powerful tool for studying tea bioactive components in complex biological matrices (Wong et al., 2009).

References

Adnan, M., Ahmad, A., Khalid, N., Hayat, I., & Ahmed, I. (2013) Chemical composition and sensory evaluation of tea (*Camellia sinensis*) commercialized in Pakistan. *Pakistan Journal of Botany, 45*, 901-909.

Arce, L., Rios, A., & Valcarcel, M. (1998). Determination of anti-carcinogenic polyphenols present in green tea using capillary electrophoresis coupled to a flow injection system. *Journal of Chromatography A, 827*, 113-120. http://dx.doi.org/10.1016/S0021-9673(98)00737-7

Arts, I. C. W., Van de Putte, B., & Hollman, P. C. H. (2000). Catechin Content of Foods CommonlyConsumed in the Netherlands 2. Tea, Wine, Fruit Juices, and Chocolate Milk. *Journal of Agriculture Food Chemistry, 48*, 1752-1757. http://dx.doi.org/10.1021/jf000026+

Balentine, D. A., Wiseman, S. A., & Bouwens, L. C. M. (1997). The Chemistry of Tea Flavonoids. *Critical Reviews in Food Science and Nutrition, 37*, 693-704. http://dx.doi.org/10.1080/10408399709527797

Baptista, J. A. B., da P. Tavares, J. F., & Carvalho, R. C. B. (1999). Comparative study and partial

characterization of Azorean green tea polyphenols. *Journal of Food Composition and Analysis, 12*, 273-287. http://dx.doi.org/10.1006/jfca.1999.0829

Baptista, J. A. B., Tavares, J. F. D., & Carvalho, R. C. B. (1998). Comparison of catechins and aromas among different green teas using HPLC/SPME-GC. *Food Research International, 31*, 729-736. http://dx.doi.org/10.1016/S0963-9969(99)00052-6

Barroso, M. B., & van de Werken, G. (1999). Determination of green and black tea composition by capillary electrophoresis. *Hrc-Journal of High Resolution Chromatography, 22*, 225-230. http://dx.doi.org/10.1002/(SICI)1521-4168(19990401)22:4%3C225::AID-JHRC225%3E3.0.CO;2-V

Baumann, D., Adler, S., & Hamburger, M. (2001). A simple isolation method for the major catechins in green tea using high-speed countercurrent chromatography. *Journal of Natural Products, 64*, 353-355. http://dx.doi.org/10.1021/np0004395

Bilia, A. R., Flamini, G., Taglioli, V., Morelli, I., & Vincieri, F. F. (2002). GC-MS analysis of essential oil of some commercial Fennel teas. *Food Chemistry, 76*, 307-310. http://dx.doi.org/10.1016/S0308-8146(01)00277-1

Blythe, C., & Pereira, L. (2006). *Selectivity Screening in the HPLC Analysis of Catechins. LC-GC* Europe.

Bokuchava, M. A., & Skobeleva, N. I. (1986). In I. D. Morton& A. J. McLeod (Eds.), *Food Flavours, Part B. The Flavour of Beverages* (p. 49), Elsevier.

Bokuchava, M. A., & Skobeleva, N. I. (1969). The Chemistry and Biochemistry of Tea and Tea Manufacture. *Advance Food Research, 17*, 215. http://dx.doi.org/10.1016/S0065-2628(08)60311-0

Bokuchava, M. A., Skobeleva, N. I., & Sanderson, G. W. (1980). The biochemistry and technology of tea manufacture. *Critical Reviews in Food Science and Nutrition, 12*, 303-370. http://dx.doi.org/10.1080/10408398009527280

Bondarovich, H. A., Giammarino, A. S., Renner, J. A., Sheppard, F. W., Shingler, A. J., & Gianturo, O. M. (1967). The Volatile Constituents of Tea. *Journal of Agricultural and Food Chemistry, 15*, 36-47. http://dx.doi.org/10.1021/jf60149a011

Bramati, L., Aquilano, F., & Pietta, P. (2003). Unfermented rooibos tea: Quantitative characterization of flavonoids by HPLC-UV and determination of the total antioxidant activity. *Journal of Agricultural and Food Chemistry, 51*, 7472-7474. http://dx.doi.org/10.1021/jf0347721

Bramati, L., Minoggio, M., Gardana, C., Simonetti, P., Mauri, P., & Pietta, P. (2002). Quantitative characterization of flavonoid compounds in Rooibos tea (Aspalathus linearis) by LC-UV/DAD. *Journal of Agricultural and Food Chemistry, 50,* 5513-5519. http://dx.doi.org/10.1021/jf025697h

British Standard BS ISO 14502-1:2005 Determination of substances characteristic of green and black tea. Part 1. Content of total polyphenols in tea – colorimetric method using Folin-Chiocalteu reagent. Part 2. Content of catechins in green tea – method using HPLC.

Bronner W. E., & Beecher G. R. (1998). Determination of Catechin Content in Brewed Tea by HPLC. *Journal of Chromatography, 805*, 137-142. http://dx.doi.org/10.1016/S0021-9673(98)00040-5

Butt, M., Ahmad, R., Sultan, M., Nasir, Q. M., & Naz, A. (2015). Green tea and anticancer perspectives: Updates from last decade. *Critical Reviews in Food Science and Nutrition, 55*, 6, 792-805. http://dx.doi.org/10.1080/10408398.2012.680205

Cao, X. L., Lewis, J. R., & Ito, Y. (2004). Application of high-speed countercurrent chromatography to the separation of black tea theaflavins. *Journal of Liquid Chromatography & Related Technologies, 27*, 1893-1902. http://dx.doi.org/10.1081/JLC-120038775

Carducci, C. N., Dabas, P. C., & Muse, J. O. (2000). Determination of inorganic cations by capillary ion electrophoresis in Ilex paraguariensis (St. H.) a plant used to prepare tea in South America. *Journal of Aoac International, 83*, 1167-1173.

Chen, C. W., Chang, Y. W., & Hwang, L. C. (1998). Rapid Analysis of Theaflavins by HPLC-MS (Atmospheric Pressure Chemical Ionization). *Journal of Food Drug Analysis, 6*, 713-718.

Chi, L. Z., Li, Z. C., Dong, S. Q., He, P. G., Wang, Q. J., & Fang, Y. Z. (2009). Simultaneous determination of flavonoids and phenolic acids in Chinese herbal tea by beta-cyclodextrin based capillary zone electrophoresis. *Microchimica Acta, 167*, 179-185. http://dx.doi.org/10.1007/s00604-009-0229-0

Cho, S. K., El-Aty, A. M. A., Choi, J. H., Jeong, Y. M., Shin, H. C., Chang, B. J., … Shim, J. H. (2008). Effectiveness of pressurized liquid extraction and solvent extraction for the simultaneous quantification of 14 pesticide residues in green tea using GC. *Journal of Separation Science, 31*, 1750-1760. http://dx.doi.org/10.1002/jssc.200800010

Ciecierska, M., & Obiedzinski, M. (2009). The GC-MS analysis of polycyclic aromatic hydrocarbons content in selected fruit and herbal teas. *Herba Polonica, 55*, 18-24.

Cimpoiu, C., Hosu, A., Seserman, L., Sandru, M., & Miclaus, V. (2010). Simultaneous determination of methyl-xanthines in different types of tea by a newly developed and validated TLC method. *Journal of Separation Science, 33*, 3794-3799. http://dx.doi.org/10.1002/jssc.201000554

Clark, T. J., & Bunch, J. E. (1997). Determination of volatile acids in tobacco, tea, and coffee using derivatization-purge and trap gas chromatography-selected ion monitoring mass spectrometry. *Journal of Chromatographic Science, 35*, 206-208. http://dx.doi.org/10.1093/chromsci/35.5.209

Clifford, M. N., Stoupi, S., & Kuhnert, N. (2007). Profiling and characterization by LC-MSn of the galloylquinic acids of green tea, tara tannin, and tannic acid. *Journal of Agricultural and Food Chemistry, 55*, 2797-2807. http://dx.doi.org/10.1021/jf0635331

Cordero, C., Canale, F., Del Rio, D., & Bicchi, C. (2009). Identification, quantitation, and method validation for flavan-3-ols in fermented ready-to-drink teas from the Italian market using HPLC-UV/DAD and LC-MS/MS. *Journal of Separation Science, 32*, 3643-3651. http://dx.doi.org/10.1002/jssc.200900369

Dalluge J. J., & Nelson B. C. (2000). Determination of Tea Catechins. *Journal of Chromatography, 881*, 411-424. http://dx.doi.org/10.1016/S0021-9673(00)00062-5

Degenhardt, A., Engelhardt, U. H., Lakenbrink, C., & Winterhalter, P. (2000). Preparative separation of polyphenols from tea by high-speed countercurrent chromatography. *Journal of Agricultural and Food Chemistry, 48*, 3425-3430. http://dx.doi.org/10.1021/jf0000833

Degenhardt, A., Engelhardt, U. H., Wendt, A. S., & Winterhalter, P. (2000). Isolation of black tea pigments using high-speed countercurrent chromatography and studies on properties of black tea polymers. *Journal of Agricultural and Food Chemistry, 48*, 5200-5205. http://dx.doi.org/10.1021/jf000757+

del Mar Caja, M., Preston, C., Menzel, M., Kempf, M., & Schreier, P. (2009). Online Gas Chromatography Combustion/Pyrolysis-Isotope Ratio Mass Spectrometry (HRGC-C/P-IRMS) of (+/-)-Dihydroactinidiolide from Tea (Camellia sinensis) and Rooibos Tea (*Aspalathus linearis*). *Journal of Agricultural and Food Chemistry, 57*, 5899-5902. http://dx.doi.org/10.1021/jf9009125

del Rio, D., Stewart, A., Mullen, W., Burns, J., Lean, M., Brighenti, F., & Crozier, A. (2004). HPLC-MSn analysis of phenolic compounds and purine alkaloids in green and black tea. *Journal Agriculture and Food Chemistry, 52*(10), 2807-2815. http://dx.doi.org/10.1021/jf0354848

Dev Choudhury, M. N., & Goswami, M. R. (1983). A rapid method for determination of total polyphenolicmatters in tea (*Camellia sinensis* L). *Two and A Bud, 30*, 59-61.

Ding, M. Y., Chen, P. R., & Luo, G. A. (1997). Simultaneous determination of organic acids and inorganic anions in tea by ion chromatography. *Journal of Chromatography A, 764*, 341-345. http://dx.doi.org/10.1016/S0021-9673(96)00910-7

Ding, Y., Yu, H., & Mou, S. (2002). Direct Determination of Free Amino Acids and Sugars in Green Tea byAnion Exchange Chromatography with Pulsed Amperometric Detection. *Journal of Chromatography, 982*, 237-244. http://dx.doi.org/10.1016/S0021-9673(02)01650-3

Ding, Z., Kuhr, S., & Engelhard, U. H. (1992). Influence of Catechins and Theaflavins on the Taste of the Brewed Black Tea. *Z. Lebensm. Unters Forsch, 195*, 108-111. http://dx.doi.org/10.1007/BF01201768

Ding, M.-Y, Chen, P.-R., & Luo, G.-A. (1997). Simultaneous determination of organic acids and inorganic anions in tea by ion chromatography. *Journal of Chromatography A, 64*, 341-345. http://dx.doi.org/10.1016/S0021-9673(96)00910-7

Donovan, J. L., Luthria, D. L., Stremple, P., & Waterhouse, A. L. (1999). Analysis of (+)-Catechin, (-)Epicatechin and their 3'- and 4'-0-Methylated Analogs. *Journal of Chromatography. B, 726*, 277-283. http://dx.doi.org/10.1016/S0378-4347(99)00019-5

Du, Q. Z., Jiang, H. Y., & Ito, Y. (2001). Separation of theaflavins of black tea. High-speed countercurrent chromatography vs. sephadex LH-20 gel column chromatography. *Journal of Liquid Chromatography &*

Related Technologies, 24, 2363-2369. http://dx.doi.org/10.1081/JLC-100105147

El-Hady, D. A., & El-Maali, N. A. (2008). Determination of catechin isomers in human plasma subsequent to green tea ingestion using chiral capillary electrophoresis with a high-sensitivity cell. *Talanta, 76*, 138-145. http://dx.doi.org/10.1016/j.talanta.2008.02.026

Fanali, C., Rocco, A., Aturki, Z., Mondello, L., & Fanali, S. (2013). Analysis of polyphenols and methylxantines in tea samples by means of nano-liquid chromatography utilizing capillary columns packed with core shell particles *Journal of Chromatography, 1234*, 38-44. http://dx.doi.org/10.1016/j.chroma.2011.12.103

Fecka, I., Cisowski, W., & Luczkiewicz, M. (2001). TLC Determination of Catechins and Epicatechins in Tea using Chemically Modified Phases. *Journal of Planar Chromatography Modern TLC, 14*, 405-408.

ISO Standards for tea. Intergovernmental group – on tea, 10th session. New Delhi, 12-14 may 2010. ISO/34 SC8 Tea.

Feng, H. T., Wang, T. L., & Li, S. F. Y. (2003). Sensitive determination of trace-metal elements in tea with capillary electrophoresis by using chelating agent 4-(2-pyridylazo) resorcinol (PAR). *Food Chemistry, 81*, 607-611. http://dx.doi.org/10.1016/S0308-8146(02)00561-7

Finger, A., Kuhr, S., & Engelhardt, U. H. (1992). Chromatography of Tea Constituents. *Journal of Chromatography, 624,* 293-315. http://dx.doi.org/10.1016/0021-9673(92)85685-M

Flaten, A. K., & Lund, W. (1997). Speciation of aluminium in tea infusions studied by size exclusion chromatography with detection by post-column reaction. *Science of the Total Environment, 207*, 21-28. http://dx.doi.org/10.1016/S0048-9697(97)00239-8

Guth, H., & Grosch, W. (1993). Identification of Green and Black Tea Aromas using Head-Space Method. *Flavour Fragrance Journal, 8*, 173-178.

Gotti, R., Furlanetto, S., Lanteri, S., Olmo, S., Ragaini, A., & Cavrini, V. (2009). Differentiation of green tea samples by chiral CD-MEKC analysis of catechins content. *Electrophoresis, 30*, 2922-2930. http://dx.doi.org/10.1002/ffj.2730080402

Gu, X. G., Yao, C. C., Zhang, Z. Z., Wan, X. C., Ning, J. M., & Shao, W. F. (2011). GC-ECD Method for Determination of Glucosidically Bound Aroma Precursors in Fresh Tea Leaves. *Chromatographia, 73*, 189-193. http://dx.doi.org/10.1007/s10337-010-1816-2

Guillarme, D., Casetta, C., Bicchi, C., & Veuthey, J. L. (2010). High throughput qualitative analysis of polyphenols in tea samples by ultra-high pressure liquid chromatography coupled to UV and mass spectrometry detectors. *Journal of Chromatography A, 1217*, 6882-6890. http://dx.doi.org/10.1016/j.chroma.2010.08.060

Guth, H., & Grosch, W. (1993). Identification of Potent Odourants in Green and Black Tea by Static Head Space Gas Chromatography. *Flavour Fragrance Journal, 8*, 173-178. http://dx.doi.org/10.1002/ffj.2730080402

Hara, Y., Luo, S. J., & Wickremasinghe, R. L. (1995). Yamanishi T. Special Issue on Tea. *Food Reviews International, 11*, 371-545.

Harbowy, M. E., & Balentine, D. A. (1997). Tea Chemistry. *Critical Reviews in Food Science and Nutrition, 16*, 415-480.

Haslam, E. (2003). Thoughts on Thearubigins: Review. *Phytochemistry, 64*, 61-73. http://dx.doi.org/10.1016/S0031-9422(03)00355-8

Heins, J. T., Maarse, H, Noever de Brauw, M. C., & Weurman, C. (1966). Durect food vapour analysis and component identification by a coupled capillary GLC-MS arrangement. *Journal of Gas Chromatography, 4*, 395-397. http://dx.doi.org/10.1093/chromsci/4.11.395

Hertog, M. G. L., Hollman, P. C. H., & van de Putte, B. (1993). The Content of Potentially Anticarcinogenic Flavonoids in Brewed Tea. *The Journal of Agricultural and Food Chemistry, 41*, 1242-246. http://dx.doi.org/10.1021/jf00032a015

Ho, C.-T., Lin, J.-K., & Shahidi, F. (2009). *Tea and Tea Products* (p. 305). London: CRS Press Taylor and Francis Group.

Ho, Y., Lee, Y. L., & Hsu, K. Y. (1995). Determination of (+)-Catechin in Plasma by HPLC with Fluorometric Detection. *Journal of Chromatography B, 665*, 383-389. http://dx.doi.org/10.1016/0378-4347(94)00535-D

Hoeffer, A. C., & Coggon, P. (1976). HPLC of the Constituents. *Journal of Chromatography, 129*, 460-469.

Hoff, J. E., & Singletion, K. I. (1977). A method for determination of tannins in foods by means of immobilized protein. *Journal of Food Science, 42*, 1566-1569. http://dx.doi.org/10.1111/j.1365-2621.1977.tb08427.x

Horie, H., & Kohata, K. (2000). Analysis of tea components by high-performance liquid chromatography and high-performance capillary electrophoresis. *Journal of Chromatography A, 881*, 425-438. http://dx.doi.org/10.1016/S0021-9673(99)01345-X

Horie, H., Mukai, T., & Kohata, K. (1997). Simultaneous determination of qualitatively important components in green tea infusions using capillary electrophoresis. *Journal of Chromatography A, 758*, 332-335. http://dx.doi.org/10.1016/S0021-9673(96)00764-9

Horie, H., Yamauchi, Y., & Kohata, K. (1998). Analysis of organic anions in tea infusions using capillary electrophoresis. *Journal of Chromatography A, 817*, 139-144. http://dx.doi.org/10.1016/S0021-9673(98)00340-9

Hsieh, M. M., & Chen, S. M. (2007). Determination of amino acids in tea leaves and beverages using capillary electrophoresis with light-emitting diode-induced fluorescence detection. *Talanta, 73*, 326-331. http://dx.doi.org/10.1016/j.talanta.2007.03.049

Hu, C. D., Liang, Y. Z., Guo, F. Q., Li, X. R., & Wang, W. P. (2010). Determination of Essential Oil Composition from Osmanthus fragrans Tea by GC-MS Combined with a Chemometric Resolution Method. *Molecules, 15,* 3683-3693. http://dx.doi.org/10.3390/molecules15053683

Hu, H., Ding, N., & Wu, L. (2007). Determination of residual ethyl acetate in green tea extract by headspace gas chromatography. *Journal of Tea Science, 27*, 67-70.

Huang, S. P., & Huang, S. D. (2006). Dynamic hollow fiber protected liquid phase microextraction and quantification using gas chromatography combined with electron capture detection of organochlorine pesticides in green tea leaves and ready-to-drink tea. *Journal of Chromatography A, 1135,* 6-11. http://dx.doi.org/10.1016/j.chroma.2006.09.027

Huang, Z. Q., Zhang, Y., Wang, L. B., Ding, L., Wang, M. L., Yan, H. F., … Zhu, S. H. (2009). Simultaneous determination of 103 pesticide residues in tea samples by LC-MS/MS. *Journal of Separation Science, 32*, 1294-1301. http://dx.doi.org/10.1002/jssc.200800605

Jiang, G., & Zhang, T. (2014). Quantitative analysis of catechins in tea by UHPLC/UV. Thermo Fisher scientific. Analytical application.

Jumtee, K., Komur, H., Bamba, T., & Fukusaki, E. (2011). Prediction of Japanese green tea (sen-cha) ranking by volatile profiling using GC-MS and multivariate analysis. *Journal of Bioscience and Bioengineering, 112*, 252-255. http://dx.doi.org/10.1016/j.jbiosc.2011.05.008

Kanrar, B., Mandal, S., & Bhattacharyya, A. (2010). Validation and uncertainty analysis of a multiresidue method for 42 pesticides in made tea, tea infusion and spent leaves using ethyl acetate extraction and liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A, 1217*, 1926-1933. http://dx.doi.org/10.1016/j.chroma.2010.01.062

Kanrar, B., Mandal, S., & Bhattacharyya, A. (2010) Validation and Uncertainty Analysis of a Multiresidue Method for 67 Pesticides in Made Tea, Tea Infusion, and Spent Leaves Using Ethyl Acetate Extraction and Gas Chromatography/Mass Spectrometry. *Journal of Aoac International, 93*, 411-424.

Kartsova, L. A., & Alekseeva, A. V. (2008). Chromatographic and Electrophoretic Methods for Determination of Polyphenolic Compounds. *Journal of Analytical Chemistry, 63*, 1126-1136. http://dx.doi.org/10.1134/S1061934808110026

Kartsova, L. A., & Alekseeva, A. V. (2009). Selective Complexation of Catechols with Fe+3 Ions in Determining Caffeine in Tea by High-Performance Thin-Layer Chromatography. *Journal of Analytical Chemistry, 64*, 930-934. http://dx.doi.org/10.1134/S106193480909010X

Kartsova, L. A., & Ganzha, O. V. (2006). Electrophoretic separation of tea flavanoids in the modes of capillary (zone) electrophoresis and micellar electrokinetic chromatography. *Russian Journal of Applied Chemistry, 79*, 1110-1114. http://dx.doi.org/10.1134/S1070427206070135

Kasai, N., & Nakatsubo, G. (2006). Size-exclusion chromatography of tea tannins and intercepting potentials of peptides for the inhibition of trypsin-caseinolytic activity by tea tannins. *Journal of Agricultural and Food Chemistry, 54*, 5149-5156. http://dx.doi.org/10.1021/jf060435r

Kayali-Sayadi, M. N., Rubio-Barroso, S., Cuesta-Jimenez, M. P., & Palo-Diez, L. M. (1998). Rapid

determination of polycyclic aromatic hydrocarbons in tea infusion samples by high-performance liquid chromatography and fluorimetric detection based on solid-phase extraction. *Analyst, 123*, 2145-2148. http://dx.doi.org/10.1039/a803967d

Khokhar, S., & Magnusdottir, S. G. M. (2002). Total Phenol, Catechin, and Caffeine Contents of Tea Commonly Consumed in the United Kingdom. *Journal of Agricultural and Food Chemistry, 50*, 565-570. http://dx.doi.org/10.1021/jf0101531

Kiehne, A., & Engelhardt, U. H. (1996). Thermospray-LC-Ms analysis of various groups of polyphenols in tea .2. Chlorogenic acids, theaflavins and thearubigins. *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung, 202*, 299-302. http://dx.doi.org/10.1007/BF01206100

Kiehne, A., Lakenbrink, C., & Engelhardt, U. H. (1997). Analysis of proanthocyanidins in tea samples. LC-MS results. Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung a-*Food Research and Technology, 205*, 153-157. http://dx.doi.org/10.1007/s002170050144

Kim, J. I., Hong, S. B., & Row, K. H. (2002). Effect of Particle Size in Preparative Reversed-Phase HPLC on the Isolation of Epigallocatechin Gallate from Korean Green Tea. *Journal of Chromatography, 949*, 275-280. http://dx.doi.org/10.1016/S0021-9673(01)01273-0

Kinugasa H., & Takaeo T. (1990). Deterioration Mechanism for Tea Infusion Aroma by Retort Pasteurization. *Agricultural and Biological Chemistry, 54*, 2537-2542. http://dx.doi.org/10.1271/bbb1961.54.2537

Kodama, S., Ito, Y., Taga, A., Nomurad, Y., Yamamoto, A., Chinaka, S., … Hayakawa, K. (2008). A fast and simple analysis of glyphosate in tea beverages by capillary electrophoresis with on-line copper(II)-glyphosate complex formation. *Journal of Health Science, 54*, 602-606. http://dx.doi.org/10.1248/jhs.54.602

Kodama, S., Yamamoto, A., Matsunaga, A., & Yanai, H. (2004). Direct enantioseparation of catechin and epicatechin in tea drinks by 6-O-alpha-D-glucosyl-beta-cyclodextrin-modified micellar electrokinetic chromatography. *Electrophoresis, 25*, 2892-2898. http://dx.doi.org/10.1002/elps.200305902

Koenig, W. A., Evers, P., Krebber, R., Schulz, S., Fehr, C., & Ohloff, G. (1989). Determination of the Absolute Configuration of Alpha Damascone and Alpha Ionone from Black Tea by Enantioselective Capillary Gas Chromatography. *Tetrahedron, 45*, 7003-7006. http://dx.doi.org/10.1016/S0040-4020(01)89167-5

Kofink, M. (2007). Paragiannopoulos M., Galensa R. Enantiomeric Separation of Catechin and Epicatechin. *European Foods Research and Technology, 225*, 569-579. http://dx.doi.org/10.1007/s00217-006-0455-1

Köhler N., Chou, E.-F. Ito, Y., & Winterhalter, P. (2004). Application of high-speed countercurrent chromatography to the separation of black teatheaflavins. *Journal of Liquid Chromatography Technology*, *27*, 1893-1902. http://dx.doi.org/10.1081/JLC-120038775

Kralj, B., Krizaj, I., Bukovec, P., Slejko, S., & Milacic, R. (2005). Speciation of aluminium in tea infusions by use of SEC and FPLC with ICP-OES and ES-MS-MS detection. *Analytical and Bioanalytical Chemistry, 383*, 467-475. http://dx.doi.org/10.1007/s00216-005-3312-3

Kuang, H., Miao, H., Hou, X. L., Zhao, Y. F., Wu, Y. N., & Xu, C. L. (2010). Simultaneous Determination of 16 Pyrethroid Residues in Tea Samples Using Gas Chromatography and Ion Trap Mass Spectrometry. *Journal of Chromatographic Science, 48*, 771-776. http://dx.doi.org/10.1093/chromsci/48.9.771

Kuhnert, N., Clifford, M. N., & Muller, A. (2010). Oxidative cascade reactions yielding polyhydroxy-theaflavins and theacitrins in the formation of black tea thearubigins: evidence by tandem LC-MS. *Food and Function 1*, 180-199. http://dx.doi.org/10.1039/c0fo00066c

Kumamoto, M., & Sonda, T. (2000). Tabata M. Enhanced Separation of Catechins by HPLC Using Mixed Solvents of Water, Acetonitrile and Ethyl Acetate as the Mobile Phase. *Analytical Science, 16*, 139-144. http://dx.doi.org/10.2116/analsci.16.139

Kumar, S. D., Narayan, G., & Hassaraiani, S. (2008). Determination of anionic minerals in black and kombucha tea using ion chromatography. *Food Chemistry, 111*, 784-788. http://dx.doi.org/10.1016/j.foodchem.2008.05.012

Lee, B. L., & Ong, C. N. (2000). Comparative analysis of tea catechins and theaflavins by highperformance liquid chromatography and capillary electrophoresis. *Journal of Chromatography A, 881*, 439-447. http://dx.doi.org/10.1016/S0021-9673(00)00215-6

Lee, S. H., Jeon, C. W., & Wabaidur, S. M. (2008). Flow-injection chemiluminescence determination of aspartic

acid in tea leaves using tris (2,2 '-bipyridyl) ruthenium (II)-Ce(IV) system. *Journal of Fluorescence, 18*, 655-660. http://dx.doi.org/10.1007/s10895-008-0387-1

Li, H. P., Li, G. C., & Jen, J. F. (2004). Fast multi-residue screening for 84 pesticides in tea by gas chromatography with dual-tower auto-sampler, dual-column and dual detectors. *Journal of the Chinese Chemical Society, 51*, 531-542.

Li, M. J., Zhou, J. Y., Gu, X., Wang, Y., Huang, X. J., & Yan, C. (2009). Quantitative capillary electrophoresis and its application in analysis of alkaloids in tea, coffee, coca cola, and theophylline tablets. *Journal of Separation Science, 32*, 267-274. http://dx.doi.org/10.1002/jssc.200800529

Li, Z. B., Huang, D. N., Tang, Z. X., & Deng, C. H. (2010). Microwave-assisted extraction followed by CE for determination of catechin and epicatechin in green tea. *Journal of Separation Science, 33*, 1079-1084.

Ligor, M., Kornysova, O., Maruska, A., & Buszewski, B. (2008). Determination of Flavonoids in Tea and Rooibos Extracts by TLC and HPLC. *Jpc-Journal of Planar Chromatography-Modern Tlc, 21*, 355-360. http://dx.doi.org/10.1556/JPC.21.2008.5.7

Lin, J. K., Lin, C. L., Liang, Y. C., Lin-Shiau, S. Y., & Juan, I. M. (1998). Review of Catechins, Gallic Acid, and Methylxanthines in Green, Oolong, Black, and Pu-erh Teas. *Journal of Agricultural and Food Chemistry, 46*, 3635-3642. http://dx.doi.org/10.1021/jf980223x

Lin, Y.-S., Tsai, Y.-J., Tsay, J.-S., & Lin, J.-K. (2003). Factors Affecting the Levels of Tea Polyphenols and Caffeine in Tea Leaves. *Journal of Agricultural and Food Chemistry, 51*, 1864-1873. http://dx.doi.org/10.1021/jf021066b

Long, H., Zhu, Y., & Cregor, M. (2001). HPLC with Multi-Channel Electrochemical Detection for the Determination of Epigallocatechin Gallate in Rat Plasma. *Journal of Chromatography B, 763*, 47-57.

Lozano, A., Rajski, L., & Bellmonte – Valles, N. (2013). Pesticide analysis in teas and chamomile by LC and GC-MS/MS using modified QuEchERS methods validation and pilot survey in real samples. *J. Chromat, 1268*, 109-122. http://dx.doi.org/10.1016/j.chroma.2012.10.013

Lu, H. P., Zhang, Q. S., Lin, Z., Wang, L., Tan, J. F., & Guo, L. (2012). Aroma characterization of Pu-erth tea using headspace – solid phase microextraction combined with GC-MS and GC-olfactometry. *Food Chem, 130*, 1074-1081. http://dx.doi.org/10.1016/j.foodchem.2011.07.135

Lv, Y., Yang, X. B., Zhao, Y., Ruan, Y., Yang, Y., & Wang, Z. Z. (2009). Separation and quantification of component monosaccharides of the tea polysaccharides from Gynostemma pentaphyllum by HPLC with indirect UV detection. *Food Chemistry, 112*, 742-746. http://dx.doi.org/10.1016/j.foodchem.2008.06.042

Mandel, S., & Yodim, M. (2004). Catechin polyphenols: Neurodegeneration and neuroprotection in neurodegenerative diseases. *Free Radical Biology and Medicine, 37*, 304-307. http://dx.doi.org/10.1016/j.freeradbiomed.2004.04.012

Manzocco, L., Anese, M., & Nicoli, M. C. (1998). Antioxidant Properties of Tea Extracts as Affected by Processing. *Lebensmittel-Wissenschaft und-Technologie, 31*, 694-698. http://dx.doi.org/10.1006/fstl.1998.0491

Matsuura, H., Hokura, A., Katsuki, F., Itoh, A., & Haraguchi, H. (2001). Multielement determination and speciation of major-to-trace elements in black tea leaves by ICP-AES and ICP-MS with the aid of size exclusion chromatography. *Analytical Sciences, 17*, 391-398. http://dx.doi.org/10.2116/analsci.17.391

Menet, M. C., Sang, S., & Yang, C. S. (2004). Analysis of Theaflavins and Thearubigins from Black Tea by MC-Maldi-TOT. *Journal of Agricultural and Food Chemistry, 52*, 2455-2461. http://dx.doi.org/10.1021/jf035427e

Michalski, R. (2006). Simultaneous determination of common inorganic anions in black and herbal tea by suppressed ion chromatography. *Journal of Food Quality, 29*, 607-616. http://dx.doi.org/10.1111/j.1745-4557.2006.00098.x

Mick, W., & Schreier, P. (1984). Additional volatiles of black tea aroma. *Journal of Agricultural and Food Chemistry, 32*, 924-929. http://dx.doi.org/10.1021/jf00124a055

Mizukami, Y., Kohata, K., Yamaguchi, Y., Hayashi, N., Sawai, Y., Chuda, Y., … Yoshida, M. (2006). Analysis of acrylamide in green tea by gas chromatography - Mass spectrometry. *Journal of Agricultural and Food Chemistry, 54*, 7370-7377. http://dx.doi.org/10.1021/jf061029a

Mo, X. R., Zheng, C. H., Chen, J. W., Zhao, D. Y., & Yang, M. M. (2009). Cloud Point Extraction Coupled with Ultrasonic-assisted Back-extraction for Determination of Pyrethroid Pesticides in Tea by Gas Chromatography with Electron Capture Detection. *Chinese Journal of Analytical Chemistry, 37*, 1178-1182.

Moinfar, S., & Hosseini, M. R. M. (2009). Development of dispersive liquid-liquid microextraction method for the analysis of organophosphorus pesticides in tea. *Journal of Hazardous Materials, 169*, 907-911. http://dx.doi.org/10.1016/j.jhazmat.2009.04.030

Naczk, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. *Journal of ChromatographyA, 1054*, 95-111. http://dx.doi.org/10.1016/j.chroma.2004.08.059

Ning, P. B., Gong, C. M., Zhang, Y. M., & Guo, K. K. (2010). La, Ce, Pr, Nd and Sm concentrations in Pu'er tea of Yunnan, China. *Journal of Rare Earths, 28*, 636-640. http://dx.doi.org/10.1016/S1002-0721(09)60170-8

Nokagawa K., & Miyazawa T. (1997). HPLC with Chemiluminescence Detection of Catechins in Human Plasma. *Analytical Biochemistry, 248*, 41-49.

Novak, I., Seruga, M., & Komorsky-Lovric, S. (2010). Characterisation of catechins in green and black teas using square-wave voltammetry and RP-HPLC-ECD. *Food Chemistry, 122*, 1283-1289. http://dx.doi.org/10.1016/j.foodchem.2010.03.084

Obanda, M., Owuor, P. O., & Taylor, S. J. (1997). Flavanol Composition and Caffeine Content of Green Leaf as Quality Potential Indicators of Kenyan Black Teas. *Journal of the Science of Food and Agriculture, 74*, 209-215. http://dx.doi.org/10.1002/(SICI)1097-0010(199706)74:2%3C209::AID-JSFA789%3E3.0.CO;2-4

Odegard, K. E., & Lund, W. (1997). Multi-element speciation of tea infusion using cation-exchange separation and size-exclusion chromatography in combination with inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry, 12*, 403-408. http://dx.doi.org/10.1039/a606153b

Oellig, C., & Schwack, W. (2013). Planar solid phase extraction clean-up for pesticide residue analysis in tea by LC-MS. *J. Chrom, 1260*, 42-53. http://dx.doi.org/10.1016/j.chroma.2012.08.088

Owuor, P. O., & Chavanji, A. M. (1986). Caffeine contents of clonal tea; seasonal variations and effects of plucking standards under Kenyan conditions. *Food Chemistry, 20*, 225-233. http://dx.doi.org/10.1016/0308-8146(86)90175-5

Owuor, P. O., Horita, H., Tsushida, T., & Murai, T. (1987). Variations in the Chemical Composition of Some Kenyan Clonal Teas. *Journal of Science Series A., 8*, 29-32.

Owuor P. O., Othieno C. O., Robinson J. M., & Baker D. M. (1991). Changes in the quality parameters of seedling tea due to height and frequency of mechanical harvesting. *Journal of the Science of Food and Agriculture, 55*, 241-249. http://dx.doi.org/10.1002/jsfa.2740550209

Owuor, P. O., Takeo, T., Horita H., Tsushida, T., & Murai, T. (1987). Differentiation of clonal teas by terpene index. *Journal of the Science of Food and Agriculture, 40*, 341-345. http://dx.doi.org/10.1002/jsfa.2740400407

Page, B. D., & Charbonneau, C. F. (1984). Headspace Gas-Chromatographic Determination of Methylene-Chloride in Decaffeinated Tea and Coffee, with Electrolytic Conductivity Detection. *Journal of the Association of Official Analytical Chemists, 67*, 757-761.

Pierce, A. R., Graham, H. N., Glassner, S., Madlin, H., & Gonzalez, J. G. (1969). Analysis of Tea Flavanols by Gas Chromatography of their Trimethylsilyl Derivatives. *Analytical Chemistry, 41*, 298-302.

Pelillo, M., Biguzzi, B., Bendini, A., Toschi, T. G., Vanzini, M., & Lercker, G. (2002). Preliminary investigation into development of HPLC with UV and MS-electrospray detection for the analysis of tea catechins. *Food Chemistry, 78*, 369-374. http://dx.doi.org/10.1016/S0308-8146(02)00112-7

Pelillo, M., Bonoli, M., Biguzzi, B., Bendini, A., Toschi, T. G., & Lercker, G. (2004). An investigation in the use of HPLC with UV and MS-electrospray detection for the quantification of tea catechins. *Food Chemistry 87*, 465-470. http://dx.doi.org/10.1016/j.foodchem.2003.12.034

Peterson J., Dwyer, J., Bhagwat, S., Haytowitz, D., Holden, J., Eldridge, A. L., … Aladesamni, J. (2005). Major Flavonoids in Dry Tea. *Journal of Food Composition and Analysis, 18,* 487-501. http://dx.doi.org/10.1016/j.jfca.2004.05.006

Pongsuwan, W., Bamba, T., Yonetani, T., Kobayashi, A. K., & Fukusaki, E. (2008). Quality prediction of Japanese green tea using pyrolyzer coupled GC/MS based metabolic fingerprinting. *Journal of Agricultural*

and Food Chemistry, 56, 744-750. http://dx.doi.org/10.1021/jf072791v

Pongsuwan, W., Fukusaki, E., Bamba, T., Yonetani, T., Yamahara, T., & Kobayashi, A. (2007). Prediction of Japanese green tea ranking by gas chromatography/mass spectrometry-based hydrophilic metabolite fingerprinting. *Journal of Agricultural and Food Chemistry, 55*, 231-236. http://dx.doi.org/10.1021/jf062330u

Price, K. R., Rhodes, M. J. C., & Barnes, K. A. (1998). Flavonol Glycoside Content and Composition of Tea Infusions Made from Commercially Available Teas and Tea Products. *Journal of Agricultural and Food Chemistry, 46*, 2017-2522. http://dx.doi.org/10.1021/jf9800211

Price, W. E., & Spitzer, J. C. (1993). Variations in the Amounts of Individual Flavanols in a Range of Green Teas. *Food Chemistry, 47*, 271-276. http://dx.doi.org/10.1016/0308-8146(93)90160-H

Quantitative tea analysis. Occupational safety health administration (OSHA). Imis code number 117 GC/TEA.

Rawat, R., Gulati, A., Babu, G. D. K., Acharya, R., Kaul, V. K., & Singh, B. (2007). Characterization of volatile components of Kangra orthodox black tea by gas chromatography-mass spectrometry. *Food Chemistry, 105*, 229-235. http://dx.doi.org/10.1016/j.foodchem.2007.03.071

Rechner, A. R., Wagner, E., Van Buren, L., Van De Put, F., Wiseman, S., & Rice-Evans, C. A. (2002). Black Tea as a Major Source of Dietary Polyphenols among Regular Tea Drinkers. *Free Radical Research, 36*, 1127-1235. http://dx.doi.org/10.1080/1071576021000006707

Reto, M., Figueira, M. E., Filipe, H. M., & Almeida, C. M. M. (2007). Analysis of vitamin K in green tea leafs and infusions by SPME-GC-FID. *Food Chemistry, 100*, 405-411. http://dx.doi.org/10.1016/j.foodchem.2005.09.016

Reymond, D., Mueggler-Chavan, F., Viani, R., Vuataz, L., & Egli, R. (1966). Gas chromatographic analysis of steam volatile aroma constituents: application to coffe, tea and cocoa aromas. *Journal of Gas Chromatography, 4*, 28-31. http://dx.doi.org/10.1093/chromsci/4.1.28

Robb, C. S., & Brown, Ph. R. (2001). Catechins in Tea: Chemistry and Analysis. *Advances in Chromatography, 41,* 379-410.

Roberts, E. A. H., Cartwright, R. A., & Wood, D. J. (1956). The leuco-anthocyanins of unprocessed tea leaf. *Journal of the Science of Food and Agriculture, 7*, 253-257. http://dx.doi.org/10.1002/jsfa.2740070406

Sakakibara, H., Honda, Y., & Nakagowa, S. (2003) Simultaneous Determination of all Polyphenols in Vegetables, Fruits, and Teas. *Journal of Agricultural and Food Chemistry, 51*, 571-581. http://dx.doi.org/10.1021/jf0209261

Sampson, L., Rimm, E., Hollman, P. C., de Vries, J. H., & Katan, M. B. (2002). Flavonol and Flavone Intakes in US health professionals. *Journal of the American Diet Association, 102*, 1414-1420. http://dx.doi.org/10.1016/S0002-8223(02)90314-7

Sanderson, G. W. (1972). Chemistry of Tea Manufacturing. In V. C. Runeckles & T. C. Tso (Eds.), *Recent Advances in Phytochemistry: Structural and Functional Aspects of Phytochemistry* (pp. 247-316). New York: Academic Press.

Sano, M., Tabata, M., Suzuki, M., Degawa, M., Miyase, T., & Maeda-Yamamoto, M. (2001). Simultaneous Determination of Twelve Tea Catechinsby HPLC with Electrochemical Detection. *Analyst-London- Society of Public Analysts Then Royal Society of Chemistry, 126*, 816-820.

Savic, I. M., Nikolic, V. D., Savic, I. M., Nicilic, L. B., & Stankovic, M. S. (2013). Development and validation of a new RP-HPLC method for determination of quercetin in green tea *Journal of Analytical Chemistry, 68*, 906-911. http://dx.doi.org/10.1134/S1061934813100080

Savic, I. M., Nikolic, V. D., Savic, I. M., Nikolic, L., & Jovic, M. (2014). The qualitative analysis of the green tea extract using ESI-MS method. *Advanced Technologies, 3*(1), 30-37.

Sereshti, H., Samadi, S., & Jalali-Heravi, M. (2013). Determination of volatile components of green, black, oolong and white tea by optimizing ultrasound-assisted extraction-dispersive liquid-liquid microextraction coupled with gas chromatography. *Journal of Chromatography A., 1280*, 1-8. http://dx.doi.org/10.1016/j.chroma.2013.01.029

Schurek, J., Portoles, T., Hajslova, J., Riddellova, K., & Hernandez, F. (2008). Application of head-space solid-phase microextraction coupled to comprehensive two-dimensional gas chromatography-time-of-flight

mass spectrometry for the determination of multiple pesticide residues in tea samples. *Analytica Chimica Acta, 611*, 163-172. http://dx.doi.org/10.1016/j.aca.2008.01.007

Shimoda, M., Shigematsu, H., Sheratsuchi, H., & Osajima, Y. (1995). Comparison of Volatile Compounds among Different Grades of Green Tea and Their Relations to Odor Attributes. *Journal of Agricultural and Food Chemistry, 43*, 1621-1625. http://dx.doi.org/10.1021/jf00054a038

Singh, H. P., Ravindranath, S. D., & Singh, C. (1999). Analysis of Tea Catechins by Two-Dimensional Paper Chromatography with Spectrophotometric Visualization. *Journal of Agriculture Food Chemistry, 47*, 1041-1045. http://dx.doi.org/10.1021/jf9807263

Stalcup, A. M., Ekborg, K. H., Gasper, M. P., & Armstrong, D. W. (1993). Enantiomeric Separation of Chiral Components Reported to Be in Coffee, Tea, or Cocoa. *Journal of Agricultural and Food Chemistry, 41*, 1684-1689. http://dx.doi.org/10.1021/jf00034a031

Steinhaus, B., & Engelhardt, U. H. (1989). Theaflavins in Black Tea. *Z. Lebensm. Unters Forch, 188*, 509-511. http://dx.doi.org/10.1007/BF01043039

Steiniger, D., Lu, G. P., Butler, J., Phillips, E., & Fintschenko, Y. (2010). Determination of Multiresidue Pesticides in Green Tea by Using a Modified QuEChERS Extraction and Ion-Trap Gas Chromatography/Mass Spectrometry. *Journal of Aoac International, 93*, 1169-1179.

Stewart, A., Mullen, W., & Crozier, A. (2005). On-line high-performance liquid chromatography analysis of antioxidant activity of phenolic compounds in green and black tea. *Molecular Nutrition and Food Research, 49*(1), 52-60. http://dx.doi.org/10.1002/mnfr.200400064

Syu, K-Y., Lin, C-L., Huang, H-C., & Lin, J-K. (2008). Determination of Theanine, GABA, and other amino acids in green, oolong, black, and pu-erh teas with dabsylation and high-performance liquid chromatography. *Journal of Agriculture and Food Chemistry, 56*(17), 7637-7643. http://dx.doi.org/10.1021/jf801795m

Tirimann, A. S. (1973). Qualitative Study of Essential Oil of Manufactured Black Tea by Thin-Layer Chromatography. *Mikrochimica Acta,* 9-14. http://dx.doi.org/10.1007/BF01220128

Tsumura, Y., Wada, I., Fujiwara, Y., Nakamura, Y., Tonogai, Y., & Ito, Y. (1994). Simultaneous Determination of 13 Synthetic Pyrethroids and Their Metabolite, 3-Phenoxybenzoic Acid, in Tea by Gas-Chromatography. *Journal of Agricultural and Food Chemistry, 42*, 2922-2925. http://dx.doi.org/10.1021/jf00048a052

Turkman, N., Velioglu, Y. S., Sari, F., & Polot, G. (2007). Effect of Extraction Conditions on Total Content of Antioxidants and Antibacterial Activities of Black Tea. *Molecules, 12*, 484-496. http://dx.doi.org/10.3390/12030484

Umashankar, D. C. (2001). Quantitative and qualitative analysis of (-)-epigallocatechin gallate from green tea by LC-MS. *Journal of Natural Remedies, 1,* 151-154.

Umegaki, K., Esashi, T., Tezuka, M., Ono, A., Sano, M., & Tomita, I. (1996). Determination of Tea Catechins in Food by HPLC with Electrochemical Detection. *Journal of Food Hygienic Society of Japan, 37*, 77-82. http://dx.doi.org/10.3358/shokueishi.37.77

USDA Database for the Flavonoid Content of Dried Teas, Release 2.1, 2007.

Venzie, J. L., Castro, J., Krishna, M. V. B., Nelson, D. M., & Marcus, R. K. (2007). Electron-impact and glow-discharge ionization LC-MS analysis of green tea tincture. *Analytical and Bioanalytical Chemistry, 387*, 321-333. http://dx.doi.org/10.1007/s00216-006-0934-z

Vovk, I., Simonovska, B., & Vuorela, H. (2005). Separation of Eight Selected Flavan-3-ols on Cellulose Thin-Layer Chromatographic Plates. *Journal of Chromatography A, 1077*, 188-194. http://dx.doi.org/10.1016/j.chroma.2005.03.096

Vuong, Q. V., Golding, J. B., Stathopoulos, C. E., Hguyen, H. N., & Roach, P. D. (2012). Optimizing conditions for the extraction of catechins from green tea using hot water. *Journal of Separation Science, 34*, 3099-3106. http://dx.doi.org/10.1002/jssc.201000863

Wang, L. F., Lee, J. Y., Chung, J. O., Baik, J. H., So, S., & Park, S. K. (2008). Discrimination of teas with different degrees of fermentation by SPME-GC analysis of the characteristic volatile flavour compounds. *Food Chemistry, 109*, 196-206. http://dx.doi.org/10.1016/j.foodchem.2007.12.054

Wang, S., Liang, Y., & Zhang, S. (2013). Separation of epigallocatechin gallate from tea polyphenol by

simulated moving bed chromatography. *Journal of Chromatography, 1265*, 46-51. http://dx.doi.org/10.1016/j.chroma.2012.09.098

Wang, H. F., & Helliwell, K. (2001). Determination of Flavonols in the Leaves of Green and Black Tea and in Brewed Teas by HPLC. *Food Research International, 34*, 223-227. http://dx.doi.org/10.1016/S0963-9969(00)00156-3

Wedzicha, B. L., Lo, M. F., & Donovan, T. J. (1990). Countercurrent Chromatography of Black Tea Infusions. *Journal of Chromatography, 505*, 357-364. http://dx.doi.org/10.1016/S0021-9673(01)93050-X

Werkhoff, P., Bretschneider, W., Güntert, M., Hopp, R., & Surburg, H. (1991). Chirospecific analysis in flavor and essential oil chemistry Part B. Direct enantiomer resolution of frans α-ionone and trans-α-damascone by inclusion gas chromatography. *Zeitschrift für Lebensmitteluntersuchung und -Forschung A, 192*, 111-115.

Wickremasingle, R. L., Wick, E. L., & Yamanishi, T. (1973). Gas chromatographic – mass spectrometric analysis of flavory and non-flavory ceylon black tea. *Journal of Gas Chromatography, 79*, 75-78. http://dx.doi.org/10.1016/S0021-9673(01)85275-4

Wickremasinghe, R. L. (1978). Tea. In C. O. Chichester (Ed.), *Advances in Food Research, 24*, 229-286. http://dx.doi.org/10.1016/S0065-2628(08)60159-7

Witzthum, O. G., Werkhoff, P., & Charambolous, G. (1978). *Analysis of Foods and Beverages Headspace Techniques*. London, New York: Academic Press.

Wong, C-C., Cheng, K-W., Chao, J., Peng, X., Zheng, Z., Wu, J., … Wnag, M. (2009). Analytical methods for bioactive compounds in tea. In book: Tea and tea products. Chemistry and Health-Promoting Properties (p. 305). London, New York: CRC Press. http://dx.doi.org/10.2174/157340709789054777

Wright, L. P., Aucamp, J. P. and Apostolides, Z. (2001). Analysis of black tea theaflavins by non-aqueous capillary electrophoresis. *Journal of Chromatography A, 919*, 205-213. http://dx.doi.org/10.1016/S0021-9673(01)00762-2

Wu, F. W., He, Z., Luo, Q. Y., & Zeng, Y. (1998). High-performance liquid chromatographic determination of oxalic acid in tea using tris (1,10-phenanthroline)-ruthenium(II) chemiluminescence. *Analytical Sciences, 14*, 971-973. http://dx.doi.org/10.2116/analsci.14.971

Wu, J., Xie W., & Pawliszyn, J. (2000). Automated In-Tube Solid Phase Microextraction Coupled with HPLC-ES-MS for the Determination of Catechins and Caffeine in Tea. *Analyst, 125*, 2216-2222. http://dx.doi.org/10.1039/b006211l

Xu, X.-M., Yu, C., Han, J.-L., Li, J.-P., El-Sepai, F., Zhu, Y., … (2010). Multi-residue analysis of pesticides in tea by online SEC GC/MS. *Journal of Separation Science, 34*.

Yamanishi, T., Kita, Y., Watanabe, K., & Nakatani, Y. (1972). Constituents and Composition of Steam Volatile Aroma from Ceylon Tea. *Agricultural and Biological Chemistry, 36*, 1153-1158. http://dx.doi.org/10.1271/bbb1961.36.1153

Yamanishi, T., Kobayashi, A., Sato, H., Ohmura, A., & Nakamura, H. (1965). Flavor of Black Tea Part II. Alcohols and Carbonyl Compounds. *Agricultural and Biological Chemistry, 29*, 1016-1020. http://dx.doi.org/10.1271/bbb1961.29.1016

Yamauchi, Y., Nakamura, A., Kohno, I., Kitai, M., Hatanaka, K., & Tanimoto, T. (2008). Simple and rapid UV spectrophotometry of caffeine in tea coupled with sample pre treatment using a cartridge column filled with polyvinylpolypyrrolidone (PVPP). *Chemical & Pharmaceutical Bulletin, 56*, 185-188. http://dx.doi.org/10.1248/cpb.56.185

Yang, T., & Koo, M. (2000). Inhibitory effect of Chinese green tea on endothelial cell-induced LDL oxidation. *Atherosclerosis, 148*, 67-73. http://dx.doi.org/10.1016/S0021-9150(99)00239-7

Yao, L. H., Jiang, Y. M., Caffin, N., Yao, L. H., & Jiang, Y. M. (2006). Phenolic Compounds in Tea from Australian Supermerkets. *Food Chemistry, 96*, 614-620. http://dx.doi.org/10.1016/j.foodchem.2005.03.009

Yashin, Ya. I., Yashin, A. Y., & Chernousova, N. I. (2005). Chromatographic Determination of Tea Chemical Composition. *Beer and Beverages, 2*, 44-49.

Yashin, Ya. I., Yashin, A. Ya., & Chernousova, N. I. (2005). Tea Chromatography. *Chemistry and Life, 3*, 50-53.

Yashin, Ya. I., & Yashin, A.Ya. (2004). Chromatographic Methods used for Analysis of Tea Chemical

Composition. *Partners and competitors, 4*.

Yashin, Ya. I., Yashin, E. Ya., Yashin, A.Ya. (2009). *Gas Chromatography* (p. 528). Moscow Trans Lit.

Yuan, L. M., Chen, X. X., Ai, P., Qi, S. H., Li, B. F., Wang, D., … Liu, Z. F. (2004). Preparative separation of alkaloids from green tea by pH-zone-refining countercurrent chromatography. *Journal of Liquid Chromatography & Related Technologies, 27*, 365-369. http://dx.doi.org/10.1081/JLC-120027106

Zeeb, D. J., Nelson, B. C., Albert, K., & Dalluge, J. J. (2000). Separation and Identification of Twelve Catechins in Tea Using HPLC-MS. *Anal. Chem, 72*, 5020-5026. http://dx.doi.org/10.1021/ac000418f

Zhai, C.-H. (2014). Analysis of pesticide residues in green tea using Agilent Bond Elut QuEChERS EN Kit by LC/MS/MS Detection. Application Note Agilent technologies.

Zuo, Y., Chen, H., & Deng, Y. (2002) Simultaneous Determination of Catechins, Caffeine, and Gallic Acid in Green, Oolong, Black, and Pu-Erh Teas by HPLC with Photodiode Detection. *Talanta, 57*, 307-316. http://dx.doi.org/10.1016/S0039-9140(02)00030-9

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Why Are Alternative Diets Such as "Low Carb High Fat" and "Super Healthy Family" So Appealing to Norwegian Food Consumers?

Annechen Bahr Bugge

[1] National Institute for Consumer Research, Norway

Correspondence: Annechen Bahr Bugge, National Institute for Consumer Research, Norway. Tel: 47-2204-3567. E-mail: annechen.bugge@sifo.no

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Abstract

Aspiring for health and fitness has become increasingly important for Norwegians. This is expressed in many ways. For instance there has been a significant increase in the proportion who states that they are very interested in having a healthy diet. Furthermore, three out of ten stated that they had tried diets to achieve weight reduction over the past twelve months. One consequence of this trend is a consumption field that requires a multitude of products and services. This includes everything from food and dietary products that help you realize the dream of a sound, slim, strong, smart and sexy body, to books, blogs and TV shows that guide the individual towards making the right food choices. Through media, books and product launches, consumers are continuously exposed to different theories and beliefs about what and how to eat. A typical characteristic of the diets that have gained wide acceptance over the past few years is that they are in conflict with the national guidelines for a healthy diet. Another tendency is that traditional products in the Norwegian diet such as bread, potatoes and dairy products, in particular, have been up for debate. The purpose of this article is to explore why these alternative and rebellious diets have become so appealing to today's food consumer. Data are derived from both quantitative and qualitative materials.

Keywords: beauty, body, consumption, diets, health, obesity, orthorexia, self-help, therapy

1. Introduction

The present focus on healthy and unhealthy lifestyles and the increased pressure on the individual to take responsibility for their own bodies and well-being has resulted in a growing preoccupation with what and how one should eat (Maurer & Sobal, 1995; Caplan, 1997; Crossley, 2004, Lupton, 1996; Lupton, 2012). A characteristic feature of the health messages that are sent to consumers is the underlying assumption that the receiver is a rational actor who makes use of new knowledge to change attitudes and behavior (Crawford, 2006; Herrick, 2009; Madsen, 2010). Within this model, the food consumer is regarded as a health-conscious actor who includes and excludes ingredients and products in accordance with the dietary advice.

Diets and dieting is a theme that has received a great deal of public attention in Norway in recent years (Bugge, 2012 & 2014). The debate has shown that diets are being used as explanations of and guides to physical and mental health. Thus diets reflect the cultural acceptance of the idea that you can create your own person and that you are responsible for becoming your own ideal. However, there is no consensus about what is right and wrong. Through media, diet books and product launches consumers are continuously exposed to a number of different theories and beliefs about what and how to eat. Many of these theories are directly contradictory. Like in other Western countries, it also seems as diets that are in conflict with national dietary policies, have become more prevalent in Norway (Bentley, 2004; Bentley, 2005; Kristensen et al., 2011; Knight, 2012; Bugge, 2012).

However, the purpose of this article is not to question the value of healthy eating, but to develop a better insight into how people think about these issues. Furthermore, the purpose is not to determine whether the various dietary theories are right or wrong, but rather to look more closely at why these alternative and rebellious diets seem so appealing to today's food consumers. Consequently, we will explore how this way of thinking about food is reflected in Norwegian consumers' preferences, priorities and practices: How many have been influenced by the alternative diets that have received public attention in recent years, such as Low carb high fat (LCHF), Super Healthy Family (diet without milk, gluten and additives) or Raw Food (a diet consisting of unheated food and minimal use of meat and fish), and which values do the consumers wish to realize through this type of eating

behavior? What ingredients and products are considered wrong, and why is it so important to avoid them? What food assets do today's food consumer consider important? By using this type of research questions, we want to contribute to and extend the knowledge of the values, dilemmas and strategies that today's food consumers are concerned with. The purpose of this paper is thus to highlight the consequences of the focus on diet, body and health.

2. Background

Decades ago, Mennell et al. (1992) expressed that sociologists should be more concerned with how health and diet regimes can be considered as responses to social pressures on people. In recent years, several studies on eating, diet and culture have explored this approach (e.g. Bentley, 2004; Bentley, 2005; Gard & Wright, 2005; Mallyon, 2010; Kristensen, 2011; Knight, 2012).

According to Crawford (2006), health as self-control and self-validation has become increasingly prominent. The message is that you can be whoever you want to be and that you are responsible for your own health, body, etc. (Rimke, 2000; McRobbie, 2009). Food also seems to offer an increasing number of therapeutic solutions. An image search on Google on the Norwegian phrase "spis deg…" ("eat yourself…") gave almost 2 million hits. Of the 30 first hits, 12 were diet books (Note 1). By eating in a certain way, you were promised everything from good health and a slim, fit, youthful and beautiful body to harmony, happiness and even pregnancy.

Among the variety of promising diets that have appeared in the recent decade, the *LCHF* diets in particular, have received immense attention in the Norwegian public debate (Bugge, 2008; Bugge. 2012). Dr. Hexeberg's book (2010) "Frisk med lavkarbo. Nytt liv med riktig mat" («Healthy with low carb. A new life with the right food») was number one on the Booksellers Association's list of top selling books through 2010 and 2011. However, it was Dr. Lindbergh who first introduced the so-called Atkins diet in Norway (Lindbergh, 2001). There is little doubt that mediators of LCHF have had a relatively large influence on Norwegians' eating habits over the last couple of years. The sale of potatoes, white bread and products made from refined grains has declined significantly. It should also be noted that the core products of the LCHF diet - such as eggs, bacon, red meat, fatty dairy products, butter and avocado - have had a significant sales growth (Bugge, 2012). This is not exclusively a Norwegian phenomenon. Studies have shown similar trends in other Western countries (Bentley, 2004; Bentley, 2005; Knight, 2005).

Recently Norwegians have also become familiar with diets such as *Super Healthy Familiy* (Mauritson, 2011), *Raw Food* (Palmcrantz & Lilja, 2011) and *Super Food* (Berge & Chacko, 2010). In order to have a super healthy family, it is important to stay away from dairy products, particularly cow milk (Mauritson, 2011). Studies have shown that the nutritional status of milk has become more problematic (Kristensen et al., 2011) Two out of ten food consumers say that they want to limit the intake of milk. Correspondingly, results have showed a significant decline in the consumption of milk in the last decade (Bugge, 2012).

The Raw Food diet involves an eating pattern where raw, unprocessed and organic food represents a large part of the diet. Depending on the interpretation of the rules of the diet, it is possible to include fish and meat on the condition that it is served raw (sushi, sashimi, carpaccio etc.). Most meals, however, consist mainly of raw vegetables, salads, smoothies and (detox) juices (Berge & Chacko, 2010; Palmcrantz & Lilja, 2011). Consumption figures show that these types of dishes are now eaten more often than before, in particular among young, urban food cultural trendsetters. According to Andrews (2006), raw fish and seaweed have gone from being exotic to being a conspicuous symbol of a trendy and healthy lifestyle among Norwegian food consumers. In 2001, only 4% of people in Oslo ate this once a month or more. In 2011 this had increased to 40%. A product that is particularly associated with Raw Food is wheat grass. This is not sold only through specialty stores, but also in supermarkets and major bakery chains (Bugge, 2012).

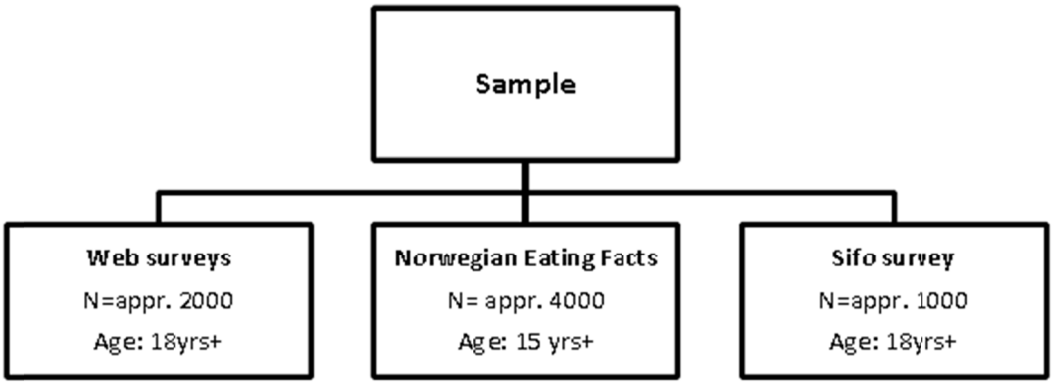
A common feature of these diets is that they recommend avoiding a number of common ingredients and foods in the Norwegian diet - such as bread, potatoes, pasta, flour, root vegetables, milk and meat. Particularly the LCHF-communicators' recommendation of a high intake of (saturated) fat has provoked many nutritional experts and Norwegian health authorities (Bugge et al., 2008). This has led to many heated debates and sensational media coverage. Another common feature of the alternative diets is that they all promise bodily and mental improvements. By committing to the LCHF diet, one can avoid everything from obesity, high blood pressure, atrial fibrillation, inflammation of the gums, migraine and fluctuating blood sugar to sugar addiction, chronic inflammations, Chronic fatigue syndrome (CFS), Bekhterev's disease, Fibromyalgia and barrenness, according to Dr. Hexeberg (2010).

In addition to the many therapeutic effects attributed to these diets, their popularity must also be seen in light of the many actors - experts, marketers, food manufacturers, publishers, program makers etc. – that continuously try

to find new and marketable products and services (Lawrence, 1999; McRobbie, 2009; Miles, 2002). An overview of the costs of advertising show that manufacturers of health foods and supplements are among those who spend most money on advertising in Norway (Note 2). There has also been a tremendous growth in sales of this type of products on the Norwegian market in recent decades (Note 3). A study of the scope and content of food commercials in Norwegian media channels showed that the prevailing message of today's commercials is that of healthiness and naturalness (Bugge & Rysst, 2013). Several theorists have discussed how advertising reflects the existing and desired knowledge and values of a society (Albers-Mills & Gelb, 1996; Schumann et al., 1991). The majority of today's food advertisements refer to health, and this can be interpreted as a reflection of society's desire for physical and mental perfection. The theme of this article is how this is expressed in food consumers' preferences, priorities and practices.

3. Materials and Methods

The data on which this article is based are derived from both qualitative and quantitative materials. Through the quantitative materials we wanted to answer questions such as: How many people try to achieve different physical changes and improvements through food? How many follow specific diets? The qualitative materials were intended to provide answers to questions such as: What characterizes the phenomenon of being on a diet? And why do alternative diets have such an appeal?



In order to gain better knowledge of Norwegian consumers' food preferences, priorities and practices, we carried out several surveys. The collection method was four WEB-interviews (May and November 2011, February 2012) and two telephone-interviews (SIFO-survey, 2006/2012). The WEB-surveys were conducted via E-mail. All the surveys were conducted by the data-collection agency Norstat Norway (Note 4). The material also consists of a survey conducted by the same company in 2010. The panel was a pre-recruited sample of persons aged 18 or older, about 80 000 people in total. Participants were recruited randomly. The selection consisted of approximately 2000 respondents aged 18 years and older. The results were weighted by gender, age, education and region. This means that the sample can be considered representative of the entire population. The response rate was approximately. 40%. We also made use of Norwegian Eating Facts (1985-2012) which is part of Ipsos MMI Norwegian Monitor. This is a comprehensive postal questionnaire with approximately 4000 respondents. The sample is aged 15 years and older. The analysis of the quantitative materials consists of simple statistics such as frequency distributions and contingency tables using the computer program SPSS. We tested level of significance using Pearson's r ($p<0.05$).

To get a better understanding of why alternative diets have such great appeal to Norwegian food consumers, we believed it was also important to look into the popular (scientific) diet discourse: What characterizes the advice given in books and blogs about diet and health? What explanation models is this advice based on? And how is it that so many "ordinary" ingredients and products have been considered "extraordinary"? The qualitative materials consist of analyses of Norway's best-selling diet books and most visited diet-blogs. The books that were analyzed were selected on the basis of the Booksellers' Association's list of top selling books in the period 2010-2011. Most of the books referred to the LCHF diet, but there were also books on Super Healthy Family, Raw Food and Super Food. Social media have become an increasingly central part of Norwegians' daily life. Data from Statistics Norway show that 6 out of 10 Norwegians regularly use social networking sites (Note 5).

Blogging is an example of an activity that is carried out in the social media. The most popular diet blog in the period 2010-2011 was The LCHF blog (Note 6), followed by "Low Carb done easily" (Note 7). The most visited blog on the top list is Fotballfrue.no (Footballwife.no) (approx. 105 000 visitors on an average day) (Note 8). Healthy eating is a central topic in this blog, too. The author has declared herself a supporter of both LCHF and Raw Food.

In the analysis the blog posts were transcribed and coded. The data analysis program Atlas.ti was used to detect and visualize the phenomenon of "being on a diet". In the diet books this was marked, coded and noted directly in the text. Just like a Swedish study on how families manage the everyday health puzzle (Johansson & Ossiansson, 2012), we, too, were inspired by Ehn and Løfgren's (2011) cultural analysis. In the analysis of the texts we looked for themes and connections, and related the data to cultural contexts. This enabled us to problematize the most prominent ideas, notions and categories that emerged from the texts.

4. A Brief Overview of the Survey Results

In the following section we will give an overview of the most significant findings from the surveys. After doing so, we will analyse both the quantitative and qualitative results in light of several important theoretical contributions to the challenges associated with food, body and health.

4.1 Increased Emphasis on a Healthy and Slim Body

In order to identify consumer preferences, priorities and practices (the three P's), we formulated various statements, and the respondents had to consider whether the statement in question matched with their own three P's.

There has been a significant increase in the proportion who reported that they tried to get a slimmer body. In 2006 56% fully or partially agreed with the statement "I try to get a slimmer body". This proportion had increased to 76% in 2012. From 2006 to 2012 the proportion which fully or partially agreed with the statement "I try to get a more muscular/fit body" had increased from 44% to 53% (SIFO Survey 2006/2012). The web survey (2011) revealed that 45% fully or partly agreed with the statement: "I aspire to have a nice shape (slim, fit)". According to figures from the Norwegian Institute of Public Health, it is estimated that about half of the population are overweight or obese (Note 9). However, our survey showed that even more perceived their own body weight as too high. Sixty six percent reported that they had a few extra pounds.

4.2 Increased Emphasis on Food's Impact on Bodily and Mental Improvements and Changes

The surveys also revealed that there is an increasing interest in healthy eating. At the beginning of the 2000s, 48% reported that they were interested in healthy eating. This proportion had increased to 58% in 2011 (Norwegian Eating Facts 2012). Similar results emerge from other surveys as well. In the web survey (2011) seven out of ten reported that they completely or partially agreed with the statement: "I am very interested in eating healthy". Only 9% said they completely or partially disagreed. The Web survey (2011) also showed that many people had been on various diets during the last 2 years. 34% had tried to lose weight or improve their health condition using certain food or diets (33%). There were also quite a few who said they had tried to prevent health problems (24%), improve performance (23%), increase wellness (22%) and improve appearance (13%).

The surveys showed that quite a few participants were regularly on different diets, for example slimming, vegetarian, milk and meat reduced diets etc. Women were more likely to answer this than men. 37% of women and 21% of men regularly or sometimes ate diet foods in order to lose weight. It also appeared that diets that were in conflict with national nutrition policies had quite a big impact. Examples include Low Carb High Fat (LCHF), Super Healthy Family, Super Food and Raw Food. In fact, surprisingly few expressed great trust in the national guidelines. Only 10% said they totally agreed with the statement: «I have great trust in the health authorities' national dietary advice», and 23% partially agreed. A significant proportion (20%) said they were very or quite interested in the Low Carb High Fat Diet. Moreover, 36% reported that they were somewhat (a little) interested in this diet. 42% reported that they replaced ordinary food products with Low Carb products regularly/sometimes. Two out of ten avoided milk because they believed they did not tolerate it (Norwegian Eating Facts 2012).

The surveys also showed that there was a significant decrease in the proportion who replace regular food with low-fat products in the period 2009 to 2011 (Norwegian Eating Facts 1985-2011). Although for decades Norwegian health authorities have strongly recommended that people should reduce their intake of saturated fats, our figures show that relatively many do not share this view. In the web survey (2011) only 22% reported that they regarded saturated fat as unhealthy. Almost as many (20%) said that fat dairy products were healthier than the lean ones. The answers to questions that dealt with opinions about fat had a relatively large proportion of

«neither/nor» or «do not know». There was, for example, 10-15% who responded that they did not know whether saturated fat was healthy or unhealthy. A similar proportion did not know whether they had changed their eating frequency of fat, saturated fat or unsaturated fat. This may indicate that consumers have relatively little knowledge of the various types of fat.

4.3 Increasing Emphasis on Eating Raw and Fresh

Out of a total of 26 quality attributes, freshness (85%) was the one that respondents emphasized most when they were doing grocery shopping. This emphasis had also increased. 57% and 50%, respectively, said the same about attributes such as healthy and low price. More than half of the respondents (52%) claimed that they *always* or *very often* avoided buying foods that contained artificial additives. In addition, 23% said they did it *sometimes*. Women (81%) were more likely to answer this than men (70%). The proportion that answered that this was something they did always or very often increased from 17% in 2003 to 23% in 2011 (Norwegian Eating Facts 1985-2012). The popularity of Raw Food can be seen in light of the food consumer's increasing emphasis on fresh, clean and natural food. In 2011, around 15% of respondents expressed interest in this eating pattern. Young people aged 15-24 years and people living in Oslo (21%) were the most interested.

4.4 Constantly New Products on the List of What You Should Avoid Eating

A typical feature of popular diets nowadays is that they often identify a type of nutrient, a product or a preparation technique that you should avoid. While the LCHF-communicators recommend avoiding carbohydrates, the communicators of the Super Healthy Family diet recommend that you leave out dairy products and gluten. If you are following a Raw Food or Super Food diet you should minimize your intake of animal products and avoid heated food. The surveys revealed that many had been influenced by these dietary guidelines.

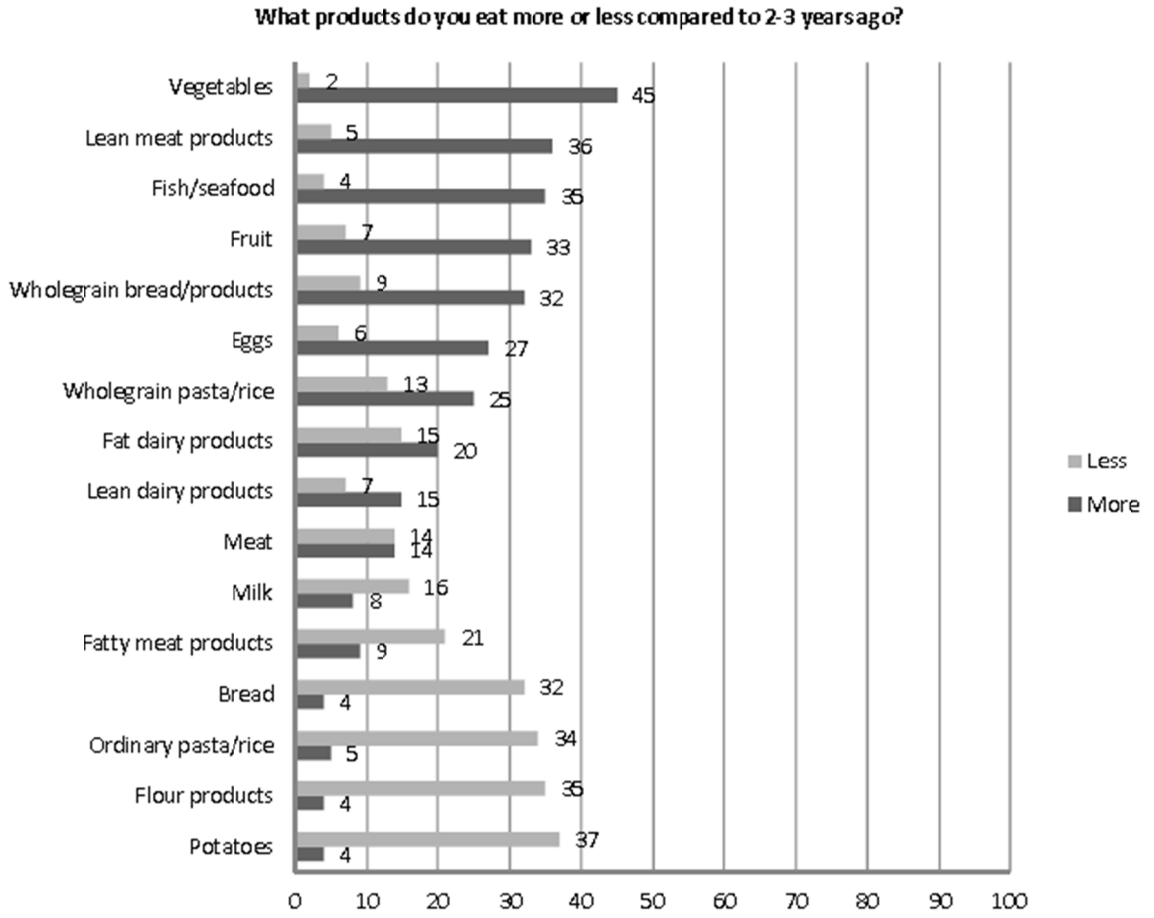


Figure 2. What products do you eat more or less compared to 2-3 years ago? N=1000.%. Web Survey 2011

As Figure 2 shows, carbohydrate rich products like potatoes, flour products, regular pasta/rice and bread top the list of foods that the respondents want to avoid. LCHF-communicators have also recommended a high intake of eggs and high-fat dairy products. 27% and 20%, respectively, said they had increased their intake of these products (Figure 2). Despite the fact that relatively few said they had reduced milk intake the past few years, our figures show that there has been a significant decline in milk consumption – about 50% (Norwegian Eating Facts 2012).

Similar results emerged when it came to changes in the intake of various nutrients. 30% said they ate fewer carbohydrates. Sugar (48%) topped the list of what respondents believed they ate less of. Fiber (33%) and proteins (25%) topped the list of what they thought they ate more of. Only 9% said they ate more fat. Figures from Norwegian Eating Facts (2012) and our WEB-survey (2011) showed that there have been significant changes in people's view on various nutrients in the period from 2009 to 2011.

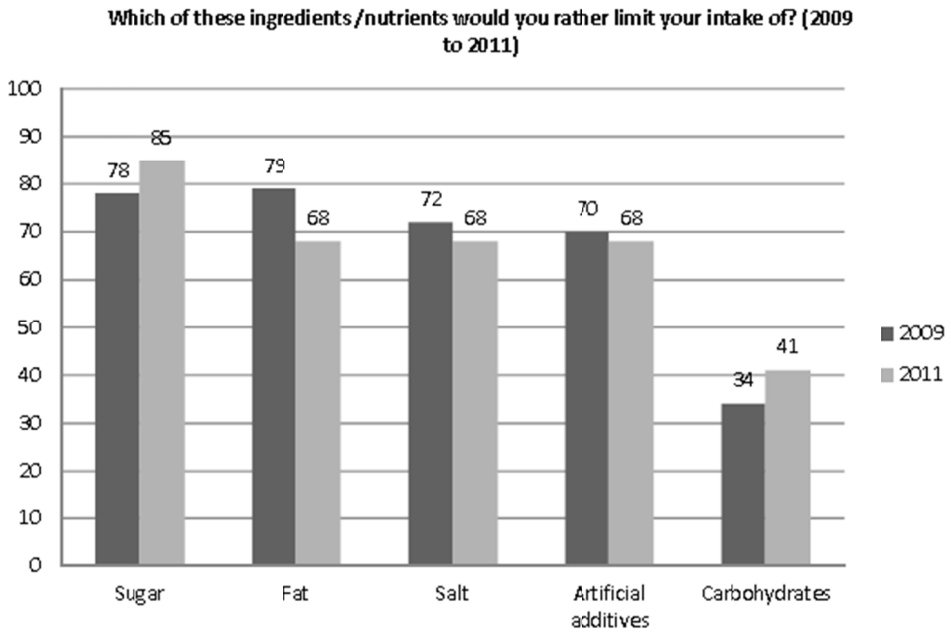


Figure 3. The Proportion of those who would rather limit their intake of the mentioned ingredients/nutrients in the period 2009* to 2011**. * N=3887.%. Norwegian Eating Facts (2010). ** N=2017.%. Web Survey 2011

There has been a significant increase in the proportion who prefer to limit their intake of sugar (from 78 to 85%) in the period from 2009 to 2011. Moreover, the percentage who said the same about carbohydrates has increased from 34% to 41% in the same period. There has also been a significant decrease in the proportion who want to limit their intake of fat (from 79% to 68%) (Figure 3).

5. Discussion

Several theorists have discussed the influence of a therapeutic mindset on the Western world (e.g. Giddens, 1991; Lupton, 1996; Crawford, 2006; Madsen, 2010). The body is not something you can take for granted, but it has become a project of its own. As the results from the surveys showed, food also seems to play an increasingly important role as a means to achieve physical and mental perfection. In the following section, we will look more closely at the various imperatives that give meaning to contemporary Norwegian eating patterns. Such imperatives reveal a lot about the moral and social pressures that operate on people's food choices (Warde, 1997). We also believe they are important for understanding why diets such as LCHF, Super Healthy Family and Raw Food have become so appealing to Norwegian food consumers.

5.1 Be Responsible

A consequence of the individualistic therapeutic view is that the individual is responsible for making the correct health choices (Lupton, 2012; Crawford, 2006; Herrick, 2009; Madsen, 2010). Food and eating habits have received an increasingly central role in achieving good health. In this light, popular diets such as LCHF, Super

Healthy Family and Raw Food can be seen as a reflection of the widespread cultural acceptance of the idea that you are responsible for whether you feel good about yourself, you are healthy, you are overweight, you are fit etc. Our material showed that many had tried to achieve certain effects – prevent obesity, improve health conditions, more wellness etc. by means of food and diets. Reducing the intake of carbohydrate-rich products was a common way to achieve these goals. The blog material also showed that carbohydrate-rich products were seen as a bad choice of foods. One of the bloggers wrote: "I can promise you that once you change your carbohydrate sources, you will experience large changes in your body composition!" Another blogger wrote: «Need help to start dieting. Any good advice?" She got the following answer "First and foremost, stay away from bread, rice, pasta, potatoes and sugar. Eat eggs, fish, meat and, naturally, enough fat – butter, sour cream, cream, avocado…."

The individualization processes have not only led to the individual being responsible for healthy eating, but also a widespread belief that what is considered proper food is very individual. One of the bloggers said: "The most important thing is to find out what works best for you". Another one wrote "I eat LCHF because it is good for MY body".

The lists of the most popular books, television programmes, blogs and media stories show how the self-help genre has gotten a far wider appeal. From 2008 to 2009, for example, Norwegian bookstores could report an increase of 30% in sales of self-help books (Madsen, 2010:88). In recent years, it is precisely books about the LCHF diet that have topped the *Booksellers Association's* sales lists. *The Language Council of Norway* even named "lavkarbo" ("low carb") the word of the year in 2011.

The distinction between the professional and the self-taught expert is rather vague in the self-help industry. For example, mediators of the popular diets clearly mark a distance to the official dietary guidelines: LCHF mediators describe the official dietary guidelines as unsuccessful and mistaken (Hexeberg, 2010; Eenfeldt, 2011). A similar skepticism is evident in Mauritson's (2010) book. In the introduction she thanks all the families who have children with "autism, ADHD, 22q11, hyperactivity, schizophrenia, NLD, Tourette's syndrome, brain damage, gastrointestinal disorders, eczema, asthma or behavioral disorders": "You are all fighting a fierce battle against skeptical doctors, teachers…" (p. 4).

Our findings also revealed that there were many conflicting and competing views on what constitutes a healthy diet. While the national guidelines recommend reduced intake of foods high in (saturated) fat, mediators of the LCHF diet recommend the reverse. Moreover, while mediators of Raw Food recommend high intake of fruit, mediators of LCHF recommend people the reverse.

The fact that so many embrace alternative diets can be interpreted as an attempt to exercise authority over oneself. This again can be seen as a consequence of the emphasis on individuality and the individual's ability to take responsibility for his/her life. It is simply irresponsible and incorrect to let others decide what you should eat. Thus, it is not surprising that Mauritson (2010) described herself as a "housewife" in her bestselling book *Super Healthy Family*. She described a modern housewife's cooking as a combination of "common sense" and "new knowledge". This is opposed to what is often perceived as either reactionary "experts" or eccentric and alternative beliefs about what and how to eat. If the diet – for example LCHF or Super Food - is wrong for "your body", there are always other opportunities: "You are in charge – and you know what's best for you" (Giddens 1991:77).

5.2 Be Better

From the material it may seem as if the more promising products and services that are launched, the higher the demands we make of bodily and mental perfection. According to Ehrenberg (2009), the greatest paradox of the individual-therapeutic view is that it has both become an area of great satisfaction and happiness, while it also poses a risk of dissatisfaction and exhaustion.

While the consumption of products and services that promise a slimmer, healthier and more beautiful body is increasing it also seems like more and more people do not feel they live up to the ideals (Lupton, 1996; Williams & Germov, 1999). As shown, more believed that they had an overweight body than what is shown by statistics from the Norwegian Institute of Public Health (Note 10). There has been a significant increase in the proportion who had tried to get a slimmer body. There was also an increasing proportion who reported that they had spent money on slimming products.

Although many men also expressed dissatisfaction with their own body weight, our study showed that this was an issue that concerned far more women than men. There were, for example, more women than men who were dissatisfied with their own body weight and appearance. Far more women than men had tried to lose weight or improve their appearance by making changes to their diet during the last two years. Bratman (2000) suggests that

dieting is a culturally accepted norm for women to seek coveted values such as beauty and slenderness without admitting it. Thus, being on a diet is not just motivated by a desire for good health, but also for a look that lives up to the aesthetic ideals: it is the slim and fit body that is perceived as sexually attractive (Germov & Williams, 1999; Sobal, 1999; McRobbie, 2009; Brewis et al., 2011).

Like Crawford's (2006) study, our results showed that health practices – such as eating habits – have become an increasingly important means of social and personal evaluation and devaluation. Those who succeed are seen as morally superiors. It is also a field that is characterized by a lot of guilt, shame and fear (Lupton, 1996). According to Beck (1986) and Giddens (1991), a side effect of the growing number of self-realization opportunities that exist in our culture is precisely higher demands on the individual and increased dissatisfaction.

5.3 Be Disciplined

Today's foodscape is characterized by an abundance of energy-rich foods and drinks, which make great demands on the individual's capacity for self-control and self-discipline (Winson, 2004). According to Lupton (1996) self-control is a prevailing value of our time. Moreover, she believes that the body is increasingly becoming a symbol of whether one possesses this virtue or not.

The increase in dieting can be seen in light of the idea that following a set of strict eating rules is just an effective way to demonstrate that you have control over your body, mind and emotions. A common feature of the various diets that have been popular in recent years is that they all have many restrictions associated with ordinary ingredients and products in the Norwegian diet – such as bread, pasta, white flour, milk, meat, vegetables that grow under the ground, heated food, frozen foods, light foods etc. The materials revealed that many were affected by the rules and restrictions that were conveyed through the various diets. Furthermore, it emerged that following such diets both demanded both careful planning of every meal and great persistence. For some the ultimate eating pattern was to conduct regular detox cures (Berge & Chacko, 2010; Palmcrantz & Lilja, 2011).

A keyword in today's health and diet message is reduction. Everybody is encouraged to eat less fat, sugar, salt, carbohydrates, additives, meat, potatoes, bread, wheat flour, processed food, fast food. One should drink less alcohol, soft drinks, milk, coffee etc. Moreover, one should reduce body weight, blood pressure, cholesterol, stomach fat etc. This pattern has been referred to as a form of cultural anorexia: "I lack nothing, therefore I eat nothing" (Baudrillard, 1986:55). Or as a famous rap artist said to a Norwegian youth magazine: "The ultimate eating pattern is to just drink health drinks" (Bugge & Lillebø, 2009:158). Our findings show that smoothies, shakes and juices have become a core product among health-conscious consumers. In all the diet books we found many recipes for such drinks. Thus, in our affluent society reduction is described as both heroic and ideal.

As with international studies, our study also showed that the value of self-control seems to be particularly prevalent among people with higher education. For the middle classes, their relation to body and health is not a question about heritage and luck, but rather an acquired status that one constantly has to work and strive for. An example of how this is expressed is how the middle class are spending more and more of their free time on healthy cooking and keeping fit (Warde, 1997; Crotty, 1999; Crawford, 2006; Bugge, 2010). Like many other studies, our study also showed that more participants with higher education than lower education were interested in having a healthy diet. Far more people with higher education than with lower education said they aimed to be slim and fit. Education also had an impact on the amount of exercise. Furthermore, educated people ate healthier than those with low education. For example, more people with higher education ate vegetables daily than people with lower education. The same was true for fish and white meat. People with higher education also had a significantly lower consumption of sugary soft drinks than those with low education (Bugge, 2010).

5.4 Be Critical

A particular feature of the most popular diets is that they undertake a critical position to modern food production and eating habits (Knight 2005, Knight 2012). Our reading of bestselling diet books shows that they usually begin with a critique of food and nutrition science, technology advances and politics. In Hexeberg's (2010:12) book we can read: "Before the agricultural revolution our ancestors lived about two million years on a diet low in carbohydrates ...". Moreover, the author of the book *Super Healthy Family* (Mauritson, 2011:24) claimed that "dietary advice in this country reflects the fact that we are an agricultural country. More emphasis is put on economic interests of farmers and dairy giants than health reasons". The authors of the bestselling books about Raw Food and Super Food also recommend "anti-industrial" and vegetarian food. Berge and Chacko (2010:154) express it in the following way: "Natural, non-processed foods are what we are meant to eat from nature's side. Super foods are just that - pure and nutritious food that makes you feel in harmony with nature".

Food consumers are putting increasing emphasis on attributes such as fresh, natural and pure. Among other

things, this means food that is produced with the least amount of additives, medications, pesticides, i.e. the product should not change too much from its original form. The growing interest in raw food and super foods is just one of several examples of how this desire for purity and naturalness is expressed. The growing skepticism of wheat flour can also be seen in light of the criticism of modern food production. By LCHF mediators this is not only described as unhealthy and fattening, but also as unnatural (Hexeberg, 2010; Eenfeldt, 2011).

Several researchers view this valorization of the natural as a result of increased awareness of the side effects of industrialization. What many fear is that we do not know how the cultivated nature will behave (Beck, 1986; Giddens, 1991; Pollan, 2008). The materials show that many suspect that the cultivated, new food production is more unpredictable than the old. For example, four in ten reported that they were anxious about the content of (artificial) additives in products on the Norwegian market. And three in ten feared that the food we eat can cause illness. This is probably an important reason why diets that involve "detoxification" and "cleaning" - such as Raw Food and Super Food – have become so appealing.

The critical consumer has also resulted in science becoming more and more necessary, yet less and less sufficient for the definition of truth. People will constantly ask questions like "yes, but …?","on the one hand, and on the other hand", and "what if…" (Beck, 1986). It is this form of skepticism that has opened up many possible truths about what to eat - from the National Council for Nutrition advisories (Note 11) to diets such as LCHF, Raw Food and Super Food to neo-religious "earthing food" (Princess, Märtha, Louise, & Samnøy, 2009). In our study it was clearly stated that health authorities' did not have a monopoly on the truth of what comprised a healthy diet.

5.5 Be Proactive

A prominent feature of late-modern society is that risk calculations have become a more important part of how we organize our lives (Beck, 1986; Giddens, 1991). Beck (1986) described this kind of calculations as not-yet-events which stimulate action. Moreover, they appeal to our rationality and our role as calculating and active individuals (agency). What many fear is that our eating habits today can have unwanted consequences in the future, for example obesity, premature aging, diabetes, heart disease, cancer. The study showed that risk reduction was not something that concerned a small group, but rather something that occupied the majority of consumers. As shown, seven in ten stated that they were interested in eating healthy. An important motivational factor was preventing health problems. It is perhaps not surprising that diets such as LCHF and Super Healthy Family has gained such popularity, if we look closely at what the mediators claim that we can avoid by eating their diet. As already mentioned this includes everything from overweight to autism, depression and psoriasis.

It was also apparent that the LCHF message had led to changes in consumers' perception of what is considered as "risky" ingredients and products in the last couple years. While somewhat fewer gave the impression of being skeptical of eating fat, the number of people who were skeptical of carbohydrates had increased significantly. The changing views had also led to a number of changes in consumers' eating habits. When respondents were asked about which products or nutrients they had consumed less of during the past few years, sugar and carbohydrates topped the list. Moreover, potatoes, bread, pasta, rice and wheat flour topped the list of products they had eaten less of in the same time period.

According to Bratman (2000), the huge number of health risks that people perceive can be avoided by using food has resulted in our food choices being driven by fear rather than actual choice. Through specific diets we are seeking to eliminate as many risks as possible. The long list of health hazards due to "wrong foods" makes it relevant to refer to Beck's (1986) assertion that there is an overproduction of risk in our time. This is due to the continuous production of knowledge about the various risks, the tendency to exaggerate risks and an insatiable desire for medical news. Crawford (2006) described this as a continuous spiral of anxiety-control-anxiety.

It is also worth commenting that many of the negative health consequences that consumers want to avoid, are not felt directly on the body, but made visible and understood through knowledge. However, when an expert's knowledge is communicated, it is, according to Beck (1986), often confused with the expert's own interpretations and value assessments. Thus, what constitutes a risk to human health is therefore not merely an academic concern, but also a moral issue. In particular, experts have pointed to overweight and obesity as significant risk factors in today's eating habits. Although rising prevalence of overweight and obesity confirms the statistical risk our eating habits involve, it is important to bear in mind that the risks a society chooses to emphasize cannot only be explained by statistics. They are also an expression of a social critique that is based on shared values and common fears (Douglas & Wildavsky, 1982; Campos, 2004; Crossley, 2004; Gard & Wright, 2005).

5.6 Be Harmonious

Several theorists have described the contemporary mood as therapeutic, not religious: we are not concerned with salvation, but personal well-being and good health (Lupton, 1996; Madsen, 2010). As Giddens (1991) wrote: "When people were unhappy in the past they went to church, now they visit the nearest therapist" (p. 179). He also pointed out that therapy has not replaced earlier religious authority. Anyone who is seeking therapy is confronted with a number of different theories and practices. While some describe this as a secularization process, there are others who believe that the therapeutic orientation has contributed to a resurgence of New Age religiosity. According to Madsen (2010), it seems that the therapeutic culture encourages many forms of individual searches for meaning.

The popular books by Princess Märtha Louise and Elisabeth Samnøy (2009, 2012) are an example of how New Age religiosity has been expressed in Norway in recent years. The core of their message is unmistakably similar to a lot of self-help literature (Madsen, 2010). To gain more control of your life and to realize your dreams, they advise the reader to include angels into their life. Moreover, you have to take responsibility for your own life. The individual herself is the key to her own success and failure. Food is one of the tools for success. Unlike diet books, the Princess and her partner's advice is camouflaged in neo-religious language. To succeed with so-called earthing (Norw. "jording") - i.e. to achieve an open dialogue with the earth from the heart - it is recommended to eat certain types of food (p. 97), for example lentils, beans and root vegetables (pp. 125-135).

The enormous preoccupation with healthy eating can thus be seen as a quest for spirituality, identity and control (Skårderud, 1991; Lupton, 1996; Bratman, 2000). Raw Food and Super Food are examples of how this is reflected in post-secular societies - both diets are characterized by a worship of nature. Moreover, they are mysterious and have their own rituals. As can be seen from Palmcrantz and Lilja's (2011) book, one of the eating rituals is detoxing the body and soul through food. Although these diets can be characterized as eating patterns for a minority of the population, many of the core ingredients and dishes have become increasingly popular in Norway in recent years, e.g. healthy juices and smoothies (Bugge, 2012).

Studies have shown that many Norwegians eat various supplements and "therapeutic products" (Bugge, 2012). The desire to provide our bodies with omega-3, proteins, vitamins, wheatgrass, detox juice, low-glycemic foods, cholesterol-reducing margarine, can also be seen as a quest for immortality - or at least a postponement of old age. One of the best-selling diet books is just about eating yourself younger by eating food that has a rejuvenating effect (Hafsteinsdottir, 2009). In order to achieve this, however, it was important to avoid sugar, dairy and gluten.

In the old days, one saw faith as a harbinger of misfortune and death. Now, on the other hand, we have a tendency to oppose a limited future (Giddens, 1991). The importance of being able to eat ones way to desired values can be seen in connection with the view of the individual's increasing ability to influence their physical and spiritual future.

5.7 Be Successful

According to Giddens (1991), the reason that eating regimes have such a great significance for the individual's identity is that they relate habits to the visible aspect of the body. On the one hand, eating habits are what he describes as a ritual display, on the other hand they also affect bodily form, indicating in this way something about the individual's personality and background as well as the kind of self the individual wants to cultivate (p. 62). Several studies have shown that being healthy, slim and fit is a central feature of modern identity (Note 12). In this health-appreciative culture, people are largely defined by how well they succeed or fail to apply the right practices.

Although people has always been concerned with health, Crawford (2006) claims that a new kind of health consciousness emerged in the 1970s. Several counter-cultural movements were established, for example movements that advocated organic and natural foods, jogging, meditating, yoga, dieting, fitness, etc. The growing health interest was especially visible among young, urban, cultural trendsetters. In 1980 Crawford introduced the term *healthism*. The aim was to describe the growing moralism over health issues in the American middle classes. When people talked about good and bad health they very often used words like self-control, self-discipline and willpower. Contrasting themes were also prominent: indulgence, overeating and of course lack of willpower, self-discipline etc.

Crawford (1980) sees this change as result of the social development in the postwar period. In the years after the 2nd World War until the 1980s emerged a consumer society that was characterized by a degree of prosperity, abundance and leisure that people never had witnessed before. This also promoted continuous upward social

mobility. For the younger generation, however, the question of how to maintain their class position became an urgent question. In Crawford's (2006) opinion the pursuit of health and fitness gave ample opportunities for the individual to maintain class position. By making the body a task, the health conscious can demonstrate both for themselves and others that they possess the core values which define their class.

Similar to other studies, our study also show that education is a variable that has a significant effect on food priorities and practices. Far more highly educated people were interested in having a healthy diet. Moreover, people with higher education were more interested in weight control and having a slim and fit body. It was also apparent that people with higher education had been more responsive to dietary advice. Examples include a higher intake of whole-grain products, fruit and vegetables among people with higher than lower education. On the contrary, people with higher education had a lower intake of sugary drinks and salty snacks than people with lower education (Bugge, 2010). People with higher education were more concerned about limiting their intake of sugars, carbohydrates and (saturated) fat than those with lower education.

5.8 Be Healthy, But Not Insanely Healthy

As pointed out, there has been a significant focus on health as self-control. Our material show that placing of responsibility on the individual to eat healthy – you are what you eat, or what you don't eat – for some people lead to becoming obsessed with what they eat. An American doctor referred to this phenomenon as orthorexia (Bratman, 2000). However, it is important to underline that being on a diet or eating healthy is not the same as being orthorectic. It is the degree of obsession, which according to Bratman, determines whether it is considered an eating disorder or simply a healthy choice of lifestyle.

Although healthy eating mainly is a reasonable choice, it is, according to Bratman (2000), also important to question how important food should be in your life. A bias of modern nutritional science is precisely that its enthusiasm for diet changes – "eat more …, eat less ..." – can result in obsessions. As already mentioned, a particular characteristic of the recent super diets is to place many restrictions on food choices. As with Bratman's (2000) study, our findings also show that the list of what you cannot eat in the name of health seems to be growing. In the book *Raw Food in Norwegian* (Palmcrantz & Lilja, 2011) the authors claim that you actually are what you do not eat. The blog material revealed that a recurring issue was discussions on how to replace ordinary ingredients and products. The products that the bloggers ate were usually described as "no" or "free from" gluten, sugar, white flour, additives, soya, milk, meat, carbohydrates etc. As shown, there was also a significant proportion who reported that they limit their intake of ordinary ingredients and products such as potatoes, bread, pasta, milk and meat.

In addition to the orthorexic's long list of "no-food", it is also extremely important for him/ her to include specific ingredients and products in their diet. The blog material revealed that for some the meals had to be planned down to the smallest detail – everything was counted, assessed and weighed. The authors of the book *Super Food* gave the reader a list of 50 different food items your diet should consist of. The also gave information about what nutrients they contained, and what effect they had on your body (Berge & Chacko, 2010).

Several doctors have discussed the increasing tendency not to tolerate weaknesses, handicap and suffering. One example of this is the stigmatization of bodies which do not fit into the ideal, e.g. overweight and obese (Johannison, 1993; Sobal, 1999; Crossley, 2004; Crawford, 2006; Lærum, 2013). Bratman (2000) thinks there is reason to criticize our increasing demands for spiritual and bodily perfection: What is healthy enough? What is slim enough? What is so harmful about bread, potatoes, flour, milk, cooked food, etc.? Why is it so important to stay away from these products? Moreover, he believes that one can easily lose perspective when focusing to a large extent on avoiding ordinary ingredients and foods. There are many factors that contribute to good health, obesity, etc. Although eating the wrong food is seen as an important reason for poor health, there are also many other factors that should be taken into consideration.

6. Concluding Remarks

Our results suggest that food and eating is an increasingly important therapeutic tool in changing and improving the body and self. New diets and products which are being marketed as especially healthy are being launched continuously. A major challenge is that the therapeutic consumption field is also characterized by a variety of theories, practices and therapies - many of them are totally contradictory. As a consequence it seems that eating habits have become an increasingly intricate and anxiety-provoking project. However, our purpose was not to question the value of healthy eating. There is no question that making the right food choices can reduce the risk of overweight, obesity, heart disease and cancer. This is a well-known and indisputable fact (Note 13). Yet, there is a need for more research on the social consequences of today's preoccupation with healthy eating. As shown in

this paper, our food choices are both a source of realizing desirable values, but also a field characterized by guilt and shame; nothing is ever good (healthy) enough. From our findings, there is much to suggest that being a picky eater now seems to be a way of expressing health awareness. All in all, our findings show that more and more have developed a strained relationship with food. As a blogger commented: "I do not know anybody who has a relaxed relationship with food!"

References

Albers-Mills, N. D., & Betay, D. G. (1996). Business Advertising Appears as a Mirror of Cultural Dimensions: A Study of Eleven Countries. *Journal of Advertising, 25*(4), 57-70. http://dx.doi.org/10.1080/00913367.1996.10673512

Andrews, T. (2006). Hva er det med rå fisk? En sosiologisk refleksjon over en økende interessen for det japanske kjøkken. *Sosiologisk tidsskrift, 2*, 171-177.

Baudrillard, J. (1986). *Amerika*. Oslo: Profil forlag.

Beck, U. (1986). *Risk Society. Towards a New Modernity.* London: Sage Publications.

Bentley, A. (2004). The Other Atkins Revolution. Atkins and the shifting culture of dieting. *Gastronomica – The Journal of Food and Culture, 4*(3), 34-45. http://dx.doi.org/10.1525/gfc.2004.4.3.34

Bentley, A. (2005). Men on Atkins: Dieting, Meat, and Masculinity. In H. Lisa, K. Mommer & C. Pineo (Eds.), *The Atkins Diet and Philosophy. Chewing the Fat with Kant and Nietzsche* (pp. 185-190). Chicago: Open Court.

Berge, T., & Sara, C. (2010). *Supermat*. Oslo: J.M. Stenersen forlag.

Bratman, S. (2000). *Orthorexia Nervosa: Overcoming the Obsession with Healthful Eating. Health food junkies*. New York: Broadway Books.

Brewis, A. (2011). Body norms and fat stigma in a global perspective. *Current Anthropology, 52*(2), 269-276. http://dx.doi.org/10.1086/659309

Bugge, A. (2010). Eating out: A multifaceted activity in contemporary Norway. *Food, Culture & Society. An International Journal for Multidisciplinary Research, 13*(2), 215-240. http://dx.doi.org/10.2752/175174410X12633934463150

Bugge, A. (2014). Kroppsrelatert matforbruk og helse. In R. Lavik & E. Borgeraas (Eds.), *Forbrukertrender 2014. SIFO Survey 2014.* Oslo: National Institute for Consumer Research (SIFO).

Bugge, A., & Mari, R. (2013). Usunne mat- og drikkereklamer rettet mot barn. En systematisk kartlegging av omfanget i utvalgte mediekanaler. SIFO Report no. 5-2013. Oslo: National Institute for Consumer Research (SIFO).

Bugge, A. (2012). Spis deg sunn, sterk, slank, skjønn, smart, sexy… - finnes det en diett for alt? SIFO Report no.12-2012. Oslo: National Institute for Consumer Research (SIFO).

Bugge, A., & Kjersti, L. (2009). «Fit, ikke fet! Forandring, forbedring og forvandlingsimperativer. In A. Kristin & E. Jacobsen (Eds). *Forbrukernes ansvar.* (pp. 145-170). Oslo: Cappelen Akademisk Forlag.

Bugge, A., Kjersti, L., & Randi, L. (2008). Nordmenns brød- og kornvaner. I stabilitet og endring. SIFO Report no.2-2008. Oslo: National Institute for Consumer Research (SIFO).

Campos, P. (2004). *The Obesity Myth. Why America's Obsession with Weight is Hazardous to Your Health*. New York: Gotham Books.

Caplan, P. (1997). *Food, Health and Identity.* London: routledge.

Crawford, R. (2006). Health as a meaningful social practice. *Health (London), 10*, 401-420. http://dx.doi.org/10.1177/1363459306067310

Crossley, N. (2004). Fat is a sociological issue: Obesity rates in late modern, "body-conscious" Societies. *Social Theory & Health, 2*, 222-253. http://dx.doi.org/10.1057/palgrave.sth.8700030

Crotty, P. (1999). Food and Class. In G. John & L. Williams (Eds.), *A Sociology of Food and Nutrition. The Social Appetite* (pp. 135-148). Oxford: Oxford University Press.

Douglas, M., & Aron, W. (1982). *Risk and Culture: An Essay on Selection of Technological and Environmental Dangers*. Berkeley: University of California Press.

Donna, M., & Jeffery, S. (1995). *Eating Agendas: Food and Nutrition as Social Problems.* New York: Aldine de

Gruyter.

Eenfeldt, A. (2011). *Matrevolusjonen. Naturlig sunn med skikkelig mat*. Oslo: Lille måne.

Ehn, B., & Orvar, L. (2012). *Kulturanalytiska verktyg*. Malmö: Gleerups.

Ehrenberg, A. (2009). *The Weariness of the Welf: Diagnosing the History of Depression in the Contemporary Age*. Montreal: McGill-Queen's University Press.

Gard, M., & Jan, W. (2005). *The Obesity Epidemic. Science, Morality and Ideology*. London: Routledge.

Giddens, A. (1991). *Modernity and Self-identity. Self and Society in the Late Modern Age*. Cambridge: Polity Press.

Hafsteinsdottir, T. (2009). *Spis deg yngre. 96 oppskrifter som smaker himmelsk og gir deg energi og vitalitet*. Oslo: Aschehoug.

Herrick, C. (2009). Shifting blame/selling health: corporate social responsibility in the age of obesity. *Socioogy of Health & Illness, 31*(1), 51-65. http://dx.doi.org/10.1111/j.1467-9566.2008.01121.x

Hexeberg, S. (2010). *Frisk med lavkarbo. Nytt liv med riktig mat*. Oslo: Cappelen Damm.

Johannison, K. (1993). Vårt behov for sjukdom. *Nordisk Medici, 108*, 92-93.

Johansson, B., & Eva, O. (2012). Managing the everyday health puzzle in Swedish families with children. *Food & Foodways, 20*(2), 123-146. http://dx.doi.org/10.1080/07409710.2012.680358

Knight, C. (2005). "The Food Nature. Intended You to Eat": Low-Carbohydrate Diets and Primitivst Philosophy. In H. Lisa, K. Mommer & C. Pineo (Eds.), *The Atkins Diet and Philosophy. Chewing the Fat wit Kant and Nietzsche* (pp. 43-56). Chicago: Open Court. http://dx.doi.org/10.1038/433797a

Knight, C. (2012). "An alliance with mother nature": Natural food, health and morality in low-carbohydrate diet books. *Food & Foodways, 20*(2), 102-123. http://dx.doi.org/10.1080/07409710.2012.680352

Kristensen, D., Heidi, B., & Søren, A. (2011). Leaving the milky way! The formation of a consumer counter mythology. *Journal of Consumer Culture, 11*(2), 195-214. http://dx.doi.org/10.1177/1469540511402449

Lawrence, M., & John, G. (1999). Future food: The politics of functional foods and health claims. In G. John & L. Williams (Eds.), *A Sociology of Food and Nutrition. The Social Appetite*. Oxford: Oxford University Press.

Lupton, D. (1996). *Food, the Body and the Self*. London: Sage Publications.

Lupton, D. (2012). *Medicine as Culture. Illness, Disease and the Body in Western Societies*. London: Sage Publication. http://dx.doi.org/10.4135/9781446254530

Lærum, O. D. (2013). Hvem er syk og hvem er frisk? Sykdommer behøver oss, uten oss kan de ikke leve. Behøver vi sykdommene for å vise at vi vantrives I kulturen? *Tidsskrift for den norsk legeforening, 15*(133), 634-637.

Madsen, O. J. (2010). *Den terapeutiske kultur*. Oslo: Universitetsforlaget.

Mallyon, A., Mary, H., John, C., & Maria, Z. (2010). I'm not dieting, «I'm doing it for science»: Masculinities and the experience of dieting. *Health Sociology Reviwew, 19*(3), 330-342. http://dx.doi.org/10.5172/hesr.2010.19.3.330

Mauritson, N., & Thorbjörg, H. (2011). *Kjernesunn mat. Forvandler familien på få uker*. Oslo: Cappelen Damm.

Mennell, S., Anne, M., & Anneke, H. (1992). *The Sociology of Food. Eating, Diet and Culture.* London: Sage Publications.

McRobbie, A. (2009). *The Aftermath of Feminism. Gender, Cultures and Social Change*. London: Sage Publications.

Miles, S. (2002). *The Changing Consumer: Markets and Meaning.* London: Taylor & Francis Ltd.

Palmcrantz, E., & Imerla, L. (2011). *Raw food på norsk. De beste oppskriftene på naturlig mat*. Cappelen Damm.

Pollan, M. (2008). *In Defence of Food: An Eater's Manifesto*. Waterville: Thorndike Press.

Princess, M. L., & Elisabeth, S. (2009). *Møt din skytsengel. En innføring i å møte din unike kraft.* Oslo: Cappelen Damm.

Rimke, H. M. (2000). Governing Citizens through Self-help Literature. *Cultural Studies, 14*(1), 61-78. http://dx.doi.org/10.1080/095023800334986

Skårderud, F. (1991). *Sultekunsnere. Kultur, kropp og kontroll*. Oslo: Aschehoug.

Sobal, J. (1999). Sociological analysis of the stigmatisation of obesity. In J. Germov & L. Williams (Eds.), *A Sociology of Food and Nutrition. The social appetite*. Oxford: Oxford University Press.

Warde, A. (1997). *Consumption, food and taste*. *Culinary antinomies and commodity culture*. London: Sage Publications.

Williams, L., & John, G. (1999). The thin ideal: Women, food, and dieting. In G. John & L. Williams (Eds.), *A sociology of food and nutrition. The social appetite.* (pp. 205-228). Oxford: Oxford University Press.

Winson, A. (2004). Bringing political economy into the debate on the obesity epidemic. *Agriculture and Human Values, 21*, 299-312. http://dx.doi.org/10.1007/s10460-003-1206-6

Notes

Note 1.
http://www.google.no/search?hl=no&site=imghp&tbm=isch&source=hp&biw=1920&bih=980&q=%22spis+deg%22&oq=%22spis+deg%22&gs_l=img.3..0j0i24l9.14483.19539.0.20093.18.13.4.1.0.0.141.1085.12j1.13.0...0.0...1ac.1.17.img.OPV57FqAgwc (20.06.13)

Note 2. AC Nielsen 1995-2011

Note 3. http://brn.no/brn.no/brnno/Bransjen/Markedsdata/ (02.01.13)

Note 4. http://www.norstatgroup.com/ (25.06.13)

Note 5. Norwegian Statistics (2012) *IKT-bruk i husholdningene. 2. kvartal 2012.* http://www.ssb.no/english/subjects/10/03/ikthus_en/ (September 20th 2012)

Note 6. http://lchf-bloggen.blogspot.no/ (September 20th 2012)

Note 7. http://www.lavkarbogjortenkelt.net/ (September 20th 2012)

Note 8. http://fotballfrue.no/ (September 20th 2012)

Note 9. FHI (2011) Overweight and obesity in Norway – fact sheet. http://www.fhi.no/eway/default.aspx?pid=238&trg=Area_5954&MainArea_5811=5895:0:15,4988:1:0:0:::0:0&MainLeft_5895=5954:0:15,4988:1:0:0:::0:0&Area_5954=5825:74991::1:5955:1:::0:0 (02.01.13)

Note 10. Norwegian Institute of Public Health (2011) Overweight and obesity in Norway – fact sheet.

http://www.fhi.no/eway/default.aspx?pid=238&trg=Area_5954&MainArea_5811=5895:0:15,4988:1:0:0:::0:0&MainLeft_5895=5954:0:15,4988:1:0:0:::0:0&Area_5954=5825:74991::1:5955:1:::0:0 (Search date: 10.10.12)

Note 11.
http://helsedirektoratet.no/publikasjoner/kostrad-for-a-fremme-folkehelsen-og-forebygge-kroniske-sykdommer/Publikasjoner/kostrad-for-a-fremme-folkehelsen-2011.pdf (29.08.13)

Note 12. E.g. Maurer & Sobal 1995, Sobal 1999, Williams and Germov 1999, McRobbie 2000, Crawford 2006.

Note 13.
http://helsedirektoratet.no/publikasjoner/kostrad-for-a-fremme-folkehelsen-og-forebygge-kroniske-sykdommer/Publikasjoner/kostrad-for-a-fremme-folkehelsen-2011.pdf (29.08.13)

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Effects of Different Storage on Qualities of Adzuki and Red Kidney Beans

Shan Wu[1,2], Shuo Feng[1,2] & Michiyuki Kojima[1]

[1] Department of Food Production Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan

[2] United Graduate School of Agricultural Sciences, Iwate University, Morioka, Iwate, Japan

Correspondence: Michiyuki Kojima, Department of Food Production Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan. Tel: 81-015-549-5547. Fax: 81-015-549-5547. E-mail: kojima@obihiro.ac.jp

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Abstract

This is the first study to report the effects of frozen soil storage on adzuki and red kidney beans. Adzuki and red kidney beans were stored for 15 months in polyethylene or paper bags under three different conditions: natural cold energy storage in frozen soil, storage in a freezer at -20 °C, and storage at a constant temperature of 25 °C. Samples were collected from every treatment group every 3 months, and qualities related to processing (bean paste yield and cooked bean hardness), seed moisture content, one-hundred seed weight, seed density, and sample physical properties (electrical conductivity and soluble solid content) were evaluated. Frozen soil storage resulted in the least deterioration of important qualities in adzuki and red kidney beans. We also showed that the electrical conductivity value and soluble solid content of the liquid used for soaking beans can be used to evaluate the processing suitability of cooked beans.

Keywords: bean, electrical conductivity, adzuki, natural cold energy, storage

1. Introduction

Adzuki beans (*Vigna angularis* (Willd.) Ohwi et H. Ohashi) are widely grown in East Asian countries such as China and Japan, as well as in the Korean Peninsula (Watanabe, 2000). These beans have high nutritional value. Their protein content is approximately 20%; anthocyanin is abundant in the seed coat, and minerals such as zinc also are abundant (Kagawa, 2010). These beans have traditionally been widely used in Japanese sweets, such as dorayaki and amanatto (a boiled bean paste of adzuki and red kidney beans), and Chinese food items, including jelly and steamed bread (Hatai, 1994).

Red kidney beans (*Phaseolus vulgaris* L.) were introduced from Latin America to Japan and are grown worldwide. These beans also have high nutritional value and are used in amanatto (Kokubun, 2010).

Hokkaido, in the northernmost region of Japan, produces 86% of adzuki beans and 94% of kidney beans in this region (Ministry of Agriculture, Forestry and Fisheries, 2010). Fluctuations in planted acreage and weather conditions affect production. Therefore, it is important to develop storage methods that can ensure a stable supply of high-quality beans (Kato, 2002). Long-term storage of red kidney beans under poor conditions leads to the hard-to-cook (HTC) phenomenon, in which the suitability of the beans for processing declines (Moscoso et al., 1984). Storage at high temperatures (30 °C) also reduced the processing suitability of adzuki beans (Yousif et al., 2002).

In recent years, storage systems that use natural cold energy, rather than freezers that require fuel to operate and cause greenhouse gases to be emitted, have been implemented as a measure to avoid global warming. About 140 such facilities were created in Hokkaido in June 2010 (Planning and Follow-up Group, Development Research Division, Development Administration, 2010). Tsuchiya et al. (1994) reported that storage systems that used natural cold energy are cost-effective, emit less carbon dioxide than freezers, and can reduce the global burden on the environment. In particular, frozen soil storage that does not use power has been used to store vegetables such as potatoes. Storage under these conditions positively affected quality and led to saccharification and sprout inhibition. According to one study, the sugar content of potato stored in frozen soil storage for 4 months

increased (Tsuchiya & Ryokai, 1990). However, no studies have reported the effects of frozen soil storage on adzuki and red kidney beans.

In this study, adzuki and red kidney beans were stored under frozen soil storage by using a heat-pipe system; stored in a freezer at -20 °C, which is known to be an effective method of maintaining food quality during long storage; and maintained at a constant temperature of 25 °C, the usual temperature used for storing beans. Processing suitability values of the beans after storage were compared across the three conditions, in order to reveal the method that most efficiently and conveniently maintained processing suitability.

2. Materials and Methods

2.1 Plant Materials

Adzuki beans and red kidney beans were harvested in October 2009 at Otofuke, Tokachi, Hokkaido. The beans were maintained at 5 °C in the dark until the assays were performed in March 2010.

2.2 Storage Conditions

The beans were stored under frozen soil storage by using a heat-pipe system (NCE; temp.: 7.1 °C ± 3.4; relative humidity (RH): 84.8% ± 4.4, Obihiro City, Hokkaido, Japan); in a freezer at -20 °C (FS; RH: 35.5% ± 17.1); and at a constant temperature of 25°C (CS; RH: 41.3% ± 5.9). There was no difference in the temperature or humidity of NCE storage, as measured in a study that spanned 1988 to 1993 (Tsuchiya & Ryokai, 1996). Temperature and humidity for each storage condition and the outside environment in Hokkaido from March 2010 to February 2011 are shown in Figure 1 (Feng, 2015; Japan Meteorological Agency, 2015). The average temperature from March 2010 to February 2011 in Obihiro City, Hokkaido, Japan, was 7.9 °C ± 10.4, and relative humidity was 72.6% ± 6.2. There was no significant difference between the average temperature of NCE storage and the external environment in Obihiro City ($p < 0.01$).

A 1.4-kg portion of the bean sample was placed in polyethylene zipper bags (sealed bags, SB) having dimensions of 270 mm × 280 mm × 0.04 mm (Daiwa Bussan Co., Ltd.). The average humidity in the SB remained stable during the storage period (RH: adzuki beans, 54.8% ± 7.1; red kidney beans, 55.0% ± 4.2). The remaining samples were stored in paper bags (PB; tertrapack; tare weight, 280 g; Japan Toyo Paper Co., Ltd.) having dimensions of 830 mm × 417 mm × 0.4 mm.

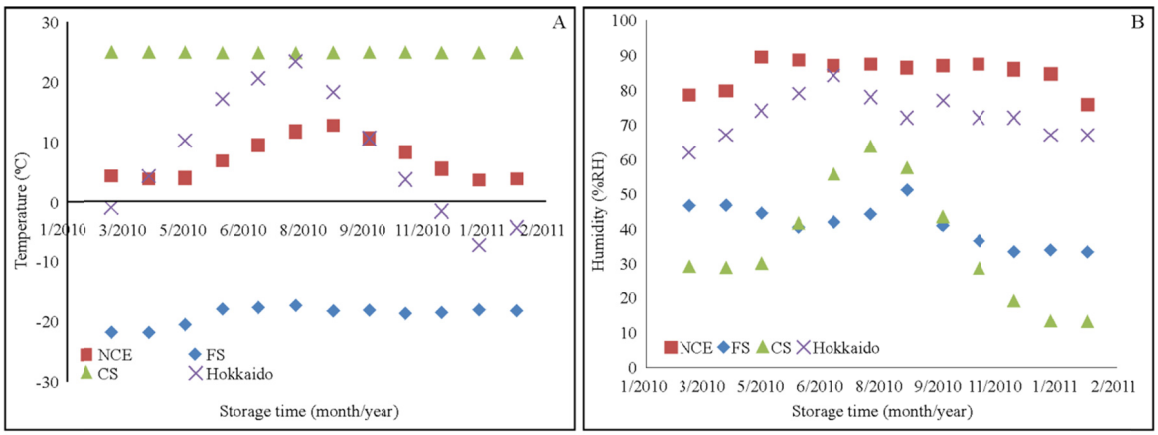

Figure 1. Changes in the temperature and humidity of each storage condition and Hokkaido, Japan from 3/2010 to 2/2011. NCE, natural cold energy (storage in frozen soil with a heat pipe type system); FS, storage in a freezer at 20 °C; CS, storage at a constant temperature of 25 °C (Feng, 2015; Japan Meteorological Agency, 2015)

2.3 Sample Collection

From March 2010 to May 2011, a sample from each of the three storage conditions was collected every three months (1.4 kg/time).

2.4 Seed Moisture Content

The seed moisture content (SMC) was measured using the AOAC method (Cunniff, 1995). The decrease in moisture content was measured after the seeds were placed in an oven at 105 ± 5 °C for 24 h.

2.5 Seed Traits

Hundred-seed weight (100 SW) and seed density were measured, separately determining the weight of 100 randomly selected seeds of each type of stored beans. In addition, 50 g beans and 250 mL deionized water were placed in a 500-mL measuring cylinder, the cylinder was sealed to prevent the entry of air, and the volume was recorded. The weight (g) and volume (mL) values were used to calculate the seed density (g/mL) (Giami & Okwechime, 1993).

2.6 Physical Properties and Processing Suitability

In order to measure the processing suitability of beans, 250 mL deionized water was added to 50 g bean seeds. The beans were soaked for 18 h in an incubator at 25 °C, removed from the soaking liquid, and weighed. The hydration coefficient was calculated (El-Refai et al., 1988). The liquid in which the beans were soaked was collected and filtered, and its electrical conductivity and soluble solid content were measured using a digital electric conductivity meter (DEC-1; Atago Co., Ltd.) and digital pocket refractometer (PAL-Patissier; Atago Co., Ltd.), respectively. Subsequently, the soaked beans were placed in a beaker, 250 mL deionized water was added, and the beans were autoclaved for 20 min at 95 °C. They were then cooled at room temperature for 15 min, and the liquid and beans were separated using a bamboo basket. The hardness of the cooked beans was measured using a texture-analyser with a flat 2-mm diameter steel punch that was applied with a crosshead speed of 30 cm min^{-1} (Stable Micro System Co., Ltd.), according to the method described by Reyes-Moreno et al. (1994). A total of 3*10 beans were punched individually for each treatment and the mean peak force was calculated in Newtons per seed (N $seed^{-1}$). Finally, the seed coat of the cooked beans was separated by passing the beans through a 0.5-mm sieve, washing them three times with 1 L deionized water, and squeezed using a bleached cotton cloth bag to prepare a raw bean paste. The yield of the paste was calculated as follows: bean paste yield = raw bean paste dry weight/bean dry weight × 100.

2.7 Statistical Analysis

All the data are expressed as means ± standard deviation (SD). Analysis of variance (ANOVA) with Tukey's studentized range (HSD) test was conducted using SAS 9.3, and differences were considered significant at $p < 0.01$.

3. Results

The changes in SMC, seed traits, and processing suitability of adzuki and red kidney beans stored under the three different conditions are shown in Tables 1-4.

3.1 Seed Moisture Content

The SMC of red kidney beans was higher than that of adzuki beans stored in PB (Tables 1 and 2). There was almost no difference in the SMC of the two types of beans stored in the SB under the three storage conditions for 15 months. The SMC of both types of beans was greatest when they were stored in PB at NCE for 15 months, and that of beans stored in PB at CS was the smallest. Thus, packaging in SB might suppress the variation in moisture content of both types of beans.

Table 1. Changes in the seed moisture content, hundred seed weight, seed density, and hydration coefficient of adzuki beans

SB			
Storage time	Seed moisture content (%)		
(months)	NCE	FS	CS
0	12.3±1.0a	12.3±1.0a	12.3±1.0a
3	11.8±0.1a	12.3±0.1a	11.4±0.1a
6	11.8±0.1a	11.6±0.1a	11.8±0.0a
9	11.8±0.0a	12.2±0.3a	11.9±0.0a
12	12.3±0.1a	12.2±0.1a	11.9±0.4a
15	12.4±0.5a	12.2±0.1a	12.0±0.1a
	Hundred seed weight (g)		
	NCE	FS	CS
0	13.2±0.1a	13.2±0.1a	13.2±0.1a
3	13.0±0.1a	13.3±0.2a	13.5±0.3a
6	13.1±0.1a	13.2±0.2a	13.1±0.2a
9	13.0±0.1a	13.1±0.1a	13.1±0.2a
12	13.1±0.1a	13.1±0.1a	12.8±0.2a
15	12.9±0.1a	13.1±0.1a	13.1±0.1a
	Seed density (g/mL)		
	NCE	FS	CS
0	1.32±0.0a	1.32±0.0a	1.32±0.0a
3	1.32±0.0a	1.29±0.0a	1.33±0.0a
6	1.32±0.0a	1.31±0.0a	1.33±0.0a
9	1.32±0.0a	1.32±0.0a	1.33±0.0a
12	1.32±0.0a	1.32±0.0a	1.32±0.0a
15	1.32±0.0a	1.32±0.0a	1.32±0.0a
	Hydration coefficient		
	NCE	FS	CS
0	235±4a	235±4a	235±4a
3	239±9a	246±5a	243±5a
6	236±5a	235±4a	237±6a
9	238±0a	236±0a	237±1a
12	237±0a	236±0a	237±2a
15	237±0a	236±0a	236±0a
PB			
Storage time	Seed moisture content (%)		
(months)	NCE	FS	CS
0	12.3±1.0a	12.3±1.0a	12.3±1.0a
3	14.1±0.2a*	12.4±0.0ab	8.6±0.2c*
6	15.3±0.3a*	12.4±0.1b	11.4±0.1c*
9	15.9±0.1a*	12.9±0.1b	9.1±0.1c*

12	16.5±0.1a*	13.3±0.2b	7.4±0.1c*
15	15.6±0.2a*	12.69±0.1b*	5.7±0.1c*
	Hundred seed weight (g)		
	NCE	FS	CS
0	13.2±0.1a	13.2±0.1a	13.2±0.1a
3	13.9±0.2a	13.4±0.2ab	12.6±0.2b
6	13.8±0.2a	13.2±0.1ab	13.1±0.1b
9	13.8±0.3a	13.2±0.2a	12.4±0.1b
12	13.8±0.2a	13.2±0.1b	12.1±0.0c
15	14.0±0.1a	13.1±0.1b	12.3±0.1c
	Seed density (g/mL)		
	NCE	FS	CS
0	1.32±0.0a	1.32±0.0a	1.32±0.0a
3	1.33±0.0a	1.33±0.0a	1.35±0.0a
6	1.32±0.0a	1.28±0.0a	1.31±0.0a
9	1.32±0.0a	1.32±0.0a	1.32±0.0a
12	1.32±0.0a	1.32±0.0a	1.32±0.0a
15	1.32±0.0a	1.32±0.0a	1.32±0.0a
	Hydration coefficient		
	NCE	FS	CS
0	235±4a	235±4a	235±4a
3	240±9a	241±10a	243±7a
6	239±7a	236±5a	236±5a
9	239±1a	239±3a	236±7a
12	240±2a	238±0a	236±5a
15	240±2a	238±0a	234±5a

NCE, natural cold energy (storage in frozen soil with a heat pipe type system); FS, storage in a freezer at 20 °C; CS, storage at a constant temperature of 25 °C; SB, sealed bags; PB, paper bags. Data are expressed as means ± SD (n = 3). The values with different superscript letters in the same row are significantly different among the storage conditions ($p < 0.01$). The superscript * indicates significant difference in the data presented in the columns compared with the values for 0 month ($p < 0.01$).

3.2 Seed Traits

The 100 SW of adzuki beans placed in SB was not significantly different across the three storage conditions (12.1-14.0 g), whereas that of adzuki beans stored at NCE in PB was the greatest, indicating the highest retention of moisture content (Table 1). On the other hand, there was no significant difference in the 100 SW of red kidney beans stored under the three storage conditions in SB and PB (67.9-72.5 g; Table 2).

There were no changes in seed density of adzuki and red kidney beans, irrespective of the storage conditions or packaging methods used (Tables 1 and 2).

Table 2. Changes in the seed moisture content, hundred seed weight, seed density, and hydration coefficient of red kidney

SB			
Storage time	Seed moisture content (%)		
(months)	NCE	FS	CS
0	13.2±0.2a	13.2±0.2a	13.2±0.2a
3	11.3±0.2a*	11.2±0.5a	11.5±0.3a*
6	12.0±0.2a*	12.5±0.2a	12.2±0.2a*
9	12.2±0.1a*	12.8±0.1a	13.3±0.2a
12	13.6±0.7a	13.3±0.8a	13.1±0.1a
15	11.8±0.2a*	11.8±0.5a	12.0±0.1a*
	Hundred seed weight (g)		
	NCE	FS	CS
0	67.9±3.3a	67.9±3.3a	67.9±3.3a
3	70.9±0.7a	68.6±2.0a	70.4±1.2a
6	70.5±0.8a	69.2±0.8a	69.3±2.0a
9	70.6±0.8a	67.9±2.0a	69.0±1.9a
12	69.9±1.1a	69.5±1.4a	67.8±0.6a
15	68.7±0.8a	67.8±1.4a	70.4±1.2a
	Seed density (g/mL)		
	NCE	FS	CS
0	1.27±0.0a	1.27±0.0a	1.27±0.0a
3	1.25±0.0a	1.25±0.0a	1.26±0.0a
6	1.25±0.0a	1.26±0.0a	1.28±0.0a
9	1.25±0.0a	1.25±0.0a	1.25±0.0a
12	1.25±0.0a	1.25±0.0a	1.25±0.0a
15	1.25±0.0a	1.25±0.0a	1.25±0.0a
	Hydration coefficient		
	NCE	FS	CS
0	225±1a	225±1a	225±1a
3	226±1a	227±1a	224±2a
6	227±1a	227±1a	225±1a
9	228±0a	224±1a	228±4a
12	229±5a	229±3a	229±1a
15	228±3a	228±3a	228±0a
PB			
Storage time	Seed moisture content (%)		
(months)	NCE	FS	CS
0	13.2±0.2a	13.2±0.2a	13.2±0.2a
3	14.6±0.3a	12.5±0.3b	7.2±0.5c
6	17.1±0.7a*	13.2±0.1b	11.5±0.1b
9	20.2±0.8a*	12.9±1.4b	9.3±3.5b

12	20.3±0.3a*	13.7±1.6b	10.2±0.2b
15	19.4±0.6a*	11.2±0.5b	9.8±0.9b
	Hundred seed weight (g)		
	NCE	FS	CS
0	67.9±3.3a	67.9±3.3a	67.9±3.3a
3	72.5±1.9a	70.8±2.0a	70.9±1.1a
6	70.1±1.0a	69.4±1.1a	71.5±0.6a
9	69.9±0.9a	66.2±1.7a	67.9±1.6a
12	70.5±1.3a	68.5±0.8a	68.8±0.7a
15	68.7±0.8a	69.7±0.8a	70.7±1.7a
	Seed density (g/mL)		
	NCE	FS	CS
0	1.27±0.0a	1.27±0.0a	1.27±0.0a
3	1.25±0.0a	1.25±0.0a	1.26±0.0a
6	1.25±0.0a	1.26±0.0a	1.26±0.0a
9	1.25±0.0a	1.25±0.0a	1.25±0.0a
12	1.25±0.0a	1.25±0.0a	1.25±0.0a
15	1.25±0.0a	1.25±0.0a	1.25±0.0a
	Hydration coefficient		
	NCE	FS	CS
0	225±1a	225±1a	225±1a
3	225±1a	227±2a	225±2a
6	225±3a	226±1a	224±1a
9	232±3a	233±1a	234±8a
12	231±1a	234±3a	232±4a
15	229±2a	226±2a	223±3a

NCE, natural cold energy (storage in frozen soil with a heat pipe type system); FS, storage in a freezer at 20 °C; CS, storage at a constant temperature of 25 °C; SB, sealed bags; PB, paper bags. Data are expressed as means ± SD (n = 3). The values with different superscript letters in the same row are significantly different among the storage conditions ($p < 0.01$). The superscript * indicates significant difference in the data presented in the columns compared with the values for 0 month ($p < 0.01$).

3.3 Physical Properties and Processing Suitability

The hydration coefficient, electrical conductivity, soluble solid content, and hardness of beans stored under NCE and FS conditions were not significantly different between the two types of beans (Tables 3 and 4). The values of all the physical properties, except the hydration coefficient of adzuki and red kidney beans stored at CS, significantly increased at 12 and 9 months ($p < 0.01$), respectively.

There was no significant difference in the paste yield of both the beans stored under NCE and FS conditions for 15 months (56.81-60.97% and 61.71-63.53%, respectively), whereas that of both the types of beans stored under CS conditions decreased significantly ($p < 0.01$; Tables 3 and 4).

3.4 Correlation Between Physical Properties and Processing Suitability

The SAS software package was used to calculate the correlation coefficients between physical properties and processing suitability. The hardness of adzuki beans was strongly positively correlated with both electrical conductivity and the soluble solid content of the soaking liquid ($r_{SB} = 0.983$, $r_{PB} = 0.935$, $r_{SB} = 0.943$, $r_{PB} = 0.944$; Figure 2). The correlation coefficients between physical properties and processing suitability of red kidney beans

were similar to those of adzuki beans, as shown in Figure 3.

Table 3. Changes in physical properties and processing suitability of adzuki beans stored at different storage conditions

SB			
Storage time	Electrical conductivity (μS/cm)		
(months)	NCE	FS	CS
0	900±0a	900±0a	900±0a
3	900±0a	900±0a	900±0a
6	900±0a	900±0a	900±0a
9	900±0b	900±0b	1067±58a*
12	900±0b	900±0b	1300±0a*
15	900±0b	900±0b	1467±58a*
	Soluble solid content (Brix%)		
	NCE	FS	CS
0	0.4±0.1a	0.4±0.1a	0.4±0.1a
3	0.4±0.0a	0.4±0.0a	0.4±0.0a
6	0.4±0.0a	0.4±0.0a	0.4±0.0a
9	0.4±0.0a	0.4±0.0a	0.4±0.0a
12	0.4±0.0b	0.4±0.0b	0.6±0.0a*
15	0.4±0.0b	0.4±0.0b	0.6±0.0a*
	Hardness of cooked beans (N/seed)		
	NCE	FS	CS
0	0.529±0.0a	0.529±0.0a	0.529±0.0a
3	0.534±0.0a	0.520±0.0a	0.505±0.0a
6	0.551±0.1a	0.535±0.1a	0.702±0.1a*
9	0.538±0.0b	0.537±0.1b	1.140±0.2a*
12	0.548±0.1b	0.505±0.1b	2.724±0.1a*
15	0.540±0.1b	0.517±0.0b	3.739±0.1a*
	Yield of bean paste (%)		
	NCE	FS	CS
0	59.43±2.3a	59.43±2.3a	59.43±2.3a
3	59.13±3.2a	59.76±2.5a	58.68±2.7a
6	59.47±3.0a	59.83±4.0a	58.47±2.1a
9	60.97±2.3a	60.83±2.6a	52.73±1.8a
12	57.87±1.6a	60.40±1.0a	38.06±2.9b*
15	58.27±2.0a	58.00±3.3a	19.63±1.3c*
PB			
Storage time	Electrical conductivity (μS/cm)		
(months)	NCE	FS	CS
0	900±0a	900±0a	900±0a
3	900±0b	900±0b	1333±58a

6	900±0b	900±0b	1400±100a
9	900±0b	900±0b	1933±58a*
12	900±0b	900±0b	2233±58a*
15	900±0b	900±0b	2600±100a*
	Soluble solid content (Brix%)		
	NCE	FS	CS
0	0.4±0.1a	0.4±0.1a	0.4±0.1a
3	0.4±0.0a	0.4±0.0a	0.5±0.0a
6	0.4±0.0a	0.4±0.0a	0.5±0.0a
9	0.4±0.0b	0.4±0.0b	0.7±0.0a
12	0.4±0.0b	0.4±0.0b	0.8±0.1a*
15	0.4±0.0b	0.4±0.0b	0.9±0.0a*
	Hardness of cooked beans (N/seed)		
	NCE	FS	CS
0	0.529±0.0a	0.529±0.0a	0.529±0.0a
3	0.532±0.0a	0.518±0.0a	0.542±0.0a
6	0.530±0.1a	0.513±0.1a	0.539±0.0a
9	0.540±0.0b	0.512±0.1b	0.949±0.1a*
12	0.528±0.0b	0.496±0.1b	1.062±0.1a*
15	0.526±0.0b	0.500±0.0b	1.139±0.1a*
	Yield of bean paste (%)		
	NCE	FS	CS
0	59.43±2.3a	59.43±2.3a	59.43±2.3a
3	58.74±2.9a	58.60±4.4a	57.63±2.6a
6	59.93±4.1a	59.31±3.0a	57.96±2.5a
9	58.85±1.8a	59.83±1.9a	53.17±3.3a*
12	57.88±2.1a	56.81±1.3a	46.18±3.2b*
15	58.55±1.1a	57.53±2.6a	44.10±0.8b*

NCE, natural cold energy (storage in frozen soil with a heat pipe type system); FS, storage in a freezer at 20 °C; CS, storage at a constant temperature of 25 °C; SB, sealed bags; PB, paper bags. Data are expressed as means ± SD (n = 3). The values with different superscript letters in the same row are significantly different among the storage conditions ($p < 0.01$). The superscript * indicates significant difference in the data presented in the columns compared with the values for 0 month ($p < 0.01$).

Table 4. Changes in the physical properties and processing suitability of red kidney beans stored at different storage conditions

SB			
Storage time	Electrical conductivity (μS/cm)		
(months)	NCE	FS	CS
0	800±0a	800±0a	800±0a
3	800±0a	833±58a	800±0a
6	800±0a	800±0a	800±0a
9	800±0b	800±0b	900±0a*
12	800±0b	800±0b	900±0a*
15	800±0b	800±0b	1100±0a*
	Soluble solid content (Brix%)		
	NCE	FS	CS
0	0.4±0.1a	0.4±0.1a	0.4±0.1a
3	0.4±0.0b	0.4±0.0b	0.5±0.0a*
6	0.4±0.0a	0.4±0.0a	0.4±0.0a
9	0.4±0.0b	0.4±0.0b	0.5±0.0a*
12	0.4±0.0b	0.4±0.0b	0.5±0.0a*
15	0.4±0.0b	0.4±0.0b	0.5±0.0a*
	Hardness of cooked beans (N/seed)		
	NCE	FS	CS
0	1.127±0.0a	1.127±0.0a	1.127±0.0a
3	1.151±0.1a	1.119±0.1a	1.175±0.3a
6	1.117±0.1a	1.107±0.1a	1.184±0.2a
9	1.113±0.1b	1.064±0.1b	3.826±0.2a*
12	1.131±0.1b	1.085±0.0b	3.939±0.2a*
15	1.109±0.2b	1.055±0.2b	5.968±0.5a*
	Yield of bean paste (%)		
	NCE	FS	CS
0	63.53±2.8a	63.53±2.8a	63.53±2.8a
3	62.70±4.1a	62.51±4.4a	61.06±4.7a
6	62.56±2.3a	62.02±2.7a	61.04±3.0a
9	61.92±2.6a	62.04±3.3a	43.03±3.6b*
12	61.90±2.9a	62.84±3.0a	41.30±1.3b*
15	62.09±1.3a	62.84±3.0a	26.13±0.8b*
PB			
Storage time	Electrical conductivity (μS/cm)		
(months)	NCE	FS	CS
0	800±0a	800±0a	800±0a
3	800±0a	800±0a	933±58a
6	800±0a	800±0a	800±0a
9	800±0b	800±0b	1100±0a*

12	800±0b	800±0b	1100±0a*
15	800±0b	800±0b	1000±0a*
	Soluble solid content (Brix%)		
	NCE	FS	CS
0	0.4±0.1a	0.4±0.1a	0.4±0.1a
3	0.4±0.0b	0.4±0.0b	0.5±0.0a
6	0.4±0.0a	0.4±0.0a	0.4±0.0a
9	0.4±0.0b	0.4±0.0b	0.6±0.0a*
12	0.4±0.0b	0.4±0.0b	0.6±0.0a*
15	0.4±0.0a	0.4±0.0a	0.4±0.0a
	Hardness of cooked beans (N/seed)		
	NCE	FS	CS
0	1.127±0.0a	1.127±0.0a	1.127±0.0a
3	1.188±0.1a	1.111±0.0a	1.262±0.1a
6	1.207±0.0a	1.066±0.1a	1.208±0.2a
9	1.199±0.0b	1.104±0.0b	2.267±0.0a*
12	1.202±0.0b	1.114±0.0b	2.275±0.0a*
15	1.075±0.0b	1.136±0.0b	2.220±0.0a*
	Yield of bean paste (%)		
	NCE	FS	CS
0	63.53±2.8a	63.53±2.8a	63.53±2.8a
3	62.86±1.1a	63.17±2.8a	61.57±5.2a
6	61.71±4.6a	62.16±4.5a	61.47±4.0a
9	63.00±1.0a	63.23±2.1a	48.61±2.8b*
12	62.00±5.0a	62.08±3.0a	47.61±4.2ab*
15	62.43±2.3a	60.51±4.0a	44.50±1.6b*

NCE, natural cold energy (storage in frozen soil with a heat pipe type system); FS, storage in a freezer at 20 °C; CS, storage at a constant temperature of 25 °C; SB, sealed bags; PB, paper bags. Data are expressed as means ± SD (n = 3). The values with different superscript letters in the same row are significantly different among the storage conditions ($p < 0.01$). The superscript * indicates significant difference in the data presented in the columns compared with the values for 0 month ($p < 0.01$).

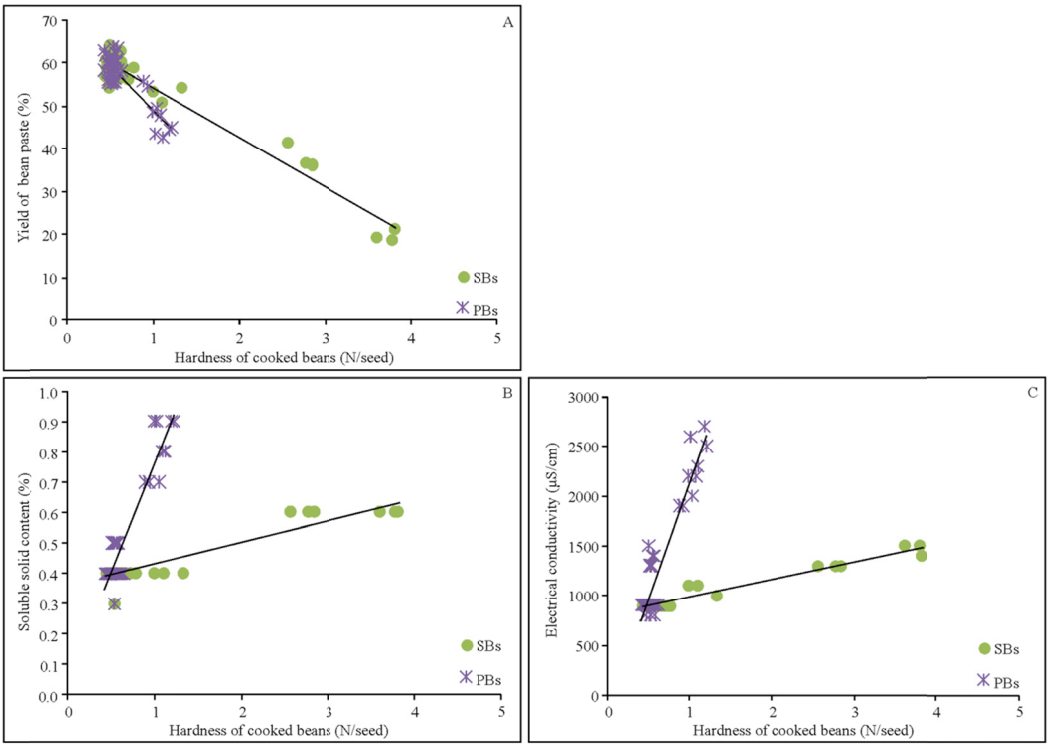

Figure 2. Correlation between yield of bean paste (A, rSB = -0.970, rPB = -0.861), soluble solid content (B, rSB = 0.943, rPB= 0.944), electrical conductivity (C, rSB = 0.983, rPB = 0.935), and hardness of cooked adzuki beans stored at different storage conditions ($p < 0.01$, $n = 48$)

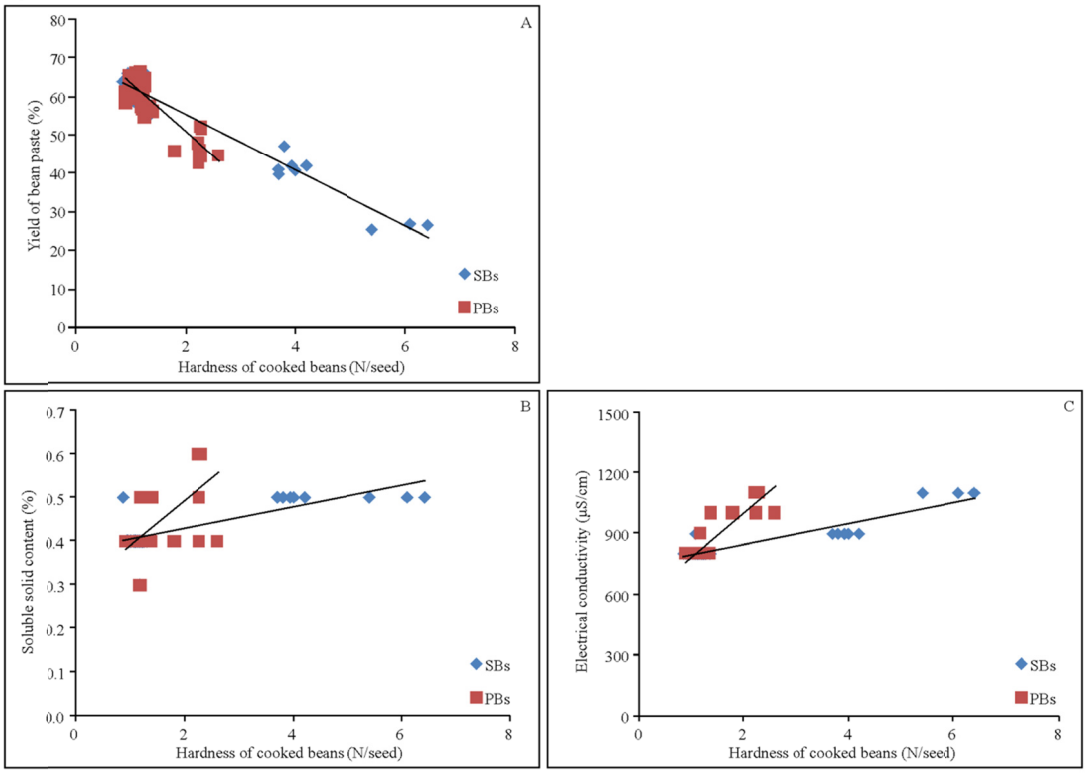

Figure 3. Correlation between yield of bean paste (A, rSBs = -0.970, rPBs = -0.875), soluble solid content (B, rSBs = 0.751, rPBs = 0.686), electrical conductivity (C, rSBs = 0.942, rPBs = 0.919), and hardness of cooked red kidney beans stored at different storage conditions ($p < 0.01$, $n = 48$)

4. Discussion

Adzuki and red kidney beans stored in SBs in frozen soil for 15 months showed good processing suitability that was similar to that obtained after storage at -20 °C. In particular, sensory evaluation of both the beans stored in SB in the frozen soil was significantly higher than that of beans stored using other methods. Therefore, a temperature of 7.1 °C and RH of 55.0% (SB in frozen soil storage) were considered the best storage conditions for adzuki and red kidney beans. In order to maintain a high RH and low temperature in frozen soil storage, a heat pipe is used to transfer cold energy to the underground storage location. This process does not require power and saves energy by using natural conditions to maintain a cold environment (Tsuchiya, 2004). It is likely that frozen soil storage can be efficiently implemented in Hokkaido, Japan, which has a cold climate.

The hardness of cooked beans and the amount of paste yield are important values that can predict whether the HTC phenomenon might occur. Frozen soil storage and storage at -20 °C efficiently maintained the hardness and paste yield of both the types of beans. However, both the bean parameters increased remarkably after constant storage at 25 °C. This finding is in agreement with that of Kato et al. (2000), who showed that adzuki beans stored for extended periods at high temperatures needed a longer time to cook to attain the same tenderness as beans stored under more suitable conditions. Liu et al. (1992) also showed that long-term storage of cowpeas at 30 °C increased the seed hardness from 15.8 to 91.2 $N \cdot g^{-1}$, whereas there was no change in seed hardness of beans stored at -18 °C. Similarly, the hardness of common beans (*Phaseolus* spp.) stored at 30-35 °C increased by 3–6 N per seed (Del-Valle & Stanley, 1995).

To determine the association between high temperature and HTC, Nakazato et al. (1986) investigated the effects of high temperature on kidney beans stored at high, medium, and low temperatures. They found a positive correlation between the hardness of beans and the insoluble pectin present in the cell walls and showed that beans stored at high temperature had the highest amount of insoluble pectin. On the other hand, Bhatty (1990) found that lignin content varied between beans that cooked quickly and those that took a long time to cook (1.2-1.7%). Therefore, insoluble pectin and lignin are considered to be responsible for the development of HTC.

The processing suitability was better when the beans were placed in SB and PB in frozen soil storage, storage at -20 °C for 15 months, or constant storage at 25 °C for 6 months. Berrios et al. (1999) reported that black beans stored constantly at 4.5 °C with an RH of 50-60% exhibited the high-quality characteristics found in fresh beans, such as shorter cooking time, smaller quantities of solid loss and electrolyte leaching, and lower percentage of hardshell, than those found in beans stored under other conditions (23-25 °C; RH, 30-50%). Storage of legumes for extended periods at high temperatures (30 °C) increases the thickness of the cotyledon cell wall, especially of the middle lamella and secondary cell wall (Hincks & Stanley, 1987). In our research, we found that constant humidity (SB storage) was associated with poor processing suitability of beans stored at 25 °C (a relatively high temperature). Beans stored at this temperature also showed poor processing suitability when the storage humidity was lower. However, humidity had no effect on the processing suitability of beans stored at -20 °C (FS) or 7.1 °C (NCE).

Electrical conductivity and soluble solid content of both types of beans showed no change after storage in frozen soil or in a -20 °C freezer for 15 months; however, constant storage at 25 °C for 3 to 9 months significantly increased the electrical conductivity and soluble solid content. Storage of legume seeds under poor conditions (high temperature and RH) for extended periods damages their cell membrane, allowing divalent cations to leak from the cells (Liu, 1995).

The SMC was retained by storing the adzuki beans in SB. Similarly, features associated with high quality (percentage weight loss, moisture content, fatty acid content, and color) of corn grains stored in sealed enclosures were well maintained with minimal damage by insects at the completion of the storage trial (Gras et al., 1990).

Bean hardness had a high positive correlation and paste yield had a negative correlation with electrical conductivity and soluble solid content of the soaking liquid of adzuki and red kidney beans. This finding suggests that the electrical conductivity and soluble solid content of the liquid used to soak beans may be used to evaluate the processing suitability of cooked beans and the paste yield of adzuki and red kidney beans. Nasar-Abbas et al. (2008) reported that solute and electrolyte leakage and the hardness of faba beans stored at 5-50 °C increased with increasing storage temperature. The soluble solid content of soaking liquid of soybeans stored under different storage conditions was correlated with processing suitability (Hira, 1983). Therefore, some researchers have hypothesized that electrical conductivity and the release of soluble solid content from bean cells might reduce their water affinity and water-holding capacity in accordance with osmotic principles, thereby affecting the processing suitability of beans (Jones & Boulter, 1983; Berrios et al., 1999; Nasar-Abbas et al.,

2008).

5. Conclusions

The results of this study indicated that the deterioration of processing suitability of adzuki and red kidney beans could be inhibited by using frozen soil storage. When both types of beans were stored in sealed bags in a natural cold storage system, their taste was retained and the HTC phenomenon was prevented. Electrical conductivity value and soluble solid content of the liquid used for soaking beans may be used to evaluate the processing suitability of cooked beans.

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References

Berrios, J. D. J., Swanson, B. G., & Cheong, W. A. (1999). Physico-chemical characterization of stored black beans (*Phaseolus vulgaris* L.). *Food Research. International, 32*, 669-676. http://dx.doi.org/10.1016/S0963-9969(99)00144-1h

Bhatty, R. S. (1990). Cooking quality of lentils: The role of structure and composition of cell walls. *Journal of Agricultural and Food Chemistry, 38*, 376-383. http://dx.doi.org/10.1021/jf00092a007

Cunniff, P. (1995). *AOAC, Official Methods of Analysis of AOAC International* (16th ed.), Arlington, VA: AOAC International.

Del-Valle, J. M., & Stanley, D. W. (1995). Reversible and irreversible components of bean hardening. *Food Research International, 28*, 455-463.

El-Refai, A. A., Harras, H. M., El-Nemr, K. M., & Noaman, M. A. (1988). Chemical and technological studies on faba bean seeds. I—Effect of storage on some physical and chemical properties. *Food Chemistry*, *29*, 27-39. http://dx.doi.org/10.1016/0308-8146(88)90073-8

Feng, S., Wu, S., & Kojima, M. (2015). Effect of storage in frozen soil on the quality of adzuki beans, soybean, red kidney beans, and buckwheat. Submitted to *Journal of Food Nutrition Research*.

Giami, S. Y., & Okwechime, U. I. (1993). Physicochemical properties and cooking quality of four new cultivars of Nigerian cowpea (*Vigna unguiculata L Walp*). *Journal of the Science of Food and Agricukture, 63*, 281-286. http://dx.doi.org/10.1002/jsfa.2740630304

Gras, P. W., Bason, M. L., Esteves, L. A., Sabio, G. C., Annis, P. C., & Graver, J. E. van S. (1990). Quality changes in maize stored in sealed bag stacks. *Journal of Stored Product Research, 26*, 199-206. http://dx.doi.org/10.1016/0022-474X(90)90022-K

Hatai, A. (1994). Cooking characters of adzuki beans. *Science of Cookery, 27*, 238-242. (in Japanese).

Hincks, M. J., & Stanley, D. W. (1987). Lignification: Evidence for a role in hard-to-cook beans. *Journal of Food Biochemistry, 11*, 41-58. http://dx.doi.org/10.1111/j.1745-4514.1987.tb00111.x

Hira, H. (1983). Quality of domestic soybean -3- correlation of processing suitability, physical properties and chemical composition. *Report of National Food Research Institute, 42*, 27-39. (in Japanese).

Japan Meteorological Agency (2015). Temperature and humidity of each year in Obihiro City, Hokkaido, Japan. Retrieved from http://www.data.jma.go.jp/obd/stats/etrn/view/annually_s.php?prec_no=20&block_no=47417&year=2010&month=&day=&view=p1 (in Japanese).

Jones, P. M. B., & Boulter, D. (1983). The cause of reduced cooking rate in Phaseolus vulgaris following adverse storage conditions. *Journal of Food Scieince, 48*, 623-626. http://dx.doi.org/10.1111/j.1365-2621.1983.tb10804.x

Kagawa, Y. (2010). *Standard tables of food composition in Japan – Fifth Revised and Enlarged Edition*. Tokyo, Japan: Kagawa Nutrition University Publications Division. (in Japanese).

Kato, J. (2002). Estimation method for characteristics for boiling of beans by using electric conductivity of soaking water. *Journal of Science of Soil and Manure, Japan, 73*, 181-184 (in Japanese).

Kato, J., Yousif, A. M., Deeth, H. C., Suzuki, M. M., Caffin, N. A., Meguro, T. (2000). Differences in the cooking quality between two adzuki varieties harvested in Australia and stored at different temperatures. *Journal of Cookery Science of Japan, 33*, 257-266.

Kokubun, M. (2010). *New revised food crops*. Tokyo, Japan:Yokendo Co. (in Japanese).

Liu, K. (1995) Cellular, biological, and physicochemical basis for the hard-to-cook defect in legume seeds. *Critical Review of Food Scieince and Nutriton, 35*, 263-298. http://dx.doi.org/10.1080/10408399509527702

Liu, K., McWatters, K. H., & Phillips, R. D. (1992). Protein insolubilization and thermal destabilization during storage as related to hard-to-cook defect in cowpeas. *Journal of Agriculture and Food Chemistry, 40*, 2483-2487. http://dx.doi.org/10.1021/jf00024a028

Ministry of Agriculture, Forestry and Fisheries. (2010). *Yield of beans (dry grain) and buckwheat*. Statistics Bureau, Ministry of Internal Affairs and Communications. Retrieved from http://www.e-stat.go.jp/SG1/estat/List.do?lid=000001081820 (in Japanese).

Moscoso, W., Bourne, M. C., & Hood, L. F. (1984). Relationship between the hard-to-cook phenomenon in red kidney beans and water absorption, puncture force, pectin, phytic acid and minerals. *Journal of Food Science, 49*, 1577-1583. http://dx.doi.org/10.1111/j.1365-2621.1984.tb12848.x

Nakazato, T., Ichikawa, T., Mitani, T., & Sasaki, I. (1986). Variation of kidney beans quality during storage: changes in pectin, calcium and magnesium. *Science of Cookery, 19*, 281-284 (in Japanese).

Nasar-Abbas, S. M., Plummer, J. A., Siddique, K. H. M., White, P., Harris, D., & Dods, K. (2008). Cooking quality of faba bean after storage at high temperature and the role of lignins and other phenolics in bean hardening. *LWT-Food Science Technology, 41*, 1260-1267. http://dx.doi.org/10.1016/j.lwt.2007.07.017

Planning and Follow-up Group, Development Research Division, Development Administration (2010). *Survey on transport that utilize the snow and ice cold of the cold storage of agricultural products*. Ministry of Land, Infrastructure, Transport and Tourism Hokkaido Regional Development. Retrieved June 2010, from http://www.hkd.mlit.go.jp/topics/toukei/chousa/h22keikaku/03.pdf (in Japanese).

Reyes-Moreno, C., Paredes-López, O., & Barradas, I. (1994). A fast laboratory procedure to assess the hard-to-cook tendency of common bean varieties. *Food Chemistry, 49*, 187-190. http://dx.doi.org/10.1016/0308-8146(94)90157-0

Tsuchiya, F. (2004). Artificial permafrost storage using heat pipes. *Journal of Japan Society of Snow and Ice, 66*, 251-257 (in Japanese).

Tsuchiya, F., Ishibashi, K., Ryokai, K., & Yoshida, H (1994). Studies on effects of the system on artificial permafrost facilities using heat pipes and abilities of storage for vegetables and rice. *Proceedings of Cold Region Technology Conference*, *10*, 619-626 (in Japanese).

Tsuchiya, F., & Ryokai, K. (1990). Low temperature storage room of artificial permafrost using heat pipes. *Journal of JSIDRE, 58*, 881-886 (in Japanese).

Tsuchiya, F., & Ryokai, K. (1996). Cold storage system surrounded by frozen soil using cold energy. *Journal of JSIDRE, 64*, 231-235 (in Japanese).

Watanabe, T. (2000) *Encyclopedia of beans: processing and utilization*. Tokyo, Japan: Saiwai Shobo Co (in Japanese) .

Yousif, A. M., Deeth, H. C., Caffin, N. A., & Lisle, A. T. (2002). Effect of storage time and conditions on the hardness and cooking quality of adzuki (*Vigna angularis*). *LWT-Food Science Technology, 35*, 338-343. http://dx.doi.org/10.1006/fstl.2001.087

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Does Spray Mango Kernel (*Mangifera indica* Linn.) Prolong the Shelf Life of Beef Sausages?

Aly R. Abdel-Moemin[1]

[1] Nutrition and Food Science, Faculty of Home Economics, Helwan University, Egypt

Correspondence: Aly R. Abdel-Moemin, Nutrition and Food Science, Faculty of Home Economics, Helwan University, Egypt. Tel: 20-233-908-130. E-mail: dralymoemin@yahoo.co.uk

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Abstract

The study was aimed to look at the effect of different forms of mango kernels (MK) on the shelf life of refrigerated beef sausages over 12 days of cold storage. The (MK) was chemically and microbiologically analyzed. Beef sausages were treated with MK in 3 states, as dry ground (1.5%), an extract (1.5%) and spray MK extract (1.5%) over minced beef of sausages. Two controls were used; BHT 0.02% and no additives. A series of analyses were performed after treatments; thiobarbituric acid reactive substances (TBARS), analysis of color, myoglobin and odor. The results indicated that different forms of MK added to the beef sausages had different effects on its shelf life. Furthermore, the sprayed MK extract has significantly ($P \leq 0.05$) lowered metmyoglobin (MMb) and TBARS and increased oxymyoglobin (MbO_2), odor score and a* (redness) than other forms. The potential effects of the sprayed MK may be due to a cloud of droplets cover the large surfaces of minced beef sausages with efficient extracted antioxidants. MK is source of flavonoids 142mg/g F.W. GAE. The spraying of MK at 1.5% showed an improvement of *E. coli* from minced beef and beef sausages that were less than 10 cfu g^{-1} Also the concentrations of yeasts and moulds were not detected at day 12 of storage. Hierarchically, sprayed MK extract gave best results than ground MK or MK extract form which shows effective inhibitor of lipid oxidation and microbial growth of beef sausages.

Keywords: sprayed mango kernels, beef sausage, lipid oxidation, myoglobin, shelf life, antioxidative and anti-microbiological

1. Introduction

Fruits wastes make a large component of solid waste residue from agro-fruit processing industries that could increase environmental pollution due to its rapid decay. It has been reported that 45% of wastes are from mangoes, compared to 50% from citrus fruits and 10% from apples (Rao, 2006). Fruit peels and seeds are the most enriched part of fruits as they act as storage sites for nutrients required by the young plants. They have also been suggested to have antioxidant properties. Fiber-rich by-products may be incorporated into food products as inexpensive, non-caloric bulking agents for partial replacement of flour, fat or sugar, as enhancers of water and oil retention (Soong & Barlow, 2004).

Mango (*Mangifera indica* Linn.) is considered as one of the most summer favorite fruits in Egypt. During processing of mango, by-products such as peel and seed kernel are produced. Seed kernels may take up about 17-22% of the fruit. Mango seed is a single flat rectangular seed that can be fibrous or hairy on the surface, depending on the cultivar. Inside the seed coat 1-2 mm thick is a thin lining covering a single embryo, 4-7 cm long, 3- 4 cm wide, and 1 cm thick.

The MK has shown to be a good source of flavonoids, phytosterols such as campesterol, sitosterol and vitamin E as well. (Kittiphoom, 2012).

Meat products such as beef sausages are highly perishable and deteriorate rapidly causing potentially dangerous health risks through microbial growth and chemical changes. During storage in the presence of oxygen the oxidation of lipids takes place with production of free radicals as consequence of the metabolic changes in a meat biological system (Lee et al., 1998; Olsen et al., 2005). Lipid oxidation products contribute to the undesirable changes such as oxidation of oxymyoglobin to metmyoglobin which results in dark brown meat color (Renerre & Labas, 1987; Lee et al., 1998).

A small number of studies had investigated the possibility of MK to extend the shelf life of meat products. However, we have not a conclusion so far about the best form of MK to prolong the shelf life of beef sausages. Gadallah and Abdel Fatah (2011) tested the antibacterial effect of MK in a powder and extracted form with methanol at different levels 1.0, 2.0, 3.0 to treat minced beef over 15 days of cold storage. While Hung (2012) studied the antioxidative effect of ground MK (1% w/w) on the shelf life of pork sausages and pork patties over 10 days of cold storage. Also Pereira et al (2011) tested the inclusion of mango seed extract (MSE) at different levels 0.1% and 0.2% in Bologna-type mortadella preparations and found the 0.2% promotes higher pH values after 14 and 21 days of storage at 2 °C. However, the researcher concluded that mango seed extract (MSE) can be used in 0.1 or 0.2% levels in Bologna-type mortadella with similar or better antioxidant effects than those of BHT 0.01%.

The objective of the present study was to optimize the best form of MK that prolongs the shelf life of beef sausage over 12 days of cold storage. For first time we investigated the effect of spraying MK extract on the shelf life of beef sausages at 1.5 % level.

2. Materials and Methods

2.1 Materials

Mango seed kernels were collected from local juice shops, Cairo suburb, Egypt after mango flesh processing from (Zebda) variety during the summer season of 2014. The average weight of mangoes 375 g, they can be described according to Kader (2008) as relatively strong, firm, green to yellow and less full cheek. Beef legs were purchased from Cairo butcher shop.

Chemicals such as trichloroacetic acid (TCA), thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropane (TMP), ethanol, sulphuric acid, hydrochloric acid, ethyl acetate, Whatman 1 filter were obtained from Sigma, Egypt. Folin–Ciocalteu's phenol reagent, sodium carbonate (Na_2CO_3) and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO). The rest of chemicals were purchased from El Gomhoria Chemical Company, Cairo, Egypt.

2.2 Proximate Analysis of MK

Three samples (5 g) from each MK were examined for lipid, protein, ash and carbohydrate contents according to the official methods AOAC (2000). Moisture and dry matter were determined in MK by using "Infrared Moisture Determination Balance (FD-610-Japan) with a 5 g sample at 80 °C for 60 min. Carbohydrates were calculated by difference. The amount of total flavonoids content was determined by using Folin-Ciocalteu reagent according to Singleton and Rossi, (1965) with little modification according to Abdel-Moemin (2014) (CT-2200 Spectrophotometer, E-Chrom Tech, Taiwan).

2.3 Extraction of MK

The freeze-dried powder of MK (Snijders Scientific-tilburg, Holland, capacity 3 kg Ice) was extracted to ethanolic solvent according to Vega-Vega et al. (2013). Ethanolic extracts of MK was carried out by weighing (10 g) of MK and placed in E-Flask with 100 mL of ethanol: water (70:30). The sample was left to infuse in a dark room for 10 days at 25-30 °C. The extract was then filtered and the solvent removed by using a rotary evaporator at reduced pressure and temperature of 45 °C. The aqueous fraction was freeze-dried, forming the dry extract, which was subject to alkaline hydrolysis (10 mL of NaOH 4 M) for 4 h in darkness. Therefore, an acid hydrolysis was conducted with HCl 4 M taken every sample to pH 2. The hydrolyzed extract was subject to liquid-liquid separation by 2 washes with 20 mL of ethyl acetate (Oboh & Rocha, 2007). The ethyl acetate phase was used and evaporated; the obtained extract was re-suspended in deionised water to a concentration of 25 mg of extract per mL.

2.4 Preparation of Beef Sausages

Three samples of beef legs were purchased after 24 hours post-slaughter beef Caracas from local butchers Cairo, Egypt. Three forms of MK were treated the minced beef suasges; ground MK at 1.5%, extract MK 1.5% and spray MK at 1.5%. For example, ground sample of MK was mixed with minced beef sausage in a food processor for 3 minutes to prepare the beef sausages. Furthermore, the spray MK extract was sprayed over minced beef sausage through household sprayer. Controls (without additives) and BHT were also blended similarly to the above. Portions used to make the beef suasges in this study were in Table 1.

Table 1. Preparation of beef sausages

Ingredients	Sausage recipes				
	Control	BHT	Extract	Spray	Ground
Minced beef (g)	600	600	600	600	600
MK (%)	-	-	Extract	Spray	Ground
*BHT (%)	-	0.02	--	-	-
Salt (g)	3	3	3	3	3
Pepper (g)	2.4	2.4	2.4	2.4	2.4

Control: Standard sausage recipe made from minced beef with no antioxidants, **BHT**: a sausage recipe made from minced beef at 0.02% BHT(control II), **Extract**: a sausage recipe made from minced beef and blended with MK extract at 1.5%, **Spray**: a sausage recipe made from minced beef and sprayed with mango kernels extract at 1.5%. **Ground**: a sausage recipe made from minced beef with ground mango kernels at 1.5% *Butylated hydroxytoluene with a concentration of 0.02%.

Each recipe was then extruded into 10cm natural sheep intestinal casings and air dried at 10 °C for 6 hours. The beef sausages (n=9 per treatment) were placed in polystyrene trays which were covered with polyvinyl chloride film and then stored at 4 °C for 1, 2, 4, 6, 8, 10 and 12 days. Sampling was performed every 2 days to assess the effect of the extract applied on beef sausages.

2.5 Proximate Analysis of Minced Beef and Beef Sausages

Moisture and dry matter were determined by using (Infrared Moisture Determination Balance FD-610-Japan). Crude fat content was determined by using Soxhlet extraction (AOAC 976.21). Crude protein was measured by determining nitrogen using the Kjeldahl method (AOAC 981.10). Nitrogen was converted to crude protein content by multiplying with the factor 6.25.

2.6 Thiobarbituric Acid Reactive Substances (TBARS)

Thiobarbituric acid reactive substances (TBARS) in minced and beef sausages were modified slightly from Maraschiello et al. (1999). Briefly, 0.5 g of meat was added to 10 mL of deionised water and homogenized (1 min). 2.5 mL of 25% TCA were added (to precipitate the protein), samples were stored for 10 min at 4 °C and centrifuged (8 min, 3000 rpm, at 4 °C). An aliquot of 3.5 mL of the supernatant was added to 1.5 mL of 0.6% TBA and incubated in water bath for 30 min at 90 °C. The intensity of the developed color was measured at 539 nm UV-visible spectrophotometer CT-2200 Spectrophotometer, E-Chrom Tech, Taiwan) against a blank consisting of 2.5 mL of deionised water, 1 mL 25% aqueous TCA, and 1.5 mL 0.6% TBA.

2.7 Determination of Myoglobin

Deoxymyoglobin (Mb), oxymyoglobin (MbO_2) and metmyoglobin (MMb) in minced beef and sausages were determined according to the procedure of Krzywicki (1982). The absorbance of each supernatant was read at 565, 545 and 525 nm in a UV-visible spectrophotometer CT-2200 Spectrophotometer, E-Chrom Tech, Taiwan. Percentages of Mb, MbO_2 and MMb in the pigment extracts were calculated.

2.8 Determination of Suasges Odor

Odor acceptability of beef sausage (n=3) for a period of 6 subplots (2, 4, 6, 8, 10, 12) was assessed by 9 panelists at room temperature (30 °C), the cut of point for acceptable odor sausage was 2.5 marks by using a 5-point hedonic scale where 1 =very unpleasant, 2 = moderately unpleasant, 3= moderately pleasant, 4 = pleasant and 5 = very pleasant (Das et al., 2011).

2.9 Determination of Sausage Color

The beef sausages samples were taken out of the refrigerator and placed on the table for 5 minutes at room temperature (30°C) before color measurement. Color measurements were made on the surface of raw beef sausages with Minolta Chroma Meter CR-310 colorimeter (Minolta Corp., Ramsey, NJ).The colorimeter was calibrated according to the manufactures instructions.

2.10 Microbiology of Beef Sausages

Microbiological profile of MK, fresh minced beef and stored sprayed beef sausages with MK were done on the day 12. The examination included TVC, *E. coli*, staphylococci, coliforms yeasts, moulds and Salmonella for

minced beef and refrigerated stored beef sausages. The MK samples were also analyzed for TVC, *E. coli*, Bacillus cereus, yeasts and moulds.

2.11 Statistics

Means and standard deviations ±SD were calculated. One Way Analysis of Variance (ANOVA) was conducted. The critical values for the Tukey HSD Test were used in significance at ($P \leq 0.05$) Innersoft stats v 0.8, version 0.8.00, copyright 2013 Innersoft.

3. Results

The percentage average of Zebda mangoes portions were of 78, 8.5,11 and 2.5% for flesh, peel, kernel and kernel crust (the hard layer that cover the seed) respectively. The average moisture content was 85.4, 74.2, 38.42 and 12% for mango flesh, mango peel, kernels and kernel crust respectively.

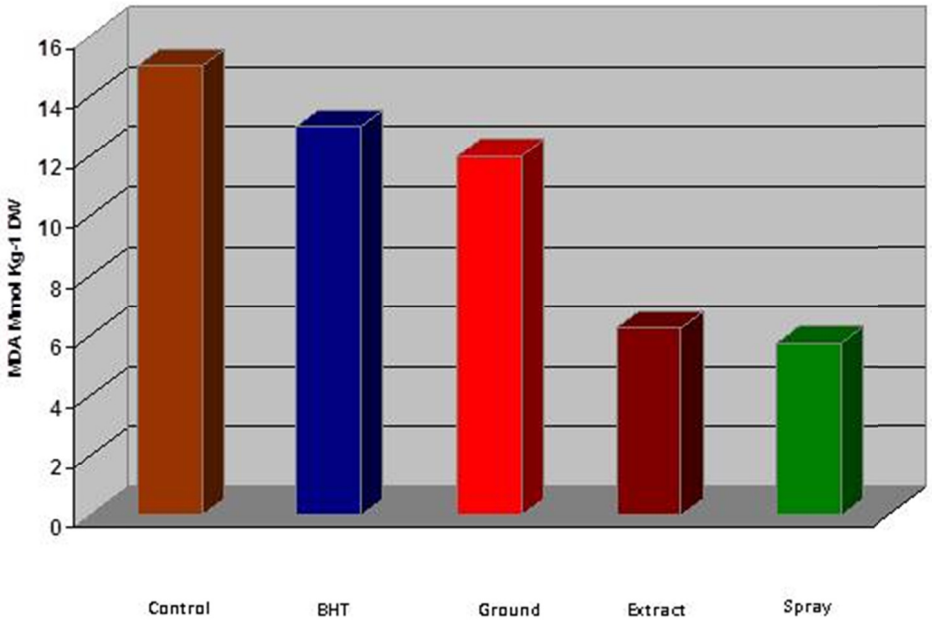

Figure 1. TBARS (MDA) values (µmol Kg^{-1}DW) in sausages treated with the 3 forms of MK at (4 °C) at day 12

Figure 2. A: Mango fruit (Zebda) (*Mangifera indica* Linn.), B: Mango Kernel, C: ground mango kernels

3.1 Proximate Analysis of Mango Kernels

The results in Table 2 indicate to the average chemical composition of MK, for example, fat content constitutes 6.94%. The MK constituted about 1.93 crude protein (Nx6.25), (1.61%) crude ash, (47.19%) crude carbohydrate, and (258.75 Kcals-1035 KJ) gross energy. The total phenolic content of dried mango kernel was 142 mg/g F.W.GAE

Table 2. Proximate analysis of MK%

Moisture %	Crude Protein %	Fats %	Crude fiber%	Ash %	Carbohydrates %	Energy KJ	Total phenolics mg/g F.W.GAE
38.42±0.4	1.93±0.6	6.94±0.8	3.91±1.5	1.61±0.2	47.19±0.8	1035	142±1.5

3.2 Microbiological Profile of Mango Kernels

The microbiological profile of MK was safe which recorded 900 cfu g^{-1} for TVC and less than for the following; 50 cfu g^{-1} for yeasts and moulds, 10 cfu g^{-1} for Bacillus and 5 Cfu g^{-1} for *E. coli*.

3.2.1 Proximate Analysis of Fresh Minced Beef

The proximate composition of fresh minced beef for moisture, crude protein, fat, ash and carbohydrates were 60.2±0.36, 17.9±3.2, 18.2±2.7, 2.48± and 1.22±3.6% FW respectively.

3.3 Odor of Sausages

The sausages, regardless of their components, showed a decrease in their odor scores over the 12 days of cold storage. The beef sausages without antioxidants (control I) decreased the odor (unpleasant odor) from 5 to 0 after 12 days of storage. Furthermore, had an undesirable score (< 2.5) and unpleasant odor after 4 days of storage. Nevertheless, the odor score of sausages with MK spray, extract and ground had acceptable score (≥ 2.5) for up to 8 days of storage whilst those with sprayed with MK had (≥ 3) for up to 10 days. However, added BHT had an acceptable margin score (2.5).

Table 3. Myoglobin pigments (%) in the beef sausages during storage period (4 °C)

Days	****Mb%**				
Days	Control	BHT	Extract	Spray	Ground
1*	30±0.2	30±1.3	42±2.2	45±1.2	32±3.1
2	32±2.3	35±1.2	37±0.3	35±0.4	35±1.5
4	45±1.1	37±0.5	35±1.2	32±0.2	36±1.8
6	38±1.3	37±2.0	33±1.4	32±1.8	37±2.2
8	42±2.1	38±2.2	31±0.9	31±1.1	37±2.4
10	53±0.1	37±0.7	34±0.2	31±1.3	40±2.2
12	55±1.4	40±0.2	34±1.6	32±2.2	41±3.2
Days	**MbO_2 %**				
	Control	BHT	Extract	Spray	Ground
1*	$29±1.2^{b}$	$44±0.8^{a}$	$22±1.6^{d}$	$25±0.6^{c}$	$31±1.2^{ab}$
2	$30±2.0^{ab}$	$37±3.1^{a}$	$23±1.2^{d}$	$30±2.3^{ab}$	$29±1.7^{ab}$
4	$22±0.7^{ab}$	$35±2.2^{a}$	$25±3.0^{c}$	$35±1.2^{a}$	$28±0.2^{b}$
6	$17±1.4^{ab}$	$33±1.2^{a}$	$28±2.4^{b}$	$34±2.5^{a}$	$27±2.4^{b}$
8	$15±0.2^{d}$	$32±1.5^{b}$	$30±0.9^{ab}$	$35±1.8^{a}$	$27±2.6^{c}$
10	$14±2.2^{c}$	$30±0.8^{b}$	$31±1.2^{b}$	$37±2.6^{a}$	$26±0.6^{ab}$
12	$11±1.4^{c}$	$27±1.3^{ab}$	$34±0.2^{b}$	$37±3.2^{a}$	$26±2.2^{ab}$
Days	**MMb %**				
	Control	BHT	Extract		Ground
1*	$23±0.3^{b}$	$13±1.9^{c}$	$17±0.1^{ab}$	$14±1.6^{c}$	$24±2.2^{a}$
2	$27±1.8^{a}$	$15±2.2^{d}$	$19±1.3^{ab}$	$16±1.2^{c}$	$23±0.8^{b}$
4	$22.5±1.1^{b}$	$22±1.6^{b}$	$14±2.4^{c}$	$16±0.9^{ab}$	$25±1.5^{a}$
6	$33.5±3.0^{a}$	$34±0.7^{a}$	$16±1.2^{c}$	$18±2.0^{ab}$	$28±1.2^{b}$
8	$39±1.4^{a}$	$32±1.2^{b}$	$14±3.1^{ab}$	$12±1.4^{c}$	$33±0.7^{b}$
10	$37±0.7^{a}$	$31±0.2^{b}$	$18±0.6^{ab}$	$12±1.1^{c}$	$37±1.6^{a}$
12	$40±0.9^{a}$	$33±1.5^{ab}$	$20±1.2^{c}$	$14±2.4^{d}$	$37±3.0^{b}$

Control: Standard sausage recipe made from minced beef with no antioxidants, **BHT**: a sausage recipe made from minced beef at 0.02% BHT. **Extract**: a sausage recipe made from minced beef and blended with MK **extract** at 1.5%, **Spray**: a sausage recipe made from minced beef and **sprayed** with mango kernels extract at 1.5%. **Ground**: a sausage recipe made from minced beef with **ground** mango kernels at 1.5%.*Myoglobin pigments were determined in day1 after 24 hours from treatments. The results of Mb, MbO_2 and MMB % were the outcome of 3 repeated measurements for each treatments. Mean values with different capital letters within a row are statistically different at P value less than or equal the significance level $P \leq 0.05$ for MbO_2 and MMb.** no statistical significant has been found for Mb pigment.

3.4 Myoglobin Pigments

Fresh minced beef at day one represent 30% Mb, 29% MbO_2 and 15% MMb respectively. Changes in the type of myoglobin in beef sausages over a 12-day storage period showed significant differences ($p \leq 0.05$) between the treatments specifically MbO_2 % and MMb %. At day 6, the proportion of Mb was almost stable in the sprayed MK of the sausages.

3.5 TBARS and Red Color during Storage Period at 4 °C

Fresh minced beef recorded 7.25 MDA (μmol $Kg^{-1}DW$) and 7.80 for redness (a*). Generally, addition of MK to the beef sausages lowerd TBARS values compared to the controls over the 12 days of storage period. In the control (I) beef sausage TBARS values were increased significantly ($P \leq 0.05$) at day 4 and continued to increase.

There were no differences in a* between the control and treated sausages at day one. All the treatments of beef sausages decreased their a* values from day 1 to day 12. There was a significant decrease ($P \leq 0.05$) in the a* values of the control (I) sausages, specifically at day 6. In the control sausages seems to continually decrease a* values to day 12 while the sausages with antioxidants, specifically those with sprayed, ground and extracts, looks to have a* up to day 12.

Table 4. TBARS (MDA) and a* values in sausages treated with the 3 forms of MK (4 °C)

Days	MDA μmol $Kg^{-1}DW$					A*				
	Control	BHT	Extract	Spray	Ground	Control	BHT	Extract	Spray	Ground
Minced beef	7.25±0.6					7.8±0.9				
1**	7.75±1.2^{a}	6.0±1.0^{b}	6.0±3.7^{b}	6.0±0.7^{b}	7.0±2.2^{c}	7.75±2.3^{a}	7.68±1.4^{a}	7.75±1.9^{a}	7.75±2.3^{a}	8.0±1.6^{a}
2	7.0±2.4^{a}	6.2±1.8^{b}	6.2±1.5^{b}	6.1±0.9^{b}	6.6±1.9^{c}	6.5±1.6^{a}	7.5±1.8^{b}	7.4±1.6^{b}	7.5±0.9^{b}	8.25±1.3^{c}
4	7.0±0.8^{a}	6.2±2.1^{b}	6.2±2.4^{b}	6.1±1.1^{b}	7.0±1.7^{a}	6.0±1.0^{a}	6.75±0.6^{b}	7.1±1.2^{b}	7.0±1.1^{b}	7.0±2.4^{b}
6	12.0±1.1^{a}	6.5±0.9^{b}	6.5±1.6^{b}	6.4±2.0^{b}	9.0±1.4^{ab}	5.0±0.4^{a}	6.65±0.2^{b}	6.75±1.7^{b}	6.75±2.6^{b}	6.25±0.9^{c}
8	13.0±3.1^{a}	9.5±1.4^{b}	6.5±1.3^{ab}	6.1±2.3^{ab}	9.1±0.8^{b}	4.25±2.5^{a}	6.5±1.2^{b}	6.65±0.5^{b}	6.75±1.8^{b}	5.75±1.7^{c}
10	13.0±6.4^{a}	10.5±1.2^{b}	5.75±1.5^{c}	5.5±1.2^{d}	10.2±1.3^{b}	3.5 ±1.9^{a}	6.70±1.3^{b}	6.5±2.7^{b}	7.0±1.6^{c}	5.5±2.8^{ab}
12	15.0±4.2^{a}	13.0±1.7^{b}	6.25±2.4^{ab}	5.75±1.6^{d}	12.0±0.6^{b}	3.0±1.1^{a}	7.0±2.2^{b}	6.5±2.1^{c}	7.5±1.2^{ab}	6.00±1.4^{d}

Control: Standard sausage recipe made from minced beef with no antioxidants, **BHT**: a sausage recipe made from minced beef at 0.02% BHT, **Extract**: a sausage recipe made from minced beef and blended with MK extract at 1.5%, **Spray**: a sausage recipe made from minced beef and sprayed with mango kernels extract at 1.5%. **Ground**: a sausage recipe made from minced beef with ground mango kernels at 1.5%, *a indicates to the degree of redness of beef sausage. **Day 1 means determinations had been done after 24 hours from treatments. (Mean values with different small letter within a row are significantly different ($P \leq 0.05$).

3.6 Microbiological Profile of Beef Sausage With MK Extract

Fresh minced beef and beef sausages with sprayed MK at 1.5% were only tested for microbiological investigation; TVC, *E. coli*, staphylococcus aureus, clostridiums, yeasts, moulds and salmonella. The microbiological investigations

were included the period of fresh state of minced beef and day 12 at 4 °C (Table 5).

Table 5. Microbiological profile of beef sausage with MK extract

Profile / Sample	TVC cfu g^{-1}	*E. coli* Cfu g^{-1}	Clostridiums cfu g^{-1}	Staphylococci cfu g^{-1}	Yeasts & Moulds cfu g^{-1}	Salmonella**
Fresh minced beef	1,000,000	>1000	< 10	< 10	2.4×10^2	Nd
*Beef sausage sprayed MK (day 12)	<100,000	< 5	< 10	< 10	Nd	Nd

*Sausage recipe made from minced beef and blended with mango kernels extract at 25 mg/mL. nd: Non-detected cfu g^{-1}: colony-forming units per gram TVC: total viable count. **Salmonella per 10 g sample.

The results of TVC, *E. coli*, Clostridiums, Staphylococci, Yeasts & Moulds and Salmonella in fresh minced beef represent 1,000,000, > 1000, < 10, < 10, 2.4×10^2 cfu g^{-1} and not detected respectively. While the results of beef sausage blended with MK extract at 1.5% (day 12) were < 100,000, < 5, < 10, < 10, nd and nd respectively. Coliforms (*E. coli*) were improved from minced beef and beef sausages were less than 10 cfu g^{-1}. Also the concentrations of yeasts and moulds in the sprayed beef sausages were not detected at day 12. Salmonella were not detected in any of the beef samples tested.

4. Discussion

The current study was focused to optimize the best form that would prolong the shelf life of beef sausages. A series of analyses were done to evaluate the best form that enhances the shelf life of beef sausages at 1.5% for a period of 12 days at 4 °C. For example, analysis of color, odor, and TBARS were conducted. The minced beef and beef sausage treated with sprayed MK were also examined for microbiological safety. Two controls were used *vs* the treated beef sausage with kernels. These controls were BHT supplemented sausages as commercial synthetic at 0.02% and beef sausages without any additives.

We have already data about the antioxidative and antimicrobial effects of extract plant origin on processed meat for example, Carpenter et al. (2007) who reported an increase in redness of cooked pork patties containing the highest concentrations of grape seed extract. Also, these authors observed that this increase in meat redness was not perceived as negative by the sensory panel.

We have also data from literature about the role of MK to extend the shelf life of meat products in a state of ground or extract. (Gadallah & Abdel Fatah, 2011; Hung, 2012; Pereira et al., 2011). As they tested the antioxidative and antibacterial effect of MK either as ground form or as an extract at different levels on the shelf life of meat products over certain days of cold storage. These studies showed extended shelf life as the antioxidative and antimicrobial effects of mango seed kernels. However, we have not yet a conclusion about the best form of MK to prolong the shelf life of beef sausages. For the first time, we have investigated the effect of spraying MK in an extract form at 1.5% over minced beef of sausage in order to testing if spraying MK may extend the shelf life of beef sausages than other forms.

Thiobarbituric acid reactive substances (TBA), analysis of color, myoglobin and odor and microbiological examination were used to assess the shelf life of the treated beef sausages.

Currently, there is a market trend to utilize from natural antioxidants as food additives because of observed safety and toxicity problems associated with synthetic antioxidants such as BHA, BHT and TBHQ (Buxiang & Fukuhara, 1997; Jo et al., 2006). MK has been suggested as potential sources of natural antioxidants since they contain polyphenols, anthocyanin, carotenoid, vitamin C and vitamin E (Soong et al., 2004; Ajila et al., 2007). There is, however, still little data on the effects of MK on lipid oxidation associated with color and odor changes in beef sausages stored in cold environment.

Soong et al. (2004) reported that MK has potent antioxidant activity with not only phenolic compounds but also lipophilic antioxidants such as phytosterols and tocopherols that possesses strong scavenging abilities to capture free radicals and chelate metals.

Furthermore, although BHT has often been used to prevent the harmful effects of lipid oxidation, sprayed MK was more effective than extract, ground, and BHT in the present study. This is probably due to the various kinds of antioxidants in the sprayed MK having a cloud of droplets cover the large surfaces of minced beef to make beef sausage and generally the sprayed form was extracted as having antioxidants as hydrophilic and hydrophobic forms therefore enhancing the antioxidative effects on muscle systems. Abdalla et al. (2007) also suggested that MK extracts could be used as a source of natural antioxidants therefore this study used this recommendation to test spraying extract of MK over beef sausage.

The findings in this study suggest that lipid oxidation influences the parameters of lipid oxidation of control (I) over the 12 days storage period. These findings suggest that TBARS, Mb and MMb increased whilst MbO_2, odor score and a* value decreased when lipid oxidation occurred.

The results can be attributed to the oxidation of MbO_2 to MMb after the first 4 days of storage. A factor responsible for meat discoloration and MbO_2 oxidation is lipid oxidation (Brown & Mebine, 1969; Gray et al., 1996) which produces free radicals and aldehydes that react with MbO_2 therefore speed up the accumulation of MMb.

The sprayed MK over minced beef sausages exhibited antimicrobial effects against *E. coli*, yeasts and moulds and TVC in the beef sausages. In our study, the effect of the sprayed extract of "Zebda" mango kernels could be increased the antioxidant and antimicrobial capacity of beef sausages. As they were treated by spraying, then packed and stored at 4 °C for 12 days.

Odor is the most important parameter that affects the consumer perception of the quality of stored cold meat. We evaluate the changes in the quality of beef sausages beside changes in TBARS, color, myoglobin and microbial test in the same samples.

Sprayed MK sausage samples were more effective in maintaining the redness in beef sausages. While all sausages tended to be less red in the storage days, those with additives MK and BHT had a redder color and maintained the redness in a stable form from day 6 to the end of the storage period.

Loss in redness may be attributed to the myoglobin (Mb) and oxymyoglobin (MbO_2) in beef products being lost by oxidation which turns the pigment to a brown color due to metmyoglobin formation. These differences may be due to the type of muscle analyzed.

The best results were obtained from sprayed MK probably extraction helped to obtain high concentration of antioxidants and reduce any unnecessary anti-nutrients. Furthermore, spraying the extract over minced beef that used in the preparation of beef sausages would help the efficiency of the antioxidants and generate droplets of extract that can be suit fat soluble antioxidants to the lipid phase and generate a potent *in vitro* antimicrobial of beef sausages.

The addition of MK and BHT may be changed the three forms of myoglobin pigments. This could be due to either the immediate effect of BHT and MK increasing MbO_2 and reducing Mb and MMb. These results were in agreement with (Hung, 2012).

Nevertheless, after day one, there were incompatible changes in Mb, MbO_2 and MMb of beef sausages at day 2 and 4, respectively due to the complex changes in the formation and loss of the three myoglobin forms inter-conversion of the three forms of pigments in the presence of oxygen (Giddings, 1974; van Laack et al., 1990; Zhu & Brewer, 1998). Also oxidation of the ferrous-species of myoglobin to the brown ferric-species MMb due to the prolonged exposure to air and the low oxygen pressure (Lindahl et al., 2006).

The microbiological results of beef sausage sprayed with MK indicate that coliforms (*E. coli*) were improved from minced beef and beef sausages less than 10 cfu g^{-1}. Also the concentrations of yeasts and moulds in the sprayed beef sausages were not detected at day 12. Salmonellae were not detected in any of the beef samples tested. These results imply that spraying of MK has a potential antimicrobial activity against TVC in beef sausages until the day 12.

5. Conclusion

Mango kernel is a good source of flavonoids (142 mg/g F.W. GAE) found to be an excellent additive to prolong shelf life of beef sausage for 12 day at 4 °C. Generally, mango kernels forms, when added at 1.5% level to beef sausages provided sufficient antioxidants to extend the shelf life of beef sausages from 2 to 12 specifically in a spray form. Similarly, MK extract and ground prolonged the shelf life of beef sausages for 2 to 6 days and from 2-8 days respectively compared to control sausages (without additives). The potential effect of sprayed MK extract to extend the shelf life of beef sausages could be due to covering the large surface of minced with the tiny

droplets of extract that inhibiting the lipid oxidation and delaying the changes in color and odor of beef sausages. Hierarchically, sprayed MK extract excelled significantly the other forms of mango kernels that mixed dried or extract with beef sausages. This study suggests the efficient antioxidative and antimicrobial properties of sprayed mango kernels extract to inhibit lipid oxidation and extend the shelf life of beef sausage.

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References

Abdalla, A. E. M., Darwish, S. M., Ayad, E. H. E., & EL-Hamahmy, R. M. (2007). Egyptian mango by-product 1. Compositional quality of mango seed kernel. *Food Chemistry, 103*(4), 1134-1140. http://dx.doi.org/10.1016/j.foodchem.2006.10.017

Abdel-Moemin, A. R., & Aborya, A. O. (2014). Innovative products of coffee and tea based on dandelion (*Taraxacum officinale*) roots and leaves. *Agricultura, 11*(1), 1-10.

Ajila, C. M., Bhat, S. G., & Rao, U. (2007a). Valuable components of raw and ripe peels from two Indian mango varieties. *Food Chemistry, 102*(4), 1006-1011. http://dx.doi.org/10.1016/j.jff.2012.11.017

Ajila, C. M., Naidu, K. A., Bhat, S. G., & Rao, U. P. (2007b). Bioactive compounds and antioxidant potential of mango peel extract. *Food Chemistry, 105*(3), 982-988. http://dx.doi.org /10.1016/j.foodchem.2007.04.052

AOAC International. (1995). Official Methods of Analysis of AOAC International, AOAC Official Method 981.10 Crude Protein in Meat: Block Digestion Method, ISBN 0-935584-54-4. Arlington, US.

Buxiang, S., & Fukuhara, M. (1997). Effects of co-administration of butylated hydroxytoluene, butylated hydroxyanisole and flavonoids on the activation of mutagens and drug-metabolizing enzymes in mice. *Toxicology, 122*, 61-72. http://dx.doi.org/10.1016/S0300-483X(97)00078-4

Carpenter, R., O'Grady, M. N., O'Callaghan, N., O'Brien, M., & Kerry, J. P. (2007). Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork. *Journal of Meat Science, 76*, 604-610. http://dx.doi.org/10.1016/j.meatsci.2007.01.021

Clydesdale, F. M. (1978). Colorimetry-methodology and applications. In T. E. Furia (Ed), *Critical Reviews in Food Science and Nutrition* (pp. 243-301). Boca Raton, Fla: CRC Press. http://dx.doi.org/10.1080/10408397809527252

Gadallah, M. G. E., & Abdel, F. A. A. (2011). The antibacterial effect of mango seed kernel powder in minced beef during refrigerated storage. *World Journal of Dairy & Food Sciences, 6*(2), 219-228.

Hung, M. L. E. (2012). Antioxidative effects of mango wastes on shelf life of pork products, Ph.D. Thesis, p.V Lincoln University.

JO, S. C., Nam, K. C., Min, B. R., Ahn, D. U., Cho, S. H., Park, W. P., & Lee, S. C. (2006). Antioxidant activity of Prunus mume extract in cooked chicken breast meat. *International Journal of Food Science and Technology, 41*, 15-19. http://dx.doi.org/10.1111/j.1365-2621.2006.01234.x

Kader, A. A. (2008). National Mango Board: Mango quality attributes and grade standards-A review of available information and identification of future research. Retrieved February 25, from http://www.mango.org/mango/sites/default/files/download/mango_grade_standards_review_full_report.pdf

Kittiphoom, S. (2012). Utilization of Mango seed. *International Food Research Journal, 19*(4), 1325-1335.

Ladikos, D., & Lougovois, V. (1990). Lipid oxidation in muscle foods: A review. *Food Chemistry, 35*(4), 295-314. http://dx.doi.org/10.1016/0308-8146(90)90019-Z

Lee, B. J., Hendricks, D. G., & Cornforth, D. P. (1998). Antioxidant effects of carnosine and phytic acid in a model beef system. J Food Sci, 63, *Journal of Meat Science, 51*, 245-253. http://dx.doi.org/10.1111/j.1365-2621.1998.tb15750.x

Madhar, A., & Pushpalatha, P. B. (2002). Quality up gradation of jellies prepared using pectin extracted from fruit wastes. *Journal of Tropical & Agriculture, 40*, 31-34.

Maraschiello, C., Sarraga, C., & Garcia, R. J. A. (1999). Glutathione peroxidase activity, TBARS, and α-Tocopherol in meat from chickens fed different diets. *Journal of Agriculture & Food Chemistry, 47*(3), 867-872. http://dx.doi.org/10.1021/jf980824o

Oboh, G., & Rocha J. B. T. (2007). Distribution and Antioxidant Activity of Polyphenols in Ripe and Unripe Tree Pepper (*Capsicum pubescens*). *Journal of Food Biochemistry, 31*(4), 456-473. http://dx.doi.org/10.1111/j.1745-4514.2007.00123.x

Pereira, A. L., Fernandes V .T., Teixeira, M. C., Fontoura, O. P., Fernandes, R. C., … Zapata, J. F. F. (2011). Antioxidant effect of mango seed extract and butylated hydroxytoluene in bologna-type mortadella during storage. *Cienc. Tecnol. Aliment., Campinas, 31*(1), 135-140. http://dx.doi.org/10.1590/S0101-20612011000100019

Raharjo, S., & Sofos J. N. (1993). Methodology for measuring malonaldehyde as a product of lipid peroxidation in muscle tissues: A review. *Journal of Meat Science, 35*(2), 145-169. http://dx.doi.org/10.1016/0309-1740(93)90046-K

Rao, K. L. (2006). *Agro-industrial parks experience from India.* Agriculturral and food Engineering Working Document. Food and Agriculture Organization of the United Nations.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology & Viticulture, 16*, 144-158.

Soong, Y. Y., & Barlow, P. J. (2004). Antioxidant activity and phenolic content of selected fruit seeds. *Food Chemistry, 88* (3), 411-417. http://dx.doi.org/10.1016/j.foodchem.2004.02.003

Soong, Y. Y., Barlow, P. J., & Perera, C. O. (2004). A cocktail of phytonutrients: identification of polyphenols, phytosterols and tocopherols from mango (*Mangifera indica L.*) seed kernel. In IFT annual meeting. July 12-16. Las Vegas, U.S.

Vasavada, M. N., & Cornforth, D. P. (2006). Evaluation of antioxidant effects of raisin paste in cooked minced beef, pork, and chicken. *Journal of Food Science, 71*(4), C242-C246. http://dx.doi.org/10.1111/j.1365-2621.2005.tb07168.x

Vega-Vega, V. (2011). Enriquecimiento de la Capacidad Anti- oxidante y. Protección Antimicrobiana del Mango Fresco Cortado Aplicando Compuestos Fenólicos de sus Sub- productos," MS.c. Thesis, Centro de Investigación en Ali- mentación y Desarrollo, A.C, Hermosillo, Sonora, Mexico.

Vega-Vega, V., Silva-Espinoza, B. A., Cruz-Valenzuela, M. R., Bernal-Mercado, A. T., González-Aguilar, G. A., Vargas-Arispuro, I., … Ayala-Zavala, J. F. (2013). Antioxidant enrichment and antimicrobial protection of fresh-cut mango applying bioactive extracts from their seeds by-products. *Food and Nutrition Sciences, 4*, 197-203. http://dx.doi.org/10.4236/fns.2013.48A024

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Microbial Decontamination of Fresh Produce (Strawberry) Using Washing Solutions

Naresh Shahi[1], Byungjin Min[1] & Eunice A. Bonsi[1]

[1] Department of Food and Nutritional Sciences, Tuskegee University, Tuskegee, AL, 36088, United States

Correspondence: Byungjin Min, Department of Food and Nutritional Sciences, Tuskegee University, Tuskegee, AL, 36088, United States. Tel:1-334-727-8393. E-mail: minbj@mytu.tuskegee.edu

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Abstract

This study was carried out to determine the effect of natural antimicrobial washing solutions against microbial growths on fresh produce specifically strawberries. Selected washing solutions used for strawberry washing, and treatments were sterile water (control), white vinegar (VI), crude lemon juice extract (LE), VI+Origanum oil (VIO), LE+Origanum oil (LEO), and VI+LE+Origanum oil (VILEO). From the preliminary study of antimicrobial activity of washing solutions in aqueous model system tested at 2, 5, 10, 15, 20 and 25 min against *S.* Typhimurium, washing time was determined as 5 min to be used for this study. After the washing, strawberries were stored at 4 °C for 5 days. Results showed that all natural washing solutions exhibited inhibitory effect against total aerobic bacteria, yeast and mold. On day 5, compared to the control, all washing solutions significantly reduced *S.* Typhimurium by 2.7 Log CFU/g ($P<0.05$). Color results showed that samples color were slightly changed by washing with VIO and VILEO. However, there was no significant difference in total color change on strawberries compared to the control ($P>0.05$). Based on the results, it is indicated that the combination of vinegar with crude lemon juice extract and essential oil might be suitable as natural sanitizer for decontamination of fresh produce.

Keywords: antimicrobial, decontamination, washing solution, essential oil, strawberry

1. Introduction

Market trend along with fresh produce consumption continues to grow in recent years due to their health promoting capabilities (Jennylynd & Tipvanna, 2010). It is suggested that daily consumption of fresh produce help to prevent degenerative and metabolic diseases such as cardiovascular disease, obesity and certain types of cancers (Rico, Martin-Diana, Barat, & Barry-Ryan, 2007). Fresh produce can be a vehicle of microorganisms from farm to point of consumption (Schuele & Snead, 2001); thus act as a source of foodborne illness (Beuchat, 1992). Fresh produce is vulnerable to potential microbial contamination at any points of the food value chain (WHO/FAO, 2006), if improper handling technique and storage occur during cultivation, harvesting, storage, transportation and distribution. Both pathogenic and/or deteriorative microorganisms may contaminate the product at any point of contact, increasing the risk of foodborne diseases (Diaz-Cinco, Acedo-Felix, & Garcia-Galaz, 2005). It was reported that consumption increase, large scale production, distribution system, international trades etc. contributed to the increase in the outbreaks of human infections associated with fresh produce (Beuchat, 2002; Olaimat & Holley, 2012). Fresh produce have been associated with outbreaks of foodborne diseases and food spoilage caused by bacteria, viruses, yeasts, molds and parasites in many countries (De Roever, 1999). Postharvest loss of fruit is a major challenge throughout the world. In the industrialized countries, it is estimated that loss can be up to 25% of harvested fruits. The situation is far more exacerbating in the developing countries, where postharvest loss can be over 50% in some areas (Nunes, 2012). Fresh produce is decayed by pathogens during post harvesting and handling (Sharma et al., 2009). Household consumers are the final link in the food supply chain (Kagan, Aiello, & Larson, 2002). Diverse bacterial communities can exist everywhere at home. Poor personal hygiene and unsanitary environment can result in cross-contamination of microorganisms in the kitchen (Sanita, 2006). According to World Health Organization (WHO, 2002), over 30 to 40% of the global populations are at risk for food borne diseases at home in every year. In the United States, from 1998 to 2008, up to 15% of food-related illnesses were from the home. In Europe, approximately one-third of foodborne diseases were associated with fresh produce at home (European Food Safety Authority, 2013).

Various decontamination methods were developed and have been applied to improve safety of fresh produce. Industrial decontamination methods include synthetic chemicals and physical techniques such as chlorine, ozone, electrolyzed water, bromine, tri-sodium phosphate, iodine, irradiation, refrigeration, pulsed light, electrostatic sprays, and cold plasma (Goodburn & Wallace, 2013). However, efficacy of decontamination is varied and none of these methods are able to ensure elimination of pathogens completely (Zivile, Irina, Kristina, Egle, & Pranas, 2012). In addition, consumption of fresh produce without proper washing may be carcinogenic from chemical residues as well as by products (Beuchat, 2000). Synthetic chemicals and sophisticated technology such as chlorine dioxide, irradiation, and pulsed light may not be reliable sources for food safety and preservation at household level. Moreover, these techniques might be difficult to establish at large scale by government especially in developing countries because of lack of infrastructure and availability and accessibility of resources (FAO/APO, 2006).

Recently, researchers have focused more on alternative methods for the preservation and safety of fresh produce such as organic acids and essential oils which are generally recognized as safe (GRAS) to minimize foodborne pathogens (Akabas & Olmez, 2007; Gunduz, Gonul, & Karapinar, 2010). Studies found that origanum oil exhibited antimicrobial activity as well as antifungal properties (Calo et al., 2015; Manohar et al., 2001). It was reported that lemon juice and vinegars showed inhibitory activities against *C. perfringens* spore germination and outgrowth on reduced salt (Li et al., 2012). Washing time and techniques are important considerations during decontamination of fresh produce (Goodburn & Wallace, 2013). Nastou et al. (2012) reported that 2% (v/v) acetic acid was shown to have some antimicrobial effect in most of the cases with immersion time of 5 or 10 min for fresh produce. Similarly, there was no significant reduction of *C. jejuni* in chicken wings after dipping in 2% acetic acids at 4 °C, even with an increase in treatment time from 15 to 45 min (Zhao & Doyle, 2006). Influences of micro-organisms by organic acids depend on several parameters including; reduction pH, chain length, ratio of un-dissociated ions, cell physiology and metabolisms. It is assumed that weak organic acids are more effective than strong acids because they are lipophilic and easily penetrate plasma membrane and destroy the cell's genetic materials (Booth & Kroll, 1989).

This study was carried out to identify and develop alternative washing solutions as sanitizer for microbial decontamination of strawberry using natural products such as vinegar, organic acids, lemon juice extract, essential oils, and their combinations. In addition, the study evaluated antimicrobial activities of natural washing solutions which contribute to the reduction of the risk of microbial contamination during refrigerated storage.

2. Materials and Methods

2.1 Collection of Fresh Produce

Fresh whole strawberries, fresh lemons and white vinegar were purchased from local grocery stores in Auburn, AL, USA and transported to the laboratory at Tuskegee University. The produce samples were chosen independently and randomly. All samples were analyzed within 24 hours of purchase while keeping them in their original storage condition. Origanum oil from *Thymus capitatus* was purchased from Sigma-Aldrich Company (St. Louis, MO, USA) and stored at 4 °C.

2.2 Preparation of Natural Antimicrobial Washing Solutions

White vinegar solution (VI) was diluted with sterile water from 2% of stock solution and final concentration was 1%. For crude lemon juice extract (LE), fresh lemons were aseptically washed with sterile water and cut with sterile knife and extracted by sterile juicer. Then crude extract was filtered by sterile Watman® filter paper (No. 1). The resultant clear solution was dissolved in sterile water to make lemon juice extract (1:1 v/v). For combined washing solutions, 0.1mL of origanum oil was dissolved in 99.9 mL of 1% white vinegar (VI) and lemon juice extract (LE) separately to obtain VIO and LEO, respectively. Similarly 0.l mL of origanum oil was dissolved in 99.9 mL of combination of VI and LE to obtain VILEO. Sterile water was used as control and five treatments VI, LE, VIO, LEO, and VILEO were used as natural washing solutions in the present study. All washing solutions were prepared on the same day of produce wash, and solutions were stored in the refrigerator at 4 °C until used.

2.3 pH Measurement of Washing Solutions

The pH of washing solution was measured before and after washing of the sample with a pH meter (Denver Instrument, Model 215). Determination of pH was performed in triplicate at room temperature (23 °C ± 2).

2.4 Storage Study of Fresh Produce

Fresh strawberries (20 g ± 0.2 g) were used for storage study. Two sets of sample were prepared and two strawberries were used for each treatment. First set was used for the determination of total aerobic bacteria, yeast

and mold counts. The second set was used for surface contamination with foodborne bacteria. Samples in the sterile sample bag were tested on day 0, 1, 3, and 5 during the storage at 4 °C in the refrigerator. On the test day, samples were taken out of storage bag and microbiological analysis was performed.

2.5 Analysis of Total Aerobic Bacteria, Yeast and Mold Counts

Fresh sample was used to determine the total aerobic, yeasts and molds count. A 20 g of sample was dissolved and diluted (1:10 w/v) in 0.1% buffered peptone water (BPW), homogenized by hand massage for 5 min and serially diluted with BPW. Diluted samples were plated on aerobic count plates and yeast and mold plates 3M® Petrifilm (3M® Microbiology, St. Paul, MN). 3M® Petrifilms were incubated at 37 °C for 48 hours to determine total aerobic bacteria. Yeast and mold counts were determined after incubation at 25 °C for 5 days. Aerobic plate counts, yeast and mold counts were determined according to the instructions by 3M® Microbiology.

2.6 Bacterial Culture

Salmonella Typhimurium (ATCC 51812) was obtained from School of Veterinary Medicine Allied Health at Tuskegee University. Stock cultures were transferred into Tryptic Soy Broth (Fulka analytical 22091, Sigma-Aldrich) and incubated at 37 °C for 18 hrs. Cultures were streaked onto TSA plates and incubated at 37 °C for 18±1 h. Subsequently, single colony of bacteria was aseptically inoculated in 5 mL Tryptic Soy Broth (TSB) and incubated at 37 °C for 18±1 h and 100 µL of bacterial suspension was inoculated into 5 mL TSB for a subsequent 18±1 h incubation at 37 °C to achieve a viable cell population of 8-9 Log CFU/mL. Salmonella was harvested by centrifugation at 5000 rpm (Brofuge 22R, Heraeus Instruments, Inc., USA) for 5 min at 4 °C. The supernatant was carefully discarded and pellet was washed and re-suspended in sterile peptone water and thoroughly mixed by vortexing. This centrifugation and washing procedure was repeated. The collected bacterial cells were diluted in peptone water for storage study. Defined numbers of inocula were determined by counting colonies from the 18 h cultured cells grown on TSA (spread 0.1 mL) from each diluent by tenfold dilution. Correspondingly, optical density was also measured with the final concentration of 6-7 Log CFU/mL.

2.7 Inoculation on Strawberry

All the samples in sterile stainless steel tray were placed in sterile aluminum foil and subjected with UV treatment under the laminar flow hood for 25 min to eliminate the micro flora on strawberries before artificial contamination. Sequentially, 10 µL/g of suspension of *S.* Typhimurium with final bacterial concentration 6-7 Log CFU/mL was inoculated separately on the outer surface of strawberries. The bacterial culture solution was spot-inoculated and spread around the surface using pipette tips to ensure homogeneous spread across the surface. Contaminated samples were co-incubated for 2 h at room temperature then stored at 4 °C for 5 days.

2.8 Sample Treatment with Dipping/Shaking

Contaminated samples were dipped in approximately 50 mL of natural antimicrobial washing solutions VI, LE, VIO, LEO, VILEO, SW (sterile water/control), and WO (without wash), respectively in sterile beakers at room temperature. The samples were continuously shaken for 5 min at room temperature using automatic shaker at 100 rpm. Strawberries were fully immersed into each natural antimicrobial washing solution. All the samples were immediately drained by sterile metal filter and placed in a sterile filter paper under bio safety cabinet and allowed to dry. One set was analyzed immediately after washing (day 0) and the other sets were stored in sterile bags at 4 °C for up to 5 days. During the washing process pH of the solution was recorded.

2.9 Retrieval of Bacteria from Artificially Contaminated Sample

Hekton Enteric Agar (HEA) was used for selection of artificially contaminated samples with *S.* Typhimurium. Samples were homogenized and diluted (1:1 W/V) with 0.1% buffered peptone water, then homogenized by hand massage and kept for 60 min before retrieving *S.* Typhimurium 0.1 mL aliquot was then pipetted from each sample and spread onto HEA plates. Isolation and identification of contaminated *S.* Typhimurium were performed, based on color of the isolated colonies. The black centered with green background colonies on HEA plates were selected as inoculated *S.* Typhimurium from strawberries. Isolated colonies were also compared with *S.* Typhimurium cultured on HEA as control plates. Isolated colony was subjected to further analysis on 3M® *Salmonella* express system as a confirmative test. Handling procedures for 3M® *Salmonella* express system was followed according to manufacturer's instructions.

2.10 Color Measurement

A Minolta Chroma Meter (Model CR-400 version 1.11, Konica Minolta, Japan) was used to measure color values of samples. The CIE L *, a*, and b* were used where L* represents lightness, and a* and b* represents redness and yellowness, respectively. The instrument was standardized using reference color before color measurement.

Strawberry samples were placed in clean plastic bag and color values were measured from three different representative spots at before and after wash and on day 5. Total color difference (Delta E) was calculated using equation (Equation 1) and compared among treatments before and after washed and at day 5. All measurements were performed in triplicate at room temperature (23 °C ±2).

Delta E of the color was calculated by equation as follows:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^{*2} + \Delta b^{*2}} \quad \text{.....} \quad \text{(Equation 1)}$$

Where: $\Delta L^2 = L^2_{(final)} - L^2_{(initial)}$, $\Delta a^2 = a^2_{(final)} - a^2_{(initial)}$, $\Delta b^2 = b^2_{(final)} - b^2_{(initial)}$,

2.11 Statistical Analysis

Two replicate trials were performed for each experiment. Each treatment was replicated. Data were analyzed by repeated measurement-analysis of variances (RM-ANOVA) using the general linear model procedure of the statistical system SAS 9.3 (SAS Inst., Cary, N.C., U.S.A.). Mean comparisons were calculated using Fisher's Protected Least Significant Difference. Plate count data was converted to logarithms scale prior to their statistical analyses. Level of significance of all tests was defined at $P < 0.05$.

3. Results and Discussion

3.1 pH Determination of Washing Solutions

The pH of the washing solutions before and after washing fresh strawberries remained the same for all treatments throughout the study period. However, the pH of the strawberries washed with sterile water decreased from 6.8 to 4.9. All washing solutions were in low acidic pH and it was ranged from 2.2 to 2.7. No reduction of pH in strawberry was observed throughout the storage period (Data was not included). Most common household sanitizers such as commercial vinegars (white vinegars, apple cider vinegars), and lemon juice reduced the aerobic bacteria without affecting the organoleptic quality (Vijayakumar & Wolf-Hall, 2002). In the study, pH range of the washing solutions was between 2.2 and 2.7, indicating that most of the acids were present in un-dissociated form (Cunningham, O'Byrne, & Oliver, 2009).

3.2 Decontamination of Microorganisms in Strawberries by Washing Treatments

The effect of dipping while shaking in different washing solutions at room temperature on the survival of total aerobic bacteria (TAB) is presented in Figure 1. Result was analyzed by comparing the effect of each treatment with storage day.

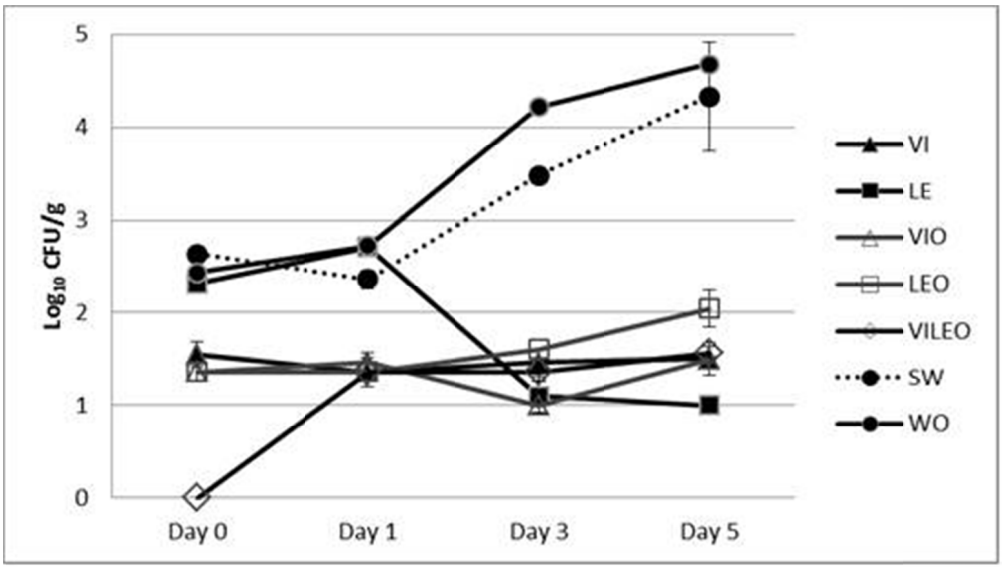

Figure 1. Effectiveness of washing solutions against total aerobic bacteria during storage at 4 °C on strawberries

(VI) White vinegar; (LE) Crude lemon juice extract; (VIO) VI+Origanum oil; (LEO) LE+Origanum oil; (VILEO) VI+LE+Origanum oil; (SW) Sterile water; (WO) Without wash

At day 0, initial population of TAB from unwashed (WO) and strawberries washed with sterile water (SW) were 2.4 and 2.6 Log CFU/g, respectively (Figure 1). Population of TAB on strawberries by WO and SW increased significantly on Day 3 and Day 5 ($P<0.05$). TAB counts on WO and SW were highest on day 5 with 4.7 and 4.4 Log CFU/g, respectively. On day 0, bacterial reduction of TAB on strawberries washed with VI, LEO, VIO, and VILEO were 1.1, 0.3, 1.3, 1.3 and 2.6 respectively. Compared to the sterile water, on day 3 and day 5, TAB counts on strawberries washed by five washing treatments was reduced by 1.9~2.4 and 2.3~3.3 respectively. The results showed that vinegars and combination of washing solutions significantly reduced TAB as compared to control ($P<0.05$). Growth of yeast and mold were high in control samples throughout the storage time from 3.8 to 4.4 CFU/g as presented in Figure 2. Effectiveness of washing solutions at different storage days showed that population of yeasts and molds were significantly reduced by washing solutions. Washing solutions VI, LEO, and VILEO reduced yeast and mold counts on strawberries 3.7, 3.6, and 3.6 Log CFU/g, respectively as compared to control (Figure 2). Four washing solutions (VI, VIO, LEO, and VILEO) among the treatments also showed antifungal activities as well. The result of our study showed that the reduction of yeasts and molds counts on strawberries were significant ($P<0.05$). However, results also revealed that washing solution LE was not suitable to remove yeasts and molds from strawberry.

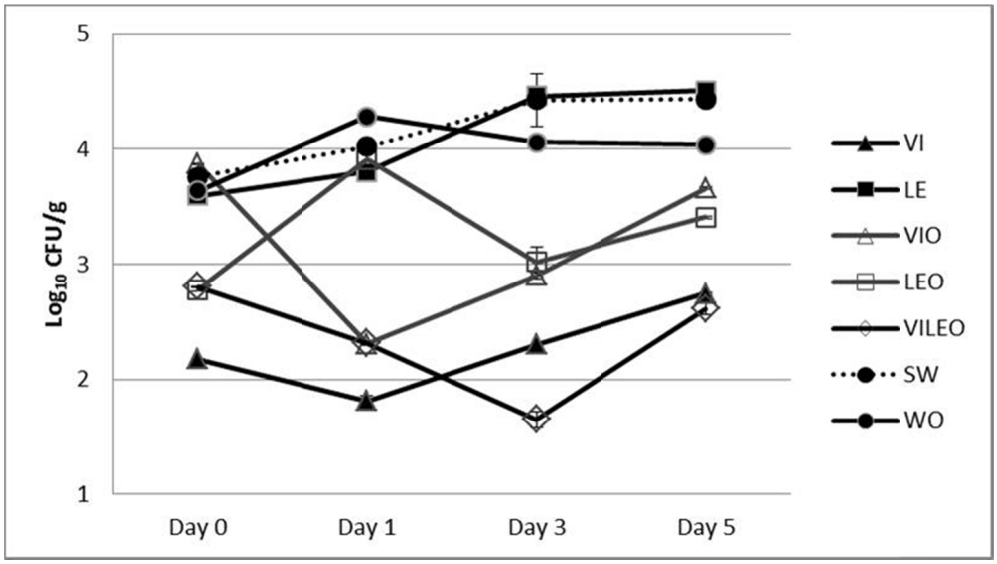

Figure 2. Effectiveness of washing solutions against fungal growth during storage at 4 °C in strawberries (VI) White vinegar; (LE) Crude lemon juice extract; (VIO) VI+Origanum oil; (LEO) LE+Origanum oil; (VILEO) VI+LE+Origanum oil; (SW) Sterile water; (WO) Without wash

It was reported that malted vinegar having concentration of 0.03 to 5% acetic acid largely reduced bacteria isolated from catfish after 15 min of dipping time; this result supported the concentration of commercial vinegars used in our study as an antimicrobial agents (Lingham, Lee, Besong, & Ozbay, 2012). Chlorine is one of widely used sanitizers to prevent cross-contamination, but some studies showed that washing solution containing chlorine within the recommended range of 50-200 ppm cannot eliminate pathogens completely but can reduce 1 to 3 Log CFU/g (Aruscavage, Lee, Miller, & Lejeune, 2006). Therefore, various alternative natural sanitizing agents for fresh produce such as citric acid, lactic acid, ascorbic acid, peroxyacetic acid, and acidified sodium chlorite have been studied (Ruiz-Cruz, Acedo-Félix, & Díaz-Cinco, 2007; Tajkarimi & Ibrahim 2011, Altintas et al., 2013; Nastou et al., 2012). The method proposed in this study could be useful at house-hold level and it might be helpful to reducing washing time and sanitizing water especially under the situation where water resource is limited. This study also focused on alternative and reliable washing solution for fresh produce. Selected washing solutions, especially combination with origanum oil in this study significantly reduced viable count of bacteria and yeasts and molds from strawberry throughout the storage period. The active components present in essential oil have a broad spectrum of antimicrobial activity, against food-borne pathogens and spoilage bacteria (Gutierrez, Barry-Ryan, and Bourke (2009). The antimicrobial activity of essential oil is due to presence of hydrophilic functional group such as hydroxyl group of phenolic compound along with lipophilicity

of essential oil (Dorman & Deans, 2000). However, combination of treatments with origanum oil in this study showed less effect at day 0, it might be due to the less dispersion and insufficient time of contact with microorganism to exhibit its antimicrobial activities at day 0 in strawberry. Nevertheless, results from this study showed that washing solutions VI, LE, VIO, LEO, and VILEO could be used as an alternative natural decontaminants washing solution for fresh produce at the house-hold level.

3.3 Decontamination of Foodborne Pathogen

Strawberries were artificially contaminated with *S.* Typhimurium and initial concentration of inoculum was 6 to 7 Log CFU/ml. Bacterial populations on the surface of strawberry after the inoculum were 3.4 and 3.9 Log CFU/g respectively. On day 0, bacterial reductions after the washing by VI, LE, VIO, LEO and VILEO were 1.8, 1.9, 2.1, 3.4 and 2.1 respectively. Compared to the sterile water, all washing solutions maintained antimicrobial activity through the storage time. Also, it was shown that all five washing treatments significantly reduced bacterial population by 2.7 logs on day 5 as compared with SW wash and unwashed samples ($P < 0.05$) as presented in Table 1. Particularly, compared to the control, *S.* Typhimurium by LEO wash on day 0, 1, 3 and 5 was reduced by 3.4, 3.6, 3.2 and 2.7 respectively. Efficacies of washing solutions can differ with food samples and types of washing solution. In the present study, washing solutions were very effective to inhibit *S.* Typhimurium on strawberries (Table 1).

Table 1. Effectiveness of washing solutions during the storage against *S.* Typhimurium on strawberries

Washing Solution	(Log_{10} CFU/g) Day 0	Day 1	Day 3	Day 5
VI[1)]	1.65	1.95	ND[2)]	ND
LE	1.54	2.18	ND	ND
VIO	1.39	1.48	1.60	ND
LEO	ND	ND	ND	ND
VILEO	1.35	1.60	1.48	ND
SW	3.44	3.63	3.15	2.73
WO	3.87	3.64	3.17	2.66

[1)](VI) White vinegar; (LE) Crude lemon juice extract; (VIO) VI+Origanum oil; (LEO) LE+Origanum oil; (VILEO) VI+LE+Origanum oil; (SW) Sterile water; (WO) Without wash.

[2)]ND=Not Detected.

It was reported that selected house-hold vinegars with concentration of 2.5 and 5% acetic acids were effective in inhibition against *L. monocytogenes, E. coli O157:H7*, and *S.* Typhimurium however, only 5% vinegar was effective against *S.* Typhimurium (Yang, Kendall, Medeiros, & Sofos, 2009). Reduction of *L. monocytogenes* and pathogens after dipping/spraying or in combination with organic acid solutions has been reported in several studies (Akbas & Olmez, 2007; Nastou et al., 2012). This study investigated the efficacy of dipping/shaking at room temperature with different natural washing solutions containing organic acids in combination with origanum oil and how these solutions reduced micro-organisms in the fresh produce under examination. Findings indicated that concentration of washing solution and selection of applied method can play an important role in effectiveness of reducing power against the microbial growths in strawberries. Natural substances exhibited the antimicrobial activities alone and sometime efficacy of the antimicrobial substances increased when combined with other substances. Fresh produce such as tomato and iceberg lettuce dipped in origanum oil solution at concentration of 15, 25, 40, 75, and 100 ppm significantly reduced the *S.* Typhimurium, and natural flora by 2.8 CFU/g at 75 ppm (Gunduz, Gonul, & Karapinar, 2010). Shredded lettuce with commercial vinegar containing 5% acetic acid (pH 3.0) for 5 min reduced *E. coli* O157:H7 and *S.* Typhimurium by 5 logs population at 25 °C (Chang & Fang, 2007). It showed that 100 ppm oregano oil reduced *S.* Typhimurium by 2.8 CFU/g (Gunduz et al., 2010). Findings in this study also demonstrated that crude lemon juice extract with concentration of 50% alone and in combination with origanum oil or vinegar significantly reduced *S.* Typhimurium. Organic compounds such as acetic acid, lactic acid and benzoic acid have antimicrobial effect; these compounds may disrupt microbial cell membrane or cell macromolecules or interfere with nutrient transport and energy

metabolism, causing bactericidal effect (Rick, 2003). It is also reported that weak organic acids are more inhibitory activity than strong acid because they have lipophilic and penetrate plasma membrane and acidify the interior cell organelles (Booth & Kroll, 1989). However, lemon juice was not effective for yeast and mold in strawberries used in this study. Yeasts and molds are more resistant at lower pH as compared to bacteria (Betts, 2013). Prior study reported that origanum essential oil has no antibacterial effect against tested human pathogenic bacteria but has antifungal activities against anthracbose-causing fungal plant pathogens in strawberry (Wedge, Mincsovics, Tabanca, & Altintas, 2013). However, this study found that washing treatments were effective in inhibition for both pathogenic bacteria and fungi. Combination of treatments can be more effective towards tested organisms in some cases. Studies showed that combination of vinegar and lemon juice (1:1) reduced the log counts of 6.0 to 5.7 of viable *S.* Typhimurium and reduced to undetectable level when treatment was applied up to 30 min (Sengun & Karapinar, 2004). Similarly, other studies found that combination of 2% organic acids (malic, lactic and citric acids) and ultrasound of 40 kHz reduced number of *E. coli* O157:H7, *L. monocytogenes* and *S.* Typhimurium on lettuce leaves by 3.2-2.3 Log CFU/g (Sagong et al., 2011). In another study, combination of alkaline electrolyte water and 1% acetic acid reduced *L. monocytogenes* and *E. coli* O157:H7 by 4 Log CFU/g on shredded carrots (Rahman at al., 2011). Combination of organic acids (citric acid and ascorbic acids) reduced internalized *E. coli* and *L. monocytogenes* on lettuce by more than 3 Log CFU/g (Olmez & Temur, 2010). This study indicated that natural antimicrobial agents and their combinations can be an effective alternative washing solution. In addition to the application of sanitizing agents on food systems, it can be applied in kitchen environments. Several parameters are important to reduce the contamination of fresh produce at house hold level such as washing temperature, agitation, immersion time, concentration of treatments and biocidal agents.

3.4 Color Analysis

Fresh strawberries showed some variation in color values during storage. There was a slight change in ΔE values in strawberries during storage. However, there was no significant difference compared with control ($P>0.05$) as presented in Table 2. Similarly, there were no significant differences in L* values ($P>0.05$) of strawberries among the treatments during the storage except VLO sample which was slightly darker (Table 3).

Table 2. Color changes (**ΔE)** of strawberries before, after washing and at day 5

Washing Solution	Color Changes		
	ΔE_{BA} [3)]	ΔE_{B5}	ΔE_{A5}
VI[1)]	6.4±2.1[a 2)]	8.9±2.5[a]	6.5±1.6[ab]
LE	6.4±2.1[a]	7.9±2.5[a]	4.9±1.6[ab]
VIO	6.6±2.1[a]	6.7±2.5[a]	4.3±1.6[b]
LEO	6.2±2.1[a]	6.8±2.5[a]	9.6±1.6[a]
VILEO	10.8±2.1[a]	8.8±2.5[a]	8.8±1.6[ab]
S/W	8.1±2.1[a]	7.9±2.5[a]	4.7±1.6[b]
W/O	7.1±2.1[a]	5.3±2.5[a]	5.4±1.6[ab]

[1)](VI) White vinegar; (LE) Crude lemon juice extract; (VIO) VI+Origanum oil; (LEO) LE+Origanum oil; (VILEO) VI+LE+Origanum oil; (SW) Sterile water; (WO) Without wash.

[2) a-d] Means±Standard Error within a same column followed by same superscript letters are not different ($P>0.05$).

[3)] $\Delta E_{BA=}$ Changes in color between before and after washed; $\Delta E_{B5=}$ Changes in color between before washed and after 5 days; $\Delta E_{A5=}$ Changes in color between after washed and after 5 days.

The CIE a* values were lower on treatments VO and VLO in strawberry samples after the wash (Table 3). It is assumed that natural sanitizers might be helpful to extend the shelf-life of fresh produce without affecting the color. Natural color of strawberries may vary by different factors such as cultivar, cultivation condition, harvesting time and etc. However, color serves a useful criterion of quality and indication of various types of deteriorative effect in fresh produce. The change in delta E, L*, a* and b* values indicated slight change in color before and after washing with solutions and during storage. It is indicated that present decontamination study is

more suitable to preserve the color of fresh produce than physical methods. However, the right combination among fresh-cut and antimicrobial activity of essential oils must be anticipated to optimize the use of essential oils as natural additive for fresh- cut produce to meet consumer's requirements (Ayala-Zavala, Gonzalez-Aguilar, & Del-Toro-Sanchez, 2009).

Table 3. Color values in strawberries before, after washed and at the end of storage day 5

	Before Wash			After Wash			Day 5		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
VI[1)]	43.5±1.9[a2)]	21.5±2.2[a]	7.8±1.4[a]	38.6±2.0[ab]	19.0±1.6[ab]	7.0±1.0[ab]	39.3±2.0[a]	18.2±2.9[a]	6.2±1.3[a]
LE	42.6±1.9[a]	20.3±2.2[a]	8.3±1.4[a]	43.9±2.0[a]	23.4±1.6[a]	9.8±1.0[a]	43.2±2.0[a]	20.0±2.9[a]	7.8±1.3[a]
VIO	44.6±1.9[a]	20.7±2.2[a]	9.0±1.4[a]	40.8±2.0[ab]	18.0±1.6[b]	6.5±1.0[b]	40.2±2.0[a]	17.0±2.9[a]	7.0±1.3[a]
LEO	40.6±1.9[a]	19.0±2.2[a]	6.4±1.4[a]	41.4±2.0[ab]	22.0±1.6[ab]	8.7±1.0[ab]	40.9±2.0[a]	24.5±2.9[a]	7.8±1.3[a]
VILEO	44.6±1.9[a]	21.4±2.2[a]	8.6±1.4[a]	37.6±2.0[b]	18.2±1.6[b]	6.3±1.0[b]	39.0±2.0[a]	19.4±2.9[a]	7.6±1.3[a]
SW	43.1±1.9[a]	19.1±2.2[a]	9.1±1.4[a]	42.4±2.0[ab]	23.5±1.6[a]	8.9±1.0[ab]	42.3±2.0[a]	22.9±2.9[a]	8.6±1.3[a]
WO	43.1±1.9[a]	16.7±2.2[a]	7.1±1.4[a]	40.9±2.0[ab]	17.0±1.6[b]	6.1±1.0[b]	39.9±2.0[a]	17.4±2.9[a]	6.7±1.3[a]

[1)](VI) White vinegar; (LE) Crude lemon juice extract; (VIO) VI+Origanum oil; (LEO) LE+Origanum oil; (VILEO) VI+LE+Origanum oil; (SW) Sterile water; (WO) Without wash.

[2) a-b] Means±SE within a same column followed by same letters are not significantly different (P>0.05).

4. Conclusion

Based on the results, it is concluded that natural antimicrobial washing solutions were effective to inhibit foodborne pathogenic bacteria, yeasts and molds in strawberries. Therefore, it is suggested that natural washing solution has potential to be used as a house-hold washing solution for safety of fresh produce. It is recommended that further study in various washing temperature and sensory tests might be helpful to maximize inhibition effect on various foods and to determine the impact of washing solutions on consumer's organoleptic sensitivities and acceptability.

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References

Akbas, H., & Olmez, M. Y. (2007). Inactivation of *Escherichia coli* and *Listeria monocytogenes* on iceberg lettuce by dip wadh tratments with organic acids. *The socity of Applied Microbiology, Letter to Applied Microbiology, 44*, 619-624. http://dx.doi.org/10.1111/j.1472-765x.2007.02127.x

Altintas, A., Tabanca, N., Tyihak, E., Ott, P. G., Móriczm, A. M., Mincsovics, E., & Widge, D. E. (2013). Characterization of volatile constituents from *origanum onites* and their antifungal and antibacterial activity. *Journal of AOAC International, 96*(6), 1200-1208. http://dx.doi.org/10.5740/jaoacint.sgealtintas

Amoah, S., Albano, E. E., & Kobina, L. (2012). Application of antagonistic microorganisms for the control of postharvest decay in fruits and vegetables. *International Journal of Advanced Biological Research, 2*(1), 1-8.

Aruscavage, D., Lee, K., Miller, S., & Lejeune, J. T. (2006). Interaction affecting the proliferation and control of human pathogens on edible plants. *Journal of Food Science, 71*, 89-99. http://dx.doi.org/10.1111/j.1750-3841.2006.00157.x

Ayala-Zavala, J. F., Gonzalez-Aguilar, G. A., & Del-Toro-Sanchez, L. (2009). Enhancing safety and aroma appealing of fresh-cut fruits and vegetables using the antimicrobial and aromatic power of essential oils. *Journal of Food Science, 74*(7). http://dx.doi.org/10.1111/j.1750-3841.2009.01294.x

Betts, R. (2013). Microbial update: Yeasts and moulds. *International Food Hygiene, 24*(4), 10-11.

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

Beuchat, L. R. (2000). Use of sanitizers in raw fruit and vegetable processing. In S. M. Alzamora, M. S. Tapia, & A. López-Malo (Eds.), *Minimally processed fruits and vegetables* (pp. 63-77). Gaithersburg, MD, USA: Aspen Publishers, Inc. http://dx.doi.org/10.1007/978-1-4615-2393-2

Beuchat, L. R. (1992). Shelf stability and safety of fresh produce as influenced by sanitation and disinfection. *Journal of food protection, 10*(55), 808-814.

Booth, I. R., & Kroll, R. G. (1989). Mechanisms events occurring in this region will not be expected of action of food preservation procedures, the preservation of and may well be reversed as the interface is crossed. In G.W. Gould (Ed.), *Foods By Low pH* (pp. 119-160) London, New York: Elsevier.

Calo, J. R., Crandall, P. G., O'Bryan, C. A., & Ricke, S. C. (2015). Essential oils as antimicrobials in food systems – a review. *Food Control, 54*, 111-119. http://dx.doi.org/10.1016/j.foodcont.2014.12.040

Chang, J. M., & Fang, T. J. (2007). Survival of *E. Coli* O157:H7 and Salmonella enterica serovars Typhimurium in iceberg lettuce and the antimicrobial effect of rice vinegar against *E. coli* O157:H7. *Food Microbiology, 24,* 745-751. http://dx.doi.org/10.1016/j.fm.2007.03.005

Cunningham, E., O'Byrne, C., & Oliver, J. D. (2009). Effect of weak acids on Listeria monocytogenes survival: Evidence for a viable but non-culturable state in response to low pH. *Food Control, 20*(12), 1141-1144. http://dx.doi.org/10.1016/j.foodcont.2009.03.005

De Roever, C. (1999). Microbiological safety evaluations and recommendations on fresh produce food. *Food Control, 9*, 321-347. http://dx.doi.org/10.1016/s0956-7135(98)00022-x

Diaz-Cinco, M. E., Acedo-Flix, E., & Garcia-Galaz, A. (2005). Principals microorganisms patogenous y de deterioro. Nuevas technologies de conservation de productos vegetables frescos cortados (pp. 216-240). CIAD, A.C. Sonora, Mexico.

Dorman, H. J. D., & Deans, S. G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol, 88*, 308-316. http://dx.doi.org/10.1046/j.1365-2672.2000.00969.x

EFSA. European Food Safety Authority, European Centre for Disease Prevention and Control. (2013). The european union summary report on trends and sources of Zoonoses, zoonotic agents and food-borne outbreaks in 2011. EFSA J., 11, 3129.

FAO/APO. (2006). Postharvest Management offruits and vegetables in the Asia- pacific Region. In R. Rolle (Ed.), FAO/APO.

Goodburn, C., & Wallace, C. A. (2013). The microbiological efficay of decontamination methodologies forfruits and vegetables: A review. *Food Control, 32*, 418-427. http://dx.doi.org/10.1016/j.foodcont.2012.12.012

Gunduz, G. T., Gonul, S. A., & Karapinar, M. (2010). Efficacy of oregano oil in the inactivation of salmonella Typhimurium on lettuce. *Food Control, 21*, 513-517. http://dx.doi.org/10.1016/j.foodcont.2009.07.016

Gutierrez, J., Barry-Ryan, C., & Bourke, P. (2009). Antimicrobial activity of plant essential oils using food model media: efficacy, synergistic potential and interaction with food components. *Food Microbiol, 26*, 142-150. http://dx.doi.org/10.1016/j.fm.2008.10.008

Jennylynd, B. J., & Tipvanna, N. (2010). *Processing of fresh- cut tropicalfruits and vegetables: A techniqual guide.* Bangkon: RAP publication.

Kagan L. J., Aiello, A., & Larson, E. (2002). The role of the home environment in the transmission of infectious diseases. *Journal of Community Health., 27*, 246-267. http://dx.doi.org/10.1023/a:1016378226861

Lingham, T., Lee, J. M., Besong, S., & Ozbay, G. (2012). Antimicrobial activity of vinegar on bacterial species isolated from retails and local channel catfish (*Ictalurus punctatus*). *J. food Process Technol*, S11-001. http://dx.doi:10.4172/2157-7110.S11-001

Manohar, V., Ingram, C., Gray, J., Talpur, N. A., Echard, B. W., Bagchi, D., & Preuss, H. G. (2001). Antifungal activities of origanum oil against candida albicans. *Molecular and Cellular Biochemistry, 228*, 111-117. http://dx.doi.org/10.1023/a:1013311632207

Nastou, A., Rhoades, J., Smirniotis, P., Makri, I., Kontominas, M., & Likotrafiti, E. (2012). Efficacy of household washing treatments for the control of Listeria monocytogenes on. *International Journal of Food Microbiology, 159*, 247-253. http://dx.doi.org/10.1016/j.ijfoodmicro.2012.09.003

Nunes, C. A. (2012). Biological control of postharvest diseases of fruit. *European Journal of Pathology, 133*(1), 181-196. http://dx.doi.org/10.1007/s10658-011-9919-7

Olaimat, A. N., & Holly, R. A. (2012). Factors influencing the microbial safety of fruits and vegetables: A review. *Food Microbiology, 32*, 1-19. http://dx.doi.org/10.1016/j.fm.2012.04.016

Olmez, H., & Temur, S. D. (2010). Effects of different sanitizing treatments on biofilms and attachment of *Escherichia coli* and *Listeria monocytogenes* on green leaf lettuce. *LWT - Food Science and Technology, 43*, 968-970. http://dx.doi.org/10.1016/j.lwt.2010.02.005

Rahman, S. M. E., Jin, Y. G., & Oh., D. H. (2011). Combination of treatment of alkaline electrolyzed water citric acid with mild heat to ensure microbial safety, shelf-life and sensory quality of shredded carrots. *Food Microbiology, 28*, 484-491. http://dx.doi.org/10.1016/j.fm.2010.10.006

Ricke, S. C. (2003). Perspectives on the use of organic acid and short chain fatty acid as antimicrobials. *Poult. Sci., 82*, 632-639. http://dx.doi.org/10.1093/ps/82.4.632

Rico, D., Martin-Diana, A. B., Barat, J. M., & Barry-Ryan, C. (2007). Extending and Measuring the quality of fresh-cut fruits and vegetables: A review. *Trends in Food and Technology, 18*, 373-386. http://dx.doi.org/10.1016/j.tifs.2007.03.011

Ruiz-Cruz, S., Acedo-Félix, E., & Díaz-Cinco, M. (2007). Efficacy of sanitizers in reducing *Escherichia coli* O157:H7, Salmonella spp. and *Listeria monocytogenes* populations on fresh-cut carrots. *Food Control, 18*, 1383-1390. http://dx.doi.org/10.1016/j.foodcont.2006.09.008

Sagong, H-G., Lee, S-Y., Chang, P-K., Heu, S., Ryu, S., Choi, Y-J., & Kang, D-H. (2011). Combined effect of ultrasound and organic acids to reduce *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on organic fresh lettuce. *International Journal of Food Microbiology, 145*(1), 287-292. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.01.010

Sanita, I. S. (2006). Safe food in domestic environment: goals and recommendations for surveillance and prevention of foodborne disease (in Italiany). Rome.

Schuele, B., & Snead, J. (2001). From Farm to fork, critical control points for food safety. *Journal of Nutriton in Recipe and Meme Development, 3*(1), 3-27. http://dx.doi.org/10.1300/j071v03n02_02

Sengun, I. Y., & Karapinar, M. (2004). Effectiveness of lemon juice, vinegar and their mixture in the elimination of Salmonella Typhimurium on carrots (*Daucus carota* L.). *International Journal of Food Microbiology, 96*, 301-305. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.04.010

Sharma, M., Ingram, D. T., Patel, J. R., Millner, P. D., Wang, X., Hull, A. E., & Donnenberg, M. S. (2009). A Novel approach to investigate the uptake and internalization of *Escherichia coli* O157:H7 in spinach cultivated in soli and hydroponic medium. *Journal of Food Protection, 72*(7), 1513-1520.

Vijayakumar, C., & Wolf-Hall, C. E. (2002). Evaluation of household sanitizers for reducing levels of Escherichia coli on iceberg lettuce. *Journal of Food Protection, 65*, 1646-1650.

Wedge, D. E., Mincsovics, E., Tabanca, N., & Altintas, A. (2013). Characterization of volatile constituents from origanum onites and their antifungal and antibacterial activities. *Journal of AOAC International, 96*(6), 1200-1208. http://dx.doi.org/10.5740/jaoacint.sgealtintas

WHO. (2002). *Global strategy for food safety.*

WHO/FAO. (2006). *Guidelines for the safe use of wastewater, excreta and grey water: Wastewater in agriculture.* Geneva: WHO/FAO.

Zhao, T., & Doyle, M. P. (2006). Reduction of *Campylobacter jejuni* on chicken wings by chemical treatments. *Journal of Food Protection, 4*, 762-767.

Zivile, L., Irina, B., Kristina, K. Egle P., & Pranas, V. (2012). High-power pulsed light for microbial decontamination of some fruits and vegetables with different surfaces. *Journal of Food, Agriculture & Environment, 10* (3&4), 162-16.

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Desorption Isotherms and Isosteric Heats of Fermented Cocoa Beans (*Theobroma cocoa*)

Djedjro C. Akmel[1], Kouassi E. Kakou[1], Kisselmina Y. Koné[1], Nogbou E. Assidjo[1] & Patrice Kouamé[2]

[1] Laboratoire des Procédés Industriels de Synthèses de l'Environnement et des Energies Nouvelles, Institut National Polytechnique Félix Houphet Boigny (INP-HB), Yamoussoukro, Côte d'Ivoire

[2] Laboratoire de Biochimie et des Procédés, Université Nangui Abroguoa (UNA), Abidjan, Côte d'Ivoire

Correspondence: Djedjro C. Akmel, Laboratoire des Procédés Industriels de Synthèses de l'Environnement et des Energies Nouvelles, INP-HB, BP 1313 Yamoussoukro, Côte d'Ivoire. Tel: 225-0557-1021. E-mail: akmeldc@gmail.com

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Abstract

Water desorption isotherms of fermented cocoa beans from Ivory Coast were determined using the gravimetric static method of saturated salts solutions at 30 °C, 40 °C and 60 °C, and isosteric heats of desorption were calculated from Clausius-Clapeyron equation. The experimental data were fitted to several isotherm equations. The best fittings were obtained for the BET equation for aw<0.50 (with an average mean relative deviation (MRD) value of 1.56%) and for the Harkins-Jura equation for aw≥0.50 (with an average value MRD equation of 4.17%). The isotherms obtained at 30 °C and 40 °C are practically coincident and overlapped for aw below 0.40. Fermented cocoa beans presented a monolayer moisture content of 0.083 dry basis (d.b.) at 30 °C and this value decreases with increasing temperature. The net isostheric heats of desorption for fermented cocoa beans for the temperature range studied (30-60 °C) were estimated as a function of moisture content. The maximum net isosteric heat of desorption for fermented cocoa beans was estimated at around 13.51 kJ/mol corresponding to a moisture content value of 2.85%. The energy requirement for maintaining the moisture content low of 8.7% (d.b.) or 8% wet basis (w.b.) for safe storage of this product or for reducing the moisture content during drying was estimated at around 9.58 kJ/mol.

Keywords: cocoa beans, isostheric heat, mathematical model; water sorption isotherm

1. Introduction

Cocoa production is the main activity of smallholders. They provide 95% of the world production on surfaces lower than 10 ha with a weak average yield estimated from 350 to 400 kg/ha cocoa beans (Barel, 2005). Ivory Coast is the world's largest cocoa grower. Its production represents 10% of GDP (Gross Domestic Product) and 40% of export earnings (Banzio, 2003). However since some years, the quality of cocoa beans has decreased slowly and gradually on the world market. Quality of cocoa beans for export-market is significantly affected by post-harvest processing method (i.e. fermentation and drying) and the problems of cocoa beans stability during storage (Anonymous, 2006; Sandoval & Barreiro, 2002; Fowler, 1995). In most cases the aerothermic conditions of storage area are unfavourable affecting beans moisture content and water activity. Water activity influences particularly micro-organisms growth, enzymatic reactions kinetic and the lipids oxidation in biological products. To maintain the stability of dried cocoa beans during storage, it is necessary to know product physical properties, in particular the relationship between water activity and equilibrium moisture content at room temperature and the relative humidity. This relationship is represented by product sorption isotherms. The effect of temperature on sorption isotherm is very important due to the fact that cocoa beans packaged in permeable jute bags are exposed to variable temperatures during storage and processing. In another hand, water activity changes with temperature (Al Muhtaseb, McMinn, & Magee, 2002). Furthermore, sorption isotherm is used in drying to determine the final moisture (Cassini, Marczak, & Norena, 2006; Noumi et al., 2004).

Unfortunately, few works is found in literature about water sorption isotherms of cocoa beans (Sandoval et al., 2002). Mercier, Tusa and Guaiquirian (1982) determined moisture desorption isotherms of cocoa beans at three temperatures (33.3 °C, 41.8 °C and 52.4 °C) using a gravimetric method. The monolayer water content and the average sorption enthalpy were calculated using the BET equation. Talib, Daud and Ibrahim (1995) determined

moisture desorption isotherms of cocoa beans using a constant environment chamber for various combinations of air relative humidity (30 to 90%) and temperature (20 °C to 70 °C) . The new modified Hasley, Henderson, and Chung equations, whose parameters are fitted to a fifth-order polynomial with respect to temperature, was used to represent satisfactorily the isotherms of cocoa beans in the temperature range investigated.

Sandoval et al. (2002) obtained no statistical differences ($p<0.01$) among the water sorption isotherms of non fermented cocoa beans (Venezuelan fine second grade) at 25 °C, 30 °C and 35 °C. All data were adjusted with a single isotherm in this temperature range and the best fit were found for BET ($aw<0.50$) and Harking –Jura ($aw\geq 0.50$) models.

More recently, GAB model gave the best fit of the sorption isotherms of cocoa and cupuassu products at 15 °C, 30 °C and 35 °C (Medeiros, 2006).

Although several mathematical models exist to describe water sorption isotherms of food materials (Iglesias, Chirife, & Lombardi, 1975), none of them gives accurate results throughout the whole range of water activities, or for all types of foods.

The main purposes of this work were to provide experimental data for the sorption characteristics of fermented cocoa beans originated from Ivory Coast to model the desorption isotherms, determine their dependence on temperature, and estimate the differential heat of sorption which is importance when designing equipment for dehydration processes of cocoa beans.

2. Material and Methods

2.1 Material

The experiments were achieved on fresh cocoa beans previously fermented. About 20 kg fermented cocoa beans with an initial moisture content around 55-60% (by wet weight) supplied by an organic agricultural farm were used.

2.2 Measurement of Water Sorption Isotherms

The method for determining water desorption isotherms was static gravimetric technique in which the weights were followed discontinuously until the equilibrium. This method was based on the COST 90 project method (Wolf, Spiess, & Jung, 1985). About 25 ± 0.001 g sample of cocoa beans was put in an open shallow glass container inside a glass jar containing diluted solutions of sulphuric acid. Each experiment was carried out in triplicate. Sulphuric acid (Fisher Scientific, UK) solutions were used to maintain the specified relative humidity inside the glass jars.

The glass jars with the samples were kept in temperature-controlled cabinets (± 0.5 °C) at 30 °C, 40 °C and 60 °C. The samples were allowed to equilibrate until there was no discernible weight change, as evidenced by constant weight values (±0.001 g). This involved a period of approximately 9 – 12 days for 60 °C, 15 – 21 days for 40 °C and 21 – 27 days for 30 °C. The long stabilization time of samples constitutes main disadvantage of this method (Medeiros, Ayrosa, Pitombo, & Lannes, 2006). To prevent microbial spoilage of samples, a small dish containing crystalline tymol was placed in the glass jar where high water activities occurred ($a_w > 0.7$) (Wolf et al., 1985; Cassini et al., 2006). In order to avoid disrupting the degree of atmospheric moisture sorption, the sample was weighted only every third day in five seconds or less. After the equilibrium had been reached, the samples were dried using the oven method at 105 °C during 24 h (AOAC, 1990; Augier, 1999). Moisture determinations were done by triplicate and the averages were calculated. Seven (7) equilibrium points were obtained in these experiments.

The isotherm models used to fit the data are presented in Table 1. The goodness of fit of each model was evaluated using the correlation coefficient (r) and the mean relative deviation (MRD). The MRD value is given in percentage and may be estimated as follows:

$$MRD(\%) = \frac{100}{N}\sum_{i=1}^{N}\frac{\left|X_{Exp,i} - X_{Cal,i}\right|}{X_{Exp,i}} \tag{13}$$

where values below 10% are indicative of good fit (Lomauro, Bakshi, & Labuza., 1985).

Table 1. Isotherm equations for experimental data fitting

Model	Mathematical expression		a_w range
BET (Brunauer et al., 1938)	$X_{eq} = X_m C a_w / [(1-a_w)(1+(C-1)a_w)]$	(1)	$a_w < 0.50$
GAB (Van den Ben & Bruin, 1981)	$X_{eq} = X_m C K a_w / [(1-Ka_w)(1+C_G K a_w - Ka_w)]$	(2)	$0.05 < a_w < 0.95$
	$C = c_0 \exp(\Delta H_C / RT)$	(3)	
	$K = k_0 \exp(\Delta H_K / RT)$	(4)	
Chung and Pfost (1967)	$X_{eq} = \frac{1}{B}[\ln A - \ln(-\ln a_w)]$	(5)	$0.20 < a_w < 0.90$
Hasley (1948)	$X_{eq} = (-A/\ln a_w)^{\frac{1}{B}}$	(6)	$0.05 < a_w < 0.80$
Harkins and Jura (1946).	$X_{eq} = [-B/(\ln(a_w) - A)]^{\frac{1}{2}}$	(7)	$a_w > 0.50$
Henderson (1952)	$X_{eq} = [-\ln(1-a_w)/A]^{\frac{1}{B}}$	(8)	$0.50 < a_w < 0.95$
Kuhn (Labuza, Mizrahi, & Kasel, 1972).)	$X_{eq} = A/\ln a_w + B$	(9)	$a_w < 0.5$
Oswin (1946)	$X_{eq} = A[a_w/(1-a_w)]^B$	(10)	$0.05 < a_w < 0.90$
Smith (1947)	$X_{eq} = A + B\ln(1-a_w)$	(11)	$0.50 < a_w < 0.95$
Freundlich (1906)	$X_{eq} = A(a_w)^{\frac{1}{B}}$	(12)	$a_w < 0.90$

Variables to measure experimentally: X_{eq}= equilibrium moisture content (% d.b.); T= temperature (K) ; a_w= water activity.

Parameters to be estimated from the data: A=constant (dimensionless), B= constant (dimensionless), C= GAB or BET model parameter (dimensionless), c_0= constant (adjusted to the temperature effect) (dimensionless), ΔH_C= difference in enthalpy between mono-layer and multi-layer sorption (Kj/mol), ΔH_K= difference between the heat of condensation of water and the heat of sorption of the multilayer (Kj/mol), K= GAB model parameter (dimensionless), k_0= constant (adjusted to the temperature effect) (dimensionless), R= universal gas constant (0.00831Kj/(mol.K)), Xm= Monolayer moisture content (% d.b.).

2.3 Measurement of Net Isosteric Heats

The net isosteric heat of desorption (q_{st}) was calculated from the Clausius-Clayperon equation (Labuza, Kaanane, & Chen, 1985; Tsami, 1991; Hossain, Bala, Hossain, & Mondol, 2001; Veltchev & Menkov, 2000; Igbabul, Ariahu, & Umeh, 2013):

$$q_{st} = -R\left(\frac{\partial \ln(a_w)}{\partial(1/T)}\right) \tag{14}$$

where T is the absolute temperature (K) and R is the universal gas constant (0.00831Kj/(mol.K)) and a_w is the water activity.

This relationship required previously that sorption isotherms were determined at the study temperatures, in order to calculate the logarithmic variation in water activity as a function of temperature inverse at constant moisture content. In practice, it is easier to determine the slope of regression lines $ln(a_w)$ *vs* $1/T$ for a specific moisture content, and then deduce the net isosteric heat of sorption (q_{st}).

The heat of desorption is a measure of the energy requirement to break the intermolecular forces between water vapour molecules and adsorbent surface (AL-Muhtaseb et al., 2002; Rao & Rizvi, 1995).

In an attempt to describe the relationship between the net isosteric heat of sorption and the equilibrium moisture content, Tsami, Maroulis, Morunos and Saravacos.*(1990)* proposed an empirical exponential correlation, which can be written as:

$$q_{st} = q_0 \exp(-X / X_0) \tag{15}$$

where q_0 is the net isosteric heat of sorption of the first molecules of water in the food and X_0 is the characteristic moisture content of food material. The values of q_0 and X_0 were determined by adjustment of net isosteric heat q_{st} data with the Equation (15).

3. Results and Discussion

3.1 Water Sorption Isotherms

The experimental data obtained for water desorption isotherms of fermented cocoa beans at 30 °C, 40 °C and 60 °C are shown in Figure 1. The desorption isotherms reveal an increase in equilibrium moisture content with increasing water activity, at a constant temperature. The effect of temperature on desorption isotherm for the total range of water activities can be observed. The Figure 1 shows clearly that the moisture contents decrease while the temperature increases at constant water activity. This behavior is typical of many food products (Vazquez, Chenlo & Moreira, 2003). Moisture sorption isotherms of most foods are nonlinear, generally sigmoidal in shape, and have been classified as Type II isotherms (Akkad et al., 2008; AL-Muhtaseb et al., 2002; Sandoval et al., 2002; Kouhila, Belghit, & Daguenet, 1999).

The isotherms obtained at 30 °C and 40 °C are practically coincident and overlapped for aw below 0.40. Indeed many researchers have observed that the sorption isotherms vary very little for temperature differences lower than 10°C. A similar behavior was observed for non fermented cocoa beans at 25 °C to 35 °C (Sandoval et al., 2002), for sultana raisin at 20 °C to 30 °C (Saravacos, Tsiourvas, & Tsami, 1986).

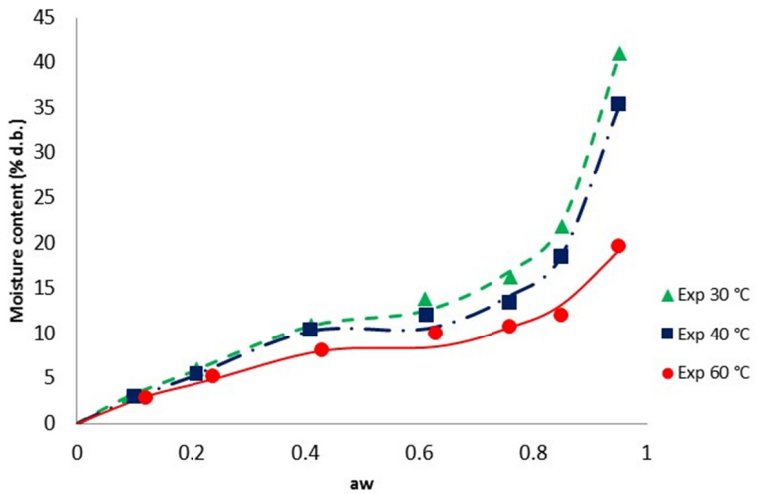

Figure 1. Desorption isotherms of fermented cocoa beans at different temperatures

Table 2 shows the fitting constants of models (presented in table 1), the correlation coefficient (r) and the mean relative deviation (MRD).

Table 2. Estimated values of constants, correlation coefficient (r) and the mean relative percentage deviation modulus (MRD) obtained for the models

Model	Constants	30 °C	40 °C	60 °C
GAB	Xm	0.0543	0.0455	0.0464
	C	27.0155	44.0684	22.5443
	K	0.9117	0.9151	0.7930
	r	0.990	0.985	0.972
	MRD(%)	14.30	16.46	13.27
BET	Xm	0.0828	0.0822	0.0554
	C	5.0030	4.1163	6.5404
	r	>0.999	>0.999	0.997
	MRD(%)	0.24	0.64	3.81
Chung-Pfost	A	2.3626	2.4923	3.2081
	B	8.8321	10.5798	17.4761
	r	0.883	0.868	0.962
	MRD(%)	32.71	31.82	10.88
Hasley	A	0.1436	0.1439	0.1442
	B	1.7608	1.7510	1.7595
	r	0.972	0.955	0.910
	MRD(%)	339.29	393.60	519.41
Harkins-Jura	A	-0.0051	-0.0080	0.049420715
	B	0.0077	0.0054	0.0037
	r	0.998	0.996	0.970
	MRD(%)	3.85	4.77	3.90
Henderson	A	8.4469	10.2375	58.2966
	B	1.0830	1.1008	1.7338
	r	0.979	0.969	0.974
	MRD(%)	23.02	24.74	9.73
Kuhn	A	-0.1103	-0.1071	-0.0708
	B	-0.0137	-0.0163	-0.0025
	r	0.998	0.999	0.989
	MRD(%)	7.51	2.58	6.81
Oswin	A	0.1050	0.0844	0.0752
	B	0.4568	0.4864	0.3191
	r	0.995	0.990	0.985
	MRD	9.86	11.13	11.54
Smith	A	0.0222	0.0220	0.0361
	B	-0.1199	-0.1012	-0.0516
	r	0.982	0.926	0.976
	MRD	13.15	14.89	14.70
Freundlich	A	0.3939	0.3311	0.1673
	B	0.4440	0.4655	1.0183
	r	0.934	0.917	0.925
	MRD	43.29	43.74	17.05

All models present correlation coefficient very close to unity indicating good fit to experimental data. However, considering the MRD, the best fittings were obtained for the BET equation for a_w <0.50 (with an average value of 1.56%) and for the Harkins-Jura equation for $a_w \geq 0.50$ (with an average value of 4.17%). The isotherms graphical representation at 30 °C, 40 °C and 60 °C with these equations and experimental data are presented in Figure 1 Sandoval et al. (2002) reported that the BET equation (a_w<0.50) and the Harkins-Jura equation ($a_w \geq 0.5$) provide good description of cocoa beans isotherms.

The fittings of these equations to experimental data generated the lowest MRD values, due probably, to the fact that these equations describe water sorption isotherms of food materials in limited range of water activities where as some models are involved in overall range. Labuza (1975) noted that no sorption isotherm model could fit data over the entire range of water activities because water is associated to food matrix by different mechanisms in different water activity regions. Furthermore Iglesias and Chirife (1982) recommended to divide in two domains the water activities range to realize sorption isotherms of food materials.

Using the Harkins-Jura equations, the high values of moisture contents, corresponding to a water activity of 0.70, for which cocoa beans remained safe for microbiological stability after drying or in storage, were 14.83%, 12.47% and 9.56% at 30 °C, 40 °C and 60 °C, respectively. Different studies reported adsorption and/or desorption isotherms of cocoa beans (Cassini et al., 2006; Sandoval et al., 2002; Talib et al., 1995). Concerning desorption isotherms of fermented cocoa beans, the values estimated from the data presented by Talib et al. (2002) for the same water activity value were 26.56%, 18.52% and 11.09% at 30 °C, 40 °C and 60 °C, respectively. These values of moisture contents were higher than the results obtained herein at the same temperatures. This could be explained by the excessive initial moisture content of cocoa beans used by these researchers for their experimentation.

The monolayer moisture content values (Xm) of fermented cocoa beans estimated with BET equation at 30 °C, 40 °C and 60 °C can observed in Table 3. Fermented cocoa beans presented a monolayer moisture content of 0.083 (d.b.) at 30 °C and this value decreases with increasing temperature. This fact on Xm is a characteristic of type II isotherms. Similar results were found by others researchers for protein (Cassini et al., 2006), for starch powders (AL-Muhtaseb et al., 2002) and for tomatoes (McLaughlin & Magee, 1998). This decrease in monolayer moisture content reflects the reduction in product's hygroscopicity which accompanies the increasing temperature. This can be explained by the reduction of the degree of hydrogen bonding in such products with the increasing temperature, thereby decreasing the availability of active sites for water binding and thus, the monolayer moisture content (AL-Muhtaseb et al., 2002).

Considering the values of monolayer moisture content estimated with BET equation, a more detailed analysis of GAB parameters can provide further valuable information about desorption. Therefore a direct non-linear regression technique was adopted, with Equations 3 and 4 being substituted into Equation 2. The results of regression analysis are summarized in Table 3. These parameters (c_0, ΔH_C, k_0 and ΔH_K) have also physical meaning in terms of sorption processes (Van den Berg, 1984). ΔH_C represents the difference in enthalpy between mono-layer and multi-layer sorption (Van den Berg, 1984). The values of ΔH_C are negatives (with an average value of -25.125 Kj/mol). These negative values show that the reaction of the water removal associated with the food matrix is endothermic. This indicates the need to bring energy to achieve the optimum moisture content of the food, for which stability is maximum. For some authors (Brunauer et al., 1938; Rockland, 196.), this optimum moisture content corresponds to the mono-layer moisture content of BET. ΔH_K represents the difference between the heat of condensation of water and the heat of sorption of the multi-layer (Van den Berg, 1984). The positive value of ΔH_K indicates that the heat of sorption of the multi-layer is lower than the heat of condensation of water, in the case of fermented cocoa beans.

Table 3. Characteristic GAB parameters for Equation 3 and Equation 4 at different temperatures

T(°C)	Xm	c0	ΔH_C (Kj/mol)	k0	ΔH_K (Kj/mol)	r	MRD(%)
30 °C	0.083	9057.49	-24.331	0.285	2.843	0.985	18.97
40 °C	0.082	8956.29	-25.514	0.198	3.838	0.977	19.36
60 °C	0.055	8871.27	-25.531	0.195	3.946	0.966	16.90

c_0= constant (adjusted to the temperature effect) (dimensionless), ΔH_C= difference in enthalpy between mono-layer and multi-layer sorption (Kj/mol), ΔH_K= difference between the heat of condensation of water and the heat of sorption of the multi-layer (Kj/mol), k_0= constant (adjusted to the temperature effect) (dimensionless), *Xm*= Monolayer moisture content (d.b.).

3.3 Net Isosteric Heats of Desorption

A graphical representation of the net isostheric heats of desorption for fermented cocoa beans is shown in Figure 2. The results illustrate a progressive increase in the heat of desorption with decreasing moisture content. Our results agrees with observations by many researchers (Cassini et al., 2006; Magda, Ana, Ronaldo, & Suzana, 2006; Fasina & Sokhansanj, 1993), since the lower the moisture content, the higher the energy required to remove water from product.

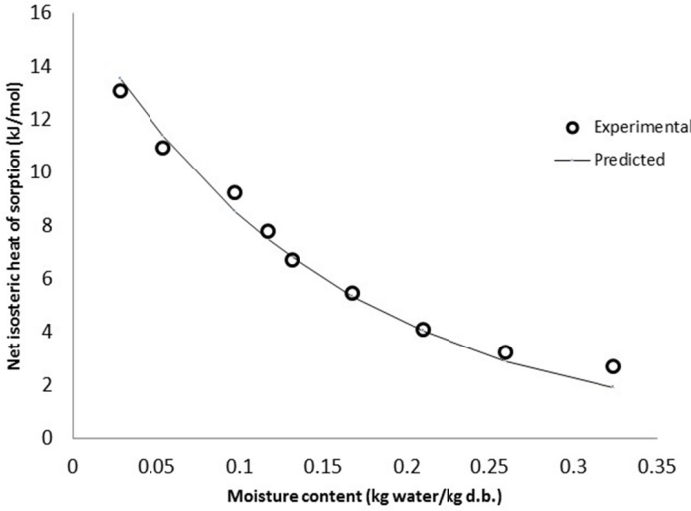

Figure 2. Net Isosteric heat of sorption of fermented cocoa beans estimated between 30 and 60 °C

The net isosteric heats of sorption have been modeled by an empirical exponential correlation given by Tsami et al. (1990):

$$q_{st} = 16.34 \exp(-X / 0.15)$$ (with r =0.996 and MRD=18.89%).

Thus, the maximum net desorption isosteric heat of fermented cocoa beans was estimated at around 13.51 kJ/mol corresponding to a moisture content value of 2.85%.

The energy required for maintaining the moisture content at 8.7% (d.b.) or 8% (w.b.) for safe storage of this product (Braudeau, 1970) or for reducing the moisture content during drying was estimated at around 9.58kJ/mol.

4. Conclusions

The experimental data for water desorption isotherms of fermented cocoa beans (Ivory Coast) were determined for three temperatures (30 °C; 40 °C and 60 °C) and adjusted to several equations. Considering the MRD, the best fittings were obtained for the BET equation for a_w<0.50 (with an average value of 1.56%) and for the Harkins-Jura equation for a_w≥0.50 (with an average value equation of 4.17%). Using the Harkins-Jura equation, the values of moistures contents considering safe for microbiological stability (a_w=0.70) were deduced at each temperature studied. Fermented cocoa beans presented a monolayer moisture content of 0.083 (d.b.) at 30 °C and this value decreases with increasing temperature.

The net isostheric heats of desorption for fermented cocoa beans for the temperature range studied (30-60 °C) were estimated as a function of moisture content. The energy requirement for maintaining the moisture content at 8.7% (d.b.) or 8% (w.b.) for safe storage of this product or for reducing the moisture content during drying was estimated at around 9.58 kJ/mol.

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References

Akkad, S., Idlimam, A., Lamharrar, A., Essaber, M., Kouhila, M., & Costa, J. (2008). Evaluation des isothermes de désorption et de la chaleur isosthérique de deux plantes aromatiques et médicinales par la méthode gravimetrique statique en vue de leur séchage. *Revue des Energies Renouvelables SMST 08 Alger,* 1-6.

AL-Muhtaseb, A. H., McMinn, W. A. M., & Magee, T. R. A. (2002). Moisture sorption isotherm characteristics of food products: A review. *Trans IChemE, 80*(2), 118-128. http://dx.doi.org/10.1205/09603080252938753

Anonymous. (2006). *Elaboration d'un plan national qualité café cacao: Etude diagnostique. Rapport final, Bourse du café et du cacao (BCC)* (p. 60), Abidjan.

AOAC. (1990). *Association of Official Analytical Chemists*. Official Methods of Analysis: 930.04. Moisture content in plants, 1, p. 949.

Basunia, M. A., & Abe, T. (2001). Moisture desorption isotherms of medium-grain rough rice. *Journal of Stored Products Research, 37*, 205-219. http://dx.doi.org/10.1016/S0022-474X(00)00022-9

Augier, F. (1999). *Transport d'eau et d'acide en milieu complexe- Application au séchage de la fève de cacao*. Thèse de doctorat, Université Montpellier II, p. 187

Banzio, D. (2003). *Comprendre et opérer dans les filières café et cacao en dix modules* (pp. 10-23). Abidjan.

Barel, M. (2005). *Le commerce du cacao dans le monde. Cirad, Chocolat et confiserie*. Magazine n 410.

Braudeau, J. (1970). *EL Cacao*. Editorial Blume, Barcelona, Spain

Brunauer, S., Emmet, P. H., & Teller, E. (1938). Adsorption of gases in multi-molocular layers. *Journal of American Chemical Society, 60*, 309-319. http://dx.doi.org/10.1021/ja01269a023

Cassini, A. S., Marczak, L. D. F., & Norena, C. P. Z. (2006). Water adsorption isotherms of texturized soy protein. *Journal of Food Engineering, 77,* 194-199. http://dx.doi.org/10.1016/j.jfoodeng.2005.05.059

Chung, D. S., & Pfost, H. B. (1967). Adsorption and desorption of water vapor by cereal grains and their products. *Transactions of the ASABE, 10*(4), 549-557. http://dx.doi.org/10.13031/2013.39726

Fasina, O. O., & Sokhansanj, S. (1993). Equilibrium moisture relations and heat of sorption of Alfafa Pellets. *Journal of Agriculture Engineering Research, 56*, 51-63. http://dx.doi.org/10.1006/jaer.1993.1060

Fowler, M. S. (1995). *Quality of cocoa beans for chocolate manufacturers* (pp. 147-153). Seminar Proceedings, Montpellier.

Freundlich, H. M. F. (1906). Uber die adsorption in losungen. *Zeitschrift fur Physikalishe Chemie, 57*(A), 385-470.

Halsey, G. (1948). Physical adsorption on non-uniform surfaces. *Journal of Chemistry Physics, 16*, 931-937. http://dx.doi.org/10.1063/1.1746689

Harkins, W. D., & Jura, G. (1946). Surfaces of Solids. XII. An Absolute Method for the Determination of the Area of a Finely Divided Crystalline Solid. *Journal of American Chemical Society, 68*, 1941. http://dx.doi.org/10.1021/ja01236a047

Henderson, S. M. (1952). A basic concept of equilibrium moisture. *Agricultural Engineering, 33*, 29-33.

Hossain, M. D., Bala, B. K., Hossain, M. A., & Mondol, M. R. A. (2001). Sorption Isotherms and Heat of Sorption of Pineapple. *Journal of Food Engineering, 48*(2), 103-107. http://dx.doi.org/10.1016/S0260-8774(00)00132-1

Igbabul, B. D., Ariahu, C. C., & Umeh, E. U. (2013). Moisture Adsorption Isotherms of African Arrowroot Lily (*Taccainvolucrata*) Tuber Mash as Influenced by Blanching and Natural Fermentation. *Journal of Food Research, 2*(3), 79-92. http://dx.doi.org/10.5539/jfr.v2n3p79

Iglesias H. A., & Chirife J. (1982). *Handbook of food isotherms water sorption. Parameters for food and food components.* New York: Academic Press. http://dx.doi.org/10.1016/B978-0-12-370380-4.50006-2

Iglesias, H. A., & Chirife, J. (1976). Isosteric heats of water vapour sorption on dehydrated foods. Part I. Analysis of differential heat curves. *Lebensm. -Wiss.- Technol, 9*, 116-122.

Iglesias, H. A., Chirife, J., & Lombardi, J. L. (1975). Comparison of water vapour sorption by sugar beet root components. *Journal of Food Technology, 10*, 385-391.

http://dx.doi.org/10.1111/j.1365-2621.1975.tb00044.x

Kouhila, M., Belghit, A., & Daguenet, M. (1999). Approche experimentale des isothermes de sorption de la menthe en vue du séchage par énergie solaire. *Revues des energies renouvelables, 2*(1), 61-68.

Labuza, T. P., Kaanane, A., & Chen, J.vY. (1985). Effect of Temperature on the Moisture Sorption Isotherms and Water Activity Shift of Two Dehydrates Foods. *Journal of Food Science, 50*, 38-391. http://dx.doi.org/10.1111/j.1365-2621.1985.tb13409.x

Labuza, T. P., Mizrahi, S., & Kasel, M. (1972). Mathématical model for optimization of flexible film packaging of foods for storage. *Trans. ASAE, 15*, 150. http://dx.doi.org/10.13031/2013.37853

Labuza, T. P. (1975). *Interpretation of sorption data in relation to the state of constituent water*. In R. B. Duckworth (Ed.), *Water Relations of Food* (pp. 155-172). New York, USA: Academic Press. http://dx.doi.org/10.1016/B978-0-12-223150-6.50014-6

Lomauro, C. J., Bakshi, A. S., & Labuza, T. P. (1985). Evaluation of food moisture sorption isotherm equations part I: Fruit, vegetable and meat products. *Food Science and Technology, 18*(2), 111-117.

Magda, L. M., Ana, M. I. B. A., Ronaldo, N. M. P., & Suzana, C. S. L. (2006). Sorption isotherms of cocoa and cupuassu products. *Journal of Food Engineering,* 77, 194-199. http://dx.doi.org/10.1016/j.jfoodeng.2005.02.002

McLaughlin, C. P., & Magee, T. R. A. (1998). The determination of sorption isotherm and the isosteric heats of sorption for potatoes. *Journal of Food Engineering, 35*, 267-280. http://dx.doi.org/10.1016/S0260-8774(98)00025-9

Medeiros, M. L., Ayrosa, A. M. I. B., Pitombo, R. N. M., & Lannes, S. C. S. (2006). Sorption isotherms of cocoa and cupuassu products. *Journal of Food Engineering, 73*, 402-406. http://dx.doi.org/10.1016/j.jfoodeng.2005.02.002

Mercier, P., Tusa, A., & Guaiquirian, H. (1982). *Adsorcion del agua en semilla de café y cacao. Acta Cientifica Venezolana, 33*(1), 398.

Noumi, G. B., Laurent, S., Ngameni, E., Kapseu, C., Jannot, Y., & Permentier, M. (2004). Modélisation de la deshydratation de la pulpe des fruits du *Canarium schweinfurthii Engl,. Tropicultura, 22*(2), 70-76.

Noumi, G. B., Laurent, S., Ngameni, E., Kapseu, C., Jannot, Y., & Permentier, M. (2004). Modélisation de la déshydratation de la pulpe des fruits du *Canarium schweinfurthii Engl Tropicultura, 22*(2), 70-76.

Oswin, C. R. (1946). The kinetics of package life. III. The isotherm. *Journal of Chemistry and Industry, 65*, 419-421. http://dx.doi.org/10.1002/jctb.5000651216

Rao, M. A., & Rizvi, S. S. H. (1995). *Engineering Properties of Foods* (Marcel Dekker Inc, New York, USA).

Ruegg, M. (1980). Calculation of the activity of water in sulphuric acid solutions at various temperature. *Lebensmittel-Wissenchaft und Technology, 13*, 22-24.

Sandoval, A. J., & Barreiro, J. A. (2002). Water sorption isotherms of non-fermented cocoa beans. *Journal of food engineering, 51*, 119-123. http://dx.doi.org/10.1016/S0260-8774(01)00047-4

Saravacos, G. D., Tsiourvas, D. A., & Tsami, E. (1986). Effect of temperature on thewater adsorption isotherms of sultana raisins*, J Food Sci, 51*, 381-387. http://dx.doi.org/10.1111/j.1365-2621.1986.tb11135.x.

Smith, S. E. (1947). The sorption of water vapour by high polymers. *Journal of the American Chemical Society, 69*, 646. http://dx.doi.org/10.1021/ja01195a053

Rockland, L. B. (1969). Water activity and storage stability. *Food Technology, 23*, 1241-1251.

Talib, M. Z. M., Daud, W. R. W., & Ibrahim, M. H. (1995). Moisture Desorption Isotherms of Cocoa Beans. *American Society of Agricultural and Biological Engineers, Transactions of the ASABE, 38*(4), 1153-1155. http://dx.doi.org/10.13031/2013.27933

Tsami, E., Maroulis, Z. B., Morunos-Kouris, D., & Saravacos, G. D. (1990). Heat of sorption of water in dried fruits, *Int J Food Sci Technol, 25*, 350-359. http://dx.doi.org/10.1111/j.1365-2621.1990.tb01092.x

Tsami, E. (1991). Net Isosteric Heat of Sorption in Dried Fruits. *Journal of Food Engineering, 14*(4), 327-335. http://dx.doi.org/10.1016/0260-8774(91)90022-K

Vazquez, G., Chenlo, F., & Moreira, R. (2003). Sorption isotherms of lupine at different temperatures. *Journal of Food Engineering, 60*, 449-452. http://dx.doi.org/10.1016/S0260-8774(03)00068-2

Veltchev, Z. N., & Menkov, N. D. (2000). Desorption Isotherms of Apples at Several Temperatures. *Drying Technology, 18*(4-5), 1127 -1137. http://dx.doi.org/10.1080/07373930008917759

Van den Berg, C. (1984). Description of water activity of foods for engineering purposes by means of the GAB model of sorption, In B. M. McKenna (Ed.), *Engineering and Food,* (Vol. 1, pp. 311-321). New York, USA: Elsevier Applied Science.

Van den Ben & Bruin. (1981). Water activity and its estimation in food systems. In L. B. Rockland & G. F. Steward (Eds.), *Water Activity: Influences on Food Quality* (pp. 147-177). New York, USA: Academic Press.

Wolf, W., Spiess, W. E. L., & Jung, G. (1985). Standardization of isotherms measurements (cost-project 90 and 90 bis). In D. Stimatos & J. L. Multon (Eds.), *Properties of water in foods in relation to quality and stability* (pp. 661-679). Dordrecht: Martinus Nijhoff Publishers. http://dx.doi.org/10.1007/978-94-009-5103-7_40

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The Diet and Behaviour Scale (DABS): Testing a New Measure of Food and Drink Consumption in a Cohort of Secondary School Children From the South West of England

Gareth Richards[1], Alice Malthouse[1] & Andrew Smith[1]

[1] Centre for Occupational & Health Psychology, Cardiff University, United Kingdom

Correspondence: Gareth Richards, Centre for Occupational & Health Psychology, Cardiff University, United Kingdom. Tel: 44-029-2087-6574. E-mail: RichardsG6@cardiff.ac.uk

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Abstract

A multitude of instruments exist to assess dietary intake. Many, however, are time-consuming to administer, focus primarily on macronutrient composition or the effects of specific micronutrients, and do not consider the effects of foods and drinks that fail to add significant nutritional contributions (e.g. energy drinks, chewing gum). In order to address these issues the current paper introduces the Diet and Behaviour Scale (DABS). This 29-item questionnaire is used to measure both the frequency and amount of consumption of common foods and drinks, with a particular onus on functional foods and dietary variables of current concern. The DABS was administered to a large cohort of secondary school children from the South West of England at two time-points. At Time 1 (December 2012) the cohort consisted of 3071 pupils, 2030 of whom responded to the questionnaire; at Time 2 (June 2013) 3323 pupils made up the cohort, and 2307 completed the questionnaire. Factor analysis yielded a four-factor solution labelled Junk Food, Caffeinated Soft Drinks/Gum, Healthy Foods, and Hot Caffeinated Beverages. When investigating how these factors were related to demographic and lifestyle variables, Chi-square analyses uncovered the following relationships: being male was associated with high Junk Food intake; sleeping for fewer hours than average, achieving low school attendance, and having poor general health were associated with high intake of Caffeinated Soft Drinks/Gum; lower school year, more sleep, more frequent exercise, and good general health were associated with high intake of Healthy Foods; and being male, having a special educational needs status, reporting fewer hours of sleep, and being in an older school year were associated with a high intake of Hot Caffeinated Beverages. Whilst controlling for demographic and lifestyle variables, logistic regression analyses determined that poor general health was predicted by high consumption of Caffeinated Soft Drinks/Gum and low consumption of Healthy Foods. Though additional studies are required to further test the questionnaire and its associated factor structure, the DABS is considered to be a useful self-report measure of certain aspects of dietary intake, and is proposed as a useful tool for future research investigating dietary influences on psychological variables such as mental wellbeing.

Keywords: caffeine, diet, diet and behaviour scale, energy drinks, nutrition, wellbeing

1. Introduction

Though it is widely understood that poor quality nutrition is associated with physical health complications such as obesity, diabetes, and the metabolic syndrome (Bonow & Eckel, 2003), it is a lesser-known fact that diet also exerts both short-term and long-term effects on cognition, mood, and behaviour. For instance, carbohydrate-rich afternoon snacks can provide acute benefits in cognitive performance (Kanarek, 1997; Kanarek & Swinney, 1990), and high vegetable consumption has been shown to protect against age related cognitive decline and Alzheimer's disease (Loef & Walach, 2012). It is likely that many such diet-induced improvements in cognitive functioning may simply reflect the reversal of a poor nutritional status (Bellisle, 2004). However, we also consume things that have little nutritional effect but also influence behaviour (e.g. caffeine). The initial aim of the present research was to develop a questionnaire that could be used to assess consumption of types of food and drink that are not always represented in food frequency questionnaires (FFQs). Other research often uses single measures of the food/drink under consideration and there is frequently little attempt to co-vary additional aspects of diet. The need for such a measure can be shown by considering some of the recent research in this area. Consumption of certain foods may have positive effects (e.g. consumption of breakfast, fruit and vegetables) whereas other eating and drinking

patterns (e.g. consumption of junk food and energy drinks) are thought to lead to more negative outcomes. Both types of effect are described here. The review is then followed by research on the initial development of the questionnaire, which can then be use in analyses examining the association between diet, academic attainment, attendance and behaviour at school.

A well-documented example of how diet can affect behaviour and cognition is the intake or omission of breakfast. Eating breakfast has been associated with acute benefits such as promoting positive mood and calmness, improving short-term recognition and spatial memory, free recall and auditory attention (Mahoney, Taylor, Kanarek, & Samuel, 2005; Smith, Clark, & Gallagher, 1999; Smith, Kendrick, & Maben, 1992; Smith, Kendrick, Maben, & Salmon, 1994). Furthermore, the benefits appear to extend beyond the short-term, with those who consume breakfast on a daily basis being found to be less depressed, less emotionally distressed, and to have lower levels of perceived stress than those who do not eat breakfast each day (Smith, 1998; for a review of the behavioural effects of breakfast, see Smith, 2011). Breakfast consumption is often measured using a single item that asks about the frequency of having breakfast (Smith, 2011). This means that most of the research has failed to remove the influence of other dietary variables. Most of the research has also been cross-sectional, which means that it is often difficult to determine causality (e.g. not eating breakfast could increase depression, or, alternatively, depression could influence consumption of breakfast). Breakfast intervention programmes have been shown to improve school attendance (Powell, Walker, Chang, & Grantham-McGregor, 1998), academic performance (Rampersaud, Pereira, Girard, Adams, & Metzl, 2005) and behaviour (Murphy et al., 1998). In addition, diet has also been found to be a significant predictor of academic performance, even after socioeconomic status and gender differences have been controlled for (Florence, Asbridge, & Veugelers, 2008). Due to such observations, a more thorough understanding of the cognitive and behavioural effects of different dietary profiles in the school environment is desirable.

Another aspect of diet that has gained considerable interest regarding its effects on behaviour is snacking (defined as consuming food or drink between meals; Chaplin & Smith, 2011a). The acute effects of snacking appear to be similar to those observed after meals; for example, cereal bars have been shown to produce similar effects to those of breakfast (Smith & Stamatakis, 2010; Smith & Wilds, 2009). However, it also appears that certain forms of snacking may be associated with negative effects. For example, a study of over 800 nurses, (Chaplin & Smith, 2011b) found snacking on crisps, chocolate and biscuits to be associated with higher stress, more cognitive failures and more injuries outside of work. Furthermore, a recent 10-day intervention study (Smith & Rogers, 2014) demonstrated snacking on chocolate once per day to lead to decreases in self-reported wellbeing. However, this study also found that snacking on fruit led to an increase in wellbeing, therefore suggesting that snacking itself may be of less importance than the foods that are chosen to snack upon, and that supplementing the right food items as snacks may be an effective way to increase subjective wellbeing.

One aspect of diet that is generally considered to be beneficial is the high intake of fruit and vegetables. Though campaigns such as 'five-a-day' are likely to have been motivated by research showing fruit and vegetable intake to have protective effects against stroke and coronary heart disease (Ness & Powles, 1997) as well as a number of cancers (Riboli & Norat, 2003), their consumption is also known to exert effects on mood and cognitive functioning. For instance, high cruciferous and green leafy vegetable intake has been associated with slower age-related cognitive decline (Kang, Ascherio, & Grodstein, 2005; Morris, Evans, Tangney, Bienias, & Wilson, 2006). Furthermore, a recent longitudinal study of elderly Taiwanese adults demonstrated high vegetable intake to be associated with significantly fewer depressive symptoms (Tsai, Chang, & Chi, 2012).

A number of dietary products of current concern do not provide significant nutritional contributions. As FFQs often focus on macronutrient composition (Rockett et al., 1997), micronutrient profiles (Watson, Collins, Sibbritt, Dibley, & Garg, 2009), or food categories (Hu et al., 1999), rather than specifically identifying factors known to influence behaviour, the effects of certain 'functional foods' may be wrongly ascribed or missed altogether. Chewing gum, for example, has been associated with positive mood, faster reaction times, and increased alertness (Allen & Smith, 2011; Smith, 2009, 2010). Another important example is caffeine. Though caffeine contributes no nutritional value in itself, it has become one of the most commonly consumed dietary ingredients (Heckman, Weil, & Gonzalez de Mejia, 2010) with around 80% of the world's population consuming it on a daily basis (Ogawa & Ueki, 2007). Due to the far-reaching effects of caffeine on mood, behaviour and cognitive function (Smith, 2002) and considering that roasted coffee beans (*Coffea Arabica* and *Coffea robusta*) and tea leaves (*Camelia siniensis*) are the world's primary sources of the substance (Barone & Roberts, 1996), it may be important to record tea and coffee consumption when assessing diet. In addition to tea and coffee, 'energy drinks' are known to provide little of nutritional value, yet deliver high levels of caffeine. These products are associated with short-term improvements in aerobic endurance, anaerobic performance, reaction time, concentration and memory (Alford,

Cox, & Westcott, 2001; Scholey & Kennedy, 2004). Though others (e.g. McLellan & Lieberman, 2012) consider there to be little evidence to ascribe these effects to ingredients other than caffeine, the fact that such products have also been associated with serious health complaints, such as arrhythmias, tachycardia, stroke, psychotic symptoms/mania, seizures, and even death (Seifert, Schaechter, Hershorin, & Lipschultz, 2011) suggests that their inclusion in dietary questionnaires is both relevant and necessary.

The above section shows that it is desirable to have a measure of consumption of food and drink that may lead to changes in cognition and behaviour. This topic has often been studied using single frequency or quantity questions and such an approach does not allow one to control for other aspects of diet. There have been comprehensive reviews that have examined the dietary assessment methods in school age children. One review (McPherson, Hoelscher, Alexander, Scanlon, & Serdula, 2002) concluded that the heterogeneity of the designs of the studies, study populations, and instruments makes comparisons between methods, and often within methods, difficult. Another review (Livingston & Robson, 2000) examined the issue of misreporting and the identification of misreporters. Correlations between reference methods and dietary assessment tools were almost always higher for food records and recall than for FFQs. Despite the superiority of techniques based on food records or recall these methods of measuring dietary intake can be problematic for several reasons. If, for example, one is using weighed food records, data collection and analysis are often extremely time consuming, expensive, and dropout rates for studies could be relatively high. Some of these problems can be removed by using estimated food records but, again, this is not an ideal method for large sample sizes. Food recall also has problems in that the observations may be a poor measure of general intake and may show biases towards recall of certain types of dietary product. Multi-pass recall removes some of these problems but, again, is memory dependent and data entry can be labour intensive. Due to these reasons, FFQs are often used as a more economical alternative.

There are studies that have shown self-administered FFQs to be able to produce similar results as food diaries (Rimm et al., 1992). However, these correlations are often present for the group as a whole but not for individuals (Rockett et al., 1997). Other studies (e.g. Willett et al., 1985) have shown poor agreement between the FFQ and recall, although the FFQ could correctly classify low, medium and high intake consumers. This suggests that studies using FFQs with children should compare these categories rather than analyzing the scores as continuous variables. Many FFQs are still relatively long and time consuming to implement. Even scales such as The Youth/Adolescent Food Frequency Questionnaire (Rockett et al., 1997), which contains 131 items, could be problematic when administered to participants who struggle to sustain concentration for long periods of time (e.g. schoolchildren). The main focus of most FFQs is the estimation of nutrient values (Willett et al., 1985; Willett, Reynolds, Cottrell-Hoehner, Sampson, & Brown, 1987), caloric consumption, and macronutrient composition (Martin-Moreno et al., 1993). However, people do not eat isolated nutrients, but meals consisting of a variety of foods with complex combinations of nutrients (Hu et al., 1999). In addition to this, certain foods and drinks (e.g. chewing gum and energy drinks) contain very little of nutritional value, yet are known to have far reaching effects on behaviour, cognition and mood.

Factor analysis is a common method used to reduce a large number of foods and drinks to take into account the fact that consumption of different items is often highly correlated. Not all studies use factor analysis; some classify the items on the basis of nutritional properties (Bertoli et al., 2005; Brunner, Stallone, Juneja, Bingham, & Marmot, 2001; Emmett, 2009; Rockett et al., 1997; Watson et al., 2009). The results of factor analyses have also been very variable. For example, some studies report a two-factor solution (Ambrosini et al., 2011; Hu et al., 1999). However, this often leads to inclusion of items with a low weighting on the factor and/or exclusion of certain factors. These methods of factor analysis also often explain very little of the variance (e.g. 20% - Hu et al., 1999). Other studies (Speck, Bradley, Harrell, & Belyea, 2001) have identified 10 factors with several only containing a small number of items. There have been a number of studies that use factor analysis to examine the dietary patterns of adolescents (Ambrosini et al., 2011; Bertoli et al., 2005; Malik et al., 2012; McNaughton, Ball, Mishra, & Crawford, 2008; Speck et al., 2001). These studies also show variable results but often identify a "Western" pattern (e.g. high intake of take-away foods, soft drinks, confectionery, French fries, refined grains, full-fat dairy products and processed meats) and a "healthy" or "prudent" pattern (e.g. whole grains, fruit, vegetables, legumes and fish). These dietary patterns are associated with lifestyle, demographic and psychosocial factors. Indeed, it is clear that dietary patterns are present in adolescents and that these may be risk factors for future disease (Malik et al., 2012; McNaughton et al., 2008).

The objective of the current paper is to describe a new, easy to administer questionnaire, which can be used in studies of the psychological effects of diet, in order to provide a solution to some of the problems associated with other commonly used measures. The questionnaire's main function is to record both the frequency and amount of consumption of common foods and drinks, with the further purpose of investigating their effects on behaviour and

cognition. It is not intended as a replacement for FFQs used to study other domains and does not provide information on all important food groups (e.g. dairy products are not covered). The current paper further aims to investigate the structure underpinning the questionnaire by using exploratory factor analysis. The paper will also then discuss relationships observed between the factors extracted and a number of demographic and lifestyle variables. This initial study was conducted with schoolchildren, as it was part of a larger programme examining associations between diet, academic attainment and behaviour. Other parallel research is also using the scale with university students and working adults.

2. Method

The Cornish Academies Project is a large-scale longitudinal programme of research designed to investigate dietary effects on school performance and wellbeing in secondary school children. Two cross-sections of data were collected from three academies in the South West of England. The first cross-section (Time 1; T1) was collected six months prior to the second cross-section (Time 2; T2), in order to allow for longitudinal analyses of dietary change over time (though such analyses will be presented in future reports).

2.1 Participants

Three thousand and seventy one secondary school pupils from three academies in the South West of England (Academy 1 $N = 954$, Academy 2 $N = 1363$, Academy 3 $N = 754$) were asked to take part in the current study. Two thousand six hundred and ten (85%) agreed to participate. Approximately 20% of the sample came from each of the five year-groups present in UK secondary education, giving an age range of 11-16 years ($M = 13.83$, $SD = 1.46$) and a relatively balanced sex ratio (51.1% males, 48.9% females). Almost all participants were White (97.3%), the majority of which spoke English as their first language (98.3%). Thirteen per cent of pupils met the eligibility requirements to receive free school meals (a proxy indication of socioeconomic status; Shuttleworth, 1995), and the prevalence of special educational needs was relatively high (21.8%).

2.2 Materials & Apparatus

The Diet and Behaviour Scale (DABS) is a 29-item questionnaire developed for the purpose of assessing intake of common dietary variables with an onus on functional foods, and foods and drinks of current concern (for individual questions included, see Tables 1 and 2). The questions were selected to cover areas of eating and drinking where there has been interest in possible effects on behaviour. Many of the questions had been used individually by the researchers or other research teams to assess the behavioural effects of coffee, tea, caffeinated soft drinks, breakfast, chewing gum, fruit and vegetables, and junk food. Individual items were also present in other FFQs and have been compared with food recall or records. The advantage of the present approach over the use of single items was that consumption of other foods and drinks could be statistically controlled for. The advantage over other FFQs was the length, and, as described in the literature, the relevance to food and drink with little nutritional value.

The first section of the DABS focuses on how frequently the respondent typically consumes common foods and drinks. Frequency of consumption of 18 dietary variables is measured on a five-point scale (1 = never, 2 = once a month, 3 = once or twice a week, 4 = most days [3-6], 5 = every day). The second section investigates the typical amounts consumed for 11 common foods and drinks. Eight of these items (energy drinks, cola, coffee, tea, crisps, chocolate, burgers/hot dogs, and chewing gum) require participants to state how much they typically consume per week, whereas three items (pieces of fruit, portions of vegetables, and water) require participants to state how much they typically consume per day.

Alongside the DABS, five questions were administered in order to assess additional aspects of lifestyle. It is considered important to address such variables as it has been suggested by some (e.g. Akbaraly, 2009) that diet simply reflects general lifestyle. Three items were used to gauge the frequency by which subjects participated in mildly energetic, moderately energetic, and vigorous physical exercise, with answers being given on a four-point scale (1 = never/hardly ever, 2 = about once to three times a month, 3 = once or twice a week, 4 = 3 times a week or more). Finally, participants were asked to state how many hours per night they typically spent sleeping, and to give an indication of their general health (1 = very good, 2 = good, 3 = fair, 4 = bad, 5 = very bad).

2.3 Design & Procedure

Schoolteachers administered the DABS, along with the aforementioned additional lifestyle questions, in the classroom to pupils from their respective academies. Demographic information relating to the participants was later acquired through the School Information Management System (SIMS) and stored within a confidential database in Cardiff. This information included age, sex, academy attended, school year, ethnicity, special

educational needs status, eligibility to received free school meals, whether or not English was spoken as an additional language, and whether the child was looked after by a non-parental guardian.

All questionnaire and demographic data were fully anonymised before being merged into a single dataset. Cardiff University's School of Psychology Ethics Committee granted ethical clearance for the study, and informed consent was acquired from all participants (as well as from their parents) prior to data collection.

2.4 Statistical Analysis

Data analysis was conducted using IBM SPSS Statistics Version 20. Initial cross-tabulations were examined to determine how representative the sample was. This was followed by factor analysis using varimax rotation. Based on the items that loaded strongly onto each factor extracted, subscales were then created, and internal consistency was tested using Cronbach's alpha. Finally, relationships between dietary factors and lifestyle and demographic variables were examined using cross-tabulations and logistic regression.

3. Results

3.1 Representativeness of the Sample at T1

A relatively high response rate of 77.8% was observed for completion of the DABS at T1. In order to investigate whether this sample was representative of the academies from which it came, Chi-square tests were used to determine if SIMS data for those who completed the DABS differed from SIMS data of those who did not. Though it was noted that there were trends for females, $\chi 2$ (1, N = 3040) = 2.935, p = .087, and those not entitled to free school meals, $\chi 2$ (1, N = 3040) = 3.218, p = .073, to be more likely to answer the questionnaire, neither achieved statistical significance. However, the school year that a participant came from was significantly related to their likelihood to complete the DABS, $\chi 2$ (4, N = 3040) = 13.076, p = .011, with fewer respondents than expected coming from Year 7, and more respondents than expected coming from Year 9. It was also found that children with a special educational needs status were less likely to answer the questionnaire, $\chi 2$ (1, N = 3068) = 21.056, $p < .001$. In addition to this, more respondents than expected came from Academy 1 and Academy 2, and fewer than expected came from Academy 3, $\chi 2$ (2, N = 3071) = 164.003, $p < .001$. Though such findings may cast doubts on the sample's representativeness, it must be noted that the variables in question were statistically controlled for in subsequent analyses.

Of those who completed the DABS at T1, 683 (33.6%) came from Academy 1, 993 (48.9%) came from Academy 2, and 354 (17.4%) came from Academy 3. Exactly 50% were male and 50% were female, and similar numbers came from each of the school years present in secondary education: Year 7 = 356 (17.8%), Year 8 = 393 (19.6%), Year 9 = 438 (21.9%), Year 10 = 398 (19.9%), Year 11 = 417 (20.8%). Two hundred and forty-five (12.2%) pupils were eligible for free school meals and 393 (19.4%) had a special educational needs status. Almost all pupils were White (1937; 97.5%), spoke English as their first language (1994; 98.2%), and were not looked after by a non-parental guardian (2018; 99.4%).

3.2 Dietary Questionnaire Data and Factor Analysis

Considerable variance in responding to the DABS was observed (for frequency of consumption data, see Table 1; for amount of consumption data, see Table 2). Table 2 shows a number of outliers that probably reflect confusion over the time period assessed. Such outliers need to be removed if the scores are treated as continuous variables. The amount of missing data was generally low (the greatest amount for frequency items being 1.2% at T1 and 1.8% at T2; the highest for amount items being 2.4% at T1 and 2.8% at T2) and probably reflects slight difficulties in understanding the questions (e.g. some children may not know what processed meat refers to, or may use metric units rather than pints).

In order to reduce data, and because the frequency and amount of consumption of many foods and drinks are known to be heavily inter-correlated (Wiles, Northstone, Emmett, & Lewis, 2009), food frequency data are often entered into a factor analysis. All 29 items of the DABS were entered into an exploratory factor analysis with the number of factors extracted being determined by examining the scree plot. The factor analysis used varimax rotation and a four-factor solution with eigenvalues greater than 1.5 was extracted. This solution accounted for 38.02% of variance within the dataset at T1 and 37.74% at T2. Due to high loadings from crisps, chocolate, chips, and sweets, factor 1 was labelled 'Junk Food'. This factor explained 11.87% of variance at T1 and 12.07% at T2 (initial eigenvalues: T1 = 4.584, T2 = 4.479). Due to high loadings from energy drinks, chewing gum, and cola, factor 2 was labelled 'Caffeinated Soft Drinks/Gum'. This factor explained 10.44% of variance at T1 and 10.26% at T2 (initial eigenvalues: T1 = 2.539, T2 = 2.547). Factor 3 explained 8.52% of variance at T1 and 8.34% at T2 (initial eigenvalues: T1 = 2.21, T2 = 2.204), and was labelled 'Healthy Foods' due to high loadings from variables measuring fruit and vegetable consumption. Factor 4 was labelled 'Hot Caffeinated Beverages' due to high

loadings from tea and coffee. This last factor explained 7.19% of variance within the dataset at T1 and 7.07% at T2 (initial eigenvalues: T1 = 1.694, T2 = 1.715). For factor loading scores at T1 and T2, see Table 3.

To verify the factor structure described in the above paragraph, separate exploratory factor analyses were conducted for each of the three academies at both T1 and T2. Very similar four-factor structures emerged in each of these analyses (for the percentage of variance explained by each factor and the initial eigenvalues, see Table 4; for all factor loading scores at T1 and T2, see Tables 5 and 6, respectively). In order to assess whether the factors discussed above measure the same underlying variables, reliability analyses were conducted for the items that loaded strongly onto each factor to test for internal consistency. It was found that the internal consistency for each of these dietary subscales was acceptable. Standardised Cronbach's α values were as follows: Junk Food (items 2, 3, 10, 17, 23, and 24) T1, 0.735, T2, 0.74; Caffeinated Soft Drinks/Gum (items 7, 8, 9, 19, and 26) T1, 0.741, T2, 0.724; Healthy Foods (items 4, 27, and 28) T1, 0.691, T2, 0.693; Hot Caffeinated Beverages (items 5, 6, 21, and 22) T1, 0.675, T2, 0.661.

Table 1. Frequency of consumption of common dietary variables as assessed by the DABS at T1 and T2

	N		Never		Once a month		Once/twice a week		Most days (3-6)		Every day	
Frequency	**T1**	**T2**	**T1**	**T2**	**T1**	**T2**	**T1**	**T2**	**T1**	**T2**	**T1**	**T2**
Q1. How often did you eat breakfast?	2022	2306	8.60%	8.20%	4.70%	5.10%	15.70%	15.60%	20.60%	23.10%	**50.40%**	**48%**
Q2. How often did you eat chocolate?	2019	2294	1.70%	1.70%	11.40%	12%	**43.50%**	**45.40%**	29.80%	30%	13.50%	10.90%
Q3. How often did you eat crisps?	2019	2298	4.30%	5.60%	10%	11.10%	30%	30.70%	**36.50%**	**36.60%**	18.60%	15.90%
Q4. How often did you eat 5 fruit or vegetables?	2011	2295	6.20%	6.40%	9.30%	7.90%	27.50%	29.60%	**42.70%**	**42.70%**	14.30%	13.30%
Q5. How often did you drink coffee?	2025	2301	**63.80%**	**65.30%**	10.30%	9.70%	10.70%	11.40%	7.80%	6.70%	7.50%	6.90%
Q6. How often did you drink tea?	2024	2303	**35.60%**	**35.80%**	11.80%	11%	17.20%	18.50%	16.40%	14.80%	19.10%	20%
Q7. How often did you drink cola?	2025	2298	11.40%	10.40%	25.90%	26.60%	**37.80%**	**41.40%**	18.30%	16.80%	6.70%	4.80%
Q8. How often did you drink energy drinks?	2004	2291	**44.10%**	**44.90%**	28.90%	30.60%	16.30%	16%	7.80%	6.10%	2.80%	2.50%
Q9. How often did you chew gum?	2006	2291	15.80%	16.10%	25.90%	25.30%	**29.30%**	**30.60%**	19.90%	20.40%	9.10%	7.60%
Q10. How often did you eat sweets?	2003	2283	3.70%	4.20%	19.90%	23.30%	**50%**	**53.10%**	21.80%	16.50%	4.60%	2.80%
Q11. How often did you eat fast-food?	2001	2285	8.30%	8.30%	**61.60%**	**61.80%**	24.80%	26.70%	4.50%	2.60%	0.80%	0.60%
Q12. How often did you eat an Indian or Chinese take-away?	2007	2293	23.40%	25.10%	**62.90%**	**64.20%**	11.90%	10%	1.30%	0.30%	0.50%	0.30%
Q13. How often did you eat pies or pasties?	2005	2292	13.90%	14.40%	**50.60%**	**53.20%**	28.80%	27.70%	5.90%	3.80%	0.70%	1%
Q14. How often did you eat processed meat?	1999	2281	**44.90%**	**46.60%**	22.50%	25.70%	20.10%	17.80%	10%	7.90%	0.60%	2%
Q15. How often did you eat fried fish?	2012	2289	29.50%	29.40%	**41.50%**	**43.30%**	24.50%	23.40%	4.20%	3.50%	0.30%	0.30%
Q16. How often did you eat oily fish?	2012	2286	**46.60%**	**47%**	33.80%	32.10%	15.90%	17.40%	3.30%	3.10%	0.40%	0.40%
Q17. How often did you eat chips?	2007	2283	3.40%	3.40%	24.70%	25.10%	**53.30%**	**56.20%**	16.10%	13.90%	0.40%	1.40%
Q18. How often did you eat beans of peas?	2006	2277	10.30%	9.90%	10.90%	12.20%	**46.90%**	**48.40%**	28.50%	27.10%	3.40%	2.50%

Note. Modal values are displayed in bold.

Table 2. Amount of consumption of common dietary variables as assessed by the DABS at T1 and T2

	N		Min		Max		Mean		SD	
	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
Q19. Cans of energy drinks per week	2008	2254	0	0	25	20	0.99	0.93	1.96	1.86
Q20. Cans of cola per week	1996	2253	0	0	36	32	1.49	1.47	2.14	2.22
Q21. Cups of coffee per week	2014	2265	0	0	40	50	1.41	1.42	3.66	4.03
Q22. Cups of tea per week	2010	2267	0	0	50	50	3.48	3.81	5.88	6.54
Q23. Packets of crisps per week	2006	2262	0	0	30	30	3.62	3.55	2.88	2.75
Q24. Bars of chocolate per week	2009	2269	0	0	70	50	3.15	3.12	3.56	3.39
Q25. Burgers/hot dogs per week	1995	2245	0	0	10	11	0.73	0.69	1.09	1.02
Q26. Packs of chewing gum per week	2005	2263	0	0	15	16	1.33	1.29	1.9	1.78
Q27. Pieces of fruit per day	2008	2263	0	0	17	18	2.82	2.74	1.91	1.82
Q28. Portions of vegetables per day	1981	2250	0	0	15	16	2.77	2.57	1.91	1.68
Q29. Pints of water per day	1964	2203	0	0	17	18	2.43	2.47	2.01	1.97

Table 3. Exploratory factor analysis of DABS items at T1 and T2

	Junk Food		Caffeinated Soft Drinks/Gum		Healthy Foods		Hot Caffeinated Beverages	
	T1	T2	T1	T2	T1	T2	T1	T2
Q1. Breakfast (F)	.124	.146	-.456	-.409	.321	.32	.031	-.016
Q2. Chocolate (F)	**.66**	**.611**	.016	-.032	-.065	-.084	.032	-.062
Q3. Crisps (F)	**.669**	**.682**	-.046	-.093	-.057	-.074	-.007	-.014
Q4. Five pieces of fruit or veg (F)	-.262	-.250	-.137	-.084	**.622**	**.623**	-.032	-.076
Q5. Coffee (F)	.013	-.052	.144	.187	.02	.019	**.734**	**.72**
Q6. Tea (F)	.001	.061	.091	.054	.103	.129	**.676**	**.656**
Q7. Cola (F)	.377	.366	**.544**	**.538**	-.039	-.123	.061	.033
Q8. Energy drinks (F)	.178	.171	**.742**	**.693**	-.02	-.077	.115	.196
Q9. Chewing gum (F)	.068	.036	**.61**	**.634**	.021	.079	.175	.044
Q10. Sweets (F)	**.525**	**.512**	.264	.305	.031	.072	-.011	-.053
Q11. Fast-food (F)	.452	.453	.342	.377	-.007	-.06	-.057	-.034
Q12. Takeaway (F)	.375	.356	.259	.214	.185	.129	.069	.062
Q13. Pies or pasties (F)	.312	.350	.229	.198	.395	.318	.048	.108
Q14. Processed meat (F)	.266	.265	.091	.118	.206	.177	-.051	.082
Q15. Fried fish (F)	.227	.239	.038	.029	.485	.457	.082	.073
Q16. Oily fish (F)	.091	.063	-.107	-.062	.497	.454	.081	.188
Q17. Chips (F)	**.531**	**.541**	.196	.138	.021	-.01	-.005	-.016
Q18. Beans or peas (F)	.09	.103	-.069	-.146	.483	.452	.064	.071
Q19. Energy drinks per week	.093	.121	**.699**	**.644**	-.011	-.084	.048	.197
Q20. Cola per week	.250	.276	.456	.472	-.087	-.097	-.034	-.003
Q21. Coffee per week	.029	-.055	.081	.139	-.037	-.052	**.714**	**.684**
Q22. Tea per week	-.005	.065	.034	-.052	.024	.068	**.683**	**.671**
Q23. Crisps per week	**.67**	**.697**	-.019	-.037	-.103	-.104	.066	.105
Q24. Chocolate per week	**.62**	**.626**	.02	.018	-.109	-.098	.03	.009
Q25. Burgers/hot dogs per week	.397	.447	.314	.323	.166	.042	-.023	.012
Q26. Chewing gum per week	-.001	-.046	**.61**	**.658**	.04	.158	.138	-.005
Q27. Fruit per day	-.237	-.231	.054	.044	**.639**	**.66**	-.045	-.1
Q28. Vegetables per day	-.195	-.151	-.02	-.006	**.616**	**.652**	-.026	-.021
Q29. Water per day	-.034	-.036	.02	.044	.401	.405	-.02	.012

Note. Factor scores are the product of varimax (orthogonal) rotation. Factor scores > .5 are displayed in bold. 'F' refers to 'frequency'.

Table 4. Initial eigenvalues and variance explained by each factor across academies at T1 and T2

		Total	Junk Food		Caffeinated Soft Drinks/Gum		Healthy Foods		Hot Caffeinated Beverages	
		Total variance explained	Initial eigenvalue	% variance explained	Initial eigenvalue	% variance explained	Initial eigenvalue	% variance explained	Initial eigenvalue	% variance explained
Academy 1	T1	39.45%	5.05	13.55%	2.62	9.32%	2.17	8.85%	1.61	7.72%
	T2	40.37%	5.11	13.12%	2.63	10.36%	2.11	8.89%	1.86	7.99%
Academy 2	T1	38.02%	2.72	10.69%	4.36	11.38%	2.29	8.7%	1.66	7.25%
	T2	36.08%	4.01	11.91%	2.64	9.43%	2.16	7.89%	1.66	6.85%
Academy 3	T1	38.9%	4.69	12.4%	2.44	10.57%	2.09	8.03%	2.06	7.9%
	T2	40.56%	2.79	11.18%	4.87	12.59%	2.29	9.02%	1.81	7.77%

Table 5. Exploratory factor analysis of DABS items at T1 for individual academies

	Junk Food			Caffeinated Soft Drinks/Gum			Healthy Foods			Hot Caffeinated Beverages		
	School 1	School 2	School 3	School 1	School 2	School 3	School 1	School 2	School 3	School 1	School 2	School 3
Q1. Breakfast (F)	.116	.117	.2	-.462	-.488	-.353	.349	.261	.417	-.029	.103	-.068
Q2. Chocolate (F)	**.688**	**.683**	**.636**	.089	-.041	.01	-.092	-.069	.063	.033	.05	.053
Q3. Crisps (F)	**.703**	**.639**	**.676**	-.156	.014	-.012	-.064	-.021	-.058	.149	-.108	-.117
Q4. Five pieces of fruit or veg (F)	-.251	-.248	-.28	-.165	-.137	-.163	**.64**	**.605**	**.633**	-.028	.034	-.092
Q5. Coffee (F)	.005	-.02	.051	.246	.14	.104	.051	.027	-.014	**.607**	**.72**	**.722**
Q6. Tea (F)	.024	.021	-.037	.029	.079	.053	.079	.09	.145	**.763**	**.66**	**.684**
Q7. Cola (F)	.435	.307	.388	.47	**.61**	**.563**	.001	-.04	-.11	.062	.047	.061
Q8. Energy drinks (F)	.219	.103	.245	**.748**	**.764**	**.689**	-.109	.023	.019	.122	.113	.158
Q9. Chewing gum (F)	.168	.075	-.031	**.469**	**.588**	**.638**	-.021	.022	.091	.323	.254	.024
Q10. Sweets (F)	**.561**	**.545**	.452	.233	.216	.349	.043	-.014	.17	.118	.015	-.077
Q11. Fast-food (F)	**.552**	.401	.398	.333	.349	.34	-.007	-.028	.067	-.127	.018	-.089
Q12. Takeaway (F)	.394	.312	.411	.257	.308	.296	.188	.173	.273	-.046	.052	.184
Q13. Pies or pasties (F)	.383	.302	.155	.208	.217	.318	.353	.44	.356	.019	.062	.141
Q14. Processed meat (F)	.232	.212	.357	.065	.11	.117	.295	.189	.103	.16	-.201	.021
Q15. Fried fish (F)	.209	.161	.285	.096	.041	.086	.45	.499	**.517**	.124	-.037	.18
Q16. Oily fish (F)	.112	.065	.046	-.013	-.132	-.06	.444	**.538**	.481	.029	.046	.131
Q17. Chips (F)	.479	**.581**	.489	.205	.213	.178	.05	.034	-.002	.035	-.023	.017
Q18. Beans or peas (F)	.161	.015	.126	-.086	-.054	-.089	.459	**.527**	.367	.087	-.025	.211
Q19. Energy drinks per week	.153	.016	.151	**.709**	**.724**	**.659**	-.051	.002	-.016	.075	.032	.071
Q20. Cola per week	.316	.168	.276	.329	**.552**	**.534**	-.066	-.098	-.131	-.018	-.058	-.095
Q21. Coffee per week	.009	-.028	.127	.2	.078	.009	.018	-.017	-.136	**.572**	**.686**	**.726**
Q22. Tea per week	.089	-.01	-.074	-.087	.06	-.025	.03	-.026	.073	**.739**	**.685**	**.688**
Q23. Crisps per week	**.688**	**.614**	**.734**	-.097	.04	-.01	-.061	-.094	-.168	.197	-.059	-.02
Q24. Chocolate per week	**.666**	**.612**	**.627**	.104	-.006	.0	-.05	-.135	-.104	-.029	.034	.08
Q25. Burgers/hot dogs per week	.442	.316	.462	.323	.371	.218	.233	.185	-.006	-.163	.016	.154
Q26. Chewing gum per week	.068	.005	-.065	.48	**.582**	**.66**	.041	.059	-.053	.33	.205	-.043
Q27. Fruit per day	-.213	-.24	-.251	.061	.034	.039	**.686**	**.658**	.468	-.068	.025	-.145
Q28. Vegetables per day	-.185	-.198	-.214	-.058	-.04	.052	**.673**	**.578**	**.571**	-.008	.024	-.11
Q29. Water per day	-.07	-.043	.04	-.093	.063	.056	.382	.392	.443	-.002	-.01	-.021

Note. Factor scores are the product of varimax (orthogonal) rotation. Factor scores > .5 are displayed in bold. 'F' refers to 'frequency'.

Table 6. Exploratory factor analysis of DABS items at T2 for individual academies

	Junk Food			Caffeinated Soft Drinks/Gum			Healthy Foods			Hot Caffeinated Beverages		
	School 1	School 2	School 3	School 1	School 2	School 3	School 1	School 2	School 3	School 1	School 2	School 3
Q1. Breakfast (F)	.097	.113	.182	-.497	-.371	-.262	.298	.346	.342	.014	-.007	-.173
Q2. Chocolate (F)	**.602**	**.587**	**.629**	.053	-.154	.104	-.151	-.024	-.025	.016	-.102	-.044
Q3. Crisps (F)	**.702**	**.642**	**.73**	-.133	-.054	-.033	-.097	-.058	.04	.067	-.061	-.153
Q4. Five pieces of fruit or veg (F)	-.234	-.271	-.261	-.104	-.017	-.112	**.612**	**.632**	**.654**	-.132	-.059	-.12
Q5. Coffee (F)	.02	-.094	-.019	.101	.3	.109	.043	.0	.002	**.655**	**.627**	**.795**
Q6. Tea (F)	.058	.083	-.059	.096	.003	.078	.107	.083	.268	**.714**	**.723**	**.523**
Q7. Cola (F)	.4	.442	.237	**.539**	.469	**.597**	-.091	-.164	-.079	-.05	.099	.04
Q8. Energy drinks (F)	.195	.227	.094	**.639**	**.721**	**.687**	-.096	-.049	-.113	.254	.142	.212
Q9. Chewing gum (F)	.079	.042	-.031	**.672**	**.583**	**.673**	.023	.04	.168	.147	.084	-.134
Q10. Sweets (F)	**.55**	**.54**	.324	.346	.141	**.524**	.139	.067	.017	.048	-.051	-.088
Q11. Fast-food (F)	**.508**	.43	.439	.414	.217	.497	-.03	-.094	-.041	-.098	.065	-.021
Q12. Takeaway (F)	.427	.319	.374	.185	.123	.295	.142	.144	.058	-.093	.221	.093
Q13. Pies or pasties (F)	.383	.373	.241	.209	.131	.256	.419	.286	.231	.058	.134	.217
Q14. Processed meat (F)	.261	.211	.272	.098	.086	.251	.281	-.008	.35	.086	.086	.037
Q15. Fried fish (F)	.233	.211	.184	.073	-.092	.132	**.509**	.389	.49	.196	.024	.149
Q16. Oily fish (F)	.06	.042	.011	-.117	-.15	.108	.42	.422	**.531**	.195	.18	.23
Q17. Chips (F)	**.528**	**.558**	.492	.163	.048	.268	.014	-.037	.03	.062	-.016	-.081
Q18. Beans or peas (F)	.123	.051	.128	-.099	-.184	-.098	.439	.444	.485	.032	.086	.096
Q19. Energy drinks per week	.171	.145	.069	**.601**	**.682**	**.633**	-.137	-.032	-.115	.234	.123	.275
Q20. Cola per week	.216	.362	.262	.433	.413	**.583**	-.069	-.11	-.101	-.157	.003	.21
Q21. Coffee per week	.052	-.138	-.02	.122	.228	.069	-.026	-.023	-.148	**.652**	**.539**	**.803**
Q22. Tea per week	.064	.065	-.015	.029	-.116	-.038	.066	.024	.202	**.718**	**.758**	.492
Q23. Crisps per week	**.722**	**.652**	**.737**	-.071	.008	-.004	-.089	-.077	-.049	.239	-.004	.0
Q24. Chocolate per week	**.667**	**.581**	**.623**	.085	-.103	.095	-.083	-.06	-.103	.109	-.065	.07
Q25. Burgers/hot dogs per week	.467	.471	.39	.273	.257	.417	.106	-.009	.042	-.025	.017	.149
Q26. Chewing gum per week	.019	-.037	-.186	**.682**	**.618**	**.687**	.107	.138	.18	.157	-.01	-.117
Q27. Fruit per day	-.228	-.219	-.241	-.031	.193	-.05	**.664**	**.657**	**.645**	-.153	-.093	-.079
Q28. Vegetables per day	-.213	-.103	-.174	-.003	.048	-.063	**.644**	**.685**	**.571**	-.09	.019	.043
Q29. Water per day	-.051	-.065	.018	-.072	.16	.045	.455	.373	.368	.095	-.04	.026

Note. Factor scores are the product of varimax (orthogonal) rotation. Factor scores > .5 are displayed in bold. 'F' refers to 'frequency'.

3.3 Lifestyle Variables

Mildly energetic exercise was common, with the majority of pupils (73% at T1, 76.7% at T2) reporting to take part three times a week or more. Likewise, 66.8% at T1 and 65.8% at T2 took part in moderately energetic exercise at least once per week. Vigorous exercise was also relative common, with 56.5% at T1 and 57.1% at T2 taking part at least once per week. The majority of pupils reportedly slept between seven and 10 hours per night, with mean scores of 8.64 (SD = 1.55) at T1 and 8.41 (SD = 1.54) at T2 being observed. General health was also deemed to be relatively high, with 95.5% at T1, and 94.9% at T2, claiming their health to have been ‘fair’ or better (72.3% at T1 and 70.6% at T2 responding with either ‘good’ or ‘very good’).

The three items relating to exercise frequency (mildly energetic, moderately energetic, and vigorous exercise) were factor analysed to provide a single factor solution. At T1 the (un-rotated) factor loadings were as follows: moderate exercise, .796, vigorous exercise, .765, mild exercise, .534. The initial eigenvalue was 1.503, and the factor extracted explained 50.12% of variance. At T2, the following (un-rotated) factor loadings were observed: vigorous exercise, .778, moderate exercise, .765, mild exercise, .56. The initial eigenvalue was 1.504, and the factor was found to explain 50.13% of the variance.

3.4 Relationships Between Dietary Factors and Lifestyle and Demographic Variables at T1

Factor scores were recoded into new dependent variables based on median splits. This provided a high consumption group and a low consumption group for each factor extracted. Relationships between these groups and demographic and lifestyle variables were subsequently investigated at T1 using Chi-square analyses. To partial out variance from confounders (e.g. socioeconomic status), any observed associations were then further investigated using forwards logistic regression. The covariates entered into the regression models were academy attended, school year, sex, eligibility to receive free school meals, special educational needs status, exercise frequency (median split of the previously discussed exercise frequency factor score), school attendance, and sleep. Ethnicity, speaking English as an additional language, and being looked after by a non-parental guardian were not controlled for in these analyses due to the numbers present in the relevant minority groups being particularly small. General health was also dichotomised, with those claiming their health to have been 'good' or 'very good' making up the good health group, and those claiming their health to have been 'fair', 'bad', or 'very bad' comprising the poor health group. It was found that poor health was associated with being in the high consumption group for Caffeinated Soft Drinks/Gum, OR = 1.388, 95% CI [1.11, 1.735], $p = .004$, and being in the low consumption group for Healthy Foods, OR = .477, 95% CI [.38, .598], $p < .001$. Once the demographic and lifestyle covariates described earlier in this paragraph were controlled for, both of these effects remained significant: Caffeinated Soft Drinks/Gum, OR = 1.326, 95% CI [1.034, 1.699], $p = .026$, Healthy Foods, OR = .537, 95% CI [.418, .689], $p < .001$.

3.4.1 Factor 1 (Junk Food)

The only demographic or lifestyle variable that was significantly related to Junk Food consumption was sex. Males were more likely than females to be high consumers, $\chi2$ (1, $N = 1674$) = 10.413, $p = .001$.

3.4.2 Factor 2 (Caffeinated Soft Drinks/Gum)

High consumption of Caffeinated Soft Drinks/Gum was related to poor general health, $\chi2$ (1, $N = 1627$) = 8.736, $p = .003$, fewer hours of sleep per night, $\chi2$ (1, $N = 1643$) = 48.678, $p < .001$, and below average school attendance, $\chi2$ (1, $N = 1674$) = 5.284, $p = .022$.

3.4.3 Factor 3 (Healthy Foods)

Consumption of Healthy Foods was related to school year, $\chi2$ (4, $N = 1674$) = 10.504, $p = .033$. This finding reflected a significant linear-by-linear trend, by which its consumption decreased with age, $\chi2$ (1, $N = 1674$) = 9.083, $p = .003$. High consumers of Healthy Foods were also found to sleep for more hours per night, $\chi2$ (1, $N = 1643$) = 17.885, $p < .001$, to exercise more frequently, $\chi2$ (1, $N = 1585$) = 28.621, $p < .001$, and to report better general health, $\chi2$ (1, $N = 1627$) = 42.252, $p < .001$.

3.4.4 Factor 4 (Hot Caffeinated Beverages)

Those in the high consumption group for Hot Caffeinated Beverages were more likely to be male, $\chi2$ (1, $N = 1674$) = 6.703, $p = .01$, to have a special educational needs status, $\chi2$ (1, $N = 1699$) = 4.282, $p = .039$, and to report fewer hours of sleep per night, $\chi2$ (1, $N = 1643$) = 6.248, $p = .012$. Consumption of Hot Caffeinated Beverages was also related to school year, $\chi2$ (4, $N = 1674$) = 10.522, $p = .033$, with a significant linear-by-linear trend showing that its consumption increased with age, $\chi2$ (1, $N = 1674$) = 9.772, $p = .002$.

3.5 Possible Methods for Scoring the DABS in Future Research

One method of scoring the DABS is to use four subscales based on the previously discussed factors extracted through exploratory factor analysis. For example, the items loading strongly onto the Junk Food factor were Q2, Q3, Q10, Q17, Q23, and Q24. Therefore these items can be used to make up a subscale for Junk Food. In order to test whether these subscales provide similar measures of diet to the factors extracted through factor analysis, relationships between the relevant variables were investigated using Pearson's correlations. Before being able to do this however, the questionnaire data needed to be converted so that the scoring systems were universal for the items that measured frequency of consumption as well as for those that measured amount. As FFQs are able to distinguish between high, medium and low consumers (Willett et al., 1985), scores from all items were recoded into tertiles (except in cases where a bimodal distribution was observed: for these variables, the smaller of the two groups was counted as one tertile, and a median split was performed on the remaining data to create the required three groups). Strong positive correlations were observed between each subscale and its respective factor score at both time-points: Junk Food: T1, $r(1697) = .744$, $p < .001$, T2, $r(1898) = .729$, $p < .001$; Caffeinated Soft Drinks/Gum: T1 $r(1697) = .747$, $p < .001$, T2 $r(1898) = .743$, $p < .001$; Healthy Foods: T1, $r(1697) = .646$, $p < .001$, T2, $r(1898) = .601$, $p < .001$; Hot Caffeinated Beverages: T1, $r(1697) = .816$, $p < .001$, T2 $r(1898) = .8$, $p < .001$.

Though the subscale scores have been shown to be reliable, and to correlate strongly with their respective factor scores, it is suggested that the factor scores should be used wherever possible during analysis as they take into account variance from items that do not load strongly onto any particular factor. However, as the factor scores cannot be considered to be exactly the same across time-points, it is necessary to use the subscale scores when undertaking change score analyses. It was therefore deemed useful to examine whether the subscales can produce consistent responses over time. To do this, Pearson's correlations (two-tailed) were conducted to determine how strongly the subscale scores from T1 correlated with those from T2. All correlations were positive and ranged from weak to moderate: Junk Food, $r(1514) = .413$, $p < .001$, Caffeinated Soft Drinks/Gum, $r(1542) = .398$, $p < .001$, Healthy Foods, $r(1535) = .295$, $p < .001$, Hot Caffeinated Beverages, $r(1594) = .475$, $p < .001$.

4. Discussion

The current study has shown that the DABS can be associated with an underlying four-factor model of diet consisting of Junk Food, Caffeinated Soft Drinks/Gum, Healthy Foods, and Hot Caffeinated Beverages. In addition to this, it was found that all four factors were significantly related to demographic variables and/or certain aspects of lifestyle. The four-factor model produced provides a useful system for exploration of dietary effects upon other areas of life. Though factor analysis of other FFQs has provided two-factor solutions, such as 'prudent dietary pattern' vs. 'Western pattern' (Ambrosini et al., 2011; Hu et al., 1999), and 'wholefoods' vs. 'processed foods' (Akbaraly, 2009), such a models are considered likely to obscure the effects of dietary items that do not contribute much of significant nutritional value. As these very items (i.e. energy drinks, cola, and chewing gum) were found to make up a unique factor in the four-factor model presented here, this model is deemed to be very relevant when regarding potential for subsequent investigation of their effects upon behaviour, cognition and mood.

It must be acknowledge that several limitations are incurred by the current study. Firstly, as the DABS has previously been untested, the results presented are somewhat preliminary, and so, need validation from future research. In addition to this, the study sample used was somewhat homogeneous (being made up almost entirely of White children from a specific age range, as well as including a high proportion of pupils with special educational needs), and came from an area of relatively low socioeconomic status. Generalisability of the results may therefore be limited.

The issue of reverse-causation is another potential limitation of the current findings. It is highly probable that, though diet is likely to affect health, health may also affect choices made regarding diet and lifestyle. For example, eating healthy foods may promote good health, but having good health may also lead towards the selection of healthy foods. It is possible therefore, that certain dietary variables, particularly those associated with the Caffeinated Soft Drinks/Gum factor, may be viewed as outcomes rather than just causes of behaviour. A healthy diet may also simply reflect an overall healthy lifestyle (Akbaraly, 2009), and so, any effects observed may not be entirely attributable to diet. Though the current study attempted to avoid such issues by controlling for lifestyle covariates such as exercise frequency and number of hours of sleep, it is likely that other variables, mental wellbeing for example, should also be taken into account.

The current paper provides evidence that the DABS can be used to measure the frequency and amount of consumption of common foods and drinks, and it is suggested that the four-factor model (as well as the relevant subscales) associated with it should be further investigated using other populations. As it has previously been demonstrated that diet can exert effects upon behaviour, cognition, and mood, it is also suggested that studies should investigate dietary effects upon psychological wellbeing in order to help identify products that are potentially beneficial or harmful. Further use of the scale may also provide information on levels of consumption that produce effects of clinical significance. In addition, comparison with other methods of assessing diet will allow further development of the measure.

5. Conclusions

The current paper has described a new measure of commonly consumed dietary variables, with an onus upon functional foods and foods and drinks of current concern, that addresses both frequency of consumption as well as amount of consumption, and may save time regarding data collection and analysis compared to other FFQs. A four-factor structure of diet was associated with the questionnaire, consisting of Junk Food, Caffeinated Soft Drinks/Gum, Healthy Foods, and Hot Caffeinated Beverages. The main finding was that Caffeinated Soft Drinks/Gum was associated with negative effects such as fewer than average sleep hours and poor general health, whereas Healthy Foods was associated with good health, frequent exercise and more than average sleep hours. Though the DABS requires further rigorous testing, it is currently considered to be a convenient tool for providing

an assessment of recent dietary consumption, and may be of additional use when investigating the effects of diet on mental wellbeing, school performance and behaviour.

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References

Akbaraly, T. N. (2009). Dietary pattern and depressive symptoms in middle age. *British Journal of Psychiatry, 195*(5), 408-413. http://dx.doi.org/10.1192/bjp.bp.108.058925

Alford, C., Cox, H., & Westcott, R. (2001). The effects of Red Bull energy drink on human performance and mood. *Amino Acids, 21*(2), 139-150. http://dx.doi.org/10.1007/s007260170021

Allen, A. P., & Smith, A. P. (2011). A review of the evidence that chewing gum affects stress, alertness and cognition. *Journal of Behavioral and Neuroscience Research, 9*(1), 7-23.

Ambrosini, G. L., O'Sullivan, T. A., de Klerk, N. H., Mori, T. A., Beilin, L. J., & Oddy, W. H. (2011). Relative validity of adolescent dietary patterns: Comparison of a food frequency questionnaire and a 3-day food record. *British Journal of Nutrition, 105*(4), 625-633. http://dx.doi.org/10.1017/S0007114510004137

Barone, J. J., & Roberts, H. R. (1996). Caffeine consumption. *Food and Chemical Toxicology, 34*(1), 119-129. http://dx.doi.org/10.1016/0278-6915(95)00093-3

Bellisle, F. (2004). Effects of diet on behaviour and cognition in children. *British Journal of Nutrition, 92*(Suppl. 2), 227-232. http://dx.doi.org/10.1079/bjn20041171

Bertoli, S., Petroni, M. L., Pagliato, E., Mora, S., Weber, G., Chiumello, G., & Testolin, G. (2005). Validation of food frequency questionnaire for assessing dietary micronutrients and calcium intake in Italian children and adolescents. *Journal of Pediatric Gastroenterology and Nutrition, 40*(5), 555-560. http://dx.doi.org/10.1097/01.mpg.0000153004.53610.0e

Bonow, R. O., & Eckel, R. H. (2003). Diet, obesity, and cardiovascular risk. *New England Journal of Medicine, 348*(21), 2057-2058. http://dx.doi.org/10.1056/NEJMp030053

Brunner, E., Stallone, D., Juneja, M., Bingham, S., & Marmot, M. (2001). Dietary assessment in Whitehall II: Comparison of 7 d diet diary and food-frequency questionnaire and validity against biomarkers. *British Journal of Nutrition, 86*(3) 405-414. http://dx.doi.org/10.1079/BJN2001414

Chaplin, K., & Smith, A. P. (2011a). Definitions and perceptions of snacking. *Current Topics in Nutraceutical Research, 9*(1-2), 53-60. http://dx.doi.org/10.1016/j.appet.2006.07.014

Chaplin, K., & Smith, A. P. (2011b). Breakfast and snacks: Associations with cognitive failures, minor injuries, accidents and stress. *Nutrients, 3*(5), 515-528. http://dx.doi.org/10.3390/nu3050515

Emmett, P. (2009). Dietary assessment in the Avon Longitudinal Study of Parents and Children. *European Journal of Clinical Nutrition, 63*(Suppl. 1), 38-44. http://dx.doi.org/10.1038/ejcn.2008.63

Florence, M. D., Asbridge, M., & Veugelers, P. J. (2008). Diet quality and academic performance. *Journal of School Health, 78*(4), 209-215. http://dx.doi.org/10.1111/j.1746-1561.2008.00288.x

Heckman, M. A., Weil, J., & Gonzalez de Mejia, E. (2010). Caffeine (1, 3, 7-trimethylxanthine) in foods: A comprehensive review on consumption, functionality, safety, and regulatory matters. *Journal of Food Science, 75*(3), R77-R87. http://dx.doi.org/10.1111/j.1750-3841.2010.01561.x

Hu, F. B., Rimm, E., Smith-Warner, S. A., Feskanich, D., Stampfer, M. J., Ascherio, A., Sampson, L., & Willett, W. C. (1999). Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire. *American Journal of Clinical Nutrition, 69*(2), 243-249.

Kanarek, R. B., & Swinney, D. (1990). Effects of food snacks on cognitive performance in male college students. *Appetite, 14*(1), 15-27. http://dx.doi.org/10.1016/0195-6663(90)90051-9

Kanarek, R. B. (1997). Psychological effects of snacks and altered meal frequency. *British Journal of Nutrition, 77*(Suppl. 1), 105-120. http://dx.doi.org/10.1079/bjn19970108

Kang, J. H., Ascherio, A., & Grodstein, F. (2005). Fruit and vegetable consumption and cognitive decline in aging women. *Annals of Neurology, 57*(5), 713-720. http://dx.doi.org/10.1002/ana.20476

Livingstone, M. B., & Robson, P. J. (2000). Measurement of dietary intake in children. *Proceedings of the Nutrition Society, 59*(2), 279-293. http://dx.doi.org/10.1017/s0029665100000318

Loef, M., & Walach, H. (2012). Fruit, vegetables and prevention of cognitive decline or dementia: A systematic

review of cohort studies. *Journal of Nutrition Health and Aging, 16*(7), 626-630. http://dx.doi.org/10.1007/s12603-012-0097-x

Mahoney, C. R., Taylor, H. A., Kanarek, R. B., & Samuel, P. (2005). Effect of breakfast composition on cognitive processes in elementary school children. *Physiology and Behavior, 85*(5), 635-645. http://dx.doi.org/10.1016/j.physbeh.2005.06.023

Malik, V. S., Fung, T. T., van Dam, R. M., Rimm, E. B., Rosner, B., & Hu, F. B. (2012). Dietary patterns during adolescence and risk of Type 2 Diabetes in middle-aged women. *Diabetes Care, 35*(1), 12-18. http://dx.doi.org/10.2337/dc11-0386

Martin-Moreno, J. M., Boyle, P., Gorgojo, L., Maisonneuve, P., Fernandez-Rodriguez, J. C., … Willett, W. C. (1993). Development and validation of a food frequency questionnaire in Spain. *International Journal of Epidemiology, 22*(3), 512-519. http://dx.doi.org/10.1093/ije/22.3.512

McLellan, T. M., & Lieberman, H. R. (2012). Do energy drinks contain active components other than caffeine? *Nutrition Reviews, 70*(12), 730-744. http://dx.doi.org/10.1111/j.1753-4887.2012.00525.x

McNaughton, S. A., Ball, K., Mishra, G. D., & Crawford, D. A. (2008). Dietary patterns of adolescents and risk of obesity and hypertension. *Journal of Nutrition, 138*(2), 364-370.

McPherson, R. S., Hoelscher, D. M., Alexander, M., Scanlon, K. S., & Serdula, M. K. (2002). Validity and reliability of dietary assessment in school-age children. In C. D. Berdanier (Ed.), *Handbook of nutrition and food*. (pp. 495-522). Boca Raton, FL, CRC Press. http://dx.doi.org/10.1201/9781420038392.ch20

Morris, M. C., Evans, D. A., Tangney, C. C., Bienias, J. L., & Wilson, R. S. (2006). Associations of vegetable and fruit consumption with age-related cognitive change. *Neurology, 67*(8), 1370-1376. http://dx.doi.org/10.1212/01.wnl.0000240224.38978.d8

Murphy, J. M., Pagano, M. E., Nachmani, J., Sperling, P., Kane, S., & Kleinman, R. E. (1998). The relationship of school breakfast to psychosocial and academic functioning. *Nutrition, 152*(9), 899-907.

Ness, A. R., & Powles, J. W. (1997). Fruit and vegetables, and cardiovascular disease: A review. *International Journal of Epidemiology, 26*(1), 1-13. http://dx.doi.org/10.1093/ije/26.1.1

Ogawa, H., & Ueki, N. (2007). Clinical importance of caffeine dependence and abuse. *Psychiatry and Clinical Neurosciences, 61*(3), 263-268. http://dx.doi.org/10.1111/j.1440-1819.2007.01652.x

Powell, C. A., Walker, S. P., Chang, S. M., & McGregor, S. M. (1998). Nutrition and education: A randomized trial of the effects of breakfast in rural primary school children. *American Journal of Clinical Nutrition, 68*(4), 873-879.

Rampersaud, G. C., Pereira, M. A., Girard, B. L., Adams, J., & Metzl, J. D. (2005). Breakfast habits, nutritional status, body weight, and academic performance in children and adolescents. *Journal of the American Dietetic Association, 105*(5), 743-760. http://dx.doi.org/10.1016/j.jada.2005.02.007

Riboli, E., & Norat, T. (2003). Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *American Journal of Clinical Nutrition, 78*(Suppl. 3), 559-569.

Rimm, E. B., Giovannucci, E. L., Stampfer, M. J., Colditz, G. A., Litin, L. B., & Willett, W. C. (1992). Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. *American Journal of Epidemiology, 135*(10), 1114-1126.

Rockett, R. H., Breitenbach, M., Frazier, A. L., Witschi, J., Wolf, A. M., Field, A. E., & Colditz, G. A. (1997). Validation of a youth/adolescent food frequency questionnaire. *Preventive Medicine, 26*(6), 808-816. http://dx.doi.org/10.1006/pmed.1997.0200

Scholey, A. B., & Kennedy, D. O. (2004). Cognitive and physiological effects of an "energy drink": An evaluation of the whole drink and of glucose, caffeine and herbal flavouring fractions. *Psychopharmacology, 176*(3-4), 320-330. http://dx.doi.org/10.1007/s00213-004-1935-2

Seifert, S. M., Schaechter, J. L., Hershorin, E. R., & Lipschultz, S. E. (2011). Health effects of energy drinks on children, adolescents, and young adults. *Pediatrics, 127*(3), 511-528. http://dx.doi.org/10.1542/peds.2009-3592

Shuttleworth, I. (1995). The relationship between social deprivation, as measured by individual free school meal eligibility, and educational attainment at GCSE in Northern Ireland: A preliminary investigation. *British Educational Research Journal, 21*(4), 487-504. http://dx.doi.org/10.1080/0141192950210404

Smith, A. P. (1998). Breakfast and mental health. *International Journal of Food Science and Nutrition, 49*(5), 397-402. http://dx.doi.org/10.3109/09637489809089415

Smith, A. P. (2002). Effects of caffeine on human behavior. *Food and Chemical Toxicology, 40(*9), 1243-1255.

http://dx.doi.org/10.1016/s0278-6915(02)00096-0

Smith, A. P. (2009). Effects of caffeine in chewing gum on mood and attention. *Human Psychopharmacology Clinical and Experimental, 24*(3), 239-247. http://dx.doi.org/10.1002/hup.1020

Smith, A. P. (2010). Effects of chewing gum on cognitive function, mood and physiology in stressed and non-stressed volunteers. *Nutritional Neuroscience, 13*(1), 7-16. http://dx.doi.org/10.1179/147683010x12611460763526

Smith, A. P. (2011). Breakfast and adult and child behaviors. In R. B. Kanarek & H. R. Lieberman. (Eds.), *Diet, brain, behaviour: Practical implications.* (pp. 53-70). London: CRC Press. http://dx.doi.org/10.1201/b11194-5

Smith, A. P., Kendrick, A. M., & Maben, A. L. (1992). Effects of breakfast and caffeine on performance and mood in the late morning and after lunch. *Neuropsychobiology, 26*(4), 198-204. http://dx.doi.org/10.1159/000118920

Smith, A. P., Kendrick, A., Maben, A., & Salmon, J. (1994). Effects of breakfast and caffeine on cognitive performance, mood and cardiovascular functioning. *Appetite, 22*(1), 39-55. http://dx.doi.org/10.1006/appe.1994.1004

Smith, A. P., Clark, R., & Gallagher, J. (1999). Breakfast cereal and caffeinated coffee: Effects on working memory, attention, mood, and cardiovascular function. *Physiology and Behavior, 67*(1), 9-17. http://dx.doi.org/10.1016/s0031-9384(99)00025-6

Smith, A. P., & Wilds, A. (2009). Effects of cereal bars for breakfast and mid-morning snacks on mood and memory. *International Journal of Food Sciences and Nutrition, 60*(Suppl. 4), 63-69. http://dx.doi.org/10.1080/09637480802438305

Smith, A. P. & Stamatakis, C. (2010). Cereal bars, mood and memory. *Current Topics in Nutraceutical Research, 8*(4), 169-172.

Smith, A. P., & Rogers, R. (2014). Positive effects of a healthy snack (fruit) versus an unhealthy snack (chocolate/crisps) on subjective reports of mental and physical health: A preliminary intervention study. *Frontiers in Nutrition 1*, 10. http://dx.doi.org/10.3389/fnut.2014.00010

Speck, B., Bradley, C. B., Harrell, J. S., & Belyea, M. J. (2001). A food frequency questionnaire for youth: Psychometric analysis and summary of eating habits in adolescents. *Journal of Adolescent Health, 28*(1), 16-25. http://dx.doi.org/10.1016/s1054-139x(00)00171-3

Tsai, A. C., Chang, T.-L., & Chi, S.-H. (2012). Frequent consumption of vegetables predicts lower risk of depression in older Taiwanese – results of a prospective population-based study. *Public Health Nutrition, 15*(6), 1087-1092. http://dx.doi.org/10.1017/s1368980011002977

Watson, J. F., Collins, C. E., Sibbritt, D. W., Dibley, M. J., & Garg, M. L. (2009). Reproducibility and comparative validity of a food frequency questionnaire for Australian children and adolescents. *International Journal of Behavioral Nutrition and Physical Activity, 6*, 62. http://dx.doi.org/10.1186/1479-5868-6-62

Wiles, N. J., Northstone, K., Emmett, P., & Lewis, G. (2009). 'Junk food' diet and childhood behavioural problems: Results from the ALSPAC cohort. *Journal of Clinical Nutrition, 63*(4), 491-498. http://dx.doi.org/10.1038/sj.ejcn.1602967

Willett, W. C., Sampson, L., Stampfer, M. J., Rosner, B., Bain, C., Witschi, J., Hennekens, C. H., & Speizer, F. E. (1985). Reproducibility and validity of a semiquantitative food frequency questionnaire. *American Journal of Epidemiology, 122*(1), 51-65.

Willett, W. C., Reynolds, R. D., Cottrell-Hoehner, S., Sampson, L., & Brown, M. L. (1987). Validation of a semi-quantitative food frequency questionnaire: Comparison with a 1-year diet record. *Journal of the American Dietetic Association, 87*(1), 43-47.

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Mitigating Production Practices and Antibiotics Use in Meat Industries Prone to Economies of Scale by Institutional Novelties, Marketing and Voluntary Actions

Ernst-August Nuppenau[1]

[1] Department of Agricultural Market Analysis, Justus-Liebig-University, Giessen, Germany

Correspondence: Ernst-August Nuppenau, Department of Agricultural Market Analysis, Justus-Liebig-University, Giessen, Germany. E-mail: Ernst-August.Nuppenau@agrar.uni-giessen.de

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Abstract

Achieving improved standards in animal husbandry (including less use of antibiotics) through appropriate interventions has become a matter of public concern. It is currently both, hotly debated and a challenge for food economics. The question is: how can one achieve change in a given environment of property rights and interests? This paper offers a novel approach intended for conflict solving in meat industries which are prone to economies of scale, which are under international and competitive pricing and which experience structural change. In particular, in case of: (i) economies of scale favouring large-scale production and high stocking densities (supported by increased antibiotic use), (ii) political power about resistance to regulate (avoiding strong interference), but also in contrast to (iii) consumers' wishes and willingness to pay (WTP for a change in production modes), there is a problem of coordination and institutions. In this article, the issue is delineated as a problem of political bargaining and creating marketing channels (broker and agency) which shall actively pursue promotion of reduced antibiotics use (specifically through reduced stocking density) as well as negotiations on compensations (for cost increase). Producers are outlined with regards to scheme participation along willingness to accept (WTA). Stocking density reduction is used as proxi for more healthy animal rearing methods. We establish interest functions and show how a bargain can be modelled in the tradition of Zusman's political economy. Bargaining involves power coefficients for brokerage (premium sharing) as well as an agency (called FSA); the agency is modelled as a bureaucracy optimizing modified costs and benefits. In fact, the agency maximizes its budget and ensures participation of willing producers to join programs. Finally we refer to ways how to solve the issue in modes of political economy models. The assumption is that asymmetric information prevails and consumers are willing to pay (WTP) for healthy food.

Keywords: food safety, market segmentation, contracting for antibiotic reduction, political economy model, bargaining model

1 Introduction

Recently intensified discussions on the use of antibiotics in animal production reveal a huge problem of market coordination in meat production of countries of concern (for instance in Europe/Germany this has recently reached mass media: Die Zeit, 2014, and bans are requested). Before, the problem had already resulted in many proposals such as: labelling, consumer enlightenment, ban on "industrial farming", etc. (again on the debate in Germany see: TAZ, 2013). However, most initiatives never went really through. Depending on interests the case is usually portrayed differently by lobbying groups, apparently as in a political game. We might realize that the case was put forward on many grounds (health, welfare, property rights, etc.; even philosophical), but limited actions occurred on the side of the public (government) and interventions are marginal (only minimal standards passed). For example, again in Germany, a law was passed which is deemed to regulate; but does not forbid antibiotics (only set minimal rules: TopAgrar Online, 2013a). In contrast public concern increases. Hereafter, pressure is big and the conflict escalates. An exception for relaxation of conflict seems to be a voluntary compromise on animal welfare which is, though not directly addressing human health, linked to advances in changes of practices. It also applies a payment scheme for compensation of costs incurred (Deutsche Bauernkorrespondenz, 2014 on Tierwohl: for instance, a recent statement, TopAgar online 2015, says that 4 Euro-Cent/kg will be collected by retailers from consumers and 64 million Euros will be offered to farmers for changes in practices).

Such willingness to find options for practice change shows fears of agribusiness for bans. Yet, in the opinion of authors there is an open debate on governance, institutions and marketing novelties. The aim of the paper is to describe such a novelty, imbedded in market segmentation.

First we have to understand the issue in a broader frame of technologies, economics, strategies, politics, and social science. To start as observation: by different strategies such as prophylactic application vs. illness related application of antibiotics (in detail) as well as modern vs. traditional husbandry (in general) farmers try to solve problems in production (animal health), minimize costs (use economies of scale), and become competitive instantly; instead "no use of antibiotics" needs real incentives. For large-scale intensive farming an association between antibiotics use and farm size was suggested as early 2002 (Tilman, Cassman, Matson, Naylor, & Polasky, 2002). Recently (Fels-Klerx, Puister-Jansen, Asselt, & Burgers, 2014) investigated this association and they confirmed in multiple regressions that those farming systems associated with size have a problem in antibiotics use; though density is giving a spot with other factors. As it was found a wide range of dosage exists; some farms are more prone to use more antibiotics and these farms seem to have hygiene problems. Yet, about technologies this is associated with economies of scale and it determines success.

Second to describe novelties one has to refer to theoretical concepts and economic practices jointly. From an economic point of view institutions such as markets should optimize allocation, but also solve conflicts and minimize transaction costs (Coase, 1960). Actually the problem might be better described by: what is an appropriate level of antibiotics as external effect and how to get it by right setting. Then a theoretical solution would be to calculate approximately a social optimum and balance costs and benefits (here willingness to pay WTP of health vs. low meat prices for consumers). Such solution requires a full set of property rights and finding markets including pricing of externality. A crucial thing is: are consumers WTP for change in meat production and do technologies exist? It seems, yes, there is WTP to change as comparable studies show for beef (White & Brady, 2014.) Then a broader conceptual work on producers' willingness to accept (WTA) is needed and markets (actions) for change (options) have to be investigated (Lusk & Norwood, 2012). But, why does it not work? Market failure seems to prevail and property rights are incomplete (yet "market failure" might be only proved for some aspect: Carlson, Frykblom, & Lagerkvist, 2007; and it is a general problem). Precisely, deliberations on complex institutional options are missing. (In contrast, it seems that simple interventions are suggested which shall force farmers to modify animal husbandry without cost recognition and this failed). As a political will which may suggest strong industry regulations is lacking (rights with consumers), a "first best" economic solution is far away; it lacks support. So, is there a second best alternative? We think that there is and we will discuss an option based on institutional change imbedded in the theory of bargaining (Zusman, 1976).

Third, for such option imbedded in institutional change we must assume: rights to technologies (antibiotics, stocking, animal health, etc.) are with producers. This seems to be current political practice. In fact, in accordance with the law (whatever volumes of different substances, antibiotics farmers have access to antibiotics under supervision of veterinarians) standards seem to be low in consumers' eyes. (They only claim a right to health). So no solution seems to exist, which serves all parties and which is Pareto superior presuming consumers claim health as right without payment. In contrast there is meat without antibiotics; but is expensive. Looking for such meats (i.e. 100% healthy given by practices, low stocking, which ought to be achieved for health, though costly), those market shares are low. Privileges are with farmers. Most consumers are not buying this meat and this is considered a problem Requillart and Soler, 2014). A survey in Germany showed: 83 % of chickens received antibiotics at least once (Top-Agrar, 2012). Even, if this is a question on technology, it is also on competiveness because farmers with modern technologies strongly dependent on economies of scale.

Even if it is problem of technology and information, another problem appears at market and public level by giving bad images to meat. As standard argument (raised in public for example by "Food Watch", a lobby group see: PEW 2009) we hear about a necessity to regulate whole production ("no industrial food") as panacea; i.e. regulations through controlling sizes and practices on antibiotics by an authority (governance) are wanted. But what are the underlying assumptions? In economic terms, it could be assumed that consumers/citizens are informed and it depends on WTP to get rid of antibiotics and control stocking indirectly. Though there is evidence on consumers' concerns, assessments of animal welfare, stocking density and antibiotics (Vanhonacker, Verbeke, Van Poucke, Buijs, & Tuyttens, 2009) as well as WTP (specific for food attributes, health claims sustainability of meat production: Lee et al. 2014) action is lacking. Yet ago, questions of market failure were raised with respect to consumer information on antibiotics use (Carlson, Frykblom, & Lagerkvist, 2007).

Fourth, with regards to WTP behind doors, why is the industry so much in favour of industrial production, in favour of no-change and does not capitalize WTP (Deutscher Bauernverband, 2013)? Perhaps it is because of fear and power? As opponents of industrial production mostly base arguments on externalities (human health,

animal welfare and environmental effects: see Meat.Org, 2013 on practices/opinions), producers do not want to surrender. At the other hand to ignore arguments of the industry for income and survival is also not realistic; it is fairly plausible that front lines exist. To recap, there is a fight about interests and how to solve it?

The author suggests: novel institutions could make a difference and solve partly the conflict. Especially by introducing new intermediaries creating incentives and reaching participation of, at least, parts of industry in terms of voluntary agreements, a perspective is opened. This perspective is grounded in contracting. For that we offer bargaining as modelling. In order to be successful in finding innovative solutions, mechanisms (Rausser, Swinnen & Zusman 2011) have to be anticipated and understood as well as the bargaining modelled. In this regard the aim of this paper is to delineate the issue from a point of bargaining theory (Zusman, 1976); and a power-interplay is presumed as well as measuring and modelling it. Further we follow Requillart and Soler (2014) about classified policy interventions and refer to supply side options on changing the market environment for getting healthier products. Especially since options for differently "controlled" markets are lacking, novelties for health payments are offered which means an indirect market on health. In particular the institution of "broker" and "agency" are proposed beside government interferences and their roles prescribed.

The paper is organized in six sections. (i) We examine the issue of antibiotics use and explain how it should be linked to farm practices, mainly stocking density. (i) The issue of finding institutions is explained by examining the background given economies of scale and intervention options. (iii) The conceptual framework is explained. (iv) I.e. positions for bargaining are delivered and (v) this is mathematically done. (vi) Finally we venture in a short description what is meant by bargaining? We start with the background giving first issues and then ideas.

2. State of the Art in Antibiotic Evidence and Problems in Meat Industries

For finding solutions we have to dig deeper into the problem. But, this paper neither intends to present a scientific review on the problem of antibiotics in animal production nor it offers a proof regarding correlations between antibiotics, animal health and farm practices. Our arguments are economic, are based on specific evidence and are as follows: Yet, at the level of science, one can say (also from a human health perspective) that there is preliminary evidence on resistance to bacteria and use of antibiotics in animal husbandry which suggest a precaution in farm practice. In conjunction to production practices, in the meat industry, there was concern already quite early (for agriculture see: Alanis, 2005 and for feed adds specifically: Wegener, 2003), here at micro-biology level. (This goes even further where scientists are now looking at antibiotics in soils and relate crop contamination to practice: Torre, Iglesias, Carballo, Ramírez, & Muñoz , 2012). At national levels recent studies (Chantziaras, Boyen, Callens, & Dewulf, 2013) using ranking methods of countries suggest statistical evidence for resistance for animals, also taking farm sizes and stocking density into consideration. To become particularly practical on negotiation terms: a focus is almost at all times on stocking density. Though at the level of correlation between microbiology and animal husbandry, there seem to be a complex story of animal health, antibiotics, hygiene and husbandry (i.e. stocking density, in agronomy, bacteria growth, animal stress and husbandry), such issues have led to recognition of stocking density as a pivotal parameter in industry. (Admittedly besides other practices to control diseases: see Archie & Theis; 2011, and on stocking density: Estevez, Andersen & Naevdal, 2007). Generally speaking there is a request to bridge the flaw between health, animal welfare (Soerensen & Fraser, 2010) and productivity (Rushen, 2003) by reducing stress (stocking, i.e. looking for better production modes).

Likewise at public level citizens look (ask) for better standards in production (lower stocking density). Farmers shall give more space to animals and improve health, but this comes at costs (lower productivity and hence lower competitiveness) for the industry. Producers think they can produce in whatever mode arguing for costs. In the language of property rights (Coase, 1960) they argue for lowest costs and additional costs due to changes in practices should be transmitted to consumer (to get efficiency in antibiotics is not their aim). But do we have a spontaneous market for citizen preferences for reduction and farmers acceptance? The author thinks: no. Remarkably, WTP analyses on labels should show concern of consumers about animal welfare (Loo, Caputo, Nayga, & Verbeke, 2014), but no effective payments exists. In that regards it is not only a debate on "science", but also on conflicting issues and consumer vs. producer interests. Many such articles (Carlson, Frykblom, & Lagerkvist, 2007) have been written on preferences and opinions of citizens about animal (welfare), health, rights and farming modes. What do they tell us? In particular stocking density plays a role. An example is the paper of Vanhonacker, Verbeke, Van Poucke, Buijs, and Tuyttens (2009). They wrote about animal welfare and industries' responsibility. Work on badly treating animals (high stocking) has gained much attention (Goodwin & Shoulders, 2013) asking for rights beyond farmers' rights. Here labelling comes in, but rights are with farmers; they do not want to change things! Labelling (effective for WTP) is requested as tool; but with no market segmentation it is difficult (Tonsor & Wolf, 2011); how to target money? Labelling requires market segmentation.

But is segmentation enough to make consumers confident? Yes, if we take stocking, this offers scope for agreement. Stocking may concern animal welfare per se!

As another observation: to a certain extent public discussions mostly end up with requests for specific bans on sizes of farms. As Deckers (2010) argues, the issue is again farm practice. In that respect it has been found that consumers put high premiums on banning big industry (Lusk, Norwood, & Pruitt, 2006), i.e. specific forms of highly stocking animals in large stables. But this is questioned by industry opinions (Bauernverband, 2013; and for moderate versions see Ngapoet al., 2004). Preferring market segmentation is an option, again. Citizens may decide on limited banning (producers with very high stocking). Admittedly, to come out of that corner there are yet initiatives of the industry as mentioned (TopAgrar, 2013b) looking at meat market segments: (i) no control, (ii) "minor control" (stocking), and (iii) premium.

Finally, if we proceed, we have to address the issue of stocking density and see antibiotics from the point of efficiency and competitiveness. At farm level, modern technology, technical efficiency and economies of scale are crucial performance indicators. In particular, economies of scale are well documented. Many papers argue that modern, large farms are more efficient than small ones (Ollinger, MacDonald & Madison, 2005), and no return please. Work on economic performance, looking at industry level, supports this view that stocking density is in particular important for business feat (Oude Lansink & Reinhard, 2004). To summarize, farmers in meat industries are reluctant to change technologies (stocking density), insist on public-private partnerships and strongly depend on current technologies as competiveness is concerned. Change shall not threaten competitiveness (Seals, 2012) and without compensation no change!

3. Reasons for Market Failure, Background, Institutional Innovation and Governance

3.1 Market Failure

Then we can go even deeper in the reasons for market failures and we should firstly ask: why is the market no solving the problem of antibiotics, though segmentation and labels are suggested; secondly why are there no spontaneous institutional innovations yet and thirdly what are appropriate suggestions fitting in a framework of market failure and politics, as well as how do consumers see it (Korzen, Sandoee, & Lassen, 2011)? The author thinks there is a lack of institutional innovations because of blocking beyond pure market failure. Generally, institutional innovations are supposed to promote exchange (noticeable under uncertainty) at low costs and they should channel WTP of consumers for health concerns to WTA of producers for actions requested. This requires trust, contracts and coordination. The opposite persists if there is no will for contracting and coordination by participants. In cases of conflict no communication, no deals, and not finding solutions are preferred (missing cooperation and contracts); these are indicators of market failure and policy quarrels (Rausser, Swinnen, & Zusman, 2011).

In contrast, in theory (i.e. in cases of aiming at self-explained solutions - as example see principal-agent concepts or incentive schemes inter alia: Furubotn & Richter, 2005) - we have instant actions/service taken by intermediaries. For instance a broker steps in in case of market search. To transfer the idea, in our case a "service" would be antibiotic reduction and a "payment" (WTP, compensation) for quality meat without antibiotics can solve the problem. How should it be organized? In particular, we see our task in increasing numbers of institutional choices by suggesting brokers and an agency as contracting units. Brokers sell meat at a certified level of no antibiotics and agreed lower stocking density. Likewise, an agency (here a "Food Safety Agency" FSA: using compensation for industry's efforts to use lower antibiotics and stocking density) may bargain on reducing density at a flexible/bargain rate. Then we have different meat markets. In result it means splitting markets. Such a suggestion may create tensions in beginning, but from an analytical viewpoint institutions of a broker and an agency are innovative choices, creating incentives and cooperation, as will be shown. How to do it?

3.2 Alternative Institutions

For a short introduction: for brokers, the description seems to be easy (see below), because they can promote meat of high quality directly referring to quality meat without antibiotics. In this paper it is assumed that brokers can create trust (Lindgren, 2003). However, it is not discussed how this works out, rather we take the position that it is costly and the broker and retailer system will do only the job if a margin can be realized. For further literature on modes to create trust, farmers' assurance we refer to discussions on the role and perspective of the meat industry (Bailey & Garforth, 2014) as well as we are fully aware that consumer trust vs. mistrust are complex issues in the meat industry (perhaps we cannot address them here: Eden, Bear, & Walker, 2008). There might be big problems of disease management (Garforth, Bailey, & Tranter, 2013) and it is assumed that QS (in Germany) is one response. However again, it is not the intention of this paper to qualify who is doing better broker/retailers of the agency/QS? Rather we want to explore the costs and benefits as well as option to get a

coordination mechanism along market segmentation and brokers and an agency/QS competing for consumers WTP in different market segments. Hereby the quality (food with no antibiotics) is exogenous for the broker, but endogenous to QS (subject to negotiations on stocking density of animals and antibiotics use).

For the agency, the task assignment is more complicated. Such an agency can negotiate with industry members on terms of conduct (farm practice in particular), and cost compensation (in general). Compensations could come from health insurance companies which act on behalf of citizens' WTP for safer food. The agency is needed as a competitor to redirect brokerage or else we are stuck as presently observable by blocking. A suggestion is: government establishes a semi-autonomous agency (FSA for instance "QS" in Germany, but with competence, money and power: QS, 2013a). We will model the agency as an entity inspecting production parameters (such as stocking density of animals linked to antibiotics) and assures correct practices. The suggested concept departs from a simple minded version of governing quality and health of food in the meat sector through checks and control of a public authority. It rather works with a hybrid system of minimal standards (on antibiotic use in terms of state control of veterinary regulations) and private sector institutional innovations which are partly financed by health insurance units.

3.3 Governance and Institutions

Our underlining issue for governance is: yes, the government can set production standards; but who controls them, what are the control costs (for the public)? For this we seek solutions which are more cost effective that public control of each and any farm. Additionally we work with the Coase (1960) perception that "good" governance is to set rights which are tradable (see below); rather than to assign the burden of health control directly to the government which would mean spending a lot taxpayers' money. We assume there is a WTP for health, but the question is how to "optimally" channel that money to agencies which are working on participatory grounds with the industry and by that "minimizing" transaction costs.

Actually the objective of the paper is not to make simple suggestions; rather we need to delineate complex interest functions and show how practices (technology standards on antibiotics) can be endogenously derived (negotiated, here based on correspondingly agreed criterions (stocking density). These standards can then be linked to marketing and governance. In fact, we will show how to develop an economic bargaining concept (model) which enables mutual contracting on parameters and spells out industry performance (Zusman, 1989). As will be shown, particular modifications to a Zusman model shall suit already contracting in meat industry. Also, reference points for bargaining to find optimal parameters must be mentioned.

On procedures: we (i) delineate explicitly novel options as derived from the institution economic theory of Zusman (1989) who proved that trust can be with brokers and agencies in agrarian societies which are serving different roles as middlemen for revenues, (ii) we show consequences for health and (iii) say what might be accepted. It is against this background that we (iv) will discuss innovation through explaining normative choices for redesigning institutions, opening up bargains on indirect terms (stocking density). (v) Options for farmers (on stocking) imbedded in institutions offer improvements for both sides (be Pareto superior); in particular a negotiation platform is discussed. Improvements are to be recognized at physical level as reduced application of antibiotics (health), but also at financial and welfare levels (gains for consumers: WTP, and producers: profits plus WTA); and costs (competitiveness) of the industry matter (farmers and processors seek viability). On governance, it has to be mentioned, that, in a tradition of self-regulation, the suggested concept is restrained to voluntary bargaining of agents in industries. The government, as said, is not directly involved, though minimum standards are introduced and we get a hybrid in governance. But no game with government is modelled. Actions to be taken are voluntary; and it can be tested how a set of rights set by government (as standards) contribute to results. For that task references of cooperation and defection are to be stated (below). We stick to a concept of minimal governance.

4 Concept and Framework

In this chapter we will explain our conceptual framework. It is based on the notion of co-existence of economies of scale in meat production with modes to encourage reduced stocking as well as to create markets for better "quality" meat. Hereby we follow the above problem statement. The assumption is larger farms with high stocking show higher infection risks, they want high compensation, and better prices are needed to receive participation in antibiotics reduction. There is a trade-off between size, density and antibiotics. It has been said before, large farmers will immediately deny this (actually to defend themselves); but we work with indications and options which constitute the conflict for which we seek solutions and negotiation.

We start with a depiction of economies of scale and stocking density (Diagram 1: for mathematical outline see next chapter). The argument is: average costs are currently low because of economies of scale and high stocking

densities. Because of the fact that antibiotics are almost free in use (though minimum standards must be recognized and veterinarians are involved) high stocking densities in the industry are feasible. By use of high antibiotics, though at legal limits, assure animal health. A reversal requires compensation, i.e. average costs and marginal costs are to be related to application (control) of antibiotics and technology. This means in absence of or in case of low antibiotics, given a certain size of the operation, additional costs accrue because of additional hygiene measures, etc. Please note this is not a criticism of industries, rather an observation of industries typically aiming at low costs in competition. Such reality is linked to missing opportunities for market segmentation (i.e. higher prices, WTP, for meat with no antibiotics in production; higher costs cannot be transmitted, etc.). Later, average and marginal costs are linked to pricing in market segments. Note, as usual in marketing analysis (Zusman, 1989) we are here not interested in changing farm structures, rather our focus is looking for marketing mechanisms and controlling animal density in terms of institutions.

Diagram 1. Farm size and capability to meet standards for antibiotics

4.1 Brokerage for Antibiotic-free Meat and Marketing Channels

A necessary next step requires us to explain how to create a linkage between production modes and marketing of products. Meat products may be produced with high stocking (and antibiotics), reduced stocking, or no antibiotics (premium). We hypothesize consumers have a WTP for premium, but seek assurances of "no antibiotics", then they relate it to "low stocking" as assurance. Here, the brokerage can help. As institutional novelty brokerage can most likely fetch a price premium and assure service (low stocking density); though the broker charges a commission. He communicates a certain stocking density as antibiotics free. As generic arrangement and as mediated for exchange (here modified stocking) farmers are WTA. Promotion with consumers (finding retailer/customers) on the other side is a broker's task. We see premium meat as a market segment in which the collaboration of farmers, brokers and retailers is promoting a ban on antibiotics, controlling diseases and, as tool stocking density is fixed according to technical and veterinarian knowledge. Technical details in production are exogenous to our study. Farmers "must" reduce stocking to become entitled to a percentage of consumers' WTP to pay a premium. The broker fixes the percentage (opposite to premium for him) for efforts (promotion) which are optimized. Brokering (see below in the consecutive modelling) is figured instead of simple retailing because brokers shall look actively for premiums. Also it creates individual costs; but finally they share premiums with producers. This, for sure, is a simplification, and brokers finally use retailers to sell the meat. A broker shall be primarily active at the level of making farmers interested in premium meat and promote the meat with selected retailers. Then, at retailing we assume a competitive market and price, and premiums are paid by consumers as "price minus unit cost" for retailing. So we do not look in retailing. Perhaps the real world is more complicated since different retailer/broker interactions may result in different consumer prices and premiums; this is beyond our analysis. Also we do not go into detail how practically brokers create trust (more or less: perhaps some retailer-farmers-consortium will get highest premium, etc.); rather modelling is of priority here.

As consumers shall have choices between 3 categories of products: (i) un-controlled (rest), (ii) controlled (following negotiated terms on stocking density), and (iii) premium (quality is assured by a broker), the premium

meat (at given stocking associated with no antibiotics) is a central element. The broker inspects attentively and is responsible for quality assurance, bears risk & trusts vice versa. In that regard the broker "makes" the market and charges % or mark-up, i.e. if he is successful, producers receive higher prices as part of a premium. The broker charge (commission) is for services; but it is not competitive, rather negotiable. The charge is agreed as a percentage to be found jointly and an interest is created on both sides. The third party is the consumer. The "controlled" market is more complex and will be elucidated soon.

4.1.1 Broker's Setting, Competition With Agency and Role of Free Market

To better understand brokering and bargaining as an institutionally regulated activity of channelling purchasing power (WTP), reference scenarios need to be established. They strongly determine bargaining modes, bargaining power and success of regulations. Hence the role of brokers cannot be seen isolated. Usually modelling of political bargaining in marketing (incl. brokerage) implies a reference of no cooperation (Zusman, 1989). So what are alternatives choices, if a producer denies the services of brokerage? First, the alternative is either a "rest market of lowest quality" and price or bargaining with an agency (see below). Second, as compared to the other choices the contract must be superior. In case of farmer-broker-agency-interaction with two marketing alternatives it means "not-cooperating" with partner "A" (broker) has a reference "cooperating with partner B" (food safety agency "FSA", next chapter). As further comment: farmers like choices and this will reduce resistance to interferences giving a feeling of freedom. Bargains on conditions (choices) are necessary to reveal preference. For brokerage this means that farmers negotiate a shared price premium at a reference which is designed being the alternative. For us (later) it is working with an agency, i.e. marketing in the "controlled", negotiated channel. Vice versa, selling with an agency FSA (see again below) has brokerage as reference. Brokerage is imbedded. A semi-competitive institutional frame emerges. To look at the FSA (different mode of payment, later) from the brokerage point of view (i.e. if compensation is the bargain), such competing channel becomes relevant.

4.1.2 Brokering Meat and Image Creation and Trust

In practice, concerning the channel for premium meat, we need ambitious activities to create trusts and images (Lindgreen, 2003) which are apparently costly and create questions of property rights. Brokerage can be seen as simple institutional aspect of dealing with image creation of antibiotics-free-meat with regards to cost and benefit sharing arrangements. If activities lift average market price a question is who bears costs and gains? The institutional innovation is explicit cost sharing as opposed to hidden cost transmission to producers. In fact, retailer-broker-interactions must be active as campaigning, labelling, assurance, etc.; but who does what; claim rights; etc.? Here brokers can take risk. If there is the issue of: cooperation, sharing, trusting, image creation etc. (also risk taking), it can be addressed through brokerage as exemplified by Zusman (1989). Anyhow, contracting is risky for both, farmer and broker; if failures (fraud) occur, images are endangered, etc. However, in cases of risky ventures it has been shown (by theory and practice) that sharing of costs and benefits is a suitable institution (Zusman, 1989). We foresee indirect communication of farmers with retailers through brokers and trust must be assured (first to retailers) by monitoring that no antibiotics are applied. Then brokerage is market creation depending on contracting with farmers. Here we do not dig in the multiple aspects of retailing, broker, and producer interaction, rather simplify as below shown.

4.1.3 Broker Operation

As indicated, in the above setting, retailing through a broker shall offer marketing activities for premium meat. Institutionally a broker charges a proportional factor (commission) on surplus (premium for no antibiotics "n" vs. standard price "s", see later for implications: $\upsilon\ [p^n\text{-}p^s]$ and υ is retention of broker, producer gets $(1\text{-}\upsilon)[p^n\text{-}p^s]$). This rule is an institutional suggestion typical for incentive schemes (Furubotn & Richter, 2005). Success of the suggested mechanism (hybrid scheme) relies on WTP and purchases of meat (share) at premium. At least there is risk sharing (by υ) and negotiations are engrafted in sharing (see below). Also the broker bears costs of inspection. The argument is about using marketing for density reduction in an industry by brokers as offering incentives for increasing market shares vs. agency. To be clear, brokers are (in this model) not negotiating standards (here antibiotics free production); rather the job is promotion and market creation for premium meat of lowest density (no antibiotics).

4.2 Agency of Controlling Stocking and Compensation for Antibiotic Control

Alternatively to brokers meat producers can (shall) contract with an agency which has money to promote negotiated changes in stocking density. The agency negotiates on flexible standards to get participation which is voluntary; here food prices are given and compensation is negotiated. Our Food Safety Agency, we call it "FSA", is yet to be initiated (though in Germany, for instance, "QS" plays partly the job: QS, 2013a). It is considered

semi-autonomous, as parastatal; i.e. it shall represent the public goals, and it anticipates public concern (public WTP for reduced antibiotics). Especially, we have to clarify how it will be equipped with money. We see a possibility to get money from health care based on public WTP for antibiotic control (not private WTP for premium meat as with brokers; rather a secondary source of money as WTP for health). How does FSA function? For the agency we assume that it "buys in technology" giving producer compensation at lowest costs (negotiates on stocking density and compensates farmers: WTA). Supposedly, in economic terms, there is a WTP for a probable reduction of health risk by changing practices (yet citizens', not consumer' money for lower stocking). The assumption is: a positive net effect in a cost-benefit (CBA) from stocking density reduction. The agency requests a budget from public health on basis of achievements and compensates farmers for cost increases due to less economies of scale (lower stocking density). We assume that property rights are with farmers. In other words, FSA shall "convince the industry" (actually as majority) that using fewer antibiotics (reduce stocking) will be of benefit to them (indirectly; this will result in overall reduced antibiotics as negotiation parameter). Note, one can go even further and talk about an index of various farm practises, but that needs new agreements (Agrarheute, 2014) and it raises issues whether in the end it will block agreements because of no clear commitments. Bargaining can be constructed as follows.

4.2.1 Bargaining Outline for the Agency

For operational feasibility a special outline of bargaining (as part of the novel institutional set-up) is suggested (i.e. created: see below). We try to reduce complexity and work with stocking density as a simple measure (eventually extended). In fact, our suggestion offers a normative outline for a quality assurance (FSA), yet to be established. It has to be constructed because it does not exist in most countries. However, offering an interest function to be pursued and bargaining (referring to economic theory) means to support real issues. Bargaining usually works by outlining bargaining parameters that are agreed upon in a mode of real exchange (Zusman (1989). For comparison and transfer of this concept: usually an agency, as a unit working with fixed price, charges a fixed rate for services (cost plus mark-up). Financial surpluses come from operating a service which is cost compensated. In our case we introduce bargaining on stocking density in exchange for payments and assume that FSA is a bureaucracy with surplus.

To spell out the underlying assumptions on establishing FSA: in a first version, i.e. in a deterministic approach by which bargaining can be conceptualized as an incentive and cap-and-trade system, compensation is a negotiated payment. In this system the control of antibiotics (stocking density) is the task to be accomplished through a self-regulatory element in industry (payment). A second version would be a probability-wise approach including "illicit" behaviour and fining; i.e. if, in cases, farmers fail to comply with stocking they are fined generating cash. Then revenues are different. Both, financial returns from fining and money by the agency/government for health can be used to pay farmers who are WTA reductions in stocking and comply with regulations. In the first case money solely comes from public WTP for health. In the second case there is a chance for an own budget where money comes also from farmers, (fined), but costs for inspection are to be included. We take the first version because it is much easier to model. Further note that bargains are individual ones, which opens up flexibility.

4.2.2 Agency Behaviour and References

As said in the introduction, our design of FSA will follow basically the concept of Zusman (1989). This concept is moreover based on reference points for creating cooperation as well as on specifying interest functions of parties (i.e. here the agency FSA and farmer, see below in the bargain outline). Starting with the first aspect, it is important to note that our references in dealing and bargaining with the FSA are either the broker (the no-antibiotics option) or not participating (zero change option). As said the project is voluntary. Then, interests of producers, as variation in regulations (practices, incl. bans or change in stocking), can be specified as follows: choices are determined by profits, i.e. on (i) sales at "free" rest markets, (ii) program or (iii) premium market. However, producers must be encouraged to cooperate with the FSA. Working with FSA at individual stocking density reductions and with brokers, it must be seen as income change. Essentially, FSA should serve those farmers with modest wishes for reduction, supplementing income. The FSA has to be imbedded in mutual (multiple) bargains offering farmers gradual choices. From a FSA viewpoint, bargaining of endogenous regulations is revenue maximizing. Hitherto we will model FSA as bureaucracy in accordance with Niskanen (1971). Since we may consider it a parastatal and not company, this modifies the interest function (see below). In such bureaucracy, it has to be assured that interest is created and FSA is not of "natural" interest. Interest will be given as striving for budget sizes.

5. Mathematical Outline

In fact, there are three marketing channels which interact, we have to look behaviour, and we have to look at costs. Producers have choices with conflicting interests: they deliver meat simultaneously (or at corner solution) to (i) a rest market at low price (or export to a world market without regulation), (ii) domestic market with competitive prices plus compensation (at negotiated stocking density) or (iii) "premium" market with high price for meat. Producers alternatively contract with brokers on surcharge in case of premium prices (no-antibiotics and low stocking) or on (endogenous) stocking density for compensation. A comparison for this is provided in Zusman (1989). Calculating ad(dis)vantages from modification of marketing choices becomes optional and broker participation is premium, though responsibility for product (meat) image is joint (active). Brokerage has to be seen in institutional mixture with FSA.

In this section we will explain how to model connectivity between the interest functions of the industry (as to be derived) and contracting variables (as achievements), i.e. in terms of: (i) stocking density (antibiotics use), (ii) premium sharing (channelling WTP from consumers to farmers, incl. commission: privately) and (iii) compensations (using health care money: publicly). The mathematical outline serves to settle the conflict; antibiotics vs. stocking density by compensation payments and negotiations based on a mathematical concept of bargaining developed by Zusman (1989). Such outline further enables us to do a quantitative analysis on power in games (as revelation mechanism). Power is needed to establish the bargain (below).

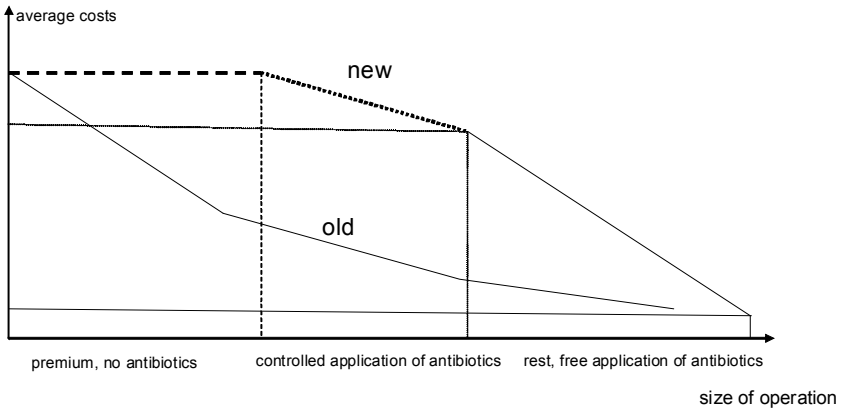

Diagram 2. Average costs

To start: produces and costs are segmented into three parts (according to economies of scale see Diagram 2). We assume that the industry's operation is separable in three sections. For instance, the first part (meat produced without antibiotics at lowest stocking density) has highest costs according to fixed regulations for animal health and welfare. (Technologies are actually considered exogenous to this paper, though they adjust). Here, in part (i) no economies of scale form changing stocking can be achieved. The part (ii) contains negotiated practices as explained above which allows slight change in scale, and the last part (iii) is free of any control and ends up at maximum stocking given by regulations as legal practices (minimum). In this section we have the strongest decline of economies of scale. We distinguish further the old and new patterns of economies of scale after and before using new institutional set-ups.

In each part or market segment, products with different consumer preferences and different economies of scale prevail. In section (i) production is based on high costs of caring for hygiene, good forage, air conditioning, etc. and perhaps it is out-door or organic farming (but this aspect does not matter here). Looking at mathematical expressions a continuous costs function is deployed; in reality, it is vital how animals are raised. In segment (ii) there is, as a result of compensation, a moderated decline in economies of scale (as compared to a reference of a previous medium scale farming incl. without compensation even thresholds change). In segment (iii), still a strong cost decline can be reached because stocking density regulations are standard only and antibiotic application still allows densities of animals beyond controlled.

Producers (or a single farm, if we allow multipurpose operation) decide on production categories and deliveries to markets according to options along segments. Two cases might be distinguished: in case one a producer delivers only to one segments, for example premium meat, while in case two a continuum is assumed. Case one is difficult to be addressed mathematically. We work with a continuum. In that case the producer can serve all

segments and decisions are internal. Given knowledge and references for economies of scale in segments, there are two modes of sector modelling: (a) it is a decision to accommodate all segments being endogenous or (b) groups of farmers decide for modes of farming (i.e. producers are going individually for "no: c", "controlled: c", or "rest: r".) To simplify (as abstraction) in "premium" stocking density is not disputable and we restrict decisions to market channel volumes. Then, the average application rate of antibiotics in relationship to average density "d^a" is determined endogenously by average segment densities and sizes of segments (market shares).

$$d^a = \varsigma_n d_n + \varsigma_c d_c + [1 - \varsigma_n - \varsigma_c] d_r \tag{1}$$

where: d: stocking density of a: average industry , n: no antibiotics (broker), c: controlled (agency), r: rest, $\varsigma_{:i}$: share of production for r: rest, c: compensation and n: no-antibiotics

The stocking densities "d" (for c and r) will soon be determined (endogenously) and we will relate the average densities to expected antibiotics involved in production. This is important for the creation of WTP. Note, in the first segment, d^n is fix, no antibiotics are applied; as said this is correlated with highest cost in animal rearing (though these higher unit costs are enabled by premiums). In the second segment, stocking density is a matter of negotiation (as bound to compensation) and in the third segment farmers freely decide. To decide freely means that no real standard prevails on stocking. The government's role is the following: it has to decide about allowed antibiotics as standard (in segment "r" farmers declare being member r). The explanatory equation contains average costs as function of shares of economies of scale costs:

$$c^a = \varsigma_n c_n + \varsigma_c c_c + [1 - \varsigma_n - \varsigma_c] c_r \tag{2}$$

where: c: average costs of a: average, n: no antibiotics (brokerage), c controlled (agency), r:

rest, ς: share of production for …

This specification parallels Equation (1). From the two Equations (1) and (2) we seek to determine the average costs. We assume the cost function for all produces has a quadratic expression as usually used in supply analysis (i.e. costs increase by produce and this is over-proportional: concave); but we blend it with economies of scale. Equation (3) states that coefficients of curvature are dependent on stocking density. Economies of scale are given as follows:

$$C^a = \gamma^a_{10} q_t + 0.5[\gamma^a_{10} + \gamma^a_{11}] q_t^2 \quad \text{and} \quad \gamma^a_{11} = \gamma^a_{110} + \gamma^a_{111} [1 - r_t \,/\, q_t] \tag{3}$$

where additionally: q: production of t: total produce, r: room for animal (space).

$$C^a = \gamma^a_{10} q_t + 0.5[[\gamma^a_{10} + \gamma^a_{110}] q_t^2 + \gamma^a_{111} r_t \, q_t] \tag{4}$$

The joint function, given in Equation 4, contains the technology parameters, economies of scale as animal density and production volume as determining variables. This cost function is based on parameter "r" depicting economies of scale in technologies as well as depends on marginal and average costs being subject to decisions on scales. For unit (average) costs it is:

$$c^a = \gamma^a_{10} + 0.5[[\gamma^a_{10} + \gamma^a_{110}] q_t + \gamma^a_{111} r_t] \tag{5}$$

Type (5) determination of average (unit) costs, in combination with Equation (2) and both equated, gives a reliance of meat industry as stated, dependent on scale technology as equilibrium:

$$\gamma^a_{10} + 0.5[[\gamma^a_{10} + \gamma^a_{110}] q_t + \gamma^a_{111} r_t] = \varsigma_n [c^c_n - c_r] \varsigma_n + \varsigma_c [c_c - c_r] \varsigma_c + 1 c_r \tag{6}$$

Further, if (as well since we have constant average costs) we have c^c_n / d^c_c, we get:

$$d^c_n \gamma^a_{10} + 0.5 d^c_n [[\gamma^a_{10} + \gamma^a_{110}] q_t + d^c_n \gamma^a_{111} r_t - d^c_n [c_c - c_r] \varsigma_c + d^c_n c_r = [c^c_n - c_r][[d_c - d_r] \varsigma_c + d_r] - d^a] \tag{7}$$

In principle, Equation (7) offers a delineation of average stocking density based on market shares; coefficients

are settled. We need this later for negotiation modelling and WTP.

$$d^{a} = \gamma_{10}^{*} + \gamma_{11}^{*}\varsigma_{c} + \gamma_{12}^{*}q_{t} + \gamma_{13}^{*} + \gamma_{14}^{*}\{c_{c} - c_{t}\} + \gamma_{15}^{*}c_{r} + \gamma_{16}^{*}d_{c} + \gamma_{17}^{*}d_{r} \quad (7')$$

For interpretation of Equation (7'): looking at average costs and stocking density simultaneously and linking those to antibiotics use (where density is a measure of probable use intensity), a linear constraint prevails for representative producers. This is important for bargaining. In modelling, Equation (7') enables us (i) to reduce the number of variables in negotiations from the side of producers, (ii) limits choices in regards to markets, and (iii) enables us to find an analytical solution. For Equation (7') all coefficients should be easily retrievable (by econometrics), i.e. from market settings in which segments are optimized for local and total economies of scale, i.e. where "d_r and c_r" are maximum and technology given. The market shares are yet free of choice and indicate sizes of operation in terms of references. In particular, the corresponding stocking density for residual "r" can be (is) set by government as maximum which is the immediate choice of producers in this segment. Stocking density in the no-antibiotics case "n" (as fixed coefficients) is also given technology-wise. Recursively we only have to determine stocking density in the controlled segment "c" as d_c by bargaining, i.e. endogenously (subject to the negotiation with the agency). In terms of institutions FSA fixes it.

Then market share "ζ_c" of controlled production will be also determined by the agency based on its cost-benefit analysis and available budget (compensation money, see below), i.e. it is in the capability of FSA to actually control the industry in size including transaction costs and its capability counts for success based on achievements, recursively, by budgets given to it. The central choice variable (i.e, variable in the negotiation) is stocking density d_c of animal population in the controlled segment and this makes calculations for achievable average cost c_c, in the segment and total industry important. This last aspect is chief for compensation calculation and the trigger to change in the system. Internally, the industry costs c_c becomes negotiable, been grounded in compensation. Compensation is envisaged as gearing the system and part of any negotiations with producers. But, since not a classical market prevails also "prices" are indirectly negotiated, i.e. overcompensated as a likely effect based on power. Power in negotiations depend on market volume q_r (expressed as ζ_r) in other segments. All production volumes and given shares (n, c, r) can be linked to sales with brokers. Because of that we start with brokerage. Now finally d^a is the policy variable (government) since it expresses antibiotics use.

6. Objective Functions of Participants (Producer, Retailer and Safety Agency)

As segmentation creates markets (options) and shares (negotiation) we have to establish objective functions. Objective functions are to be specified for (i) producers, (ii) FSA, and (iii) brokers (working with retailers. In our bargaining, i.e. as retrievable game, consumers, retailers and government are passive. Also it is not a game in an analytic fashion of Myerson (1991); rather it looks for recovering parameters from revealed negotiations by reconstruction of objective function and optimization: Zusman, 1989, the game is indirect). A priority is given to interaction and negotiations. At the same time certain variables are to be put in a position to be parameters for negotiation. Parameters are to be forged as mutually conflicting and are expressed in modes enabling calculations of instant costs and benefits. It means, at times, producers receive offers from brokers and the FSA, which are to be informed on acceptance.

6.1 Objective Function of the Producer

We start with a producer who makes choices on marketing (channels), delivers meats designed for segments "n, c, r", i.e. priority choices in marketing on basis of cost-benefit calculations, as well as negotiated parameters (compensation for controlled produce and sharing premiums).

$$\Pi = R^{n} - C^{n} + R^{c} - C^{c} + R^{r} - C^{r} \quad (8)$$

where additionally R: revenue: n: no antibiotics (brokerage), c controlled (agency), r: rest

Again note as assumed in segment "n", here no economies of scale do prevail towards stocking density and decision is on market shares. In segment "c" economies of scale are moderated through limitations (reduction through bargained control for lower stocking density which is reducing antibiotics use) which increase costs and in segment "r" there is no control.

With respect to the idea of having primarily a bargain on stocking density in segment "c" this has to be modelled negotiating space (density) per animal. For simplicity, industry decisions are taken in combination with each other. This means we assume that each producer produces can serve potentially all market segments. Then we can write the objective as function (8):

$$\Pi = R^n - c^n q^n + R^c - c^c q^c + R^r - c^r q^r \tag{8'}$$

Inserting of the above cost specification (Equation 3) gives:

$$\Pi = R^n - c^n q^n + R^c - [0.5[[\gamma_{10}^c + \gamma_{110}^c] + \gamma_{111}^c 1/r_0^c[1 - r^c/q^c]]][q^c]^2 + R^r - c^r q^r \tag{8'}$$

Consecutively, Equation (8') then delivers:

$$\Pi = R^n - c^n q^n + R^c - 0.5[[\gamma_{10}^c + \gamma_{110}^c] + \gamma_{111}^c[q^c/r^c]]q^c + R^r - c^r q^r \tag{9}$$

And the objective can be further explained in shares of the market segments

$$\Pi = [p^n + p^c + p^r - [c^n \varsigma^n - 0.5[[\gamma_{10}^c + \gamma_{110}^c] + \gamma_{111}^c[q^c/r^c]]\varsigma^c - c^r[1 - \varsigma^n - \varsigma^c]]q^t \tag{10}$$

Objective function (10) reduces the no. of options for negotiation towards: market shares of meat in the premium ζ_{ci}, stocking density r_{ci}, and share in premium increase 1-υ_i (for individual producers knowing υ_i is commission). Stocking density is linked to compensation. Finally, some remarks on residual q_r and total production q_t: they are implicitly integrated as free optimization and as outside to the negotiation. Since the intention is to model bargaining and not decisions in total, we consider production decision secondary to industry and consider it exogenous. This not applies to premium's market share where quantities are determined by consumers and government will. To complete the analysis, the task of a broker is to negotiate that share, i.e. yet it has to be integrated. This implies that price p_n (as been observed by producers) is variable (in fact a market analysis is needed); it is part of negotiation (optimization in bargaining). Because price increases are shared (see above), finally, the objective can be stated as:

$$\Pi_i = [(p^c + [p_s^n - p^c][1 - \nu_i] - c^{n,r})\varsigma^n + (p^c - c_{10}^c + \gamma_{11}^c d^c)\varsigma^c - p^{r,net}]q^t \tag{11}$$

In other words: variables in negotiations are (i) market share of meat ς^n (as 1-ς^c-ς^r: sold with no-antibiotics), (ii) stocking density (now as d^c, derive from r^c and c^c linked to d^c) and (iii) share of controlled produce ς^c (determined by FSA: where we see interaction and sequential optimization). Likewise (iv) there is scope for improved revenue (1-υ) that is dependent on brokers' activities in marketing/promotion. The rest of production (share in production capacity) is implicit and the prices for the rest and compensated market are unchanged (export) prices.

6.2 Introductory Remark on the Role of Marketing Units for Reduction

Remember we spoke of creating marketing channels and promoting negotiations as a tool to reduce stocking density and antibiotics, i.e. going for "less antibiotics" (synonym for having less production of "rest meat") at industry level. Now issues in "designing bargains" emerge. First, a question is: how to include public concern for health? Second, channel 3 (rest) which produces uncontrolled food is the problem. It indirectly matters and the aim is to reduce its shares. So how can a broker provide incentives and how is this mathematically retrievable? He is a master player in terms of premium (given in Equation 12 in vein of Zusman, 1989); i.e. channelling better meat prices to producers. His interest is gaining commissions. He ensures (i) no antibiotics are used, (ii) promotes sales and (iii) offers farmers premiums. The intermediary FSA instead is controlling (stocking, not markets); FSA is needed to calibrate alternatives at lower level commitments and to offer alternatives in negotiations by giving fixed payments.

6.3 Broker

Additionally to revenues (i), which are determined by the shares which can be achieved in premium meat, the broker's objective function (working through retailing) shall comprise (ii) costs of promotion, inspection, handling, etc. and market share (iii) determine volumes:

$$B = (p_s^n - p_c)\nu_i[\sum_i \varsigma_i^n q_i^t)] - \zeta_b s_b - C_b(\sum_i \varsigma_i^n q_i^t)\varsigma_n^n \tag{12}$$

where additionally: ν: sharing of price increases

s: search and promotion (costs)

C_b: cost of control by the broker for the purpose of quality assurance (no antibiotics)

To a large extent, the objective function (12) fits with that of Zusman (1989) for a broker. An amendment is control costs of antibiotics (on stocking density and animal health as transaction cost). In general terms the broker takes care of inspection and internalizes transaction costs. We assume brokers receive a licence by government through label approval. Then the average application can be accumulated and additional costs are included based on "d[a]":

$$B = (p_s^n - p_c)v_i\,[\sum_i \varsigma_i^n q_i^t)] - w_g^n d^a - \zeta_b s_b - C_b(\sum_i \varsigma_i^n q_i^t)\varsigma_n^n \quad (13)$$

To supplement the approach the premium price is a function of "sensitizing markets": $p_s^n = f_s(s_b)$ (promotion and guaranteeing). Such sensitizing is similar to Zusman's (1989) search task. Yet, negotiations with farmers are on market shares ς^n which shall increase. The issue involved is solidifying consumers' trust in no-antibiotics meat or reducing average use.

6.4 Food Safety (Assurance) Agency

The objective function of the FSA is more complicated to establish than the brokers' one. "Establishing" refers to behavioural arguments which are not "natural" in this case. FSA shall work on behalf of a government, but may as well be constituted formally as non-profit organization (eventually association: as legal entity) and a question is how it "behaves". This means that meat processors and traders can join an association which is professionally managed by a board along interest. The board has a budget. Budgets improve status and set rules for trading.

So what modelling option do we have for FSA? FSA can be considered as a bureaucracy licensed by government and being supported by industry as long as interests are met. We assume FSA receives a budget derived from WTP for reducing antibiotics (health care) based on technology (stocking density). I.e. there is a causality chain from health [less antibiotics], along performance [technologies] to characteristics [stocking density]. FSA maximizes its budget given the condition of balancing costs and benefits (Niskannen, 1971: see in Diagram 3). FSA makes no profits; instead revenues shall be equated with costs. Revenues are modelled as incoming WTP for reduction of antibiotics specified as dependent on stocking density. To exemplify: Diagram 3 shows a downward sloped marginal WTP, i.e. offers are optimized (negotiable/monitored). The issue behind in modelling is how to obtain a mathematical outline linking budget maximization and compensation to stocking density. Note, it is not a social optimum; but interests shall be created. We look for improvement and action instead of blocking.

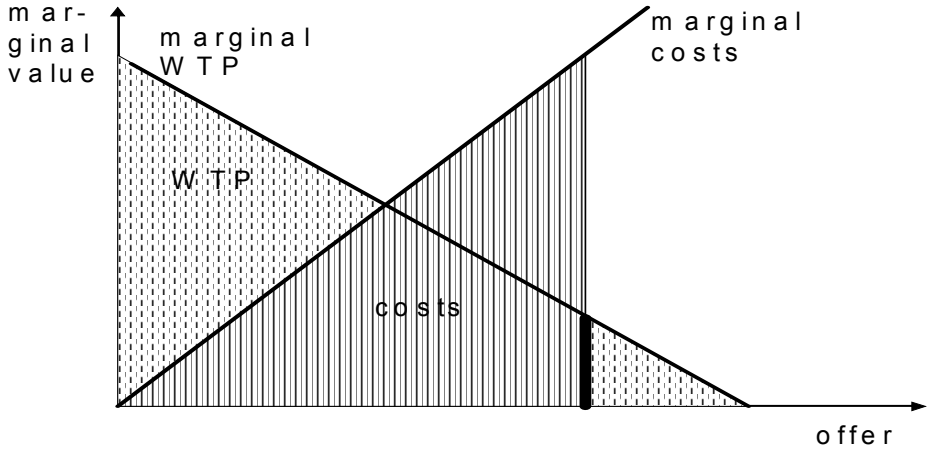

Diagram 3. Bureaucracy behaviour according to niskannen

In order to make things operational: WTP can be enumerated within two scenarios: (a) where stocking density reduction is feasible based on quantified measures (compensated against current state of high antibiotics and its costs; i.e. it changes by improved farming practices complying with administrative control), or (b) a scenario of WTP for straight low future antibiotics use which would be per unit and achieved by transparent accounts of antibiotics at farms. For both, marginal WTP in principle could be formulated and this gives a "welfare" function,

i.e. if marginal payment is stated. We take (a) assuming lower transaction cost involvement. We here base WTP on stocking density as reduction (from current average level of industry, giving government checks, easy measuring, approval, etc.) for a release of the budget:

$$WTP = -\theta_{10}^{w}[\sum d_i^a - \sum d_i^r] + 0.5\theta_{11}^{w}[\sum d_i^a - \sum d_i^r]^2 \quad (14)$$

Revenue (from public WTP) and cost (compensation as WTA plus transaction costs: personal, monitoring costs, etc.) can be modelled for FSA by the thought as equating costs and revenue. If stocking density reduction shall be $D=\Sigma d_i$, it gives money along a Niskanen (1971) concept (as in bureaucracies) expenditures. Cash receipts equal cost (now excl. own costs of operation, labour costs, laboratories, etc. being proportional to units) if no profits prevail. Whence the budget is maximized; cost equal revenue is a constraint. In actuality costs incurred may be more complicated to measure. Let us stick to a simple mathematical outline, though we are aware there are further costs (information, special control costs, new laboratory costs, etc., not only compensations and expenditures as in accounting). Nevertheless we summarize them all into transaction costs of FSA: $C_A = C_A(D^a,S)$. Also to make things less complicated for modelling we assume that costs are linear, i.e. revenues and costs are a function of D and w, where w is unit cost: this allows us to split services of FSA (measured as reduction of density $[D-D_r]$) and compensation; all multiplied with w (unit costs), i.e.: $D \cdot w$. For expression see Equation (15):

$$R - E = S_0 D_{b0} + [S_0 - S\ ]D_{b0} + S\ [D_b - D_{b0}] - c_c^a D_i \quad (15)$$

where additionally: D: average density in the industry. S or s_a: subsidy equivalent for unit costs which can be expressed as average subsidy.

Establishing the objective function as integral over marginal revenue (WTP), giving the constraint as well as maximizing, we apply a Lagrange expression (16, derived from reduction from WTP, as measure for D). This is firstly done for the agency at the summarized level $D = \Sigma d_i$:

$$A = -\theta_{10}^{w}[D_b^a - D_b^r] + 0.5\theta_{11}^{w}[D_b^a - D_b^r]^2 + \lambda[s_{a,0}D_{b0}^r + [s_{a,0} - s_a]D_{b0}^r + s_a[D_b^a - D_{b0}^r] - c_c^a D_b^a] \quad (16)$$

where (16) is the FSA's objective. For optimization of D and shadow price we get:

$$\partial A / \partial D_b^a - \theta_{10}^{w} + \theta_{11}^{w}[D_b^a - D_b^r] + \lambda[s_a - c_c^a] = 0 \quad (16a)$$

$$\partial A / \partial \lambda = [s_{a,0}D_{b0}^r + 0.5[s_{a,0} - s_a]D_{b0}^r + s_{a,0}[D_b^a - D_{b0}^r] - c_c^a D_b^a] = 0 \quad (16b)$$

and the shadow price is a function of a reduced form

$$\lambda = \theta_{10}^{*} + \theta_{11}^{*}c_c + \theta_{12}^{*}s_{a,0} + \theta_{13}^{*}s_a + \theta_{14}^{*}D_{b0}^r + \theta_{15}^{*}D_{b1}^r \quad (16c)$$

Hereby λ and D are simultaneously optimized (solved). Inserting them in the initial objective function provides a residual objective function of FSA which is ready for bargaining with individual producers' reduction of density "Δd_i" as dependent on parameters set in bargaining.

Optimizing Equations (16) for FSA, i.e. dealing with multiple producers, was yet at industry level; but also it can serve as reference for individual bargaining. Industry-wide optimization tells the FSA (as reference) what can be achieved in terms of budget accumulation based on getting WTP for average reduction from the government. Hereby, FSA is an agent on behalf of the government and to a certain extent government is a principal, but it has delegated matters to FSA. We must further admit Equations (16a to c) do not provide the "social optimum". Nevertheless, because our societal "approach" is based on interests, it is "material" and pecuniary. Note if trading with individuals it is below possible achievements, some WTP is lost.

For (re-)construction of the FSA's interest function (i.e. bargaining with producers) on stocking density and also compensation of average costs, it is necessary to insert the average density (in reduced form as above) into the objective A (see 17):

$$A = -\theta_{10}^{*}[D_b^a - D_b^r] + 0.5\theta_{11}^{*}[D_b^a - D_b^r]^2 \quad (17)$$

Then, for the sake of later depiction of individual bargains (given generalized interdependencies between

average costs, stocking reduction and bargaining variables (such as individual average costs c_{ci}, reduction request d_{ci} and market share ζ_{ci}), the changes on density have to be broken down to individual contributions. Adding them and using market-wide relationships

$$D_b^a = \gamma_{14}^* \sum_i \{c_{ci} - c_t\} + \gamma_{16}^* \sum_i d_{ci} + \gamma_{10}^* + \gamma_{11}^* \varsigma_{ci} + \gamma_{12}^* Q_t + \gamma_{13}^* + \gamma_{15}^* c_r + \gamma_{17}^* d_r \quad (17')$$

we can now determine the objective function of FSA for bargaining at individual level. In respect of interactions with average costs, we get Equation (18). It brings in individual compensation for farmers as directed subsidy for producers in eye of society:

$$\begin{aligned} A = \theta_{10}^* [\gamma_{14}^* \sum_i \{c_{ci} - c_t - s_{ci}\} + \gamma_{16}^* \sum_i d_{ci} + \gamma_{10}^* + \gamma_{11}^* \varsigma_{ci} + \gamma_{1x}^* x_i - D_b^r] + \\ 0.5\theta_{11}^* [[\gamma_{14}^* \sum_i \{c_{ci} - s_{ci} - c_t\} + \gamma_{16}^* \sum_i d_{ci} + \gamma_{10}^* + \gamma_{11}^* \varsigma_{ci} + \gamma_{1x}^* x_i - D_b^r]^2 \end{aligned} \quad (18)$$

Yet Equation (18) is a function of (i) average costs c_{ci}, (where the spread of average costs shall be compensated by s_{ci}, which is the subsidy paid for density reduction Δd_i), (ii) individual reduction request Δd_i (associated with increase of costs), and (iii) market share ζ_{ci}. All these parameters can be determined in negotiations with individual producers on basis of delineating the marketing alternatives and jointly negotiating and fixing compensation in competition.

In fact, at individual level, the FSA shall address c_{ci} as dependent on Δd_i and look at changes fitting producers: c_{ci} - $c_{ci,0}$ = γ_{ci} [d_{ci}-$d_{ci,0}$]. There might be overcompensation and market shares are affected; but having additional information on how s_{ci} stimulates ζ_{ci} (at given [ζ_{ci}-$\zeta_{ci.0}$]=γ_{ci} s_{ci}) this helps finding solutions. Here we have to see interfaces with brokers. The broker's ζ_{bi} and residual market share ζ_{ir} add to (1-ζ_{ci}). It means ζ_{bi}+ζ_r =1-ζ_{ci}. Producers negotiate with partners indirectly and a quasi-competitive situation for service of stocking reduction is found.

7. Bargaining

Having clarified the interest functions and modes of conduct (i.e. how interests can be expressed for bargaining) procedures as dependent on parameter, features and routines of bargaining have to be specified. This can be done and exemplified as well as modelled as system optimization. In fact, it has to be shown how bargaining helps in achieving public goals (reduction of antibiotics and a cost-benefits), and we have to be put goals into an operational perspective (i.e. within a theory of political economy). We hereby refer to the theory of Zusman (1976 and 1989) which is based on cooperative games (Harsanyi, 1993). This theory links individual interest functions to a weighted "interests" of society. In his example on referenced bargain Zusman has shown how to put marketing decisions and negotiation on contracts into a mathematical framework. Though real simulations are needed for application, here we only can sketch them as illustration. In the paper we have worked out interest functions which fit the frame of Zusman (1989). So the coining of interest functions is ready for application. Any actor (broker, agency and producer) is modelled as having opposed interests on mutually exclusive parameters given choices and references for parameters, i.e. of competing options. In regards to producer/broker and FSA/producer it links objectives as references.

To sketch the idea as has been outlined by Zusman (1989), one can convey bargaining as a modelling (interactive optimization) with weights. Bargaining offers contract parameters in terms of measureable agreements and it shows how one can retrieve power coefficients from observable bargains (cooperation vs. non-cooperation, which is given in Diagram 4; the weights are the power coefficients). Technically bargaining is modelled as optimization and deriving a power function is done simultaneously. The aim of the power analysis is to obtain both, contract parameters and a power λ function (weights) based on positions of actors as references. Yet, referring to the political economy bargaining theory of Zusman (1976) for detail, it can come to an analytical solution of the above problem. In particular Zusman put emphasis on setting alternatives (references in Diagram 4) done as pairwise comparison. Usually, an outline on bargaining modelling is very theoretical. But such models offer simulations for compromises as dependent on variations in government regulations and institution.

As it is observable in Diagram 4, we need cooperation. Bargaining starts from a limited allocation of property rights (as opposed to a market solution of full rights) and it depicts power relationships involved in cooperative games. To reveal more of the theoretical background, note power and weighted cooperative actions (not social welfare) are derived from individual interests (prepared above). In cooperative games (Harsanyi 1993) a solution is offered where contracts (parameters) are endogenous. In particular, we need corner solutions to establish the

slope λ which is the power indicator and which serves for weighting interests. Corners are established by alternatives. Interest creation is broadly defined as: (i) "maximizing net gains from cooperation"; and (ii) cooperation with one marketing unit reduces cooperation with the other (a perspective of individual producers as well as links to markets by broker and the FSA, a "driving force" for cooperation becomes into play). This is enough for a construction of a joint welfare function (not a social one). This "welfare function" (19) is a weighted one.

In practice, negotiations (either of the broker/retailer and farmer for premium meat without antibiotics on sharing or of FSA and farmer for compensation for reduced stocking) are conducted based on knowledge of reference points gained in negotiations. They are chosen by producers mutually. Producers depict (know) choices with alternative trading partners entering new ones. Hereby, we see a similarity to Zusman, 1989. Finally power coefficients are calculated, given reference points for an ultimately explained situation of a Nash game. The crucial point is that such political bargaining models work along exchange of contractual parameters.

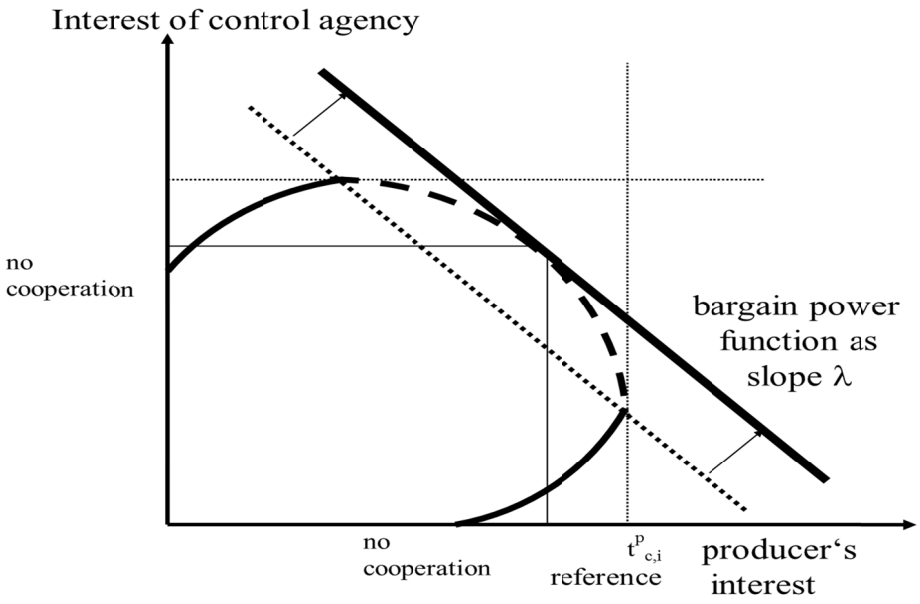

Diagram 4. Political bargain model and power measurement

Diagram 4 illustrates how power coefficients for "social welfare" are assembled as lines.

$$W = [\sum_i \Pi_i] + \theta_B B + \theta_A A \tag{19}$$

additionally: θ_i: power coefficients of A agency and B broker: The power of producers is (1- θ_A- θ_B

Such "social welfare function (19)" is technically obtainable; it is endogenous to the bargaining, not assumed. Power is given by the slope of the diagonal which intersects corner solutions in modelling of reference points for bargaining (given different initial rights as in a principal agent model). Partial optimization means slopes are zero for corners. If FSA has the right to set terms for a contract as a principle and a producer is an agent, we have the upper left corner and vice versa we have lower left corner. Finally, the result is a public "societal welfare function" which is optimized (Zusman, 1989). The optimization itself is thorny. Still, the important task here was to show how to find power coefficients. In other words, the crucial thing is to determine references in bargaining which can be done using the above interest functions.

To finally comment, there is no simple version of an institution which guarantees a social optimum per se; rather in case of collusion bargaining is a way. We have to work with second best institutions in reality; though it is feasible to find improvements. Lastly scenarios of bargaining, as in case of Zusman's marketing models, can be delineated and they are bilateral negotiations with references, constituted by marketing opportunities and governance (minimum standards on antibiotics). Hereby government can take an indirect position in creating power for the agency FSA. The proposition is: Bargaining equilibriums can be established which are superior to no-cooperation; i.e. selling of meat to corresponding market segments or alternatives is a partial solution and it is welfare improving. Those who have worked with Zusman (1989) know it is challenging to get drafts, but we can get it with mathematical procedures.

8. Summary and Discussion

This paper has developed a framework for bargaining on stocking density in the meat industry which is voluntary and aiming at reducing antibiotics use in case of economies of scale. It is involving marketing options and power. For this we developed a new institutional setup which is (i) based on existing literature of bargaining. It was argued that stocking density and antibiotics are correlated. (ii) Then we looked at market segmentation as option to stimulate interest in reduction of stocking density, i.e. in a cooperative game frame. (iii) As potential actors to negotiate with producers on stocking density, we introduced a broker (linked to retailing) and an agency (FSA) which, on behalf of a government, compensates farmers for not fully exploring economies of scale (stocking density). We elaborated on how to create interests by using market choices, share premiums and get compensation. It has been developed for markets with discretionary choices of farmers (market channels) and intermediaries (brokers & agency) and it is applied to the case of a meat industry. (iv) Then we showed how interest functions can be carved formally to obtain a game which can be and is partly mathematically presented.

Finally, we suggested how to proceed in a mathematical formulation and explained that the suggested frame fits into existing bargaining concepts such as those of Zusman, (1989). As a result of the paper we can say that there is scope to think about creating private and semi-autonomous institutions for reducing stocking density and antibiotics use in meat industries. A test would be whether stocking density works as agreeable proxy. As remark on stocking and negotiating parameters, alternatively one can think about an index comprising several other, weighed measures (changes: Agrarheute, 2014). This would imply making further agreements and control of measures and weights. Also, alternatively there are suggestions about differently financing a FSA (by surcharge for better images of meat), then from retailers (Germany: QS, 2013b; however this is not explored in this contribution, though feasible to model). It implies that FSA works similar, but cost-benefits change. The problem is then to re-specify WTP as public and private? It increases complexity, but is feasible. Especially the issue of transaction cost minimization should be further considered in searching for alternatives to govern the meat industry for health reason. One next task, for food safety policy research, would be to obtain simulations on likely parameter and link them to derivations from practice in bargains.

References

Agrarheute. (2014). Tierwohlinitiative: Erste Details bekannt. Retrieved from http://www.agrarheute.com/tierwohl-initiative-details. Internet accessed: 08.01.14.

Alanis, A, J. (2005). Resistance to Antibiotics: Are We in the Post-antibiotic Era? *Archives of Medical Research, 36*, 697-705. http://dx.doi.org/10.1016/j.arcmed.2005.06.009

Archie, E. A., & Theis, K. (2011). Animal behaviour meets microbial ecology. *Animal Behaviour, 82*, 425-436.

http://dx.doi.org/10.1016/j.anbehav.2011.05.029

Bailey, A.P., & Garforth, C. (2014). An industry viewpoint on the role of farm assurance in delivering food safety to the consumer: The case of the dairy sector of England. *Food Policy, 45*, 14-24. http://dx.doi.org/10.1016/j.foodpol.2013.12.006

Carlson, F., Frykblom, P., & Lagerkvist, C. J., (2007). Consumer willingness to pay for animal welfare: mobile abbatorirs versus transportation to slaughter. *European Review of Agricultural Economics, 34*, 321-344. http://dx.doi.org/10.1093/erae/jbm025

Chantziaras, I., Boyen, F., Callens, B., & Dewulf, J. (2014). Correlation between veterinary antimicrobial use and antimicrobial resistance in food producing animals. A report on seven countries. *Journal of Antimicrobial Chemotherapy, 69*, 827-834. http://dx.doi.org/10.1093/jac/dkt443

Coase, R., H. (1960). The problem of social costs. *Journal of Law and Economics, 2*, 1-44. http://dx.doi.org/10.1086/466560

Deckers, J. (2010). Should the consumption of farmed animal products be restricted, and if so, how much? *Food Policy, 35*, 97-503. http://dx.doi.org/10.1016/j.foodpol.2010.06.003

Deutscher, B. (2013). Bundesrat verabschiedet Arzneimittelgesetz: DBV warnt vor mangelhafter Praktikabilität und viel Bürokratie. Retrieved from http://www.bauernverband.de/bundesrat-verabschiedet-arzneimittelgesetz. Press-Information, accessed: 05.07.2013

Deutsche, B. (2014). Initiative Tierwohl geht an den Start. Brochure.

Die, Z. (2014). Tiermedizin Dauernd Stoff vom Arzt. Retrieved from http://www.zeit.de 2014/49/antibiotika-im-fleisch-tiermedizin

Eden, S., Bear, C., & Walker, G., (2008). Understanding and (dis)trusting food assurance schemes: consumer confidence and the 'knowledge fix'. *J. Rural Stud, 24*, 1-14. http://dx.doi.org/10.1016/j.jrurstud.2007.06.001

Estevez, I., Andersen, I.-L., & Naevdal, E. (2007). Group size, density and social dynamics in farm animal. *Applied Animal Behaviour Science, 1003*, 185-204. http://dx.doi.org/10.1016/j.applanim.2006.05.025

Fels-Klerx, H. J. V. D., Puister-Jansen, L. F., Asselt, E. D. V., & Burgers, S. L. G. E. (2014). Farm factors associated with the use of antibiotics in pig productions. *Journal of Animal Science, 89*(6), 1922-1929. http://dx.doi.org/10.2527/jas.2010-3046

Furubotn, E. G., & Richter, R., (2005). *Institutions and Economic theory. The Contribution of the New Institutional Economics* (2nd ed., p. 653). Ann Arbor: The University of Michigan Press.

Garforth, C., Bailey, A., & Tranter, R. (2013). Farmers' attitudes to disease risk management in England: a comparative analysis of sheep and pig farmers. *Prev. Vet. Med, 110*, 456-466. http://dx.doi.org/10.1016/j.prevetmed.2013.02.018

Goodwin, J. N., & Shoulders, C. W. (2013). The future of meat: A qualitative analysis of cultured meat media coverage. *Meat Science, 95*, 445-450. http://dx.doi.org/10.1016/j.meatsci.2013.05.027

Harsanyi, J. C. (1993). A simplified bargaining model for the n-person cooperative game. *International Economic Review, 4*(2), 194-220.

Korzen, S., Sandoee, P., & Lassen, J. (2011). Pure meat – Public perceptions of risk reduction strategies in meat production. *Food Policy, 36*(2), 158-165. http://dx.doi.org/10.1016/j.foodpol.2010.10.005

Lindgreen, A. (2003). Trust as a valuable strategic variable in the food industry: Different types of trust and their implementation. *British Food Journal, 105*(6), 310-327. http://dx.doi.org/10.1108/00070700310481694

Lusk, J. L., Norwood, F. B., & Pruitt, J. R. (2006). Consumer demand for a ban on Antibiotic Drug Use in Pork Production. *American Journal of Agricultural Economics, 88*(4), 1015-33. http://dx.doi.org/10.1111/j.1467-8276.2006.00913.x

Lusk, J. L., & Norwoord, F.B. (2012). Speciesism, altruism and the economics of animal welfare. *European Review of Agricultural Economics, 39*(2), 189-212. http://dx.doi.org/10.1093/erae/jbr015

Loo, E. J. van, Caputo, V., Nayga, R. M. (jr.), & Verbeke, W. (2014). Consumers' valuation of sustainability labels on meat. Food Policy*, 49*(1), 137-15. http://dx.doi.org/10.1016/j.foodpol.2014.07.002

Meat.Org. (2013). Retrieved from http://www.meat.org/ video

Myerson, R. (1991). *Game Theory: Analysis of Conflict*. (p. 569) Harvard University Press Cambridge, MA., First Harvard University.

Ngapo, T. M., Dransfield, E., Martin, J.-F., Magnusson, M., Bredahl, L., & Nute, G. R. (2004). Consumers perceptions: pork and pig production. Insight from France, England, Sweden and Denmark. *Meat Science, 66*(1), 125-134. http://dx.doi.org/10.1016/S0309-1740(03)00076-7

Niskanen, W. A. (1971). *Bureaucracy and representative government*. New Jersey.

Ollinger, M., MacDonald, J. M., & Madison, M. (2005). Technological Change and Economies of Scale in U.S. Poultry Processing. *American Journal of Agricultural Economics, 87*(1), 116-129. http://dx.doi.org/10.1111/j.0002-9092.2005.00706.x

Oude Lansink, A., & Reinhard, S. (2004). Investigating technical efficiency and potential technological change in Dutch pig farming. *Agricultural Systems, 79*, 353-367. http://dx.doi.org/10.1016/S0308-521X(03)00091-X

QS. (2013a). Retrieved from http://www.q-s.de

QS. (2013b). Mehr Tierwohl in der Schweine- und Geflügelwirtschaft. Pressemitteilung. Press Release Accessed 05.09.13. QS Qualität und Sicherheit GmbH.

Pew Commission. (2009). On Industrial Farm Animal Production. Retrieved from http://www.ncifap.org/

Rausser, G., Swinnen, J., & Zusman, P. (2011). *Political Power and Economic Policy. Theory, analysis and Emprical Apllications* (p. 624). Cambridge: Cambridge University Press. http://dx.doi.org/10.1017/CBO9780511978661

Requillart, V., & Soler, L-G. (2014). Is the reduction of chronic diseases related to food in the hands of the food industry? *European Review of Agricultural Economics, 41*(3), 375-403. http://dx.doi.org/10.1093/erae/jbu010

Rushen, J. (2003). Changing concepts of farm animal welfare: bridging the gap between applied and basic research. *Applied Animal Behaviour Science, 81*, 199-214. http://dx.doi.org/10.1016/S0168-1591(02)00281-2

Seals, M. (2012). Developing a Government-Industry Partnership on Animal Health and welfare Policy in England. *Eurochoices, 11*(2), 45-48. http://dx.doi.org/10.1111/j.1746-692X.2012.00234.x

Soerensen , J. T., & Fraser, D. (2010). On-farm welfare assessment for regulatory purpose: Issues and possible solutions. *Livestock Science, 131*, 1-7. http://dx.doi.org/10.1016/j.livsci.2010.02.025

Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., & Polasky, S. (2002). Agricultural Sustainability and intensive production practices. Review Article. *Nature, 418*, 671-677. http://dx.doi.org/10.1038/nature01014

TAZ. (2013). Fleisch aus Massentierhaltung: Ein Siegel für mehr Stallfläche. Retrieved from http://www.taz.de/Antibiotikum-im-Grundwasser/!149216/ accessed 12.03.15

Tonsor, G. T., & Wolf, C. A. (2011). On mandatory labelling of animal welfare attributes. *Food Policy, 36*, 430-437. http://dx.doi.org/10.1016/j.foodpol.2011.02.001

Top Agrar online. (2013a). Bundesrat verabschiedet neues Arzneimittelgesetz. Retrieved from http://www.topagrar.com/news/Home-top-News-Bundesrat-verabschiedet-neues-Arzneimittelgesetz-1192220.html

Top Agar online. (2013b). Erste Details zur Initiative Tierwohl bekanntgegeben. Retrieved from http://www.topagrar.com/news/Home-top-News-Erste-Details-zur-Initiative-Tierwohl-bekanntgegeben-1245739.html

Top Agar online. (2015). Tierwohl-Initiative: Das sind die Kriterien für die Teilnahme . Retrieved from http://www.agrarheute.com/tierwohl-initiative-das-sind-die-kriterien-fuer-die-teilnahme.

Top-Agrar. (2012). Antibiotika-Studie: Hat Remmel Betriebe mit Hähnchen verwechselt? Retrieved from http://www.topagrar.com/news/Home-top-News-Antibiotika-Studie-Hat-Remmel-Betriebe-mit-Haehnchen-verwechselt-710715.html, accessed 082.12.12

Torre, A. de la, Iglesias, I., Carballo, M., Ramírez, P., & Muñoz, M. J. (2012). An approach for mapping the vulnerability of European Union soils to antibiotic contamination. *Science of the Total Environment, 414*, 672-679. http://dx.doi.org/10.1016/j.scitotenv.2011.10.032

Vanhonacker, F., Verbeke, W., Van Poucke, E., Buijs, S., & Tuyttens, F. (2009). Societal concern related to stocking density, pen size and group size in farm animal production. *Livestock Science, 123*, 16-22. http://dx.doi.org/10.1016/j.livsci.2008.09.023

Wegener, H.C. (2001). Antibiotics in animal feed and their role in resistance development. *Current Opinion in Microbiology, 6*, 439-445. http://dx.doi.org/10.1016/j.mib.2003.09.009

Zusman, P. (1989). Peasants' Risk Aversion and the Choice of Marketing Intermediaries and Contracts: A Bargaining Theory of Equilibrium Market Contracts. In P. Bardahn (Ed.), *The Economic Theory of Agrarian Institutions* (pp. 297-310). Oxford. http://dx.doi.org/10.1093/0198287623.003.0015

Zusman P. (1976). The incorporation and measurement of social power in economic models. *International Economic Review, 7*(2), 447-462. http://dx.doi.org/10.2307/2525712

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