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Evaluation of Surface Enhanced Raman Spectroscopy (SERS) for Detection of Acetone in the Context of Food Safety and Quality Application

Punyatoya Mohapatra
Department of Agricultural and Biosystems Engineering
North Dakota State University, Fargo, ND 58108, USA
Tel: 1-701-367-4904 E-mail: punyatoya@gmail.com

Suranjan Panigrahi (Corresponding author)
Department of Agricultural and Biosystems Engineering
North Dakota State University, Fargo, ND 58108, USA
&
Department of Electrical and Computer Engineering Technology
Purdue University, Knoy 155, 401 N. Grant Street, West Lafayette, IN 47907, USA
Tel: 1-765-494-6908 E-mail: spanigr@purdue.edu

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Abstract

Development of sensors for detection of contaminated food products is important to ensure safety of the community. This paper focuses on detection of a compound, acetone associated with pathogen contamination and spoilage of beef in packaged condition. Surface Enhanced Raman Spectroscopy (SERS) technique was used to develop an integrated sensing system. This sensing system utilized laboratory prepared silver sol as the SERS substrate to detect acetone at low ppm and high ppb concentration. A set of eight experiments with two different experimental conditions were conducted. The first four set provided an estimated lower detection limit (LDL) ranging between 69.63 ppm and 630.17 ppm corresponding to the 1711 cm^{-1} and 532 cm^{-1} Raman shift (cm^{-1}) regions, respectively. The second four sets of experiments provided a lower estimated LDL ranging between 9.78 ppm and 43.45 ppm corresponding to 1222 cm^{-1} and 532 cm^{-1}, respectively. This study demonstrated the ability of this sensing system to detect the indicator compound.

Keywords: Surface enhanced Raman spectroscopy, Lower detection limit, Acetone, Food safety, Beef

1. Introduction

Physical and biological activities of pathogenic and spoilage bacteria that cause food safety problems produces metabolites. The metabolites generated in liquid and gaseous form accumulate in the headspace and absorbent sheath of the packaged beef. These metabolites consist of multiple compounds and these compounds can be used as indicators for determination of spoilage and contamination in packaged beef. It is to be noted that many of these compounds are present in trace in parts per million or parts per billion. Hence, detection of these compounds present in parts per million (ppm) or parts per billion (ppb) levels can help consumers detect quality and safety issues associated with packaged beef and reduce the risk of contamination and spoilage in food products. The current sensing techniques are time consuming, tedious, and prone to human error. Therefore, there is a need to develop a robust, reliable, and accurate sensing method to detect indicator compounds present

inside packaged beef.

This study was a part of a large project where we conducted a systems approach to address these issues from multiple directions. Thus, our larger study focused on developing sensors based on the compounds present in the gaseous and liquid metabolite. Some of our research related to the development of olfactory sensors based on gaseous metabolite are reported in (Balasubramanian et al., 2008), (Balasubramanian, Panigrahi, Logue, Gu, & Marchello, 2009), and (Amamcharla, Panigrahi, Logue, Marchello, & Sherwood, 2010). These sensors could constitute an integrated sensing system.

For the development of sensors related to detecting compounds present in liquid metabolites, we postulate that a versatile, portable, low cost SERS based sensor capable of detecting the individual compounds associated with pathogen contamination in beef would be beneficial. The proposed sensing system will be a component of the integrated sensing system consisting of multiple sensing components where each component will respond to different key indicator compounds. A preliminary study from our group indicated acetone as one of the compounds of interests associated with spoilage and pathogen contamination of beef inside the packaged beef (Bhattacharjee et al., 2010). Hence, development of a sensing system to detect acetone at low (10^2–10^3) ppm concentrations could be one of the components of an integrated sensor system. In this study, SERS technique using silver sol as SERS substrate has been used as a sensing system to detect target indicator compound (acetone) in the context of food safety application.

SERS is a rapid and a reliable technique that requires no sample preparation. Several studies have been conducted on identification of different types of liquid analytes for different applications. Silver-doped sol gel films coated on glass substrate exhibited strong enhancement (24000 fold) of Raman scattering of benzoic acid over a period of 48 hours (Y.-H. Lee, Dai, & Young, 1997). Another application of SERS involved the determination of cyanide in wastewater by low-resolution SERS on a sol-gel substrate. Laboratory prepared gold sol-gel substrate using 785 nm diode laser provided a limit of detection of 10 ppb (Premasiri, Clarke, Londhe, & Womble, 2001). Another study conducted by Norrod *et al.*(1997) compared five SERS substrates in terms of sensitivity and limit of detection. Vapor deposited silver films, electrochemically roughened silver electrodes, nitric acid etched silver foil , Tollens reagent produced silver film and photoreduced silver films on TiO_2 (Titanium oxide) were compared. Out of all of these, post deposition annealed silver films exhibited a limit of detection of 0.36 ± 0.02 femtomoles for trans-1, 2-bis (4-pyridyl) ethane (BPE). Another interesting application was the application of SERS for the identification of anthraquinone dyes used in works of art (Chen et al., 2006). Ag-Al_2O_3 SERS substrate was prepared by spin coating an alumina nanoparticle layer onto a glass slide followed by deposition of a layer of thermally evaporated silver nanoparticles on top of the alumina layer. This substrate provided an estimated limit of detection of 7 x 10^{-15} g for alizarin used as dye in the works of art. Recent applications reported the use of SERS for detection and discrimination of polychlorinated biphenyl (PCB), a commonly known environmental contaminant. Partition layer-modified nanostructured substrates were used to develop reusable SERS sensing mechanism for detection of PCB congeners (Bantz & Haynes, 2009). Studies showed that it would be possible to detect 50 pM of PCB without any instrument or substrate optimization.

A silver-coated polypropylene filter (AgPPF) was used as a highly sensitive and promising SERS substrate (Bhandari, Walworth, & Sepaniak, 2009). The study reported the use of SERS for the development of a reproducible SERS substrate for detection of environmentally significant compounds, which includes selected pharmaceuticals, personal care products, and possible endocrine disruptors. In this study, it was found that endocrine disruptors such as Apigenin and Daidzein, were detected down to 5 x 10^{-8} M and 1 x 10^{-7} M, respectively, without a preconcentration step. An important study conducted by Yaffe & Blanch (2008) discussed the anomalies in the SERS spectra of biological molecules caused by the use of a wide range of aggregating agents for hydroxylamine-reduced and citrate-reduced silver colloid SERS substrates. The study reported the effect of aggregating agents resulting in anomalous bands in the spectra of analytes. The study recommended the acquisition of SERS spectra of silver colloids and aggregating agent prior to the analyte to observe any peaks due to the silver colloid.

A recent study reported the use of SERS using silver colloidal nanoparticles for detection of azole, widely used as a corrosion inhibitor in copper (Pergolese, Muniz-Miranda, & Bigotto, 2008). This study compared the interaction of azole with silver colloidal nanoparticles with the normal Raman spectra. It was observed that analyte molecule reacted with the silver nanoparticles producing a shift in the Raman spectrum. This phenomenon was analyzed by density functional theory (DFT) calculations to detect azole.

From the above studies, it was observed that SERS provided fingerprint information about specific chemical compounds. This technique has been widely used for the detection of compounds at different concentrations.

However, no research has been reported on application of SERS for detection of specific indicator compound associated with pathogen contamination. Therefore, the objective of this study is the evaluation of a low cost Raman spectrometer sensing system using silver sol as SERS substrate for detection of target indicator compound, acetone present in low ppm concentration.

2. Materials and Methods

2.1 Preparation of Silver Sol (SERS substrate)

Silver sol was synthesized in the laboratory following a standard protocol described by Lee & Meisel (1982). Deionized water (100 ml) was heated on a stirrer hot plate to 45^{o}C. 18 mg of 99% silver nitrate ($AgNO_3$) was added to deionized water and the solution was brought to boiling. Upon further heating, 2ml of 1% trisodium citrate was added to the boiling solution. The solution was boiled for another 15 minutes. The resulting chemical reaction produced a greenish-grey colored sol. The silver sol was allowed to cool to room temperature before use. The sol had a maximum absorbance at 420nm with an average particle size of 50nm.

2.2 Design of Experiment

Four different concentrations of acetone; 800 ppm, 500 ppm, 200 ppm, and 100 ppm were prepared using the series dilution method. The blank was used as a standard to determine the lower detection limit.

A total of eight experiments, with two different experimental conditions were conducted. Each condition had four sets of experiments. The detailed experimental design is shown in Figure 1. An equal volume of acetone was mixed with silver sol and placed inside a quartz cuvette (Starna Cells Inc., CA, USA).The content of the quartz cuvette was shaken vigorously using a vortex mixer (VWR Scientific, IL, USA) to ensure proper mixing of the two components. Figure 2 shows the integrated Raman system for acquisition of SERS spectra of acetone with a schematic representation of the system alignment. The sample was placed in a cuvette and kept in proper position under the fiber optic probe. The incident light from the laser passed through the fiber optic probe and caused the excitation inside the sample. The excited beam was carried through the fiber optic probe and the raw SERS spectrum was collected. Figure 3 shows a flow chart describing the process to acquire the SERS spectra using the integrated SERS system. SERS intensity of peak height corresponding to eight peak wavenumbers was identified as a suitable feature for determination of LDL. A standard curve of acetone obtained from Enwave Optronics, Inc., CA, USA ("Enwave Optronics, Inc.," n.d.). was used to select peak wavenumbers as a feature for determination of LDL. Table 1 shows the eight different peak wavenumbers selected from the standard acetone curve as promisible feature for estimating LDL. The peak wavenumbers were 532 cm^{-1}, 766 cm^{-1},898 cm^{-1}, 1069 cm^{-1}, 1222 cm^{-1}, 1353 cm^{-1}, 1427 cm^{-1}, and 1711 cm^{-1} respectively. An estimated lower detection limit (LDL) was determined using peak height corresponding to the peak wavenumber as the feature. The detailed logic to estimate the LDL is described below:

The detailed logic to estimate the LDL is described below:

(1) The raw SERS spectrum (R) was collected for a wavenumber region λ^{-1}(0-1976cm^{-1}) for all the samples using *Spectra Suite* Software provided by (Ocean Optics Inc, USA).
(2) The raw spectrum (R) was smoothed by 5–point**,** Savitzky- Golay algorithm using *GRAMS-32* software. The smoothed spectrum (S) was saved for further processing.
(3) A zero offset was performed on the smoothed spectra (S) using the in-house developed Macro.
(4) Then offset spectrum (O) was divided into eight peak wavenumbers ($\lambda^{-1}_{i=1\text{-}8}$) based on standard acetone curve described in the above paragraph. A range of ±10 $cm^{-1}$was used to calculate the peak height (h) corresponding to the peak wavenumber (λ^{-1}).
(5) The peak height (h) corresponding to the peak wavenumber was used as a feature to calculate the estimated LDL.

Out of the several analytical figures of merit, lower detection limit, (LDL) is one of the most commonly used parameters to interpret the minimum detectable concentration. Various researches have reported the use of LDL to quantify the amount of analyte. Silver nanoparticles were used for detection of folic acid in water and human serum by Stokes *et al.* (2008). A LDL of 0.018 µmolar was reported at 514.5 nm. The LDL could be further lowered to 0.001 µmolar by increasing the accumulation time of the sample. A study conducted by Tripp *et al.* (2008) reported the limit of detection of virus ranging between 1–10 pfu by considering peak area under the curve for a stretching band of a functional group. Bao *et al*. (2003) have reported the use of silver–doped sol-gel film as a Surface- Enhanced Raman substrate for detection of uranyl and neptunyl ions. The calibration curve provided a detection limit of 8.5 x 10^{-8} M. Silver colloid in sol-gel derived xerogel was used for analysis of phenylacetylene and biphenyl. The substrate provided a lower detection limit of 9 ppb and 135 ppb for phenylacetylene and biphenyl respectively (Lucht, Murphy, Schmidt, & Kronfeldt, 2000). From the above

information, it can be observed that lower detection limit (LDL) is one of the commonly used analytical figures of merit to quantify a given concentration of the chemical. Hence, in the current work, LDL has been selected to determine the lowest concentration of acetone in a detectable region.

As per the qualitative definition, LDL refers to minimum concentration or mass of analyte that can be detected at a known confidence level. This limit depends upon the ratio of the magnitude of the analytical signal to the size of the statistical fluctuations in the blank signal (Long & Winefordner, 1983).The lower detection limit (LDL) was calculated using the following equation:

$$LDL = \frac{3\sigma b}{m} \quad (1)$$

where, LDL = lower detection limit, σb = Standard deviation of blank, m = slope of the curve

3. Results and Discussion

This study focused on detection of acetone at different concentration using silver sol as the SERS substrate. Silver sol produced an approximate enhancement factor of 56. The normal Raman spectrum of acetone showed a lower peak intensity of 750 intensity units as compared to higher peak intensity of $42x10^3$ intensity units corresponding to the same wavenumber of 786cm^{-1} (Figure 4) using silver sol as the SERS substrate. It was evident that SERS spectra of acetone showed more pronounced peaks as compared to the normal Raman spectrum of acetone.

3.1 Determination of estimated LDL for condition-1

As described in the flow chart provided in Figure 1, in condition-1, three replications were obtained on three separate quartz cuvettes. A sample plot of estimated LDL for condition-1 has been provided in Figure 5. It can be observed that a correlation coefficient of 0.91 exists between the SERS intensity and acetone concentration in, ppm. There is variability amongst the replications for a given concentration of acetone. This is explained by the use of three separate cuvettes for three different replications. The position of the cuvette below the fibre optic probe could also vary from one replication to another resulting in higher variation amongst the replication.

It can be observed from Table 2 that Expt-1, 2, and 4 provided lowest estimated LDL of 254.11 ppm, 198.04 ppm, and 69.63 ppm respectively corresponding to peak wavenumber of 1711cm^{-1}. However, Expt-3 provided the lowest estimated LDL of 189.25 ppm corresponding to 1069 cm^{-1} peak wavenumber. The correlation coefficients ranging between 0.55–0.90 were obtained across the four experiments performed under condition-1.

It was observed that 532cm^{-1} wavenumber of the spectrum provided the highest estimated LDL of 630.17 ppm and 1711cm^{-1} wavenumber provided the lowest estimated LDL of 69.63 ppm for condition-1.

3.2 Determination of estimated LDL for condition-2

As described in the flow chart provided in Figure 1, in condition-2, three replications were obtained on same quartz cuvettes. Estimated LDL was calculated for individual experiments corresponding to eight peak wavenumbers. Table 3 shows the estimated LDL for four different experiments conducted under condition-2. It can be observed that Expt 1, 2, and 4, provided the lowest estimated LDL of 29.05 ppm, 27.95 ppm, and 17.9 ppm respectively corresponding to peak wavennumber of 898 cm^{-1} . Figure 6 shows the lowest estimated LDL of 69.63 ppm obtained in condition-1 at 1711 cm^{-1} as compred to 9.78ppm LDL obtained in condition-2 at 1222cm^{-1}. Since, condition-2 had less variability amongst the replications; it resulted in a higher correlation coefficient ranging between 0.92–0.96 across the four different experiments.

It can be observed that particular wavenumber (532cm^{-1}) of the entire spectral region provided the highest estimated LDL and another specific region (898cm^{-1}) provided the lowest estimated LDL for condition-2.

3.3 Comparative study of condition-1 and condition-2 and implications on real world applications

A study was performed between the two experimental conditions to observe the variations of estimated LDL. It was observed that for a given peak wavenumber, 532cm^{-1}; condition-1 provided a higher estimated LDL of 630.17 ppm as compared to 43.45 ppm for condition-2. Comparison of data obtained from Table 2 and 3 shows that; condition-1 provided higher estimated LDL for all peak wavenumbers than condition-2.There are several possible reasons resulting in the higher variation in estimated LDL. One of the major reasons is the high sensitivity of the Surface Enhanced Raman spectroscopy technique. The reason for lack of reproducibility can be divided into two components; (a) instrument component and (b) substrate component.

(a) The instrument component consisted of the alignment of the sample, optimum focal length between the

fiber optic probe and the sample, and the lighting conditions. The cuvette was placed at an angle of 45^o with respect to the fiber optic probe to acquire the spectra. This angle was measured manually during each replication. The slightest displacement in the cuvette position can result in displacing the optimum focal length resulting in lack of reproducibility of the spectra. Proper lighting condition could be another factor responsible for widely scattered observations between the replications. Diffused light from other sources could interfere with the performance of the system resulting in poor reproducible spectra.

(b) The substrate component consisted of the nature and property of the SERS substrate. In this study, silver sol was used as the SERS substrate. Silver sol was prepared in a batch process. Hence, each experiment was conducted with a different batch of SERS substrate. This could have resulted in lack of reproducibility from sample to sample.

4. Summary and Conclusions

The present study was conducted to detect an indicator compound (acetone) associated with contamination and spoilage inside the packaged beef. Surface Enhanced Raman spectroscopy was used as a novel sensing technique to detect acetone at different concentrations. Silver sol was used as a SERS substrate to enhance the spectral signature and a customized portable Raman Spectroscopy system was integrated.

This study showed the potential ability of SERS as a sensing technique to detect acetone at different concentrations. A comparative study of two different conditions showed that condition-2 provided a lower estimated LDL of 9.78 ppm than 69.63 ppm for condition-1 .The variation within the replications due to separate cuvettes resulted in higher estimated LDL for condition-1.This variability was avoided in condition-2 by using single cuvette to acquire the replications for a given concentration. This study showed the potential of SERS sensing technique to detect acetone to an estimated LDL of 9.78 ppm. However, further scope of this study will involve evaluating the performance of other SERS substrates to further lower the LDL. Additional studies are recommended to further validate this finding on a larger data set and in real world packaged beef samples.

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Table 1. Peak wavenumbers of acetone obtained from standard Raman spectral signature. Source: http://www.enwaveopt.com/spectra.htm

Sl.no.	**Peak Wavenumber (cm^{-1})**
1	532
2	766
3	898
4	1069
5	1222
6	1353
7	1427
8	1711

Table 2. Estimated Lower detection limit (LDL) in ppm of acetone and the correlation coefficient (r) corresponding to eight peak wavenumbers for experiment 1, 2, 3 and 4 under condition-1(Three-replication three separate cuvette)

Peak wavenumber (cm^{-1})	**Est LDL (ppm) Expt-1**	**Corr Coeff (r)**	**Est LDL (ppm) Expt-2**	**Corr Coeff (r)**	**Est LDL (ppm) Expt-3**	**Corr Coeff (r)**	**Est LDL (ppm) Expt-4**	**Corr Coeff (r)**
532	**630.17**	0.74	461.69	0.80	198.49	0.86	508.60	0.85
766	592.71	0.55	356.53	0.85	204.99	0.84	443.56	0.88
898	448.00	0.74	311.88	0.87	190.51	0.84	347.29	0.90
1069	387.48	0.76	296.83	0.87	**189.25**	0.82	311.44	0.91
1222	330.60	0.77	274.31	0.90	201.58	0.76	258.29	0.91
1353	340.26	0.75	415.27	0.89	238.79	0.71	268.51	0.89
1427	336.69	0.75	278.06	0.91	216.29	0.75	258.30	0.90
1711	**254.11**	0.72	**198.04**	0.88	319.52	0.75	**69.63**	0.85

Table 3. Estimated Lower detection limit (LDL) in ppm of acetone and the correlation coefficient (r) corresponding to eight peak wavenumbers for experiment 1, 2, 3 and 4 under condition-2. (Three-replication single cuvette)

Peak wavenumber (cm^{-1})	**Est LDL (ppm) Expt-1**	**Corr Coeff (r)**	**Est LDL (ppm) Expt-2**	**Corr Coeff (r)**	**Est LDL (ppm) Expt-3**	**Corr Coeff (r)**	**Est LDL (ppm) Expt-4**	**Corr Coeff (r)**
532	**43.45**	0.92	35.5	0.95	27.06	0.95	26.89	0.96
766	40.79	0.92	32.91	0.95	24.15	0.94	24.03	0.95
898	**29.05**	0.93	**27.95**	0.94	13.76	0.93	**17.9**	0.94
1069	32.33	0.92	34.26	0.94	13.5	0.93	22.22	0.94
1222	33.54	0.93	29.62	0.95	**9.78**	0.93	19.18	0.95
1353	31.34	0.93	29.78	0.95	12.47	0.93	18.35	0.95
1427	30.33	0.93	30.37	0.94	12.6	0.94	18.53	0.95
1711	32.66	0.93	30.55	0.95	20	0.94	19.26	0.95

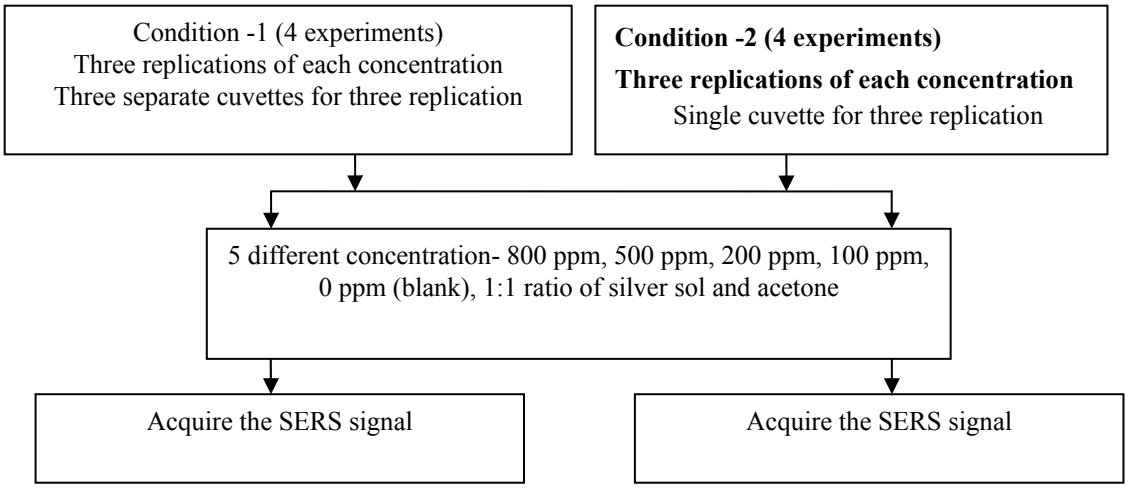

Figure 1. Experimental design for condition-1 and condition-2 of experiments

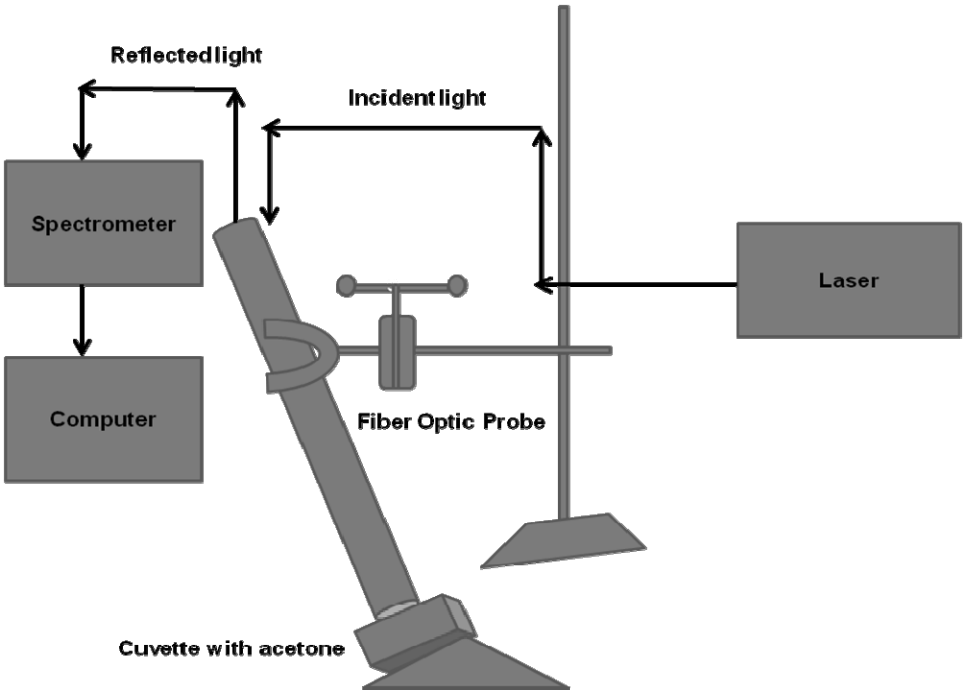

Figure 2. Schematic diagram of the SERS unit

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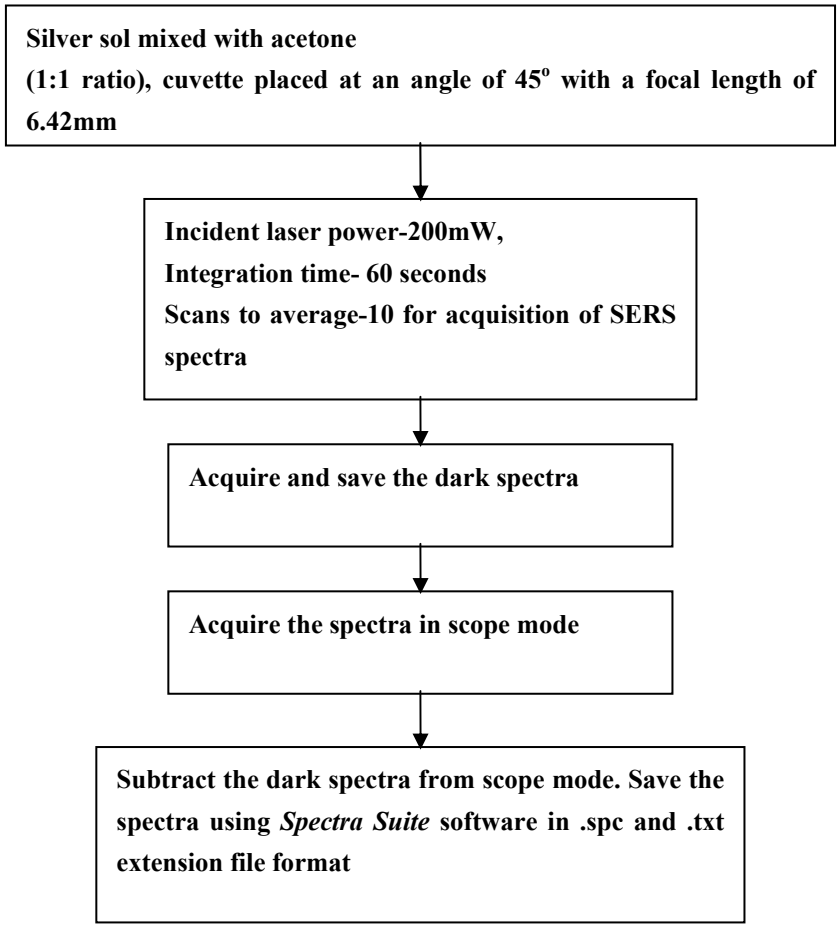

Figure 3. Flow chart for acquisition of Surface Enhanced Raman spectroscopy spectra using *Spectra Suite* software

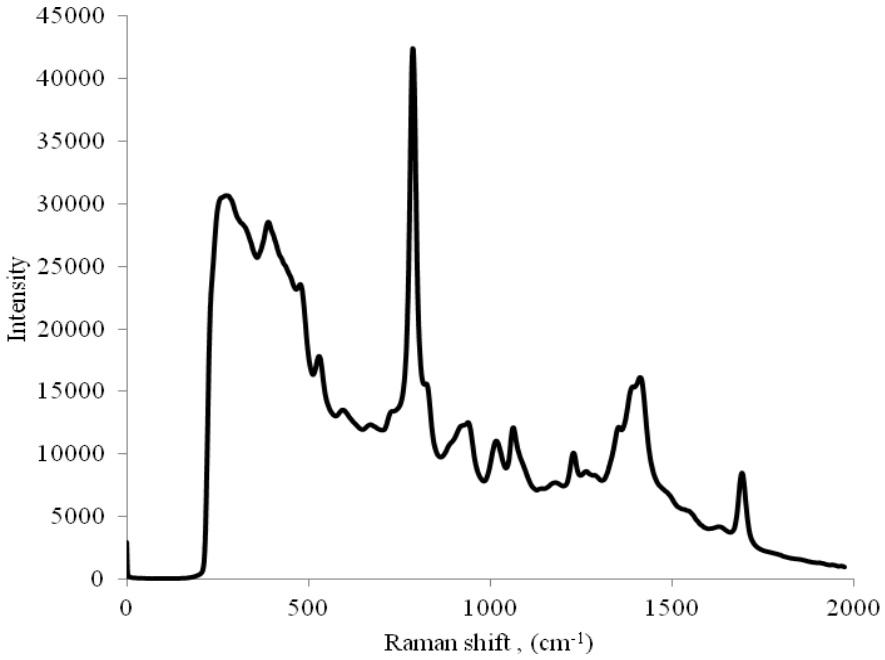

Figure 4. Spectral signature of acetone obtained using silver sol as SERS substrate

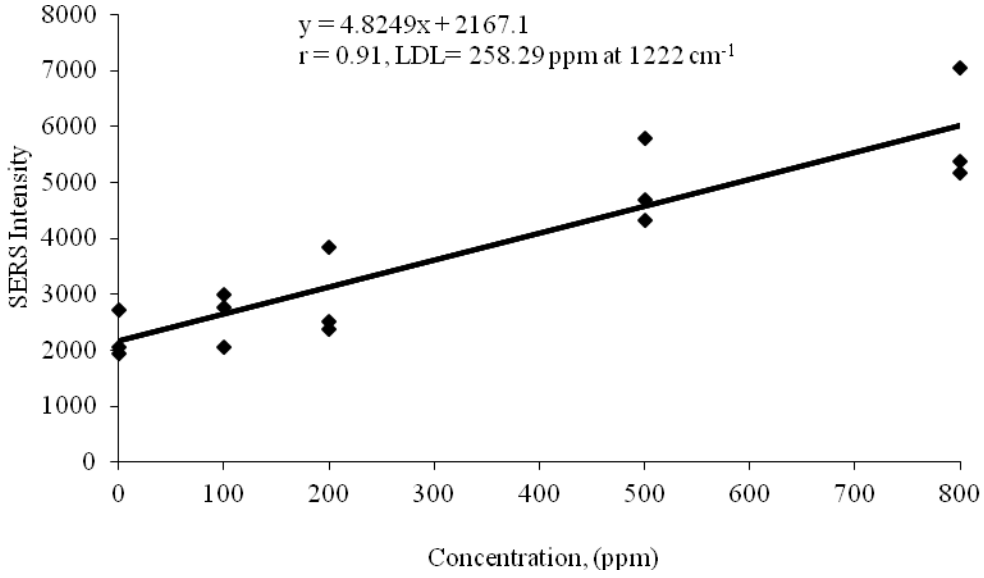

Figure 5. Estimated LDL of acetone at 1222 cm^{-1} for experiment 4 under condition-1

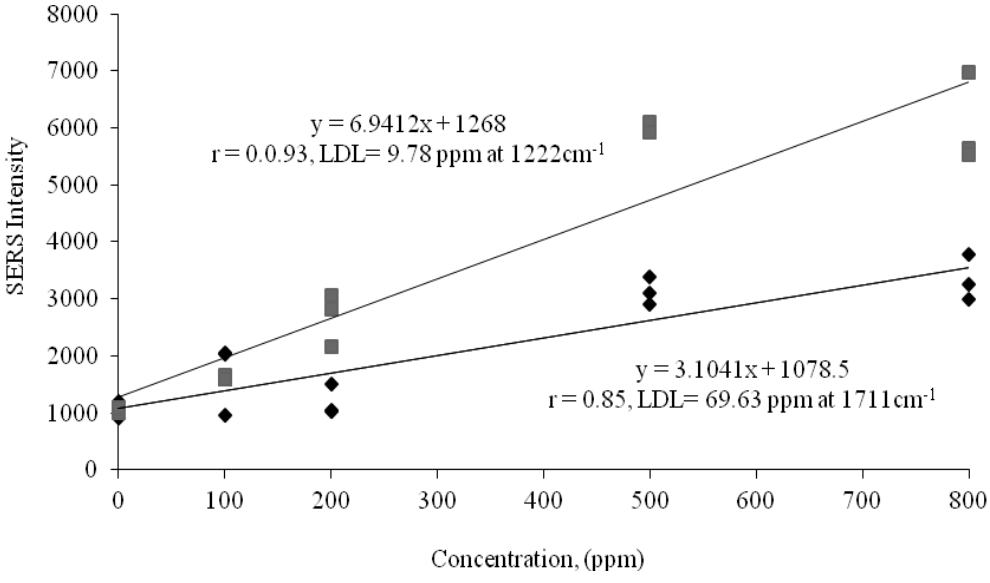

Figure 6. Lowest estimated LDL of acetone at 1711 cm^{-1} and 1222$cm^{-1}$for under condition-1 and 2 respectively

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Mixing Properties and Gluten Yield of Dough Enriched with Pea Protein Isolates

Samuel Mercier, Sébastien Villeneuve (Corresponding author), Martin Mondor, Hélène Drolet, Denis Ippersiel, François Lamarche & Louis-Philippe Des Marchais

Food Research and Development Centre

Agriculture and Agri-Food Canada

3600 Casavant Blvd West, Saint-Hyacinthe

Quebec J2S 8E3, Canada

Tel: 1-450-768-3335 E-mail: sebastien.villeneuve@agr.gc.ca

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Abstract

Over the last few years, many studies were carried out on the use of legume-based ingredients to supplement cereal-based matrices and produce nutritionally enhanced products. However, little is known about the influence of supplementation on the mixing properties of the enriched cereal-based matrices. The objective of this work was to study the impact of supplementing cereal-based matrices with commercial pea protein isolate or pea protein isolate produced by ultrafiltration/diafiltration using a 50 kDa membrane on the dough mixing properties. Studies were performed using a Perten® Glutomatic to estimate gluten yield, namely in terms of gluten index, wet gluten, dry gluten and water binding capacity, and using a Brabender® Farinograph to estimate water absorption, dough development time, stability, mixing tolerance index and minimum and maximum water content for dough formation. Four levels of pea protein isolate enrichment were considered: 0, 5, 10 and 15%. Results indicated that level of enrichment has little effect on measured mixing properties compared to the pea protein isolates considered. Isolate processed by membrane technologies takes part to the dough formation which does not seem to be the case with commercial isolate. Higher amount of water is required for dough formation with matrices enriched with commercial pea isolate compared to membrane processed isolate, while stronger dough properties are observed for matrices enriched with membrane processed isolate. This is attributable to the properties of the isolate, namely solubility and state of the proteins (native or denatured), which could impact how they interact with wheat proteins.

Keywords: Farinograph, Glutomatic, Mixing properties, Gluten yield, Pea protein, Durum wheat semolina

1. Introduction

Over the last few years, many studies were carried out on the use of legume based products to supplement durum wheat semolina and produce nutritionally enhanced pasta (Nielsen, Sumner & Whalley, 1980; Bahnassey, Khan & Harrold, 1986; Yanez-Farias, Bernal-Aguilar, Ramirez-Rodriguez & Barron-Hoyos, 1999; Zhao, Manthey, Chang, Hou & Yuan, 2005; Sabanis, Makri & Doxastakis, 2006; Shogren, Hareland & Wu, 2006; Torres, Frias, Granito, Guerra & Vidal-Valverde, 2007; Wood, 2009; Gallegos-Infante, Rocha-Guzman, Gonzalez-Laredo, Ochoa-Martinez, Corzo, Bello-Perez, Medina-Torres & Peralta-Alvarez, 2010; Petitot, Boyer, Minier & Micard, 2010; Mercier, Villeneuve, Mondor & Des Marchais, 2011). Supplementation with legume protein ingredients allows to improve pasta protein content and compensates wheat semolina deficiency in lysine and threonine, two essential amino acids (Kies & Fox, 1970; Abdel-Aal & Hucl, 2002; Sánchez-Lozano & Martínez-Llorens, 2009).

It was observed that enrichment affects pasta quality, namely in terms of texture (Nielsen *et al.*, 1980), color (Gallegos-Infante *et al.*, 2010), cooking quality (Zhao *et al.*, 2005), sensorial aspect (Alireza Sadeghi & Bhagya, 2008) and engineering properties (Mercier *et al.* 2011). The impact of fortification is dependant on the level of supplementation and on the legume from which origins the product (Zhao *et al.*, 2005; Petitot *et al.*, 2010).

However, little is known about the influence of the processing of the protein ingredients. Indeed, these ingredients can be made using various processes which can influence proteins structure and as a result the proteins functional properties including their solubility. For example, it is well known that soy protein isolate produced by isoelectric precipitation are more denatured and as a result have lower solubility than isolates produced by membrane technologies (Petruccelli & Anon, 1994; Wagner, Sorgentini & Anon, 2000; Rao, Shallo, Ericson, & Thomas, 2002). As observed by Ribotta, Edel Leon, Pérez & Anon (2005) in the case of soy proteins, the state of the proteins (native or denaturated) can impact their solubility and therefore their interaction with wheat proteins.

The objective of this work was to study the impact of supplementing cereal-based matrices with commercial pea protein isolate or pea protein isolate produced by ultrafiltration/diafiltration using a 50 kDa membrane. Effect of enrichment was studied on dough mixing properties and gluten yield, which are properties that are highly correlated to the final product quality, namely in terms of texture and cooking characteristics (Dexter & Matsuo, 1980; Ohm & Chung, 1999; Mason, Navabi, Frick, O'Donovan, Niziol, & Spaner, 2006; Oak, Sissons, Egan, Tamhankar, Rao & Bhosale, 2006).

2. Materials and Methods

2.1 Raw materials

Commercial pea protein isolate (CPPI) was purchased from Nutri-pea Limited (Portage La Prairie, MB, Canada). Isolate produced by membrane technologies (MTPPI) was made from certified #1 Eclipse Yellow peas purchased from Wagon Wheel Seed Corporation (Churchbridge, SK, Canada). Roller-milled durum wheat semolina was purchased from Horizon Milling (Montreal, QC, Canada).

2.2 Production of pea protein isolate by membrane technologies

Pea protein isolate was produced by membrane technologies as previously described by Taherian *et al.* (2011). The peas were first soaked in water (ratio 1:8 w/w) at room temperature for 90 minutes to swell them and detach the grain hull or cuticle so they would be easier to dehull afterwards. After soaking for 90 minutes in water, sodium hypochlorite (NaOCl) was added to the mixture until a concentration of 0.7 g/L was reached and the soaking was carried out for an additional 30 minutes. The peas were then recuperated and rinsed with water (ratio 1:8 w/w) to remove any residual sodium hypochlorite. They were dried overnight in a forced air 4-plate dryer (The National Drying Machinery Co., Philadelphia, Pennsylvania, USA, model 52979) operated at room temperature. Upon removal from the oven, the peas were ground using a Quadro Comil model 196 (Quadro Engineering Inc., Ontario, Canada) equipped with screen 002 for a first pass and with screen 009 for a second pass. The broken peas and their hulls were then passed through a multi aspirator (Kice, Wichita, Kansas, USA, model 6F6), where the hulls were removed from the top while the dehulled peas fell into the reservoir through the force of gravity. The dehulled peas were ground again using the Quadro Comil equipped with screen 106 to produce flour. The flour was placed in aluminium pouches which were hermetically sealed and stored at 4 °C until used.

For the production of the pea protein isolate, 7 kg of flour was dispersed in 105 kg of water (ratio 1:15 w/w) at room temperature and the dispersion was agitated for 30 minutes to allow the hydration of the flour. The pH of the dispersion was then adjusted to 7.5 using 2 M NaOH and the extraction was carried out for 45 minutes under agitation. If required, the pH was readjusted during the extraction. Once the extraction was completed, the insoluble matter was removed using a Basket Centrifuge (Western States, Hamilton, Ohio, USA, model STM-1000). In a first pass, a filter bag with a molecular weight cut-off of 60 μm was used while in a second pass, a filter bag with a molecular weight cut-off of 1 μm was used. The solution obtained after removal of the insoluble matter was then purified by ultrafiltration/diafiltration (UF/DF) using an home-made module equipped with two 50 kDa hollow fibre membranes with a surface of 2.3 m^2 for each membrane (Romicon (KOCH) model CTG 3", HF 25-60-PM50). The UF/DF sequence used to purify the pea extract was a UF step with a VCR 5 carried out at the extraction pH (i.e. 7.5) and a discontinuous DF step with a re-VCR 5 again at the extraction pH. The resulting pea protein isolate was lyophilized and placed in aluminium pouches which were hermetically sealed in stored at 4 °C until used.

2.3 Solubility of pea protein isolates

Protein solubility of both isolates was determined at pH 7 as follows: dry samples were rehydrated in bi-distilled water in order to obtain a 5% w/w solution (1 g of isolates in 19 g of bi-distilled water). The solutions were vigorously agitated for a few seconds using a vortex mixer and allowed to rest at room temperature for approximately two hours, with a second agitation after one hour. The samples were then centrifuged at 4000 g

for 15 minutes at room temperature. An aliquot of approximately 5 ml of the supernatant was taken, weighed with precision, and transferred to a Kjeldahl flask. Supernatant samples were mineralized and then titrated in the Kjeldahl flask, an official method for proteins determination. Protein relative solubility was determined by multiplying the fraction of protein in the supernatant over total protein content (dry basis) by 100. Moisture content was determined using AOAC Methods 925.09 (AOAC International, 1995) by drying samples overnight in a vacuum oven at 92 °C.

2.4 Farinograph analysis

Farinograph analysis was performed using two methods. The first method was the classical constant dough weight AACC Approved Method 54-21.02. This method measures and records the resistance of dough to mixing. It is used to evaluate the absorption of flours and to determine stability and other characteristics of dough during mixing. Results were expressed in terms of 6 parameters: (1) water absorption, which is the amount of water required to bring the dough to 500 FU; (2) dough development time, which is the time from water addition to the first sign of dough weakening in the range of maximum consistency; (3) stability, which is the difference in time between the points where the top of the curve reach and leave a constant torque line tangent to the middle curve at the point of maximum consistency; (4) mixing tolerance index, which corresponds to the decrease in Farinograph units (FU) between the top of the curve at maximum consistency and the top of the curve four minutes after maximum consistency was reached; (5) overmixing tolerance index, defined here as the decrease in FU between the top of the curve four and eight minutes after maximum consistency was reached; (6) bandwidth, which is the difference in torque between the top and bottom line as measured four minutes after peak time.

The second Farinograph method was a modified version of the continuous water addition method developed by Landillon, Cassan, Morel & Cuq (2008). Water was continuously added to the dough at a rate of 4 mL min^{-1} with a Masterflex peristaltic pump. Results were expressed as resistance to mixing (in FU) *versus* dough water content. Three parameters were determined: (1) minimum water content for dough formation, which was arbitrarily defined as dough water content when a 100 FU torque level was reached; (2) water content at maximum torque, which corresponds to dough water content at maximum torque; (3) maximum torque, which was the highest value of FU reached.

Both methods were conducted using a Brabender-E Farinograph equipped with a 50-g bowl (Model FA-R/2; Brabender Co., South Hackensack, NJ, USA). All Farinograph tests were conducted in duplicate with durum wheat semolina substituted at a 5, 10 and 15% level with CPPI and MTPPI.

2.5 Gluten yield

Gluten yield analysis was performed according to AACC Approved Method 38-12.02 using a Glutomatic (Perten Instruments, Huddinge, Sweden). Results were expressed in terms of gluten index, wet gluten, dry gluten and water binding capacity. Tests were conducted in triplicate with durum wheat semolina substituted at a 0, 5, 10 and 15% level with CPPI and MTPPI.

2.6 Statistical analysis

Analysis of variance was performed *a priori* on each parameter using SAS software (version 8.2, SAS Institute Inc.. Cary, NC, USA). Multiple comparison procedures (Least Significant Difference, $p = 0.05$) were performed *a posteriori* to compare parameters whose variance was significantly different.

3. Results

3.1 Pea isolates protein content and solubility

The protein content of the CPPI is 84.8 ± 0.4% dry basis, which is lower than the protein content of the MTPPI (96.1 ± 0.2% dry basis). However, the solubility of the CPPI is 6.1 ± 0.2%, which is approximately 13.8 times lower than the one of the MTPPI (85.1 ± 0.8%). This indicates that MTPPI proteins are mostly in their native states, while CPPI proteins are denatured.

3.2 Mixing properties

Typical data obtained using the Farinograph constant dough weight method, with 10% enrichment level, are presented in Figure 1. Dough with substituted CPPI showed a characteristic sharp peak and noise around dough development time (indicated by the vertical line below the curve), while data of dough containing MTPPI were smoother, without sharp peak. This suggests that the protein solubility has a more important impact on dough formation than the protein content of the isolates since the behaviour (shape of the curve in Figure 1) was strongly affected. It is well known that difference in protein content alone would not have affected the shape of the curve but only the intensity of the various parameters (Irvine, Bradley & Martin, 1961).

Measured Farinograph parameters are presented in Figure 2-7. Increasing durum wheat substitution level with both CPPI and MTPPI resulted in higher water absorption (Figure 2) compared to control ($P < 0.01$). This is the trend typically observed when flour or semolina is enriched with proteins (Wood, 2009; Sabanis *et al.*, 2006; Mashayekh, Mahmoodi & Entezari, 2008). Although MTPPI had higher protein content, it overall resulted in dough with lower water absorption than CPPI at an enrichment level of 15% ($P < 0.05$), but not at 5 or 10% level.

Dough development time (Figure 3) increased when CPPI or MTPPI was added to semolina ($P < 0.01$), but only at 5% substitution level, which is similar to the results reported by Ribotta *et al.* (2005) with soybean flour. However, the gluten network may not have been completely formed at the measured dough development time for semolina enriched with high level of CPPI. Indeed, curves were very unstable and wide around this point and another small increase in consistency was observed 3-5 min after dough development time for 15% CPPI enrichment level.

Enriching semolina with CPPI at a 5% level induced a sharp increase in stability (Figure 4) and a diminution of mixing tolerance index (Figure 5) compared to control ($P < 0.05$). Both of those properties are associated to strong flour or semolina. However, a reverse trend was observed at 10 and 15% enrichment level. Indeed, the Farinograph curves for 10 and 15% CPPI level showed just as good stability in the long run after the peak compared to 5%. This is supported by the decrease in overmixing tolerance index (Figure 6) for 10 or 15% CPPI level compared to control ($P < 0.05$). The opposite was observed with the MTPPI: overmixing tolerance index was higher than control when a substitution level of 15% was used ($P < 0.05$).

Dexter and Matsuo (1980) reported that bandwidth correlates well with gluten strength and spaghetti cooking and overcooking quality. Their results indicated that wide bandwidth is linked to strong gluten with good cooking and overcooking qualities. In this study, enrichment with both isolates resulted in lower bandwidth ($P < 0.05$) than control (Figure 7), which might be due to gluten dilution. Decline in bandwidth was more pronounced with MTPPI compared to CPPI ($P < 0.05$).

Dough mixing properties under continuous water addition are presented in Table 1. While the classical Farinograph method allows analysing dough resistance to mixing over time, this procedure allows to study the behaviour of dough at different moisture content. Minimum water content for dough formation was affected by the isolate ($P < 0.01$). MTPPI supplementation caused a decrease of this parameter compared to CPPI or to isolate-free semolina. This effect was enhanced at 10 or 15% enrichment level ($P < 0.01$). This indicates that semolina enriched with MTPPI requires less water for the development of the gluten network. Petitot *et al.* (2010) also reported a decrease in minimum water content for dough formation with durum wheat flour enriched with split pea of faba bean flour.

Similar results were obtained for dough water content at maximum torque. Enrichment with MTPPI resulted in a decrease in water content at maximum torque compared to control ($P < 0.05$), while enrichment with CPPI did not have a significant effect. The decrease in water content at maximum torque with MTPPI was independent of the level of substitution.

In terms of the maximum torque obtained, effects were observed for both the type of isolate and substitution level ($P < 0.01$). The addition of isolate resulted in dough with higher resistance to mixing at the water content of maximum torque, especially in the case of MTPPI.

3.3 Gluten yield

Parameters from gluten yield experiments are presented in Table 2. No results are available for CPPI at a 15% enrichment level because no gluten network developed during the mixing step in the Glutomatic. Similar observations were made by Ribotta *et al.* (2005) with durum wheat flour enriched with soy protein flour. However, a gluten network developed for each substitution level when using MTPPI. This indicates that the CPPI is more harmful to gluten formation than MTPPI especially at higher level of supplementation.

Gluten index represents the ratio of strong gluten to total gluten. Matsuo, Dexter, Kosmolak & Leisle (1982) and D'Egidio, Mariani, Nardi, Novaro & Cubadda (1990) indicated that gluten strength is an important factor influencing pasta quality. In the present study, gluten index was significantly influenced by the protein isolate ($P < 0.01$). Overall, gluten was stronger when semolina was enriched with MTPPI compared to control or dough enriched with CPPI. This effect was independent of the level of substitution. This result is due to the fact that proteins of the MTPPI have more interactions with the gluten proteins compared to CPPI, causing more important changes in the network properties.

Wet and dried gluten, when measured in terms of percentage of dry wheat semolina, were influenced by both the

type of isolate and substitution level ($P < 0.01$). Less gluten was retrieved when semolina was enriched with the MTPPI compared to CPPI or isolate-free semolina. Moreover, the difference was enhanced with high MTPPI substitution level. Thus, MTPPI retrieves some gluten from the network, causing it to pass through the Glutomatic screen with water and starch, which is not the case or at lesser extent for CPPI.

Water binding capacity, when expressed in terms of percentage of wet gluten, was not affected by the protein isolate or enrichment level. Thus, enriching semolina with pea proteins did not affect gluten capacity to hold water.

4. Discussion of the Impact of Supplementing Durum Wheat Semolina with Pea Protein Isolate

Enriching durum wheat semolina with protein isolate altered most dough properties as measured by Farinograph and Glutomatic. However, the impact was dependant on which isolate was used. Enrichment with CPPI did not impact gluten development, the minimum water content for dough formation and the water content at maximum torque while it increased the maximum torque. Enrichment with MTPPI resulted in an increase in gluten strength, and decreases in wet and dry gluten. It also required less water for the development of the network while it increased the maximum torque. In practice, these observations suggest that the MTPPI will take part to the network formation of pasta matrices while the CPPI would remain entrapped into the network limiting the amount of CPPI that can be supplemented into the pasta. In this context, previous work on the bread-making potential of the MTPPI clearly showed that bread matrix can support 10% substitution with MTPPI without harmful effect on the network (Des Marchais, Foisy, Mercier, Villeneuve, & Mondor, 2011). This resulted in bread with $20.9 \pm 0.1\%$ of protein which can be considered as high protein bread.

The impact of the two protein isolates is different due to the conformation state in which their proteins are. MTPPI making process leads to soluble proteins, while CPPI proteins were very less soluble. This indicates that the proteins presents in the MTPPI are less denatured than the ones in the CPPI. This impacts how pea and gluten proteins interact, similarly to what is observed in the case of wheat-soy protein interactions (Ribotta *et al.*, 2005; Roccia, Ribotta, Pérez & Leon, 2009). Pea proteins in their native state interact more directly with wheat proteins, thus explaining the increase in maximum torque and gluten index observed with MTPPI. Moreover, decreases in wet gluten and dry gluten are due to wheat-pea proteins interactions causing an increase in gluten solubility. On the contrary, proteins from CPPI alter dough properties more indirectly because of water competition and gluten dilution. To strengthen these observations, it would be of interest to perform some chemical analysis on the dough during its formation in order to assess the nature of the interactions between proteins. For example, the quantitative measurements of sulfhydryl (SH) and disulfide (SS) bonds would be of interest.

5. Conclusion

Level of dough enrichment with pea protein isolates (0, 5, 10 and 15%) has little effect on measured mixing properties compared to the pea protein isolate considered for enrichment. Pea protein isolate processed by membrane technologies takes part to the dough formation, which does not seem to be the case with commercial isolate. Higher amount of water is required for dough formation with matrices enriched with commercial pea isolate compared to membrane processed isolate or isolate-free dough, while stronger dough properties are observed for matrices enriched with membrane processed isolate.

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Table 1. Mixing properties of control and enriched dough under continuous water addition Farinograph method

Parameters			CPPI				MTPPI			
		Control	5%	10%	15%	Mean	5%	10%	15%	Mean
MWCDF	g-water	0.54	0.51	0.51	0.51	0.51	0.42	0.38	0.37	0.39
	g-semolina^{-1}	±0.00	±0.01	±0.03	±0.01	±0.02	±0.01	±0.02	±0.00	±0.03
WCMT	g-water	0.76	0.76	0.79	0.76	0.77	0.65	0.62	0.62	0.63
	g- semolina^{-1}	±0.02	±0.02	±0.03	±0.01	±0.03	±0.02	±0.01	±0.01	±0.02
MT	FU	521	539	608	687	611	787	>950	>950	>896
		± 17	±49	±48	±100	±86	±63			

Where MWCDF is the minimum water content for dough formation, WCMT is the water content at maximum torque and MT is the maximum torque.

Table 2. Gluten yield of control and enriched dough

Parameters			CPPI				MTPPI			
		Control	5%	10%	15%	Mean*	5%	10%	15%	Mean
GI	%	72.7 ±0.8	71.8 ±0.4	73.9 ±2.2	-	72.9 ±1.3	83.2 ±2.5	85.1 ±1.9	85.6 ±2.3	84.7 ±0.3
WG	%	48.3 ±1.0	49.1 ±0.6	47.7 ±2.2	-	48.4 ±1.1	44.4 ±0.3	44.4 ±0.5	40.0 ±1.1	43.0 ±0.3
DG	%	16.8 ±0.2	16.9 ±0.3	16.4 ±0.9	-	16.6 ±0.4	15.6 ±0.1	15.4 ±0.1	13.6 ±0.2	14.8 ±0.1
WBC	%	65.1 ±1.1	68.7 ±3.6	65.7 ±0.3	-	66.7 ±2.3	64.7 ±0.7	65.4 ±0.4	65.9 ±0.6	65.3 ±0.2

Where GI is the gluten index, WG is ratio of wet gluten to dry durum wheat semolina, DG is ratio of dry gluten to dry durum wheat semolina and WBC is the water binding capacity as % of wet gluten.

* Mean value was calculated from 5 and 10% data since no data were available at 15%.

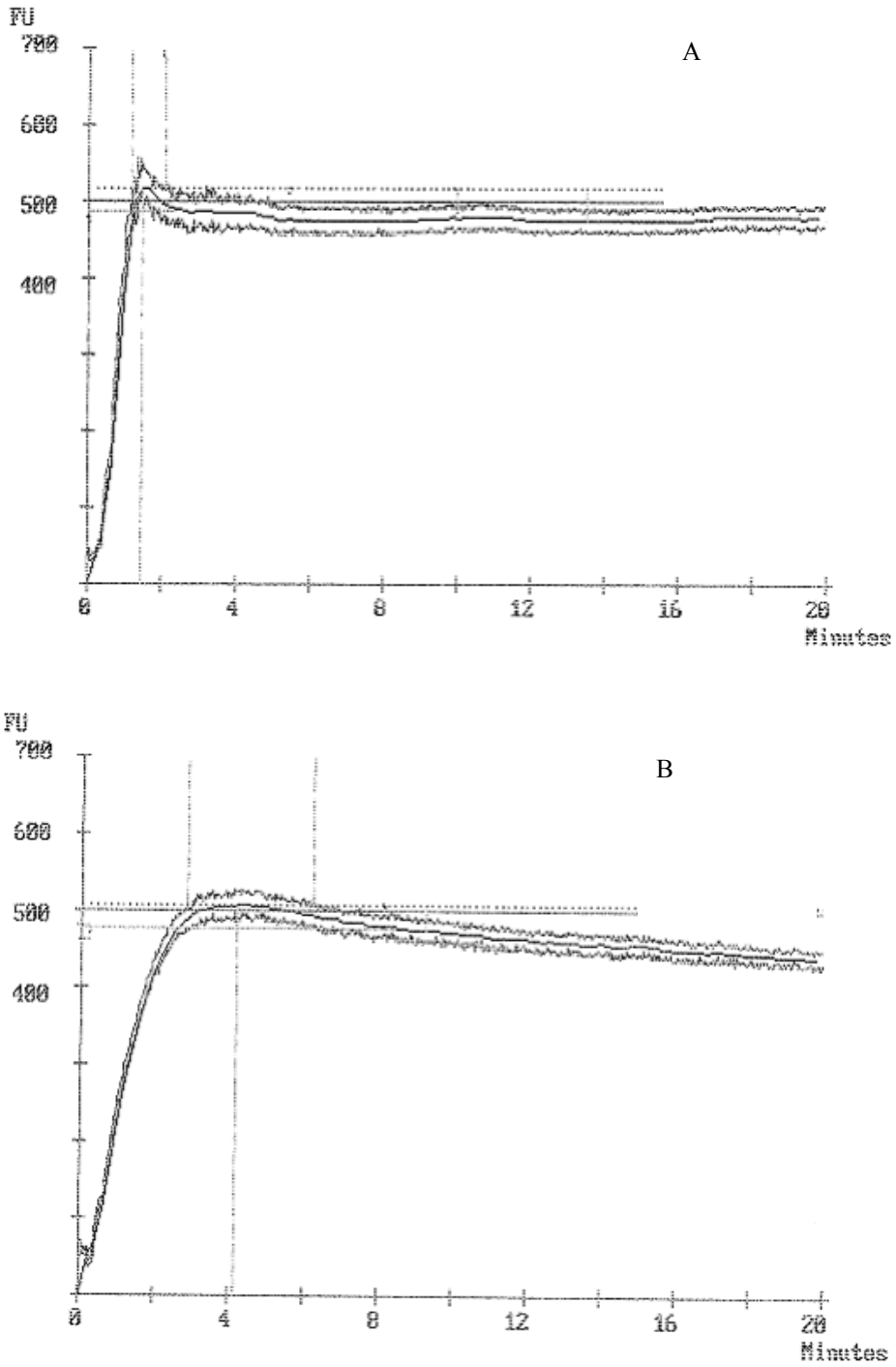

Figure 1. Typical experimental plot of resistance to mixing *versus* time for constant dough weigh Farinograph test with durum wheat semolina enriched with 10% CPPI (a) and MTPPI (b)

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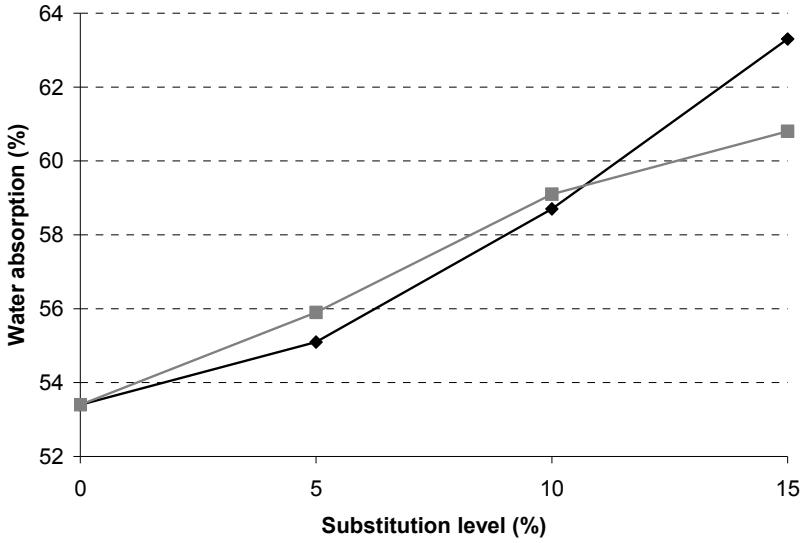

Figure 2. Experimental results of water absorption for Farinograph constant dough weight procedure with durum wheat semolina enriched with CPPI (black line) and MTPPI (grey line) at 0, 5, 10 and 15% level

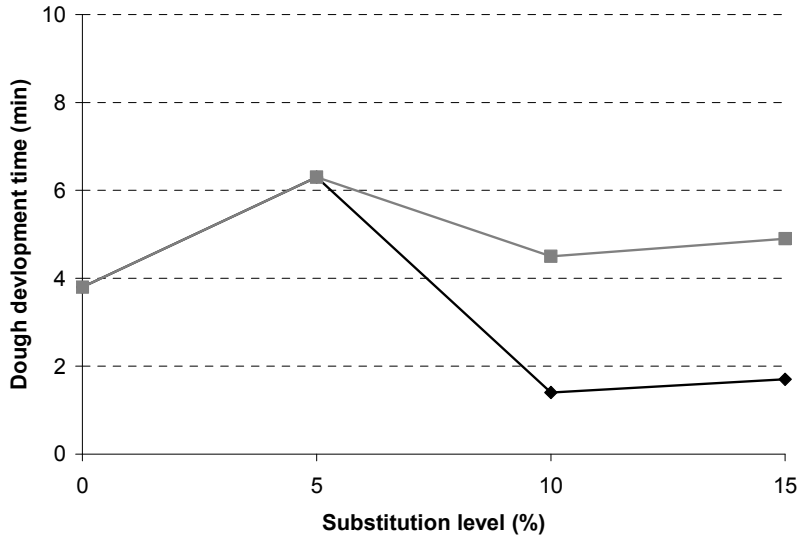

Figure 3. Experimental results of dough development time for Farinograph constant dough weight procedure with durum wheat semolina enriched with CPPI (black line) and MTPPI (grey line) at 0, 5, 10 and 15% level

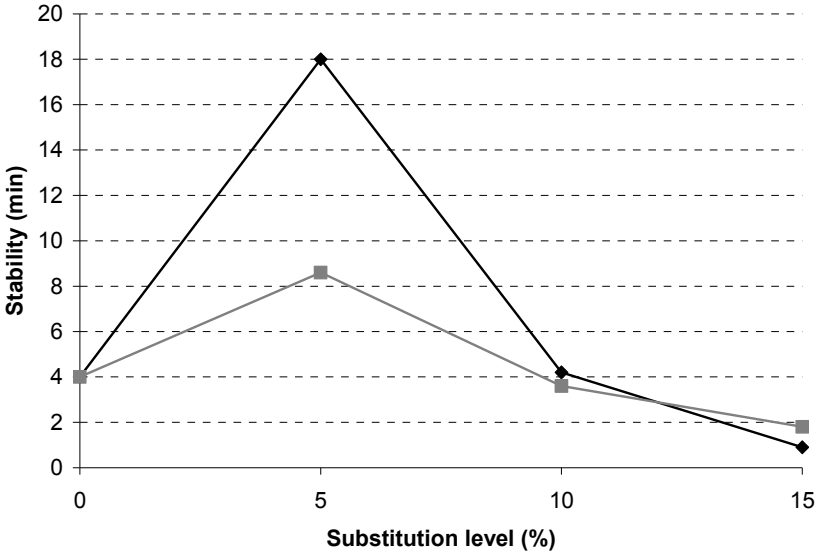

Figure 4. Experimental results stability for Farinograph constant dough weight procedure with durum wheat semolina enriched with CPPI (black line) and MTPPI (grey line) at 0, 5, 10 and 15% level

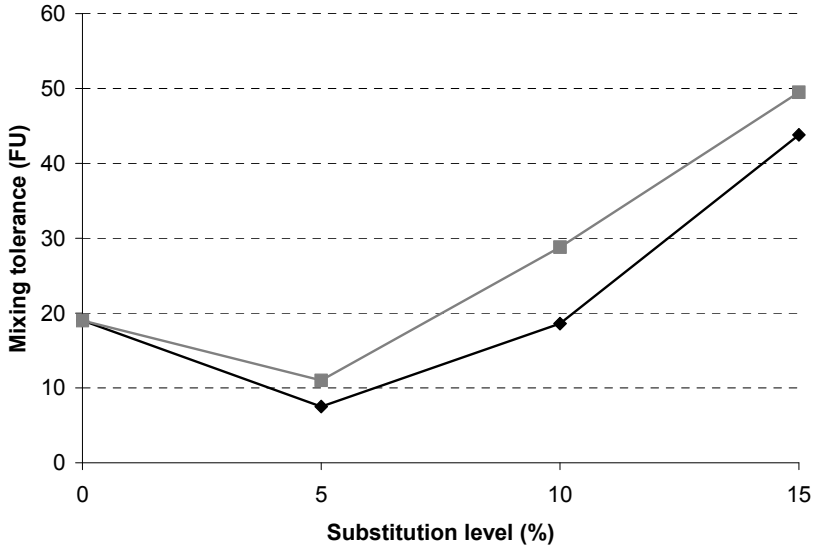

Figure 5. Experimental results of mixing tolerance index for Farinograph constant dough weight procedure with durum wheat semolina enriched with CPPI (black line) and MTPPI (grey line) at 0, 5, 10 and 15% level

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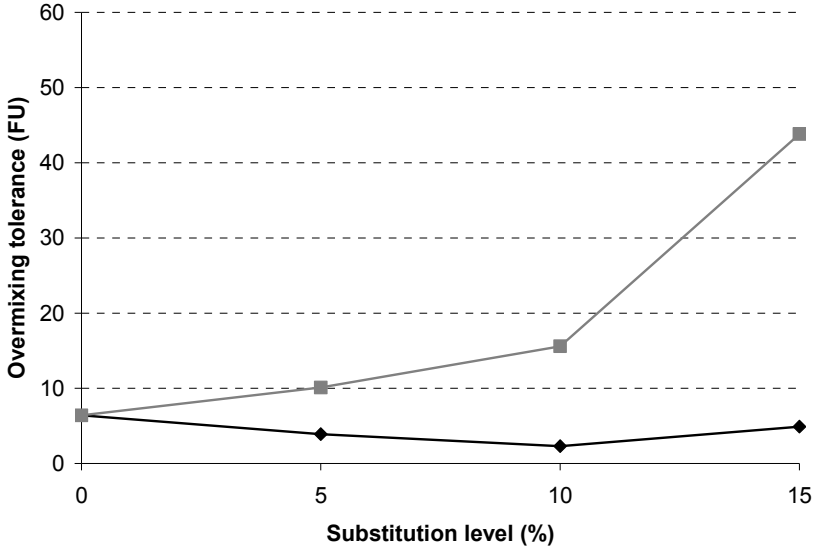

Figure 6. Experimental results of overmixing tolerance index for Farinograph constant dough weight procedure with durum wheat semolina enriched with CPPI (black line) and MTPPI (grey line) at 0, 5, 10 and 15% level

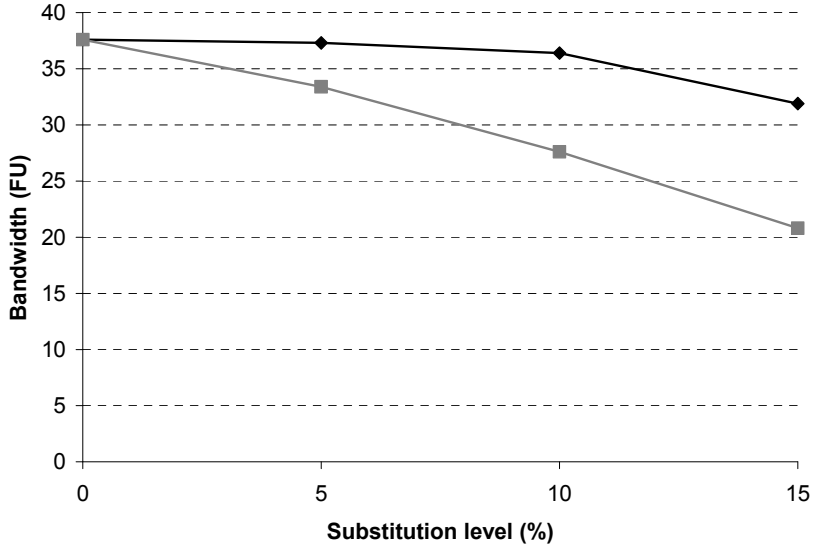

Figure 7. Experimental results of bandwidth for Farinograph constant dough weight procedure with durum wheat semolina enriched with CPPI (black line) and MTPPI (grey line) at 0, 5, 10 and 15% level

Impact of Instant Controlled Pressure Drop (DIC) Treatment on Drying Kinetics and Caffeine Extraction from Green Coffee Beans

I. Kamal
University of La Rochelle
Laboratory of Engineering Science for Environment(LaSIEFRE-CNRS 3474)
Avenue Michel Crepeau
La Rochelle 17042, cedex 01, France
E-mail: imustaph@univ-lr.fr

A. Gelicus
University of La Rochelle
Laboratory of Engineering Science for Environment(LaSIEFRE-CNRS 3474)
Avenue Michel Crepeau
La Rochelle 17042, cedex 01, France
E-mail: anthony.gelicus@univ-lr.fr

K. Allaf
University of La Rochelle
Laboratory of Engineering Science for Environment(LaSIEFRE-CNRS 3474)
Avenue Michel Crepeau
La Rochelle 17042, cedex 01, France
Tel: 33-546-458-766 E-mail: kallaf@univ-lr.fr

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Abstract

The present work is directed towards the impacts of Détente InstantanéeContrôléeDIC (French, for instant controlled pressure-drop) in terms of decaffeination and drying of Ethiopian green coffee beans (GCBs).DICconsisted in subjecting the product to a high-pressure saturated steam during some seconds and ended with an abrupt pressure drop towards a vacuum. A conventional aqueous extraction and a hot air-drying took place after DIC treatment. Inthis study, Response Surface Method (RSM) was used withDIC saturated steam pressure P, thermal treatment time t, and initial moisture content W asthe independent variables. Both direct DICextract recovered from the vacuum tank and the aqueous extracts wereanalyzed and quantified using the reversed phase-HPLC. With decaffeination ratiosas dependent variables, P and Wwere the most significant operating parameters; whilet was much weaker.Total decaffeination ratio could reach 99.5% after DIC treatment at specificconditions of W=11.00% db, P=0.1 MPa, and t=35swhile it was only 58% when achieved with untreated raw material.

The effective diffusivity D_{eff} and the starting accessibility δW_s were calculated from the diffusion/surface interaction kinetic model of hotair drying after DIC treatment. They dramatically increased with P and t while W had a weak impact.Thus, at the optimized DICconditions, D_{eff} and δW_s increased from 0.33 to 12.60 10^{-10}m² $s^{-1}$and from 0.75 to 11.53 g/100 g db, respectively. Drying time needed to reach 5% db became 60 min instead of 528 min for untreated raw material.

Keywords: Green coffee beans (GCBs), Instant Controlled Pressure Drop (DIC), Drying kinetics, Caffeine extraction, Reversed phase-HPLC, Response Surface Methodology (RSM)

1. Introduction

Coffee isthesecondpopular beverageworldwide, and is oneof the mostimportant commodities in international trade. The twowell-known species of coffee trees, which are Coffee Arabica and Coffee Robusta, present high commercial importance. They differ in chemical composition; Arabica contains more lipids and Robusta contains more caffeine and sucrose as well as the polyphenols antioxidant chlorogenic acid and its derivatives(Parliament and Stahl, 1995).

The term "green coffee beans" GCBsrefers to un-roasted mature or immature coffee beans. Caffeine content in green coffee beans varies markedly between species and within species (Robusta vs. Arabica), mainly depending on geographic origins (Rodrigues et al., 2007). It is reported that it ranges from 1.22% in Arabica to 2.54% dry basis (or g/100 g of dried coffee) in Robusta (Kyet al., 2001). Huge number of studies focused on coffee, its composition, flavor and quality characteristics (De Maria et al., 1996; De Maria et al., 1994; Sarrazinet al., 2000; Franca et al., 2005).

Caffeine (1,3,7-trimethylxanthine) paid lot of attention. Caffeine is one of the most notorious compounds because of its many physiological effects on the human body, and scientific studies have linked caffeine to the prevention of some related-age disease (Glade, 2010). However, caffeine acts as a central nervous system stimulant and also has negative withdrawal effects (Smith, 2002); as a result, decaffeinated coffee is being used to overcome its negative effects. Thus, from marketing point of view, the decaffeinated coffee green beans have an important economical value. Conversely, it was found that caffeine has had particular interest for pharmaceutical, cosmetic and satiety applications. Both caffeine extract and coffee intakes were modestly and inversely associated with weight gain (James et al., 2006).

Decaffeination processes are usually carried out in order to dramatically reduce the caffeine level.Decaffeinated coffee beverage should reach a caffeine level nearly 0.04 g/100 g of dried coffee (Fujioka et al., 2008). Literature survey showed huge information on the attempts used for decaffeination. Different factors having an impact on the decaffeination process include blend composition, brewing extraction rates, grind or water temperature (Maeso et al., 2006). Processes such as organic solvent extraction, with or without ultrasound treatment, have been used for decaffeination. However, it is difficult to completely remove the traces of solvent from end-decaffeinatedproducts and the highest level admitted by the international standards is increasingly restrictive (Wang & Weller, 2006). The final distillation stage would also lead to a great loss of light compounds. Other operations have used the supercritical CO_2 as solvent (Icen & Guru, 2009). Although attractive with low operating costs, the disadvantages of this extraction process are the costly facilities required and a cumbersome installation. Note that supercritical CO_2 process as well as microbial and enzymatic methods (Gokulakrishnan et al., 2005) are not selective and can extract not only caffeine but also volatile molecules (Clarke, 2003). The energy costs, together with low kinetics, render the main part of these extraction processes unattractive from an economic point of view.

Other caffeine extraction processes such as microwave-assisted extraction have been proposed (Wang et al., 2011). They offer some advantages regarding kinetics and preservation of the chemical composition, but as far as we know, no industrial development has been achieved and all applications are still at the laboratory scale. No work has used high temperature processes for decaffeination, although such operations would be interesting in coffee industry. Conversely, no steam decaffeination process has been defined or highlighted probably because of the relatively high boiling temperature of caffeine. However, it was noted that the roasting process altered the amount of caffeine in coffee bean. A common misconception is that the darker the roast level, the lower the caffeine content. A small amount of caffeine is lost due to sublimation. This occurs in caffeine at approximately 178 °C,however,thethermodynamic parameters of caffeine have been the subject of various studies (Griesser et al., 1999; Cuong et al., 2010; Emelyanenko & Verevkin, 2008).

In this context, it was proposed to carry out a study of the possible extraction of caffeine from the Ethiopian Green Coffee Beans GCBs using the innovative technology of Détente InstantanéeContrôléeDIC (French, for instant controlled pressure-drop) through a direct DIC extraction and an intensification of the environmentally friendly aqueous extraction thanks to the DIC-expansion.

DIC was defined in 1988 (Allafet al., 1988) as a high temperature-short time stage usually carried out by establishing high-pressure saturated steam-high temperature (0.1 to 0.7 MPa relating with 100 to 165 °C during 5 to 60 s). This is followed by an instant pressure drop towards a vacuum at about 5 kPa at a rate $\Delta P/\Delta t$ higher than 0.5 MPa s^{-1}. It simultaneously provokes an autovaporization of a part of the water and other volatile molecules within the product, and induces a swelling effect. An instant cooling of the products is capable to immediately stop

thermal degradation. Depending on the severity of the treatment, the structure can be slightly or greatly modified. Also, the operating conditions can preserve or break the cell walls. DIC has been studied, developed, optimized and used at industrial scale. DIC ensues as specificunit operations of decontamination (Mounir & Allaf, 2009), texturing (Kamal et al., 2008), and volatile compounds extraction from plants (Kristiawanet al., 2008). DIC also involves as a particular treatmentachieved in order to intensifyhot air drying, solvent extraction of non-volatile molecules such as flavonoids (Berka-Zougali et al., 2010), and other chemical or enzymatic reactions.

The technology was introduced to different sources of coffee beans prior to the roasting process. DIC treatment showed an interesting impact on expanding the bean microstructure (Kamal et al., 2008).

DIChas madeavery relevantextractionof essential oils,in spite of thehigh boiling pointof certain moleculesto about220°C. The most instructive studies were reported by(Kristiawanet al., 2008;Berka-Zugaliet al., 2010;Besombeset al., 2010), total extraction could be obtained in less than 4 min. Other research works proved that DIC used as a texturing pretreatment could dramatically intensify solvent extraction (Benamor & Allaf, 2009).

Works regarding the detection and quantification of caffeinein complex media can be found in the literature(Belay et al., 2008). Among them are theultraviolet-visible spectroscopy and its derivatives (Belay, 2010), Liquid Chromatography (Pistos & Stewart, 2004; Hywelet al., 2010), and gas chromatography (Espinoza-Perez et al., 2007). However, these works are focused on extraction and monitoring methods.

Finally, in terms of experimental design, we did not use in this study the one-factor-at-a-time approach, because it is time-consuming, expensive and doesn't allow evaluating the interaction effects among the various operating parameters. RSM (Response Surface Methodology) could overcome these difficulties by accounting the possible interaction effects between operating parametersin terms of various outputs. RSM has been successfully used to model and optimize biochemical and biotechnological processes related to food systems(Silva et al., 2007; Pompeuet al., 2009;Berkaet al., 2010).

2. Materials and Methods

2.1 Reagents and chemicals

All the materials used in this work are of analytical grade obtained fromFlukaChemie GmbH,Buchs, Switzerland.

2.2 Plant material

In this study, we used locally available Ethiopian green coffee beans.The first pretreatment involved grinding to 7-mm particles, whichwere then soaked in water bath at ambient temperature to reach predefined values of water content. Moisture content was measured using the oven method.Themeasurementswere triplicated and conducted on a 3 g of GCBs, placed in a thin layer glass capsule and dried in the oven at 105.5°C for 24 h. The moisture content was determined between the initial raw material value of11.00% ± 0.2% db (dry basis: g H_2O/100 g dry matter)up to 35 ± 0.2% dbfor soaked grains.

2.2.1 Protocol of treatment

In the present study, the treatment design was achieved as illustrated in Figure1.

2.2.2 Instant controlled pressure drop DICtreatment and equipment

DIC reactors have been presented in numerous articles (Besombeset al., 2010; Kamal and Allaf, 2011; Ben Amor et al., 2009).In the present work, the reactor used was a new developed version of DIC apparatus (Micro-DIC provided fromABCAR-DIC Process, La Rochelle, France) (Figure2). It is a 30 cc processing vessel, a 7-liter vacuum tank connected with a water ring vacuum pump allowing the pressure to reach 5 kPa. A pneumatic valve ensures an "instant" connection between the vacuum tank and the processing vessel; it can open in less than 50 ms.Other valves control the flow of steam and compressed air within the processing vessel. Figure 3 shows the different stages of a DIC treatment.

Preliminary experiments were performed to define the range of various operating parameters such as saturated steam pressure P, total thermal treatment time t andGCBinitialwatercontent W.In a second stage, DIC extraction was carried out using a specific experimental design to identify and compare the effects of saturated steam pressure, process (heating) time and initial moisture content, chosen as operating parameters (or independent variables). The extracted product was collected as an emulsion of water and other compounds from the vacuum tank. The amount of caffeine present in this emulsion was quantified and defined as the "direct DIC extract" to be considered as a dependent variable (response) of the operation.

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2.2.3 Solid – liquid extraction procedure

Reflux water distillation–extraction was used as a solid-liquid extraction technique to extract caffeine from the untreated and DIC treated GCBs. A mixture of 1 g of concerned beans, distilled water (20 ml) was heated inside a round button flask (50 ml) using a thermostatic bath set at 97 °C. The reflux distillation set-up was provided by magnetic agitation to ensure that the concentration of the extracted beans and the temperature of the solution were uniform within the flask. The crude extracts were filtered immediately after 20-min extraction using R-C membrane filter 0.45 µm (Sartorius Stedim Biotech GmbH/Germany) and tested for caffeine quantification using HPLC. This amount of caffeine we defined as the "20-min-97 °C aqueous extract" was considered as a dependent variable (response) of the operation.

2.2.4 Instrumentation and chromatographic conditions

A reversed phase HPLC system (Agilent Instruments, 1100 series, Massy, France) was used. The Chromatographic system consisted of a vacuum degasser on line with three solvent delivery pumps linked to a thermostatted column compartment via auto-sampler. The detection was carried out using a diode array UV detector (200–400 nm). Data was collected and processed using HPLCChemstation software program (Agilent, version B.04.01, Massy, France).

Caffeine crude extracts were separated on a Pursuit XRs Ultra C18 (2.8 µm), 150 ×2 mm (Varian - Agilent). The mobile phase A was a mixture of water/acetic acid and the mobile phase B was acetonitrile. Both of them were filtered through a 0.45 µm membrane filters. The elution program is outlined in table 1. The flow-rate was set at 0.4 ml/min and the column compartment was kept at a controlled temperature of 50±0.5 °C during all runs. The absolute pressure was 0.341MPaand the capillary voltage was 3 kV.

Quantification was carried out using calibration curve obtained with standard caffeine at 272 nm. For the spectrophotometric procedure, a standard curve was determined over y range of (0.05–9) µg/ml as analyte concentration*versus* x as the experimental measured area, with $R^2 = 0.99994$showing good adherence to Beer's Law:

$$y = -5 * 10^{-28}x^4 + 7 * 10^{-21}x^3 + 3 * 10^{-14}x^2 + 4 * 10^{-7}x - 7 * 10^{-4} \quad (1)$$

The experimental results were mentioned as average ± standard deviation (SD) of three measurements.

2.2.5 Drying process

AfterDICand aqueous extraction of GCBs, a conventional drying was achieved on a static-tray type with air flux at initial temperature of 40±1 °C, velocity of 1±0.2 m s^{-1}, and initial humidity of 265 Pa. A precision balance (AdventureOhaus Europe GmbH, Greifensee, Switzerland) with an accuracy of 0.1 mg was used for weighing the untreated and DIC treated GCBssamples. Before beginning the experiments, the dryer system was started in order to achieve desirable steady state conditions. The samples weight were measured after sequential drying intervals and the drying continued until no further changes in the sample mass were observed, e.g. to the final moisture content of about 5% db which was then taken as the equilibrium moisture content.

3. Fundamentals

3.1 High temperaturevolatile extraction

Some authors noted that roasting of GCBs is usually linked to a slight lose of caffeine by evaporation or sublimation. Such phenomenon never has been considered as complete heat extraction process.Fundamental approach carried out by Mounir and Allaf, (2009) assumes that heat transferredinto theplant considered as porous material mainly assures a phase change (liquid/vapor) with a partial pressure strictly depending on the temperature. The mass transport of volatile molecules from the grain surface to the surrounding environmentcan be intensified. Thatlet the internal heat and mass transfers become the limiting process.

The internal heat transfer is mainly processed by a similar conduction phenomenon. The porous structureincluding air,water and volatile molecules,allows vaporization-condensation phenomena inside the product holes.The global phenomenon is a conduction-type transfer where the gradient of temperature is conducted as the driving force with a higher value of effective conductivity. The main part of such heat flow is used to evaporate water and volatile compounds within the product holes:

$$-\lambda_{eff}\vec{\nabla}.\vec{\nabla}T + s_{abs}M_cL_c\frac{\partial}{\partial t}\left[\frac{p_c}{RT}\right] = 0 \quad (2)$$

The internal transfer of volatile compoundsis assumed to be negligible under a liquid phase, and achieved as puregas phase diffusion. It can be revealed by a Fick-type's law with the gradient of the partial pressure per temperature of each volatile compound as driving force. (Allaf, 1982):

$$\frac{p_c/T}{\rho_s}(\vec{v}_c - \vec{v}_s) = -D_{eff}\vec{\nabla}\frac{p_c/T}{\rho_s} \quad (3)$$

whereD_{eff} is the effective mass diffusivity.

3.1.1 Paradox of coupled internal heat and gas diffusion transfers

The partial pressures of water and other volatile compounds inside the holes of the plant considered as porous material closely depend on the temperature through the thermodynamic liquid/gas equilibrium of the molecule mixture. By neglecting the possible shrinkage phenomena, one can assume:

$$(p_c/T)\vec{v}_c = -D_{eff}\vec{\nabla}(p_c/T) \quad (4)$$

The gradient of p_c/T is then the driving force ofgasdiffusion (which is controlled by) and the transfer of heat to change the liquid into gas phase within the porous material,can be coupled to become, with one x dimension, as:

$$-\lambda\frac{\partial^2 T}{\partial x^2} + s_{abs}M_cL_c\frac{\partial\left[\frac{p_c}{RT}\right]}{\partial t} = 0 \quad (5)$$

$$(p_c/T)v_c = -D_{eff}\left(\frac{\partial(p_c/T)}{\partial x}\right) \quad (6)$$

However, as (p_c/T) is as higher as the temperature is higher,

$$\frac{\partial(p_c/T)}{\partial T} > 0 \quad (7)$$

the gradient of mass transfer is then directed from the surface to the sample core; the result is a paradoxical situation implying a motion completely opposite to that required for the sought extraction operation. In the usual heat extraction of volatile molecule from plant oils, the process is achieved by slow "front progression" kinetics.

3.1.2 Specificity of the DIC treatment

Depending on thehigh temperature level of DIC, the total pressure of the mixture vapor and volatile molecules in the porous material is, just after the pressure drops, much higher than the external vacuum pressure. Therefore, whatever the structure of the porous plant, the mass transfer operation within this porous matrix has to be performed from the core towards the surrounding medium through the gradient of the total pressures (Darcy-type law):

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$$\rho_v v_v = -\frac{K}{v_v}\frac{\partial P}{\partial x} \tag{8}$$

By considering mass balance and continuity laws, and by integrating between the hole (whose radius R_o) and the external radius R_s of the spherical shape, Allafet al., 2011 calculated the part m_m of vapor mixture removed as:

$$m_m = \frac{4}{3}\pi R_o^3 \frac{< M >}{RT}(P_v - P_{ext}) \tag{9}$$

Usually, the time t_v of the final vacuum DIC stage just after pressure drops is defined to allow the mixture vapor to be transported towards the surrounding medium and then collected.

DICis fundamentally relevant to remedy the disadvantages of the paradoxical stage and can greatly intensify the thermal extraction of volatile compounds.

3.2 Fundamentals of hot air drying kinetics

When external heat and mass transfers are not the limiting phenomena of the operation kinetics thanks to adequate air flow temperature and velocity, only internal transfers are considered as controlling and limiting the whole operation (Al-Haddad et al.; 2008). Mounir and Allaf (2009) havemodeled such dehydration kinetics, in the case of porous medium by couplingthe internal transfer processes with an initial superficial drying assumed to be the limiting process during the first part of the operation.

As usually internal mass transfer in vegetables is much slower than conduction heat transfer within the product, the drying kinetics is claimed as controlled bywater diffusion within the solid structure. GCBs can be assumed as isotropic structure in terms of water flow within the material; by assuming a constant temperature and homogeneous structure, different 1-D mathematical solutions may be proposed, depending on the boundary conditions (Pakowski and Mujumdar, 2006; Crank, 1975); limited to the first term approximation, a straight-line equation may be obtained through the logarithmic transformation of the adimensional water content dry basis:

$$Y = LN\left(\frac{W_{ss} - W_o}{W_{ss} - W}\right) = kt + cte \tag{10}$$

GCBcan be assumed to be spherical shape with d_p as radius. The effective diffusivity is:

$$D_{eff} = \frac{kd_p^2}{\pi^2} \tag{11}$$

The experimental data used to determine the effective diffusivity D_{eff} from the Fick's model exclude the ones concerning the points close to t=0; the extrapolation of the model thus obtained leads to evaluate W_o as, generally, distinct from the initial humidity content W_i. The difference at the starting point between W_i and $W_o$revealsthe starting accessibility δW_s, which is the superficial water capable to be quickly removed from the surface, independently from diffusion process.

4. Statistical and Experimental Design Protocol

Several authors (Benoistet al., 1994; Kamal et al., 2008) developed RSM (response surface methodology) in order to identify the effects of each factor (independent variables or operating parameters) and target the optimal treatment conditions with the possibility of maximizing the information and obtaining the most relevant responses from the lowest number of trials. In the present work, this experimental design was defined after some preliminary trials to test the behavior of the plant (structure expansion, thermal degradation etc.) versus the operating parameters. The results of these preliminary experiments allowed us to select only three factors that seemed to have a significant influence in our case. These were saturated steam pressure P, initial water content W, and total thermal processing time t. They ranged from 0.1 to 0.6 MPa, 11 to 35% db, and 16 to 84 s, respectively (Table 1). Other parameters, such as the final pressure in the vacuum tank and the pressure drop rate were kept constant. A full central composite design with the three independent variables and seven replicates at the central point, i.e. a total of 21 trials, was employed for evaluating the effect of steam DIC treatment on GCBsdecaffeination and

drying kinetics. The trials were run randomly to minimize the effects of an unexpected variability of responses due to unrelated and uncontrolled factors.

The statistical analysis of the results was carried out using the analysis design procedure of Statgraphics Plus software for Windows (1995, version 5.1, Levallois-Perret, France). Variance (ANOVA) was performed to determine significant differences between independent variables ($P \leq 0.05$). The effects of the independent variables or operating parameters on each parameter response were revealed under Pareto chart, which is usually very effective when introduced to describe a phenomenon in which 95% of the variation observed in experimental processes can be explained by a mere 5% of the causes of that variation.

Pareto chartis introduced together with the main trends, response surface, empirical model coefficients and R^2. The response parameter (dependent variable Y) is expressed through a second-order polynomial empirical model of independent variables:

$$Y = \beta_o + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i=1}^{n} \beta_{ij} x_i x_j + \varepsilon \quad (12)$$

where β_o, β_i, β_{ii} and β_{ij} are the regression coefficients, x_i the independent variables, $\varepsilon$the random error, i and j the indices of the factors. Response Surface Methodology can be used to optimize the operating parameters by coupling various studied responses. The dependent response variables were the amount of DIC-direct caffeine extract"DIC-DEC", the amount of 20-min-97 °C aqueous caffeine extract "ACE",the total decaffeination ratio "TDR", and the direct decaffeination ratio "DDR", as well as the drying kinetic parameters such as the drying time to reach a water content level of 5% db "$t_{5\%}$", the effective diffusivity "D_{eff}" and the starting accessibility "W_s".

5. Results and Discussion

5.1 Extraction processes

The process of DIC-direct-extraction of caffeine should involve the autovaporization of volatile compounds. Thisphenomenon strictly appears when the pressure drops instantaneously. Furthermore, the increase in porosity and the appearance of holes due to the possible expansion of the matter allow water and other solvents to easily diffuse within the new structure; rupture of cell walls can occur at high severity DIC treatment.

The liquid resulted from the condensation of the vapor expelled into the vacuum tank by dropping the DIC pressure was clear, homogeneous, with a pleasant coffee odor. Caffeine present in this liquid was quantified and reported to the quantity of dry treated GCBs. The 20-min-97°Caqueous caffeine extracted from the untreated and DIC-treated GCBs was measured and quantified reported to dry matter.DIC treatment was designed to assess and identify the efficacy of the two operations by quantifying the amount of DIC-Extract Caffeine (DIC-DEC) and GCBAqueous Extract Caffeine (AEC).

5.1.1 Direct extraction of caffeine by DIC treatment

The DIC-direct extract caffeine expelled in the vacuum tank had a concentration in the condensed water between 0.005and 0.133% db.

RSM analysis for direct DIC-DEC revealed that the effects of the processing time (t) and the saturated steam pressure (P) reflecting the processing temperature were both significant; whereas the initial water content (W) had a lower impact (Figure 4a). It is worth noting that the higher the saturated steam pressure (P) and the processing time (t), the higher the DIC-DECvalue (Figure4b).

RSM analysis generated a second order polynomial model, and estimated response surfaces shown in (Figure4c).The regression coefficients of the model for caffeine directly expelled by DIC were obtained by multiple linear regressions. The polynomial empirical modelcould be estimated and expressed with R^2=0.788952as the following:

$$(DIC-DEC)\%db = -0.037\% + 0.730\%W - 0.021\%P - 0.002\%t - 1.864\%W^2 + 0.307\%WP + 0.001\%Wt - 0.105\%P^2 + 0.003\%Pt + 1.6\,10^{-5}\%t^2 \quad (13)$$

The highest direct caffeine extraction DIC-DEC was calculated as 0.1622% db, at optimal conditions of 0.6MPa saturated steam pressure, 27.53% db water content and 84 s thermal treatment time. This was higher than the content of lightly roasted coffee (0.06 % as was reported by Hecimovic et al., (2011). This increase can be explained by the expulsion of some volatile compounds thanks to the gradient of the total pressure between GCB

core and the surrounding vacuum medium. Indeed, in a similar situation even with essential oil extraction, Al-Haddad et al., (2007) and Besombeset al., (2010) indicated that Darcy-similar process could effectively remedy the paradoxical stage.

The values of DIC-DEC were systematically underestimated, because a certain amount of caffeine should have been retained on the vacuum tank wall and vacuum pump.However, the results proved the ability of DIC to quickly remove a certain amount of caffeine from GCBs, with low energy consumption and water vapor as proved byMounir and Allaf (2008).

5.1.2 Aqueous extraction of caffeine

20-min-97 °C aqueous extraction was operated on untreated and DIC-treated GCBs. Each experiment treatment was carried out in triplicate and the average of aqueous extract caffeine "AEC" expressed in %db was taken as the response.

RSM analysis for aqueous extract caffeine AEC revealed that the effects of the saturated steam pressure (P) reflecting the processing temperature, and the initial water content (W) were the most significantDIC operating parameters; whereas the processing time (t) had a too low impact (Figure5a).

In the considered ranges, the higher the values of water content (W), saturated steam pressure (P), and processing time (t), the lower the aqueous extract caffeine AEC (Figure5b).However, at high P level (about 0.6 MPa) and at high W level (about 35% db), AEC kept constant, independently of W and P, respectively (Figure5c).

The regression coefficients of the model for caffeine directly expelled by DIC were obtained by multiple linear regressions. The polynomial empirical model could be estimated and expressed with R^2=0.71 as the following:

$$AEC = (3.670 - 7.197W - 4.925P - 0.007t + 2.388W^2 + 12.642WP - 0.003Wt + 0.946P^2 + 0.005Pt + 105\ 10^{-5}t^2)\% \quad (14)$$

Although increasing the DIC processing pressure is correlated with increasing the steam temperature, the dissolution and extraction of caffeine in the aqueous solution seemed to decrease with increasing DIC temperature, possibly because a higher retention of caffeine. The highest theoretical value of AEC was obtained at P=0.1 MPa, W=11.0% db, and t=35 s; its optimized value was calculated as 2.35% db.

5.2 Decaffeination process

The new decaffeination process was triggered as two stages issued from:

a. DIC direct caffeine extraction; the crude directly extract was collected in the extract container of the DIC apparatus,
b. Aqueous caffeine extraction.

The first stage allows a part of caffeine to be directly expelled towards the vacuum tank once the DIC pressure abruptly dropped, while the second stage was greatly intensified thanks to the DIC expanded structure. Indeed, by expanding GCBs, DIC treatment could dramatically increase water diffusion and solvation.

The average amount of total extracted caffeine in the first experimental design could then reach up to 1.891±0.04 g of caffeine/100 g dry GCBs, which represented a decaffeination ratio up to 80%, while it was only 1.3640 ± 0.04 g/100 g dry matter representing a decaffeination ratio of 58% when the aqueous extraction was achieved with untreated GCBs. Research works on DIC treatment of various plants highlighted similar behavior revealing greater availability of compounds to be extracted normally because of expansion and possible broken cell walls.

Statistical analyses of total decaffeination ratio TDRillustrated in (Figure 6-a,b,c),showed the effects of DIC operating parameters such as the saturated steam pressure (P), the initial water content (W) of GCBs, and the DIC processing time t on total decaffeination ratio TDR. P and W were the most significant variables with a negative impact; that means that TDR decreased with increasing P and W.TDR were revealed through the following polynomial expression established with R^2 = 0.70:

$$TDR = (190.241 - 338.641W - 259.029P - 0.450t + 27.408W^2 + 678.102WP - 0.101\%Wt + 44.036P^2 + 0.416Pt + 0.0014t^2)\% \quad (15)$$

The optimalTDRcorresponded to a complete decaffeination, which was estimated at 99.5%at optimized DIC conditions of W=11.00% db, P=0.1 MPa, and t=35 s.

Moreover, it worth to be noted that, as expected, despite itslow level, the direct contribution ofDICtodecaffeinationby the expulsionof caffeinetowards the vacuum tank should be very important because of the short operation time, the high concentration level, and the low energy consumption reported to the amount of extract.

From experimental data, it was possible to establish the response analysis as shown in (Figure7-a,b,c), and an empirical model of the DDR versus the DIC processing parameters. The R^2 value $(R^2 = 0.789)$ directly proved that the DIC treatment was a relevant extraction process with caffeine:

$$DDR = (1.926 + 38.235W - 1.105P - 0.104t - 97.635W^2 + 16.064WP + 0.070Wt - 5.487P^2 + 0.167Pt + 0.001t^2)\% \quad (16)$$

The highest value of DDRof 8.5% could be obtained with initial water content W=27.5%db, at a saturated steam pressure P=0.6MPa during processing time t=84 s.

5.3 Drying kinetics

After the decaffeination process, and just before roasting, GCBs have to be dried. The impact of DIC on drying kinetics should be correlated with DIC-expansion. At first, it was evident to note that the drying time needed to let GCBs reach moisture of 5% dbwas much lower with DIC-treated grains than raw material (Table 2).

In our case, the lowest value of $t_{5\%}$ was obtained with the following values of DIC operating parameters: P=0.6 MPa, W=23% db and t=50 s with $t_{5\%}$=60 min instead of 528 min for untreated raw material.

On the other hand, response surface method RSM analysis confirmed that DIC processing pressure P and time t had negative linear effects on $t_{5\%}$ that means the higher the DIC operating parameters P and t, the more effective the drying kinetics. (Figure 8-a,b,c) displays the effects, while the empirical mathematical model was established with R^2=68.636 percent as following:

$$t_{5\%} = 204.006 + 980.295W + 501.661P - 5.218t - 687.756W^2 - 1655.62WP - 6.107Wt - 218.591P^2 - 4.034Pt + 0.057t^2 \quad (17)$$

5.3.1 Drying model

In the present work, the mathematical diffusion-drying model coupled with the initial surface interaction was fitted to experimental data using non-linear regression analysis techniques. The experimental data of moisture (% dry basis) versus time were used in order to assess a suitable form of the drying curve for GCBs. They allowed determining the starting accessibility W_s and the effective diffusivity D_{eff} adapted to each drying curve of untreated and DIC treated GCBs. The statistical treatment of these results revealed the remarkable improvement in the starting accessibility and the moisture diffusivity that could be achieved when coffee beans are pre-treated by DIC prior to drying. In the present case of DIC treated samples, they ranged from 5 to 17.8 g/100 g db and from 5.4 to 28.4 10^{-10}m² s^{-1}, respectively; as against 0.8 g/100 g db and 1.3 10-10 m² s^{-1}, respectively for the raw material. These last values correlate with those reported for coffee cherries by Sfredoet al., (2005); Hernandez-Diaz et al., (2008) of an effective moisture diffusivity between 110^{-11} and 1 10^{-10} m² s^{-1}at 45 °C and between 3 10^{-11} and 3 10^{-10} m² s^{-1} at 60°C.

DIC textured beans with increased pore size and greater exchange surface enhance the powerful raise of drying kinetics through effective responses. The results are in agreement with other DIC experiments carried out on various food products (Albitar et al., 2011).

It was then possible to establish the response surface analysis (Figure9-a,b,c and Figure 10-a,b,c), and the empirical mathematical models of the effective diffusivity D_{eff} and the starting accessibility δW_s versus the DIC processing parameters P, t and W. The regression coefficients of the models were obtained by the multiple linear regressions, and the polynomial equations estimated are expressed as the following:

$$D_{eff} = 13.6381 - 24.103W - 34.969P - 0.226t - 37.860W^2 + 95.280WP + 0.423Wt + 10.770P^2 + 0.303Pt + 0.001t^2 \quad (18)$$

$$\delta Ws = (9.160 + 18.213W - 27.998P - 0.068t - 79.385W^2 + 38.643WP + 0.147Wt + 27.155P^2 + 0.1Pt + 0.001t^2)\% \quad (19)$$

with R^2 values 0.72 and 0.55, respectively. Though these R^2 values of D_{eff} and δW_s reveal that these equations reflect a general trend rather than a precise relationship, but all of these relations shows the great impact of DIC pretreatment in the improvement of the drying kinetics; possibly through the porous structure.

From these second order polynomial models generated by response surface analysis, optimal drying conditions of GCBs were obtained using the predictive equations generated by RSM:

Thehighest valuesof D_{eff} at 1.9994 $10^{-9}m^2$ $s^{-1}$and δW_s at 13.901% were obtained at the same DIC operating parameters P=0.6 MPa and t=84 s, while the water contents slightly differed to be W=33.5% db and 25.6% db, respectively.

Finally, it worth to note that the same optimal DIC treatment parameters were defined for DDR/Total extracted caffeine. General correlations(Figure 11) were recognized between $D_{eff,}$andDDR/Total extracted caffeine with 79.5% as correlation factor. Both of these response parameters should be directly linked to porosity and porous structure.

6. Conclusions

The results obtained throughout this work concludethata new decaffeination process could be defined and applied on green coffee beans (GCBs). It is a two-step operation of Détente InstantanéeContrôléeDIC (French, for instant controlled pressure-drop)and an aqueous extraction, normally followed by a drying step, prior to storing and roasting. The first stage of thermo-mechanical process DIC leaded to an expulsion of a part of caffeine possibly by autovaporization; it is the “direct decaffeination” step. DIC treatment can also allow the structure to be expanded and solvent extraction to be dramatically intensified. A “20-min-97 °C aqueous extraction” was defined as the second step of this solvent-free, environmentally decaffeination. In the present work, both operations of decaffeination and drying were achieved on Ethiopian green coffee beans. The highest value of the direct decaffeination ratio DDRcould be 8.5% of total caffeine content and corresponded to a DIC saturated steam pressure P=0.6 MPa, for a DIC processing time t=84s with an initial moisture content W=27.5%. Untreated and DIC-treated GCBs were then submerged for 20 min in water bath at 97 °C.Thus, a total decaffeination ratio TDRwas obtained.With optimized DIC treated GCBs of W=11.00% db, P=0.1 MPa, and t=35 s, TDRcorresponded to a complete decaffeination, which was estimated at 99.5% while only 58% could be extracted from the untreated raw materials.

Using the classical diffusion coupled with surface interaction model of hot-air drying kinetics, DIC expansion dramatically improved the drying kinetic parameters. Thus, the effective diffusivity D_{eff} and the starting accessibility δW_s increased 3780% and 1537%, respectively. Drying time needed to reach 5% db became 60 min instead of 528 min for untreated raw material.

From a technological point of view, thisnew route for coffee decaffeination is very promising because of various drawbacks related to the traditional decaffeination processes.

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Nomenclature

DIC	Détente InstantanéeContrôlée (French for "Instant controlled pressure drop")
GCB	Green coffee bean
RSM	Response Surface Method
P	DIC saturated steam pressure (in MPa)
t	DIC thermal treatment time
W	Initial moisture content of GCB
D_{eff}	Effective diffusivity from the diffusion/surface interaction kinetic model of hot air drying after DIC treatment
δW_s	Starting accessibility from the diffusion/surface interaction kinetic model of hot air drying after DIC treatment
db	Dry basis
p_c	Partial pressure of volatile compounds
R	Gas constant, (8.314 J $mol^{-1}K^{-1}$)
T	Temperature (K)
λ_{eff}	Effective heat conductivity (W m^{-1} K^{-1})
$<M_c>$	Averageof molar mass of volatile compounds
$<L_c>$	Averagelatent heat Length of volatile compounds
v_c	Velocity of volatile compound flux
P	Total pressure
K	Darcy coefficient (m^2)
m_m	Mass of vapor removed
R_o	Poreradius
P_o	Internal total pressure
P_{ext}	Externaltotal pressure
W_∞	Equilibrium moisture content
W_o	Computed initial moisture content (hypothesis of diffusion model)
W	Moisture content at certain drying time
k	Drying rate
t	Drying time
cte	Constant
d_p	Sphere radius
ρ	Density of water (kg m^{-3})

Table 1. HPLC elution conditions

Solvent gradient (acetic acid 6.7% : acetonitrile% : water%, v/v/v)	Time (min.)
10.0% : 0.0% : 90%,	0
10.8% : 0.2% : 89%	2
11.0% : 1.0% : 88%	6
11.0% : 2.0% : 87%	10
11.0% : 4.0% : 85%	15
12.0% : 8.0% : 80%	20
12.0% : 0.0% : 88%	25
10.0% : 0.0% : 90%	40

Table 2. Independent variables and their coded and natural levels employed in a 5-level central composite design to optimize the effect of DIC on decaffeination and on drying kinetics of the green coffee beans

	DIC parameters					
Trial	Saturated steam pressure P		Water content dry basis W		Total processing time t	
N°	Coded level	Natural level (MPa)	Coded level	Natural level (% db)	Coded level	Natural level (s)
1	+α	0.6	0	23.00%	0	50
2	0	0.35	0	23.00%	+ α	84
3	0	0.35	0	23.00%	0	50
4	+1	0.5	+1	30.00%	+1	70
5	0	0.35	+ α	35.00%	0	50
6	0	0.35	0	23.00%	0	50
7	+1	0.5	+1	30.00%	-1	30
8	+1	0.5	-1	16.00%	+1	70
9	0	0.35	0	23.00%	0	50
10	+1	0.5	-1	16.00%	-1	30
11	-1	0.2	+1	30.00%	+1	70
12	0	0.35	0	23.00%	0	50
13	-1	0.2	+1	30.00%	-1	30
14	-1	0.2	-1	16.00%	+1	70
15	0	0.35	0	23.00%	0	50
16	0	0.35	- α	11.00%	0	50
17	- α	0.1	0	23.00%	0	50
18	0	0.35	0	23.00%	0	50
19	-1	0.2	-1	16.00%	-1	30
20	0	0.35	0	23.00%	- α	16
21	0	0.35	0	23.00%	0	50

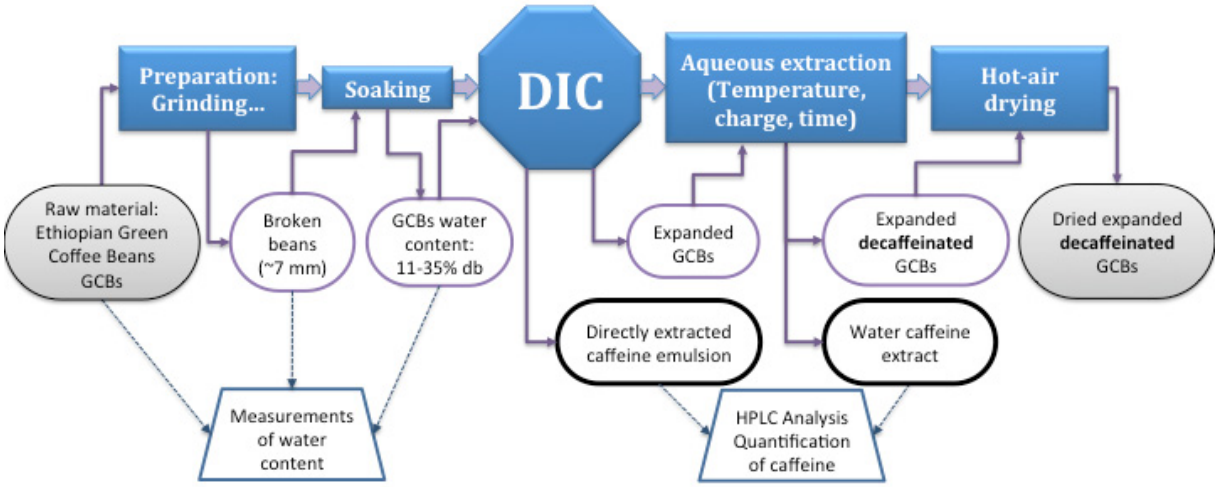

Figure 1. Treatment protocol and assessments of GCBs (Green Coffee Beans) through instant controlled pressure drop DIC

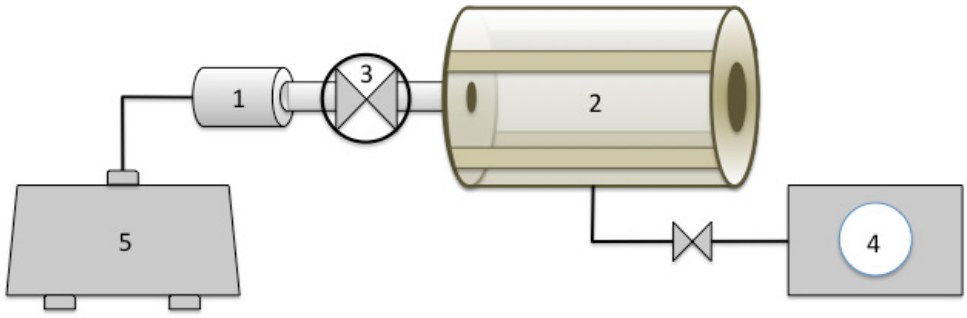

Figure 2. Schematic presentation of DIC reactor: 1- treatment vessel; 2- vacuum tank with transparent Jacket; 3- controlled instant pressure drop valve; 4- water ring vacuum pump; 5- steam generator

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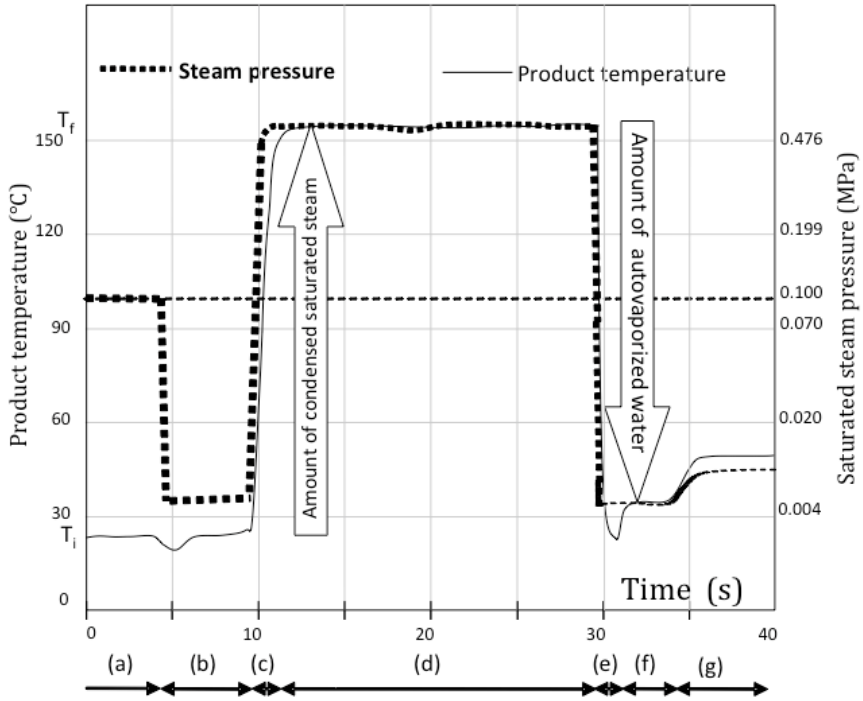

Figure 3. Different stages of a DIC treatment: a- Product to be place in the treatment vessel; b- First vacuum stage; c- saturated steam to be injected in the treatment vessel; d- High-pressure/high-temperature stage; e- Pressure to be abruptly dropped toward a vacuum; f- Second vacuum stage; g- Releasing toward Atmospheric pressure

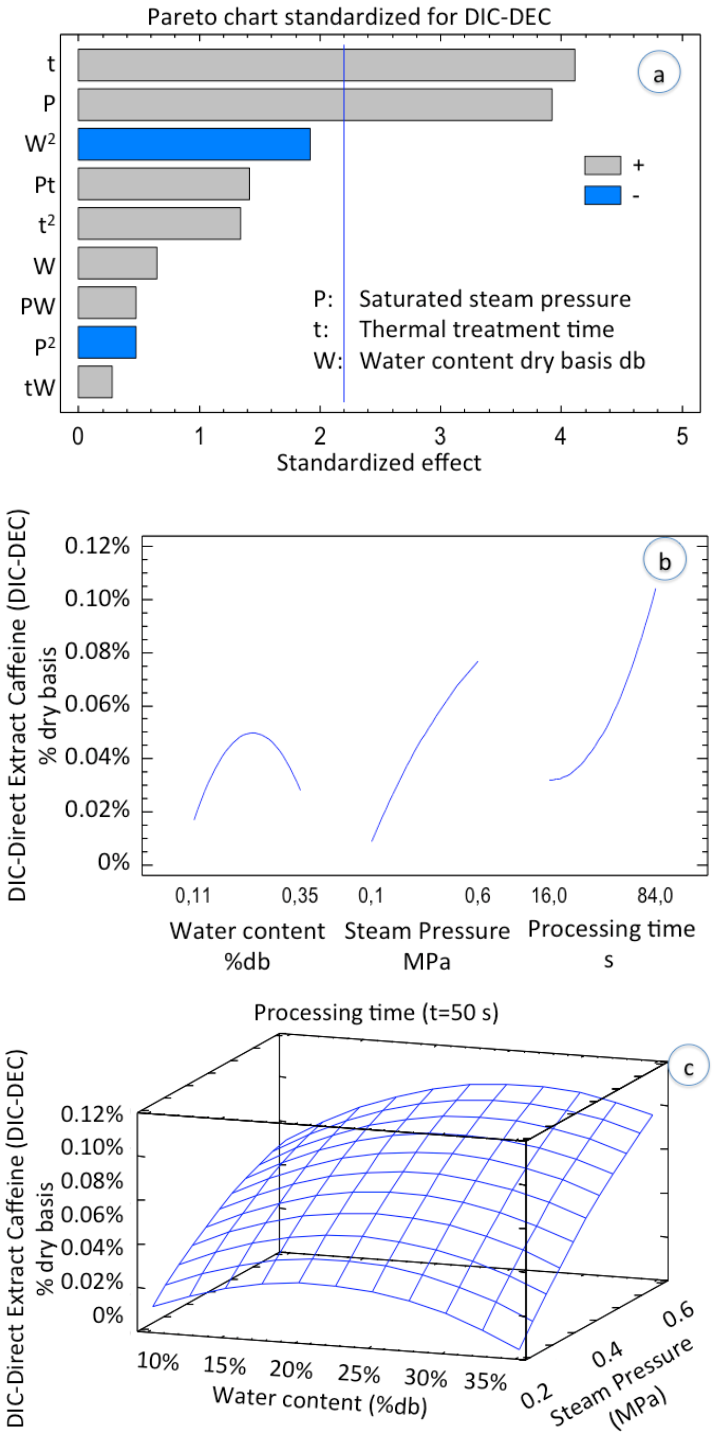

Figure 4. Pareto chart of DIC operating parameters (a), general trends (b), and response surface (c) for caffeine directly extracted by DIC (DIC-DEC)

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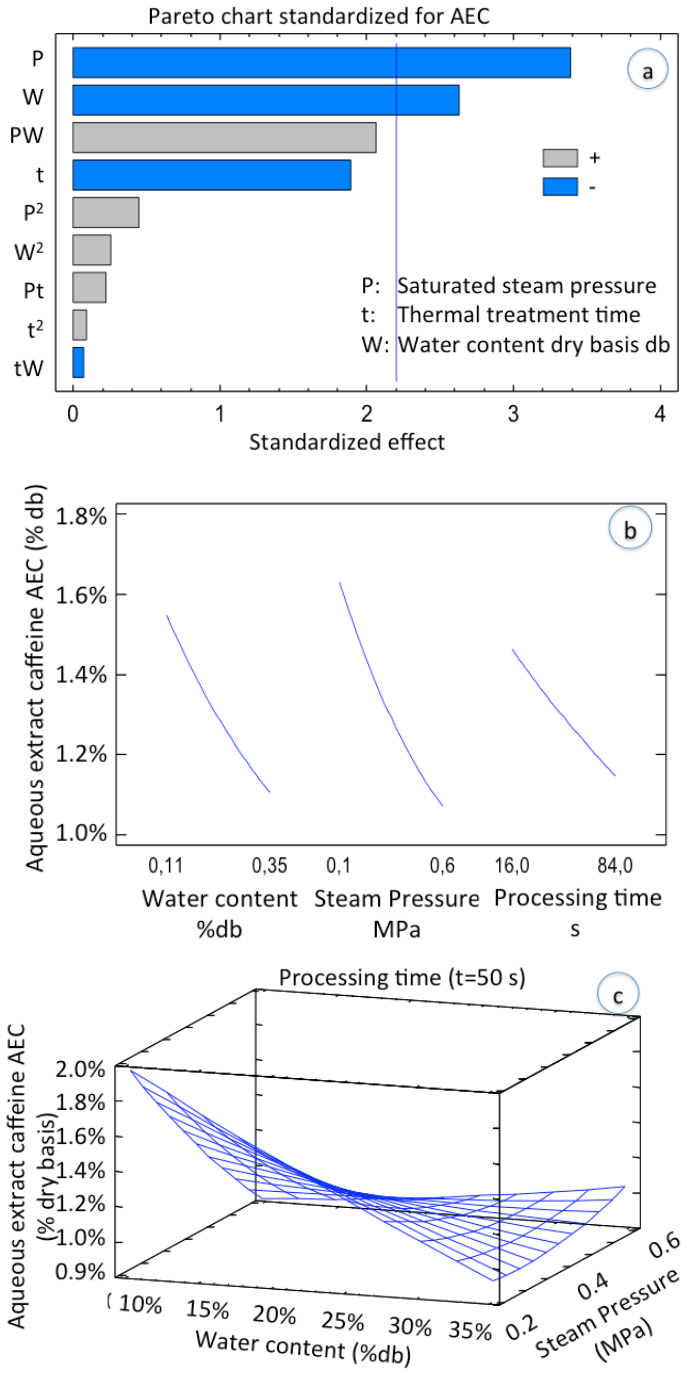

Figure 5. Pareto chart (a), general trends (b) and response surface (c) of DIC operating parameters for caffeine extracted during 20 minutes in water at 97 °C, after DIC treatments (AEC)

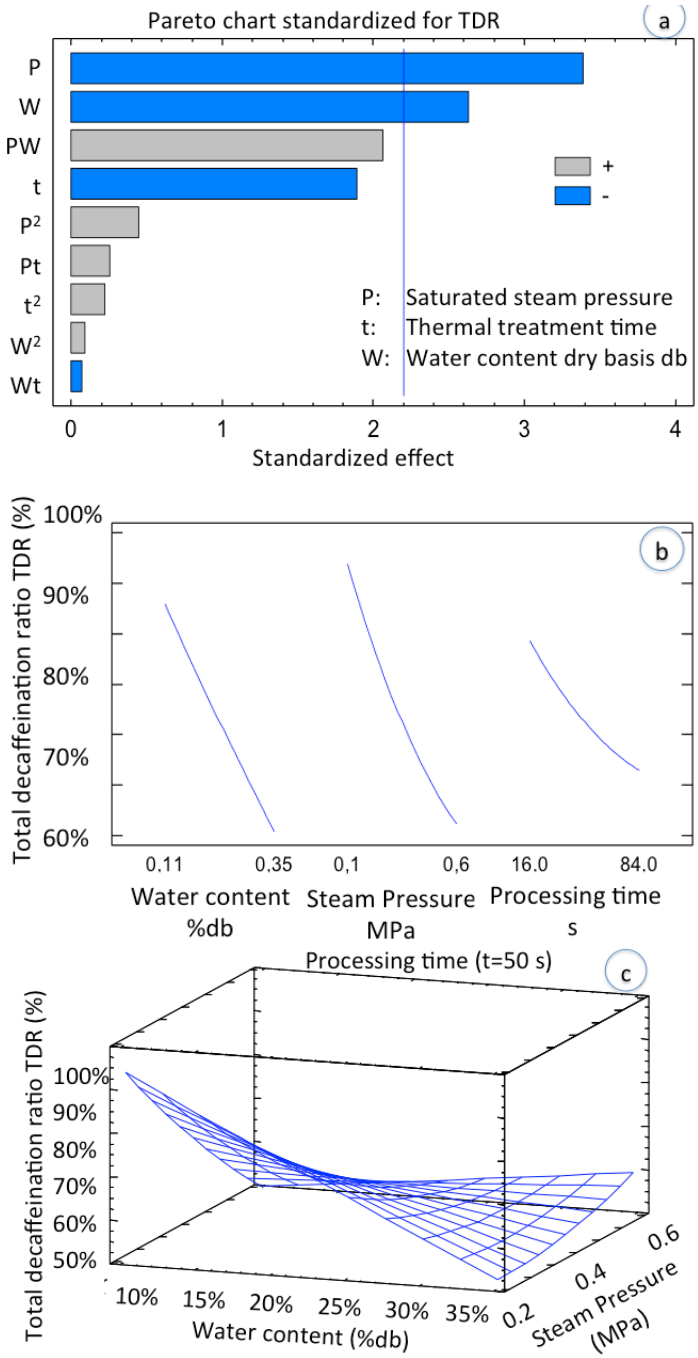

Figure 6. Pareto chart, General trends, and response surface analysis results for total decaffeination ratio (TDR)

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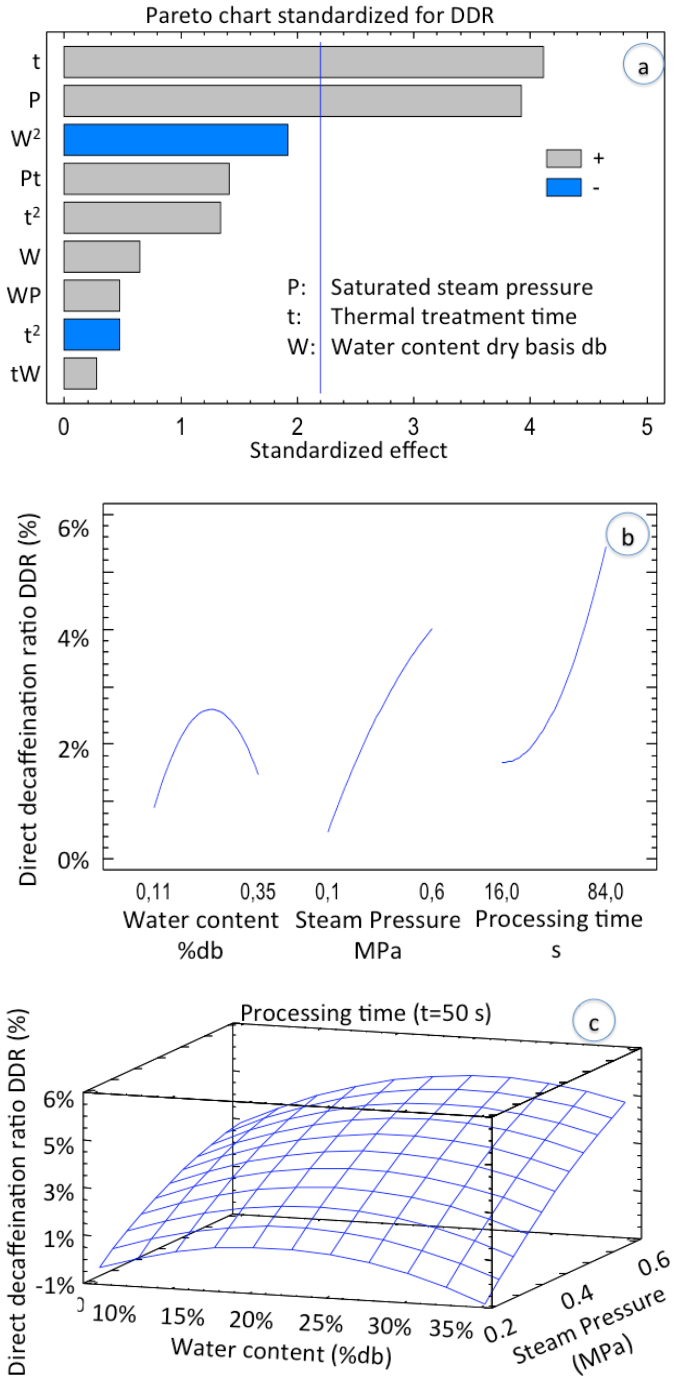

Figure 7. Pareto chart, general trends, and response surface for the DIC-Direct Decaffeination Ratio DDR

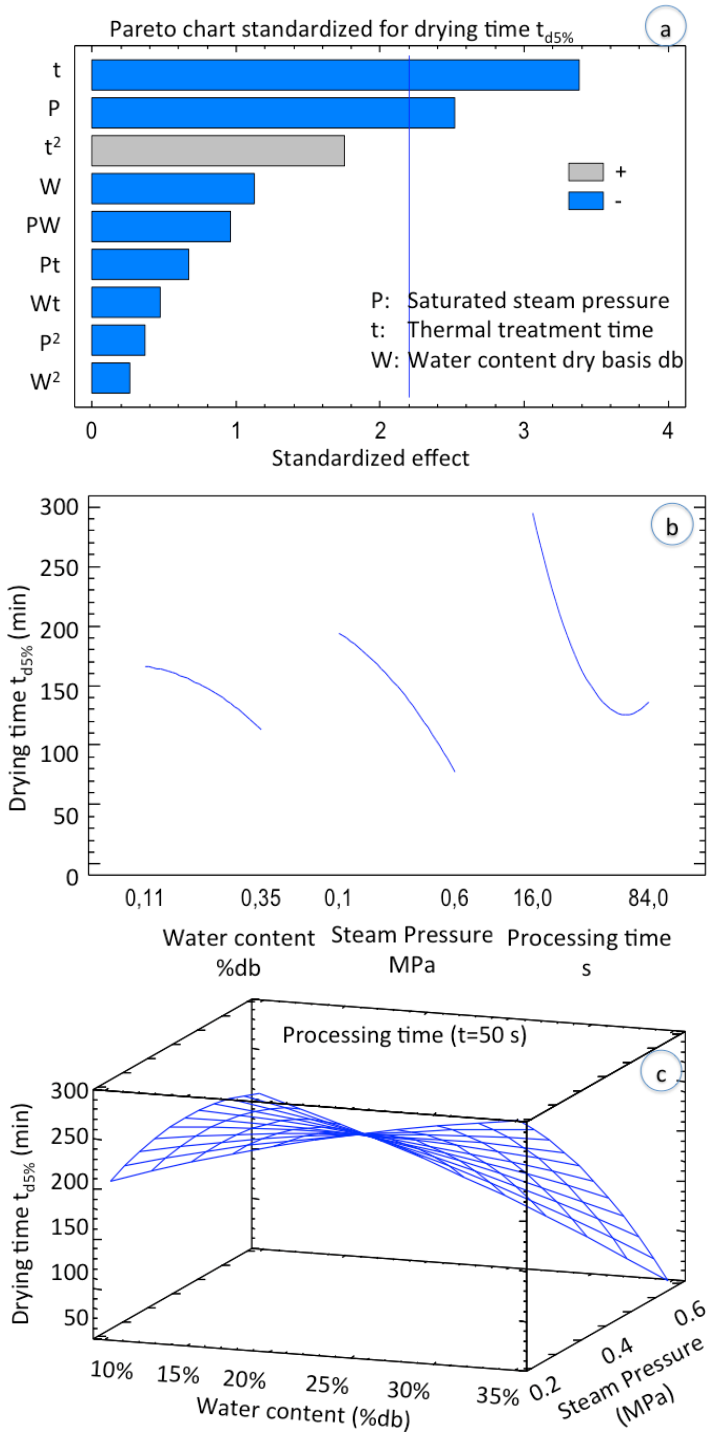

Figure 8. Pareto chart, general trends, and response surface for the drying time t5% of DIC treated GCBs

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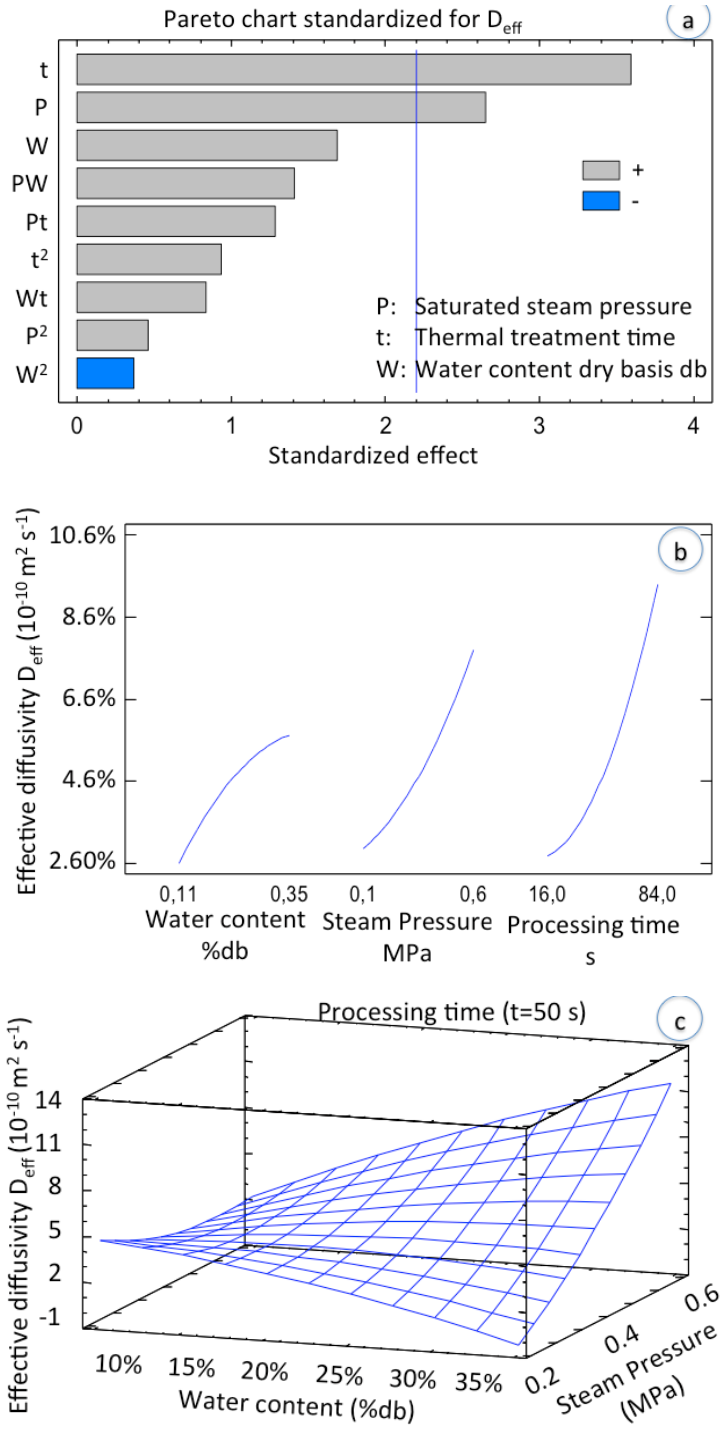

Figure 9. Pareto charts, general trends and response surfaces showing the effects of the DIC processing variables on the effective diffusivity D_{eff}

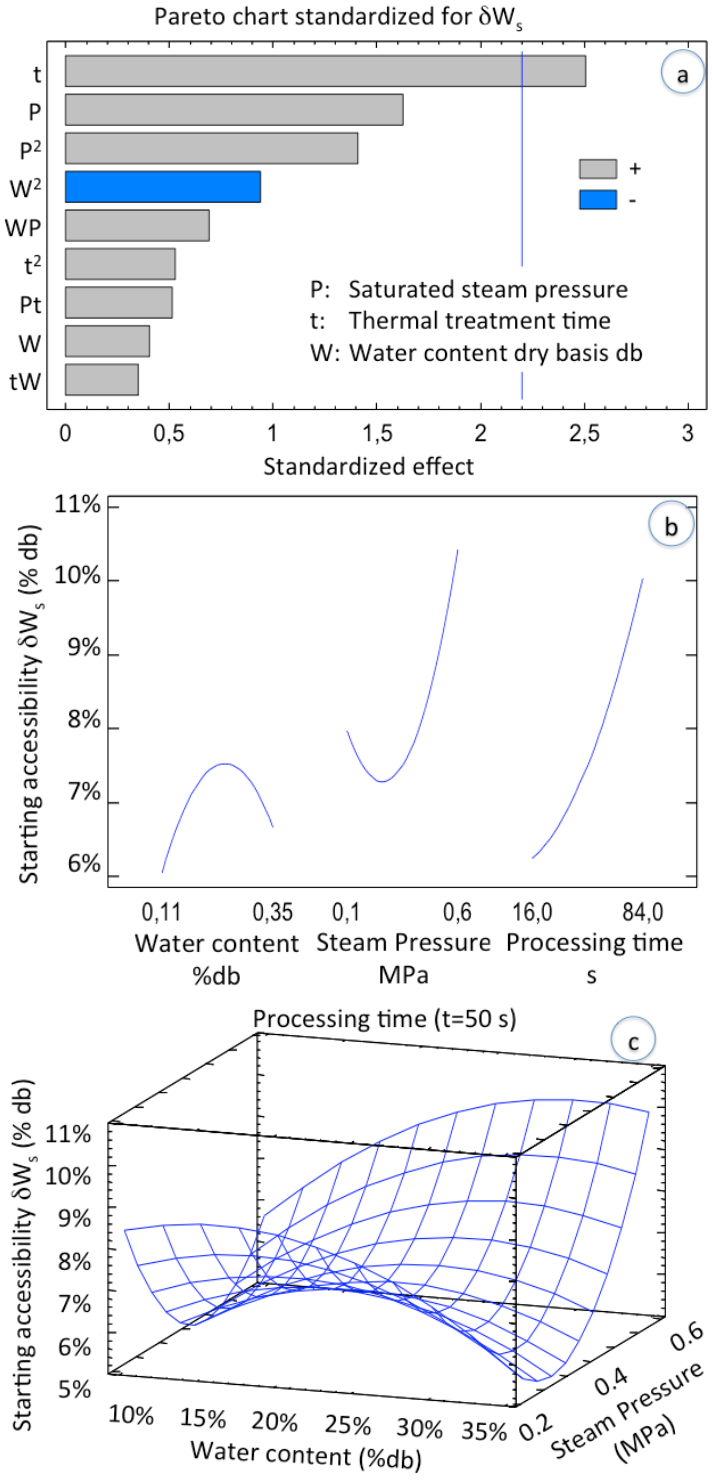

Figure 10. Pareto charts, general trends and response surfaces showing the effects of the DIC processing variables on the starting accessibility W_s

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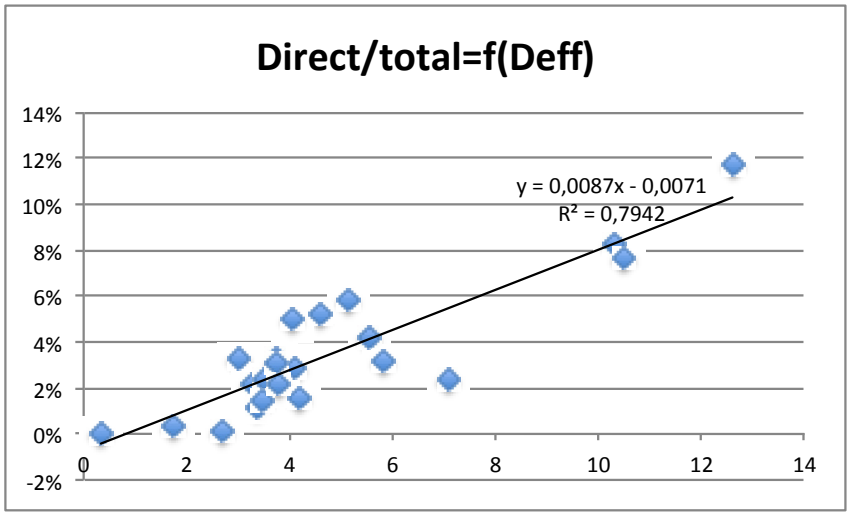

Figure 11. Correlation between drying effective diffusivity and DIC direct decaffeination to total decaffeination ratio (DDR)

Finite Element Modeling and Physical Property Estimation of Rheological Food Objects

Zhongkui Wang (Corresponding author) & Shinichi Hirai
Department of Robotics, Ritsumeikan University, Japan
Tel: 81-907-498-3398 E-mail: steven_wang@hotmail.co.jp

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Abstract

The purpose of this study is to accurately simulate the rheological behaviors of food objects undergoing a loading-unloading operation using finite element (FE) model. Due to the presence of residual deformation, it is difficult to model rheological behaviors. Especially, it is hard to accurately reproduce both rheological force and residual deformation simultaneously. In this study, objects made of food materials were tested. Force and deformation measurements were recorded for parameter estimation. Constitutive models were investigated for describing rheological behaviors. A parallel five-element model including two dual-moduli viscous elements was proposed to accurately predict both rheological force and residual deformation simultaneously. 2D/3D FE model was formulated for simulating rheological behaviors. To estimate the parameters, an effective four-step method was established based on nonlinear optimization which aimed at minimizing the differences of forces and deformation between simulation and experiments. The proposed FE model and parameter estimation method were validated in both 2D and 3D cases and good agreements were achieved in both rheological forces and deformation between numerically simulated and experimentally measured data.

Keywords: Modeling, Simulation, Physical property, Finite element, Rheological behavior

1. Introduction

The modeling and simulation of deformable objects, such as biological organs and tissues, cloth, clay, and various kinds of food products, has been studied more than twenty years. Many important applications have been involved, including computer graphics [Terzopoulos & Fleischer (1988)], surgical simulation [Bro-Nielsen (1998), Cotin *et al*. (1999)], robot manipulation [Inoue & Hirai (2006)] and food engineering [Liu & Scanlon (2003), Martins (2006)]. Three important issues should be considered during the modeling and simulation of deformable objects: 1) the constitutive model, which normally is used to govern the physical behaviors of the material, 2) the modeling method, which is used to construct the geometry of the object and formulate the dynamic equations, and 3) parameter estimation, which is dedicated to find appropriate physical parameters involving in the model.

Among various constitutive models, the ones consisting of a number of elastic and viscous elements connected in a certain configuration were widely used in literatures. Such models have explicit physical meaning, simple formulation, and are easy to be constructed, but they are linear models. Conventional modeling methods are the mass-spring-damper method (MSD) [Waters (1987)], the finite difference method (FDM) [Terzopoulos *et al*. (1987)], the boundary element method (BEM) [James & Pai (1999)], and the finite element method (FEM) [Beo-Nielsen & Cotin (1996)], in increasing order of computation cost and simulation accuracy. Most recently, meshfree particle methods, such as smoothed particle hydrodynamics (SPH), have also been used to model solid deformable objects [Zhu *et al*. (2010)]. A combination of a constitutive model and a modeling method can be employed to construct a two-dimensional (2D) or three-dimensional (3D) dynamic model. In addition, to simulate real-world deformable objects, important physical parameters need to be estimated in advance. So far, the optimization-based methods have been the most popular ones for the purpose of parameter estimation.

In our definition, we roughly divided deformable objects into three categories: elastic, plastic, and rheological objects, depending on their behaviors after a loading-unloading operation. Rheological objects have both elastic and plastic properties and always yield residual deformation after unloading. Residual deformation is important for some applications, such as manufacture of pottery and food products, where a desired final shape is required during forming process. Therefore, it is necessary to investigate the modeling and parameter estimation of such rheological objects.

Early work on the modeling of rheological objects dates back to Terzopoulos *et al*. (1987) and Terzopoulos & Fleischer (1988), who have proposed a four-element model to describe rheological behaviors. Detailed studies on the modeling and parameter estimation of rheological objects have been investigated by Noborio *et al*., who employed a three-element model to describe rheological behaviors and an MSD modeling method to construct food dough. The authors explored the lattice [Noborio *et al*. (2003), Nogami *et al*. (2004a)], the truss [Nogami *et al*. (2004b)], and the hierarchical [Ikawa & Noborio (2007)] structures, with decreased MSD elements connected between nodes to reduce the computation cost. Two optimization methods, modified randomized algorithm [Noborio *et al*. (2003)] and genetic algorithm [Yoshida *et al*. (2005)], were used to estimate the physical parameters. The authors successfully captured the deformation behaviors, but failed to reproduce the force responses at the same time [Yoshida *et al*. (2005)]. The MSD modeling method has advantages of simple formulation and relatively low computation cost, but the formulation is not based on continuum mechanics and the geometrical topology significantly affects the simulation accuracy.

Two layered Maxwell model [Sakamoto *et al*. (2007)] and Fung's viscoelastic model [Tsai *et al*. (2008)] have been used respectively to simulate the force response of 'Norimaki-sushi' when it was grasped by a robot hand. Good agreements of rheological forces were achieved. Unfortunately, both models are 1D cases and the residual deformation was not considered during the modeling and parameter estimation. FE method has been utilized to model and simulate the indentation of bread crumbs [Liu & Scanlon (2003)]. It has also been used to evaluate food quality and safety losses during processing, storage, and distribution [Martins (2006)]. In addition, FE simulation has been employed to investigate the dependence of temperature and water content on processing time during meat cooking [Purlis and Salvadori (2005)]. It has been reported that the most critical barrier against the application of robotics and automation in food industry is a lack of understanding of the food product properties as an "engineering" material for handling operations [Chua *et al*. (2003)]. Therefore, it is necessary to investigate the methods for modeling and parameter estimation of rheological objects.

In this study, three kinds of rheological food materials: commercial available clay (food-like), Japanese sweets, and bacon, were tested with a loading-unloading operation. Rheological force and deformation, including the residual deformation, were recorded. To find an appropriate model for describing these behaviors, the constitutive laws of generalized models were formulated and theoretical analysis was done. A parallel five-element model with two dual-moduli viscous elements was proposed to accurately predict both rheological force and residual deformation simultaneously. The 2D/3D FE model was then formulated by imposing a five-element model onto each triangle (2D) or tetrahedron (3D) to govern its behaviors. Based on the analytical expressions of rheological forces, an efficient approach was proposed to estimate the physical parameters involving in the FE model. This method aimed at minimizing the difference in both force and deformation between simulation results and experimental measurements. The proposed FE model and parameter estimation method were then evaluated through a series of comparisons of experimental measurements and predicted results from FE simulation.

There are some potential applications of this research in food industry. Using the proposed model and method, we are able to estimate the physical properties of food products and then establish a relationship between the physical parameters and the taste of the food product. As we know, the taste of a food product includes not only the flavor but also the chew feeling, such as the hardness and the viscosity of the product. We can use the estimated parameters to evaluate the taste of food products. The second potential application is to simulate and predict the deformed shape of food product. For example, we may need to perform a pick-and-place operation in a sushi manufacturing line. In order to grasp the sushi stably, a certain grasping deformation is required. On the other hand, large deformation may affect the final appearance of the product. There is a compromise between enough grasping deformation and appropriate final appearance. We can determine an appropriate grasping deformation by performing a series of experiments which may be time-consuming and troublesome. Instead, we are able to use the FE model of the sushi product to simulate such grasping operation to find an optimal grasping deformation. In addition, the developed FE model can be also utilized to establish a virtual scenario of food making process with haptic feedback since good reproduction of force response can be achieved using the proposed parameter estimation method. Such a virtual scenario can serve as a digital demonstration to show the

skillful making process of some traditional food products.

2. Materials and Methods

The goal of this study is to develop an FE model and establish a parameter estimation method to accurately predict rheological force and deformation behaviors simultaneously. To this end, indentation experiments were performed using typical rheological materials. Experimental measurements of force and deformation were used to estimate the physical parameters. The FE model and estimated parameters were then used to predict rheological behaviors of same materials.

2.1 Experimental materials

Three typical rheological materials, *i.e.*, commercial available clay, Japanese sweets, and bacon, as shown in Figure 1, were used in our indentation experiments. Clay is made of flour, salt, and water and supposed to be played by children over 3 years old. Three kinds of sweets materials were provided by OIMATU (a sweets company in Kyoto) and used to make traditional Japanese sweets products. Each sweets material is made of flour, water, and one kind of bean powder mixed at a specific ratio. Bacon was bought from a supermarket and normally was cut in pieces and eaten with bread for breakfast. Several object samples made from each material were prepared and indented by a linear stage.

2.2 Indentation experiments

For each material, two kinds of indentation experiments were performed. The first kind is for parameter estimation. The sample object was indented from one side using a motorized linear stage (KX1250C-L, SURUGA SEIKI Co.), as shown in Figure 2a. A push-keep-release displacement function, as shown in Figure 2b, was used to deform the object. The input deformation is assumed to be plane stress. The force response on the other side of the object was recorded using a tactile sensor (I-SCAN100L, NITTA Co.). Three static images, which denote the initial, keep, and final shapes respectively, were recorded using a digital camera (EOS Kiss X2, Canon Inc.). The deformation during keep phase and residual deformation were then calculated based on these images. As an example, Figure 3 shows the measured rheological behaviors of object made by clay material. These measurements of force and deformation were used to estimate the physical parameters.

The purpose of the second kind of experiments is to evaluate the performance of estimated parameters for predicting the behaviors of each material with different indentation operations, non-homogeneous, and irregular-shaped objects respectively. For clay material, sample objects were indented from the center part of one side (Figure 4a) instead of the entire side. Such an operation may often be used in the grasping of an object. In terms of Japanese sweets materials, non-homogeneous three-layered objects were deformed from both entire and center part of the side (Figure 4b). Irregular shaped objects (Figure 4c) were used to evaluate the parameters of bacon material. A push-keep-release procedure was also used in these experiments. Detailed information of all experimental trials is given in Table 1. In addition, it is worth to be mentioned that we performed the deformation measurements in 2D scenario and ignored the buckling or bulging behaviors which slightly happened during the deformation.

2.3 Constitutive models

The constitutive models, such as Maxwell and Voigt models, were widely used to describe the behaviors of deformable objects. In such models, we commonly utilize an elastic element (denoted by Young's modulus E) and a viscous element (denoted by viscous modulus c) to describe the elastic and viscous behaviors, respectively. By connecting several such elements into different configurations, we can construct various models for simulating rheological behaviors, such as the three-element and four-element models used in [Wang *et al*. (2009), Wang & Hirai (2009)]. In order to investigate the capability of the constitutive models and to establish a criterion for selecting appropriate models, we have summarized such models into two categories: serial and parallel models, as shown in Figure 5. The constitutive laws of generalized serial and parallel models were derived in [Wang & Hirai (2010a)] and summarized in Table 2. We found that the constitutive laws of serial and parallel models have the same forms and therefore yield the same behaviors. In other words, for a certain serial model $\boldsymbol{A}$, we are always able to find a corresponding parallel model $\boldsymbol{B}$, which has the same constitutive law and yields the same behaviors with model $\boldsymbol{A}$, and vice versa. In addition, we can always obtain analytical expressions of strain (displacement) over time by solving the constitutive laws of serial models due to the serial connections between elements. On the other hand, parallel models always result in straightforward calculation of analytical stress (force) expressions due to the parallel connection. This suggests us the way to select appropriate one from serial and parallel models. If we are interested in the deformation, it is better to choose a serial model. On the contrary, we should go with parallel model if the force is of concern.

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In our case, we are more interested in rheological force since deformation measurements only include static images. We have therefore chosen parallel models. In [Wang & Hirai (2009)], we also found that at least two exponential terms in force expressions are necessary to accurately reproduce force relaxation behavior during keep phase (Figure 3a). Consequently, we need at least two Maxwell elements in the constitutive model, *e.g.*, a parallel five-element model (the bottom one in Figure 5b with $n = 2$). By using such a model, we are able to accurately reproduce the rheological force behaviors. However, we failed to predict the residual deformation at the same time. We found a contradiction between the reproductions of rheological force and residual deformation simultaneously because the linear viscous elements (denoted by parameter c_i in Figure 5b) affected both force and residual deformation [Wang & Hirai (2010a)]. One single set of parameter c_i can guarantee a good reproduction of either force or residual deformation. However, it cannot cover both at the same time. We have therefore proposed a dual-moduli viscous element, as shown in Figure 6a, to solve this problem. The governing equation of this element can be formulated as

$$\sigma^{dual}(t) = (\kappa\alpha + c)\dot{\varepsilon}^{dual}(t), \tag{1}$$

where σ^{dual} and ε^{dual} are stress and strain vectors generated on the element, scalars α and c are parameters to be determined, switch variable κ takes the following values

$$\kappa = \begin{cases} 1 & \text{criterion is satisfied,} \\ -1 & \text{otherwise.} \end{cases} \tag{2}$$

The dual-moduli viscous element works as a switch to change the viscous coefficient from one value to another during simulation whenever a criterion is satisfied. The physical meaning of this element can be explained as the property change of a material during loading and unloading operations. For example, elastic materials experience a hysteresis phenomenon during which the material properties are slightly changed. Some metal materials also demonstrate strain hardening behavior when they are strained beyond the yield point [Shames & Cozzareli (1992)]. The properties of the materials also changed after strain hardening. In rheological materials, both hysteresis and strain hardening may also happen and may be in a stronger way. In other words, the physical parameters of rheological object may be continuously changing during loading and reach another set of values once loading operation finishes. Unfortunately, continuous change of parameters brings troubles in parameter estimation. Therefore, we suppose that the parameters take one set of values during loading operation and switch to another set once the operation finishes.

The criterion used in Eq. 2 has different options depending on applications. If the operation time is available, the simulation time can be a criterion. In some applications, the simulation time may be not available in advance. Fortunately in most cases, an interaction often happens between the object and external instruments. This interaction can also serve as a criterion for switching parameters, as presented in [Wang & Hirai (2010b)]. By introducing the dual-moduli viscous elements, we finally end up with an effective model (Figure 6b) for predicting both rheological force and residual deformation simultaneously.

2.4 FE dynamic model

The FEM is the most successful method for numerical computation of object deformation. In FE modeling, an object is described by a set of elements (e.g., triangles in 2D and tetrahedra in 3D cases). Dynamic behaviors of the object are then determined by analyzing the behaviors of individual element. In this paper, we assume that the behaviors of individual element are governed by the model given in Figure 6b. Note that the constitutive law of a Maxwell model with a dual-moduli viscous element located at the first row of the five-element model (Figure 6b) is formulated as

$$\dot{\sigma}_1 + \frac{E_1}{\kappa\alpha_1 + c_1}\sigma_1 = E_1\dot{\varepsilon}_1. \tag{3}$$

By performing a series of replacements as done in [Wang & Hirai (2011a)], Eq. 3 can be transformed to 2D/3D force-displacement relationship as

$$\dot{\boldsymbol{F}}_1 + \frac{E_1}{\kappa\alpha_1 + c_1}\boldsymbol{F}_1 = \left(\lambda_1^{ela}\boldsymbol{J}_\lambda + \mu_1^{ela}\boldsymbol{J}_\mu\right)\boldsymbol{v}_N, \tag{4}$$

where $\boldsymbol{F}_1$ is the force vector generated on the first row of the five-element model, vector $\boldsymbol{v}_N$ consists of velocity components of all nodes in the FE mesh, $\boldsymbol{J}_\lambda$ and $\boldsymbol{J}_\mu$ are referred to as connection matrices that are constant matrices and only depend on geometric quantities, say, initial coordinates of nodes, scalars λ_1^{ela} and μ_1^{ela} denote Lamé's constants and can be calculated as:

$$\lambda_1^{ela} = \frac{E_1\gamma}{(1+\gamma)(1-2\gamma)}, \qquad \mu_1^{ela} = \frac{E_1}{2(1+\gamma)}, \tag{5}$$

where parameter E_1 is Young's modulus and γ is Poisson's ratio which denotes the compressibility of the material. For an incompressible material, parameter γ usually takes a value smaller than but very close to 0.5, e.g., $\gamma = 0.499$. For compressible materials, parameter γ should take a value much smaller than 0.5. Similarly, the force-displacement relationships at the second and third row of five-element model (Figure 6b) can be formulated as

$$\begin{aligned} \dot{\boldsymbol{F}}_2 + \frac{E_2}{\kappa\alpha_2 + c_2}\boldsymbol{F}_2 &= \left(\lambda_2^{ela}\boldsymbol{J}_\lambda + \mu_2^{ela}\boldsymbol{J}_\mu\right)\boldsymbol{v}_N, \\ \boldsymbol{F}_3 &= \left(\lambda_3^{vis}\boldsymbol{J}_\lambda + \mu_3^{vis}\boldsymbol{J}_\mu\right)\boldsymbol{v}_N, \end{aligned} \tag{6}$$

where parameters λ_3^{vis} and μ_3^{vis} describe the model's viscosity and are defined as

$$\lambda_3^{vis} = \frac{c_3\gamma}{(1+\gamma)(1-2\gamma)}, \qquad \mu_3^{vis} = \frac{c_3}{2(1+\gamma)}. \tag{7}$$

Due to the parallel connections, the total force $\boldsymbol{F}^{rheo}$ generated on the model can be calculated by summing up the contributions of each row as

$$\boldsymbol{F}^{rheo} = \boldsymbol{F}_1 + \boldsymbol{F}_2 + \boldsymbol{F}_3. \tag{8}$$

Supposing that one side of the object is fixed on the ground and the other side is pushed downward with a displacement function of $\boldsymbol{d}(t)$. Two constraints on the nodes of both sides are formulated as follows using the constraint stabilization method (CSM) [Baumgarte (1972)]

$$\begin{aligned} \boldsymbol{A}^{\mathrm{T}}\dot{\boldsymbol{v}}_N + \boldsymbol{A}^{\mathrm{T}}\left(2\omega\boldsymbol{v}_N + \omega^2\boldsymbol{u}_N\right) &= 0, \\ \boldsymbol{B}^{\mathrm{T}}\left[\dot{\boldsymbol{v}}_N - \ddot{\boldsymbol{d}}(t)\right] + \boldsymbol{B}^{\mathrm{T}}\left\{2\omega\left[\dot{\boldsymbol{v}}_N - \dot{\boldsymbol{d}}(t)\right] + \omega^2\left[\boldsymbol{u}_N - \boldsymbol{d}(t)\right]\right\} &= 0, \end{aligned} \tag{9}$$

where matrices $\boldsymbol{A}$ and $\boldsymbol{B}$ denote the nodes to be constrained on each side, respectively, scalar ω is a predetermined angular frequency and is set to 2000 for both constraints.

Let $\boldsymbol{M}$ be an inertia matrix and $\boldsymbol{\lambda}_1$ and $\boldsymbol{\lambda}_2$ be the Lagrange multipliers that denote a set of constraint forces corresponding to both geometric constraints (Eq. 9). Applying Lagrange equations of motion, a set of dynamic equations of nodes is formulated as

$$-\boldsymbol{F}^{rheo} + \boldsymbol{A}\boldsymbol{\lambda}_1 + \boldsymbol{B}\boldsymbol{\lambda}_2 - \boldsymbol{M}\dot{\boldsymbol{v}}_N = 0. \tag{10}$$

Combining Eqs. 4, 6, 8, 9, 10 and considering $\boldsymbol{v}_N = \dot{\boldsymbol{u}}_N$, we have a set of differential equations for simulating the dynamic behaviors of a rheological object. Note that the above formulations are applicable in both 2D and 3D scenarios. The main difference between the 2D and 3D models is the calculation of connection matrices $\boldsymbol{J}_\lambda$ and $\boldsymbol{J}_\mu$, which depend on the geometrical meshes.

2.5 Parameter estimation

In order to accurately predict the behaviors of real-world objects, physical parameters involved in the FE model need to be determined. The FE model presented in the previous section includes the following eight physical parameters: Young's moduli E_1, E_2, viscous moduli α_1, α_2, c_1, c_2, c_3 and Poisson's ratio γ. In [Wang & Hirai (2010a)], we found that parameter c_3 accounts for attenuating the vibration in both force and deformation during object recovery. Simulation showed that a small value of c_3 is sufficient to attenuate the vibration and without

significantly affecting the amplitudes of force and deformation. Therefore, we preassigned a value of 10^2 Pa·s to c_3. It is quite small comparing with c_1 and c_2 which normally have a magnitude around 10^6 Pa·s. After determining parameter c_3, we have seven physical parameters to be estimated. We therefore propose an estimation method based on inverse FE optimization, which aims at minimizing the difference in force and deformation between simulation or theoretical calculation and experimental measurements. The measurements used here include force data during loading (includes push and keep phases) and three static images denoting the initial, keep, and final shapes of the object. The parameter estimation method proposed in this paper is summarized into the following four steps:

(1). Estimation of parameter γ by optimizing the keep-shapes,

(2). Estimation of parameters E_1, E_2, c_1^{load}, and c_2^{load} by minimizing the force difference during loading,

(3). Estimation of parameters c_1^{rel} and c_2^{rel} by optimizing the final-shapes,

(4). Calculation of parameters α_1, c_1, α_2, and c_2 based on estimated c_1^{load}, c_1^{rel}, c_2^{load}, and c_2^{rel}.

2.5.1 Estimation of Poisson's ratio γ

In [Wang & Hirai (2009)], we found that only Poisson's ratio γ affects the keep-shape and all the other parameters do not have contributions to keep-shape. This coincides with the definition of Poisson's ratio which indicates a ratio of lateral strain over normal one and it happens in all constitutive models constructed by linear elements shown in Figure 5. Therefore, we are able to estimate Poisson's ratio γ separately by minimizing the difference in keep-shapes. The objective function is formulated as

$$E(\gamma)=\sum_{i=1}^{m}\left\|x_i^{sim}(\gamma)-x_i^{keep}\right\|^2, \tag{11}$$

where $x_i^{sim}(\gamma)$ and x_i^{keep} are the displacement vectors in the keep phase from simulation and experiment, respectively. Scalar m=2N with N being the total number of nodes in the FE mesh. The optimization is terminated when the tolerance on function value $E(\gamma)$ is less than 1×10^{-12} or the tolerance on parameter γ is less than 1×10^{-6}.

2.5.2 Estimation of parameters E_1, E_2, c_1^{load} and c_2^{load}

Considering the force response during loading, let variable κ in Eq. 2 take 1 and we have $c_1^{load}=\alpha_1+c_1$ and $c_2^{load}=\alpha_2+c_2$. Thanks to the parallel configuration of the constitutive model, we are able to derive the analytical expression of rheological force during loading. By solving Eq. 4 in push phase ($0\le t\le t_p$), we have the force expression at the first row of the five-element model as

$$\boldsymbol{F}_1(t)=c_1^{load}\left(1-e^{-\frac{E_1}{c_1^{load}}t}\right)\boldsymbol{M}_\gamma\boldsymbol{v}_N^{push},\qquad \left(0\le t\le t_p\right), \tag{12}$$

where

$$\boldsymbol{M}_\gamma=\frac{\gamma}{(1+\gamma)(1-2\gamma)}\boldsymbol{J}_\lambda+\frac{1}{2(1+\gamma)}\boldsymbol{J}_\mu.$$

Vector $\boldsymbol{v}_N^{push}$ consists of velocities of all nodes in push phase. We assume that it consists of constant values if the object was deformed with a small and constant velocity. Therefore, it can be easily calculated using the displacements of nodal points divided by time period t_p.

Similarly by solving Eq. 6, we can formulate the force expressions at the second and third row as

$$\begin{aligned}\boldsymbol{F}_2(t)&=c_2^{load}\left(1-e^{-\frac{E_2}{c_2^{load}}t}\right)\boldsymbol{M}_\gamma\boldsymbol{v}_N^{push},\\ \boldsymbol{F}_3(t)&=c_3\boldsymbol{M}_\gamma\boldsymbol{v}_N^{push}.\end{aligned} \tag{13}$$

Combining the forces of three rows, the rheological force generated on the five-element model during the push phase ($0\le t\le t_p$) is then given by

$$\boldsymbol{F}(t)=\left[\sum_{i=1}^{2}c_i^{load}\left(1-e^{-\frac{E_i}{c_i^{load}}t}\right)+c_3\right]\boldsymbol{M}_\gamma\boldsymbol{v}_N^{push}. \tag{14}$$

In the keep phase ($t_\mathrm{p}\le t\le t_\mathrm{p}+t_\mathrm{k}$), solving Eqs. 4 and 6 with $\boldsymbol{v}_N=0$, we can formulate the force as

$$\boldsymbol{F}(t)=\sum_{i=1}^{2}c_i^{load}\left(1-e^{-\frac{E_i}{c_i^{load}}t_\mathrm{p}}\right)e^{-\frac{E_i}{c_i^{load}}(t-t_\mathrm{p})}\boldsymbol{M}_\gamma\boldsymbol{v}_N^{push}, \tag{15}$$

Using Eqs. 14 and 15, we are able to calculate the forces in both push and keep phases. The difference between the calculated forces and experimentally measured ones is then minimized to estimate the parameters. The objective function is formulated as

$$E(\boldsymbol{\theta})=\sum_{i=1}^{n}\left\|\boldsymbol{F}_i^{cal}(\boldsymbol{\theta})-\boldsymbol{F}_i^{\exp}\right\|^2, \tag{16}$$

where vector $\boldsymbol{\theta}=[E_1,\ E_2,\ c_1^{load},\ c_2^{load}]^\mathrm{T}$ consists of the parameters to be determined. Vector $\boldsymbol{F}_i^{\exp}$ is the force measurements from experiments at the *i*-th sampling time and vector $\boldsymbol{F}_i^{cal}(\boldsymbol{\theta})$ is the calculated forces. The termination threshold is the tolerance on $E(\boldsymbol{\theta})$ or $\boldsymbol{\theta}$ less than 1×10^{-6}. Instead of using the calculated forces, we can also obtain a set of rheological forces by running FE simulations during the optimization process. However, the optimization will become very time-consuming since four unknown parameters are involved and the FE simulation has to be iterated many times.

2.5.3 Estimation of parameters c_1^{rel} and c_2^{rel}

In [Wang & Hirai (2010a)], we found that it is hard to accurately predict both rheological force and the final-shape simultaneously by using a linear FE model. There is a contradiction between the reproduction of force and the approximation of final-shape. We have therefore introduced a dual-moduli viscous element (Figure 6a) to deal with this issue. This element switches the viscous moduli from one value (c_i^{load}) to the other (c_i^{rel}) during simulation. To accurately reproduce the final-shape, two more parameters c_1^{rel} and c_2^{rel} need to be estimated. Since force is out of concern after releasing, we only focus on the deformation and we can determine these two parameters by minimizing the difference in final-shapes between simulation and experiment. The objective function is formulated as

$$E(c_1^{rel},c_2^{rel})=\sum_{i=1}^{m}\left\|x_i^{sim}(c_1^{rel},c_2^{rel})-x_i^{final}\right\|^2, \tag{17}$$

where $x_i^{sim}(c_1^{rel},c_2^{rel})$ and x_i^{final} are the residual displacement vectors from simulation and experiment, respectively. The optimization is terminated when the tolerance on the function $E(c_1^{rel},c_2^{rel})$ is less than 1×10^{-12} or the tolerance on parameter $[c_1^{rel},c_2^{rel}]$ is less than 1×10^{-6}.

2.5.4 Calculation of parameters α_1, c_1, α_2, and c_2

After having c_i^{load} and c_i^{rel}, we can easily calculate α_1, c_1, α_2, and c_2 using the following equations:

$$\begin{aligned} &c_i+\alpha_i=c_i^{load},\\ &c_i-\alpha_i=c_i^{rel}, \qquad i=1,2. \end{aligned} \tag{18}$$

For some applications, we may only concern about the forces but not residual deformation. In such cases, it is not necessary to include the dual-moduli viscous elements in the constitutive model and we should also skip the third and fourth steps during parameter estimation. Note that the parameter estimation method proposed in this section can be applied in both 2D and 3D models.

3. Results

3.1 Parameter estimation results

Generally, the material property of an object will not differ even though the object may be subjected to different operations or has different shapes or sizes. This feature allows us to use regular-shaped objects with simple loading operations to estimate their parameters. As a result, the estimated parameters should be able to simulate arbitrary shaped objects with any operations. In our experiments, we used flat-squared objects pushed from one

side with constant velocity to estimate the parameters. Using the method proposed in Section 2.5, we estimated the physical parameters for the experimental trials given by Table 1 with 'Est-' in the trial names. Estimation results are listed in Table 3.

Note that all objects made of clay material are supposed to have the same properties since they were bought in the same pack and at the same time. The tendency of estimated parameters relative to different compressing velocities is shown in Figure 7. We found that there is no clear tendency of parameters γ, E_1, and E_2 relative to compressing velocities. On the other hand, parameters c_1 and c_2 are slightly increased along with the decreasing of compressing velocities. This is reasonable since the viscous moduli are normally used to describe the velocity related behaviors.

Because three kinds of sweets materials are made of different bean powders and with different mixture ratios, the estimated parameters are also quite different among one another. For the bacon material, due to the non-homogeneous and anisotropic properties, different compressing directions affected the physical parameters, which can be seen from the estimated parameters of bacon trials listed in Table 3. 3D mesh information and time cost for estimating parameters of two bacon trials are given in Table 4. We can see that time cost in the second step is very low comparing with the first and third steps by taking the advantage of analytical expressions of rheological force. In the first and third steps, the FE simulation was iterated several times and it costs longer time to reach an optimal solution. Comparing with the first step, two variables (c_1^{rel} , c_2^{rel}) are involved in the third step and yield higher cost in parameter estimation. The estimated parameters listed in Table 3 will be evaluated in the next subsection by predicting unknown behaviors of each material under different operations and with different shaped object.

3.2 Evaluation results of clay material

Evaluation trials of clay materials were performed using white colored objects with compressing from the top-center area and different compressing velocities. Detailed experimental information of these trials is given in Table 1 with 'Eva-' in the beginning of the trial names. The average values of estimated parameters are used to predict the rheological behaviors of these trials. Simulation results comparing with experimental measurements are shown in Figure 8. We found that both keep-shapes and final-shapes are pretty well matched between simulations and experiments but the predictions of rheological forces showed some errors in all trials.

3.3 Evaluation results of sweets material

To evaluate the estimated parameters of sweets materials, several non-homogeneous layered objects were tested. Each of them consists of three layers with two different kinds of sweets materials. Two kinds of compressing operations were performed on these objects. One is from entire top side of the objects and the other one is from the center area of the top side. The FE modeling of non-homogeneous rheological object was presented in [Wang & Hirai (2011b)]. The idea is to virtually separate a non-homogeneous object into several homogeneous ones with their own physical properties. The behaviors of the non-homogeneous object were then simulated by combining the behaviors of individual homogeneous ones using a set of boundary constraints. The estimated parameters for each sweets material listed in Table 3 are used to simulate the non-homogeneous objects.

Simulation results comparing with experimental measurements are shown in Figures 9 and 10. Figure 9 shows that both deformed shapes and forces are pretty well matched between simulation and experiment. In Figure 10, however, the force profiles experience certain errors comparing with the results in Figure 9. This is because the compressing operation. In Figure 9, the objects were compressed from the entire top sides which are the same with the operation used for parameter estimation. On the other hand, the compressing operation in Figure 10 is different from the one used in parameter estimation. Therefore, we can conclude that similar operations used for parameter estimation will yield better performance in prediction. Even with different operations as shown in Figures 8 and 10, we still can obtain acceptable prediction results, which denote that our FE model and parameter estimation method are able to predict both rheological force and deformation behaviors simultaneously, even for non-homogeneous object with different operations.

3.4 Evaluation results of bacon material

Evaluation results presented above are all based on 2D FE model. To validate our model and method in 3D cases, we performed 3D parameter estimation and evaluation using bacon material. Two flat-squared bacon slices were firstly tested with a push-keep-release procedure for estimating the parameters. Experimental data consist of force and static images, which are same with 2D case. However during parameter estimation, 3D FE model was used instead of the 2D one. 3D tetrahedra meshes are generated using an open-source mesh generator named 'TetGen' [Si (2009)], which creates a 3D mesh based on the information of the object boundary. The estimated

parameters are listed in Table 3.

Two experimental trials were performed with irregular shaped (trapezoid and diamond) bacon slices for evaluating the estimated parameters. The muscle fiber textures of trial 'Eva-B-V-T' and 'Eva-B-P-D' are vertical and parallel to the compressing direction respectively. To show the effects of the muscle fiber texture, both sets of estimated parameters are used to simulate the two evaluation trials. Simulation results comparing with experimental ones are shown in Figures 11 and 12, respectively.

Figure 11 shows that the simulated deformation behaviors matched the experimental ones quite well with both sets of estimated parameters. Unfortunately, the force experiences certain errors with both sets of parameters. We can see that the simulated force profiles are quite similar for both sets of parameters but the force amplitudes are quite different. The parameter set 'Est-B-V' yields larger force amplitude (Figure 11b-1) comparing with 'Est-B-P'. From Table 3 we also found that the parameter set 'Est-B-V' has larger values than parameter set 'Est-B-P'. This means that we feel harder if we compress the bacon slice in a direction vertically to the muscle fiber texture. From Figure 11, it is hard to say which set of parameters is better for predicting the force profile.

On the other hand, from Figure 12 we can easily tell that the parameter set 'Est-B-P', whose muscle texture direction is the same with the evaluation trial 'Eva-B-P-D', yields much better predictions of force profile. This tells us that the compressing direction affects the parameter estimation results for anisotropy objects and further affects the prediction results as well. Therefore, it would be better if we could perform the experiments for estimation using the same operation, especially the compressing direction, with the one using in prediction.

The simulated deformation behaviors shown in Figures 11 and 12 are 2D surface view projected from 3D simulations for the convenience of comparison. Figure 13 shows the 3D simulation snapshots for both evaluation trials. The 3D views of keep-shapes for both trials are shown in Figures 13a and 13b. Figures 13c and 13d are used to demonstrate the change in object thickness of both trials. To accurately approximate the experimental condition (Figure 2), one surface of the objects (left sides in Figures 13c and 13d) is constrained to make sure no deformation happens during simulation and only the other side of the objects is allowed to deform freely. Evaluation results shown in Figures 11, 12, and 13 suggested that our FE dynamic model and parameter estimation method are applicable in 3D cases as well.

4. Conclusions

In this paper, we presented 2D/3D FE dynamic model and a parameter estimation method for accurately simulating the behaviors of rheological objects. Due to the presence of residual deformation, modeling of rheological object is more difficult than doing an elastic one. Especially, it is hard to accurately predict both rheological force and residual deformation simultaneously. Therefore in this paper, we proposed a parallel five-element model with two dual-moduli viscous elements to deal with this issue. The dual-moduli viscous element has an ability to switch the viscous coefficient from one value to the other during simulation. This makes the object behave differently under loading and unloading operations, which can be physically explained as property change caused by the deformation. By imposing a parallel 5-element model onto each triangle or tetrahedron to govern its behaviors, 2D/3D FE dynamic model was formulated to simulate homogeneous objects and it can be easily extended to simulate non-homogeneous objects as well. There are 7 unknown physical parameters in this model. To determine these parameters, a four-step method for parameter estimation was proposed. This method aims at minimizing the difference in rheological force and deformation between simulation and experiments. By taking the advantage of parallel configuration of the constitutive model, we can analytically solve the force expressions. Consequently, the efficiency of the estimation method was improved significantly. It can reach an optimal solution in the second step within several seconds. This method can be also applied in other FE models as long as a parallel model is used.

Three typical rheological materials: clay, Japanese sweets, and bacon, were tested to validate the proposed FE model and parameter estimation method. Several square-flat shaped objects made of each material were indented and the measurements of force and deformation were recorded. Parameter estimations were performed with 2D FE model for clay and Japanese sweets materials and with 3D model for bacon material. Estimated parameters show that the indentation displacement and velocity do not affect the estimation results much but the indentation directions for an anisotropic object do affect the estimated parameters. To evaluate the estimated parameters, indentation experiments were performed for each material with different compressing operations, non-homogeneous, and irregular-shaped objects, respectively. Evaluation results show that we can predict the deformation quite well but the predictions of rheological forces experience certain errors for some trials. Using same compressing operations and same indentation directions in both estimation and evaluation always yield better prediction results. Due to the difference in object shapes, properties, and compressing operations, we think

the prediction errors in rheological forces shown in Figures 8, 9, 10, 11, and 12 are acceptable and the prediction results are accurate enough for some applications, such as haptic display of object manipulation. Evaluation results finally suggested us that our FE model and parameter estimation method are suitable for simulating rheological objects and can successfully reproduce both rheological force and deformation behaviors simultaneously, even for non-homogeneous, anisotropic, and 3D objects.

This paper is a study on methodology of object modeling but not on particular application. The proposed FE model and parameter estimation method can be easily applied in various applications as long as the measurement data of force and deformation field are available. They are also not limited to model rheological objects but can be used to model elastic, viscoelastic, and plastic objects as well with slight change in the constitutive model. Note that the dual-moduli viscous element is not necessary to be used in modeling elastic or viscoelastic objects since there is no residual deformation happens in such objects. Accordingly, the third and fourth steps in parameter estimation method can be skipped for dealing with elastic or viscoelastic objects.

In this paper, only compression loadings and isotropic properties were considered. In the future, tensile loading, orthotropic, and anisotropic properties will be considered as well to better understand the behaviors of rheological objects.

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Table 1. Detailed information of all experiments trials

Material	Trial	Thickness of object (mm)	Type of push	Velocity of push (mm/s)	Time	
					tp (s)	tk (s)
Clay	Est-C-R-06	12.0	top	0.5	12.07	303.78
	Est-C-R-08	10.5			16.10	304.78
	Est-C-R-10	10.5			20.12	311.82
	Est-C-B-06	10.0		0.2	30.17	311.83
	Est-C-B-08	10.0			40.24	321.88
	Est-C-B-10	10.0			49.29	342.00
	Est-C-Y-06	11.0		0.1	58.34	502.94
	Est-C-Y-08	10.0			79.46	500.94
	Est-C-Y-10	11.5			98.58	609.57
	Eva-C-W-t1	12.0	center	0.5	16.09	369.16
	Eva-C-W-t2	10.5		0.2	40.24	400.34
	Eva-C-W-t3	10.0		0.1	79.46	601.52
Sweets	Est-S-W	12.0	top	0.2	28.87	182.06
	Est-S-Y	11.0			29.68	181.26
	Est-S-P	11.0			29.97	181.46
	Eva-S-Y+P	11.0			49.49	181.47
	Eva-S-W+P	11.0			49.49	181.97
	Eva-S-W+Y-C	11.0	center		49.69	181.86
	Eva-S-Y+P-C	11.0			39.13	182.07
Bacon	Est-B-P	14.0	top	0.1	8.0	97.60
	Est-B-V	14.0			8.0	95.80
	Eva-B-P-D	10.5			7.3	92.80
	Eva-B-V-T	11.0			6.8	69.00

The first term in the trial name denotes that the trial is used to estimate the parameters (‘Est-’) or evaluate the parameters (‘Eva-’), respectively. The second term denotes different materials with ‘-C-’ standing for clay, ‘-S-’ for sweets, and ‘-B-’ for bacon materials, respectively. The third term in clay and sweets trials denotes the object color with ‘-R-’ for red, ‘-B-’ for blue, ‘-W-’ for white, ‘-Y-’ for yellow, and ‘-P-’ for purple, respectively. The third term in bacon trials denotes that the pushing direction is parallel (‘-P-’) or vertical (‘-V-’) to the texture of muscle fiber. The remaining terms in different trials are some parameters used to distinguish between one another, for example, the numbers (‘-06’, ‘-08’, and ‘-10’) in the clay trials for parameter estimation denote rough indentation displacements during experiments, the letters (‘-D’ and ‘-T’) in last two trials of bacon denote that the objects have irregular shapes of diamond and trapezoid, respectively.

Table 2. The constitutive laws of generalized models

Models	Type	The constitutive law
Serial	1 (no free elastic)	$\sum_{j=0}^{n} B_j^{s1} \frac{\partial^j \sigma}{\partial t^j} = \sum_{i=1}^{n+1} A_{i-1} \frac{\partial^i \varepsilon}{\partial t^i}$
	2 (free elastic)	$\sum_{j=0}^{n+1} B_j^{s2} \frac{\partial^j \sigma}{\partial t^j} = \sum_{i=1}^{n+1} A_{i-1} \frac{\partial^i \varepsilon}{\partial t^i}$
Parallel	1 (free viscous)	$\sum_{i=0}^{n} A_i \frac{\partial^i \sigma}{\partial t^i} = \sum_{j=1}^{n+1} B_j^{p1} \frac{\partial^j \varepsilon}{\partial t^j}$
	2 (no free viscous)	$\sum_{i=0}^{n} A_i \frac{\partial^i \sigma}{\partial t^i} = \sum_{j=1}^{n} B_j^{p2} \frac{\partial^j \varepsilon}{\partial t^j}$

In serial models, 'free elastic' indicates that an elastic element appears without a viscous element connected in parallel. In parallel models, 'free viscous' indicates that a viscous element appears without an elastic element connected in serial.

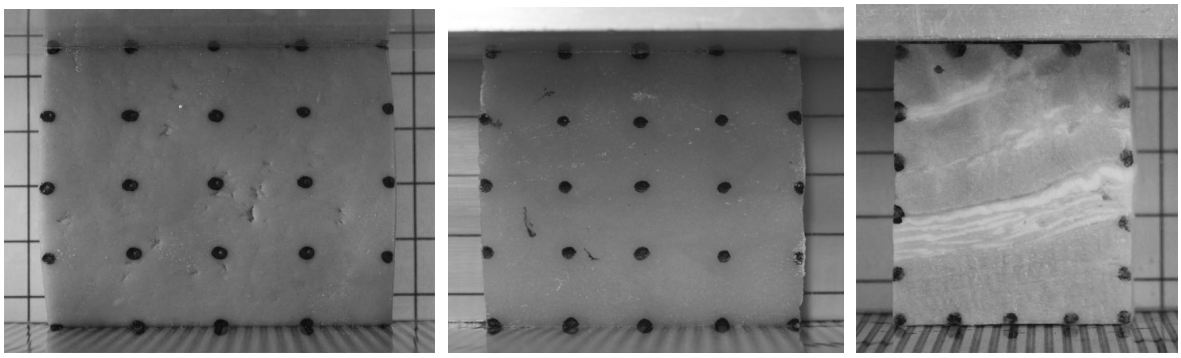
Table 3. Estimated parameters for trials with 'Est-' in the trial names

Material	Dim.	Trial name	γ	E_1 (Pa)	E_2 (Pa)	c_1 (Pa·s)	c_2 (Pa·s)	α_1 (Pa·s)	α_2 (Pa·s)
Clay	2D	Est-C-R-06	0.2672	2.8650×10^4	6.0364×10^4	4.4433×10^6	2.7411×10^5	4.3890×10^6	2.7408×10^5
		Est-C-R-08	0.2902	3.1753×10^4	7.2147×10^4	6.6600×10^6	3.5334×10^5	6.6310×10^6	3.4397×10^5
		Est-C-R-10	0.2367	2.1954×10^4	6.7528×10^4	4.2494×10^6	3.4789×10^5	4.2225×10^6	3.3506×10^5
		Est-C-B-06	0.2537	1.6582×10^4	4.2801×10^4	3.0272×10^6	3.1220×10^5	3.0032×10^6	2.9812×10^5
		Est-C-B-08	0.2292	2.2164×10^4	6.0319×10^4	4.4116×10^6	4.8374×10^5	4.3764×10^6	4.7677×10^5
		Est-C-B-10	0.2602	2.2424×10^4	7.1494×10^4	4.6666×10^6	6.2660×10^5	4.6432×10^6	6.1250×10^5
		Est-C-Y-06	0.2593	1.7273×10^4	3.6229×10^4	5.3295×10^6	4.3810×10^5	5.3065×10^6	4.1135×10^5
		Est-C-Y-08	0.2479	2.1804×10^4	4.2930×10^4	9.7251×10^6	6.9993×10^5	9.7039×10^6	6.6577×10^5
		Est-C-Y-10	0.2494	1.5206×10^4	4.1475×10^4	7.3118×10^6	7.5971×10^5	7.2902×10^6	7.1849×10^5
		Average	0.2549	2.1979×10^4	5.5032×10^4	5.5361×10^6	4.7729×10^5	5.5073×10^6	4.5957×10^5
Sweets	2D	Est-S-W	0.3746	1.3468×10^4	2.4695×10^4	1.4820×10^7	5.3855×10^4	1.4811×10^7	1.8527×10^4
		Est-S-Y	0.3353	1.0553×10^4	3.7276×10^4	6.6096×10^6	7.8271×10^4	6.6034×10^6	3.7659×10^4
		Est-S-P	0.3267	9.1565×10^3	5.0802×10^4	4.0958×10^6	8.2198×10^4	4.0851×10^6	5.2072×10^4
Bacon	3D	Est-B-P	0.4928	5.8250×10^4	1.1640×10^4	2.8537×10^7	1.8828×10^4	2.8513×10^7	1.7652×10^4
		Est-B-V	0.4923	6.7607×10^4	1.2988×10^4	3.7292×10^7	2.4118×10^4	3.7243×10^7	2.2872×10^4

Parameters of clay and sweets materials were estimated using 2D FE model and parameters of bacon were estimated using 2D measurements but 3D FE model.

Table 4. 3D mesh information and time cost for parameter estimation of bacon trials

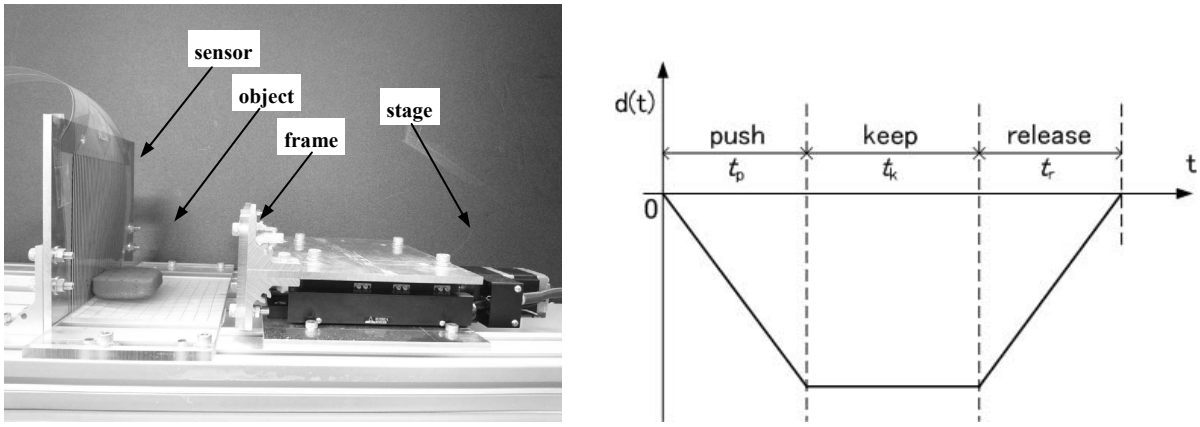
Trial name	Node number	Tetrahedra number	Time cost (s)		
			Step 1	Step 2	Step 3
Est-B-P	171	492	1898	1.5495	14530
Est-B-V	147	414	725	1.1635	18315



(a) Clay (b) Sweets (c) Bacon

Figure 1. Objects made by three typical rheological materials used in experiments

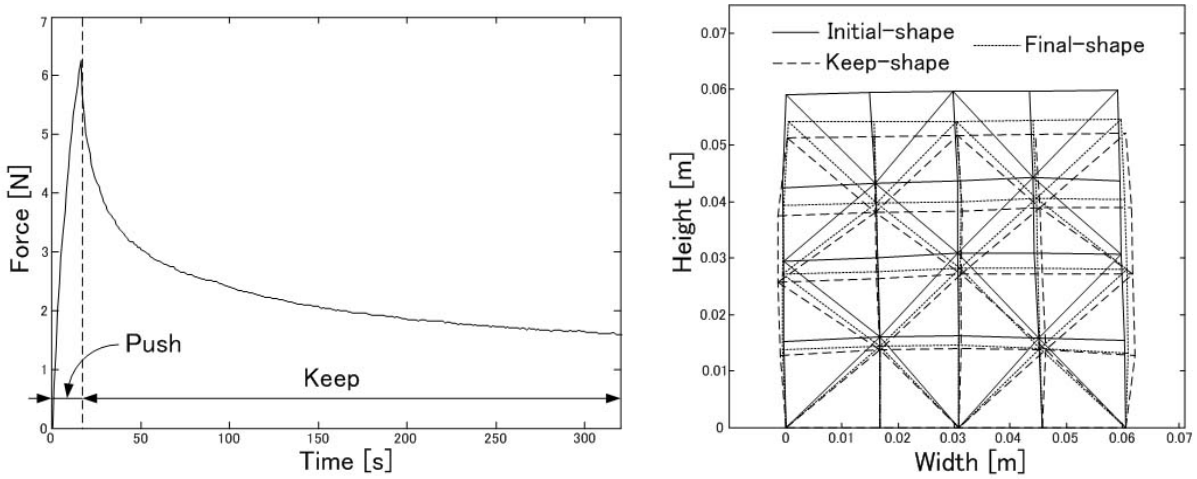
The black dots on the surface of the objects were manually drawn before experiments for the convenience of making FE meshes and clearly capturing the deformation.



(a) Experimental setup (b) Displacement input

Figure 2. Experimental setup (a) and input displacement function (b) for the indentation experiments

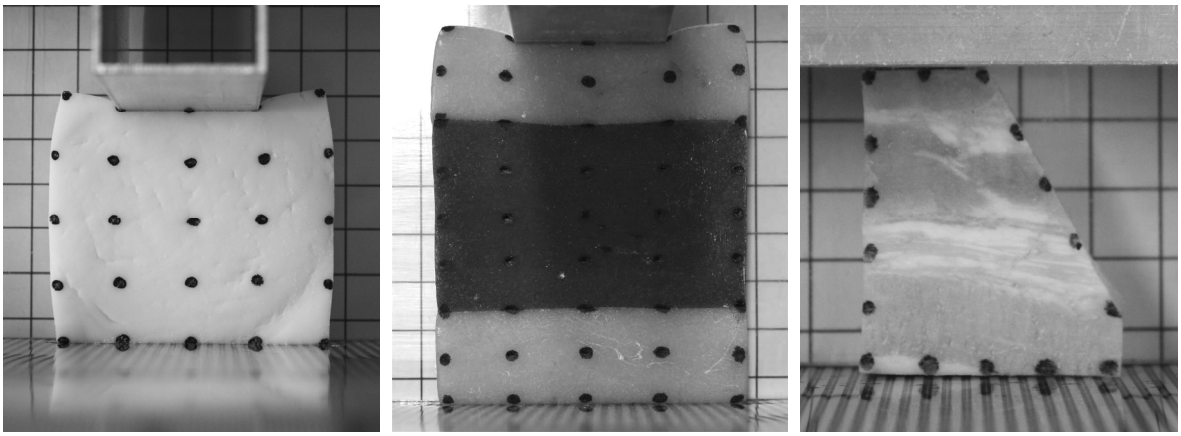
The object is pushed from time 0 to t_p by the linear stage with a constant velocity and this time period is called push phase. During time t_p to t_p+t_k (keep phase), the linear stage is stopped and keep the deformation unchanged. Accordingly, the deformed shape during this period is called keep-shape. After time t_p+t_k, the linear stage is moved back to the original position and the deformation generated inside the object is allowed to recover freely and finally reach a permanent shape, which is called final-shape in this paper.



(a) Rheological force (b) Deformed shapes

Figure 3. Experimental measurements of rheological behaviors

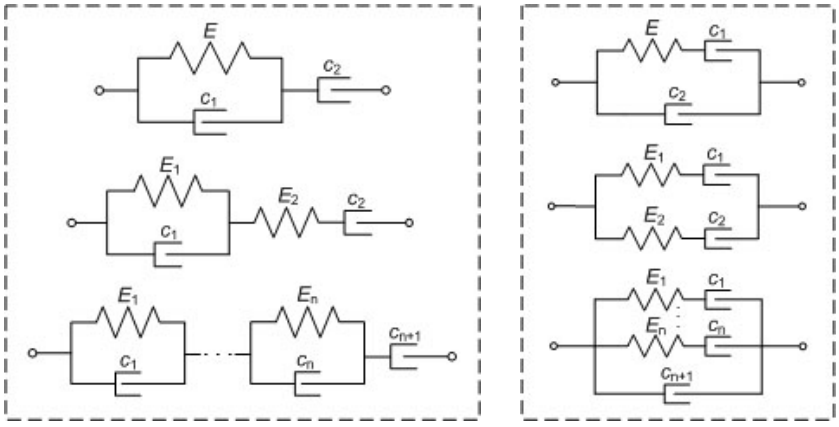
During push phase, the rheological force is increasing in an almost linear manner. During the keep phase, the deformation is kept unchanged (keep-shape) but the force response is decreasing in a nonlinear manner, which is referred to as force relaxation. After releasing, the rheological force goes to zero but the deformation starts to recover and finally reach a permanent shape called final-shape.



(a) Clay (b) Sweets (c) Bacon

Figure 4. Different pushing operations and different shaped objects used in evaluation experiments.

In clay case, the square-shaped objects were deformed from the center part of the top sides instead of entire top sides in estimation trials. In the case of Japanese sweets, non-homogeneous layered objects were used instead of homogeneous objects in estimation trials. In bacon case, irregular-shaped objects were used instead of regular-shaped objects in estimation trials.



(a) Serial models (b) Parallel models

Figure 5. Two categories of rheological constitutive models: the serial (a), and parallel (b) models.

Firstly, we introduce four basic elements: the elastic, viscous, Maxwell, and Kelvin elements, as presented in [Wang & Hirai 2010a]. Then, the serial model can be defined as a model consisting of a set of basic elements connected in serial. Similarly, the parallel model is defined as a model consisting of a set of basic elements connected in parallel.

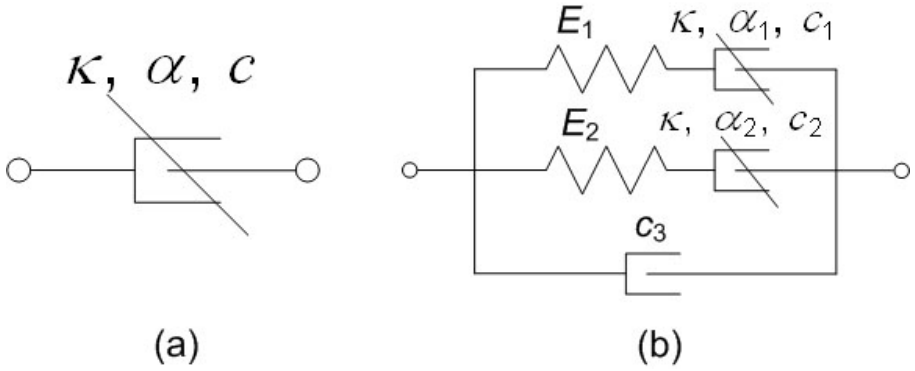


Figure 6. The dual-moduli viscous element (a) and the parallel five-element model (b) with two dual-moduli viscous elements, which is used in this paper to model rheological objects

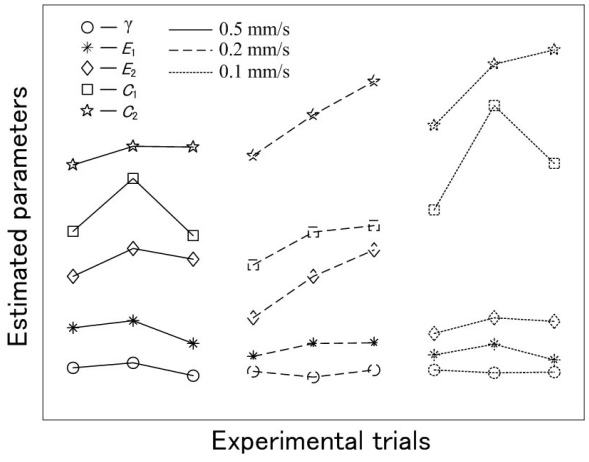


Figure 7. Tendency of estimated parameters relative to different compressing velocities

Different line types denote different compressing velocities and different markers denote different parameters.

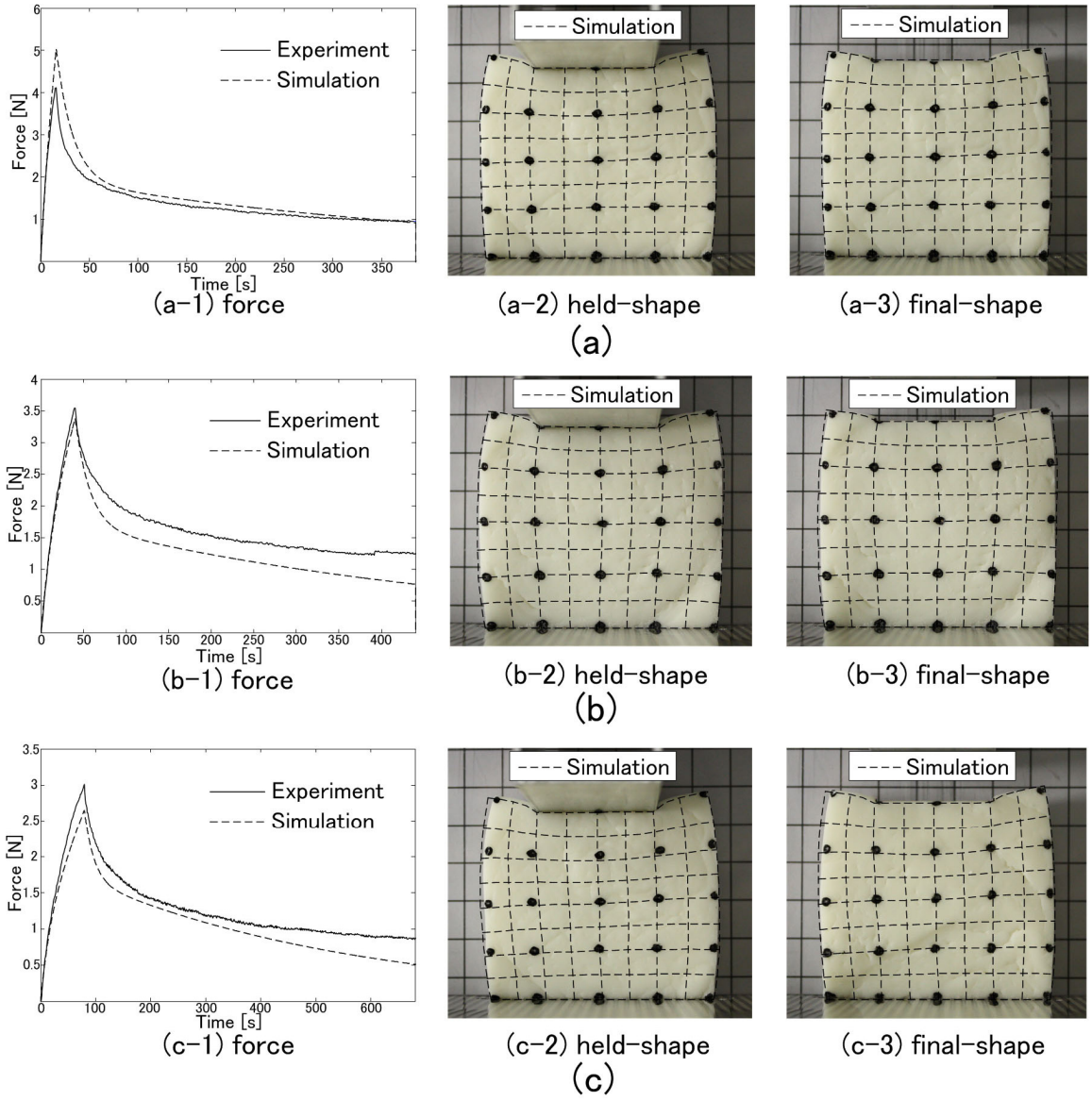


Figure 8. Evaluation results of clay objects with a compressing velocity of (a) 0.5m/s, (b) 0.2m/s, and (c) 0.1m/s, respectively

Because the deformation behaviors are complicated (especially in the contact corners), a triangular mesh with 17×17 nodal resolution was used in simulations, but only quadrilateral meshes (each quadrilateral consists of eight triangles) were demonstrated for the convenience of comparisons.

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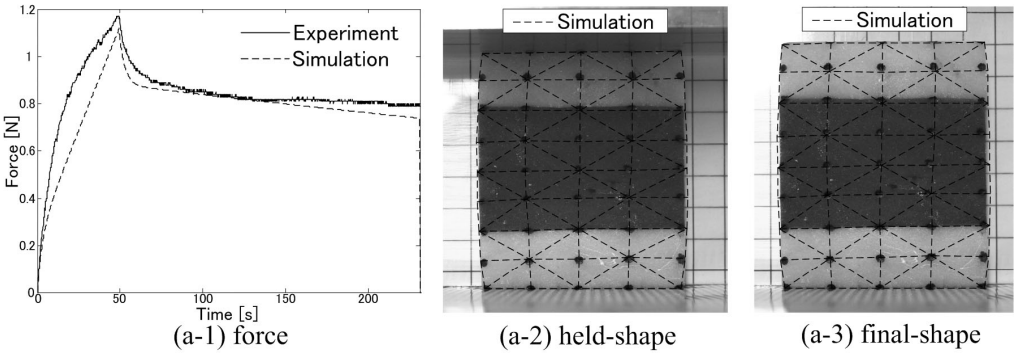
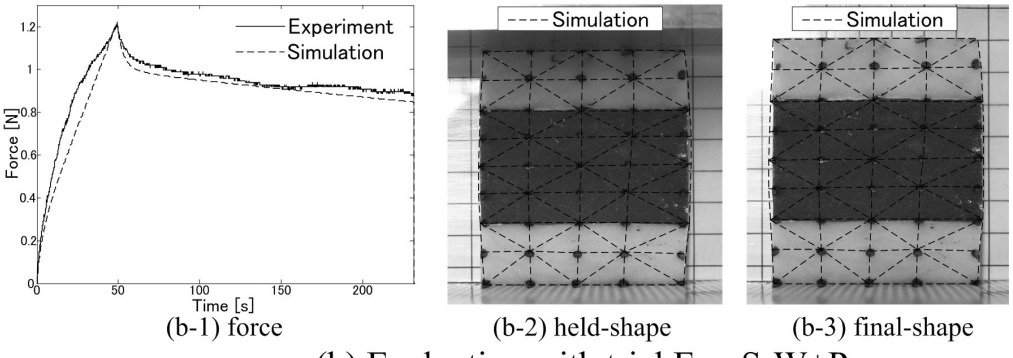


(a-1) force (a-2) held-shape (a-3) final-shape

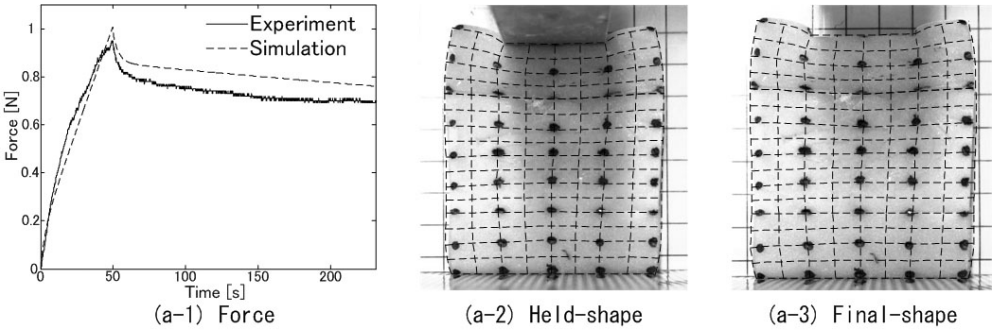
(a) Evaluation with trial Eva-S-Y+P



(b-1) force (b-2) held-shape (b-3) final-shape

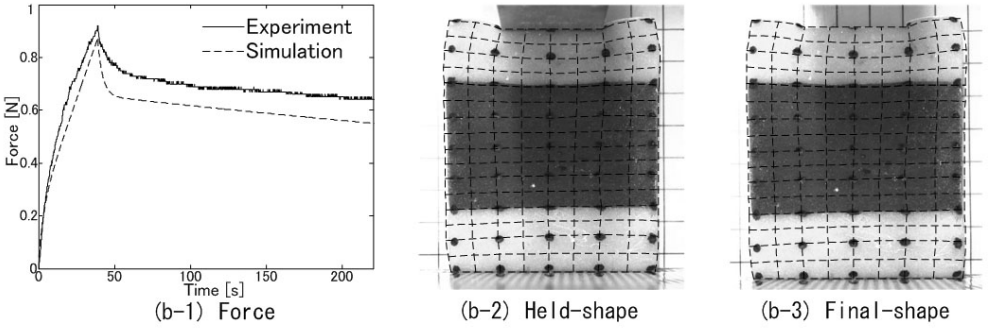
(b) Evaluation with trial Eva-S-W+P

Figure 9. Evaluation results of non-homogeneous sweets objects with a top-side compression



(a-1) Force (a-2) Held-shape (a-3) Final-shape

(a) Evaluation with trial Eva-S-W+Y-C

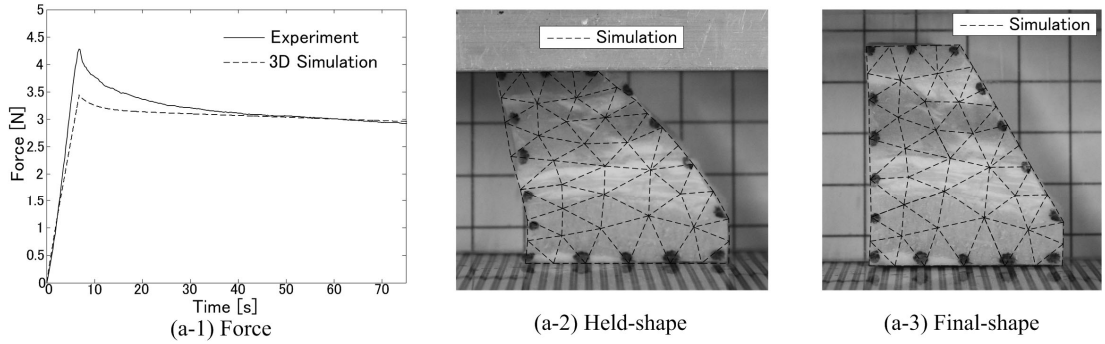


(b-1) Force (b-2) Held-shape (b-3) Final-shape

(b) Evaluation with trial Eva-S-Y+P-C

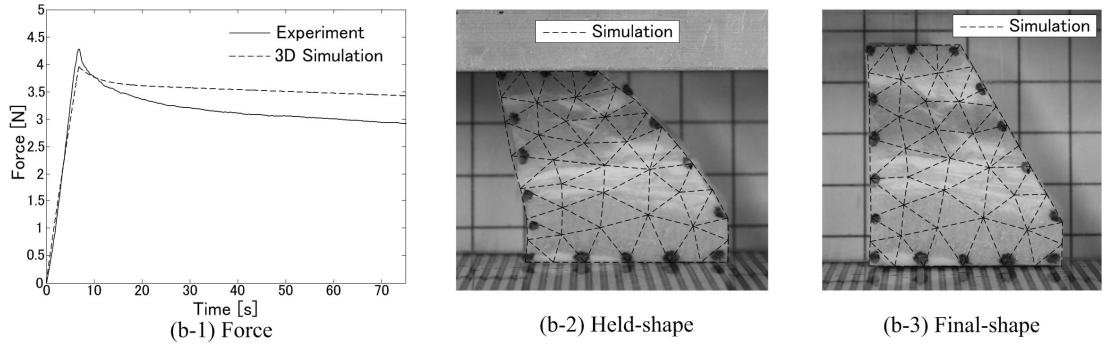
Figure 10. Evaluation results of non-homogeneous sweets objects with a center compression

The objects were simulated with triangle mesh in a nodal resolution of 17×33, but only displayed as quadrilateral meshes (each quadrilateral consists of eight triangles) for the convenience of comparison.



(a-1) Force (a-2) Held-shape (a-3) Final-shape

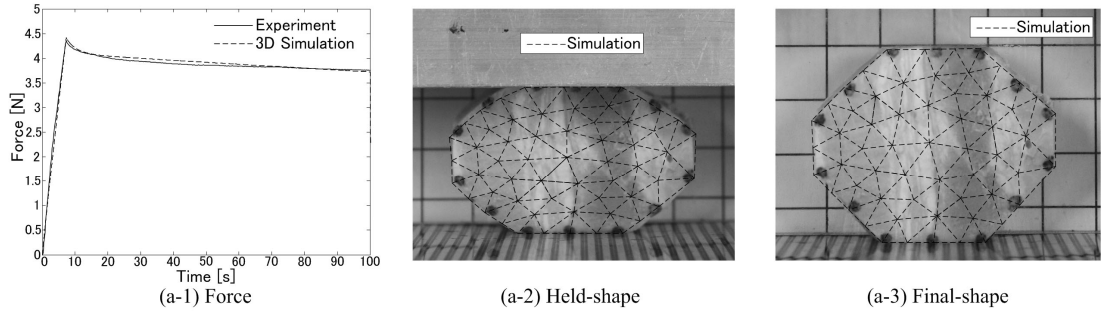
(a) Evaluation of Eva-B-V-T with parameters from Est-B-P



(b-1) Force (b-2) Held-shape (b-3) Final-shape

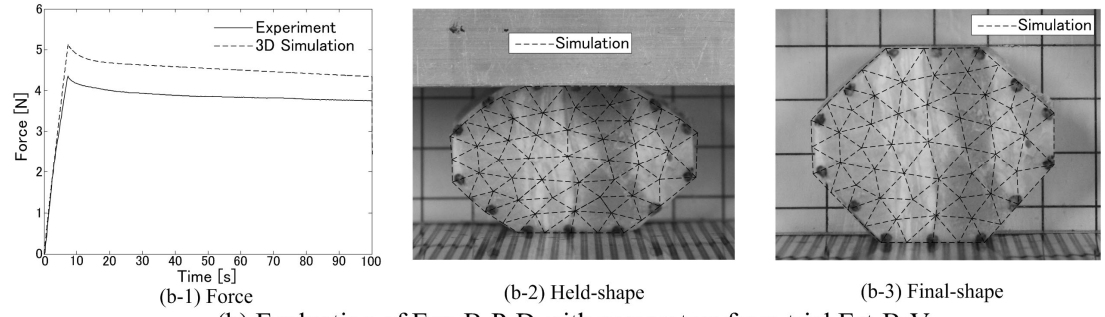
(b) Evaluation of Eva-B-V-T with parameters from Est-B-V

Figure 11. 3D evaluation results of bacon object with a trapezoid shape (Eva-B-V-T). The estimated parameters used in both (a) and (b) are from 3D estimation results of trial Est-B-P and Est-B-V, respectively



(a-1) Force (a-2) Held-shape (a-3) Final-shape

(a) Evaluation of Eva-B-P-D with parameters from trial Est-B-P



(b-1) Force (b-2) Held-shape (b-3) Final-shape

(b) Evaluation of Eva-B-P-D with parameters from trial Est-B-V

Figure 12. 3D evaluation results of bacon object with a diamond shape (Eva-B-P-D). The estimated parameters used in both (a) and (b) are from 3D estimation results of trial Est-B-P and Est-B-V, respectively

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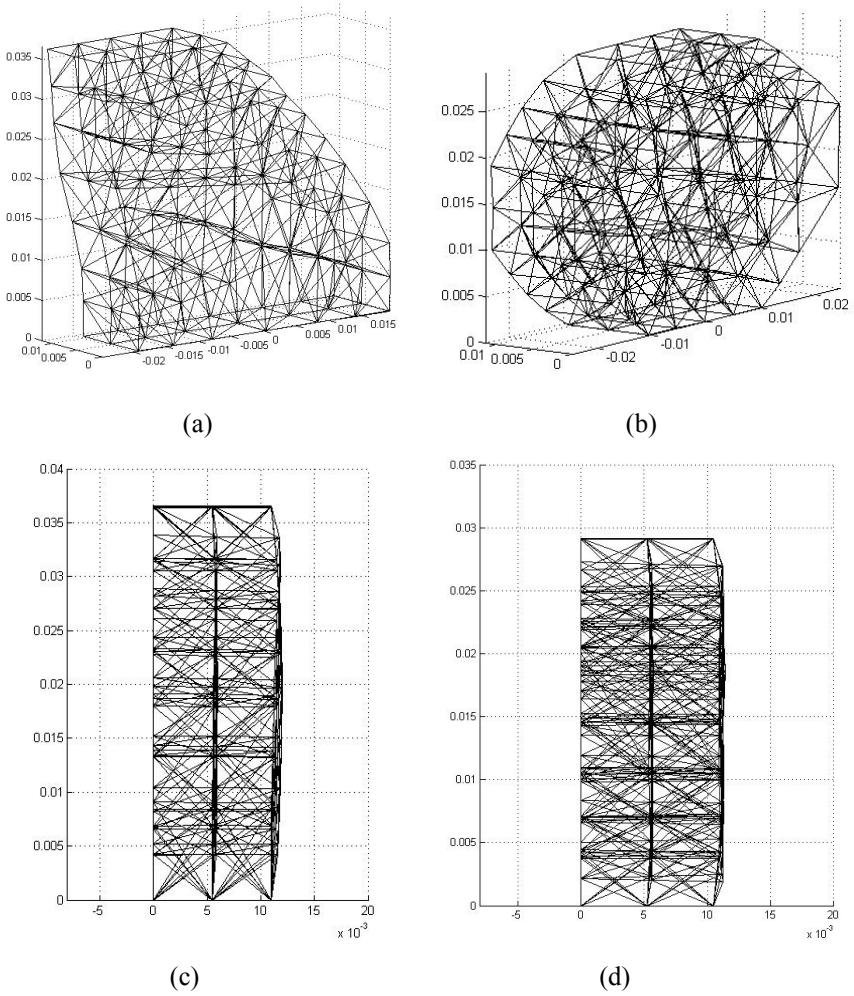

Figure 13. 3D simulation snapshots: (a) trial Eva-B-V-T in 3D view, (b) trial Eva-B-P-D in 3D view, (c) trial Eva-B-V-T in 2D lateral view, and (d) trial Eva-B-P-D in 2D lateral view

The Experiences of Elderly People Living at Home Related to Their Receiving Meals Distributed by a Municipality in Sweden

Zada Pajalic (Corresponding author)
School of Health and Society, Kristianstad University
Elmetorpsvägen 15, Kristianstad 29188, Sweden
&
School of Medical and Health Sciences
Örebro University, Örebro 70182, Sweden
E-mail: zada.pajalic@hkr.se

Lena Persson
School of Health and Society, Kristianstad University
Elmetorpsvägen 15, Kristianstad 29188, Sweden
E-mail: lena.person@hkr.se

Albert Westergren
School of Health and Society, Kristianstad University
Elmetorpsvägen 15, Kristianstad 29188, Sweden
E-mail: albert.westergren@hkr.se

Berggren Vanja
School of Health and Society, Kristianstad University
Elmetorpsvägen 15, Kristianstad 29188, Sweden
E-mail: vanja.bergren@hkr.se

Kirsti Skovdahl
School of Medical and Health Sciences
Örebro University, Örebro 701 82, Sweden
E-mail: kirsti.skovdahl@oru.se

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Abstract

The purpose of the study was to describe the experiences of elderly people, living at home who receive hot meals that are distributed by their municipality. Qualitative content analysis was used to analyse the (n=13) interviews. The results showed that feelings of dependency, loneliness and gratitude were expressed by the participants in the study related to their meals being delivered home. Dependency was expressed as not having influence over the food products the meals were made from. Loneliness was expressed as being isolated and being confined at

home alone due to difficulties getting out of the house, which was associated with the costs of taxis transportation. Gratitude was expressed by the sincere thanks for the possibility of receiving traditional meals delivered daily.

The major conclusion of the study was the indication that greater attention should be paid to meet both the practical and psychological needs of elderly people.

Keywords: Action research, Elderly people, Distribution of meals, Home-living

1. Introduction

In Sweden the "Food Distribution" (FD) system of meal distribution which may be compared to the international concept of "meals on wheels", is a service offering prepared meals delivered to elderly people or persons with health related impairments who live in their own home. The FD in Sweden is a social and care service assistance system organised by a municipality in accordance with the Swedish Social Services Act (Grönwall & Holgersson, 1998).

Swedish municipalities have the statutory obligation to provide social and care service assistance and this is regulated by two laws: the Social Services Act (Grönwall & Holgersson, 1998) and the Health and Medical Services Act (Raadu, 2011). Neither of these laws have detailed information about how FD should be organised, which allows each municipality to organise their FD services according to its own circumstances (Rothenberg & Lewald, 2006). For a person to be granted the FD service from any municipality, an assessment of the individual's requirements has to be made by community officers.

The fundamental requirement for being granted FD is that the individual is unable to do their own shopping and prepare their own meals, and the foremost reasons for this situation are illness related physical or psychical limitations (Grönwall & Holgersson, 1998). The number of elderly persons (≥65 years), in Sweden, receiving food distributed by municipally organised FD is estimated at 60,000. The requirement for this service is expected to increase in the future (Statistical Central Office, 2009).

The organisation of FD in Sweden differs from that in other welfare states where the FD, is mostly organised by either private companies or voluntary organisations, Examples of this are the USA (Frongillo et al., 2010), the UK (McKie, Clark, MacLellan, & Skerratt, 1998), Canada (Timonen & O'Dwyer, 2010), Australia (Krassie & Smart, 2000), Germany (Sneeuw, Stam, de Graaf, & van Staveren, 1991), Japan (Nakura, Tatara, Shinsho, Fukuda, & Nakajima, 1994), The Netherlands (Aagaard Nielsen & Svensson, 2006), France (Michaud et al., 1998) and Italy (Inelmen et al., 2000). In most of these countries the charges for all care and services are related to the client's income/pension. In some countries, such as Finland, the UK, Germany and France, those who cannot pay are referred to the state social assistance system. In The UK, Germany and Spain the receivers of all care and services pay 20–30% of the total cost. The fee for FD in Sweden is regulated and is based on the elderly person's ability to pay, however they pay a maximum of 10% of the total cost (Elmér, 2000).

To date, research focusing on elderly people's experiences related to food and eating hasmainly been concerned with eating as seen from a social and cultural perspective (Sidenvall, 1995). There are studies showing that meals-on-wheels receivers are at risk of malnutrition (Coulston, Craig, & Voss, 1996; Johansson, Bachrach-Lindstrom, Carstensen, & Ek, 2009; Payette, Boutier, Coulombe, & Gray-Donald, 2002). Other studies show that FD is an important service but that the consumer's experience of the service and products depends upon a range of factors including nutritional status (Krassie & Smart, 2000; Roberts, Wolfson, & Payette, 2007), demographic and socio-economic factors, and menus that accommodate the individual consumers' requirements and preferences. This highlights the importance of assessing the consumers' experiences in order to improve the FD services (Almanza, Namkung, Ismail, & Nelson, 2007; Krassie & Smart, 2000). Elderly people who receive their meals distributed through an FD service have important experiences and knowledge to share that can be used for improving the FD service (Gustafsson, Ekblad, & Sidenvall, 2005; L. Johansson, Sidenvall, Malmberg, & Christensson, 2009). However, there is, a lack of studies focusing on elderly people's experiences of receiving meals distributed by a municipality. This knowledge is of importance in order to provide and ensure the high-quality of the FD services and that the services meet the client's individual requirements.

2. Aim

The purpose of the study was to gain insight into, and describe the experiences of elderly people, living at home who receive hot meals that are distributed from the municipality.

3. Research Method

This qualitative, descriptive study is a part of larger research project using an action research (AR) approach (Bondas, 2003; Meyer, 1993; Stringer & Genat, 2004) focusing on meal distribution to elderly persons (≥65 years) living at home.

3.1 The context

The project was conducted in a medium-sized municipality of about 80,000 inhabitants situated in southern Sweden. Approximately 650 elderly people living in the municipality used the FD service, but this number varied over the weeks as some pensioners only used the FD service on some days of the week. The meals were produced at a municipally owned kitchen. The menu consisted of two hot dishes each day and an extraordinary meal once a month. The most common meal delivered was lunch, which corresponded to 30% of the clients' daily nutritional needs. The possibility to also have evening meals delivered did exist, but was not commonly used. The lunch also included raw vegetables and fruit or dessert all inclusive. Assistant nurses or taxi drivers were responsible for the transportation and delivery of the meals. The meal box was supposed to be delivered personally to the customer, that is to say "into their hands", and not simply left outside their door.

3.2 Study participants

The inclusion criteria for the participants in the study were that the informants should be aged ≥ 65 years, receiving FD, living in their ordinary home, and able to communicate verbally in Swedish. During July 2009 an information letter about the study and a request for informed consent and an addressed reply envelope was sent, with a meal box delivery, to all those persons who met the inclusion criteria. Those elderly persons who agreed to participate in the study replied, giving their informed consent and information noting when they wished to be contacted by the first author (ZP). Thirteen elderly persons agreed to participate in the study: three men aged between 75 and 92 years (average 83.5 years) and ten women aged between 68 and 89 years (average 78.5 years). All were living alone and received meals from the FD service delivered either by assistant nurses (n=9) or by taxi drivers (n=3).

3.3 Data collection

Individual semi-structured interviews were conducted (Kvale, 2007). The interviews began with the interviewer encouraging the participants to talk about what they felt was important relating to their receiving meals distributed by the municipality and what were their experiences of the service. The interviews were conducted, by the first author (ZP), in the homes of the participants, during September–December 2009, and each lasted about 45 to 60 minutes. The interviews were tape-recorded and transcribed verbatim.

3.4 Analysis process

Manifest and latent qualitative content analysis (Graneheim & Lundman, 2004) was used to analyse the interviews. The transcripts were read by authors (ZP, VB & AW) as openly as possible to gain a sense of the whole and to get ideas for the further analysis with the study aim as a basis. Meaning units were identified and condensed into codes. A manifest analysis was used to describe that which was obvious and visible in the text. Next, a latent analysis was used to grasp deeper meaning in the text. Questions related to what the text was talking about and what this meant were "addressed to the text" in order to reveal the deeper structural meaning. The latent analysis consisted of dialectical movements between the whole and the parts, between understanding and explanation and between explanation and comprehension. The codes were critically reflected on and compared in relation to the research question and sorted into categories and one theme. Lastly, all authors reflected and discussed the findings in relation to the research question and were agreed concerning the final interpretation of the theme and categories. A main theme "The meal box as a symbol of feeling dependent and lonely but grateful" arose from three categories: "Being dependent but able to exert influence on a small scale", "Feeling lonely" and "Feeling grateful"

3.5 Ethical considerations

The study was performed in accordance with the World Medical Association Declaration of Helsinki (Saif, 2000) and has been examined by the regional ethical review board (LU09/365). All the participants received written information explaining the nature of the study, what was required of them, that their participation was voluntary, and that they had the right to end their participation at any time without any consequences for them. When the participants gave their informed consent to participate in the study the first author contacted them and booked a time and place for the interview. The information given earlier about the purpose of the study, voluntary participation, the right to withdraw from participation in any interview at any time, without any consequences for them, was given to the participants again, verbally, before each interview.

4. Results

4.1 The meal box as a symbol of feeling dependent and lonely but grateful

The participant's experiences of receiving meals from the FD were interpreted as interplay between mental, social, emotional and existential needs. Feelings of dependency and loneliness were expressed by the elderly in relation to the timing of the delivery of their meals, as this could vary about one hour from day to day. Dependency could also be interpreted in situations when the elderly felt they had no influence over the food products from which the meals were made or the type of the fruit delivered with the meal. For instance, some participants wanted fruit that was easy to chew and digest, like melon, grapes or bananas etc, rather than hard fruit such as apples and in some cases pears. If hard fruit was served, the possibility to have it grated should be offered. Loneliness was interpreted from situations where the elderly described their involuntary isolation due to their being confined to their home; where they found themselves physically and economically prevented from breaking their isolation; or when they felt insecure and dissatisfied with life. Gratitude was described in terms of; the possibility to choose between two different menu alternatives and their variety; daily delivery of fresh and newly prepared hot meals with the possibility to cancel the meal service when they were not at home.

4.2 Being dependent but able to exert influence on a small scale

The experience of being dependent but still able to actively influence food-related activities

was described by the participants in different ways. They knew that they had the possibility to influence, despite their being dependent and they put this possibility into practice by personally planning and writing a grocery list, choosing the dishes on the menu, and participating in meal distribution evaluation surveys. A central indication in all of the interviews with the participants was that their feeling of being active made a difference to them even in their otherwise dependent position, which in turn helped to increase their self-esteem.

"I have written a list of dishes that I would like to have on the menu…"

Having the possibility to choose meal menus they preferred was valued highly by the participants. From the menu, they had the possibility to choose between two dishes, and of these two, the traditional food was often the most popular. The participants noted that it was difficult to accept that they could not themselves handle food preparation activities in the same way as before. For them, the decrease in their bodily functions and the development of dependency came gradually, for instance, after injuries, caused by dizziness, decreased optical function or difficulty with their sense of balance. It appeared that changes or losses in physical function were hard to accept as they now found that they had to accept help. The study results indicated that dependency on others in connection with food related activities were also associated with the participant's personal privacy. Therefore, it was a difficult experience for the participants to be forced to accept help from strangers, often in the form of the various professionals involved in public home care.

"From the beginning I refused to eat but now I have agreed to do so. I used not to feel comfortable with the staff but now I am pleased with them. It feels horrible to have strangers in your own home when you are old and helpless."

The findings in the study indicated that the participants needed time to learn to accept being dependent on help and that acceptance appears to be a process in which they began by refusing the possibility of delivered meals, but accepted them after a while. The participants noted that they felt positive about being relieved of having to shop and prepare food at home. One male participant noted that he felt he had a better life since he began receiving his meals, at home through the FD, and went on to say that this was due to the fact that he no longer needed to spend his energy preparing his meals. From the statement above it would appear that some participants accepted the help offered by the meal service if it meant their not having to deal with practical issues, such as having to prepare their own meals.

Another experience that was noted by the participants was their reflection on life. One woman said that she had finally accepted help through the FD system and that she considered that this was a part of life's cycle. Food and food-related activities were experienced by the participants as some kind of action or technique aimed at giving them energy. This indicated that food was eaten simply for the sake of eating, to fend off hunger rather than, for enjoyment and sensation. Some participants said that they do not like all the dishes but they tried to eat them anyway if only for the nutritional value.

"When I get food in the meal boxes that I do not like to eat, I still try to force it down, most of the time."

The participants expressed that the fact that they were not able to influence the time of the day that the meals were delivered made it difficult for them. Sometimes their meal was delivered too early or too late for them.

They said that the taxi drivers only delivered the meal and then left. On the other hand, there were the assistant nurses who always asked if they could help by opening the meal box or putting the food on a plate. Some of the women participants spoke of the importance of meal time situations being treated as an entirety. For this reason they felt it was important that they were asked if they needed help to put the food on a plate.

"At first when I got food delivered it came around 11.30, but now it is almost 12.35, the assistant nurses are usually more on time than the taxi drivers are."

Some of the participants offered suggestions for how the service could be improved, based on their personal experiences. For example "proper food" should be prepared from natural products and not from semi-manufactured food products. According to their experience the food from institutional kitchens could well taste like home-cooked food since it was based on natural food products .They further noted that for those people who have problems with their teeth alternative types of fruit should be offered, for example soft and hard fruits with the choice of having the hard fruits grated.

"Apples and pears should be soft or grated into smaller pieces, because if I take a wrong bite my teeth will fall out."

4.3 Feeling lonely

The participant's narratives dealt with several aspects of the meal box as a symbol of their dependency in relation to their being isolated or alone in their home and their having feelings of sorrow and insecurity. They further described how loneliness was connected to their involuntary isolation in their home due to difficulties in getting around as they would wish which was due to financial restraints. All the activities they would like to do, such as shopping, meeting other people or eating at restaurants, were associated with additional costs. Regarding these limitations, the participants noted that they needed taxi transportation to get to the nearest store. They further noted that shopping activities gave them the possibility to meet other people which helped them fulfil their social needs even though such meetings were often short hopping by themselves and also gave them the opportunity to see "first hand" the articles they might wish to purchase, Taxi transportation entailed extra costs which they had difficulty in affording. The participants expressed how they felt sad about their loss of ability to shop for food by themselves, to keep updated with new products, and to eat together with significant others.

"Since last September I was honestly quite out of my mind, since I put my dogs to sleep when I had to stay at the hospital for three months and then when I came home I ate alone. I couldn't talk to the kitchen walls. I have no living relatives."

Feelings of sorrow were emphasised even more by elderly people whose family members had died. One of the participants described how some dishes reminded her of her late daughter who had been disabled. As a mother she had cared for her daughter, among other ways by cooking her favourite meals.

"I loved to cook and my favourite was cabbage soup that I made for my daughter who was disabled."

Or as one participant, a widower put it: *"It feels terrible to eat alone; I can't even talk about it."*

When the category loneliness was reflected upon it seemed that this was also connected to feelings of insecurity, discomfort with a new situation, and the fact that the participants did not know who they should contact if they had any questions. Their narratives also expressed their feelings of being exposed if they should be confronted with difficulties due to having insufficient information from the professionals responsible for the distribution of meals, or from the taxi drivers. Some of the elderly spoke about difficulties accepting that the taxi drivers were unable to answer their questions concerning meal distribution. It appeared that the participants tried to convey the message that they should not have to continually ask for service, or as one participant described her communication with a taxi driver

"Last month I didn't get any fresh fruit so I asked him if he had any fruits or vegetables for me to which he replied "no" so I complained to him every single day"

The participants also described loneliness in the context of the symbolic value of the meals as being something important and essential in their lives. For them, this was the first time since their childhood that they became dependent on others for help and they did not find it easy to articulate their feelings of despondency that this situation brought about. It appeared as if some of their independence had been lost as a result of receiving this kind of help. Women especially, who traditionally had the responsibility for food-related tasks for their families, expressed such feelings of despondency. Earlier, social status had influenced the participant's views on loneliness, much depending on whether they earlier had a partner or not. It seems that those among the participants who had lived alone, when they were younger, did not experience being alone as something negative.

The participants used different strategies to compensate for their social needs related to meal times, such as visiting day-centres, simply accepting that they were alone, or watching television or listening to the radio.

"I have no problems eating alone because I can take my time and enjoy the food as long as it is good, no matter whether I am alone or I have company."

4.4 Feeling grateful

The participants expressed their sincere gratitude for the meal service they received from the municipality. All of them noted that they were well aware that food was essential for their health. They described the meals they received through the FD as being varied, healthy and a good alternative to home-cooked food. They found the FD food tasty. They also thought the food was worth the price, especially since a dessert and raw vegetables were also included in the price. Some felt that the portions were too large and that they sometimes split the delivery into two meals. Saving food and feeling a sense of gratitude for having their meals delivered to their home was something crucial for them. This was something that they had learnt during their lifetime.

"I think my life is good in terms of the service I get from the municipality.

From the participants perspective, it was an advantage to have the possibility to choose their

meals from a menu, and they compared the food distribution services between different

municipalities from information they could find in the media. Based on the information they had, the majority of them were grateful for the daily delivery of hot meals rather than the weekly delivery of frozen meals. It seemed that many of the participants greatly valued the

possibility to receive one or two hot meals every day, since they had heard that elderly people in other municipalities received only frozen food. They expressed negative views about the idea of only receiving frozen or cold food once a week and called this a "punishment". Furthermore, the possibility to cancel meal deliveries, when they were away, was appreciated as this meant that they could freely plan their time. It was also obvious that the participants had seen positive changes in the organisation of meal deliveries, which had resulted in a better service that was based on their input, such as the request for specific dishes on their menus. According to the participants, traditional food was most appreciated and regarded by them as "proper food".

"Some dishes I don't like but that's life."

It seemed that the variation in menus was considered as positive.

"I think that variation is good and I could not have come up with more variation myself."

5. Discussion

5.1 Methodological considerations

The purpose of the study was to gain insight into, and describe elderly people's experiences of receiving food distributed from the municipality. The experiences related by the participants in the study indicated that they were, in general, satisfied with the FD system from their municipality. As participation in the study was voluntary there is a risk that people receiving meal distribution, but who, at the same time are dissatisfied, were missed in the sample. If and how such experiences affected the findings remains unanswered in this study. Findings from the study can be evaluated in terms of trustworthiness: credibility, dependability, conformability and transferability (Lincoln & Guba, 1985). Credibility was assured by presenting views from different participants in order to capture the different experiences, and by the fact that the co-authors were involved in the analysis. Dependability was assured by the fact that the same researcher, the first author ZP, carried out all the interviews and transcriptions. The use of a tape-recorder and verbatim transcripts, as well as referring back to, and rereading the transcripts during the analysis process, allowed the researchers to remain close to the text. The citations make it possible to assure conformability. Transferability can be considered to have been achieved if the present results can be recognised and transferred to comparable contexts (Lincoln & Guba, 1985)

5.2 Discussion of the findings

The study indicates that the meal box was experienced by the elderly persons participating in the study as a symbol of dependency and loss of identity as it became evident to them that they could no longer manage to shop and prepare hot food by themselves. Feelings of loneliness were reinforced when the participants did not know who to contact if they had questions about the municipal meal distribution service. Walker and Beauchene (1991) described that, for elder people, loneliness related to meals in the context of a social event was a result of sparse contact with their children and/or the loss of their spouse (Walker & Beauchene, 1991). Further, that the

feeling of loneliness is common even when people have others around them. This is due to the fact that it is important for a person to personally choose their company (Åhgren, 2003).

Involuntary loneliness is most often caused by widowhood (Hughes, Bennett, & Hetherington, 2004), while voluntary isolation is a common strategy to avoid feelings of being observed by others when eating (Walker & Beauchene, 1991). Aberg et al. (2005) showed that elderly people have different strategies for maintaining life satisfaction in relation to the care and social services. One strategy is to try to be active and independent. In situations they could not influence, elderly people used mental activities as an adaptation strategy, such as by recalling past memories in an effort to achieve a sense of satisfaction with their life. This strategy was described as having a potential risk for passive concealment of the dissatisfaction they felt with their current conditions (Aberg, Sidenvall, Hepworth, O'Reilly, & Lithell, 2005). The results from this study confirms other studies that show that elderly people express a high level of understanding of the dangers of becoming introverted, alone and depressed (de Jong-Gierveld, Kamphuis, & Dykstra, 1987). The FD services should focus on individual needs, and this implies that meals should be seen as a social event, a symbol of a good life.

Further, it was shown in a study by Odencrants, Ehnfors and Grobe (2005) that solitude was sometimes chosen actively as some elderly people felt as if they were being observed while they were eating and found it hard to follow new socio-cultural codes (Odencrants, Ehnfors, & Grobe, 2005). Another study showed that elderly people chose to eat alone because they do not feel comfortable having company during meals they had not chosen freely, for example in the presence of assistant nurses (Leppänen, 2008). It is not unusual that many people choose some form of media as artificial company during meals (Sobal & Nelson, 2003). Chosen or enforced loneliness at mealtimes was identified as a risk factor as it is related to inadequate food intake and less variety of food which can eventually lead to low food intake (Hughes, et al., 2004).

Findings in this study also indicated that it took time for the elderly participants to accept FD as they appeared to convey that they had an inner struggle with themselves over whether or not to accept the service. This can be interpreted as an adaptation to their new life situation (Kallio, Koskinen, & Prattala, 2008). This is confirmed by similar findings that describe the process of being dependent on care as being a transformed relationship to oneself and others, adapting to a new situation, experiencing obstacles and opportunities along the way towards dependence (Locher et al., 2005). Being old and dependent, illustrated here in the form of a meal box; is a part of the life cycle and something that can take time to accept. The life cycle was conceptualised by Meleis (Meleis, 2010) as transitions which entail searching for meaning in new situations. The transitions denote a change in life, in physical and psychological health status, and new roles in a socio-cultural context. The transitions associated with health/illness can be accompanied by existential struggles related to identity as they require the individual to modify their behaviour.

This depends on an individual's values, beliefs, expectations and feelings about their situation (Andersson & Sidenvall, 2001). The results of this study agree with another study by them that found that great emphasis is placed on the importance of having and showing interest in things and not vegetating (Andersson & Sidenvall, 2001; Sidenvall, 1997). Throughout their life, people go through several transitions between the different phases of life, including the ageing process (McKie, MacInnes, Hendry, Donald, & Peace, 2000). One example is the transition from being independent to being dependent on help from outsiders, which can be hard to manage, and this appears to correspond to the way that the elderly people in this study reasoned.

The individual opportunity to influence and negotiate dependency on care seems to limit the negative sense of being dependent (Rochat et al., 2010). This indicates that it is important to involve elderly people in the whole process, from the assessment of their needs to the decision about their receiving meal boxes. In earlier studies, being in need for support or help from other people for meals has been described as being phased out as a human being (Bülow et al., 2007). Being dependent on others can cause feelings of shame for the dependent person (Hayder & Schnepp, 2008). Such feelings arise as result of a sense of being inferior, a burden, having low self-esteem and low self-confidence. Receiving meals through the FD seemed to be harder for women to accept possibly due to their traditional responsibility for preparing food for their family. Other reasons for these feelings can have a historical background, as today's elderly people grew up with norms and values requiring them to participate in the activities of daily living and to not be a burden on anyone (Gustafsson, Andersson, Andersson, Fjellstrom, & Sidenvall, 2003). However the participants in this study noted that they felt increased self-esteem when they had the possibility to exert influence, even on a small scale, such as the possibility to choose their meals from a menu.

The participants in this study expressed sincere gratitude for the hot prepared meals they received on a daily basis from the municipality. The participants relation to food was rooted in their childhood and was guided by

experiences gained throughout their life, for example, in terms of access to food, culture, religion, and the cost and quality of food (Maynard et al., 2006). This indicates that it is crucial to note and respect those elderly people, who live in their own homes, as individuals with their personal socio-cultural history, as this may have an important influence on how they experience their current life situation. It is confirmed by the findings in another study that described how many elderly people had witnessed massive changes in their lifetime, such as those in food production, access to, and the cost of food, besides having experienced a substantial change in their financial situation when leaving working life and becoming a pensioner (Sidenvall, 1995).

The results of this study showed that the participants were sincerely grateful for the FD service. This gratitude could have its roots in their experiences of the years of hardship and economic depression between the world wars and might be the reason why many elderly believe that food is a gift from God, that it should not be wasted, and that food is something to be grateful for (Meinow, Kåreholt, & Lagergren, 2005). Family morality had instilled into them the norm of being grateful for the food they received, and it was not acceptable for them to say that they did not like some types of food (Sidenvall, Fjellstrom, & Ek, 1994). Viewed from a social constructivist perspective, the elderly people who participated in this study created and formed social collective actions in their interactive exchange with others. These actions are based on the socially accepted cultural norms within the society they are part of, and although they may differ from individual to individual, they still provide an overall common collective picture (Burr, 2003).

6. Conclusion

This study indicates that greater attention needs to be paid to the experiences and views of elderly people who live in their own home, related to their receiving meals supplied by their municipality. Their experiences indicated that food distribution should not be seen as a fragmentary social support but as a starting point for empowering elderly people in order to prevent them having growing feelings of dependency and loneliness. This study shows that the meals distributed by the municipality have a symbolic value for the elderly. The professionals involved in FD can use the knowledge gained from this study to develop and improve their encounters with FD recipients. However, this study also reveals the need for further studies focusing on problems experienced by FD recipients.

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Relevance to Practice

This study indicates that greater attention needs to be paid towards meeting both the practical and psychological needs of elderly people. Food distribution should not be seen as a fragmentary social support but as a starting point towards breaking the elderly's social isolation and to actively involve them in decision making in order to prevent them having feelings of dependency and loneliness. Furthermore, the FD organisation should be continuously improved towards offering an individually adapted service.

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Predicting Wine Consumption Based on Previous 'Drinking History' and Associated Behaviours

Lauro Melo
CSIRO Food Futures National Research and CSIRO Food and Nutritional Sciences
PO Box 10041, Adelaide BC SA 5000, Australia

Greg Evans
CSIRO Food Futures National Research and CSIRO Food and Nutritional Sciences
PO Box 10041, Adelaide BC SA 5000, Australia

Nicolas Le Pollès
AgroParisTech, 16 rue Claude Bernard 75231 Paris cedex 05, France

Conor Delahunty
CSIRO Food Futures National Research and CSIRO Food and Nutritional Sciences
PO Box 54, North Ryde, NSW 1670, Australia

David N. Cox (Corresponding author)
CSIRO Food Futures National Research and CSIRO Food and Nutritional Sciences
PO Box 10041, Adelaide BC, SA 5000, Australia
Tel: 61-883-038-899 E-mail: david.cox@csiro.au

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Abstract

Associative learning processes may be related to and shape consumers' current consumption and preferences. Past consumption, experiences and behaviour with food and beverage products are likely to have an important role. Data were collected in order to predict current wine consumption based on past experiences. Longitudinal quantitative data (N=564) on past alcoholic beverage consumption behaviour ('drinking history') was collected retrospectively. Results of multiple linear regression analysis showed that previous 'drinking history' explained 40-70% of the variance in later wine consumption patterns and that red wine consumption is a more complex behaviour compared to white wine consumption. In addition, belief-evaluations towards intrinsic product characteristics are more likely to be drivers of red wine consumption ($p<0.05$), but not of white wine. Results indicated how past behaviour and experiences influence current behaviour, and identified possibilities for influencing specific wine consumption patterns.

Keywords: Past experiences, Linear regressions, Factor analysis, Current behaviour, Wine involvement, Habit formation

1. Introduction

Past consumption, experiences and behaviour with food and beverage products are likely to shape consumers' current consumption and preferences through associative learning processes, described by the theory of evaluative conditioning (De Houwer et al., 2001, Walther et al., 2005). Ratchford (2001) proposes that consumers will direct their consumption and search activities in ways that maximize the impact of their

accumulated expertise. Particular past experiences (and associations) may be related to current wine consumption or particular wine choice. According to Ouellette and Wood (1998) and Olson and Fazio (2001), past behaviour guides future responses. Frequency of past behaviour reflects habit strength and has a direct effect on future responses/reactions.

Most past wine studies provide only cross-sectional data on alcoholic beverage consumption for different age groups, or so-called generations (Beverland, 2001, Dodd et al., 2010, Olsen et al., 2007, Teagle et al., 2010). The studies that have collected data on past wine consumption and behaviour, retrospectively, so that different generations could be compared over wider age periods, were limited in terms of sample size and qualitative data (Thach & Olsen, 2004; Wilson, Rungie, & Lockshin, 2003; Wilson, 2007).

In this sense, collecting reliable quantitative data on consumers' past alcoholic beverage consumption and behaviour is the first step to better understand changes in consumption patterns, possible associations with those changes and current preferences. The broad objective of this research was to better understand the relationships between past experiences and current preferences. To meet this objective, an adaptation of validated dietary history and alcohol dependency methods was undertaken. There is evidence that past alcohol consumption is particularly well remembered (Melo et al., 2010c). Indeed Chu et al. (2010) recently reported that recalled alcohol intake after 15 and 23 years of follow-up is remarkably reliable. Therefore, a new tool that retrospectively measures consumption patterns and behaviours associated with wine, beer, and liquor consumption with an emphasis on understanding wine preferences was created (Melo et al., 2010c). The recent development and validation of this tool, a questionnaire eliciting the recall of past alcoholic beverage consumption and behaviour during consumers' lifetimes (their 'drinking histories'), is described in Melo et al. (2010c).

The preliminary findings (Melo et al., 2010a) reinforced the suggestion that past alcoholic beverage consumption, behaviours and experiences are likely to shape current wine consumption. Recently Cox (2009) reported that wine knowledge and underlying psychological personality traits predicted wine involvement, which, in turn, was a modest predictor of wine consumption. Whilst knowledge and involvement are potentially malleable, personality traits may not be, therefore seeking relationships between 'attitudes' (belief-evaluations) and involvement may be more useful. The specific objectives of the current project are to seek an understanding of past alcoholic beverage consumption and attitudes, and how these may relate to current wine consumption.

Behavioural theory, such as the Expectancy-Value Theory (Conner and Armitage, 2006), the Theory of Reasoned Action (Shepherd and Sparks, 1999) and the Theory of Planned Behaviour (Shepherd and Raats, 1996; Fishbein, 1967, Ajzen and Fishbein, 1980, Ajzen, 1988), considers 'attitudes' as products of behavioural beliefs and outcome evaluations. Individuals' attitudes are composed of three components: 'cognitive' (beliefs), 'affective' (emotions and preferences) and 'behavioural' (actions) (Ajzen and Fishbein, 1980, Eagly and Chaiken, 1993, Fishbein and Ajzen, 1975) and finding malleable predictors of consumption (beliefs) might help to guide future consumption (actions). Smith and Swinyard (1983) demonstrated that when attitudes are based on actual product trials (as opposed to advertising) they predict purchase very well and that cognitive and affective components were important in forming attitudes through advertising. Indeed, it has been argued that what finally determines a specific food choice will depend on a combination of general lifestyle attitudes, product-related attitudes and situational factors (Brunsø and Grunert, 2007).

From a very large dataset, a focus was made on predictive models that included potentially malleable attitudes (the belief-evaluation items summarized as 'attitude' factors) in order to explain wine consumption. There is evidence that attitudes are malleable variables compared to personality traits (Ajzen, 2005) or most socio-demographics. Additionally, previous alcoholic beverage consumption could be indicators of early exposure and possible habit formation (Carrasco et al., 2005, Dynan, 2000, Naik and Moore, 1996) that, in themselves, describe the acquisition of alcoholic beverage and wine preferences. Ultimately, only the identification of more malleable variables that may be used to shape specific patterns of wine consumption has practical application, as well as understanding current behaviours less resistant to change.

The aim of the current paper was to test if past alcoholic beverage consumption, belief-evaluations towards wine and wine involvement are significant predictors of current red and white wine consumption behaviour. This process included factor analysis of 29 belief-evaluation items and a series of multiple linear regressions to find predictors of wine consumption, based on previous drinking experiences. Two age groups (30-40y and 50-60y) were selected in order to test if predictors were stable or differed by age cohorts. In addition, the approach provides information that traditional cross-sectional studies cannot, such as comparing the two groups when they were at the same age (period).

2. Methods

Data was collected using a validated computer based questionnaire. The previous validation process (test–re-test) indicated reliability (Melo et al., 2010c); for instance, for specific items for each time-phase including frequency, quantity and percentages of alcoholic beverages, most items had high internal consistency and no statistically significant differences over time (p>0.05). Unreliable items, tended to be least consumed wines (i.e. sparkling, fortified and dessert) in contrast to red and white wines. These results showed that it is easier for participants to recall average consumption patterns from the past when consumption was more frequent and more regular. Measures of current average weekly red and white wine consumption were also validated (see more details in Melo et al., 2010c).

2.1 Sample

Six-hundred wine drinkers (divided into two age groups: 30-40y and 50-60y) were recruited throughout metropolitan Adelaide, South Australia. Study inclusion criteria included reporting consumption of bottled wine at least once a week and exclusion criteria included employment in the wine industry and possible alcohol dependency problems, detected using the validated Cage questionnaire (Ewing, 1984, King, 1986).

2.2 Data collected

Participants were asked to divide their past alcoholic beverage consumption into phases, based upon individual life-grid timelines previously completed (Melo et al., 2010c). For each phase they were asked to report frequency of alcoholic beverage consumption (days/month), quantity (drinks/drinking day), type of beverage (% of wine, beer and liquor) and type of wine (% of red, white, sparkling, fortified and dessert).

For each reported drinking phase, participants answered socio-demographic questions on employment situation, level of education, number of people at home and living situation. Socio-demographics income (only for the current situation), gender and age were asked as well. In addition, participants answered, for each drinking phase, belief-evaluation questions (antecedents of attitudes) (Conner and Armitage, 2006), in order to determine the main associations for different types of beverage consumption. For instance, 'taste' as an association for wine consumption was measured by the belief statement 'I usually drank wine at that time because of its taste' and by the evaluation 'At that time taste was important to me when choosing an alcoholic beverage'. Participants indicated on 7-point labelled scales how much they agreed or disagreed with the first statement and how unimportant or important the second statement was. The belief-evaluation was calculated as a multiple of the two responses. A validated measure of wine involvement (Mittal and Lee, 1989) was also undertaken for each reported drinking phase.

Finally, participants also answered questions about their current wine consumption patterns, regarding average glass volume, weekly frequency and quantity, and number of bottles purchased/month at home, restaurants and bars.

2.3 Data management and statistical analysis

As participants were free to report any number of drinking phases (and any duration for each), in order to facilitate data analysis and comparisons, participants had their drinking phases condensed into three common age periods: Phase 1 (beginning-29y), Phase 2 (30-45y) and Phase 3 (46-60y). With this approach, at the time of data collection, all 30-40y age group participants were in drinking Phase 2 and all 50-60y age group participants were in Phase 3.

Belief-evaluations towards wine (29 items) were factor analysed for each 'drinking history' phase using Principal Components Analysis with a varimax rotation. To predict what factors and consumption variables in previous drinking phases were driving wine consumption a series of multiple linear regressions were undertaken, including collinearity checks. The method chosen was simultaneous/block entry (Harrell, 2001).

In these analyses, it is important to establish the difference between current wine consumption variables and 'ongoing' 'drinking history' phase variables. The former were collected and calculated from participants' answers to questions about their current wine consumption patterns – relating to their current 'average week'. The latter (ongoing phase variables), were calculated from participants reported 'drinking history' phases and are the phase they were at the time of the study ('drinking history' Phase 2 for the 30-40y group and Phase 3 for the 50-60y group, see section 2.2 and Figure 1). Thus, ongoing phase variables cover behaviours and belief-evaluations over a wider period of years. Both (current wine consumption and ongoing 'drinking history' phase variables) were investigated and are presented below with the aim of eliciting whether, using past behaviour, it is possible to explain more about current consumption behaviour (current average week

consumption) or longer term (ongoing phase) consumption behaviours (consumption patterns). An additional aim was to understand how ongoing phase behaviour may influence current behaviour.

3. Results

3.1 Participants

After data cleaning, 564 participants remained: 282 participants in each age group, 53% male in the 30-40y age group (mean 35.3y, SD 3.27) and 51.5% male in the 50-60y age group (mean 54.8y, SD 3.10).

3.2 Factor analysis

The preliminary statistics indicated that the data matrix was factorable (Kaiser-Meyer-Olkin Measure of Sampling Adequacy was 0.834 and the Bartlett's Test of Sphericity was 3588, df=406; $p<0.001$). Inspection of the factors indicated that there was a high degree of overlap between both age groups and phases. However, in order to make the structure logically coherent and to simplify the data interpretation, the few non-common items (factor components) throughout 'drinking history' phases were allocated from the original factors to other factors. The chosen final common (for all 'drinking history' phases) factors were then calculated to be used in linear regressions. In summary, the 29 belief-evaluation variables were grouped into 6 factors labelled: 'Social & Pleasure', 'Detail', 'Regular', 'Intimate', 'Sensory', and 'Value' (see belief-evaluation items within each factor in Table 1).

In addition, reliability among belief-evaluation items within each wine factor over time (phases) was tested using the intraclass correlation coefficient (ICC). ICC estimates the squared correlation of average scores and is the most widely used psychometrics measure for estimating the internal consistency of multi-item scales (McGraw and Wong, 1996). ICC was applied to check the internal consistency of each factor in the three 'drinking history' phases (Table 1). An ICC of 1 represents a perfect reliability into one category and an ICC higher than 0.5 is arbitrarily acceptable (Bak, 2001, Cox and Evans, 2008, Meiselman et al., 1999). In the factor named 'Sensory' (factor 5, see Table 1), the 'Sweet wines' item was the opposite of the 'Dry wines' item whilst the 'Light wines' item was the opposite of the 'Heavy wines' item. Thus, 'Sensory' factors (and their ICCs) for the three 'drinking history' phases were calculated using the opposite signals for 'Sweet wines' and 'Light wines' items.

Results showed that the wine factors determined were consistent (reliable) over the three 'drinking history' phases (i.e. over time) and therefore had the potential to be used in linear regression models to find predictors of wine consumption based on different previous drinking phases.

3.3 Multiple linear regressions

Table 2 summarizes all regression models (cases) undertaken for both age groups: predicting average red or white wine consumption during a given 'drinking history' phase based on the previous phase (cases 1 and 2), predicting current red or white wine consumption based on the previous phase (cases 3 and 4), and predicting current red or white wine consumption based on the ongoing phase (cases 5 and 6).

In general, wine consumption in a drinking phase based on the previous phase (cases 1 and 2) presented higher variance explanations (R^2 values) as opposed to current wine consumption (cases 3 to 6) – and current wine consumption presented higher explanations by the ongoing drinking phase (cases 5 and 6) than by the previous phase (cases 3 and 4). Also, the models for white wine consumption dimensions had higher explained variance compared to the red wine consumption models.

3.3.1 Predicting red and white wine consumption (number of drinks/year) in a 'drinking history' phase based on the previous phase

Red or white wine consumption (in number of drinks/year) in a given drinking phase was regressed against 'current' socio-demographics (gender, age and income), socio-demographics at previous phases (employment situation, level of education, number of people at home and living situation), consumption variables at the previous phase (total number of years drinking, total number of drinks of beer, liquor (spirits), red wine and white wine), and belief-evaluation factors (Table 1) and total wine involvement at the previous phase.

Table 3 shows the cases that had at least one belief-evaluation factor amongst the significant predictors ($p<0.05$) of wine consumption in a 'drinking history' phase. Generally, socio-demographic variables did not appear to be consistent drivers of wine consumption applicable to any type of wine, phase or age group.

Participants' alcoholic beverage consumption patterns in the previous 'drinking history' phases (the alcoholic beverage consumption variables) were important predictors ($p<0.05$). More importantly, participants' previous wine belief-evaluations (the belief-evaluation factors, see Table 1) were important in defining their red or white wine consumption in a given phase (Table 3).

In addition, previous alcoholic beverage consumption patterns also influenced ongoing phase red and white wine consumption. In general, red and white wine consumption at the previous 'drinking history' phase was associated with increased red and white wine consumption, respectively. However, for the older group at Phase 3 as predicted by the previous Phase 2, earlier consumption of one type of wine was associated with changes in another type. Specifically, white wine consumption at Phase 2 was associated with increased red wine consumption at Phase 3; whilst red wine consumption at Phase 2 was associated with decreased white wine consumption at Phase 3. Moreover, a pattern across types of alcoholic beverages was found with the number of drinks of liquor at Phase 1 (beginning-29y) positively associated with the younger group's red wine consumption at Phase 2.

3.3.2 Predicting current average weekly red and white wine consumption (total mL/week) based on the previous or ongoing 'drinking history' phase

Table 4 shows the cases that provided significant models (p<0.05) with at least one belief-evaluation factor amongst the significant predictors (p<0.05) of current red or white wine consumption (total mL/week), regressed against 'current' socio-demographics (gender, age and income), socio-demographics at the previous or ongoing phase (employment situation, level of education, number of people at home and living situation), consumption variables at the previous (total number of years drinking, total number of drinks of beer, liquor, red wine and white wine) or ongoing (total number of years drinking, number of drinks per year of beer, liquor, red wine and white wine) phase, and belief-evaluation factors and total wine involvement at the previous or ongoing phase.

Socio-demographic variables did not appear amongst the significant predictors (Table 4). Belief-evaluation factors were rarely significant predictors of current average weekly wine consumption based on the previous phase, whilst they were significantly predictive (p<0.05) of wine consumption in a 'drinking history' phase based on the previous phase (Table 3). Again, white wine consumption at the previous phase was positively associated with white wine consumption; whilst the older group's red wine consumption was positively associated with the red wine consumption over the ongoing 'drinking history' phase. Liquor consumption at the previous phase was associated with the older group's increased white wine consumption.

4. Discussion

Given that it is widely recognized that the result of behavioural studies are quite variable (Stevens, 2002), the predictive models' R^2 values of between 0.3 and 0.7 (Tables 2 to 4) would appear to be moderate to good. They demonstrated the importance of collecting longitudinal (recalled) data on alcoholic beverage consumption patterns and behaviour covering personal 'drinking histories' in order to better understand consumption and behaviour changes during people's lifetimes.

Results showed that, in general, specific interventions may not change consumers' current wine consumption patterns immediately; however, they might influence consumers' average wine consumption in the next 'drinking history' phase, with stronger results if they are applied in earlier stages (highest R^2 values were found for a previous phase explaining the ongoing phase in contrast to lower explained variance for the current average weekly wine consumption, Table 2). Also, the highest R^2 values were found for white wine (as opposed to red wine), confirming preliminary results showing that consumers were found to have stronger associations between white wine consumption patterns, wine belief-evaluations and involvement compared to the red wine data (Melo et al., 2010b).

It was decided to present models that included belief-evaluation factors because they are more malleable (Ajzen, 2005). Comparison of two age groups would show whether belief-evaluation influences changed from when the older group experienced a given 'drinking history' phase to a younger group's experiences. Moreover, a 'time'/consumption effect was found, described by red and white wine consumption in an earlier phase significantly influencing ongoing phase red and white wine consumption (cases 1a2, 1b1, 2a and 2b, Table 3), respectively, indicating reinforcement effects and habit formation. Khare and Inman (2006) stated that food consumption habits can free a person from exerting effort in repetitive decisions and, as found by Naik and Moore (1996) for food consumption, the present study's results suggest habit formation and acquisition/reinforcement of liking for alcoholic beverages over time, consumption and exposure. White wine consumption reinforcing red wine consumption (case 2a, Table 3) confirmed the previously suggested 'drinking history' evolution, from white to red wine (Melo et al., 2010b). In other words, when a consumer starts consuming wine, he/she is likely not only to keep consuming the product (habit formation) but also to increase the consumption over time and, more specifically, in the direction of red wine consumption.

Influences across types of alcoholic beverage (case 1a1, Table 3) may be reflecting different historic moments, considering the age groups and alcoholic beverages availability. For instance, 'ready-to-drink' (RTD)

flavoured/sweetened liquor-based products, or 'alcopops', were launched in the Australian market in mid-90's (Barnard and Forsyth, 1998, Shakeshaft et al., 2009) and might have influenced the younger group's consumption patterns, the demographic focus of this beverage category (Chikritzhs et al., 2009, Copeland et al., 2007, Shakeshaft et al., 2009, Smith et al., 2005, Stevenson et al., 2007), as evidenced by the reported higher liquor consumption during 'drinking history' Phase 1 (beginning-29y, see Figure 1) in contrast to the older group in the same age period (Melo et al., 2010b). It is often assumed that habits constitute an important component in human behaviour. However, Davidov (2007) showed that there was no effect of habits on behaviour in a new context, in accordance with the definition of habits (Khare and Inman, 2006, Neal et al., 2006, Orbell and Verplanken, 2010, Wood and Neal, 2007). Therefore, the new offer of RTD liquor-based beverages has possibly changed the younger group's alcoholic beverage consumption habits, which later influenced their wine consumption patterns.

This early encounter with liquor (possibly flavoured/sweetened RTD) consumption appears to have led to their later red wine consumption suggesting early flavour/post-ingestive consequence pairing learning responses (Yeomans, 2006, Yeomans, 2010) to alcoholic beverages. In contrast, the older group reported a higher liquor consumption at Phase 2 (30-45y) (Melo et al., 2010b), and this liquor consumption was associated with their current white wine consumption (case 4b, Table 4) suggesting again liquor consumption leading to later wine consumption. Therefore, it appears to be easier to derive wine consumers from liquor consumers whereas beer consumers seem to be more loyal to beer preferences. Those 'progressions' may be noteworthy for the wine industry.

Participants' previous wine belief-evaluations were important to define their red or white wine consumption in a given phase. Past studies have shown that attitudes towards specific food products may predict willingness to use and accept those products (Urala and Lahteenmaki, 2004, Verbeke, 2005). In addition, other studies showed the importance of intrinsic product characteristics on consumers' wine purchase decision (Batt and Dean, 2000, Skuras and Vakrou, 2002). However, results in the present paper are the first to indicate that intrinsic product characteristics, such as those underlying 'Detail' (factor 2) and 'Sensory' (factor 5), are more likely to be drivers of red wine consumption not of white wine consumption (Table 3).

Belief-evaluation factors from the previous phase, significantly predicted wine consumption in a 'drinking history' phase. This demonstrates the importance of collecting longitudinal (recalled) data on alcoholic beverage consumption patterns and behaviour over wide age periods, instead of collecting only current wine consumption variables, to better understand consumption.

According to Wood and Neal (2007), one challenge to regulating habits is that they do not merge readily with conflicting goals, and therefore habit dispositions are not changed simply by adopting new goals or engaging in short-term behaviour change. Habit dispositions undergo minimal change to reflect current goals or occasional counterhabitual responses. Only with extended repetition in stable contexts are behaviour patterns likely to be represented in habit learning (Wood and Neal, 2007). Thus, behaviour-change campaigns still fail. The popular explanation for this tendency is that no habits were actually developed for the new behaviour. The problem of changing everyday behaviour does not involve the breaking of old habits but rather the lack of habits for the new behaviour. Until these new habits are developed, measures such as reminders and implementation intentions are necessary for the behaviour to be performed frequently (Tobias, 2009). Therefore, if the wine industry applies the present study's results, it should consider the time needed for the new habit formation.

The belief-evaluations were reflected in some weaker, however still statistically significant ($p<0.05$), predictors of wine consumption (Tables 3 and 4). More importantly, as the past alcoholic beverage consumption variables were strong predictors (standardized betas), it seems that the context is characterized by 'motivated cuing', instead of 'direct cuing'. In the motivated cuing, habit associations arise from the reward value of response outcome (Wood and Neal, 2007), which for wine consumption would be the post-ingestive psychoactive effects of alcohol (Rogers, 1996).

In addition, habits are informative when people reflect on how they have acted in the past in order to make inferences about their goals and related dispositions, such as attitudes and personality traits. In the model suggested by Wood and Neal (2007), habits are repeated responses that come to be cued by recurring features of contexts. Context cues refer broadly to the many elements of the performance environment that potentially can recur as actions are repeated, including physical locations, other people, and preceding actions in a sequence. Once a habit is formed, perception of contexts triggers the associated response without a mediating goal. In the current study models were found with belief-evaluation factors as statistically significant predictors of red and white wine consumption confirming that context is important for the habit of alcoholic beverage consumption.

One limitation of the study was possibly the age groups and ranges. An 'age effect' was previously found cross-sectionally with significant differences between age groups in total wine and red wine consumption and no significant differences were found when comparing age groups' wine consumption when they were in the same age periods; for example, when the two age groups were at 'drinking history' Phase 2 (Melo et al., 2010b). However, age was not a driver of red and white wine consumption for models within each age group, possibly because the age range (10 years for each age group) was not long enough to cause a significant effect in multiple linear regressions. However, it may be possible to find an age effect if the sample age were continuous, for instance, from 30 to 60 years old.

The relationship between current and past consumption may reflect habits and implies a condition relating the strength of habits to the evolution of consumption over time (Carrasco et al., 2005, Dynan, 2000). The present study demonstrates consumption habit formation in the wine domain (characterized by the stronger beta weights of past consumption, rather than belief-evaluation factors, on current and ongoing phases' wine consumption). This has been previously described by Naik and Moore (1996) within the food domain whereby increases in previous food consumption significantly increased current food consumption. The importance of habit formation has also been demonstrated in other domains, such as water consumption (Gregory and Di Leo, 2003), consumption of food nutrients (Khare and Inman, 2006) and college students' demand for alcohol (Williams, 2005). This latter study showed that high-school drinking has a significant and positive impact on college drinking, indicating the existence of habit formation. The author also suggests that habit formation has a stronger effect on the decision on how much to drink rather than on the decision on whether to drink.

In general, the basic techniques described above may have application in other consumer choice domains. However questionnaires must be adapted and validated for new product domains and particular cultures. The next step of the project is to evaluate whether different 'drinking histories' lead to specific current wine sensory preferences. Previous results showed that belief-evaluations related to some specific wine sensory properties – namely 'Sweet wines', 'Dry wines', 'Light wines', and 'Heavy wines' – longitudinally changed over participants' 'drinking histories' and were different across age groups when they were in the same age periods (Melo et al., 2010b). This observation together with the generally good explanations found in this study between past alcoholic beverage experiences and current wine consumption (including 'Sensory', as a significant predictor of wine consumption), suggests that past alcoholic beverage experiences may shape wine sensory preferences. With this in mind, additional work evaluated a sub-sample of the participants for their current wine sensory preferences regressed on their 'drinking history' (Melo, Delahunty, & Cox, 2011).

5. Conclusions

This is the first study to demonstrate relations between experiences (in terms of alcoholic beverage consumption, belief-evaluations towards wine and wine involvement) and current wine consumption patterns. Results showed that previous 'drinking history' phases influence later wine consumption patterns, with red wine consumption being a more complex behaviour compared to white wine consumption. On the other hand, intrinsic product characteristics are more likely to be drivers of red wine consumption, but not of white wine. Additionally, it is easier to derive wine consumers from liquor consumers whereas beer consumers seem to be more loyal to beer preferences. The main findings of this study, especially in terms of the malleable belief-evaluation factors and wine involvement, indicated how past behaviour and experiences influence current consumption patterns. In general, interventions are likely to be more effective in earlier stages of consumers' 'drinking history'.

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Table 1. Phase 1 belief-evaluations grouped into 6 factors and intraclass correlation coefficients (ICC) for each 'drinking history' phase[a], using the same set of factors

Factors (% of variance)	Belief-evaluations towards wine (loadings)	ICC		
		Phase 1	Phase 2	Phase 3
1) 'Social & Pleasure': (22.1%)	Social life (0.800) Party/celebration (0.784) To be part of a group/culture (0.729) Bars/hotels (0.718) Fun/relax/enjoy time (0.709) Outdoor-BBQ picnics (0.651) Business related meals (0.410)	0.830	0.811	0.845
2) 'Detail': (15.5%)	Region of origin (0.806) Wine new experiences (0.786) Knowledge of wine (0.739) Grape variety (0.688) Experts' opinions (0.683) Country of origin (0.678)	0.874	0.870	0.874
3) 'Regular': (6.04%)	Regular meals at home (0.767) Complement food (0.674) Health benefits (0.598) Routine/habits (0.504) Taste (0.432) Treat (0.429)	0.727	0.778	0.777
4) 'Intimate': (5.59%)	Friends' and/or families suggestions (0.689) Higher prices for special occasions (0.658) Intimate dinner (0.630) Meal with friends (0.611)	0.748	0.731	0.732
5) 'Sensory': (4.93%)	Dry wines (0.708) Sweet wines (-1) (-0.707) Light wines (-1) (-0.511) Heavy wines (0.352)	0.551	0.593	0.603
6) 'Value': (4.61%)	Price (0.811) Value for money (0.663)	0.696	0.593	0.597

[a] Phase 1: beginning-29y; Phase 2: 30-45y; Phase 3: 46-60y.

Table 2. Multiple linear regressions for red and white wine consumption depending on the 'drinking history' phases[a] and age groups

	Wine consumption (number of drinks/year) in a 'drinking history' phase					
Case	Type of wine	Age group	At	Based on	p	R^2
1a1	Red wine	30-40y	Phase 2	Phase 1	0.000	0.509
1a2	Red wine	50-60y	Phase 2	Phase 1	0.000	0.676
1b1	White wine	30-40y	Phase 2	Phase 1	0.000	0.606
1b2	White wine	50-60y	Phase 2	Phase 1	0.000	0.651
2a	Red wine	50-60y	Phase 3	Phase 2	0.000	0.702
2b	White wine	50-60y	Phase 3	Phase 2	0.000	0.762
	Current average weekly wine consumption (total mL/week)					
Case	Type of wine	Age group		Based on	p	R^2
3a	Red wine	30-40y		Phase 1 (previous)	0.093	0.368
3b	White wine	30-40y		Phase 1 (previous)	0.050	0.499
4a	Red wine	50-60y		Phase 2 (previous)	0.001	0.350
4b	White wine	50-60y		Phase 2 (previous)	0.000	0.575
5a	Red wine	30-40y		Phase 2 (ongoing)	0.000	0.530
5b	White wine	30-40y		Phase 2 (ongoing)	0.000	0.538
6a	Red wine	50-60y		Phase 3 (ongoing)	0.000	0.419
6b	White wine	50-60y		Phase 3 (ongoing)	0.000	0.532

[a] Phase 1: beginning-29y; Phase 2: 30-45y; Phase 3: 46-60y (only 50-60y age group reached its 'drinking history' Phase 3).

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Table 3. Significant predictors ($p < 0.05$) of red and white wine consumption (number of drinks/year) in a 'drinking history' phase[a] based on the previous phase using multiple linear regressions (significant models that presented belief-evaluation factors[b] as predictors)

Case[c]	Dependent variable	Significant predictors	Un-standardized coefficients (SE)	Standardized betas	t score	*p*
1a1	**Red** wine consumption -Age group: 30-40y (N = 282) -At Phase: 2 -Based on: Phase 1 - model $p < 0.001$ - model $R^2 = 0.509$	'Sensory' (factor 5)[b]	15.597 (5.111)	0.365	3.052	0.003
		Total number of drinks of liquor	0.021 (0.007)	0.341	2.864	0.006
		Wine involvement	2.674 (1.301)	0.275	2.056	0.044
		Age	-10.207 (4.589)	-0.223	-2.224	0.030
1a2	**Red** wine consumption -Age group: 50-60y (N = 282) -At Phase: 2 -Based on: Phase 1 - model $p < 0.001$ -model $R^2 = 0.676$	Total number of drinks of red wine	0.089 (0.021)	0.595	4.253	0.000
		'Detail' (factor 2)[b]	8.296 (3.554)	0.263	2.334	0.023
		Income	13.534 (6.190)	0.228	2.286	0.033
		Employment situation (not working)	-216.931 (100.224)	-0.228	-2.164	0.035
		Number of people at home	-29.406 (13.029)	-0.220	-2.257	0.028
1b1	**White** wine consumption -Age group: 30-40y -At Phase: 2 -Based on: Phase 1 -model $p < 0.001$ -model $R^2 = 0.606$	Total number of drinks of white wine	0.053 (0.010)	0.539	5.244	0.000
		Gender (female)	59.615 (26.901)	0.293	2.216	0.030
		Living situation (alone)	-62.897 (30.283)	-0.202	-2.077	0.042
		'Value' (factor 6)[b]	-3.137 (1.567)	-0.188	-2.002	0.050
2a	**Red** wine consumption -Age group: 50-60y -At Phase: 3 -Based on: Phase 2 -model $p < 0.001$ -model $R^2 = 0.702$	Total number of drinks of red wine	0.037 (0.004)	0.637	9.557	0.000
		Total number of drinks of white wine	0.023 (0.005)	0.270	4.350	0.000
		'Value' (factor 6)	5.855 (2.282)	0.171	2.566	0.012
		Age	9.762 (4.005)	0.139	2.438	0.016
2b	**White** wine consumption -Age group: 50-60y -At Phase: 3 -Based on: Phase 2 -model $p < 0.001$ -model $R^2 = 0.762$	Total number of drinks of white wine	0.056 (0.004)	0.857	15.445	0.000
		'Regular' (factor 3)[b]	-9.716 (2.664)	-0.276	-3.646	0.000
		Total number of drinks of red wine	-0.006 (0.003)	-0.141	-2.347	0.019

[a] Phase 1: beginning-29y; Phase 2: 30-45y; Phase 3: 46-60y (only 50-60y age group reached its 'drinking history' Phase 3).

[b] See belief-evaluation factors in Table 1.

Table 4. Significant predictors ($p < 0.05$) of current average weekly red and white wine consumption (total mL/week) based on the previous or ongoing 'drinking history' phase[a] using multiple linear regressions (significant models that presented belief-evaluation factors[b] as predictors)

Case[c]	Dependent variable	Significant predictors	Un-standardized coefficients (SE)	Standardized betas	t score	*p*
3b	**White** wine consumption -Age group: 30-40y (N = 282) -Based on: Phase 1 (previous) -model $p < 0.05$ -model $R^2 = 0.499$	Total number of drinks of white wine	0.193 (0.089)	0.378	2.164	0.037
		'Value' (factor 6)[b]	-33.085 (15.038)	-0.324	-2.200	0.034
4b	**White** wine consumption -Age group: 50-60y (N = 282) -Based on: Phase 2 (previous) -model $p < 0.001$ -model $R^2 = 0.575$	Total number of drinks of white wine	0.153 (0.040)	0.644	3.859	0.000
		'Social & Pleasure' (factor 1)[b]	72.420 (21.273)	0.495	3.404	0.001
		'Regular' (factor 3)[b]	-58.141 (27.913)	-0.364	-2.083	0.042
		Total number of drinks of liquor	0.080 (0.031)	0.320	2.542	0.014
6a	**Red** wine consumption -Age group: 50-60y -Based on: Phase 3 (ongoing) -model $p < 0.001$ -model $R^2 = 0.419$	Number of drinks of red wine per year	0.208 (0.042)	0.510	5.001	0.000
		'Social & Pleasure' (factor 1)	87.804 (22.344)	0.447	3.930	0.000
		Wine involvement	-20.240 (7.858)	-0.296	-2.576	0.011
		'Sensory' (factor 5)[b]	40.557 (19.151)	0.189	2.119	0.036

[a] Phase 1: beginning-29y; Phase 2: 30-45y; Phase 3: 46-60y (only 50-60y age group reached its 'drinking history' Phase 3).

[b] See belief-evaluation factors in Table 1.

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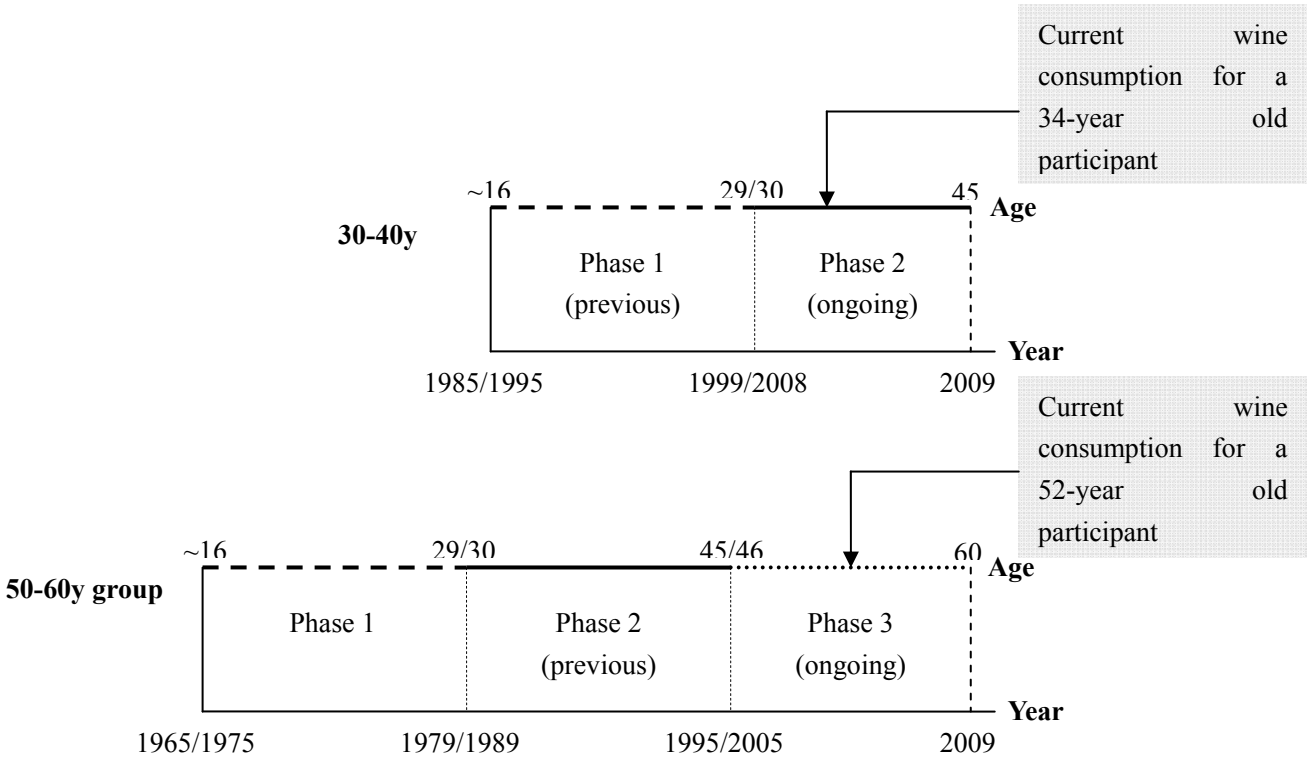

Figure 1. 'Drinking history' phases and current wine consumption

Microbial and Physical Properties of Probiotic Fermented Milk Supplemented with Lentil Flour

Fatemeh Zare & Valerie Orsat
Bioresource Engineering Department
McGill University (Macdonald Campus)
21111 Lakeshore Road, Ste.-Anne-de-Bellevue
QC H9X 3V9, Canada

Claude Champagne
Agriculture and Agri-Food Canada, 3600 Casavant Boul
West, Saint-Hyacinthe, QC J2S 8E3, Canada

Benjamin K. Simpson
Food Science & Agricultural Chemistry Department
McGill University (Macdonald Campus)
21111 Lakeshore Road, Ste-Anne-de-Bellevue
QC H9X 3V9, Canada

Joyce I. Boye (Corresponding author)
Agriculture and Agri-Food Canada, 3600 Casavant Boul. West
Saint-Hyacinthe, QC J2S 8E3, Canada
Tel: 1-450-768-3232 E-mail: joyce.boye@agr.gc.ca

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Abstract

Skim milk (9.5 % w/v solid content) was supplemented with 1-3% (w/v) lentil flour or skim milk powder, inoculated with *Lactobacillus rhamnosus* AD 200 at 37 ˚C and stored at 4 ˚C. Acid production during the fermentation, microbial growth, physical (pH, syneresis, and color) and rheological (dynamic oscillation temperature sweep test at 4-50 ˚C) properties, were studied after production and during 28 days. Milk supplementation with 1-3% lentil flour enhanced acid production, and the microbial population (CFU) of *L. rhamnosus* was comparable with that of the non-supplemented control after production. After 28 days, the CFU of 2% and 3% lentil-supplemented probiotic were as high as 1% skim milk supplemented sample. All lentil flour-supplemented samples had significantly lower "L" values and higher "b" and "a" values. Probiotic products with 1-3% lentil flour showed higher storage (G′) and loss (G″) moduli compared with 1-3% skim milk supplemented samples and the non-supplemented control.

Keywords: Lentil, Probiotic, Microbial and physical properties

1. Introduction

Probiotics are "live microorganisms which when administered in adequate amounts confer health benefits to the host" (Araya et al., 2002). Indeed, humans have been consuming probiotics in the form of fermented foods for many years (Ranadheera, Baines, & Adams, 2010). Most common types of probiotics are lactic acid bacteria (LAB), however, probiotics include species from the *Lactobacillus, Pediococcus* and *Bifidobacterium* genera.

Lactobacillus rhamnosus and *Bifidobacterium* have been the predominant species used as probiotics over the years (Tamime & Robinson, 1999; Ranadheera et al., 2010).

The health benefits of probiotics have been known for a long time, dating from the very early ages when Hippocrates and other scientists reported that fermented milk could heal certain digestive disorders (Ranadheera et al., 2010). Élie Metchnikoff is, however, considered in recent history to have discovered probiotics (Heller, 2001). He noticed with greater longevity in Caucasian populations who frequently consumed fermented milks and proposed that the acid-producing organisms in fermented dairy products stopped "fouling" in the large intestine, consequently leading to prolongation of a healthy life (Heller, 2001). Probiotics are resistant to bile and survive passage through the gastrointestinal tract without induction of systemic immune or inflammatory reactions. Recent studies have indicated that probiotic bacteria can provide several therapeutic advantages, such as modification of the immune system, blood cholesterol reduction, lessening of lactose intolerance, maintained remission of Crohn's disease, healing of diarrhea, and prevention of infections of uro-genital organs (Hekmat, Soltani, & Reid, 2009).

Prebiotics, on the other hand, are non-digestible food ingredients that alter the functionality and/or growth of one, or a limited number of probiotic bacteria in the colon (Prado, Parada, Pandey, & Soccol, 2008). Oligosaccharides such as lactulose, galactooligosaccharides, inulin, fructooligosaccharides, and other food carbohydrates are some well known examples.

There is an obvious potential for a symbiotic effect of probiotics and prebiotics, since prebiotics promote the growth and activities of probiotics. Several studies have shown that growth and viability of *L. rhamnosus* could be increased in the presence of resistant starch, inulin, fructooligosaccharides, polydextrose and oligofructose in fermented food products such as yogurt, fermented milk, cheese and ice cream (Ranadheera et al., 2010). Apart from nutritional benefits of prebiotics, addition of these ingredients provides techno-functional benefits that can improve the various quality parameters of the final products. Some plant-based matrices are very rich in prebiotic compounds and inulin-containing chicory is probably the best example of this. Inulin and oligofructan derived from inulin improve the viscoelastic properties of yogurt and fermented milk products. They have been shown to increase firmness and viscosity and decrease syneresis (Bozanic, Rogelj, & Tratnik, 2001 and 2002; Dello Staffolo, Bertola, Martino, & Bevilacqua, 2004; Debon, Prudencio, & Petrus, 2010; De Castro, Cunha, Barreto, Amboni & Prudencio, 2008). Several studies have also indicated that the physico-chemical characteristics (pH, acid production, color and water activity) of fermented products such as yogurt, soy yogurt and probiotic fermented milk products improve due to supplementation with prebiotics such as inulin, resistance starch, fiber and calcium, date fiber, β-glucan, glucose and raffinose. This could be due to the nutritional benefits of prebiotics in enhancing the growth of probiotics and promoting acid production during fermentation and storage, as well as their techno-functional properties which could enhance the physical properties of the products (Donkor, Nilmini, Stolic, Vasiljevic, & Shah, 2007; Aportela-Palacois, Sosa-Morales, & Velez-Ruiz, 2005; Vasiljevic, Kealy, & Mishra, 2007; Hashim, Khaul, & Afifi, 2009).

There is great economic interest in finding other prebiotic-rich food matrices. The nutritional composition of lentil which includes complex carbohydrates (e.g., resistant starch, oligosaccharides, sucrose, raffinose, stachyose and verbascose), protein, important vitamins and minerals as well as antioxidants, and only very small amounts of unsaturated fats could make this ingredient a very good source of prebiotic components for human nutrition and probiotics bacteria such as *L. rhamnosus* (Zare, Boye, Orsat, Champagne, & Simpson, 2011; Wang, & Daun, 2004). In a previous study, we showed that yogurt starter culture *(S. thermophilus* and *L. bulgaricus*) grow better in milk supplemented with lentil flour and acid production during fermentation and storage improved with the addition of lentil flour. This study expands on our previous studies and explores lentil supplementation of milk in the presence of a specific probiotic species, *L. rhamnosus.* We investigate the effect of supplementation of fermented milk with 1–3% lentil flour on acid production during fermentation, growth of *L. rhamnosus*, pH, syneresis, color and rheological properties of the final product immediately after production and during 28 days of storage. For comparison, skim milk as the base media for fermentation with and without supplementation with 1–3% skim milk powder was similarly analyzed.

2. Materials and Methods

2.1 Cultures and ingredients

Non-fat skim milk powder used was from Agropur (Quebon brand; St. Laurent, QC, Canada); lentil flour was from K2 Milling Company (Tottenham, ON, Canada); *Lactobacillus rhamnosus* AD200 was purchased from ABIASA Inc. (St. Hyacinthe, QC, Canada). The cultures were obtained in freeze-dried form, packaged in laminated foils and were stored at 4 °C until used. Skim milk powder mixed in distilled water (9.5 % w/v)

served as the base for supplementation and is referred to as "control". In two series of experimental assays 1-3% (w/v) of lentil flour or 1-3% of skim milk powder were added separately to the skim milk base (control).

2.2 Fermentation

The Lactobacillus rhamnosus AD200 culture contained a microbial concentration of 2 x 1011 CFU/g. It was re-hydrated at 37 ^{0}C in the sterilized skim milk to obtain 2 x 109 CFU/mL. Subsequently 1 mL of this dilution was added to 100 mL media which represented an inoculation level of approximately 2 x 107 CFU/mL. The experimental protocol used for probiotic supplementation and production are shown in Figure 1. Acidification trend in fermented milk by L. rhamnosus was measured during fermentation according to the method described by De Brabandere and De Baerdemaeker (1999) using a FACS (Fermentation Acquisition and Control System) installed in a Forma Scientific (OH, US) programmable incubator.

2.3 Product characterization

The buffering capacity of the different blends was estimated by acid titration and pH measurements using a pH meter (Accumet AP61, Fisher Scientific Inc, ON, Canada) and a 50 mL digital burette (Brinkmann Instruments Ltd., ON, Canada). For viable counts culture media; MRS agar from Difco Company (KS, USA) was used for quantifying the L. rhamnosus. Viable counts were obtained after 48 hours incubation at 37 °C under anaerobic conditions.

pH was measured in the probiotic fermented milk using a pH meter (Accumet AP61, Fisher Scientific Inc, ON, Canada).

Syneresis was determined as the amount of spontaneous whey separation from the fermented product according to the method described by Lucey, Munro and Singh (1998), with some modifications. The volume of whey drained from 100 mL of undisturbed set yogurt prepared in cylindrical tubes was measured and reported as percentage syneresis.

Color was determined as lightness (L), red/greenness (a), and yellow/blueness (b), using a colorimeter (Konica Minolta, CM-503 c, NJ, US).

Dynamic oscillation tests were conducted to determine the flow behaviour and characterize the viscoelastic properties of the yogurt, using a rheometer (TA Instruments, SR-2000, DE, US) fitted with a 40-mm-diameter cone and 0.04 radian degree cone angle and plate geometry with a 4 mm gap. To ascertain the applicable stress and frequency in which storage modulus (G') and loss modulus (G") parameters of yogurt would demonstrate a linear constant rate, dynamic frequency ramp tests (frequency from 1 to 10 Hz and stress set at 3 Pa) and dynamic stress ramp tests (stress from 1 to 10 Pa and frequency set as 2.5 Hz) were conducted at 25 °C. Dynamic temperature ramp tests were done at a stress and frequency of 3.0 Pa and 2.0 Hz, respectively, in a temperature range of 4 to 50 °C (heating) and 50 to 4 °C (cooling), at a rate of 10 °C/min. Aliquots of the samples were carefully removed from the undisturbed yogurt cup and placed on the center of the rheometer plate; the top plate was slowly lowered on the top of the sample prior to analysis.

Viable counts, pH, syneresis, color and rheological parameters were measured after fermentation as well as during 28 days storage at 4 ◦C at 7 days interval.

2.4 Statistical analysis

Statistical test was conducted using ANOVA analysis (SAS 9.1, SAS Institute Inc. NC, US). Comparisons were made using the Student–Newman-Keuls test and the two sample t-test for comparison of two means.

3. Results and Discussion

3.1 Acidification by L. rhamnosus

Acidification pattern of the samples after inoculation with L. rhamnosus is presented in Figure 2. For both skim milk powder and lentil flour, supplementation enhanced the acidification rate. The difference in pH between the supplemented sample and the control became statistically significant after 8 hours of incubation. After 8 hours, the pH in the 2% and 3% skim milk-supplemented products was significantly ($P<0.05$) lower than the lentil flour-supplemented media. However, after 12 hours of incubation, the 2% and 3% lentil flour-supplemented samples had the lowest pH. As a result, the products with lentil flour reached a pH of 4.5 significantly earlier than skim milk-supplemented and control sample (Figure 2). This constitutes important time and energy savings in the manufacturing process. These data confirm our preliminary study (Zare, Champagne, Simpson, Orsat, & Boye, 2012). As milk has greater buffering capacity in comparison with lentil flour (Table 1), the greater acidification rates in products supplemented with lentil flour could have been partially due to their lower

buffering capacity when compared to the corresponding skim milk-supplemented probiotic products. This, however, does not appear to be the case when the data for lentil flour is compared to the control. Acid production in the media, which is mainly due to lactic acid, is often linked to the growth of lactic acid bacteria (i. e: L. rhamnosus) (Tamime & Robinson, 1999). The acidification data (especially for the control and lentil flour-supplemented samples), therefore, suggests that growth of the microorganism in the lentil flour-supplemented samples was likely stimulated by the lentil flour. Although, viable counts were not followed during the fermentation to assess this hypothesis, further analyses were carried out after production as well as during storage as shown below.

3.2 Microbial growth and survival in the supplemented products after production and during storage

There is no universally accepted number of viable cells required to obtain a health benefit (Reid, 2008). In yogurt products, in order to provide health benefits targeted towards lactose digestion using probiotics, a minimum of 108 CFU per serving is required (EFSA, 2010). Canadian legislation (CFIA, 2009) allows limited non-strain linked claims if the product contains a billion viable cells per portion, and there is a trend, at least in Canada, towards this CFU level in yogurt and probiotic fruit juices available on the market. To maintain these numbers, it is important to follow the probiotics viability during manufacture and storage (Damin, Minowa, Alcântara, & Oliveira, 2006).

Viable counts of *L. rhamnosus* in fresh (day 0) products supplemented with lentil flour or skim milk powder varied from log 8.11 to log 8.71 (Table 2). Although important CFU reductions occurred in some samples after 28 days of storage, all samples supplied the minimum 1 billion per portion amount of probiotics required for a non-strain related health claim (CFIA, 2009), presuming a portion to represent about 100 mL. Furthermore, many had the 10^8 CFU/ mL threshold required to market the high-density probiotic products such as DanActive™ (Danone) or BioBest Maximmunité™ (Parmalat), which contain 10 billion CFUs per portion. These counts are, however, lower than those obtained for *L. rhamnosus* in fruit-based media, which were well above 10^9 CFU/mL (Champagne and Gardner, 2008). Growth and acidification of *L. rhamnosus* in milk is rather slow (Gaudreau, Champagne, & Jelen, 2005), and low CFU counts in milk-based products are also encountered with other probiotic cultures (Champagne, Tompkins, Buckley, & Green-Johnson, 2010). Therefore, the data on growth and acidification with *L. rhamnosus* in milk is in line with that of the literature. Supplementation with 1 to 3% skim milk powder significantly improved *L. rhamnosus* CFU levels in the fresh products (Table 2).

Addition of 2 and 3% lentil flour also increased CFU counts in the fresh products (Table 2). The high CFUs in the fresh supplemented milk powder and lentil flour samples remained higher than the control treatment during the 28 days of cold storage (Table 2). Interestingly, although the addition of lentil flour accelerated the rate of acidification, it did not increase the CFU values in the fermented probiotic products (Table 2) as much as the skim milk powder-supplemented samples.

The nature of the stimulatory factors in lentil flour, thus, remains unknown and it is hypothesized that lentil flour could serve as a prebiotic source due to its nutrients components such as protein, resistant starch, sucrose, raffinose, stachyose, verbascose and oligosaccharides for *L. rhamnosus* in probiotic products (Wang & Daun, 2004). Also, supplementation with lentil flour significantly maintained the stability of the *L.rhamnosus* during storage (Table 2).

Antioxidants (Dave & Shah, 1997) and carbohydrates (Silva, Carvalho, Pereira, Teixeira, & Gibbs, 2004) have been shown to improve the stability of lactobacilli during storage. It remains to be determined which compounds in the lentil flour have this protective benefit towards the lactobacilli.

A regression analysis was carried out to ascertain the role of the buffering capacity of the ingredients (Table 1) on the viable counts obtained at the end of the fermentation (Table 2). There was a positive correlation (R^2 = 0.79) between the CFU data after fermentation and the buffering capacity of the skim milk supplementation samples. Also, there is a positive correlation (R^2 = 0.88) between the CFU data after fermentation and the buffering capacity of the lentil flour-supplemented samples. This high correlation data suggest that, for *L. rhamnosus* AD200, the buffering capacity is a strong regulator of growth, in both skim milk and lentil flour at this range of milk solids. Thus, the very different nutritive content of milk and lentil flour had a negligible effect on final CFU levels when added to milk in the 1-3% supplementation range. The importance of the buffering capacity and biomass levels is in agreement with the literature (Badran & Reichart, 1994). However, data on acidification rates showed a different picture. One would expect that the higher buffering capacity would slow the rate of pH reduction. A regression analysis between pH values at time = 18 h (hour) and the buffering capacity of the media showed absolutely no correlation (R^2 < 0.01). Therefore the acidification rate itself is completely independent of the buffering capacity of the ingredients, and is presumably directly linked to the

nutrient content. It can be concluded, however, that the nutrients brought by lentil flour accelerated the acidification rate of L. *rhamnosus* AD200 much more than that of skim milk.

3.3 Change in pH during storage

The pH reduction is due to the acid produced by L. rhamnosus during fermentation which continues slightly during storage. In all samples, the pH decreased by 0.1 to 0.5 units over the 28 days storage period (Table 2). This drop in pH was greater in products supplemented with 1-3 % lentil flour in comparison with 1-3% skim milk-supplemented and control samples. The level of skim milk supplementation did not affect the pH reduction after 28 days of storage ($P<0.05$). However, this was not the case in products containing the lentil flour. Indeed, the pH in the 3% lentil-supplemented product was more stable during storage (Table 2) than the products containing 1-2% lentil flour, and this difference actually became statistically significant ($P<0.05$) after 14 days of storage (data not presented). It was examined if there was a link between these pH data and that from buffering capacity or viable counts (Table 1). No correlation was found between the pH at the end of storage and the buffering capacity of the medium ($R2 < 0.1$) nor with the viable counts at the beginning of storage ($R2 = 0.26$). These data are in line with other studies which show that acidification during storage does not necessarily parallel CFU counts (Seo, Lee, Chang, & Kwak, 2009). According to Kailasapathy, Harmstorf, & Phillips, 2008, the higher the buffering capacity of the media, the smaller the change in pH due to changes in acid content of the food system. This was not the case in our study; the skim milk control had the lowest buffering capacity (Table 1) but was the product having the most stable pH during storage (Table 2). Supplementation with lentil flour, therefore, seems to increase the acidifying ability of the lactobacilli during storage.

There was a concern with respect to the stability of the probiotics during storage, because a higher buffering capacity of the medium tends to increase the survival of live culture bacteria (Kailasapathy et al., 2008). This was not a problem in this study. Indeed, the viable counts in the lentil flour-supplemented products dropped on average by 0.3 log CFU/g while that of the skim milk-supplemented samples had viability losses of approximately 0.5 by log CFU/g (Table 2). Although, the increased acidification rates during storage did not negatively affect the losses in viability, pH is known to affect texture. Therefore, analyses of color and texture were carried out on the fresh products as well as on the stored ones.

3.4 Color

Color is one of the most important factors for marketability of food products and consumer acceptance. Although a probiotic product could provide several health benefits to consumers, they will not be marketable if consumers do not find them to be visually appealing.

The color of lentil flour-supplemented probiotic product should, therefore, be comparable to non-supplemented or skim milk-supplemented probiotic or other fermented dairy products. Furthermore, the color of the supplemented products should ideally remain unchanged after production and during storage. Figure 3 (a, b and c), shows differences in the color (a, b and L values) of the 1-3% lentil flour and 1-3% skim milk-supplemented and control probiotic beverages at day 1 and 28 days after production. On the first day of production all lentil flour-supplemented samples had significantly lower “L” value and higher “a” and “b” values in comparison with the other samples. Also, the level of supplementation significantly affected the “L” and “b” values in skim milk-supplemented samples, but for lentil flour-supplemented samples, the level of supplementation only affected the “b” values ($P<0.05$). After 28 days, the “L” values for the lentil-supplemented probiotic beverage samples decreased less than the “L” values for the skim milk-supplemented samples; this means that the skim milk-supplemented probiotic beverages became darker during storage significantly more than the lentil flour-supplemented samples. However, after 28 days of storage, the 3% lentil flour-supplemented sample had the lowest “L” value and the highest “a” and “b” values in comparison with the other samples ($P<0.05$). “L” value represents lightness (100) and blackness (0); “a” represents red (+ve) to green (-ve) hues, whereas “b” represents yellow (+ve) to blue (-ve) hues (Sanz, Salvador, Jimenez, & Fiszman, 2008); thus, the color measurements indicate that immediately after production the lentil flour-supplemented yogurt were darker and had less greenness and more yellowness hues in comparison with the skim milk-supplemented samples. After 28 days, the 1-2% lentil flour-supplemented probiotic beverages were comparable to the 2% skim milk-supplemented and control samples, in terms of lightness.

3.5 Syneresis

Acid production due to growth of L. rhamnosus during fermentation, results in lowering of the pH to the isoelectric point of casein (4.5), which results in protein coagulation and gel formation. Syneresis provides an indication of the non-homogeneities in the gel system; thus, higher water separation (syneresis) is related to gel instability (Lucey et al., 1998). Figure 4 shows the syneresis of lentil flour and skim milk-supplemented

probiotic and control beverage samples immediately after production and after 14 and 28 days of storage. On day 1, the 1-3% lentil flour showed the lowest syneresis, which was significantly lower than for the 1-3% skim milk-supplemented and control samples ($P<0.05$). The level of supplementation affected syneresis in lentil flour-supplemented samples (i.e., the higher the supplementation level, the lower the syneresis), whereas this factor had a lesser effect on skim milk-supplemented samples. After 14 days of storage, although the volume of liquid separated from the gel dramatically increased in the 2-3% lentil flour-supplemented samples, the 3% lentil flour-supplemented sample still showed the least syneresis compared to all other samples ($P<0.05$). Most of the increase in syneresis occurred between days 1 and 14 of storage.

It is hypothesized that the greater changes in syneresis in lentil flour supplemented samples were potentially due to the greater decrease in pH. Indeed, acidification during storage was highest in the lentil flour-supplemented products (Table 2) and this parameter is directly linked to syneresis (Tamime & Robinson, 1999).

Supplementation of probiotic products with an increase in the total solid content, especially protein content, results in stronger texture and less whey separation (Peng, Serra, Horne, & Lucey, 2009). This may explain the lowest syneresis in the 3% lentil flour and 3% skim milk-supplemented samples. Also, considering the starch and fiber contents of lentil flour, which have hydrocolloidal properties, it could be suggested that lentil flour confers a more homogenous texture to the samples in comparison with skim milk powder.

3.6 Rheological properties

Rheometry is a practical technique for measuring the textural properties of foods. Viscoelastic property measurements give knowledge of the rheological characteristics of foods and can help to give an assessment of the initial experience of a consumer (Kealy, 2006). Oscillatory tests have been used to assess the rheological properties of fermented milk products in several studies (Ozer, Robinson, Grandison, & Bell, 1997; Remeuf, Mohammed, Sodini, & Tissier, 2003; Sodini, Lucas, Tissier, & Corrieu, 2005). When a product is taken out of the refrigerator for consumption and then stored again, the rheological properties could be expected to change. Dynamic temperature ramp test allows the study of the rheological properties during heating and cooling processes.

Results of storage modulus (G´)(elasticity) and loss modulus (G˝)(viscosity) as a function of temperature for the 1-3% lentil flour-supplemented, 1-3% skim milk-supplemented and control probiotic samples at days 1, 14 and 28 of storage are presented in Figures 5, 6 and 7. According to the results, G' and G" parameters followed a hysteresis loop during heating and cooling and decreased with increasing temperature and increased back with decreasing temperature in all samples, both after production and during storage. Also, over the range of temperatures studied all supplemented and control samples demonstrated a predominantly elastic behaviour (G'>G").

Furthermore, as the level of supplementation increased, either with skim milk powder or lentil flour, the values of G' and G" both after production and during storage increased. Thus, both the supplementation level and total solids content may be important factors which alter gel structure in probiotic beverage products. This finding is in agreement with others reported in the literature (Sendra et al., 2010).

Additionally, when comparing the responses at certain temperatures during heating and cooling, the lentil flour-supplemented probiotic samples behaved differently from the skim milk-supplemented yogurt. Although both G' and G" values decreased as a result of heating and subsequent cooling for all samples, the reduction for the lentil flour-supplemented samples was not as big as that in the skim milk-supplemented and control samples. In other words, the gel structure, especially for the 1% and 2% skim milk-supplemented probiotic samples, almost collapsed since G' and G" values were close to zero during the cooling process (from 50-4 °C).

This suggests that the gel structure in the lentil flour-supplemented probiotic samples were more stable under temperature stress conditions than the skim milk supplemented probiotic beverages. This appears in line with the data on syneresis, where supplementation with lentil flour improved the water-binding property of the gel.

4. Conclusion

This study showed that supplementation of probiotic beverages with 1-3% lentil flour results in a significantly faster lowering of the pH than 1-3% skim milk powder, suggesting that the fermentation process was significantly faster in 1-3% lentil-supplemented probiotic samples. This may partially be attributed to the lower buffering capacity of lentil flour, as compared to skim milk as well as to the nutrient enrichment. Indeed, the viable counts of L. rhamnosus in the fermented products enriched by the lentil flour were higher than the non-supplemented control sample, for the same final pH level (pH = 4.5). The stability of L. rhamnosus during storage at 4 °C for 28 days in lentil-supplemented samples was at least as good as for the skim

milk-supplemented probiotic samples. Our results also showed that probiotic supplementation with 1-3% lentil flour improved the physical and rheological properties of the products in terms of their viscoelastic properties and syneresis and they minimally altered the color of the samples in comparison with the 1-3% skim milk-supplemented and control probiotic samples after production and during storage. The pH in the 1-3% lentil-supplemented probiotic sample was lower than for the 1-3% skim milk-supplemented samples after 28 days storage, which could not solely be attributed to the lower buffering capacity of the lentil-supplemented media. In terms of syneresis, after 28 days of storage, lentil flour-supplemented probiotic samples had lower syneresis than the corresponding skim milk powder supplemented-products. At any given level of supplementation, the G' and G" value of the lentil flour-supplemented probiotic samples were higher than the values for the skim milk-supplemented probiotic and control sample. The higher G' and G" values may be due to a stronger gel structure attributable to the high fiber and complex carbohydrate content of lentil flour.

Overall, on the basis of the microbial, physico-chemical and rheological properties investigated, the results suggest that lentil flour could be potentially considered as a source of prebiotic and texture improvement ingredient for use in the production of L. rhamnosus fermented milk products.

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Table 1. Amount of HCl (1 M) required to acidify 100 mL of 1-3 % lentil flour and 1-3 % skim milk from pH 6.5 to 4.0 (SM: skim milk, LF: lentil flour; means followed by the same letter are not significantly different ($P<0.05$))

Sample	Titrable HCl (mL)
	Average ± SD
1 % SM	6.84 ± 0.00 b
2 % SM	7.58 ± 0.07 c
3 % SM	9.14 ± 0.00 d
1 % LF	6.51 ± 0.01 a
2 % LF	6.83 ± 0.21 b
3 % LF	7.06 ± 0.03 b
Control	6.38 ± 0.00 a

Table 2. Effect of milk supplementation with skim milk powder (SM) or lentil flour (LF), on viable count of *L. rhamnosus* and pH after fermentation and during 28 days of storage at 4 °C

Medium	***L. rhamnosus* (Log CFU/mL)**					**pH**	
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 0	Day 28
1% SM	8.44 c	8.28 b	8.22 b	8.00 bc	7.96 bc	4.57 b	4.44 a
2% SM	8.55 b	8.51 a	8.28 b	8.04 b	8.02 b	4.51 d	4.42 a
3% SM	8.71 a	8.58 a	8.35 a	8.21 a	8.15 a	4.53 cd	4.42 a
1% LF	8.15 de	8.03 d	8.00 cd	7.88 d	7.83 d	4.53 cd	4.00 c
2% LF	8.21 d	8.14 c	8.03 c	7.94 cd	7.92 c	4.55 cb	4.10 bc

3% LF	8.21 d	8.14 c	8.04 c	8.00 bc	7.99 bc	4.59 a	4.15 b
Control	8.11 e	8.10 cd	7.96 d	7.91 d	7.71 e	4.56 b	4.53 a

Means followed by the same letter are not significantly different; for a given column ($P < 0.05$).

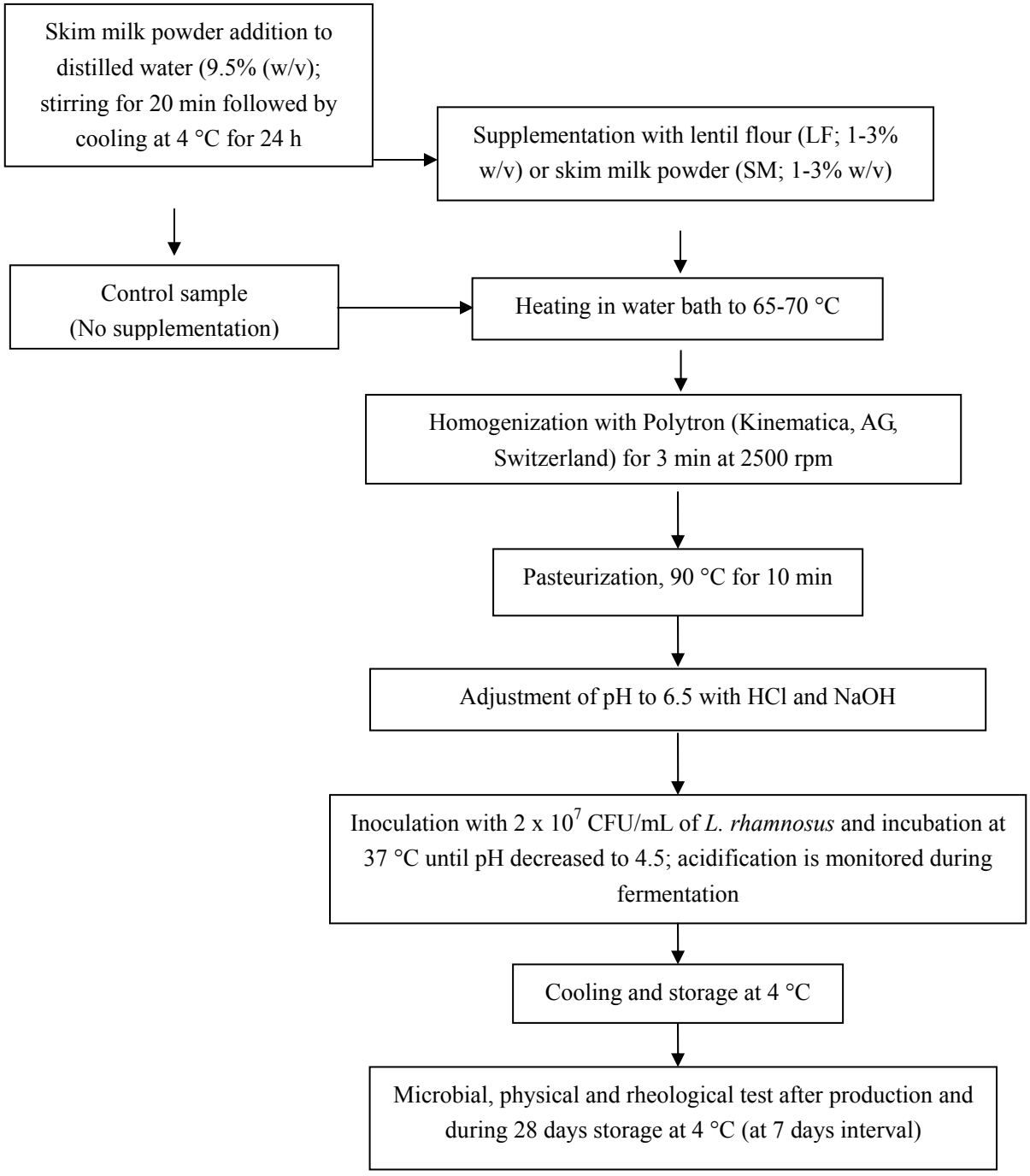


Figure 1. Schematic presentation of the process used for the preparation of *L.rhamnosus* probiotic beverage supplemented with skim milk powder (SM) or lentil flour (LF) and the control sample

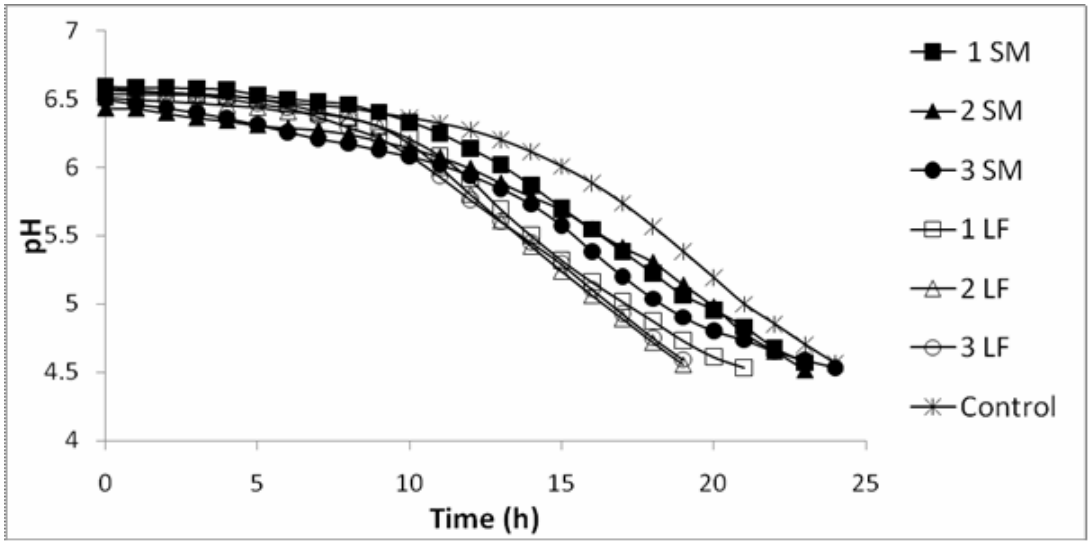
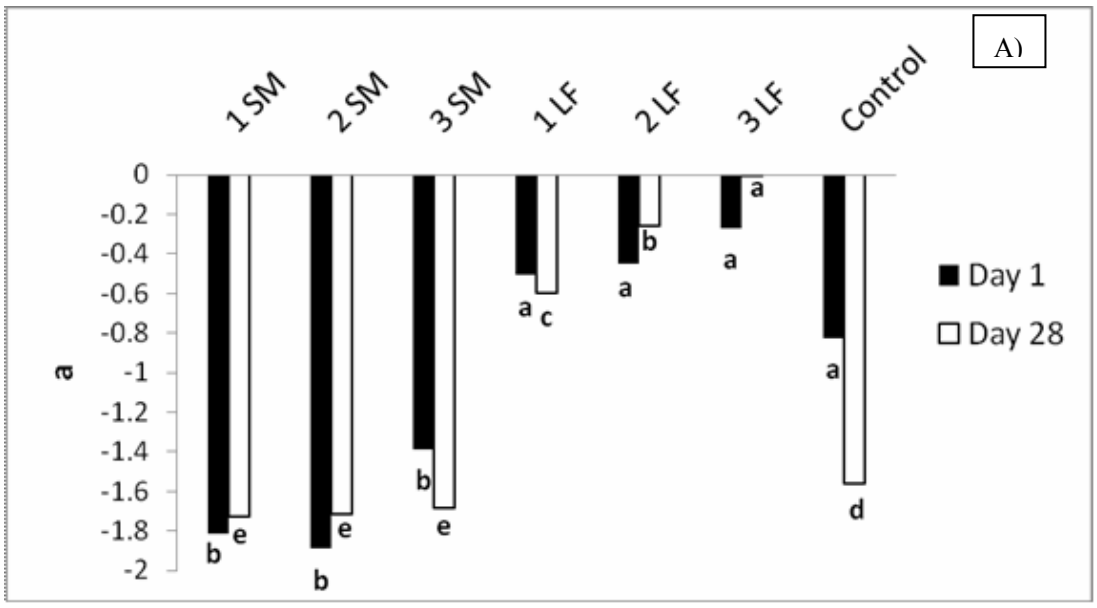


Figure 2. Acidification trend of skim milk (SM) containing *L. rhamnosus* AD200 supplemented with 1 to 3% lentil flour (1 LF, 2 LF and 3 LF - treatments) or 1-3% skim milk (1 SM, 2 SM and 3 SM - treatments)



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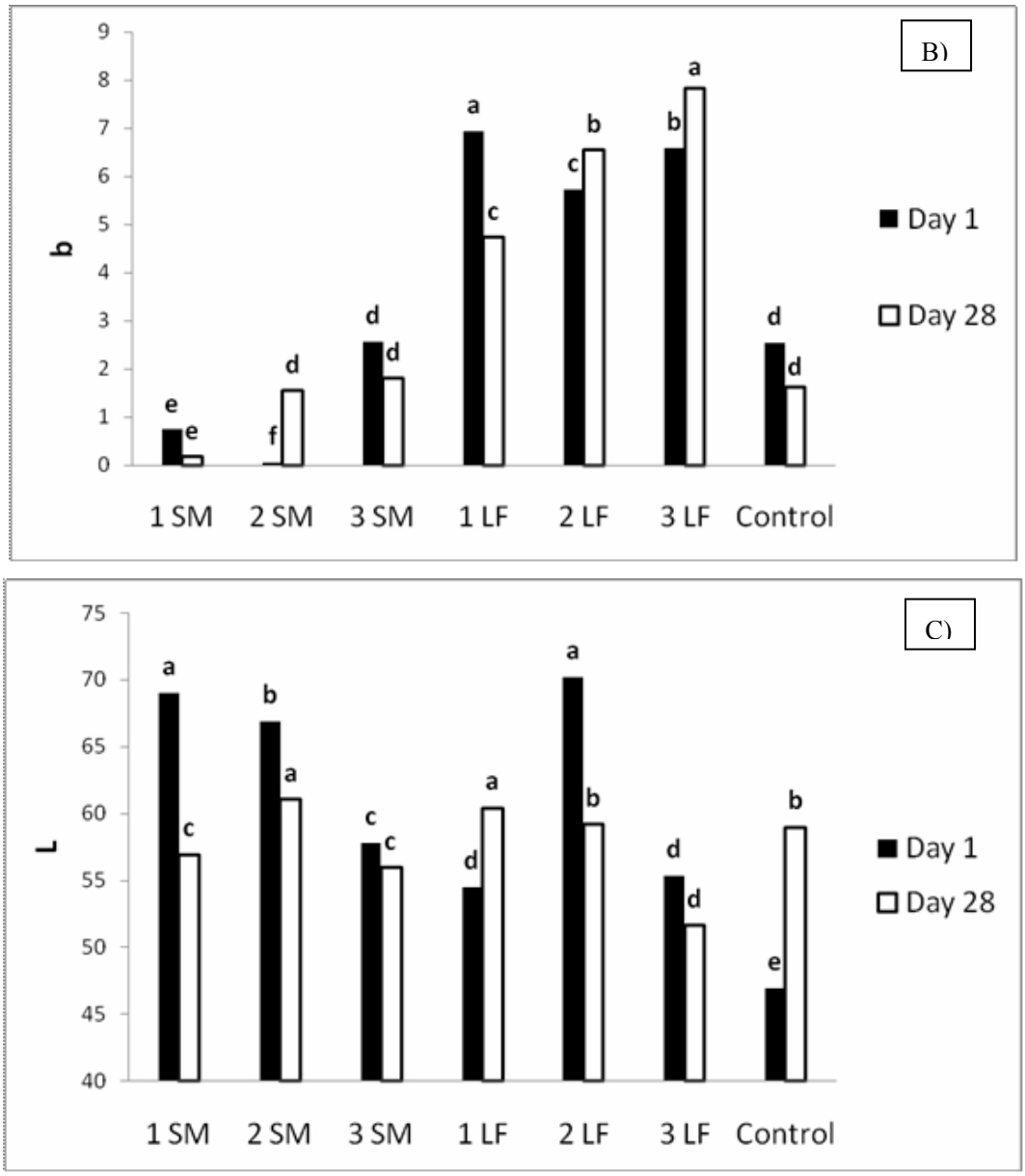

Figure 3. Color profile of probiotic beverages supplemented with 1-3% lentil flour or 1-3% skim milk and control sample (no supplementation) after production and after 28 days storage (SM: skim milk, LF: lentil flour; a (a value) +ve red, -ve green; b (b value) +ve yellow, -ve blue; c (L value): -0 to 100, black to white), means followed by the same letter are not significantly different ($P < 0.05$)

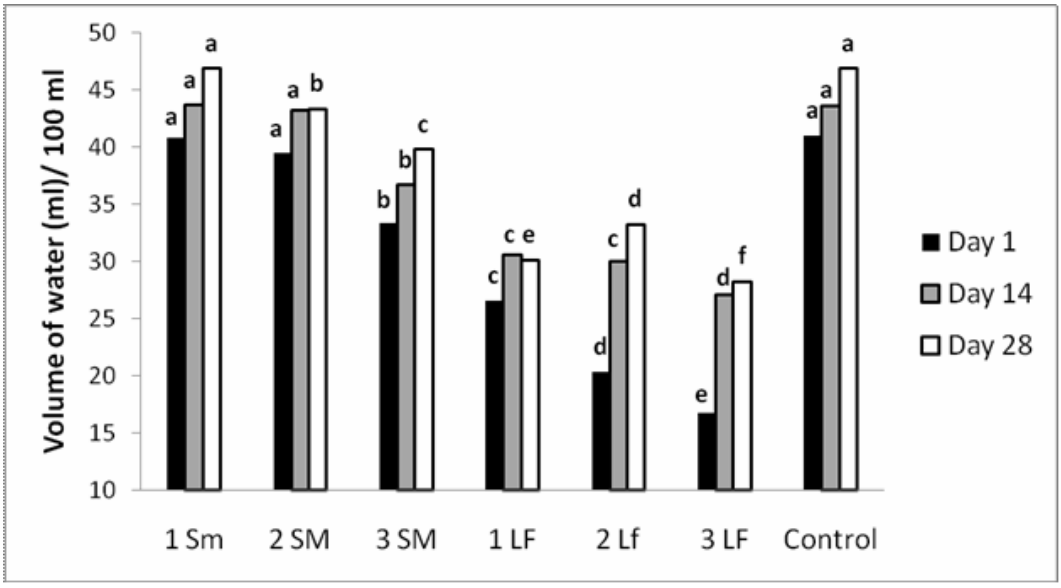

Figure 4. Syneresis in probiotic beverages supplemented with 1-3% lentil flour, 1-3% skim and control sample (no supplementation) during 28 day storage (SM: skim milk, LF: lentil flour), means followed by the same letter are not significantly different ($P < 0.05$)

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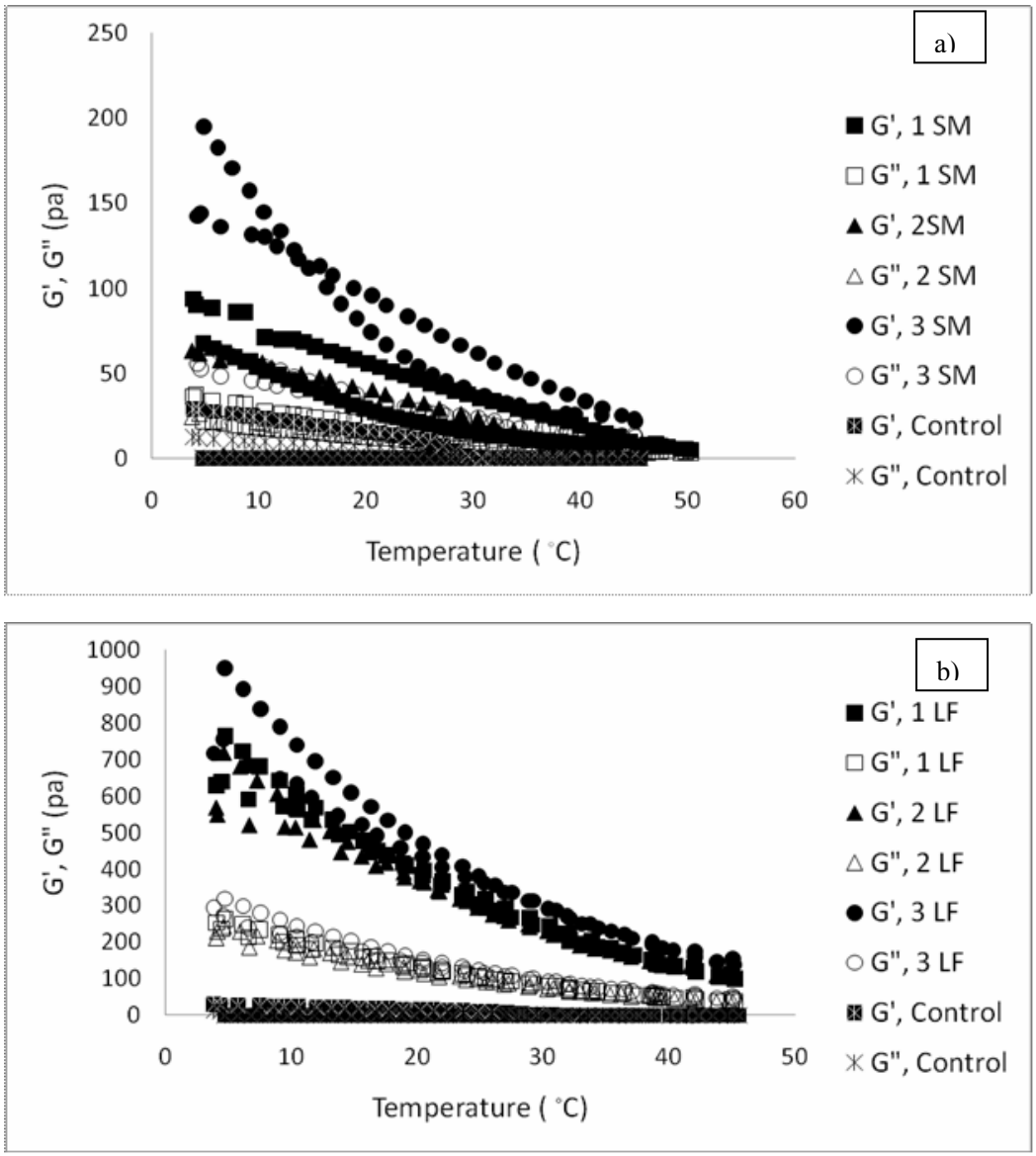

Figure 5. Storage (G´) (elasticity) and loss (G˝) (viscosity) moduli of fermented probiotic beverage products supplemented with (a) 1-3% skim milk and (b) 1-3% lentil flour heated from 4 to 50 ˚C and cooled back from 50 to 4 ^{o}C at day 1, (SM : skim milk, LF: lentil flour)

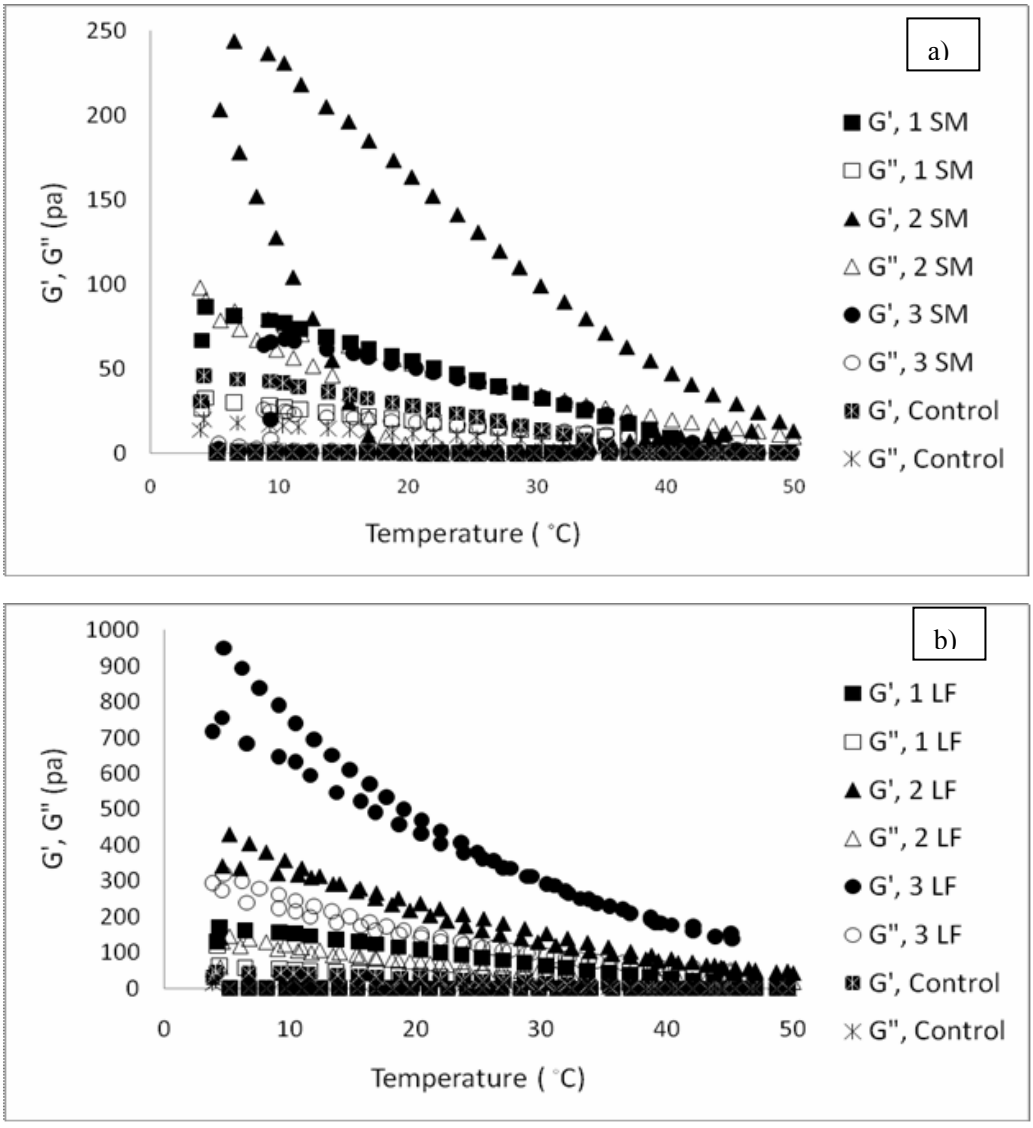

Figure 6. Storage (G´) (elasticity) and loss (G˝) (viscosity) moduli of fermented probiotic beverage products supplemented with (a) 1-3% skim milk and (b) 1-3% lentil flour heated from 4 to 50 ˚C and cooled back from 50 to 4 ^{o}C after 14 days of storage, (SM : skim milk, LF: lentil flour)

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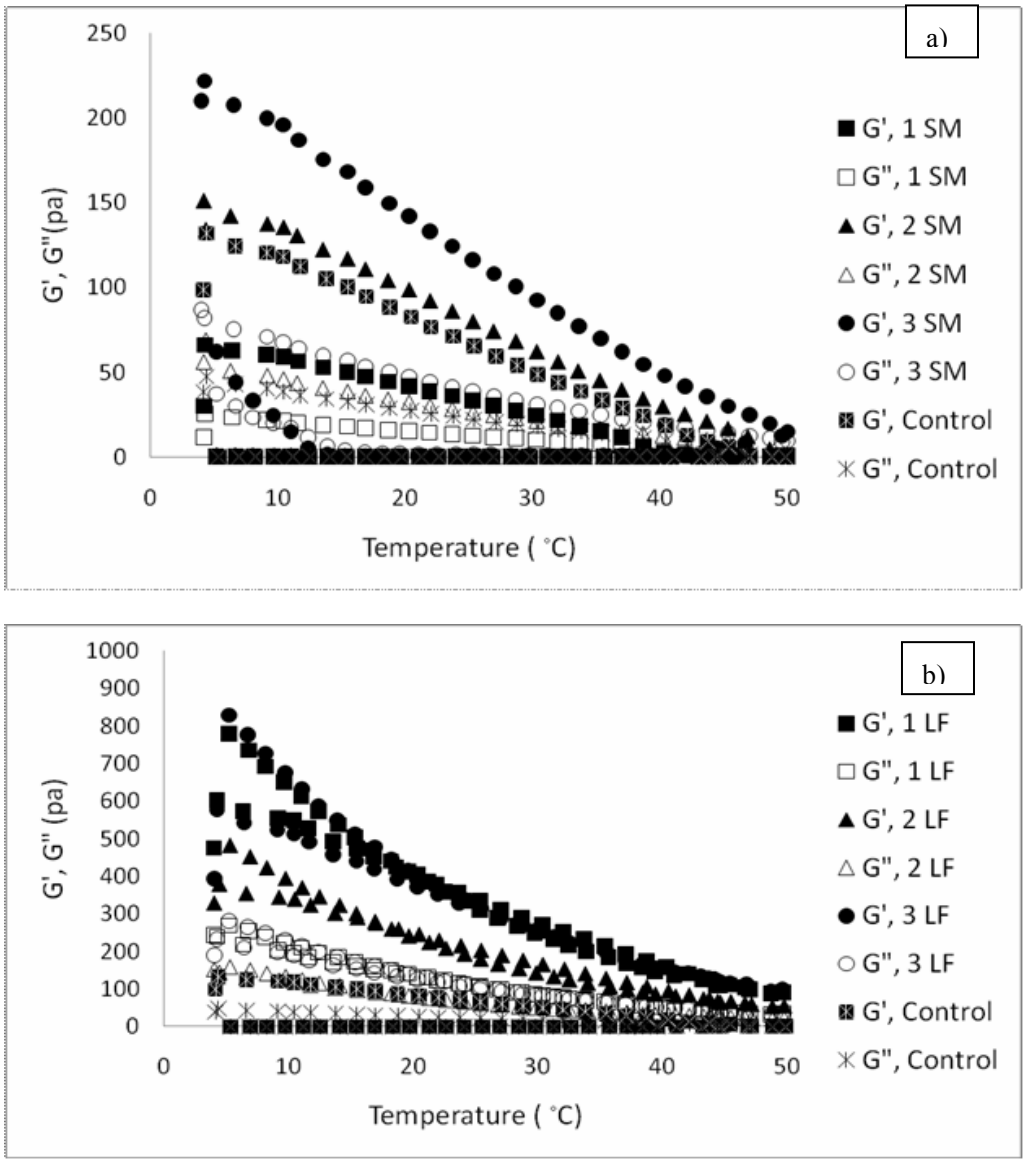

Figure 7. Storage (G´) (elasticity) and loss (G˝) (viscosity) moduli of fermented probiotic beverage products supplemented with (a) 1-3% skim milk and (b) 1-3% lentil flour heated from 4 to 50 ˚C and cooled back from 50 to 4 ^{o}C after 28 days of storage, (SM : skim milk, LF: lentil flour)

Malaysian Isolates of Lactic Acid Bacteria with Antibacterial Activity against Gram-Positive and Gram-Negative Pathogenic Bacteria

Belal J. Muhialdin (Corresponding author)
Faculty of Science and Technology
Universiti Sains Islam Malaysia (USIM)
Bandar Baru Nilai 71800, Nilai Negeri Sembilan, Malaysia
Tel: 60-163-817-246 E-mail: belaljamal.mahialdin@yahoo.com

Zaiton Hassan
Faculty of Science and Technology
Universiti Sains Islam Malaysia (USIM)
Bandar Baru Nilai 71800, Nilai Negeri Sembilan, Malaysia
Tel: 60-192-554-029 E-mail: drzaiton@usim.edu.my

Mohamed Muftah Ahmed Imdakim
Faculty of Science and Technology
Universiti Sains Islam Malaysia (USIM)
Bandar Baru Nilai 71800, Nilai Negeri Sembilan, Malaysia
Tel: 60-173-507-380 E-mail: emdakim1@yahoo.com

Fredy Kesnawan Shah Abdul Kahar
Faculty of Science and Technology
Universiti Sains Islam Malaysia (USIM)
Bandar Baru Nilai 71800, Nilai Negeri Sembilan, Malaysia
Tel: 60-137-485-085 E-mail: pembm.fst6@usim.edu.my

Mohamed Mustafa Aween
Faculty of Science and Technology
Universiti Sains Islam Malaysia (USIM)
Bandar Baru Nilai 71800, Nilai Negeri Sembilan, Malaysia
Tel: 60-176-046-716 E-mail: mmaaween@yahoo.com

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Abstract

Contamination of foodstuff with foodborne and pathogenic bacteria are global issue and it is serious hazard for

the health of the human. Lactic acid bacteria are well known for their health properties and their antimicrobial activity against spoilage and pathogenic bacteria. In this study, three isolates *Lactobacillus fermentum* Te007, *Pediococcus pentosaceus* Te010, *L. pentosus* G004 isolated from Malaysian fermented foods and fruits such as (tempeh, tempoyak, guava and banana) were evaluated for their antibacterial activity and antibiotic resistant against Gram-positive and Gram-negative bacteria by dual agar overlay method. The three isolates inhibited the growth of indicator bacteria and the activity was varied between weak and strong. All the isolates were resistant to the antibiotic nalidixic acid and vancomycin. The tested bacteria can be added to food as antibacterial agents to prevent the growth of harmful microorganisms.

Keywords: Tempeh, Lactic acid bacteria, Antimicrobial activity, Antibiotic resistance, Fermented foods

1. Introduction

The food safety is important concern in the processed foods industry because of the contamination with food borne pathogenic bacteria. The consumers demand for minimal processed food with less chemical additives (Cleveland *et al*., 2001). These reasons have led to the increase of interest to introduce the biopreservatives to food to replace chemicals with natural preservation agent (José *et al*., 2007). The foodborne and pathogenic bacteria, especially anaerobes and facultative anaerobes, even when they are present in low numbers they can grow during storage at low temperatures as in the refrigerators. These pathogens can multiply and cause risk to the safety of raw, processed and bakery products. Biopreservation is the extension of shelf life and food safety by the use of natural or controlled microbiota and/or their antimicrobial compounds (Stiles, 1996). Among the biopreservatives is lactic acid bacteria (LAB). They are known to produce variety of antibacterial **substances** as reported by several researchers (Moreno *et al.*, 2000; Mallesha *et al*., 2010; Akpinar *et al*., 2011; Muhialdin & Hassan, 2011a). LAB are safe and they have the status of general recognized as safe (GRAS), and have an important role in the preservation of foods and fermented products (Cintas *et al*., 2001). The inhibition activity of LAB to the growth of pathogenic bacteria is most likely due to the production of organic acids and bacteriocin (De Vos, 1993; Klaenhammer, 1993).

There are more than a few reports about the antimicrobial activity of LAB. A total of 16 strains (5 *Enterococcus faecium*, 5 *Enterococcus mundtii*, 4 *Pediococcus pentosaceus*, 1 *L. coryniformis* and 1 *Lactococcus garvieae*) were reported to have inhibition activity to the growth of *Listeria innocua*. Bacteriocin-like inhibitory substances were also reported to maintain the inhibition activity against non-pathogenic and pathogenic food-associated and human pathogenic bacteria (Corsetti *et al*., 2005). *L. pentosus* TV35b found to have inhibitory activity against the growth of *Clostridium sporogenes*, *Cl. tyrobutyricum*, and *Listeria innocua*, among others, the active compound produced was **produced** a bacteriocin-like peptide (pentocin TV35b) (Okkers *et al*., 1999). Moreno *et al*., (2002) reported that LAB isolated from fermented food Tempeh produced bacteriocins that inhibited the growth of Gram-positive indicators, including *Listeria monocytogenes*. Liasi *et al*., (2009) reported that Malaysia isolates (*L. casei* LA17, *L. plantarum* LA22 and *L. paracasei* LA02) isolated from the fermented fish Budu inhibited the growth of (*B. cereus, S. aureus, Salmonella enterica, Listeria monocytogenes, E. coli* and *Lactococcus lactis*). The objective of this study is to evaluate the antimicrobial activity of lactic acid bacteria isolated from Malaysian fermented foods and fruits and their antibiotic susceptibility.

2. Materials and Methods

2.1 Cultures and isolates

L. fermentum Te007, *Pediococcus pentosaceus* Te010, *L. pentosus* G004 were isolated from Malaysian fermented fruits and foods and evaluated for their antibacterial activity against (*Bacillus subtilis, Serratia marcescens, Enterobacter aerogenes, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Shigella sonnei, Klebsiella pneumonia* and *Salmonella* Typhimurium) obtained from the Microbiology Laboratory, Faculty of Food Science, University Putra Malaysia. The pathogenic bacteria were chosen to represent potential food borne bacteria. They were grown on nutrient broth (NB, Oxoid) at 30 °C for 24 h (Table 1).

2.2 Growth inhibition of pathogenic bacteria by LAB

The inhibition activities of the isolates were determined by using the dual agar overlay method as described by Magnusson & Schnurer (2001) with some modification. LAB isolates were inoculated in 2-cm lines on MRS agar plates and were grown at 30 °C for 24 h in anaerobic jars. The plates were then overlaid with 10 ml of nutrient agar containing 10^5 CFU/ml of each bacterium separately. The zone of inhibition was measured after 24 h of aerobic incubation at 30 °C; the inhibition was measured by depending on the size of the inhibition zone. The scale was used is: + weak inhibition, ++ moderate inhibition and +++ strong inhibition. The test was done in

duplicate.

2.3 Antibiotic resistant of lactic acid bacteria

LAB isolates were tested for their resistant against several antibiotics using disk diffusion method (Herreros *et al*. 2004) with MRS agar. The antibiotics tested were vancomycin (5μm), nalidixic acid (30 μm), gentamycin (10 μm), streptomycin (10 μm), tetracycline (30 μm), penicillin G (10 μm) and chloramphenicol (30 μm) (Sigma). The antibiotic disc were placed on each plate and incubated at 37^{O}C for 24 hours in an anaerobic jar. The results were taken by detection the inhibition zone around the antibiotic discs. The test was done in replicate.

3. Results

3.1 Growth inhibition of pathogenic bacteria by LAB

The three isolates of LAB (*L. fermentum* Te007, *P. pentosaceus* Te010, *L. pentosus* G004) showed antibacterial activity against tested strains of Gram positive and Gram negative bacteria. The results showed very strong inhibition activity from the isolate *L. pentosus* G004 and *L. fermentum* Te007 and moderated activity from the isolate *P. pentosaceus* Te010 (Table 2). The isolate *L. pentosus* G004 had very strong activity against all the tested bacteria except (*Salmonella* Typhimurium and *Bacillus subtilis*) which the isolate had moderated inhibition activity.

In contrast, with the isolate *L. fermentum* Te007 that showed very strong activity against (*Bacillus subtilis, Serratia marcescens, Escherichia coli* and *Staphylococcus aureus*) and moderated activity against (*Enterobacter aerogenes, Staphylococcus epidermidis, Shigella sonnei, Klebsiella pneumonia and Salmonella* Typhimurium). The isolate *P. pentosaceus* Te010 showed fair and good inhibition activity against all the examined bacteria. The three LAB isolates had moderated activity against *Salmonella* Typhimurium but no isolate showed very strong activity against it. In addition, *Bacillus subtilis* was sensitive for the isolate Te007 and the sensitivity was less for the other two LAB isolates.

3.2 Antibiotic resistant test of LAB isolates

All LAB isolates were resistant to nalidixic acid and vancomycin. Additionally, isolate *L. pentosus* G004 was resistant to Gentamycin, Streptomycin, Tetracycline and Penicillin G.. Isolate *L. fermentum* Te007 was sensitive to Gentamycin, Tetracycline and Penicillin G activity to gentamycin. Isolate *P. pentosaceus* Te010 showed resistance against nalidixic acid and vancomycin but sensitive to the other examined antibiotics. All LAB isolates were sensitive to Chloramphenicol (Table 3).

4. Discussion

The LAB isolates inhibited the growth of all tested bacteria including the pathogenic strains in different spectrum range of activities and the activity range was between weak to strong depends on the LAB strain. The highest activity was from the isolate *L. pentosus* G004 which show high activity against the tested microbes, followed by the isolate *L. fermentum* Te007 and *P. pentosaceus* Te010, respectively. Moreno *et al*., (2002) reported that LAB isolated from fermented food Tempeh produced bacteriocins that inhibited the growth of Gram-positive indicators, including *Listeria monocytogenes*.

S. aureus, B. subtilis, S. typhimurium and *E. coli* are pathogens that have been involved in outbreaks of food-borne disease in the several foodstuffs (Lindqvist *et al*., 2001; Matarante *et al*., 2004). *K. pneumonia, S. epidermidis Shigella sonnei* and *Serratia marcescens* are human pathogens and involve in many serious infectious diseases (Wang *et al*., 1998; Ammor *et al*., 2006). The concern of food safety is on increase and this comes with big responsibility of finding new, natural and inexpensive methods such as the use of the biopreservatives from safe microbes such as LAB. There are many reports described the success of the LAB isolates to inhibit the growth of pathogenic and foodborne bacteria *in vivo* and *in vitro* (Herreros *et al*. 2004; Ammor *et al*., 2006; Al-Allaf et al., 2009; Akpinar *et al*., 2011).

In this study the selected LAB isolates showed good inhibition activity against indicator strains of the pathogenic bacteria and this promote the use of these isolates as antimicrobial agent that can be added to food to prevent the growth of spoilage and foodborne bacteria and prevent the production of the different toxins in foodstuff by these bacteria. The inhibition was fast after 24 h; we could observe the inhibition zone around the two lines of the LAB isolates on the MRS agar plates. Liasi *et al*., (2009) reported Malaysia isolates (*L. casei* LA17, *L. plantarum* LA22 and *L. paracasei* LA02) to have inhibition activity against (*B. cereus, S. aureus, Salmonella enterica, Listeria monocytogenes, E. coli* and *Lactococcus lactis*), The LAB was isolated from the fermented fish Budu.

In this experiment, the selected isolates were resistant to antibiotic vancomycin and nalidixic acid. Vancomycin

resistance is of major concern because it is one of the antibiotics that broadly efficacious against clinical infections caused by multidrug-resistant pathogens (Johnson *et al*., 1990; Woodford *et al*., 1995). It is very important for LAB that added to food to be not carry transmissible antibiotic resistance genes, there are reports which indicate that LAB from fermented products may act as a vehicle of antimicrobial-resistance genes that could be transferred to pathogens, either in the food matrix or in the gastrointestinal tract (Mathur and Singh, 2005). This can lead to the development of new antibiotic-resistant pathogens and that is why those strains of LAB undesirable to be added to foodstuff (Morelli and Wright, 1997; Salminen *et al.,* 1998; Saarela *et al.,* 2000). In this study, all the isolates showed resistance to vancomycin and nalidixic acid, and the isolate *L. pentosus* G004 was resistant to the seven tested antibiotic except of Chloramphenicol. This study demonstrates that these isolates can be added to food as conservative's agents against the foodborne pathogenic bacteria and to improve the human's health especially the isolate *L. pentosus* G004.

5. Conclusion

Lactic acid bacteria isolated from different Malaysian environment inhibited the growth of (*Bacillus subtilis, Serratia marcescens, Enterobacter aerogenes, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Shigella sonnei, Klebsiella pneumoniae,* and *Salmonella* Typhimurium). The three isolates had inhibition activity against tested microorganisms and the spectrum range was varied between fair and strong. The isolates were antibiotic resistant against vancomycin and nalidixic acid. LAB isolates from Malaysian fermented foods and fruits have inhibition activity against foodborne and pathogenic bacteria and it have potential to be use as food preservatives because of their ability to eliminate the growth of spoilage microorganisms.

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Table 1. Bacterial strains and media of growth used in this study

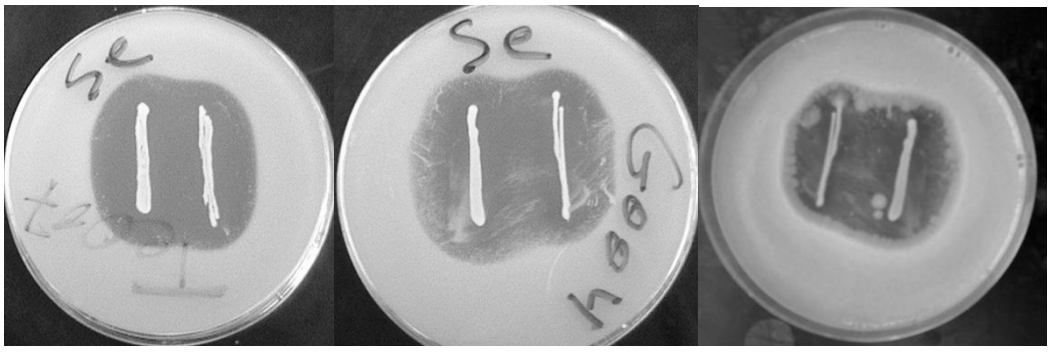
Strain	**Media**	**Incubation temperature**	**Origin or reference**
Bacillus subtilis 21332	Nutrient broth	30 °C	ATCC (American Type Culture Collections)
Serratia marcescens 13880	Nutrient broth	30 °C	ATCC (American Type Culture Collections)
Enterobacter aerogenes 13048	Nutrient broth	30 °C	ATCC (American Type Culture Collections)
Escherichia coli 25922	Nutrient broth	30 °C	ATCC (American Type Culture Collections)
Staphylococcus aureus 25923	Nutrient broth	30 °C	ATCC (American Type Culture Collections)
Staphylococcus epidermidis 12228	Nutrient broth	30 °C	ATCC (American Type Culture Collections)
Shigella sonnei 29930	Nutrient broth	30 °C	ATCC (American Type Culture Collections)
Klebsiella pneumonia 13883	Nutrient broth	30 °C	ATCC (American Type Culture Collections)
Salmonella Typhimurium 13311	Nutrient broth	30 °C	ATCC (American Type Culture Collections)
L. fermentum Te007	MRS agar	37 °C	Muhialdin *et al*., 2011b
Pediococcus pentosaceus Te010	MRS agar	37 °C	Muhialdin *et al*., 2011b
L. pentosus G004	MRS agar	37 °C	Muhialdin *et al*., 2011b

Table 2. Growth inhibition of Gram positive and Gram negative bacteria after 24 h incubation at 30ºC by dual agar overlay method

Tested organism	**Inhibition activity**		
	L. pentosus G004	*L. fermentum* Te007	*P. pentosaceus* Te010
Bacillus subtilis	++	+++	++
Serratia marcescens	+++	+++	++
Bacillus subtilis	+++	++	+
Escherichia coli	+++	+++	++
Staphylococcus aureus	+++	+++	++
Staphylococcus epidermidis	+++	++	+
Shigella sonnei	+++	++	+
Klebsiella pneumoniae	+++	++	+
Salmonella Typhimurium	++	++	++

Table 3. Antibacterial activity of selected antibiotics against lactic acid bacteria measured by diameter of inhibition zone (mm) around the discs

		LAB	
Antibiotics	*L. pentosus* G004	*L. fermentum* Te007	*P. pentosaceus* Te010
Nalidixic acid	0	0	0
Gentamycin	0	5	13
Streptomycin	0	0	11
Tetracycline	0	11	9
Vancomycin	0	0	0
Penicillin G	0	7	8
Chloramphenicol	19	9	13



A B C

Figure 1. Clear zone of growth inhibition of *Staphylococcus epidermidis* formed around the streak lines of lactic acid bacteria (LAB) (Te007) incubated at 30 ºC for 24 h by dual agar overlay method A. Te007, B. G004 and C. Te010

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Assessing Barriers to Expansion of Farm-to-Chef Sales: A Case Study from Upstate New York

Todd M. Schmit (Corresponding author)
Charles H. Dyson School of Applied Economics and Management
Cornell University, 437 Warren Hall, Ithaca, NY 14853, USA
Tel: 1-607-255-3015 E-mail: tms1@cornell.edu

Stephen E. Hadcock
Cornell Cooperative Extension, Columbia County
479 Rte. 66, Hudson, NY 12534, USA
Tel: 1-518-828-3346 E-mail: seh11@cornell.edu

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Abstract

Columbia County Bounty is a local organization made up of farmer and culinary business members, with a mission that includes promoting connections between local agricultural producers and culinary businesses. A case study was conducted to address questions raised by CCB related to expanding farm-to-chef marketing in their area. Common barriers for restaurants included larger time commitments, inconvenience, and consistency in product volumes and quality; however, satisfaction with local wholesale distributors may create new opportunities for farmers to work collaboratively with them in including more local products in their distribution. A closer inspection of channel performance by farms in the study will drive changes in future channel strategies and utilization of farm-to-chef marketing, as farms are already benefiting from strong direct marketing channels and restaurants procuring local products from these channels.

Keywords: Farm-to-chef, Local food, Marketing channels

1. Introduction

It was the Taste of Columbia County banquet in the fall of 2008, the first of what would eventually be an annual event organized by Columbia County Bounty (CCB) in upstate New York to celebrate the year's harvest of local agricultural products and to promote awareness of local foods in the community. CCB's Executive Director attended, along with approximately 25 local farmers, 20 chefs, and 150 community members. CCB members include both farmers and culinary business representatives, with a collective mission to promote networking connections between local agricultural producers and culinary businesses, as well as to educate the community about local and regional food products. The Executive Director was considering future changes in CCB operations to improve on its mission and increase the utilization of local agricultural products in the region's culinary businesses.

Unfortunately, CCB lacked the information and resources needed to address these issues. Since the formal creation of CCB in 2007, the group knew of only anecdotal evidence of the success of enhancing business-to-business relationships of farmers and chefs. A better understanding of the current utilization of farm-to-chef marketing channels by local farmers and culinary businesses was needed, along with how this channel fits into the overall sales and procurement channel strategies of its members. The county has a relatively

strong influx of tourists and part-time residents in the summer and fall. Knowing how these consumers view the use of local foods at restaurants is an important demand-side component in understanding consumer dining patterns and the potential premiums for local food offerings. Finally, a current examination of potential barriers to entry or expansion in this channel was needed to help capitalize on growing local marketing opportunities.

Previous literature has shown that marketing of farm products to local restaurants can present opportunities for increased farm sales and broadened consumer exposure to local farming operations (Lawless, 2001; Thilmany, 2004; Curtis & Cowee, 2009). Ancillary benefits of selling to restaurants have also been identified; e.g., insight into current market trends and changing consumer demands for food products (Pepinsky and Thilmany, 2004), and enhancing brand development and product differentiation for producers whose operations and products are featured on their clients' menus (Curtis & Cowee, 2009). However, generalizing the success of these types of efforts is difficult given differences in farmer goals and expectations, and performance-relevant heterogeneity in spatial market conditions. Specialized and/or spatially unique market conditions often necessitate these types of evaluations on a case-by-case basis.

These issues are the focus of this case study. In the summer of 2009, a study using data collected in Columbia County was initiated to address the issues brought forward by CCB. While the focus area was relatively small and consideration of unique market conditions in the area will be important, it was hoped that a broader understanding of this marketing channel as a component of overall marketing strategies adopted by farms could be achieved. By gathering and analyzing information from all relevant stakeholders (i.e., farmers, culinary business managers, and restaurant patrons), we hoped to provide a more comprehensive perspective than previous studies. While the results are likely not generalizable across areas with sufficiently different market conditions, our results should provide some evidence of the resiliency (or lack thereof) of barriers identified in previous work.

The approach includes an evaluation of farm-to-chef activities within the context of other local food market institutions. Specifically, information was gathered on the relative importance of farmer sales in different market channels, on alternative procurement sources used by restaurants, and on the utilization of alternative local food outlets by residents in the study area. Such an approach should facilitate a more comprehensive assessment necessary for these types of marketing institutions.

2. Case Study Area

Columbia County is located in the Hudson Valley region of New York State (NYS) (Figure 1). Given its diversity of agriculture and geographic location to large metropolitan markets, agricultural industries appear well positioned to take advantage of growing local food demands. Soils and climate allow for wide variety of crop and animal production. Of the 62 counties in NYS, the county ranks in the middle for total agricultural receipts, including both crop and livestock products (USDA, 2009). It ranks ninth in sales of fruits, tree nuts, and berries, thirteenth in poultry and eggs, and first in sheep and goat products. The county also has the sixth highest acres of apples. Within the ten-county southeast region of NYS (the first 'upstate' region above the New York City metropolitan area), Columbia County ranks third in agricultural receipts, fifth in number of farms and second in the total land on farms (USDA, 2009).

(Figure 1)

Its relatively close proximity to major metropolitan areas also makes Columbia County an attractive area for downstate tourists to visit and some to live in the area for part of the year with second homes. Columbia County median household income is about eight thousand dollars higher than NYS counties of similar population size (61,618 ± 10,000 residents) (U.S. Census Bureau, 2010). The area is also easily accessible to visitors and residents by a good road system and rail services to and from the metropolitan area downstate. These factors have opened up additional marketing opportunities for farmers and other businesses in the local food system.

Columbia County has shown particularly strong growth in direct to consumer (D2C) sales by farmers relative to the state and U.S. in aggregate (Note 1). Specifically, the number of farms selling at least a portion of their farm sales D2C increased by 29% from 2002 to 2007 (USDA, 2009). These numbers compare with 15% and 17% for NYS and the U.S., respectively. By 2007, nearly one-quarter of all farms in the county (23%) participated in D2C sales channels that contributed over 6% of all reported farms sales. In comparison, 15% and 6% of farms in NYS and the U.S., respectively, had D2C sales that made up less than 2% and 0.5% of total farm sales.

3. Previous Evidence

The development of defined purchasing specifications and delivery schedules, and a sufficient level of communication skills among all parties to facilitate and maintain information exchange are well-established

components of effective wholesale marketing. In addition, some wholesale channels (e.g., distributors or larger food service providers) may provide a timely outlet for producers with excess supplies of perishable products in a high-production season (Thilmany, 2004). Smaller product commitments through smaller restaurants may facilitate easier entry into local food markets for some farmers.

Studies focusing on a restaurant's decision to purchase local products generally find that product attributes related to taste or quality and the dependability of farmer suppliers were most important (Benepe, *et al*., 2001; Thilmany, 2004; Curtis and Cowee, 2009). Inconvenient ordering, ineffective communication skills, and higher product costs have also been cited as important impediments (Feenstra, *et al.*, 2003; Curtis & Cowee, 2009). Purchasing patterns of local food products can vary significantly by the type of restaurant; e.g., Curtis & Cowee (2009) found that smaller or independently owned restaurants should be targeted due to higher purchases of local products.

Seasonality in cropping seasons, weather-dependent harvest quantities and quality, and unexpected changes in buyer demands are often concerns expressed by farmers that can be problematic when trying to establish marketing agreements with restaurants (LeRoux, *et al*., 2010). Culinary businesses must oftentimes procure products from multiple sources to meet demands of their clientele. While this can represent competition for local producers, some ingredients are simply not available from local sources given local growing conditions or time of the year. However, this multiple-buyer dimension can provide opportunities for local producers to better differentiate their products through unique attributes, including freshness and quality (Thilmany, 2004).

4. Data Collection

Mail surveys were designed and distributed to agricultural producers and culinary businesses in Columbia County in summer 2009. Restaurant patrons were also surveyed at four participating restaurants, located in different towns within the county. Surveys were distributed in June and follow-up contacts were made throughout the summer to aid in survey response (Note 2). Surveys were distributed to 120 producers that utilized wholesale and/or retail marketing channels beyond traditional homogenous commodity-based channels. In total, 25 surveys were returned (21%), of which eight marketed farm products to restaurants.

Surveys were distributed to 92 restaurants, including full- and limited-service restaurants and specialty/catering food service establishments. In total, 10 surveys were returned (11%), of which nine bought directly from farmers (Note 3). Finally, patron surveys were conducted in four participating restaurants; each of the restaurants were located in different towns within the county; consumers were limited to completing one survey (Note 4). In total, 36 surveys were returned.

4.1 Farm summary

Farms returning surveys were generally smaller than the average farm size in the area, but more typical of farms concentrating on direct market sales. Farms employed a median of about two employees, N=22, and had 23 acres under cultivation, N=21, (Note 5). The types of products marketed across farms that did and did not sell to restaurants were very similar. This was expected given that many of the same products are marketed across multiple channels. Across all farms (N=25), 18 farms sold fruits and vegetables, 11 sold meat or egg products, and nine sold processed foods, e.g. preserves, breads, wine, cider. Only two farms marketed dairy products. Several farms marketed products in multiple categories.

The number of years farms had been selling to restaurants (N=8) ranged from one to 15 years, and included between one and six individual restaurant customers. The value of sales and number of weeks sold per year were equally varied; some sold to restaurants only a few times per year with limited sales, while others sold to restaurants for more than 40 weeks per year. With available data, we cannot disentangle the sources of this variation; e.g., specific products marketed (fresh versus processed/frozen products), technology adoption by farmers (high tunnels, greenhouses), or management/marketing skills and effort.

To better understand channel utilization strategies adopted by producers, respondents were asked to identify all market channels they participated in and their relative contributions to total farm sales. Of the 25 farms surveyed, farm stands and farmers markets were the most commonly utilized; i.e., used by 20 and 13 farms, respectively (Table1). In addition to the eight farms selling to restaurants, six farms sold to packers/distributors, grocery/specialty stores, or to other farmer vendors. With the exception of sales to other farmer vendors, retail channels, on average, contributed more to total farm sales than wholesale channels for this sample (Table 1). However, individual farm results varied greatly (e.g., percentages of total farm sales at farmers markets ranged from 5% to 85%; while packer/distributor sales ranged from 2% to 30%).

(Table 1)

The total number of channels utilized by farms was about three; farms selling to restaurants utilized between four and five total channels (on average), while those that did not utilized between one and two. Thus, for our sample, farms that sold to restaurants were also farms that tended to adopt more diversified channel strategies in general. The distribution of sales was weighted more heavily on direct channels compared to a similar study of farmers market vendors in Northern New York (Schmit & Gomez, 2011). However, the socio-economic and demographic statistics of the market areas were quite different.

The relatively strong reliance on direct marketing channels for this sample may also influence the low utilization of restaurant channels. While higher prices often attract farmers to direct marketing channels, marketing labor costs tend to be higher as well (LeRoux, *et al*., 2010; Hardesty & Leff, 2010). For a group of farms already involved in a fair amount of time-consuming direct marketing, expanding sales into the restaurant channel may be less feasible given time constraints, particularly if expected sales volumes are low. For the eight farms in our sample utilizing this channel, the average contribution to total farm sales was only 6% (the median was 2%) (Table 1).

4.2 Restaurant summary

Responding restaurants (*N*=10) represented a range of ages, sizes, and local farm product purchasing styles. Six of the culinary businesses were full-service restaurants, with the remainder either limited-service restaurants or specialty food service providers (e.g., caterers). The average age of the businesses was about 10 years (the median was 5), but ranged from first-year operations to those operating for more than 25 years. Businesses served around 300 customers per week, on average, and ranged from as few as 75 to more than 500.

Four of the eight restaurants buying from local farmers consistently purchased from them for as long as they have been in operation, the rest for about one-half of that time. Delivery frequencies ranged from once per month to six times per week. The number of farmers restaurants purchased from did not appear to be correlated with restaurant size, and ranged from as few as two to as many as twenty. How restaurants choose the farms and number they purchase from is beyond the scope of the data available; however, in general, will depend on a number of factors beyond restaurant size; e.g., types of meals and clientele, volume and quality of products required, availability and costs of local products, and philosophy in using local products.

Most restaurants purchased directly from farmers for a limited number of weeks per year (around 20), similar to annual fresh product marketing windows for local direct marketing channels in the area; however this ranged from as low as one to every week per year. Consistent with the farmer results, fresh fruits and vegetables purchases were most common; however, some restaurants purchased meat, dairy, and other processed food products (e.g., sauces, preserves, breads) that supported higher numbers of purchase weeks.

A number of procurement sources were used by the restaurants in this sample (Table 2). Importantly, all restaurants used at least one kind of wholesale distributor, including nine that used local distributors and six that used regional or national distributors. With the exception of direct farmer arrangements, wholesale distributor channels were associated with the largest shares of ingredient purchases. Purchases by restaurants using local distributors were, on average, 55% of all product purchases, regional or national distributors were about 25%.

Restaurants buying from farmers used a number of different channels to procure food products – six used direct farmer arrangements, four purchased from roadside stands, and one was a member of a CSA. However, these other sources of local products represented much lower shares of total product purchases (6% to 12%, on average) than direct purchase arrangements with farmers (27%, on average).

(Table 2)

4.3 Restaurant patron summary

Responding patrons (*N*=36) were roughly 40% male and 60% female, and represented a broad age range. The group was relatively well educated, with nearly one-half completing undergraduate or graduate degrees, and nearly three-quarters with annual household incomes above $75,000. To help understand consumer attitudes for this sample towards the use of local foods in restaurants, patrons were asked how strongly they agreed with a variety of statements. A selection of those statements and their average agreement scores are shown in Table 3 (Note 6). The average scores reflecting patron ‘wishes’ that restaurants utilize more local food products was 1.7, and, expectedly, was not statistically different than the average agreement score that customers ‘prefer’ to eat at restaurants that serve foods prepared with local ingredients (1.6). This makes sense as both statements reflect consumer preferences more generally, perhaps getting at normative or altruistic expectations.

(Table 3)

Average agreement scores dropped more precipitously when action-specific statements were proposed. Specifically, the average agreement score drops to 1.3 when customers were asked whether they were 'willing to pay more' for meals prepared with local products (Table 1), and to 1.1 when asked if they 'eat more frequently' at restaurants that serve foods prepared with local products. While the willingness-to-pay and eating frequency statement scores are not statistically different from one another, they are both below the scores when wishes and preferences were proposed.

These results provide evidence, at least modestly given sample size considerations, that while customers generally supported increased utilization of local foods at restaurants, they were more resistant to actually changing dining frequencies or willingness to pay for restaurant meals based on this factor alone. This may be due, in part, to the availability of local food products through other direct marketing channels in the area. Of the 36 restaurant patrons, 29 purchased local food products in grocery or specialty stores, 25 at farmers markets, 20 at roadside stands, 16 at u-pick operations, and 9 through CSAs.

5. Channel Barriers

No clear consensus emerged from farmers when asked whether existing barriers were preventing them from expanding (or starting) sales through the farm-to-chef market channel. The number that agreed (7) was only slightly above those that did not (6), and the rest were indifferent (8). However, the results appear consistent with responses from those currently selling to restaurants in that four expected sales though this channel to increase next year, two expected no change, and two expected sales to decrease.

Alternatively, restaurants appeared more concerned - seven agreed that barriers were preventing them from expanding farm purchases, and only two disagreed. Interestingly, only three of the nine restaurants reported that using local food products in their businesses actually increased overall restaurant sales; the rest said it had no effect. While consistent with the patron survey results discussed earlier, this small sales effect would seem to question if expanding purchases through this channel would improve overall returns. We lack sufficient information to address this question fully. Choosing the appropriate marketing mix for farmers includes consideration of many factors such as sales, profits, risk, and lifestyle preferences; the weights assigned to these factors are unique to the individual (LeRoux *et al*., 2010). The same can arguably said for the restaurants in our sample.

Farmers and restaurants were asked to identify what barriers currently exist to expanding (or initiating) farm-to-chef marketing/procurement for their operations. The responses are summarized in Table 4. For restaurants, time constraints and the inconvenience of dealing with multiple farmers was cited most frequently (6), followed by product quality and consistency concerns (5) and the availability of sufficient volumes of product (4). These results are similar to previous studies. However, in deference to previous literature, issues related to costs and communication problems appear less problematic for this sample. In addition, four of the nine restaurants said they were simply satisfied with their current distributors and were not actively seeking new suppliers.

(Table 4)

Similar to recent literature, farmers in our sample commonly cited barriers involving time constraints and inconvenience of delivering to (multiple) restaurants (10), as well as concern whether adequate prices could be received, particularly when delivery costs are considered (8). However, restaurant interest/availability in the area (4) and inflexible delivery requirements regarding volumes and quality were of lower concern overall relative to other areas. Unique attributes of the study area discussed above likely drives this result, particularly since the most highly cited barriers for our sample involved farmers that were satisfied with their existing market strategies (12) and/or were already selling all of their production to existing buyers.

6. Discussion

The area of Columbia County has seen strong growth in demand for local foods as reflected in D2C census data and the number of direct marketing channels employed by the surveyed farmers. Higher household incomes and tourist activity in the area likely contributes to farms in this sample weighing more heavily on direct marketing channels, particularly farmers markets and farm stands. Notably, these were also venues frequented by some of the surveyed restaurants as a place to procure local farm ingredients. While eight of our surveyed farmers utilized the wholesale restaurant channel, it tended to contribute a relatively low amount to total farm sales. Wholesale distributors were used by less farms in total, but tended to contribute more to farm sales. Notably, the largest use of wholesale channels, in terms of sales, was to other farmers who presumably used the products to expand their direct market offerings.

Sampled restaurants tended to utilize local food products as a relatively larger share of their total product purchases than would be implied by our farm survey results, presumably made up by the use of multiple farmer arrangements and/or integration with farm purchases available in other direct marketing outlets. That said, distributors (local, regional, or national) were still needed to augment local purchases in meeting the demands of their clientele. A sample of restaurant patrons at these types of restaurants also revealed some resistance to altering dining purchase locations or frequencies and willingness to paying higher prices for restaurants using local food products. In fact, most restaurants surveyed indicated that including local food products on their menus had no effect on sales whatsoever. This may be due to a wider presence and availability of local food purchasing opportunities for consumers in the area.

Common barriers cited by both agents involved a lack of time and inconvenience of dealing with multiple buyers/sellers and lower product volumes. Product availability, quality, and consistency surfaced as relatively strong barriers to restaurants expanding purchases, but these issues were much less problematic for farmers since they were experiencing strong sales through other outlets and satisfied with their existing channel strategy. Concern about changing processing procedures or the types of products offered for restaurants was not appealing to farmers when considering expansion to a channel where prices offered may not cover the additional costs, including delivery. Paying higher prices to farmers was less problematic for restaurants, relative to one-stop-shopping and availability provided by current distributors.

7. Conclusions

Relative to other areas of the state and country, Columbia County is unique in its strong presence of direct marketing opportunities for local food products. Purchasing patterns by restaurants with farmers varied greatly in our sample and stronger conclusions explaining these variations would benefit from a larger sample of culinary businesses in the area. Similar to other studies, however, common barriers such as larger time commitments, inconvenience, and consistency in product volumes and quality were revealed. The minimal effects on restaurant sales from using local ingredients and consumer willingness to pay also need further investigation for an area with presumably strong consumer attitudes towards utilization of local foods.

Restaurant satisfaction with current wholesale distributors may create opportunities for local farmers to work collaboratively with local distributors by including more food products from local farmers in their distribution. However, this will require time and attention to volume requirements and delivery schedules, as well as revising post-harvest processing practices to meet food safety regulations and procurement procedures with larger wholesale clients.

Given farmers are generally satisfied with existing marketing channels in the area, the restaurant channel would be more likely to enter into a farm's channel strategy as a replacement to an existing channel, rather than an addition to the channels already utilized. Expected lower volumes at restaurants and insufficient prices to cover the additional marketing costs will likely hinder those expectations, particularly in an area where restaurants have alternative sources from which to procure local food product ingredients.

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Notes

Note1. Direct-to-Consumer sales (D2C) represent the value of agricultural products sold directly to individuals for human consumption from roadside stands, farmers' markets, pick-your-own sites, etc. It excludes non-edible products such as nursery crops, flowers, and wool but includes livestock sales. Sales of agricultural products by vertically integrated operations through their own processing and marketing operations are also excluded (USDA, 2009).

Note 2. Copies of the restaurant, farmer, and patron surveys are available from the corresponding author upon request.

Note 3. The authors recognize the limitations of empirical analyses afforded by the small sample sizes; representativeness to the population of NYS should not be inferred and generalizations of the results beyond the sampled firms are necessarily restricted.

Note 4. Each restaurant was given a packet of surveys that they distributed based on their own preferences; e.g., some left them by the register or at the entrance to the restaurant and asked patrons to fill them out before they left; others had servers hand them to the patrons at their table at the end of the meal.

Note 5. We generally report median values since averages can be strongly influenced by outlier observations in small samples.

Note 6. Response categories included strongly disagree, disagree, neither agree nor disagree, agree, and strongly agree. Numeric values were assigned to each category as -2, -1, 0, 1, and 2, respectively, to compute average scores. Confidence intervals (CI) were computed assuming mean responses are normally distributed; i.e. 90% CI = average score ± 1.645*standard error.

Table 1. Utilization of Alternative Sales Market Channels by Farmers [a]

	Number	Percent of Total Farm Sales	
Channel	of Farms	Median	Mean
Retail Channels:			
Famers market	13	62	50
Farm stand	20	40	47
U-pick	4	20	34
CSA	3	40	54
Other	3	3	3
Wholesale Channels:			
Restaurant	8	2	6
Packer/Distributor	6	10	18
Grocery/Specialty	6	4	5
Other farm vendors	6	40	39

[a] Includes all responding farms, $N = 25$. Sales percentages are of utilizing farms only.

Description of the utilization of alterative market channels and their relative contribution to total farm sales.

Table 2. Utilization of Alternative Procurement Channels by Restaurants [a]

	Number	Percent of Total Purchases	
Channel	of Restaurants	Median	Mean
Local distributors	9	55	54
Regional/National distributors	6	25	36
Direct farmer arrangements	6	25	27
Roadside stands	4	10	12
Farmers markets	3	6	7
CSAs	1	10	10

[a] Includes all responding restaurants, $N = 10$. Purchase percentages are of utilizing restaurants only.

Description of the utilization of alternative procurement channels and their relative contribution to total restaurant ingredient purchases.

Table 3. Average Agreement Scores of Alternative Local Food Restaurant Statements [a]

Statement	Average Score	90% Confidence Interval
I wish restaurants would utilize more local food products in their menus	1.71	[1.57, 1.86]
I prefer to eat at restaurants that have food prepared with local food products.	1.57	[1.40, 1.74]
I am willing to pay more for meals prepared with local food products.	1.26	[1.05, 1.46]
I eat more frequently at restaurants that have foods prepared with local foods.	1.10	[0.86, 1.33]

[a] Includes all responding restaurant patrons at four participating restaurants, N = 36. Average agreement scores computed using five-category scale: strongly agree (2), agree (1), neither agree nor disagree (0), disagree (-1), and strongly disagree (-2). Confidence intervals assume responses are normally distributed.

Description of restaurant patron opinions towards local foods and impacts on their dining decisions.

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Table 4. Barriers Cited by Restaurants and Farmers Limiting Restaurant Channel Expansion and Utilization [a]

Barrier	Number of Responses
Restaurants ($N = 9$)	
Do not have time for several farmers, inconvenient	6
Unsure of product quality or consistency	5
Unsure of product volumes, insufficient volume	4
Satisfied with current distributors	4
Unsure of available farmers, poor communication	2
Prices are too high	2
Farms are too far away or do not offer delivery	2
Farmers ($N = 22$)	
Can sell all that I produce now	12
Satisfied with existing markets, would have to change processing or products offered	12
Do not have time to deliver to several restaurants, inconvenient	10
Unsure if prices will cover extra costs of delivery	8
Restaurants are not interested or are too far away	4
Cannot deliver year-round supplies with consistent quality and volume (weather)	3
Delayed payment for product deliveries	1

[a] Includes all responding restaurants and famres, the questions were not answered by one restaurant and three farmers.

Description of the barriers given by farmers and restaurants in expanding sales and purchases through the farm to chef restaurant channel.

Figure 1. Location of study area, Columbia County, NY, USA

Highlighting the focus area for the case study.

Oxalate Content of Stir Fried Silver Beet Leaves (*Beta Vulgaris* Var. Cicla) with and without Additions of Yoghurt

Evelyn Teo
Food Group, Department of Wine
Food and Molecular Biosciences, Agriculture and Life Sciences
Lincoln University, Canterbury, New Zealand
Tel: 64-3349-9263 E-mail: Evelyn.teo@lincolnuni.ac.nz

Geoffrey Savage (Corresponding author)
Food Group, Department of Wine
Food and Molecular Biosciences, Agriculture and Life Sciences
Lincoln University, Canterbury, New Zealand
Tel: 64-3349-9263 E-mail: Savage@lincoln.ac.nz

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Abstract

Total and soluble oxalic acids were extracted and analysed by HPLC chromatography following Asian cooking methods, which involved soaking, boiling and stir frying of silver beet (*Beta vulgaris* var. cicla) leaves. Autumn-grown silver beet leaves contained 1658 ± 114 mg/100 g dry matter (DM) of total oxalates, 954 ± 49 mg/100 g DM of soluble oxalates and 704 ± 98 mg/ 100 g DM insoluble oxalates. Soaking and boiling before stir frying reduced the soluble oxalate contents to a mean of 455 mg/100 g DM. Addition of standard or low fat yoghurt following the pre-treatments of soaking, boiling, stir frying and soaking, boiling and stir frying further reduced the soluble oxalate content to a mean of 190.8 ± 49.8 and 227.5. ± 47.0, respectively, for the standard and low fat yoghurt mixes.

Keywords: Silver beet, Swiss chard, Total, Soluble and insoluble oxalates, Calcium, Yoghurt

1. Introduction

A number of commonly eaten foods such as silver beet, spinach, rhubarb, nuts, multi-grain flours, chocolate, black tea and parsley contain high levels of total and soluble oxalate (Zarembski & Hodgkinson, 1962; Fasett, 1973; Brinkely, McGuire, Gregory, & Pak 1981; Noonan & Savage, 1999: Savage, Vanhanen, Mason, & Bush, 2000; Hönow & Hesse, 2002; Chai & Liebman, 2005; Siener, Hönow, Seidler, Voss, & Hesse, 2006). Brogen and Savage, (2003) showed that many green leafy vegetables commonly grown in the Indian sub-continent such as spinach, purple and green amaranth and colocasia, also contain very high levels of oxalates, while a range of other commonly eaten vegetables and spices such as coriander, curry leaves, dill and fenugreek contain moderate levels of oxalates. Savage et al. (2000) reported values for a range of other common European vegetables that were usually cooked before eating. In contrast, salad mixes, which included young leaves of green beet, spinach and red chard, also contained high levels of oxalates but were eaten raw (Jèhanno and Savage, 2009). It was fortunate that many mature leafy plant foods which contained high levels of oxalates require cooking to improve flavour, taste and palatability before serving. Oxalate levels in these vegetables were considerably reduced when these foods were boiled (Savage et al. 2000). Pre-treatment, such as soaking in cold water, can reduce the oxalate content of raw leaves. Soaking taro leaves for different time periods showed a 6% reduction in soluble oxalate content after 30 min and a 26% reduction following an 18 hour soak (Savage and Dubois, 2006). Soaking vegetables before cooking is a common practice in Asian style cooking. Pacific Islanders also soaked taro corms and leaves prior to cooking (Mårtensson and Savage, 2008).

Cooking treatments can significantly alter the oxalate concentration in the final product (Savage et al.2000; Savage and Dubois, 2006). Chai and Liebman (2005) clearly demonstrated that the soluble oxalate contents of a range of common vegetables could be effectively reduced by boiling and steaming. Soluble oxalate contents of silver beet and spinach leaves were reduced by 84-87% after boiling and 42-46% after steaming. Essentially, soluble oxalate leached into the boiling water (Savage et al., 2000) but oxalate can also be lost during steaming in the hot water dripping from the food. In contrast, baking and drying tended to concentrate the oxalate content in the plant tissues due to moisture loss (Noonan and Savage, 1999).

There was some variation in the parts of plants cooked and eaten by people in different countries. For example, silver beet (*Beta vulgaris* var. cicla) (also known as Swiss chard) leaves were more commonly eaten in the Mediterranean region while the stems are favoured by people in Northern Europe (Kiple and Kreimhild, 2000). New Zealanders preferred to boil the leaves while the stems were boiled and served in a white sauce that contained dairy products (Savage et al. 2004). Silver beet leaves contain higher soluble oxalates, 117 mg/100 g fresh weight (FW) than the stems (19 mg/100 g FW), even after considerable amount*s* of oxalates had been lost during boiling (Savage et al. 2000). The oxalate content of the leaves may depend on the growing conditions, type of soil, season and time of harvesting (Hodgkinson, 1977). Recently published values (Simpson et al., 2009) for total oxalate content of silver beet leaves ranged from 436 to 1614 mg total oxalate/100 g FW. Young leaves contained significantly lower levels of oxalates than mature leaves. Re-growth tissue contained higher levels of soluble oxalate than mature leaves and ranged from 58% of total oxalate for the mature leaves up to 89% for re-growth tissue (Simpson et al., 2009). Raw silver beet leaves contained 792.7 ± 22.9 mg oxalate/100 g FW and 350.0 ± 24.1 mg of soluble oxalate/100 g FW (Savage et al. 2004). Siener et al. (2006) reported the oxalate content in raw silver beet leaves grown in Europe to be 874 mg/100 g total oxalate FW and 327 mg/100 g FW for total and soluble oxalate, respectively.

The soluble oxalate content of foods can also be reduced by the addition of foods containing high levels of soluble calcium. Examples include adding sour cream to baked yams, adding milk to cooked taro leaves and serving cooked spinach with milk, cream or cottage cheese and consuming ice cream with rhubarb (Savage, 2002). Albihn and Savage (2001) showed that 100 g baked oca (containing 403.4 mg total oxalate) consumed with 100 g of sour cream effectively reduced the urinary oxalate to zero which strongly suggests that no soluble oxalate was absorbed from the baked oca. Oscarsson and Savage (2007) showed that baking taro leaves with milk reduced the soluble oxalate three-fold compared to the raw leaves (72.4 to 23.7%). More recent studies carried out by Simpson, Savage, Sherlock and Vanhanen*,* (2009) confirmed that addition of standard milk, low fat milk and cream to boiled silver beet leaves were each very effective at reducing the soluble oxalate content of the final mix. It was interesting to note that low fat milk (0.5% fat) had the same levels of calcium as the other two milk sources but was more effective at reducing the total soluble oxalate content. In contrast, there appeared to be little or no correlation between fat addition in a diet and oxalate absorption in the experiments carried out by Brogen and Savage (2003) or Mårtensson and Savage (2008).

This experiment sets out to investigate the effects of an Asian method of cooking silver beet which involves soaking, boiling and then stir-frying in a wok followed by the addition of standard and low fat yoghurt to identify the most effective way to reduce the soluble oxalate content of silver beet leaves.

2. Materials and Methods

2.1 Harvesting and cooking

Silver beet seeds (*Beta vulgaris* var. cicla), cultivar Fordhook giant, were sown in the early summer (November, 2009) in Wakanui silt loam in plots in the Horticulture Research Area at Lincoln University, Canterbury, New Zealand (43°38’52.88”S, 172°27’31.40”E), 19 m above sea level. The soil was fertilised with chicken manure prior to sowing the seeds and the plots were irrigated as required throughout the growing period. In late autumn, early May 2009, mature silver beet plants (height between 200-300 mm) were harvested and the mid-veins and petioles were removed. The leaves were shredded into small pieces then divided into one of four processes, soaking, boiling, stir frying, stir frying after soaking and boiling, each process was carried out in quadruplicate.

Shredded silver beet leaves were soaked in tap water at 12˚C for 30 minutes or boiled in tap water for 2 minutes, allowed to stand for 1 minute, then drained and subdivided into 115 g serving sizes (the amount that would fit into a 200 ml cup). Single servings of raw, soaked or boiled silver beet leaves were then stir-fried for 2 minutes in a wok (model: EW 30 Breville Health Smart Wok, Australia) at 200°C together with 28 ml of canola oil (Pam’s Salad and Cooking Oil, Pam’s Products Ltd., Mt. Roskill, Auckland, NZ) with constant stirring. The cooked silver beet leaves were allowed to cool in the wok for 1 minute and then transferred into individual

plastic containers and stored deep frozen at -20°C. Four separate samples were prepared for each pre-treatment method and standard and low fat yoghurt additions.

2.2 Preparation of yoghurt

Both standard yoghurt (EasiYo´s Real Base & Culture, Natural, 3.6% oil; EasiYo Products Ltd., Albany, North Shore, Auckland, NZ) and low fat yoghurt powders (EasiYo´s Real Base & Culture, Low Fat Greek, unsweetened, 1.3% oil; EasiYio Products Ltd., Albany, North Shore, Auckland, NZ) were prepared following the manufacturer's instructions.

2.3 Preparation of silver beet and yoghurt mixes

Pre-treated (soaked, boiled, stir fried and soaked, boiled and stir fried) silver beet leaves (115 g) were mixed with 115 g of either standard or low fat yoghurt and allowed to cool. All the cooked and processed silver beet leaves were stored frozen until freeze drying in a Cuddon freeze dryer (W.G. Cuddon Ltd., Blenheim, Marlborough, NZ) and then were subsequently ground to a fine powder in a coffee mill (Sunbeam, model: EM0400, China).

2.3 Proximate analysis

Dry matter of all samples was determined, in quadruplet (AOAC, 2002), by drying the processed silver beet leaves to constant weight in an oven at105°C for 24 hours. Total fat of each sample was determined by the Soxhlet method (AOAC, 2002) using petroleum ether (Shell X4) in a Foss Tecator Soxtec extraction unit HT6 (Foss Pacific Ltd., Hamilton, NZ).

2.4 Oxalate determination

The total and soluble oxalate contents of each fat-extracted sample were determined using the method outlined by Savage et al., (2000). Four separate 0.5 g samples of dried ground cooked silver beet leaves leaves were placed in a 100 ml flask, 40 ml Nanopure water added and incubated in a water bath at 80°C for 15 min with agitation to extract soluble oxalates. Total oxalates were extracted using 40 ml 0.2 M HCL at 80°C for 15 min. The extracts were allowed to cool and then transferred quantitatively into 100 ml volumetric flasks and made up to volume. The extracts were centrifuged at 2889 rcf for 15 min. The supernatant was filtered through a 0.45 mm cellulose nitrate filter. The chromatographic separation was carried out using a300 x 7.8 mm Rezex ROA ion exclusion organic acid column (Phenomenex, Torrance, CA, USA) attached to a cation H+ guard column (BioRad, Richmond, California, USA). The analytical column was held at 25°C. The equipment consisted of an auto sampler (Hitachi AS-2000, Hitachi Ltd., Kyoto, Japan), a ternary Spectra-Physics, SP 8800 HPLC pump (Spectra-Physics, San Jose, California,USA), a Waters, U6K injector (Waters Inc., Marlborough, Massachusetts, USA), a UV/VIS detector Spectra-Physics SP8450 (Spectra-Physics, San Jose, California, USA) set on 210 nm. Data capture and processing were carried out using a peak simple chromatography data system (SSI Scientific Systems Inc, State College, PA, USA). The mobile phase used was an aqueous solution of 25 mM sulphuric acid. Samples (20 ml) were injected onto the column and eluted at a flow rate of 0.6 ml/min. Insoluble oxalate content (calcium oxalate) was calculated by difference (Holloway, Argall, Jealous, Lee and Bradbury, 1989). The final oxalate values were converted to mg/100 g DM of the original test meal, taking into account the fat percentage of each sample.

2.5 Statistical analysis

The results are presented as mean values ± standard error. Statistical analysis of the oxalate data for the pre-treatments and cooking experiment was performed using one-way analysis of variance (Minitab version 15.1, Coventry, UK). Statistical analysis of the soluble oxalate data resulting from the pre-treatment and cooking with additions of yoghurt were performed using a general linear model using Minitab version 15.1 (Minitab Ltd., Brandon Court, Progress Way, Coventry, UK).

3. Results

Table 1 contains a summary of the dry matter, total fat and oxalate contents of the mixes with additions of either standard or low fat yoghurt. The four processing and cooking methods increased the dry matter content of the cooked silver beet leaves by a mean of 16%. Stir frying the raw silver beet leaves led to a 40% reduction in soluble oxalate content while soaking the leaves in cold tap water for 30 minutes followed by stir frying reduced the soluble oxalate content by 49%. When the leaves were boiled for 2 minutes and allowed to drain for a further minute a 56% reduction in soluble oxalates occurred; this was the most effective way to reduce the soluble oxalate content. Stir frying and stir frying soaked and boiled silver beet leaves showed the lowest reduction in soluble oxalates of 40 and 49%, respectively. The mean soluble oxalate content of the four treatment diets was 492 ± 30 mg/100 g DM which confirmed that these treatments were effective in reducing the soluble oxalate

content of the raw leaves when compared to the soluble oxalate content of the raw leaves. In all cases, the four treatments of the silver beet leaves led to a mean reduction in total oxalate of 42% and a mean reduction of 48% in soluble oxalate content.

Overall, the addition of 115 g of standard or low fat yoghurt to 115 g samples of each of the processed and stir fried silver beet leaves changed the overall composition of the yoghurt-based test mixes and reduced the overall total oxalate contents on a DM basis. After the yoghurt additions the mean soluble oxalate content of the stir fried mixes were 334 and 340 mg/100 g DM, respectively, significantly higher than the mean of the other three cooking treatments of 143 mg/100 g DM for standard yogurt and 190 mg/100 g DM for the low fat yogurt additions. Overall, the addition of both standard and low fat yoghurt reduced the mean soluble oxalate content for the four pre-treatments to 190.8 mg/100 g DM on the addition of standard yogurt and 227.5 mg/100 g DM on addition of low fat yoghurt.

4. Discussion

Silver beet leaves have little taste after steaming or boiling. Traditional Asian cooking involves rapid, high temperature frying in a wok with a small amount of oil, known as stir frying. Stir frying improves the flavour of many foods. However, Asian cooking normally involves pre-treatments such as soaking and/or boiling before stir frying. It was, therefore, interesting to determine whether Asian cooking methods could also effectively reduce the soluble oxalate content of the leaves. Stir frying alone, without prior soaking and boiling reduced the total oxalate content by 37% and reduced the soluble oxalate content by 40% when compared to the raw leaves (Table 1). All four methods showed an overall mean reduction of 42% of total oxalate and 49% of soluble oxalate when compared to the levels in the raw leaves. Boiling for 2 minutes was the most effective method, resulting in a 56% reduction in soluble oxalate content compared to the level in the raw leaves. In contrast, soaking, boiling and stir frying the leaves only led to a mean 49% reduction in soluble oxalate content.

The total, soluble and insoluble oxalate content of the silver beet leaves used in this study were much lower than those reported by Savage et al. (2004), Savage et al. (2000) and Simpson et al. 2009) for plants grown in a similar location. Simpson et al. (2009) noted that the maturity of the plants and the time of harvest had a considerable effect on the levels of oxalates in the leaves. Franceschi and Nakata (2005) have suggested that many environmental affects, which are difficult to control from year to year, may all affect plant growth and oxalate synthesis in plants. The addition of the two yoghurts to the leaves showed a reduction in the oxalate content of the mixes, by dilution, with the addition of dry matter from the yoghurt. This was not the only effect, as the data shows that the mean soluble oxalate content of the silver beet leaf and yoghurt mixes were significantly ($p < 0.01$) reduced when combined with the calcium in the yoghurt mix. The mean soluble oxalate content of the stir fried and pretreated silver beet leaves was 492.0 ± 29.9 mg/100 g DM, which fell to190.8 ± 49.8 mg/100 g DM for standard yoghurt addition and 227.5 ± 47.0 mg/100 g DM for low fat yoghurt addition, respectively a 61.3% and 53.8% reduction compared to the mean soluble oxalate content of the four pre-treatment values. Brogren and Savage (2003) showed that the addition of milk products were effective to reduce the absorption of oxalate contained in baked spinach in a short term a feeding experiment. The most effective treatment in their experiment was the addition of sour cream and Calci-Trim milk (Meadow Fresh, Goodman Fielder, Auckland, NZ). The effectiveness of the addition of standard and low fat yoghurt to stir fried silver beet leaves has been confirmed in a short term feeding experiment (Johansson and Savage, 2011). The mean absorption of oxalate from stir-fried silver beet leaves was 2.41%, which reduced to 1.10 and 0.89% when consumed with standard yoghurt and low fat yoghurt, respectively

In this experiment all samples of silver beet leaves were stir fried with 28 ml of canola oil resulting in a mean total oil content of 38.5 ± 1.0 g/100 g DM for the four pre-treatments. The mean oil contents of the silver beet leaves with standard yoghurt and low fat yoghurt were 28.3 ± 0.6 and 22.8 ± 1.7 g/100 g DM, respectively. This reduction of oil content in the mixes occurred because the standard and low fat yoghurt contained relatively low levels of oil, 3.6 and 1.3%, respectively. Some reports have stated that fat may increase the oxalate absorption from such foods as chocolate and peanuts which contain about 30% of fat (Finch, Kasidas, and Rose, 1981). It was suggested that dietary fat can bind to free calcium and so more soluble oxalate was available for absorption (Chadwick, Modha, and Dowling, 1973; Earnest, 1974). In this experiment the fat contents of the processed silver beet leaves were relatively high but reductions in soluble oxalate occurred following additions of standard and low fat yoghurt even after the dry mater dilution effect had been taken into account

Addition of standard or low fat yoghurt to the silver beet leaves provided enough calcium to potentially bind to all the soluble oxalate in the leaves. The resulting increased levels of insoluble oxalate (principally calcium oxalate) do not pose any health concerns because it would be not absorbed in the intestine and would eventually

be eliminated in the faeces. Advice to patients suffering from kidney stone disease who might eat silver beet as part of their diet should include the importance of cooking methods and the consumption of calcium rich sources along with high oxalate containing foods.

5. Conclusions

Pre-treatment of silver beet leaves by soaking and boiling effectively reduced the soluble oxalate in the stir fried leaves. Further reduction in soluble oxalate content can be enhanced through the mixing of either standard or low fat yoghurt into the cooked silver beet leaves.

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Table 1. Mean dry matter, total fat (g/100 g DM ± SE) and total, soluble oxalate and insoluble contents (mg/100 g DM ± SE) of processed silver beet leaves with and without addition of standard and low fat yoghurt

Treatments	Silver beet leaves					Silver beet leaves with added standard yoghurt					Silver beet leaves with added low fat yoghurt				
Cooking	Dry matter	Total fat	Total oxalate	Soluble oxalate	Insoluble oxalate	Dry matter	Total fat	Total oxalate	Soluble oxalate	Insoluble oxalate	Dry matter	Total fat	Total oxalate	Soluble oxalate	Insoluble oxalate
Raw silver beet	10.6 ± 0.4	0.6 ± 0.4	1658 ± 114	954 ± 49	704 ± 98	-	-	-	-	-	-	-	-	-	-
Stir fried	11.2 ± 0.5	36 ± 1	1040 ± 67	569 ± 23	471 ± 74	22.9 ± 1.37	28 ± 2	866 ± 37	334 ± 42	531 ± 79	20.6 ± 0.28	19 ± 2	785 ± 42	340 ± 17	445 ± 47
Soaked and stir fried	17.2 ± 0.8	41 ±1	1096 ± 34	487 ± 44	609 ± 59	22.5 ± 0.62	27 ± 1	578 ± 19	135 ± 16	443 ± 26	25.7 ± 0.39	22 ± 2	904 ± 39	263 ± 28	640 ± 40
Boiled and stir fried	18.6 ± 1.2	39 ± 2	915 ± 62	423 ± 29	492 ± 64	19.4 ± 0.32	28 ± 2	835 ± 62	179 ± 33	656 ± 73	23.3 ± 0.71	23 ± 2	478 ± 80	127 ± 14	351 ± 79
Soaked, boiled and stir fried	22.4 ± 0.7	38 ± 2	801 ± 34	489 ± 53	312 ± 87	21.5 ± 0.16	30 ± 1	704 ± 90	115 ± 13	615 ± 80	24.3 ± 0.50	27 ± 3	575 ± 44	180 ± 26	395 ± 63

		Significance		
Analysis of variance,	d.f	Total oxalate	Soluble oxalate	Insoluble oxalate
Cooking	3	**	**	NS
Treatments	2	**	**	NS
Cooking x treatments	6	**	NS	**
LSD (5%) within cooking		90.5	50.9	110.2
LSD (5%) within treatments		78.4	44.1	95.4
LSD (5%) between cooking		156.8	88.1	190.8

Redox Power Changes of Caramels and Sugar Reductones in Beer

Jan Savel (Corresponding author)
Budejovicky Budvar, N. C.
K. Svetle 4, C.Budejovice 37021, Czech Republic
Tel: 420-387-705-118 E-mail: jan.savel@budvar.cz

Petr Kosin
Budejovicky Budvar, N. C.
K. Svetle 4, C.Budejovice 37021, Czech Republic
Tel: 420-387-705-123 E-mail: petr.kosin@budvar.cz

Adam Broz
Budejovicky Budvar, N. C.
K. Svetle 4, C.Budejovice 37021, Czech Republic
Tel: 420-387-705-400 E-mail: adam.broz@budvar.cz

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Abstract

Aerated or nitrogenated solutions of various sugars (10 % w/w) in deionized water, brewing water or phosphate buffers with dichlorophenolindophenol (DCIP) addition (10 mg/l) were heated 45 min at 60 °C and absorbance at 520 nm (λ_{max} of DCIP at pH 4.6) and 610 nm (λ_{max} of DCIP at pH >6) was measured. Reduction power of the solutions increased with the absence of oxygen, increasing pH value and the kind of sugar (FRU>LAC,MAL,GLU>SUC). Aerated solutions of various sugars were also autoclaved (10 min at 121 °C) and the absorbance at 420 nm was measured. The color of caramels from sugars was dependent on the kind of sugar (FRU> LAC,MAL,GLU>SUC), and it increased with increasing pH value. The formation of caramel pigments from ascorbic acid or maltose by oxidation with potassium peroxodisulfate or hydrogen peroxide was also compared. The formation of colored pigments is considered to be the mark of irreversible aging of food.

Keywords: Caramel, Reductone, Spectroscopy, Food aging, Beer, Reduction power

1. Introduction

Beer production comprises both aerobic and anaerobic part. Growing barley and hops, malting and starting phase of fermentation need air, while fermentation and maturation take run under anaerobic condition. Air is considered as the main reason for beer instability after filtration and filling (Briggs et al., 2004).

Although oxygen measurement during malting and brewing is well established, the determination of reduction capacity meets many difficulties (Galic & Palic, 1994, Chapon & Krestchmer, 2001; Moll, 2001). Nearly all the tests are carried out in the presence of oxygen. Little care is devoted to anaerobic processes taking part in aging beer.

The changes of malt and beer color during theirs production can be explained by various enzymatic or nonenzymatic mechanisms, which were generally studied in food science and industry (Friedman, 1996). Yellow, orange and brown pigments formed by thermal degradation of sugars are usually named caramels, although other authors use the term only for group of compounds, which are produced by heating reducing sugars with ammonia and its salts. Various anions influenced the color intensity, e.g. phosphate anion could catalyze the caramel formation (Agyei-Aye & Chian, 2002).

Term melanoidins is often used for substances, which are created by reaction between sugars and α-aminoacids

(O´Brien & Nursten, 1998; Perez-Magarino & Rivero, 2000). Melanoidins are generally considered to be strong reactive substances with important technological and physiological properties (Hayashi & Namiki, 1986; Rufian-Henares & Morales, 2007).

The term reductone is usually used for all reducing compounds present in beer such as polyphenols, cysteine or sulfur dioxide. Sugar reductones comprise of colored or colorless substance with an endiol group. Ascorbic acid is an example of model sugar reductone, which is used for beer stabilization.

Sugar reductones undergo gradual degradation under both aerobic and anaerobic conditions. Although the process has not been completely described, the presence of simple sugar α-dicarbonyls was proved (Bravo & Herrera, 2008). The thermal sugar degradation products can take part in advanced glycation end products (AGE) formation (Xanthis & Hatzitolios, 2007). The AGE breakers and inhibitors play important role in fight against modern diseases (Reddy & Beayz, 2006).

Similar dicarbonyls can be also found in degradation products of ascorbic acid (Deutsch, 2000). Many of them gradually create colored oligomers and polymers. The final degradation products are usually distillable furfural (FF) or nonvolatile hydroxymethylfurfural (HMF) although they can be further oxidized (Lewkowski, 2001).

The majority of reports on nonenzymatic sugar browning of beverages have focused on fruit juice browning, where they are connected to natural reductone degradation. The role of oxygen in ascorbic acid degradation in fruit has been repeatedly reviewed (Garcia-Torres & Ponagandia, 2009). Ascorbic acid can induce browning in wine in the presence of (+)-catechin in a model wine solution (Bradshaw and Prenzler, 2001).

Dehydroascorbic acid (DHA) played the most important role in this process. The efficiency of L-cysteine and sodium sulfite as antioxidant was examined in the browning of DHA solution. The browning was suppressed at higher antioxidant concentration but it increased in the presence of low concentration of those agents (Sawamura & Nakagawa, 2000).

Both ascorbic acid and DHA provided the same species after oxidation with hydrogen peroxide. Some of resulted reaction products were more susceptible to hydrogen peroxide oxidation than ascorbic acid itself (Deutsch, 1998).

The other part of beverages browning concentrates on polyphenol oxidation. It is very difficult to distinguish between sugar or polyphenol ratio in beverage browning during its aging. Measurement of absorption spectra of beer in the VIS and UV region was used in advanced analysis of the beer color as well as in study of beer aging by differential spectrophotometry (Weeranantaphan & Downey, 2010). The addition of various organic dyes to beer extended the capability of this method (Savel & Kosin, 2009).

Aging beer is usually associated with irreversible oxidation process which can not be completely reversed but only slowed by reducing agents such as ascorbic acid, sulphite or dithionite. Neither yeast reductases can convert sugar or aminoacid oxidation products into initial compounds.

In the previous paper we studied caramel pigments as natural redox and alkali indicators (Savel & Kosin, 2010). The goal of this paper is to study reductones and caramel color changes in the beer aging and find new methods and possibilities for beer redox state determination.

2. Materials and Methods

2.1 Chemicals

All chemicals including components for the preparation of phosphate buffers (1/15 mol/l) were purchased from Sigma Aldrich. Sugars: D(-)-fructose (FRU), D(+)-sucrose (SUC) and D(+)-glucose (GLU) were anhydrous, (D+)-maltose (MAL) and D(+)-lactose (LAC) were monohydrates. Indicators were methylene blue (MB), thionine acetate (THIO), indigocarmine (INDC), N,N,N´,N´-tetramethylphenylenediamine (TMPD), dichlorophenolindophenol (DCIP), triphenyltetrazolium chloride (TTC) and methyl red sodium salt (METR). The original color of oxidized or reduced form of indicators in phosphate buffers is shown in Table 1.

2.2 Preparation of solutions and their spectroscopy

Conductivity of the deionized water (DI) was less than 0.2 μS/cm, soft brewing water (BW) contained Ca^{2+} and Mg^{2+} in total content 0.7 – 0.8 mmol/l, total Fe (0.1 mg/l), total Cu (below 0.05 mg/l). Sugar solutions contained sugars (10 % w/w), the final concentration of indicators was 10 mg/l.

Single beam spectrophotometer (Hach Lange DR 5000) equipped with cylindrical glass cells (cuvettes) and square glass or quartz cuvette, both with an optical path of 1 cm, which were filled with liquid samples (4 ml). Samples with high absorbance (> 3.5) were diluted appropriately. Data were obtained as an average of repeated

experiments.

2.3 Reductone formation from sugars

Solutions of various sugars (10 % w/w) in deionized or brewing water or phosphate buffers with DCIP addition (10 mg/l) were aerated or deaerated by air or nitrogen bubbling (10 min) in cylindrical cuvettes which were then tightly closed with rubber stoppers. The cuvettes were heated (45 min, 60 °C) and absorbance at 520 nm (λ_{max} of DCIP at pH 4.6) and 610 nm (λ_{max} of DCIP at pH >6) was measured after cooling to the room temperature. The absorbance value of DCIP solution (10 mg/l) was 0.2 AU at pH 4.6, 0.5 AU at pH 7 and 0.6 AU at pH 8.

2.4 Caramel formation from various sugars

Aerated solutions of various sugars (10 % w/w) in deionized water, brewing water or phosphate buffers in cylinder cuvettes were heated up to 121 °C (15 min), hold at 121 °C for 10 min, and cooled to 80 °C (60 min). Before opening the autoclave air was carefully introduced into the autoclave (Systec, Germany). The absorbance at 420 nm was measured after cooling to room temperature.

2.5 Caramel formation by oxidation of maltose and ascorbic acid

Maltose (10 % w/w) or ascorbic acid (10 % w/w) was dissolved in deionized water with hydrogen peroxide or potassium peroxodisulfate addition (0, 0.01, 0.1 and 1 %), the solutions were autoclaved (121 °C, 10 min) and the absorbance (420 nm) was measured after cooling.

2.6 Visualization of caramel aging

For the preparation of semisolid medium with or without redox indicator (10 mg/l) the agar (0.15 % w/w) was added to the sugar solutions before autoclaving. Hot medium (10 ml) was shortly mixed, pipetted into test tubes and transferred into refrigeration to get semisolid consistence.

Autoclaved semisolid medium containing sugar, buffer and redox indicator (alternatively) was stored for three days at room temperature and the changes of color at the top (depth 1 cm), middle part and the bottom (height 1 cm) of agar medium were recorded.

3. Results and Discussion

The reduction power of sugar solution increased during heating even at relatively low temperature. The lower the absorbance at both wavelengths after heating, the higher the reduction power was. Reduction power increased with the absence of oxygen, increasing pH value and the kind of sugar (FRU>LAC,MAL,GLU>SUC). The highest reduction power was obtained with fructose dissolved in deaerated buffer (pH 8), and the lowest with sucrose (pH 8) (Figure1,2,3).

Although various redox tests based on color changes of various inorganic or organic indicators have been broadly used in brewing, the product composition of these reactions was rarely mentioned. The redox reaction is supposed to be reversible e.g. ascorbic acid can be oxidized by DCIP to dehydroascorbic acid which can be again reversibly reduced (Deutsch 1998). On the other hand the dehydroascorbic acid undergoes also irreversible degradation which is not usually solved in the tests. Similarly the color indicators can be irreversibly destroyed during redox reaction.

The most of the tests mentioned in the literature are carried out in the presence of oxygen which usually influences the course of reaction, but only more sophisticated tests such as ESR can recognize the role of oxygen. Reactive oxygen species (ROS) may be formed which decreases the reduction power of tested compounds. Maillard and caramelization products take part in oxidative reactions in lager beer (Nøæddekaer and Andersen 2007).

It is difficult to distinguish the degree of sugar decomposition during redox test based only on the color change, which can be associated with oxidation of aldehydic group of sugar (Kunz and Lee 2011).

The color of caramels formed by heating in the autoclave was depended on the kind of sugar (FRU> LAC,MAL,GLU>SUC), and it increased with increasing pH value. Color was higher in the brewing water compared to the deionized one (Figure 4).

The autoclaved solutions were diluted (1:50) with deionized water and the absorbance at 280 nm was measured, which was considered as a degree of sugar degradation (λ_{max} of furfural and hydroxymethylfurfural). The absorbance depended on the kind of sugar (FRU>LAC,MAL,GLU>SUC) and it increased with increasing pH value (Figure 5).

Hydroxymethylfurfural (HMF) and furfural (FF) are typical products of sugar degradation (Lewkoski, 2001). They usually serve as typical markers of their degradation under acid or alkali condition (Pereira & Albuquerque,

2011). Sugar dicarbonyl compounds are Maillard reaction intermediates generated by the decomposition of 3-deoxy-2-hexosulose as a direct precursor of HMF (Bravo & Sánchez, 2002).

We suppose a reductone formation as the first step of sugar degradation, where the reduction power can paradoxically increase. In brewing technology the wort is boiled usually at 100 °C, but the temperature on the brewing kettle wall can reach 120 °C or more. During boiling the reduction power and concentration of FF or HMF increase. These compounds are reduced during fermentation but after bottling the reduction power decreases and HMF and FF concentration grow again, which is caused by sugar reductone degradation (Shimizu & Nakamura, 2001).

Brewing process is based on reductone formation and its oxidation, where the results depend on temperature and presence of oxygen. The color of caramels formed from maltose and ascorbic by oxidation with potassium peroxodisulfate during heating increased with increasing concentration of oxidation agent even at low pH value of ascorbic acid solution. The color of caramel pigments formed from ascorbic acid was higher than that of maltose, where oxidation with potassium peroxodisulfate was efficient, whereas hydrogen peroxide provided only slightly colored solution (Figure 6).

Ascorbic acid can be considered as a sugar reductone, of which degradation pathway is nowadays known in the substantial steps, but there is still much unclear. Many of the degradations products e.g. dehydroascorbic acid or diketogulonic acid show more efficient reducing effect than ascorbic acid itself, they can bind oxygen and therefore they are classified to antioxidants (Deutsch, 1998). Sugars and ascorbic acid degradation in the presence phenylalanine provided similar degradation products (Seck & Crouzet, 1981). The strong reductones are also formed during acid hydrolysis of sugars.

The strongest reducing effect is usually attributed to simple triose reductone, which was prepared by alkali hydrolysis of glucose (Abe & Horii, 1986). The oxidation of sugar reductone by oxygen in air is usually responsible for the yellow and brown pigments from sugars or ascorbic acid. Coincidence between sugars and ascorbic acid during its degradation was also found (Rojas & Gerschenson, 2001).

We observed the overoxidation of such pigments but the process needed high concentration of hydrogen peroxide. The cocktail of various degradation products including α–hydroxycarbonyl and α–dicarbonyl was found after oxidation of various sugars with potassium peroxodisulfate in acid or alkali environment (Novotny & Cejpek 2007, 2008).

The spontaneous hydrogen peroxide formation was estimated in naturally buffered drinking water with ascorbic acid in the presence of various metals with various buffering capacity (Jansson & Lindquist, 2005). Metals can also take part in hydrogen peroxide decomposition (Fenton reaction). Ascorbic acid/Cu(II) system serve as a source of oxygen free radicals (OFR) in many tests e.g. in studies searching for antioxidants and medicines (Rahbar & Figarolla, 2003). Hydrogen peroxide formation followed by its degradation was proved during beer aging (Chapon & Chapon, 1979). Organic radicals were formed during beer aging (Andersen & Skibsted, 1998).

The negative oxygen concentration gradient occurs from the top to the bottom in the semisolid agar layer (Table 2). The oxygen gradient in the semisolid medium enables the observation of the spontaneous oxidation of reductone. The sharp interface between methylene blue or thionine acetate and the rest of semisolid medium shows the zone of oxidizied dye caused by oxygen. The brown pigment formation in the top layer is probably caused by strong oxidation of yellow maltose caramels.

Thionine was newly used for the visualization of beer oxidation by air. This test is similar to methylene blue test, but the thionine is easily decolorized. The formation of ROS from oxygen is supposed to reoxidize reduced form of the dye.

Brown top layer in the case of INDC is caused by irreversible oxidative degradation of INDC together with reductone oxidation while the yellow color at the bottom of the test tube is associated with reversible reduction of INDC in the absence of oxygen. TMPD or DCIP were reduced to their colorless forms even in the presence of oxygen. On the other hand INDC undergoes air oxidation which is connected to its splitting into colorless isatin (Kettle & Clark, 2004, Savel & Kosin, 2009).

The red color of reduced TTC could be caused by strong reductive species such as superoxide radical formed from oxygen or by the strong reductone formation. Another example is irreversible degradation of methyl red, which can be achieved by heating it with fructose in phosphate buffer (pH 4.6) above 110 °C although methyl red solution without fructose remains red. The irreversible degradation of MR in the absence of air was observed before (Savel & Kosin, 2009). Colored pigments from sugars can be regarded as compounds with indicator properties (Savel & Kosin, 2010).

DCIP or TMPD can be overoxidized by strong oxidation agents e.g. potassium peroxodisulfate, while methyl red overoxidized or overreduced by strong oxidative or reductive agent was observed before (Savel & Kosin, 2009). Common feature of such irreversible/reversible processes can be expressed as single mechanism of aging (Figure 7).

We suppose that degradation of sugar forms chain of these simple reversible/irreversible reactions. In the case of ascorbic acid the final product furfural is formed mainly under anaerobic condition while under aerobic condition the oxidation continues up to 2-furoic acid and 3-hydroxy-2-pyrone (Yuan & Chen, 1998; Shi & Zhan, 2007).

In aerobic processes the oxidation agent is usually oxygen, which can provide oxygen radicals while sugar reductone ascorbic acid gives ascorbyl radical. We suppose that it might take part in MR degradation. The radical formation maintains the rapid course of reaction in several steps. The sugar oxidation by radical initiators and sugar radical formation has been also described (Luo & Qi, 2001).

In beer production the color of caramels can increase e.g. during wort boiling or decrease with subsequent oxidative degradation under aerobic condition (Savel & Kosin, 2009). Which of the both processes prevails depends probably on oxygen concentration.

The formation of colored pigments is considered to be the mark of irreversible aging of food and there is little evidence of reversibility of this reaction. In the case of sugar the main proof of irreversibility is the presence of final degradation products such as furfural and HMF which can be only partially reversible reduced to furfurylalcohol or hydroxymethylfurfurylalcohol but not to the original sugar. Reversible color changes of synthetic indicators is therefore good marker of reductone aging, providing that strong reductive agent such as yeast dehydrogenase or strong oxidative agent e.g. hydrogen peroxide are not present. On the other hand the irreversible changes of indicators show the presence of strong oxidative or reductive species.

The thermal degradation of dry sugar is connected to reductone formation (Savel a Kosin, 2009). The recent studies have confirmed coincidence between thermal fructose or sucrose degradation and HMF formation (Lee & Thomas, 2011).

4. Conclusion

Sugars dissolved in deionized or brewing water or phosphate buffers (pH 4.6-8) undergo spontaneous degradation. The sugar degradation is associated with reductone and caramel formation and solution browning.

Reduction power of the reductones depends on kind of sugar, pH value, temperature, heating time and the presence of oxygen.

Anaerobic or aerobic sugar degradation is connected with UV active (λ_{max} = 280 nm) compounds formation, probably furfural and hydroxymethylfurfural and their derivates.

Caramel pigments were also obtained by ascorbic acid or maltose oxidation with potassium peroxodisulfate or hydrogen peroxide.

Two steps oxidation mechanism of sugars or reductones degradation based on hydrogen peroxide formation followed by Fenton reaction is supposed.

Reversible/irreversible mechanism was designed to explain redox changes during aging.

Oxygen gradient in semisolid sugar medium containing redox indicators is useful tool to study sugar decomposition.

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Table 1. Color changes of redox indicators in phosphate buffers

	Reduced form			Oxidized form		
Indicator	pH 4.6	pH 7	pH 8	pH 4.6	pH 7	pH 8
MB	colorless	colorless	colorless	blue	blue	blue
THIO	colorless	colorless	colorless	blue	blue	blue
INDC	colorless	colorless	colorless	blue	blue	blue
TMPD	colorless	colorless	colorless	blue	blue	blue
DCIP	colorless	colorless	colorless	red	blue	blue
TTC	red	red	red	colorless	colorless	colorless
METR	red	yellow	yellow	red	yellow	yellow

Table 2. Color changes of redox indicators in the layer of autoclaved semisolid medium during storage in the air (three days at 25 ºC)

	Maltose, pH 7			Sucrose, pH 7		
Indicator	Top	Middle	Bottom	Top	Middle	Bottom
none	brown	yellow	yellow	colorless	colorless	colorless
MB	blue	yellow	yellow	blue	blue	blue
THIO	blue	yellow	yellow	blue	blue	blue
INDC	brown	blue	yellow	colorless	blue	blue
TMPD	brown	yellow	yellow	colorless	colorless	colorless
DCIP	brown	yellow	yellow	colorless	blue	colorless
TTC	brown	red strip	yellow	colorless	pink strip	colorless

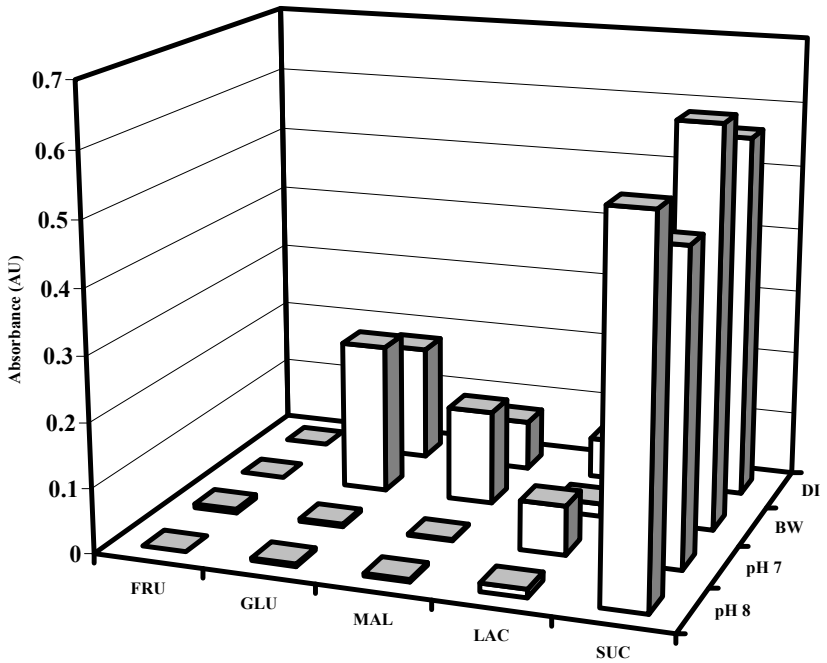

Figure 1. Absorbance (610 nm) of various sugars (10 % w/w) with DCIP (10 mg/l) dissolved in nitrogenated deionized (DI), brewing (BW) water and phosphate buffers (pH 7 and 8) after 45 min pasteurization at 60 °C, FRU – fructose, GLU – glucose, MAL – maltose, LAC – lactose, SUC – sucrose

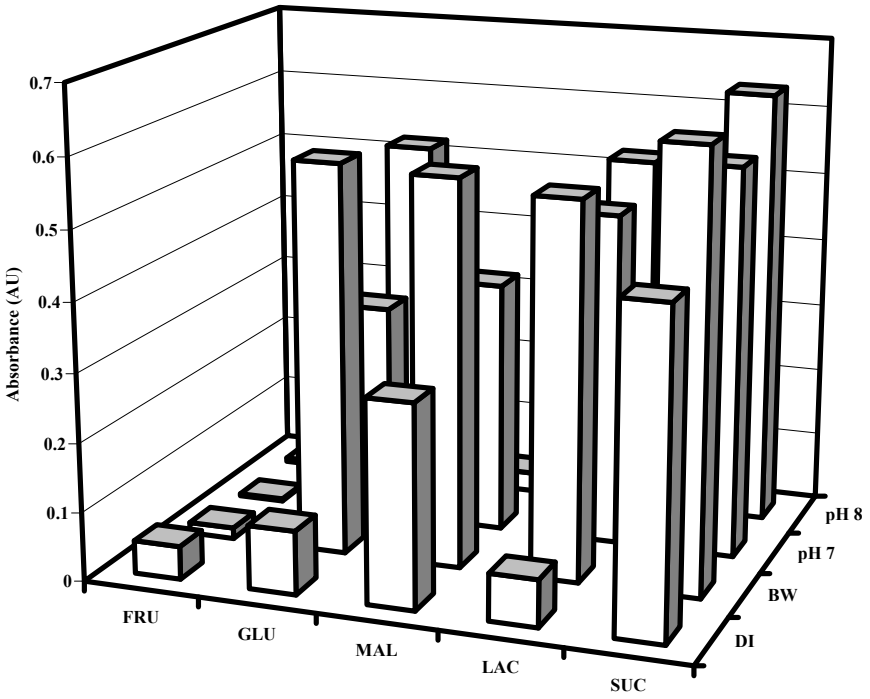

Figure 2. Absorbance (610 nm) of various sugars (10 % w/w) with DCIP (10 mg/l) dissolved in aerated deionized (DI), brewing (BW) water and phosphate buffers (pH 7 and 8) after 45 min pasteurization at 60 °C, FRU – fructose, GLU – glucose, MAL – maltose, LAC – lactose, SUC – sucrose

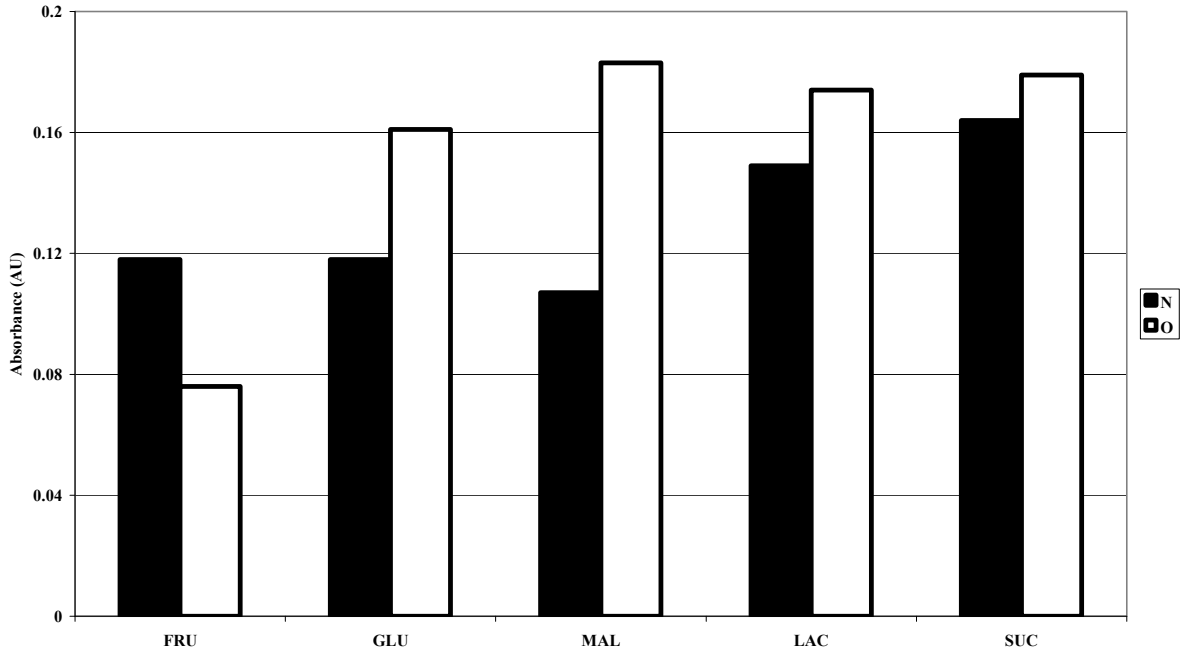

Figure 3. Absorbance (520 nm) of various sugars (10 % w/w) with DCIP (10 mg/l) dissolved in nitrogenated (N) or aerated (O) phosphate buffer (pH 4.6) after 45 min pasteurization at 60 °C, FRU – fructose, GLU – glucose, MAL – maltose, LAC – lactose, SUC – sucrose

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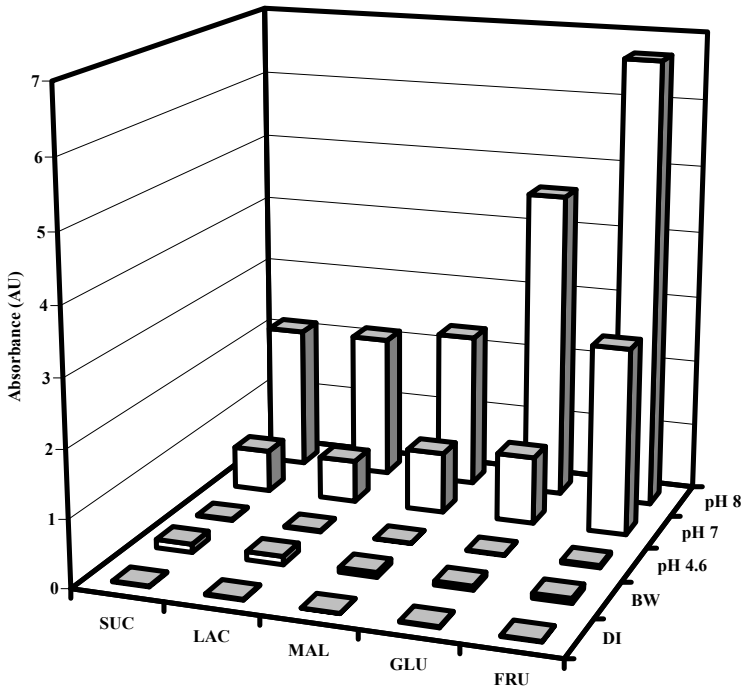

Figure 4. Absorbance (420 nm) of various sugars (10 % w/w) dissolved in aerated deionized (DI), brewing (BW) water and phosphate buffer (pH 4.6, 7 and 8) after autoclaving (10 min, 121 °C)

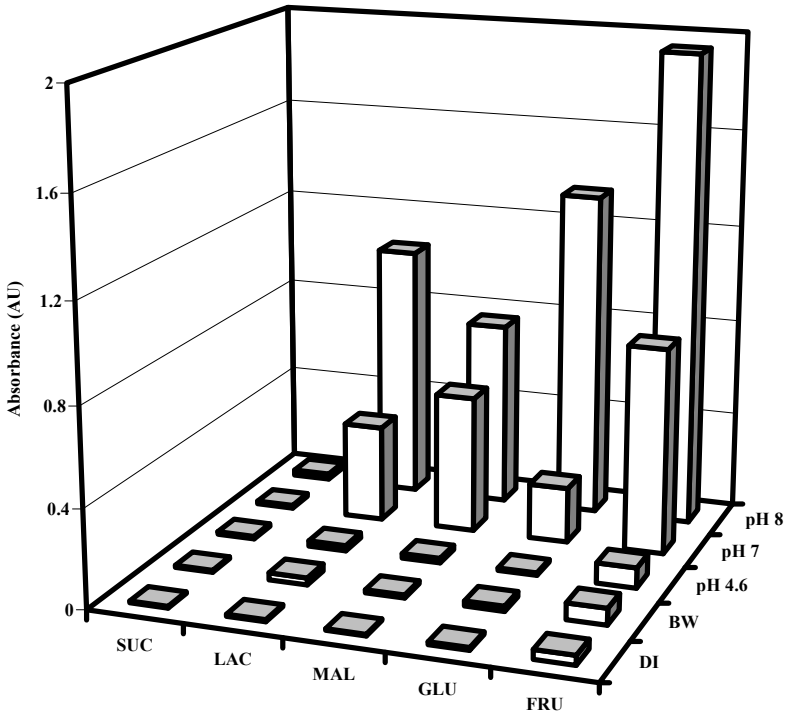

Figure 5. Absorbance (280 nm) of various sugars (10 % w/w) dissolved in aerated deionized (DI), brewing (BW) water and phosphate buffer (pH 4.6, 7 and 8) after autoclaving (10 min, 121 °C) and dilution (1:50)

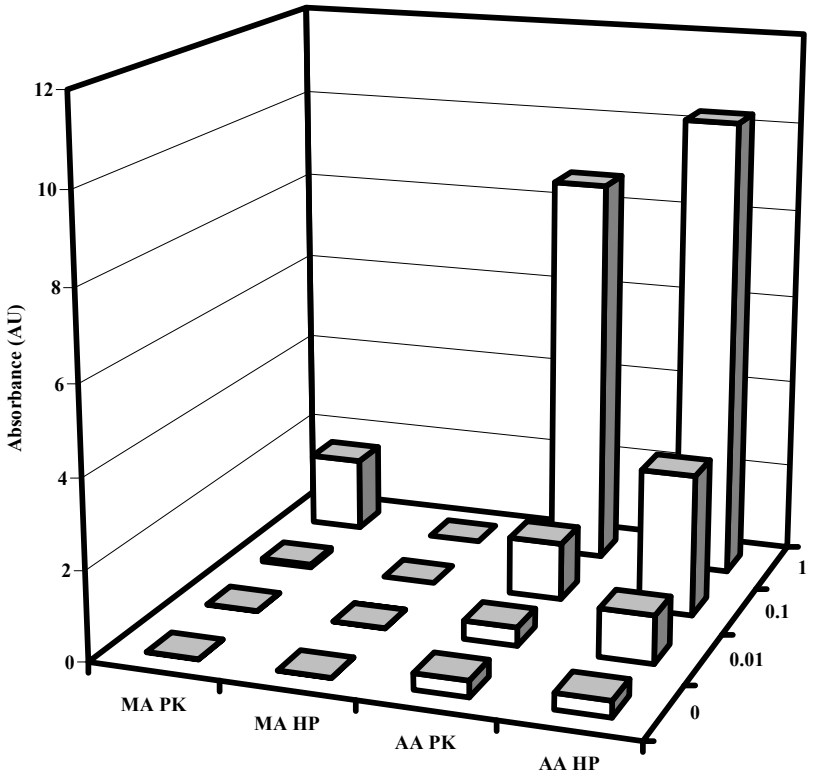

Figure 6. Absorbance (420 nm) of autoclaved solutions of maltose (MA, 10 %) or ascorbic acid (AA, 10 %) dissolved in aerated deionized water with potassium peroxodisulfate (PK, 0 – 1 %) or hydrogen peroxide (HP, 0 – 1 %)

SOURCE COMPOUND

↓

irreversible process

e.g. sugar degradation or indicator synthesis

↓

REDOX INDICATOR or

SUGAR REDUCTONE

↓

$A_{ox} + ne- = A_{red}$

irreversible product ← (reversible redox pair) → irreversible product

overoxidation, polymerization overreduction, polymerization

Figure 7. Mechanism of reversible/irreversible redox changes during sugar degradation

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Comparative Study of Antioxidant Activity between Basic and Convenience Foods

Irina Ioannou (Corresponding author)
ENSAIA-INPL-LIBio, 2 avenue de la forêt de Haye
B. P. 172, Vandoeuvre Les Nancy 54505, France
Tel: 33-383-595-814 E-mail: irina.ioannou@ensaia.inpl-nancy.fr

Aude Hyardin, Céline Charbonnel & Mohamed Ghoul
INPL-ENSAIA - Laboratoire d'Ingénierie des Biomolécules
2 avenue de la Forêt de Haye, B. P. 172, Vandoeuvre lès Nancy Cedex 54505, France

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Abstract

The behaviour of the antioxidant capacity (TEAC) and the total phenol content (TPC) of basic foods and convenience foods were analysed along the different steps of food preparation. The major loss of TEAC happens during the processing of convenience foods. A database has been constituted on the TEAC and the TPC of basic and convenience foods, commonly consumed by the French population. It showed that the addition of ingredients with high antioxidant activity to a complex preparation contributes to an increase in the total antioxidant capacity, but not in a proportional way. Differences between expected and measured values are 31 % for two ingredients and 14 % for three ingredients. For the TPC, the increase corresponds to the sum of the TPC of the ingredients. It also showed that microwave reheating and food storage, in most cases, did not lead to variations on TEAC and TPC of convenience foods.

Keywords: Additive effect, Database, Food functionality, Formulation, Reheating, Storage, Total Phenolic Content (TPC), Trolox Equivalent Antioxidant Capacity (TEAC)

1. Introduction

Over the last decade, there has been a great increase in consumer interest in the active role of foods beyond basic nutrition. Foods may provide health benefits identified as food functionalities (Diplock et al., 1999). Among emergent functionalities, antioxidant activity is the most used. Natural phenols, present in plant food could exert their beneficial health effects mainly through their antioxidant activity (Proteggente et al., 2002; Zhou and Yu, 2006) and it is well known that antioxidants may have the capacity to prevent health problems such as cancer, heart and neurological diseases. Indeed, antioxidants can prevent or delay the oxidation process triggered by free radicals and reactive oxygen species in biological systems.

Several basic foods such as fruits and vegetables are rich in anti oxidant molecules and are good for consumer health. However, consumers often have a food diet of cooked or heated products composed of a mixture of several ingredients. Data, available in the literature, on the antioxidant capacity and phenol content of foods, concern mainly basic foods without taking into account either the formulation or the process effects (Menezes et al., 2002). So it would be relevant to evaluate and to follow the evolution of the total phenol content (TPC) and the antioxidant capacity (TEAC) during life steps of convenience foods from its formulation to its consumption.

The measurement of antioxidant capacity has received much attention, but values obtained are often different due to a lack of standardisation of the antioxidant activity assays and to a variation of the composition of the raw materials used. So, to compare TEAC values, the development of a database on the antioxidant activity of some basic foods and also of some convenience foods is needed. This database would be an effective tool for estimating antioxidant consumption (Pellegrini et al., 2003). Thus, the first purpose of the present study was to provide data on the antioxidant capacity of basic and convenience foods commonly consumed by the French population.

The available data are mostly representative of foodstuffs consumed in their raw state. They cannot take into consideration the fact their activity may be changed by both environmental variables (Stahl et al., 2009, Volden et

al., 2008) and processing (Igual et al., 2010; Murakami et al., 2004). This aspect is of great importance considering that only small amounts of food are consumed in their raw state, whilst most of them need to be processed for safety, quality and economic reasons. Therefore, the second part of this study was designed to evaluate the effect of i) formulation, ii) culinary reheating, and iii) storage on the antioxidant capacity and the total phenol content of convenience foods.

To establish the effect of the formulation, the antioxidant capacity and the phenol content of basic foods and their mixture were evaluated. In fact during the step of formulation, many interactions can occur. However, these interactions have been very little studied. They can be either a negative synergism by means of a possible depletion of the antioxidant activity upon addition of certain species (Pinelo et al., 2004, Wang et al., 2000) or an additive effect (Philpott et al., 2004). The amplitude of these effects is variable depending on the physico-chemical environment (pH, ionic forces…) and matrix composition.

The effects of culinary reheating and storage steps were then studied. The evolution of antioxidant capacity and phenol content were realized for five convenience foods. The effect of these two operations is still a matter of debate. For some authors, culinary reheating or storage has little impact on antioxidant properties depending on the process and on the foods (Olsson et al., 2010; Price et al., 1997; Tudela et al., 2002). For others, these steps can have a drastic effect (Viña et al., 2007). The verification of these effects was carried out for microwave reheating and refrigeration.

2. Materials and Methods

2.1 Chemical reagents

The 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azimobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) , Folin-Ciocalteu's phenol reagent, sodium carbonate and gallic acid monohydrate (GA) (purity ≥ 98.0%), ethanol (purity ≥ 95.0%) and all other solvents and chemical were purchased from Sigma- Aldrich chemicals (France). High-purity water was produced in the laboratory using an Alpha-Q system (Millipore, MA).

2.2 Basic foods and convenience foods

2.1.1 Basic foods

Basic foods are defined as ingredients which can be eaten alone or cooked with other ingredients. The basic foods chosen here are the ingredients of the convenience foods analysed later. We found: vegetables (n=4; fresh tomato, yellow onion, green pepper, green olive) and other different foods (n=5; prepared lentils (D'Aucy), fresh parsley, olive oil, red wine and mustard). Foods were purchased from a local market. Prior to analysis, the samples were prepared as for conventional consumption: fresh vegetables were cleaned, trimmed of inedible portions; lentils were cooked in boiling water.

2.1.2 Convenience foods

Convenience foods are defined as a mix of basic foods requiring some processing, designed for ease of consumption. The samples used are sold in France under the brand Marie S.A. The convenience foods chosen are those most sold by the company. Dishes were supplied directly by the factories (5 days after manufacturing, in cooled containers). The details of the recipes can be seen in **Annex Materials.**

- Fresh dishes: prepared dishes (n= 12) are based on fish or meat with accompaniments (rice, potatoes, pasta or others) or based on vegetables. They are sold in individual portions, averaging 300g. They are already cooked and only need reheating in the microwave-oven.

- Fresh dishes: pies (n= 6) are all made of flaky pastry and a binder based on egg and cream. They essentially contain fish or vegetables. They are already cooked and require reheating.

- Deep-frozen dishes (n= 8) are based on pasta, rice or potatoes with various seasonings. They are sold in family-size bags, of 1kg on average. They are pre-cooked or cooked and only require reheating. Family bags were defrosted at ambient temperature.

2.3 Methods

2.3.1 Sample preparation

The foods were prepared according to a single procedure. 200 ± 0.1g of all the food samples were homogenized in a high speed blender (150 rpm) for 5 minutes, 20 ± 0.1 g of this preparation was mixed with 50ml ethanol and kept under agitation (250 rpm) at 25°C for 30 min for the extraction of antioxidant compounds (Moure et al., 2001). Then, the mix was centrifuged at 6000 g for 15 min, the supernatant was collected, and was filtered at 22µm.

Antioxidant activity was determined as soon as possible (within 30 minutes of preparation). 10 ml of the filtrate was evaporated (37°C, 14mbar) and the residue was solubilised in 50 % mono-propylene glycol and 50 % distilled water (v/v). The aliquot was stored for 24h at -18°C and then used for phenol determination.

2.3.2. Analytic methods

■ TEAC (Trolox Equivalent Antioxidant Capacity) assay: Radical cation ($ABTS^{\bullet+}$) scavenging activity

The free radical scavenging activity of foods was determined by ABTS radical cation decolorization assay (Re et al., 1999) with minor modifications. The method is based on the ability of antioxidant molecules to quench the blue green chromophore with a characteristic absorption of 734 nm, as compared with Trolox, a water-soluble vitamin E analogue. The addition of antioxidants to the preformed radical cation reduces it to ABTS, determining a decolorization. A stable stock solution of ($ABTS^{\bullet+}$) was produced by reacting a 7mmol/L aqueous solution of ABTS with 2.45 mmol/L potassium persulfate. The solution was stored in the dark at room temperature for 12-16h before use.

For the study of food samples, the $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0. 70 (± 0.02) at 734 nm then balanced at 30 °C. After the addition of 1.0 ml of diluted ($ABTS^{\bullet+}$) solution to 20µl of food extract or standard Trolox (final concentration 0-15 µM) in ethanol, the absorbance reading was taken at 30°C, exactly 1min after initial mixing (A_0) and again at 15 min (A_t). The inhibition percentage of absorbance at 734 nm was calculated between A_0 and A_t, according to the equation: percentage of inhibition = $(A_0 - A_t) / A_t$ with A_0 as initial optical density and A_t as final optical density. Appropriate solvent blanks were run in each assay. All determinations were carried out at least three times. The data presented are an average of three measurements. The intra and inter-assay coefficients were 2.1 % and 3.3 % respectively.

■ Total phenol contents (TPC)

The TPC of food extracts were determined using the Folin-Ciocalteu reagent assay (Singleton et al., 1998) with Gallic acid (GA) as a standard. The absorbance was measured at 660 nm. The TPC was expressed in GA equivalents (mg of Gallic acid equivalents per 100g of fresh sample) through the calibration curve of GA. All values expressed are a mean of the three replicates. The intra and inter-assay coefficients were 1% and 5.3 % respectively.

2.4 Effect of the combination of different foods on total antioxidant capacity and phenol content

Combinations of two or three different types of basic foods were used to evaluate the effect of mixtures on total antioxidant capacity and phenol content. The estimated antioxidant capacity and that measured in the mixtures were compared. Estimated antioxidant capacity and phenol content of the mixture represented the "mathematical" sum of antioxidant capacities and phenol contents of the different foods of the mixture. Measured antioxidant capacity and phenol content represented the total antioxidant activity and phenol content measured in the mixture.

Mix 1 is a mixture of pie and parsley. Mix 2 is a mixture of fish, sauce and vegetables.

2.5 Effect on storage and reheating

To evaluate whether storage can affect TEAC and TPC on a tart/pie, 2 parameters were measured at different consumption times. The convenience foods can be stored for 28 days after manufacturing, the storage conditions are without light and oxygen (heat packed in hermetic packaging), at a temperature of 4°C. We measured TEAC and TPC at days 9, 13, 15, 16 and 23 days after manufacturing. The 13^{th}, 15^{th}, and 16^{th} days cover times at which the food is the most likely to be consumed, the 9^{th} represents the shortest conservation and the 23^{rd} represents the sell-by date. The effect of preserving on the properties (antioxidant activity and phenol content) of the food matrix was analyzed on one tart.

To determine whether reheating at home can affect the TEAC and TPC, the 2 parameters were measured for five complex dishes before and after reheating. The dishes are stored in their packaging at 4°C during 9 days. They were prepared using the method the most used by consumers to reheat dishes: the microwaving (700W, 3 minutes).

2.6 Statistical analysis

Statistical analysis was performed using the freeware R 2.11.0. The differences between samples were analysed using the Student's *t-test* or analysis of variance for more than two samples. They were considered significant at $P < 0.05$. All experiments were repeated at least three times. Standard deviations are given in tables and represented as error bars on figures.

3. Results and Discussion

3.1 Database on the TEAC and the TPC of basic foods

In this study, the Total Phenol Content (TPC) and the Trolox Equivalent Antioxidant Capacity (TEAC) of 9 basic foods were studied, as shown in table 1. These results indicated that the mean values of phenols ranged from 41.37±3.80 for tomato to 368.89±8.69 mg gallic acid/100g for fresh parsley.

(Table 1)

For the total antioxidant activity of basic foods, determined by TEAC assays, the mean values ranged from 2.05±0.13 for tomato to 19.00±0.15 µmol trolox/g for fresh parsley. The comparison of these results to the literature data shows that there is variability between the measurements of TEAC and TPC. To illustrate this variability, examples of red wine and onion are taken. For red wines, some authors found total phenol content between 172.4 and 182.7±8 mg gallic acid/100g and an antioxidant activity between 42.9 and 50 ± 4µmol trolox/g (Paixao et al., 2007) whereas others found for total phenol content between 101 and 326± 80 and for antioxidant activity between 7.9 and 24.2± 0.8 (Seruga et al., 2011). Results depend on the type of wine and winemaking technologies. For onions, the mean antioxidant activity of all the onion samples analyzed was 0.579 ± 0.304 µmol/g, ranging between 0.349 ± 0.084 µmol/g for the cultivar San Juan de la Rambla and 0.696 ± 0.371 µmol/g for the Guayonje cultivar (Lorenzo León et al., 2009). The reasons to explain these differences are that vegetables or fruits are different according to the cultivar, the cultivation method and also to methods used for extraction and measurement of phenols or antioxidant molecules.

3.2 Effect of the combination of different foods on antioxidant capacity and phenol content

To verify interactions between antioxidant compounds and the food matrix, the antioxidant activity (TEAC) and phenol content (TPC) of various foods have been studied separately and in mixtures to mimic food formulation. Figure 1 shows the evolution of the TPC and the TEAC measured and estimated, for the 2 mixtures studied.

(Figure 1)

Details are given on the first mix which is composed of fresh parsley and fresh pie. Individual TEAC are 13.3 µmol trolox for 1 gram of fresh parsley and 21.5 µmol trolox for 25 grams of fresh pie. Thus estimated TEAC of the mix is 34.8 µmol trolox for 26 g of mix. The measured TEAC is 24.1 µmol trolox for 26 g, a significant difference of 31% is noticed.

Individual TPC are 3.7 mg gallic acid for 1 gram of fresh parsley and 5.4 mg gallic acid for 25 g fresh pie, thus estimated TPC of the mix is 9.3 mg gallic acid for 26 g of mix. The measured TPC is 8.8 mg gallic acid for 26 g of mix, the difference is not significant.

For the two mixtures, there is a significant difference between the TEAC measured and estimated ($P<0.05$), but not between the TPC measured and estimated ($P>0.05$). The measured TEAC of mixtures is inferior to the sum of antioxidant capacities of individual ingredients, while the measured TPC are identical. The results can be explained by possible synergic effects. The antioxidant activity of a complex dish cannot be predicted from the antioxidant activity of the ingredients. The masking of the total antioxidant activity is defined as a percentage difference between the measured increase in antioxidant capacity due to the addition of an antioxidant compared to the calculated increase based on an additive effect (Arts et al., 2001).

The total antioxidant activity measured was lower (31% in the combination of two ingredients (mix 1) and 14 % in the combination of three ingredients (mix 2)), than the accumulated antioxidant activities of individual ingredients at the known concentration. Conversely, the total phenol content of mixtures was almost equal to the sum of phenol content of separate ingredients.

The antioxidant activity of the polyphenols being due to their capacity to give up hydrogen, the availability of the hydroxyl groups depends on their chemical and spatial structures. The presence of the food matrix can modify the accessibility of the active group and thus the ability to react of molecules (Pinelo et al., 2004). It seems that the total antioxidant activity cannot be predicted only from the raw materials and that it is necessary to determine this experimentally, while the phenolic content concentration of each ingredient could be estimated in complex systems.

Our results, in complex food matrix, contradict the results found for individual phenolic mixtures. Authors (Heo et al., 2007) found that the total antioxidant activity in the phenolic mixture was equal to the sum of the antioxidant activity of its individual phenolics. However, our results confirm those of Pinelo et al. (2004) who showed that the addition of a new polyphenol to a complex phenolic system does not always promote a positive effect on its overall

antioxidant activity. Events, which mainly take place when different food matrixes are mixed together, have almost unpredictable consequences on the overall antioxidant properties and food stability (Nicoli et al., 1999).

In conclusion, the only way to know the implications of the addition of a new ingredient with high antioxidant activity is a specific experimental study of the mixture considered. In addition, from a nutritional point of view, the understanding of the consequence of food processing on food composition is one of the most important steps towards a reliable interpretation and evaluation of study results regarding dietary habits and human health.

3.3 Database on the TEAC and the TPC of convenience foods

Data of antioxidant capacity and the phenol content of 26 convenience foods were determined and are summarized in Table 2.

(Table 2)

The analysis of these data shows variations in the content of antioxidants and phenols in different types of dishes, TEAC ranged from 0.77 ±0.02 for "Rigatoni with tomatoes and beef " to 2.34±0.11 µmol trolox per gram of sample for "Greek-style cooked fish with rice" and TPC ranged from 32.27± 2.80 for "Cod with lemon sauce and rice" to 99.84± 6.65 mg gallic acid per 100 grams of sample for "Provence- style cooked Aubergines with corn semolina". No differences are noticed between fresh dishes and frozen dishes, the frozen step would not lead to a loss of antioxidant activity, for example TEAC for Andalusia-style cooked fish with rice is 1.38 and for frozen paella 1.88.

The dishes containing the highest antioxidant activity and phenol content are Greek-style cooked fish, Provence-style cooked aubergines and Indian-style cooked courgettes. These results can be explained by the fact that such dishes contain a majority of vegetables in their recipe, as shown in **Annex Materials**. Greek-style cooked fish has the highest antioxidant activity and phenol content. This is not surprising as this dish is rich in vegetables (aubergines, potatoes, tomatoes, onions, artichokes) and contains spices (coriander, turmeric and paprika). Vegetables, spices and herbs are well-known for their high antioxidant activity.

We also observe that chicken in mustard sauce has a higher antioxidant activity than dried tomatoes in oil with basil and rice, although this dish contains fewer vegetables. Chicken in mustard sauce contains only 3% mustard and 8% carrot, whereas the dish based on dried tomatoes contains 53 % of tomatoes, broccoli, carrots, chickpeas, and sweet pepper. Mustard brings a concentration of antioxidant compounds which has a higher activity than vegetables. This result shows that we did not merely consider vegetables in our approach to antioxidant content, and that condiments can also have a high antioxidant activity. Where deep-frozen dishes are concerned, those based on potatoes have a higher antioxidant capacity than dishes based on pasta and rice with the exception of paella. This result corresponds to the high antioxidant activity and phenol content of potatoes compared with pasta and rice (Halvorsen et al., 2002). Tarts vary only slightly, the garnish apparently having only a slight impact on TEAC and TPC. However, the three tarts which have the highest antioxidant activity are composed of vegetables: tomatoes and spinach.

We observed much smaller variations between the TEAC of convenience foods (from 0.77±0.01 to 2.34±0.11µmol trolox equivalents) than in that of more basic foods (from 2.05±0.02 to 19.00±0.15 µmol trolox), even though there was a great variation in the composition of the recipes studied. Moreover values of TEAC are higher for basic foods than for convenience foods. This can be explained by different interaction phenomena in the food mixture, which leads to a decrease of the antioxidant activity of the product (Wang et al., 2000). Thus, it is difficult to predict the antioxidant activity of food mixtures when only the antioxidant activity of their ingredients is considered.

Our findings suggest that whole diets ought to be considered when addressing the role of dietary antioxidants in health. Studies of only individual ingredients may underestimate or overestimate the TEAC consumed (Saura-Calixto and Goni, 2006). There is growing scientific evidence that dietary antioxidants may be a critical mediator of the beneficial effects of the Mediterranean Diet (Trichopoulou and Lagiou, 1997). Saura-Calixto and Goni (2006) determined the total dietary antioxidant capacity of a Spanish Mediterranean diet using the same methods. The results obtained were 3549 µmol trolox with the ABTS method, and 1171 mg gallic acid/person/day with the Folin-Ciocalteu method. In this diet, the consumption of fruit, vegetables, and cereals represents only 782 µmol trolox equivalents and 502 mg of gallic acid. For instance, Greek-style fish cooked can provide 702 µmol trolox equivalents per serving and 263 mg gallic acid which represents a major part of estimated TEAC. Individual foods known to have a high antioxidant capacity per gram (for instance parsley) may contribute very little to the antioxidant activity of a whole diet.

Two factors should not be neglected: (i) Much is known about the properties of each antioxidant, but there is only limited data on intakes of antioxidant mixtures. Interactions between ingredients have an impact on their antioxidant properties. (ii)The influence of food processing on naturally occurring antioxidants is a key factor in finding those technological conditions necessary to preserve or improve their original activity

3.4 Effect of culinary reheating and storage

3.4.1 Effect of reheating

The effect of reheating by microwave was studied on five complex dishes by measuring the TPC and the TEAC. Results are shown in Figures 2 and 3. There is no significant difference between values before and after reheating for Mixes 2, 3, 4 and 5 ($P>0.05$), but there is a significant difference for Mix 1 ($P<0.05$).

(Figure 2)

As can be seen on figure 2, TEAC of mixture 1 (Provence-style-cooked aubergines with corn semolina) decreased, which can be explained by a loss of natural antioxidants (carotenoids, ascorbic acid, polyphenols, etc) or may be due to the formation of novel compounds having pro-antioxidant activity (i.e. Maillard reaction products). The results observed for the other 4 dishes can be explained by a balancing of the simultaneous formation of compounds with novel or improved antioxidant properties (for instance Maillard reaction products). The great variety and complexity of compounds present in food matrixes (other antioxidants and polyphenols, oxidative enzymes, metals, etc) make it impossible to carry out an exhaustive study of their reactivity and their consequences (Pinelo et al., 2004).

(Figure 3)

On figure 3, we notice an increase of phenol content, which can be explained by a concentration of material due to thermal heating. In light of these considerations, the changes in the overall antioxidant properties of food can be attributed to the sum of the different and sometimes opposing events previously mentioned. Authors (Lo Scalzo et al., 2004) obtained the same conclusions with orange juices-thermal treatment led to an increase of phenol compounds due to the polymerisation of phenols and the liberation of bound phenols, and a decrease of antioxidant activity due to a high loss in ascorbic acid.

3.4.2 Effect of storage

The effect of storage on a savoury pie was studied by measuring the TPC and the TEAC at different consumption times. We observed no effect on these two parameters during storage ($P>0.05$). Similar observations were previously made on some fruits and vegetables stored at room temperature or in the refrigerator. The result of the study by Kevers et al. (2007) indicated that in most fruits and vegetables, storage did not negatively affect the antioxidant capacity. In contrast, Nicoli et al. (2000) reported an increase of more than 50% of the initial antiradical activity of a catechin solution during the first 2 days of storage at 25°C. In only a few cases the antioxidant capacity decreased during storage. In apricots for instance, a decrease in the antioxidant activity during storage was observed at low temperatures (Bartolini et al., 2006). Storage times can promote or enhance the progressive enzymatic or chemical oxidation of phenolic compounds. These reactions proceed at different rates depending on some intrinsic food variables as well as on processing conditions (a_w, pH, time, temperature, oxygen availability, etc).

The antioxidant potential of a polyphenol–containing food can vary greatly depending on its history or, at least, on when it is consumed (Nicoli et al., 1999).

4. Conclusion

Functional foods, beneficial for consumers, must keep a high antioxidant activity despite the phenomena undergone before consumption. Among the phenomena studied (formulation, reheating, storage), the step of formulation leads to a great loss of antioxidant activity. A database on antioxidant activity of basic and convenience foods, often consumed by the French population, has been constituted. We noticed that convenience foods having the higher TEAC are not automatically the dishes based mainly on vegetables, as condiments also have an important contribution. Moreover, TEAC of convenience foods (0.77±0.01 to 2.34±0.11µmol trolox equivalents) are lower than TEAC of basic foods (from 2.05±0.02 to 19.00±0.15 µmol trolox). Nevertheless, a comparison with the data in the literature was problematic due to the lack of standardization of the products and assays used.

The findings of this study suggest that we can predict the TPC of a food mixture from the TPC of their ingredients. The mechanisms are more complex with the antioxidant activity due to many interactions between antioxidant

compounds and/or with the food matrix. Values measured of antioxidant capacity were slightly lower than expected values- between 14 and 31 % depending on the number of mixture ingredients.

Methods of reheating and storage have no effect on the TPC and TEAC of foods studied. In the literature, many contradictory results can be found concerning the evolution of the antioxidant activity during food processes. This disagreement is essentially due to the behaviour of antioxidant compounds which is different according to the situation: process conditions, food matrix, and presence of other antioxidant compounds. Indeed, the study of a single antioxidant may be of limited value because the possible interaction in a food mixture is not taken into account.

Determining the total antioxidant capacity of convenience foods is particularly representative of contemporary dietary studies. However, the determination of each antioxidant compound is not sufficient when determining the beneficial potential of antioxidants. Thus, to develop knowledge about the prediction of antioxidant properties, further studies will have to deal with the understanding of interactions between antioxidant compounds and other ingredients, in order to develop a prediction tool of the antioxidant activity of complex foods.

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Annex Materials. Description of 26convenience foods

Type	Name of the recipe	Recipe *
Fresh dishes	Greek-style cooked fish with rice	Eggplants 23.5%. potatoes 20%. white fish 19%.tomatoes 8%. water. onions. ewe's milk cheese 4%. artichokes. tomato puree. olives 1.5%. garlic. raisins. coriander. modified starch. olive oil. salt. sugar. sweet pepper and dried tomatoes. spices (turmeric and paprika) and flavouring.
	Provence- style cooked Eggplants with corn semolina	Tomatoes 27%. corn semolina 14.5%. water. aubergines French fries (of sunflower oil) 7%. aubergines 7%.sweet pepper 6.5%. onion. courgettes 4%. carrots 3%. raisins. tomato puree. olive oil. garlic paste. salt. basil. sugar. modified starch. flavouring. thyme. pepper.
	Indian-style cooked zucchinis with rice	Courgettes 34%. cooked rice 19.5%. water. fresh cream. carrots. chilli pepper. tomato puree. peas. boiled wild rice 1.3%. twists of lemon 1%. curry. flavouring. chive. olive oil. coriander. salt. garlic. cumin. modified starch.
	Chicken in mustard sauce with rice	White rice 30%.chicken 25.5%. water. carrots 8%. fresh cream. shallots. Dijon mustard 3%. peas 3%. Sunflower oil. white wine. wild rice 1%. vinegar. salt. wholegrain mustard 0.3%. natural aromas. modified starch.
	Dried tomatoes with basil and rice	White rice 27%. tomatoes 25%. broccoli 7.5%. carrots 6.5%. dried tomatoes 6.5%. water. chickpeas 4.5%. sweet pepper 3%. tomato puree. black olives. sugar. parmesan. olive oil. garlic. basil. salt. tarragon. flavouring. modified starch. dextrose. pepper.
	Andalusia-style cooked fish with rice	White rice 34%. courgettes 23.5%. white fish 19%. sweet pepper 8.5%. green peas. fried onion (sunflower oil) olive oil. tomato puree. natural flavouring. garlic. salt. spices (turmeric. saffron). flavouring. modified starch. chilli pepper and dried tomatoes. wheat. pepper.
	Minced beef and mashed potatoes gratin	Mashed potatoes 65%. water. cooked beef 8.5%. onions 2.5%. carrots. chicken stock. modified starch. chives. garlic. salt. thickener: Xanthane gum. natural pepper extract.
	Italian-style cooked fish	Pasta 48%. white fish 19%. tomatoes 12%. water. courgettes. shallots. tomatoes 1.5%. carrots. olive oil. Parmesan 1%. garlic. tomato puree. black olives. modified starch. salt. basil 0.3%. dehydrated sweet pepper. spices and herbs/flavourings. palm oil. vinegar. sugar. pepper.
	Lasagna bolognaise	Water. lasagna 20%. cooked beef 11%. tomato puree. onions 3.5%. carrots. low fat fresh cream. modified starch. sunflower oil. basil 0.8%. corn flour. celery. flavouring. sugar. lactose and milk proteins - cream. salt. garlic. natural extracts: nutmeg - pepper - clove - thyme.
	Gratin dauphinois	Water. potatoes 44%. fresh cream. ham 7.5%. cheese l 3%. modified starch. salt. natural extracts of pepper and nutmeg.
	Quenelles of pike with rice	Pike quenelles 26.5% . white rice 21%. water. tomatoes 12.5%. carrot puree 7% . courgettes 4.5%. carrots 4.5%. shallots. sunflower oil. tomato puree. olive oil. flavours (milk. celery). modified starch. Sherry vinegar 0.3%. garlic. sugar. chicken stock. flavourings. pepper.
	Cod with lemon sauce and rice	Cod 31%. cooked basmati rice 29.5%. carrots 12%. water. fresh cream. sunflower oil. broccoli 2%. shallots. lemon juice and peel 1.3%. salt. modified starch. sugar. natural flavours (shellfish. celery). turmeric.
Deep-Frozen dishes	Savoy-style cooked potatoes	Potatoes fried 44% (potatoes. vegetable oil). water. onions. cheese 8%. white cheese. bacon 5%. mozzarella. low fat fresh cream. modified starch. flavouring. salt. thickener: Xanthane gum. natural pepper extract.
	Paella	Saffron rice 46.5% (rice. turmeric). water. cooked chicken 9.5% . shrimps 6%. fish 6%. tomatoes. peas. onions. pepper. chorizo 1.5%. tomato puree. flavouring. salt. paprika. shrimp extract.

	Tuna and potato gratin	Potatoes fried 38% (potatoes. palm and sunflower oil). tomatoes 17%. tuna 10% (sunflower oil). sweet pepper 7.5%. edam. tomato puree 6%. water. onions. olive oil. basil. modified starch. garlic. sugar. gelling agent: pectin. salt. dextrose. natural extracts of pepper and thyme.
	Savoy-style baked gratin	Potatoes fried 39.5% (potatoes. palm and sunflower oil). water. fresh cream 8%. fried onions 8%. ham 5.5%. emmental cheese 4%. reblochon cheese 2.5%. butter. modified starch. natural flavouring. lactose and milk proteins – cream. powdered egg white and yolks. salt. pepper. nutmeg.
	Basque chicken	White rice 42.5%. chicken 13%. water. tomatoes 9%.sweet pepper 8.5%. onions. tomato puree. Sherry vinegar. sunflower oil. garlic. modified starch. salt. basil. natural Cayenne pepper extracts.
	Conchiglie with tuna	Pasta (conchiglie) 46 % . tomato puree. tuna 11.5 %. sunflower oil. onions. tomatoes. olive oil. garlic. sugar. basil. salt. sherry vinegar. black olives 0.5 %. modified starch. oregano. extracted essence of pepper.
	Farfallini with gorgonzola	Pasta (farfallini) 50 % . water. fresh light cream 13 %. mushrooms. ham 9 % . Gorgonzola cheese 1.5 %. modified starch. basil. salt. yeast extract. colza oil - milk protein - cream. natural pepper and nutmeg extract.
	Rigatoni with tomatoes and beef	Pasta (rigatoni) 48 %. meat of beef 14 %. tomato purees 11.5 %. tomatoes 10 %. water. hard cheese 2.5 %. onions. olive oil. modified starch. basil. salt. shallots. sugar. garlic. pepper.
Tarts	Tomatoe and mozzarella tart	Flaky pastry 32%: corn flour . margarine. water. chicory fibre. salt. L cysteine.
		Garnish 68%: water. tomatoes 19%. mozzarella 10.5%. mushrooms. ham 8% . lactose. syrup of glucose. dextrose. natural flavour. eggs. fresh cream. white egg. modified starch. shallots. skimmed milk powder. olive oil 1%. basil. salt. origan. natural pepper extract.
	Goat's milk cheese and spinach tart	Flaky pastry 39%: corn flour. margarine. water. salt. L cysteine.
		Garnish 61%: spinach 25%. soft white cheese. low fat fresh cream. goat's milk cheese 11.5%. eggs. skimmed milk powder. thickener: E 1422. garlic. salt. sunflower oil. flavouring. cumin. curry.
	Provencal tomatoes tart	Flaky pastry 39%: corn flour. margarine. water. chicory fibre. salt. L cysteine.
		Garnish 61%: tomatoes 21%. water. soft white cheese. zucchinis. onions. eggs. 4.5%. white of egg. fresh cream. emmental cheese .dried breadcrumbs. skimmed milk powder. garlic. modified starch. tomato puree. olive oil . basil 0.9%. parsley chicory fibre. shallots. Sherry vinegar. salt. natural extracts of pepper and nutmeg.
	Leek tart	Flaky pastry 39%: corn flour. margarine. water. chicory fibre. salt. L cysteine.
		Garnish 61%: water. leek 29%. spinach. eggs. white egg. fresh cream. emmental cheese 4%. shallots. modified starch. egg yolk powder some. chicory fibre. salt. sunflower oil. natural nutmeg and pepper extracts.
	Salmon and sorrel tart	Flaky pastry 39%: corn flour. margarine. water. fibre of chicory. salt. L cysteine.
		Garnish 61%: water. salmon 31.5%. eggs. fresh cream 7%. white wine. sorrel 3%. spinach. modified starch. skimmed milk powder. cream – milk proteins. chive. natural flavouring. salt. shallots. lemon juice. natural nutmeg and pepper extracts.
	St Jacques tart	Flaky pastry 39%: corn flour. margarine. water. of chicory fibre. salt. L cysteine.
		Garnish 61%: water. Saint Jacques 18%. carrots. fresh cream. tomatoes. celery. white egg. eggs. emmental cheese. skimmed milk powder. vermouth (Noilly Prat) 2.5%. modified starch. shallots. parsley. sunflower oil. salt. natural extracts of pepper and nutmeg. natural flavouring (shellfish)

*percentages included in the list of ingredients are the ones found on the packaging and are based on wet weight.

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Table 1. The total antioxidant capacity (μmol trolox per gram of fresh weight and per serving size) and the total phenol content (mg gallic acid per 100 grams of fresh weight and per serving size) of 9 basic foods

Food sample name	Total phenol content (mg gallic acid /100 g fresh weight)	Total antioxidant capacity (μmol trolox /g fresh weight)
Olive Oil		**1.70±0.06**
Tomato	**41.37±3.80**	**2.05±0.13**
Onion	**151.61±0.68**	**3.10±0.19**
Mustard	**719.34±8.69**	**10.27±0.24**
Lentils	**67.52±1.59**	**4.23±0.10**
Sweet pepper	**108.97±2.10**	**4.31±0.04**
Green olives	**110.73±8.55**	**4.97±0.03**
Red wine	**338.59±29.72**	**27.35±0.71**
Parsley	**368.89±8.69**	**19.00±0.15**

Table 2. The total antioxidant capacity (μmol trolox per gram of fresh weight and per serving size) and the total phenol content (mg gallic acid per 100 grams of fresh weight and per serving size) of 26 convenience foods

Type of dish		Food sample name	Total antioxidant capacity (μmol trolox /g fresh weight)	Total phenol content (mg gallic acid /100 g fresh weight)
Fresh dishes	**Prepared dishes**	Greek-style cooked fish with rice	2.34 ± 0.11	87.73 ± 12.37
		Provence- style cooked Eggplants with corn semolina	2.13 ± 0.04	99.84 ± 6.65
		Indian-style cooked zucchinis with rice	1.94 ± 0.02	85.09 ± 3.25
		Chicken in mustard sauce with rice	1.87 ± 0.07	69.00 ± 2.23
		Dried tomatoes with basil and rice	1.43 ± 0.10	71.58 ± 7.23
		Andalusia-style cooked fish with rice	1.38 ± 0.07	66.1 ± 8.34
		Minced beef and mashed potatoe gratin	1.38 ±0.07	52.98 ± 7.59
		Italian-style cooked fish	1.37± 0.06	54.26 ± 2.06
		Lasagna bolognaise	1.35 ± 0.05	51.04 ± 6.21
		Gratin Dauphinois	1.19 ± 0.03	61.29 ± 3.91
		Quenelles of pike with rice	1.14 ± 0.08	48.29 ± 1.84
		Cod with lemon sauce and rice	0.81 ± 0.02	32.27 ± 2.80
	Tarts	Tomato and mozzarella tart	1.24 ± 0.12	34.93 ± 0.10
		Tart in goat's milk cheese and spinach	1.19 ± 0.1	41.81 ± 2.54
		Goat's milk cheese and spinach tart	1.14 ± 0.06	48.00 ± 1.42
		Tart in leeks	1.13 ± 0.08	41.19 ± 1.42
		Provencal tomatoes tart	0.97 ± 0.09	33.77 ± 3.82
		Tart in St.Jacques	0.91 ± 0.07	48.12 ± 3.63
Deep-Frozen dishes		Savoy-style cooked potatoes	1.89 ± 0.02	78.34 ± 2.36
		Paella	1.88 ± 0.01	63.49 ± 1.10
		tuna and potato gratin	1.68 ± 0.06	59.49 ± 6.55
		Savoy-style baked gratin	1.63 ± 0.05	76.41 ± 1.63
		Basque chicken	1.50 ± 0.01	43.56 ± 1.28
		Conchiglie with tuna	0.89 ± 0.21	52.59 ± 7.70
		Farfallini with gorgonzola	0.78 ± 0.01	34.77 ± 1.92
		Rigatoni with tomatoes and beef	0.77 ± 0.01	44.71 ± 1.40

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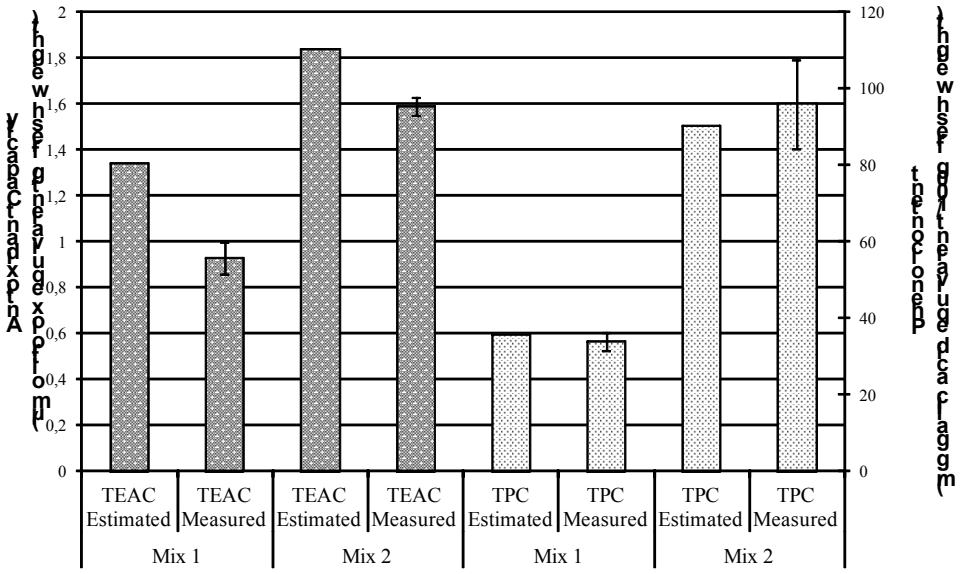

Figure 1. Interaction with food matrix: comparison between values estimated and measured. Mix 1 is a mixture of pie and parsley. Mix 2 is a mixture of fish, sauce and vegetables

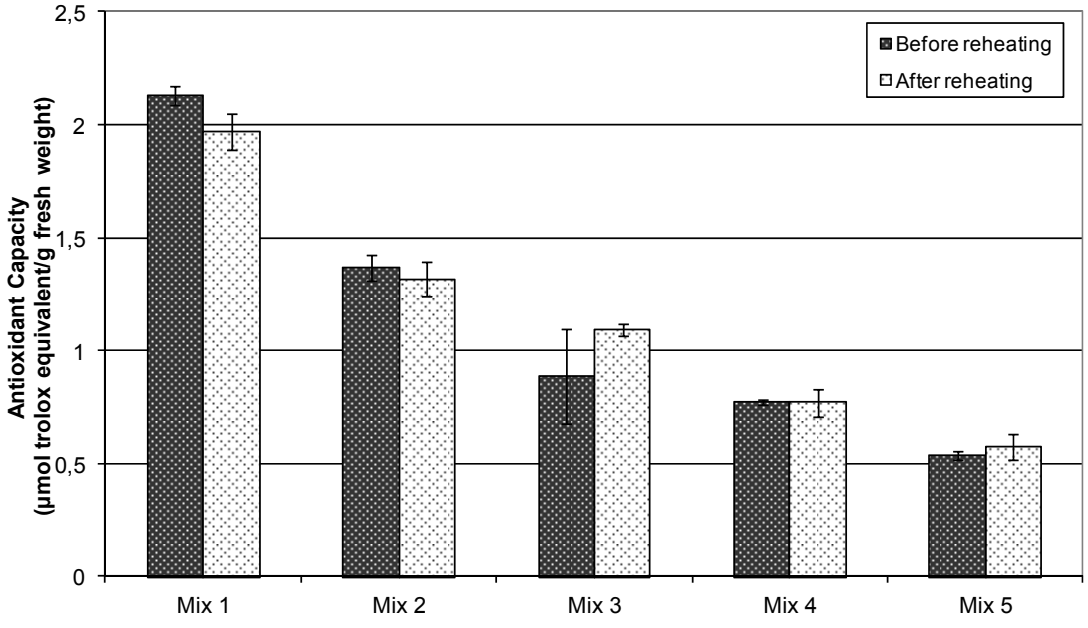

Figure 2. Effect of reheating on antioxidant capacity (TEAC) of convenience foods commonly consumed

Mix 1 is a Provencal eggplant recipe. Mix 2 is Italian-style fish. Mix 3 is pasta with tuna. Mix 4 is pasta with beef and tomatoes. Mix 5 is a tuna and potato bake.

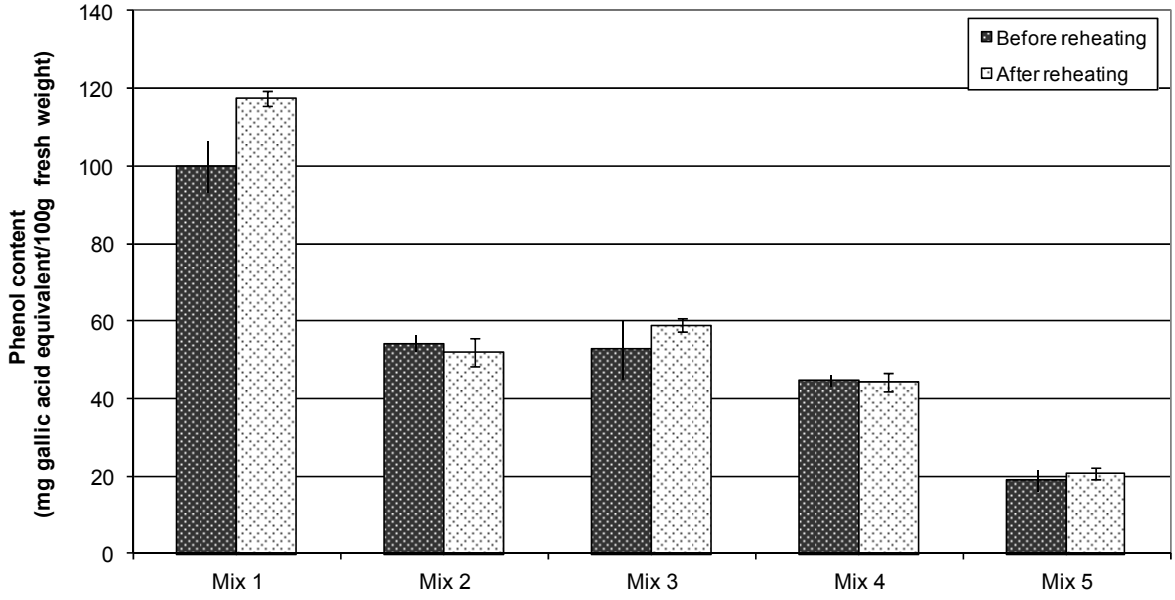

Figure 3. Effect of reheating on the Total Phenol Content of convenience foods commonly consumed

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Fractionation of *Trans* from Partially Hydrogenated Soybean Oil Fatty Acids

Ram Chandra Reddy Jala & Zheng Guo
Department of Molecular Biology, Aarhus School of Engineering
Aarhus University, DK-8000, Aarhus C, Denmark

Xuebing Xu (Corresponding author)
Department of Molecular Biology, Aarhus University
Gustav Wieds Vej 10, DK-8000, Aarhus C, Denmark
Tel: 45-8942-5089 Email: xu@mb.au.dk

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Abstract

A multi-stage, low temperature solvent fractional crystallization process was developed in this work for the removal of *trans* fatty acids (TFA) and saturated fatty acids in free fatty acids (FFAs) obtained from hydrolysis of partially hydrogenated soybean oil (PHSO). The effects of host solvents, concentration of FAs, crystallization temperature and time on separation of TFA and SFA were studied. Among all the three solvents examined namely acetone, hexane and ethanol, acetone proved to be promising solvent. By employing acetone as a solvent TFA can be reduced to 11.6% from an initial content of 19.5% and SFA can be drop down to 3% from an initial content of 20%, by applying stepwise crystallization temperatures from 5ºC to -20ºC. Crystallization temperatures lower than -30ºC such as -35ºC and -40ºC can further lower the TFA content in liquid fraction down to 5-8% by employing acetone as a solvent. TFA content can be reduced down to 12.2% and 14%; and SFA content could be reduced to 3.3% and 3.1% from an initial content of 20% when hexane and ethanol were employed as solvents, respectively. Optimum FAs to solvent ratio was 1:5 (w/v) and with a time period of 12 h for each step of crystallization. The crystallization behavior of TFA strongly depends on the type of solvents employed and crystallization temperature, while SFA crystallization depends mostly upon temperature rather than nature of solvent. Even though total removal of TFA could not be achieved in the present study, a road map has been developed for a fractionation of TFA and SFA from partially hydrogenated oil FFAs.

Keywords: *Trans* fatty acids, Elaidic acid, Partially hydrogenated soybean oil (PHSO), Fractional crystallization

1. Introduction

Trans fatty acids (TFAs) have been reported to be risk factors involved in cardiovascular diseases as well as many other negative effects for human (Buckle K, 2010; Valenzuela A & Morgado N, 1999; Semma M, 2002). It is well accepted that TFAs should be removed or minimized from our food, where the labeling of TFA content is well ruled in many parts of the world. TFAs arise in our foods mainly from industrial partial hydrogenation and natural ruminant sources. For the industrially produced TFAs, a broad mixture of TFAs is formed with elaidic acid (9*t*-18:1) as the major isomer (European Food Safety Authority [EFSA], 2004; Stender S, Dyerberg J & Astrup A, 2006). In general TFAs are present in different amounts in a broad range of foods, including most foods made with partially hydrogenated oils, while partial hydrogenation is traditionally a key technology for oil modification in frying oil and plastic fat industries.

Even though the main mode now is to rule out the use of partial hydrogenation, the possible removal of TFAs from partially hydrogenated soybean oil (PHSO) is still of interest for part of the industrial sectors as well as for academic documentation since this has not been conducted before. Initial attempts had been made to decrease the TFAs directly from PHSO TG by means of fractional crystallization as did for palm oil (Timms R.E, 2005, 1983; Hamm W, 1986; Zaliha O *et al*, 2004; Nissim G & Kiyotaka S, 2001; Kiyotaka S, 2001). Due to the randomized

distribution of TFAs in PHSO, the fractionation of PHSO triglycerides could result in little difference between the fractions in terms of TFA content (data not shown). As part of a package strategy including enzymatic selective hydrolysis and following further fractionation, the fractionation of fatty acid mixture is in central concern for this study. Therefore, the objective of the present study was to separate TFAs from PHSO fatty acids, a mixture was formed during the *trans* selective hydrolysis of PHSO.

2. Experimental

2.1 Materials

PHSO was provided by Archer Daniels Midland Company (ADM, Decatur, IL). *Candida Antarctica* A, immobilized on resin, was obtained from Codexis, Inc., (Pasadena, CA, USA) with marked assayed activity of transesterification of ethyl laurate with 1-butanol in isooctane (7.0 U/g). Fatty acid methyl ester standard was purchased from Sigma Aldrich Chemie GmbH, Steinheim, Germany. All the solvents and chemicals used were of analytical grade.

2.2 Methods

2.2.1 Preparation of fatty acid mixture from PHSO

The mixture was prepared by means of PHSO hydrolysis with *Candida Antarctica* A (5 wt % of oil weight) under conditions, viz. 100 mM *tris*-HCl buffer [pH 7.0, with 1:1 ratio (w/v, based on oil weight)] which contains 10 mM $CaCl_2$, and the contents magnetically stirred at 40ºC for 24 h, where the fatty acids were recovered by short path distillation under conditions namely feed temperature, 160-180ºC; evaporator temperature, 200-220ºC; condensor temperature, 40ºC; heat exchange temperature, 80ºC and vacuum less than 0.001 mbar. The mixture has purity of ~98%. The fatty acid composition of the fatty acid mixture used is as follows, Palmitic, 13.6%; Stearic, 6.4%; TFA (*t*-18:1) 16.3%; CFA (*c*-18:1), 35.6%; TFA (*t*-18:2), 2.7%; CFA (*c*-18:2), 22.4%; TFA (*t*-18:3), 0.5% and CFA (*c*-18:3), 2.5%, where TFA, CFA indicates *trans* fatty acids and *cis* fatty acids, respectively.

2.2.2 Fractional crystallization of PHSO fatty acids

A series of crystallizations were conducted by employing solvents namely acetone, hexane and ethanol. The cooler used for the crystallization was a COMFORT Heto Chill Master (Holm & Holby, Copenhagen, Denmark). The fatty acid mixture was dissolved in the solvent in separate glass vials and heated for 30 min at 35-40°C with vigorous shaking under N_2 atmosphere until all the fatty acids were dissolved homogenously. The solutions were cooled to room temperature. The crystallization process was carried out by changing temperature from 5°C to -20°C by decreasing 5°C each step. Each step of crystallization was carried out for 12 h and crystals were separated by means of a Buchner funnel and vacuum based filtration at the same temperature. The crystals were washed twice with pre-cooled fresh solvent at the same temperature as the crystallization. After each crystallization, the resulting liquid fraction was employed to perform the next step of the crystallization process. Hence, the sample was fractionated into 5-6 consecutive fractions. If no crystallization was found in the solution, the same solution was continuing to the next temperature step. After completion of each step of crystallization, aliquots were collected from the liquid and crystal parts for fatty acid composition analysis.

2.2.3 Gas chromatographic analysis

The AOCS Official Method Ce 1h-05 was employed to analyze the fatty acid composition of the samples (AOCS, 2005). The samples were methylated into methyl esters as follows. To 100 mg sample, 1 mL BF_3-methanol solution was added; the resulting solution was vigorously shaken for 2 min and kept for 30 min under mild heating. Subsequently, 3 mL n-heptane were added and the reaction mixture again shaken for 1 min and then set aside for 1-2 min to achieve phase separation. The upper phase was dried with anhydrous sodium sulfate and used for GC analysis. The analysis was carried out on a Thermo Fisher Scientific Gas Chromatograph system (TRACE GC Ultra, Pittsburgh, USA), equipped with a FID detector and capillary column (SLB-IL 100, 60 m X 0.25 mm X 0.2 μm film thickness). The following optimal GLC conditions were set up for the sample analysis by gas chromatograph, a split ratio adjusted at 1:100, the injector and detectors were maintained at 250 and 275 ºC. The oven temperature was maintained at 170ºC for 40 min run, and the flow rate of carrier gas (N_2) was 1.5 mL/min. The area percentage was recorded with a Thermo Fisher data system and the fatty acids were identified with standards. The responses were calibrated with standard as well. The analysis was conducted in duplicates and the average was used for evaluation.

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3. Results and Discussion

3.1 Determination of operation mode: crystallization equilibration and multi-step operation

Crystallization consists of nucleation and crystal growth stages. The time to reach equilibration is a basic parameter to examine before investigation of effects of other variables. As expected, the crystallization of TFAs from solution continuously progressed with time from 4 h to 12 h (Figure 1). Starting from 19.5% in PHSO, TFA content could be reduced to 11.6%, 12.2% and 14.0% with acetone, hexane and ethanol as solvent, respectively. Nevertheless, prolonging crystallization time from 12 h to 24 h did not result in significant reduction of TFA content in solution, with ≤0.3% reduction for all the three solvent systems. Therefore, 12 h could be regarded as the time period in which equilibrium of the fractional crystallization of TFA was essentially reached. As a result, 12 h was adopted as an optimum for the operation time of fractional crystallization.

The crystallization process is governed by thermodynamic and kinetic factors. In principle, the crystallization equilibrium could be broken by removing crystals (Glynn P.D & Reardon E.J, 1990). However, due to complex composition of the samples, interference effects may exist among the various fatty acid components. As presented in Table 1, a single step of the run at 12 h, 24 h and 36 h crystallization time period conducted in parallel, resulted in similar fatty acid composition for both liquid and crystal fractions, which agreed well with the observation in Figure 1. However, with stepwise removal of crystals, the fatty acid composition for crystal fraction significantly changed. For instance in the crystal fractions, the content of *trans*-18:1 was 26.3% (step 1), 37.6% (step 2) and 30.0% (step 3); the content of 18:0 was 21.0% (step1), 4.6% (step 2) and 5.2% (step 3); and oleic acid 6.0%, 18.3 and 28.2%, respectively.

A general observation should be noticed. In the resulting crystal fractions obtained from the stepwise operation, all the cis fatty acid content continuously increased (from step 1, 15.1% to step 2, 39%), which means the cis fatty acids were significantly lost with further stepwise operations. The removal of total TFAs from liquid fractions was only improved with 4.9%. A compromise between the goal of TFAs removal and recovery of the rest should be made.

(Table 1 & Figure 1)

3.2 Effect of temperature on fractional crystallization of TFAs

Lower temperature provides the means to form the crystallization driving force – super saturation of the solute. The cooling profile is an important operation variable to manipulate crystallization pattern. Figure 2 shows fatty acid composition changes in liquid fractions with the temperature varied from 5°C to -40°C. Until the temperature reached -5°C there was no decrease in TFA while less than 50% TFA can be reduced even by -20°C. On the other hand, saturated fatty acid content reduced more significantly. At a temperature of -20°C, saturated fatty acid content could be decreased to 3%. Even by -40°C, the TFA and saturated fatty acid levels could decrease to 6.0% and 0.8%, respectively. Therefore, TFA is very challenging to be completely removed in the current system. However, a certain reduction can be achieved with the better reduction of saturated fatty acids.

(Figure 2)

3.3 Effects of solute/solvent ratio on fractional crystallization of TFAs

At a given temperature, the degree of super saturation depends on the concentration of solute in solution. For the fractionation purposes, the concentration of the solute mixture also influences the crystallization rate and packing lattice of the crystals which affects separation. As shown in Table 2, whenever the experiments were carried out with 1:3 and 1:4 (w/v) ratios with all the three solvents, none of the solvents tested gave very good fractionation. For instance, at the ratios of 1:3 and 1:4, the content of *trans* 18:1 decreased from 16.3% to 14.3% and 13.5%, respectively. On the other hand, with 1:5 and 1:10 (w\v) ratios, all the solvents exhibited significant improvement in separation. The possible reason could be, a meta-stable solution occurs at higher concentration of fatty acids, in which spontaneous crystal growth is favored. High solute concentration might produce supersaturated solutions where nucleation was favored against crystal growth, thus decreasing the efficiency of fractionation. Among all the solvents tested, acetone gave the best results, while the ratio of 1:5 is recommended considering the cost and practicability.

A further examination is made for the three solvents. Table 3 presented more results for this purpose. Compared with hexane and ethanol, acetone proved to be a better solvent in general as shown in Table 2. On the other hand, we can see saturated fatty acids can be almost equally separated from all the three solvents while TFAs can be better separated by employing acetone as a hosting solvent.

3.4 Fractional crystallization of saturated fatty acids vs TFA

Figure 3A is drawn on crystal fractions for the 3 solvents used. This confirms the conclusion that acetone shows better performance. As a general observation in the data presented in Table 1-3, and Figure 1, the crystallization of saturated fatty acids occurred more readily than TFAs. To have a quantitative understanding, we plotted the fractionation efficiency of the crystallization process for the removal of saturated fatty acids vs TFA in acetone, hexane and ethanol (Figure 3B). As can be seen, the plot strongly deviated from non-selective crystallization to the saturated fatty acids. Until nearly 50% removal of saturated fatty acids, there was no significant reduction in the content of TFA in liquid fractions. The maximum removal of TFAs achieved were 40% by acetone at -20°C, meanwhile up to 85% of saturated fatty acids had been reduced. This is understandable as the higher m.p. fatty acids start to crystallize first. Pure stearic acid has a m.p. about 69°C while *trans* 18:1 has a m.p. about 46°C. Due to the intermediate m.p. of TFAs, they hardly crystallized in the presence of higher concentrations of saturated fatty acids (Benjamin M.O, Michael S.K, Nikova A.T & Schwartz D. K, 2002). Furthermore, unsaturated fatty acids have higher solubilities in hydrophilic solvents than saturated fatty acids, which enable easier separation of saturated fatty acids.

4. Conclusions

In general, this initial study shows that the reduction of TFA from the fatty acid mixture is possible in certain extent, but with high challenge to remove further below 10%. Significant low temperature has to be used to drive further low level so that makes the practical operation uneconomically favorable. From the study, the crystallization behavior of TFA strongly depends on the type of solvent employed and crystallization temperature, conversely the crystallization behavior of SFA is mainly temperature dependent and independent of solvent used. Fatty acid to solvent ratio also influences the efficiency of fractional crystallization and requires an optimum ratio (1:5, w/v) to give better fractionation. Stepwise crystallization is preferable rather than single step separation. Crystallization time at each step is important and an optimum time period (12 h) is required to stabilize crystal formation. It is certainly true that the crystallization process examined in the present study has to be refined and optimized; however, the current study may provide a roadmap for the future studies.

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Table 1. Fatty Acid Composition Change of Liquid and Crystal Fractions in Multi-Step Operations

Fatty Acid Composition (w %)								
Sample	16:0	18:0	18:1, TFA	18:1, CFA	18:2, TFA	18:2, CFA	18:3, TFA	18:3, CFA
L F-1	3.20 ±0.06	1.10 ±0.02	11.50±0.23	44.00±0.88	2.50 ±0.05	32.60 ±0.65	0.60 ±0.01	4.50 ±0.09
C F-1	35.40 ±0.70	21.00±0.42	26.30±0.53	6.00 ±0.12	1.90 ±0.04	5.80±0.12	0.40±0.00	3.20 ±0.06
L F-2	2.00 ±0.04	0.50 ±0.01	9.20 ±0.18	45.40 ±0.90	0.90 ±0.02	35.20±0.70	0.50 ±0.01	6.30±0.13
C F-2	17.00 ±0.34	4.60 ±0.09	37.60±0.75	18.30 ±0.37	4.30 ±0.09	13.20 ±0.26	1.30±0.03	3.70 ±0.07
L F-3	1.70 ±0.03	0.30 ±0.00	8.20±0.16	46.00±0.92	0.20 ±0.00	37.90±0.76	0.30 ±0.00	5.40 ±0.11
C F-3	15.70 ±0.31	5.20 ±0.10	30.00 ±0.60	28.20 ±0.56	6.70 ±0.13	9.50 ±0.19	3.40 ±0.07	1.30 ±0.03
LF-24 h	2.70 ±0.05	0.90 ±0.02	10.90±0.22	46.30±0.93	2.40±0.05	31.40±0.63	0.60 ±0.01	4.80±0.10
CF-24 h	36.10 ±0.72	21.60±0.43	26.90 ±0.54	5.10 ±0.10	2.10±0.04	4.70 ±0.09	0.50±0.01	3.00±0.06
LF-36 h	2.60 ±0.05	0.80±0.02	10.90 ±0.22	46.90±0.94	2.20 ±0.04	31.90 ±0.64	0.60 ±0.01	4.10±0.08
CF-36 h	36.40 ±0.73	21.90±0.44	26.60±0.53	4.90±0.10	2.30 ±0.05	4.50 ±0.09	0.60±0.01	2.80±0.06

Abbreviations: TFA, *trans* fatty acid; CFA, *cis* fatty acid; LF-1, 2, 3 indicates liquid fraction of 1, 2 and 3 steps; CF-1, 2, 3 indicates crystal fraction of 1, 2 and 3 steps; solvent, acetone; sample to solvent ratio,1:5 (w/v); each crystallization step carried out for 12 h; crystallization temperatures were maintained at -35ºC.

Table 2. Fatty Acid Compositions of Liquid Fractions by Fractional Crystallization at Different Sample/Solvent Ratios

Solvent	Ratio (w/v)	Fatty Acid Composition (w %)							
		16:0	18:0	18:1, TFA	18:1,CFA	18:2,TFA	18:2, CFA	18:3,TFA	18:3,CFA
Original		13.6	6.4	16.3	35.6	2.7	22.4	0.5	2.5
Acetone	1:3	11.50±0.23	4.40±0.09	14.30±0.29	40.60±0.81	2.50±0.05	23.50±0.47	0.50±0.01	2.70±0.05
Acetone	1:4	8.50±0.17	2.50±0.05	13.50±0.27	42.30±0.85	1.50±0.03	28.10±0.56	0.40±0.01	3.20±0.06
Acetone	1:5	2.70±0.05	0.30±0.00	8.90 ±0.18	45.00±0.90	1.90±0.04	36.70±0.73	0.30±0.01	4.20±0.08
Acetone	1:10	2.10±0.04	0.20±0.00	8.40 ±0.17	45.00±0.90	1.90±0.04	37.40±0.75	0.30±0.01	4.70±0.09
Hexane	1:3	12.50±0.25	4.70±0.09	15.70±0.31	37.60±0.75	2.80±0.06	23.10±0.46	0.80±0.02	2.80±0.06
Hexane	1:4	7.60 ±0.15	1.70±0.03	12.70±0.25	41.80±0.84	2.90±0.06	29.10±0.58	0.90±0.02	3.30±0.07
Hexane	1:5	2.90 ±0.06	0.40±0.00	9.10 ±0.18	44.90±0.90	2.30±0.05	36.00±0.72	0.80±0.02	3.60±0.07
Hexane	1:10	2.40 ±0.05	0.40±0.00	8.90 ±0.18	44.80±0.90	1.90±0.04	36.40±0.73	0.60±0.01	4.60±0.09
Ethanol	1:3	10.50±0.21	5.40±0.11	15.30±0.31	39.60±0.79	3.50±0.07	22.60±0.45	0.50±0.01	2.60±0.05
Ethanol	1:4	4.80 ±0.10	2.50±0.05	14.70±0.29	40.90±0.82	2.70±0.05	30.60±0.61	0.60±0.01	3.20±0.06
Ethanol	1:5	2.60 ±0.05	0.50±0.01	11.10±0.22	44.10±0.88	2.50±0.05	35.70±0.71	0.40±0.01	3.10±0.06
Ethanol	1:10	2.50 ±0.05	0.50±0.01	10.90±0.22	43.90±0.88	2.70±0.05	36.80±0.74	0.50±0.01	2.20±0.04

Abbreviations: TFA, *trans* fatty acid; CFA, *cis* fatty acid; Operation conditions: temperature, -20ºC; crystallization time period, 12 h.

Table 3. Fatty Acid Compositions of Liquid Fractions by Fractional Crystallization in Different Solvents

Temp (ºC)	Acetone			Hexane			Ethanol		
	SFA	TFA	CFA	SFA	TFA	CFA	SFA	TFA	CFA
Original	20.0	19.5	60.5	20.0	19.5	60.5	20.0	19.5	60.5
5	12.90±0.26	19.50±0.39	67.60±1.35	13.00±0.26	19.50±0.39	67.50±1.35	13.80±0.28	19.50±0.39	66.70±1.33
0	10.90±0.22	19.50±0.39	69.60±1.39	10.00±0.20	19.50±0.39	70.50±1.41	10.00±0.20	19.50±0.39	70.50±1.41
-5	7.80±0.16	17.50±0.35	74.70±1.49	7.80±0.16	18.50±0.37	73.70±1.47	7.80±0.16	18.50±0.37	73.70±1.47
-10	5.50±0.11	16.30±0.33	78.20±1.56	4.00±0.08	17.00±0.34	79.00±1.58	4.10±0.08	17.50±0.02	78.40±1.57
-15	3.50±0.07	12.40±0.25	84.10±1.68	3.50±0.07	14.00±0.28	82.50±1.65	3.20±0.06	16.10±0.32	80.70±1.61
-20	3.00±0.06	11.60±0.23	85.40±1.71	3.30±0.07	12.20±0.24	84.50±1.69	3.10±0.06	14.00±0.28	82.90±1.66

Abbreviations: TFA, *trans* fatty acid; CFA, *cis* fatty acid; Operation conditions: temperatures varied from 5ºC to -20ºC; sample to solvent ratio, 1:5 (w/v); crystallization time period is 12 h.

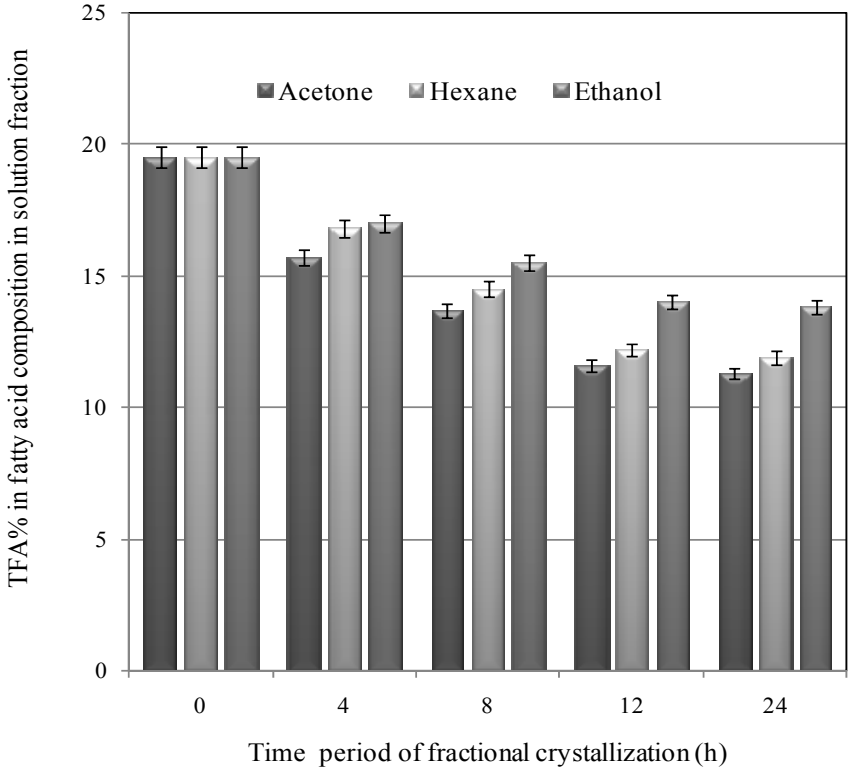

Figure 1. Fractional Crystallization of TFA with Different Time Periods. Operation conditions: sample to solvent ratio, 1:5 (w/v); and temperature maintained at -20ºC. Abbreviations: TFA-*trans* fatty acid

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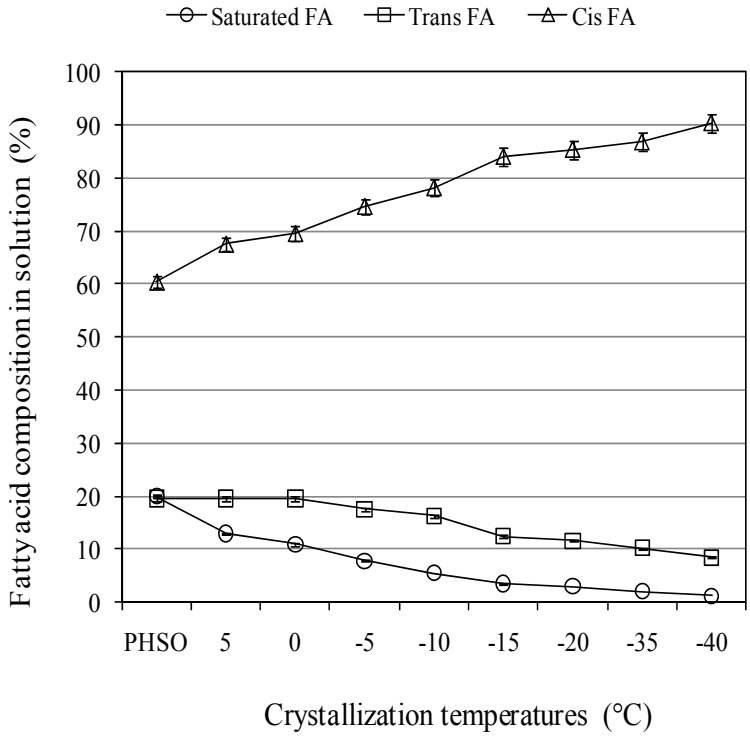

Figure 2. Effects of Temperatures on Fractional Crystallization of TFA in Acetone

Operation conditions: temperature varied from 5 to -40ºC, sample to solvent ratio, 1:5 (w/v); each step of crystallization carried out for 12 h. Abbreviations: FA-fatty acid, TFA-*trans* fatty acid, PHSO-partially hydrogenated soybean oil.

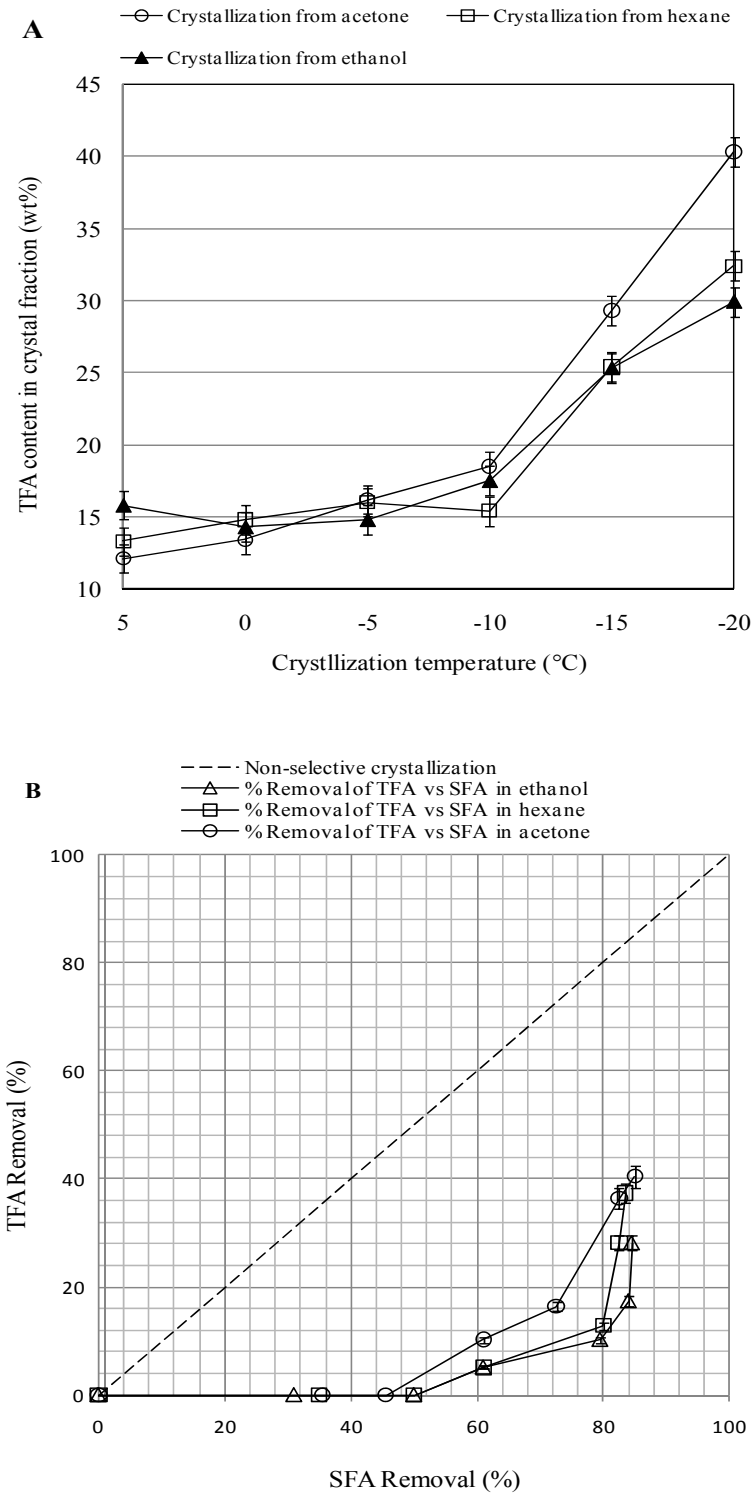

Figure 3. Solvent Dependency of Fractional Crystallization of SFA vs TFA

(A) TFA Percentage in Crystal Fractions from Different Solvents; (B) Relative Removal Content of SFA vs TFA in Different Solutions. The relative removal content of SFA or TFA was calculated as the percentage of fatty acids removed from solution of its original content (SFAs, 20% and TFAs, 19.5%). Operation conditions: temperature varied from 5 to -20ºC, each crystallization step carried out for 12 h; sample to solvent ratio, 1:5 (w/v). The plotted points in Fig. 3B corresponding to operation temperature in the order of 5, 0, -5, -10, -15 and -20ºC, respectively. Abbreviations: SFA, saturated fatty acid; TFA, *trans* fatty acid.

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Legumes and Well-Being in the Elderly: A Preliminary Study

Andrew Paul Smith
Centre for Occupational and Health Psychology
School of Psychology, Cardiff University
PO box 901, Cardiff CF113YG, UK
Tel: 44-292-087-4757 E-mail: SmithAP@Cardiff.ac.uk

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Abstract

There has been considerable recent interest in associations between diet and subjective reports of wellbeing (stress, mental health, cognitive and physical functioning). Such effects have been considered across the lifespan and benefits often observed in all age groups. Effects of diet are often easier to detect in the elderly, either because of the longer period of consumption or the decline in functioning associated with aging. The present paper reports results from surveys examining associations between frequency of consumption of peas and beans and wellbeing. No significant associations were observed in younger samples but in the elderly more frequent consumption of peas and beans was associated with reduced stress, emotional distress, anxiety, depression and somatic symptoms. Further research is now required to examine underlying mechanisms and assess the practical implications of these findings.

Keywords: Legumes, Well-being, The elderly

1. Introduction

Recent research suggests that diet influences wellbeing (Smith, 2005). Initial research has been based on epidemiological findings and shown that breakfast is associated with subjective reports of better mental and physical health and functioning across different age groups (e.g. Smith, 2001). Such research has also examined effects of macronutrients (e.g. fibre – (Smith, 2010)) micronutrients (Smith, et al., 1999) and whole diets (McMillan, et al. 2011). The aim of the present research was to examine whether frequency of consumption of legumes was associated with increased wellbeing in different age groups.

Legumes include all forms of peas and beans. They provide a range of essential nutrients including protein, low glycaemic carbohydrates, dietary fibre and minerals and vitamins (Munro 2007). Dietary guidelines recognize the favorable nutrient profile of legumes and they are considered both as vegetables and alternatives to lean meat, fish and poultry. Evidence is strengthening for the role legume consumption can play in disease protection. However, overall legume intake is low in many countries especially in children. This has limited research on legumes and health, and it is certainly the case that the scientific literature on this topic is not as extensive as that for cereal grains. The emerging picture is that eating legumes can play a role in preventing chronic diseases such as cardiovascular disease, diabetes and obesity (Kushi, et al. 1999).

The benefits of legumes may depend on them being part of other dietary profiles (e.g. the Mediterranean diet). Indeed, results from the ATTICA study show that the Mediterranean diet was associated with reduced levels of biomarkers associated with metabolic syndrome (Panagiotakos, et al., 2007). The EPIC study supports these findings with clinical data: the Mediterranean diet was associated with 14% lower mortality and high legume consumption was calculated to contribute about 10% of this protective effect of the diet (Trichopoulos, et al., 2009). A seven year longitudinal study of older people in different cultures (including Japan, Sweden, Greece and Australia) found a 7-8% reduction in the risk of death for every 20g increase in daily legume intake (Darmadi-Blackberry, et al., 2004). Similar benefits have been in epidemiological studies in the USA (e.g. NHANES – (Bazzano, et al., 2001). The reduced risk of cardiovascular disease associated with the consumption of legumes may reflect the hypocholesterolaemic effect of their fatty acid profile, dietary fibre, isoflavones and antioxidants. Indeed, legumes are good sources of saponins and phytosterols which may assist with decreasing absorption of cholesterol from the gut.

A systematic review and meta-analysis of randomized controlled trials of legumes and diabetes (Sievenpiper, et al., 2009) concluded that legumes lowered fasting blood glucose and insulin. Legumes may, therefore, reduce the risk of type 2 diabetes by effectively lowering the GI of diets through slowed absorption. Improved glycaemic control may also explain findings linking legume consumption with weight loss and obesity with low ingestion of legumes. Legume consumption has also been associated with reduced risk of certain cancers although the mechanisms underlying such effects are not clearly understood.

One mechanism through which diet may influence chronic disease is through wellbeing. Research shows that those with mental health problems are at a greater risk of developing subsequent chronic disease (Stansfeld, et al., 2002). The aim of the present research was, therefore, to examine associations between frequency of consumption of peas and beans and subjective reports of mental and physical health. This was done in three groups: young adults, a middle-aged working sample and a more elderly sample.

2. Method

A common methodology was used across the three studies. Consumption of peas and beans was assessed using a food frequency questionnaire (Yarnell, et al., 1983) with the participants being asked:

How frequently do you eat peas or beans?

Responses were measured using a 5-point scale with the following categories: Never; Less than once a week; once or twice a week; most days; and every day.

The following questionnaires were administered to measure mental and physical health:

Stress :	Perceived Stress Scale (Cohen & Williamson 1988)
Depression :	Beck Depression Index (Beck 1967)
Anxiety :	Spielberger Trait-State Anxiety Inventory (Spielberger et alk., 1970)
Emotional Distress :	Emotional distress scale of Profile of Fatigue Related States (Ray et al., 1992)
Somatic symptoms:	Somatic symptoms scale of Profile of Fatigue Related States (Ray et al., 1992)

All studies were carried out with the informed consent of the volunteers and approval of the local ethical committee.

Study 1:

This study used a student sample (N=189; 49% Female; age range: 18-30 years). There were no significant associations between frequency of legume consumption and the wellbeing measures (see Table 1).

(Table 1)

Study 2:

This study used a general population sample (N= 126; 63% Female; age range: 25 to 55 years). There were no significant associations between frequency of legume consumption and the wellbeing measures (see Table 2).

(Table 2)

Study 3:

This study used an older sample (N=205; 55% Female; age range: 60 to 80 years). There were significant associations between frequency of legume consumption and the wellbeing measures (see Table 3). Those with more frequent consumption of peas and beans reported lower levels of stress, emotional distress, anxiety, depression and somatic symptoms

(Table 3)

3. Discussion

The present studies demonstrated that higher consumption of legumes is associated with increased wellbeing in the elderly but not younger samples. Such a result, suggesting a cumulative benefit of a higher frequency of consuming legumes, could be explained by a number of the mechanisms underlying other health effects (Smith, 2005; Munro 2007; Kushi et al., 1999; Sievenpiper et al., 2009). Further research is now required to exclude other possible explanations of the findings. For example, consumption of peas and beans may be associated with other dietary features and it may be these correlated attributes that are responsible for the observed effects. The next step, therefore, is to conduct multi-variate analyses considering many aspects of diet, both alone and in combination (Smith, 2005). A second requirement is for better measures of consumption. Food frequency clearly gives no indication of amounts consumed and this needs to be rectified in further research. It is also important to obtain

better information on causality. In cross-sectional analyses there is always the possibility that it is wellbeing that is influencing diet rather than the other way around (Smith, 2005). Intervention studies are now required to investigate this further (Smith, 2010). It is also important to extend the research by considering other outcomes. Reduced wellbeing is related to chronic disease and it is important to determine whether the effects observed here are an initial part of a diet-health pathway. Aging is also associated with cognitive decline and it is important to investigate both acute and longer term effects of consuming legumes on cognitive functioning. Legumes are often considered in the context of whole grains. The methodology used here can now be used to investigate associations between frequency of consuming whole grain foods and wellbeing.

In conclusion, the present studies demonstrate that a higher frequency of consumption of peas and beans is associated with less stress, anxiety, depression and physical symptoms in the elderly but not in younger groups. Further research is required to extend these findings and elucidate underlying mechanisms and practical implications of the effects.

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Table 1. Associations between legume consumption and well-being measures (scores are means, s. e. s in parentheses) (Study 1. Students)

	Low consumption (once or twice a week at most)	High Consumption
Perceived Stress	20.3 (0.6)	21.2 (0.9)
Emotional Distress	30.0 (1.2)	30.6 (1.5)
Anxiety	37.1 (0.7)	37.3 (1.0)
Depression	4.4 (0.4)	5.0 (0.7)
Somatic Symptoms	22.1 (0.7)	22.2 (0.7)

Table 2. Associations between legume consumption and well-being measures (scores are means, s. e. s in parentheses) (Study 2. General Population)

	Low consumption (once or twice a week at most)	High Consumption
Perceived Stress	23.2 (1.0)	21.5 (1.1)
Emotional Distress	32.6 (1.8)	32.3 (2.2)
Anxiety	31.2 (1.0)	30.6 (1.1)
Depression	7.3 (0.7)	7.4 (0.9)
Somatic Symptoms	22.3 (0.7)	25.1 (1.6)

Table 3. Associations between legume consumption and well-being measures (scores are means, s. e. s in parentheses) (Study 3. Elderly Population)

	Low consumption (once or twice a week at most)	High Consumption	
Perceived Stress	16.0 (0.6)	13.6 (1.0)	$p<0.05$
Emotional Distress	28.0 (1.0)	24.6 (1.2)	$p<0.05$
Anxiety	31.2 (0.7)	28.6 (1.0)	$p<0.05$
Depression	6.3 (0.3)	4.7 (0.5)	$p<0.05$
Somatic Symptoms	23.2 (0.6)	20.8 (0.6)	$p<0.01$

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Effect of Inulin on the Physical, Chemical and Sensory Quality Attributes of Polish Chicken Burgers

Aneta Cegiełka (Corresponding author)
Warsaw University of Life Sciences-SGGW
Department of Food Technology
Nowoursynowska 159c, Warsaw 02-776, Poland
Tel: 48-225-937-532 E-mail: aneta_cegielka@sggw.pl

Krzysztof Tambor
Warsaw University of Life Sciences-SGGW
Analytical Center of SGGW
Ciszewskiego 8, Warsaw 02-786, Poland
Tel: 48-225-935-810 E-mail: krzysztof_tambor@sggw.pl

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Abstract

Four formulations of chicken burger were prepared: control product without inulin, and products with 1.0, 2.0 or 3.0 % of inulin (in relation to the weight of meat, fatty raw material, and water), respectively. Physical, chemical and sensory analyses were made to evaluate the effect of inulin on the quality of cooked burgers. The results showed that the application of inulin did not cause significant decrease of the thermal processing yield nor the shear force of the products. However, the addition of inulin resulted in slight, but significant differences ($P < 0.05$) in chemical composition and energy value of burgers. Burgers of all formulations were acceptable in sensory characteristics. The addition level of inulin not higher than 1.0 %, did not result in deterioration of physical, chemical and sensory quality characteristics of chicken burgers. To improve the nutritional value of chicken burgers, the modification of fatty acid composition is recommended.

Keywords: chicken burgers, Inulin, Quality characteristics

1. Introduction

In recent years fast food and convenient-type products gained a permanent position on Polish market of processed meat products. Among them burgers are still very popular, and their commercial offer expanded with pork and poultry products. Analyses of meat burgers available on Warsaw (Poland) market showed that their quality was very diversified and mostly depended on the formula. For example, many of the products were relatively low in protein and high in saturated fat and cholesterol. The use of a wide range of non-meat additives was also common (Krygier & Maksimowicz, 2008).

Contemporary consumers are increasingly looking for both traditional and convenient meat products with improved nutritional value. Manufacturing of "healthier" meat products is not always easy since they must taste good and be reasonably priced so that consumers will regularly purchase them (Decker & Park 2010; Fernández-Ginéz & others, 2005). To satisfy the needs of consumers producers may use different strategies. One of them is the incorporation of functional additives into processed meat products. Research results indicate that in production of burgers such additives as soy protein (Hoogenkamp, 1997), wheat fiber (Cegiełka & Pęczkowska, 2008; Cegiełka & Młynarczyk, 2010), lycopene (García & others, 2009), folic acid (Galán & others,

2010), and plant oils (Forell & others, 2010) can be applied.

Among novel ingredients used in food processing, a vegetable carbohydrate inulin offers a unique combination of nutritional properties and important technological benefits. Inulin-type fructans [ß-(2, 1)-fructans] – industrially obtained from chicory roots – are both components of soluble dietary fiber and prebiotic food ingredients (Chawla & Patil, 2010, Franck, 2002). The incorporation of inulin, due to its ability to bind water, form gel, and imitate the sensorial and technological properties of animal fat, allows upgrading of both taste and mouthfeel in a wide range of processed meat products. Although even the whole amount of animal fatty raw material could be replaced with 25% inulin gel in meat product formula, the substitution level is usually lower (Jánávary, 2005; Nitsch, 2006). The usefulness of rehydrated inulin (inulin gel) as a fat substitute was demonstrated in a wide range of processed meat products: scalded sausages (García & others, 2006; Tröger & others, 2005), canned meat products (Florowski & Adamczak, 2010), meat balls (Flaczyk and others, 2009), liver pâté (Florowski & others, 2008), and fermented sausages (Mendoza & others, 2001). However, inulin, when applied as functional additive, that is dietary fiber or prebiotic ingredient – not a fat replacer – may help to improve the nutritional value without deterioration of sensorial characteristics of meat product (Beriain & others, 2011; Ergönül & others, 2009).

So far most studies on the application of inulin in meat processing were focused on substitution of animal fatty raw material with inulin gel. Furthermore, not much research was done on convenience meat products. Therefore, the aim of this study was to evaluate the effect of addition of inulin on the quality characteristics of burgers from chicken meat. Inulin was used as nutritionally functional additive, not as replacer of animal fatty raw material. This work is a part of the research project on improving the quality and shelf-life of chicken burgers with modified nutritional profile (that is formulated with different type of dietary fiber and enriched with plant oil). The obtained results should provide a basis for further research.

2. Materials and Methods

2.1 Preparation of chicken burgers

Raw materials for the preparation of burgers were collected from "SuperDrob" SA meat processing plant (Karczew, Poland). Chilled chicken meat (about 5 kg) was purchased each time prior to the each replication of experiment. Chilled pork jowl (about 6 kg) was purchased once, before the experiment started, then cut into pieces and coarse ground using a laboratory grinder (Mesko, Skarżysko-Kamienna, Poland). The ground meat was thoroughly mixed and divided into 4 portions, which were vacuum packed and frozen at -28 ± 2 °C.

Four formulations of chicken burgers were manufactured. The composition of burgers differed by the addition level of inulin Orafti®HPX (Beneo-Orafti Ltd., Tienen, Belgium) (Table 1). Control product (PC) did not contain inulin. In burger formulations P1, P2, and P3, respectively, 1.0, 2.0, and 3.0 % of inulin was incorporated. The addition level of inulin to the batter was calculated in relation to the weight of chicken meat, pork jowl, and water. The inulin was added to the batter in a rehydrated form (1 part of inulin powder: 3 parts of water), that is as a inulin gel. The gel was prepared about 24 h prior to the production of burgers, using water provided in the formula. Inulin powder was dissolved in water using an electric blender, and then the solution was heated to boiling. Heating was continued for a while until a clear solution was obtained. Inulin solution was chilled at a room temperature (18 ± 2 °C) for 60 min, then placed in a laboratory refrigerator at 4 ± 1 °C.

About 24 h prior to the production of burgers pork jowl was thawed at 4 ± 1 °C. Directly before preparation of burgers, chicken thigh meat and pork jowl were ground using a laboratory grinder with a plate having 5-mm-dia orifices. Soy protein isolate SPI 733

(Solae™, St. Louis, Mo, U.S.A.) was rehydrated (1 part of dry preparation: 4 parts of water) using water provided in the recipe.

Batters were prepared in laboratory mixers (Kenwood Ltd., Havant, England). After mixing of chicken tight meat with table salt (about 5 min) other ingredients were added: pork jowl, soy protein isolate, seasoning and - depending on product formula - inulin gel and the remaining amount of water. Mixing was continued until ingredients were thoroughly distributed (about 15 min). Burgers were formed to a shape of flat 100-g discs (about 9-cm dia and 0.9-cm thick) by use of manual mould. Burgers were frozen at - 28 ± 2 °C for 30 min in order to maintain the shape. Then they were cooked on an electric grill (Unox S. p. A., Vigodarzere-Padova, Italy) equipped with two ceramic plates: upper and lower. Temperature of the plates was 200 ± 5 °C. Cooking was continued until the temperature of 72 °C was reached in the centre of the product (about 7 min). Internal temperature was monitored using a portable skewer digital thermometer (Hanna Instruments®, Woonsocket, RI, U.S.A.). Burgers were chilled at room temperature for 30 min, and then in a laboratory refrigerator (4 ± 1 °C).

After about 24 h burgers were subjected to analyses. Measurement of texture, analysis of basic chemical composition, and evaluation of sensorial attributes of cooked burgers were carried out. Energy value of burgers was calculated. Fatty acid composition of burgers was determined in products manufactured in the last replication of experiment. The experiment was replicated four times on four different days using different batches of poultry meat and one batch of pork jowl.

2.2 Determination of thermal processing yield

Ten raw burgers from each formulation were weighted immediately after forming. After chilling the products to room temperature, the same burgers from each formulation were weighted again to measure the weight loss by the difference and to calculate the processing yield, which was expressed as percentage.

2.3 Textural analysis

The textural properties of cooked and chilled (4 ± 1 ^{o}C, 24 h) burgers were evaluated using the universal testing machine Zwicki 1120 (Zwick GmbH & Co., Ulm, Germany) equipped with the Warner-Bratzler blade. Shear force (N) - the maximum value of the force registered during moving the blade through the sample - was calculated at the speed of crosshead of 50 mm/min. The tests were carried out on cuboidal-shaped samples cut out of burgers (3 cm x 9 cm x 0.9 cm; width x length x height). Five replicates were measured from five burgers of each formulation. The reported results are calculated mean values.

2.4 Chemical analyses

Chemical analyses were carried out on cooked and chilled (4 ± 1 ^{o}C, 24 h) burgers according to Polish Standards. For chemical analyses three pieces of burgers of each formula were randomly collected and ground twice in an electric mini food grinder (Zelmer SA, Rzeszów, Poland) with a plate having 2-mm-dia orifices.

Moisture (g water /100 g sample) was determined by drying a 3 g sample at 105 ^{o}C to constant weight according to PN-ISO 1442: 2000 (Polish Standard, 2000b). Protein (g protein /100 g sample) was evaluated by Kjeldahl method in accordance with PN-A-84018: 1975 (Polish Standard, 1975) using a Kjeltec System 1026 Distilling Unit (Foss Tecator, Höganäs, Sweden). Fat (g fat /100 g sample) was assayed by Soxhlet method according to PN-ISO 1444: 2000 (Polish Standard, 2000c) using a Büchi Extraction System B-811 (Büchi Labortechnik AG, Flawil, Switzerland). Fat content was calculated by weight loss after a 30-cycle extraction with petroleum ether. Chlorides (g chlorides /100 g sample) were determined by Mohr method according to PN-A-82112: 1973 /AZ1:2002 (Polish Standard, 1973). Ashing of sample (g ash /100 g sample) was performed at 550 ^{o}C to constant weight according to PN-ISO 936: 2000 (Polish Standard, 2000a).

All determinations were performed in duplicate. The reported results are calculated mean values.

2.5 Energy value calculation

Energy value of burgers was calculated basing on the assayed content of protein and fat, and the assumed amount of the inulin addition. Energy conversion factors corresponding to protein and fat were based on the recommendation of Council Directive 90/496/EEC (1990). For 1 g of inulin - used as dietary fibre - energy conversion factor of 8 kJ (2 kcal) was adopted, which was consistent with the recommendations of the Commission Directive 2008/100/EC (2008).

2.6 Analysis of fatty acid composition

Fatty acid composition of chicken burgers was determined only ones, that is in products obtained in the last replication of experiment. The analyses of fatty acids were carried out in cooked and chilled (4 ± 1 ^{o}C, 24 h) products. The method modified by Folch and others (1957) was used for lipid extraction from product sample. One hundred milligrams of the obtained lipids was esterified to obtain fatty acid methyl esters (FAMEs). FAMEs were separated by gas chromatograph Agilent Technologies 7890A equipped with a split injector, capillary column Restek 2330 (length 105 m, internal diameter. 0.25 mm, film thickness 0.2 μm), flame ionization detector (FID), and work station. Analysis parameters were as follows: helium as carrier gas at a constant flow of 1,2 ml /min, inlet and detector temperature: 250 °C and 300 °C respectively, inlet split 1:50. The temperature of oven was programmed as follows: initial temperature was 100 °C for 4 min, then rose to 240 °C at 3 °C /min. The final temperature of oven was held till the elution of the last peak on chromatogram. The FAMEs were indentified by comparing their retention times with those of the commercial standard FAMEs (Supelco 37 Component FAME Mix, Sigma-Aldrich, St. Louis, MO, U.S.A.). Quantification was as area percentages. The analyses of fatty acid composition were performed in duplicate in each sample. The mean values were calculated and expressed as the results.

2.7 Sensory evaluation

Sensory evaluation of burgers was conducted in accordance with PN-ISO 4121: 1998 (Polish Standard, 1998). Burgers were evaluated by eight panelists, who were recruited from the stuff and students of the Warsaw Univ. for Fife Sciences-SGGW (Warsaw, Poland). All the panelists were experienced in sensory evaluation of meat products. The selection of assessors was based on recommendations of PN-ISO 8586-1: 1996 (Polish Standard, 1996). The analyses performed in individual booths under white fluorescent lights. The burger samples were served to the panelists immediately after warming up on the electric grill to an internal temperature of 55 - 60 °C. Using a 10-point line scale (0 = very undesirable, 10 points = very desirable) the acceptability of colour, aroma, taste, hardness, juiciness, and consistency was evaluated. At the end of the test, panelists were asked to give a score for general quality of the products from 0 to 10.

2.8 Statistical analysis

Statistical analysis (one-way analysis of variance) was applied to the data to determine differences ($P<0.05$). To discover where there were significant differences between the levels of the main factor, contrasts (Tukey test) between means were made. Pearson's correlation coefficient (r) was calculated to determine the linear correlation between chosen quality attributes of chicken burgers. The statistical analyses were done using the statistical package Statgraphics Plus 4.1 (Manugistics, Inc. Rockville, MD., U.S.A.).

3. Results and Discussion

3.1 Thermal processing yield and texture of chicken burgers

The thermal processing yield of burgers of all formulations was approximately 80 % (Table 2). Increasing the level of inulin did not significantly ($P > 0.05$) decrease the yield of the products.

The obtained results were in agreement with results of other authors, who had applied inulin in order to improve the nutritional value and sensory characteristics of processed meat products. Ergönül and others (2009) found that the application of powdered inulin as a prebiotic ingredient to turkey meat balls did not increase the thermal loss. On the contrary, Beriain and others (2011) reported that inulin may affect the production yield of raw fermented low-salt sausages, due to slowing mass losses during ripening process.

In other studies inulin was used as the replacer of animal fatty raw material to lower the energy value of processed meat products. Substitution of animal fat with inulin gel usually caused an increase in cooking loss (that is decreased the production yield), what was found in canned meat products (Makała, 2003, Florowski & Adamczak, 2010) and pâtés (Florowski & others, 2008).

The addition of inulin did not differentiate significantly ($P > 0.05$) the shear force of burgers (Table 2). Only the slight decrease of this texture parameter was found with increasing addition level of inulin. The insignificant differences in shear force of cooked burgers may be explained by the fact, that inulin was not used as the substitute of animal fatty raw material. That means that no additional quantity of water was added to the meat batters.

The results obtained in this work agreed with results published by Ergönül and others (2009) who reported no negative effect of inulin – when used as a prebiotic - on instrumentally measured hardness of meat balls. However, addition of 2.5 or 5.0 % of powdered inulin into the batter caused a slight increase of hardness, when compared to the control product. Mendoza and others (2001) also found that addition of inulin did not affect hardness of raw sausage, but might decrease springiness of the final product. However, textural analysis conducted by García and others (2006) indicated that application of powdered inulin increased hardness in both low-fat and full-fat mortadella. When inulin was incorporated into the meat batters as a gel (that is in rehydrated form), the sausages were softer than the control products.

It should be noted that inulin may have a significant influence on the texture of processed meat products, especially when it is used for replacement of animal fatty raw material in the recipe. Florowski and Adamczak (2010) found the decrease of instrumentally measured hardness, cohesiveness, springiness and chewiness of canned meat products formulated with different quantities of inulin gel. Makała (2003) also reported that partial replacement of animal fatty raw material may change the rheological parameters of canned meat product, for example plasticity, but not slice strength.

3.2 Chemical analyses and energy value of chicken burgers

Compositional analysis of chicken burgers revealed slight differences in all the chemical components (Table 3). Except for chlorides, increasing the level of inulin in the formula significantly ($P < 0.05$) increased the content of moisture and ash, and decreased ($P < 0.05$) the content of fat and protein, when compared with control product

(PC). The differences for chemical components between the four burger formulations were most likely due to effect of inulin on the thermal processing yield. However, no significant correlation between moisture content and thermal processing yield was found in this study.

The results obtained in this study are similar to results of Beriain and others (2011) who found that addition of inulin – used as nutritional additive – may slightly, but significantly decrease the content of fat and protein, and increase the moisture content of raw fermented sausage. Although the ripening process of sausages formulated with inulin was not disturbed, the drying process was slowed down. On the contrary, Ergönül and others (2009) reported that the application of inulin did not significantly differentiate the content of moisture, protein, and fat in baked turkey meat balls in comparison to the control product.

As it was found in other studies, incorporation of inulin into the formula of processed meat product may significantly differentiate its chemical composition, especially when animal fatty raw material is replaced with inulin gel. As had been expected, the inverse relation between content of fat and moisture was observed in different types of meat products formulated with inulin: pork meat balls (Flaczyk & others, 2009), mortadella (Brauer, 2005; García & others, 2006; Nowak & others, 2007), scalded and liver sausages (Nitsch 2006, Tröger & others, 2005), baked pâté (Florowski & others, 2008), canned pork product (Makała, 2003) and dry fermented sausage (Mendoza & others, 2001).

Although in this study inulin was not used as the partial substitute of animal fatty raw material, that is the quantity of the pork jowl was the same in all burger formulations (Table 1), the energy value of chicken burgers decreased significantly ($P < 0.05$) with increasing the level of inulin to 2.0% or more (Table 4). The reduction of the energy value did not exceed 7% in product P3 when compared with the control product (PC). The differences for energy values of burgers may be explained by differences in thermal loss and chemical composition between all the four product formulations. The obtained results should provide a basis for further research.

The results obtained by Makała (2003), Brauer (2005), Nowak and others (2007), and Flaczyk and others (2009) showed that the energy value of processed meat products may be reduced to a much higher extent (even up to 70%), provided that the inulin gel is used to replace the animal fatty raw material in the formula.

3.3 Fatty acid composition of chicken burgers

The aim of the fatty acid composition analysis was to obtain the preliminary results for further research into improvement of the nutritional value of this chicken burgers. One of the objectives of the research project is to modify the fatty acid composition of burgers according to the recommendations of dietary guidelines.

The obtained results indicated that independent of the inulin addition level the burgers were characterized by similar content of all the groups of fatty acids (Table 5). In burgers of all the four formulations palmitic acid (C16:0) and stearic acid (C18:0) dominated among saturated fatty acids (SFAs). Palmitic acid is – in addition to myristic acid (C14:0) and lauric acid (C12:0) – used by the human organism for the synthesis of cholesterol, why the stearic acid has a neutral effect on blood cholesterol level (Simopoulos, 2000).

All the products were quite rich in unsaturated fatty acids (UFAs). In burgers of all formulations oleic acid (C18:1) dominated among monounsaturated fatty acids (MUFA), whereas the main polyunsaturated fatty acids (PUFAs) were: linoleic acid (C18:2, n-6) and α-linolenic acid (C18:3, n-3). The PUFAs are important components of all cell membranes in the human body. The two classes of PUFA: n-6 and n-3 have opposing physiological functions. Therefore, their balance is important for homeostasis, normal growth and development (Simopoulos, 2000). The ratio of PUFAs to SFAs, and the ratio of PUFAs n-6 to PUFAs n-3 are often used to characterize the nutritional value of lipids in foods. As recommended, these ratios should not be lower than 0.4, and not exceed 4.0, respectively (Givens & others, 2006). The obtained results suggest that the nutritional value of fat of chicken burgers might be improved, in terms of the relationship between PUFAs n-6 and PUFAs n-3. Due to the deficiency of PUFAs n-3 in the diet of average European, nutritionists postulate to increase consumption of food products both naturally rich in PUFAs n-3 and enriched with these components (Dybkowska & others, 2004, Givens & others, 2006).

Lack of information about the effect of inulin on fatty acid profile of processed meat products may be explained by fact that the application of inulin is not intended to modify the composition of fatty acids, but rather to lower the energy value of the product and to give the product the acceptable sensorial characteristics. Jiménez-Colmenero (2007) points out that useful method for improving the fatty acid profile of processed meat products is, for example, partial substitution of the animal fatty raw material with plant oil. In products enriched with oil inulin may help to achieve the desired sensory quality, what was confirmed by Beriain and others (2011) in raw fermented sausages.

3.4 Sensory evaluation of chicken burgers

The results of sensory evaluation of burgers showed that products of all the formulations were acceptable in sensory characteristics (Table 6). The application of inulin slightly, but significantly ($P < 0.05$) differentiated all the sensory attributes of burgers. In comparison to control product (PC), burgers formulated with 1.0 or 2.0 % of inulin (formulations P1 and P2, respectively) were scored significantly ($P < 0.05$) better for color, aroma and taste.

The products PC and P1 were scored significantly better ($P < 0.05$) for hardness than the two others, what means that the addition level of inulin higher than 1.0 % had negative effect on the hardness of burgers. Decrease of shear force of burgers with increasing addition level of inulin to the batter was detected by the panelists. However, there was no significant relationships ($r = 0,31$) between the hardness and the instrumentally measured shear force of chicken burgers.

Addition of inulin gel did not enhance the acceptability of juiciness of burgers. On the contrary, juiciness of the products P1, P2, and P3 deteriorated slightly, but significantly ($P < 0.05$) in comparison to the control product. The panelists found burgers with inulin to be "too watery". The juiciness of burgers was negatively, but not significantly correlated with the water content in products ($r = - 0,18$).

The products PC and P1 got the highest general quality scores in the sensory evaluation, being scored significantly ($P < 0.05$) better than the two others. The results also indicated that the product with 1.0 % of inulin was scored the highest notes for the five sensory attributes.

Ergönül and others (2009) also reported that addition of inulin did not decreased the general sensory quality of turkey meat balls. The results of other studies showed that inulin – when used as the animal fatty raw material replacer - may have negative effect on sensorial characteristics of processed meat products (Jánávary, 2005; Makała, 2003). On the contrary, Florowski and others (2008), and Flaczyk and others (2009) found the positive effect of animal fat replacement with inulin on the juiciness of pâtés and the texture of meat pork meat balls, respectively.

4. Conclusion

In conclusion, acceptable chicken burgers with 1.0 % of inulin (addition level as powdered inulin in relation to the weight of meat, fatty raw material, and water) can be produced. The application of inulin did not cause significant decrease of the thermal processing yield nor the shear force of the products. Although all the burgers were sensory acceptable, the product with 1.0 % of inulin was scored the highest notes for the color, aroma, taste, hardness, and juiciness. To improve the nutritional value of burgers the modification of fatty acid profile is also recommended.

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Notes

Note 1. [a] in relation to the mass of chicken meat and pork jowl (total raw materials).

Note 2. [b] in relation to the mass of chicken meat, pork jowl and water for the above table.

Table 1. Formulations of chicken burgers

Ingredient	Formulations of burgers			
	PC	P1	P2	P3
Chicken thigh meat (%)	85.0	85.0	85.0	85.0
Pork jowl (%)	15.0	15.0	15.0	15.0
Total raw materials (%)	100.0	100.0	100.0	100.0
Water [a] (%)	15.0	15.0	15.0	15.0
Sodium chloride [b] (%)	1.8	1.8	1.8	1.8
Soy protein isolate [b] (%)	1.5	1.5	1.5	1.5
Black & herbal pepper mixture [b] (%)	0.3	0.3	0.3	0.3
Inulin [b] (%)	-	1.0	2.0	3.0

Table 2. Thermal processing yield and shear force of chicken burgers

Formulations of burgers	Thermal processing yield (%) ± SD	Shear force (N) ± SD
PC	81.9 a ± 1.6	29.1 a ± 3.8
P1	81.0 a ± 1.7	29.1.a ± 4.0
P2	80.2 a ± 1.2	27.5 a ± 5.8
P3	78.8 a ± 2.1	27.2 a ± 5.3

PC - control product, P1 – product with 1.0% of inulin, P2 – product with 2.0% of inulin,. P3 – product with 3.0% of inulin.

Values in the same column bearing the same letters are not significantly different ($P > 0.05$);

SD - Standard Deviation.

Table 3. Chemical composition of chicken burgers

Formulations of burgers	Moisture (%) ± SD	Protein (%) ± SD	Fat (%) ± SD	Chlorides (%) ± SD	Ash (%) ± SD
PC	63.9 a ± 0.60	19.3 c ± 0.32	12.8 c ± 0.06	2.2 a ± 0.05	1.7 a ± 0.17
P1	64.5 ab ± 0.36	18.4 b ± 0.24	12.5 bc ± 0.30	2.1 a ± 0.06	2.0 ab ± 0.16
P2	64.8 b ± 0.26	17.8 ab ± 0.38	12.1 b ± 0.25	2.2 a ± 0.08	2.3 b ± 0.12
P3	65.1 b ± 0.26	17.5 a ± 0.31	11.5 a ± 0.37	2.0 a ± 0.05	2.5 b ± 0.23

PC - control product, P1 – product with 1.0% of inulin, P2 – product with 2.0% of inulin,. P3 – product with 3.0% of inulin.

Values in the same column bearing the same letters are not significantly different (P > 0.05);

SD - Standard Deviation.

Table 4. Energy value of chicken burgers

Formulations of burgers	Energy value (kJ/100 g) ± SD	Caloric value (kcal/100 g) ± SD
PC	803.6 c ± 3.54	192.9 c ± 0.82
P1	775.7 bc ± 8.61	188.6 bc ± 2.09
P2	754.3 ab ± 4.89	184.1 ab ± 1.59
P3	734.9 a ± 2,66	179.3 a ± 4.40

PC - control product, P1 – product with 1.0% of inulin, P2 – product with 2.0% of inulin,. P3 – product with 3.0% of inulin.

Values in the same column bearing the same letters are not significantly different (P > 0.05);

SD - Standard Deviation.

Table 5. Fatty acid composition of chicken burgers (values are expressed as g /100 g total fatty acids)

Fatty acid/ Fatty acid group	Formulations of burgers			
	PC	P1	P2	P3
C16:0	21.74	22.05	21.93	21.94
C18:0	9.95	10.79	10.56	10.13
Total saturated fatty acids (SFAs)	33.52	34.74	34.46	33.93
C18:1 *trans-9*	0.22	0.26	0.25	0.23
C18:1 *cis-9* Oleic	40.32	40.30	40.62	40.23
C20:1 *cis-11*	0.77	0.78	0.79	0.76
Total monounsaturated fatty acids (MUFAs)	44.64	44.29	44.69	44.46
C18:2 *all,cis-9,12(n-6)* Linoleic	13.37	12.81	12.97	13.71
C18:3 *all,cis-6,9,12(n-6)*	0.10	0.09	0.09	0.09
C18:3 *all,cis-9,12,15(n-3)* α-Linolenic	1.14	0.84	0.91	1.12
C20:2 *all,cis-11,14(n-6)*	0.62	0.65	0.67	0.64
C20:3 *all,cis-8,11,14(n-6)*	0.12	0.13	0.12	0.11
C20:3 *all,cis-11,14,17(n-3)*	0.11	0.11	0.12	0.12
C20:4 *all,cis-5,8,11,14(n-6)*	0.71	0.84	0.65	0.51
C20:5 *all,cis-5,8,11,14,17(n-3)* EPA	0.05	n.d.	n.d.	0.05
C22:6 *all,cis-4,7,10,13,16,19(n-3)* DHA	0.13	n.d.	n.d.	0.10
Total polyunsaturated fatty acids (PUFAs)	16.35	15.52	15.58	16.54
Total unsaturated fatty acids (UFAs)	60.99	59.81	60.27	61.00
PUFAs : SFAs	0.49	0.47	0.45	0.49
PUFAs *n-6*	14.92	14.52	15.20	15.06
PUFAs *n-3*	1.43	0.95	1.03	1.48
PUFAs *n-6* : PUFAs *n-3*	10.43	15.28	14.76	10.17

PC - control product, P1 – product with 1.0% of inulin, P2 – product with 2.0% of inulin,. P3 – product with 3.0% of inulin.

n.d. - not detected (the content of the fatty acid was lower than 0.05 g animal fatty raw material /100 g of total fatty acids).

Table 6. Sensory evaluation of chicken burgers

Formulations of burgers	Color ± SD	Aroma ± SD	Taste ± SD	Hardness ± SD	Juiciness ± SD	General quality ± SD
PC	6.0 a ± 0.12	6.6 a ± 0.12	5.9 a ± 0.09	6.9 c ± 0.09	7.1 b ± 0.09	6.5 a ± 0.33
P1	6.4 b ± 0.14	7.0 b ± 0.08	6.6 b ± 0.08	7.0 c ± 0.08	6.9 a ± 0.09	6.4 a ± 0.31
P2	6.3 b ± 0.09	6.9 b ± 0.08	6.4 b ± 0.26	6.6 b ± 0.13	6.8 a ± 0.08	6.2 b ± 0.20
P3	6.2 b ± 0.17	6.9 b ± 0.09	6.1 a ± 0.19	6.4 a ± 0.11	6.8 a ± 0.08	6.2 b ± 0.21

PC - control product, P1 – product with 1.0% of inulin, P2 – product with 2.0% of inulin,. P3 – product with 3.0% of inulin.

Values in the same column bearing the same letters are not significantly different ($P > 0.05$);

SD - Standard Deviation.

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Sensory Analysis of Pawpaw (*Asimina triloba*) Pulp Puree: Consumer Appraisal and Descriptive Lexicon

Robert G. Brannan (Corresponding author)
School of Applied Health Sciences and Wellness, Ohio University
E334 Grover Center, Athens, OH 45701, USA
Tel: 1-740-593-2879 E-mail: brannan@ohio.edu

Dane E. Salabak
Federal Hocking High School
8461 State Route. 144, Stewart, OH 45778, USA
Tel: 1-740-662-6691 E-mail: dsalabak@fedhock.com

David H. Holben
School of Applied Health Sciences and Wellness, Ohio University
E334 Grover Center, Athens, OH 45701, USA
Tel: 1-740-593-2875 E-mail: holben@ohio.edu

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Abstract

Consumer and descriptive sensory analysis was performed on pawpaw pulp. Consumer sensory analysis showed that mango was preferred compared to the pawpaw, but that only one-third of those who preferred the mango were correctly able to identify it. Consumers generated 25 flavor descriptors for pawpaw pulp, with banana and mango being the most identified. Descriptive sensory analysis was performed on pawpaw pulp that was stored frozen in the presence or absence of air and with and without heat treatment. Differences in color were detected, however no differences in any of the sensory attributes were detected during 12 months of frozen storage, suggesting that the flavor of pawpaw pulp is stable during frozen storage. The comprehensive analysis of the sensory and quality of pawpaw pulp described in this paper, including the development of a defined, standardized pawpaw sensory lexicon, is an important step in the evolution of pawpaw research.

Keywords: Pawpaw, Consumer sensory analysis, Descriptive sensory analysis

1. Introduction

Pawpaw [*Asimina triloba* (L.) Dunal] fruit grow on large trees in the eastern United States in a range that covers parts of northern Florida to southern Ontario and as far west as Nebraska, (K. W. Pomper, Layne, & Peterson, 1999), an area that comprises all of Appalachia. Among the families in the order Magnoliales, the pawpaw belongs to the Annonaceae family, the tropical custard-apple family. All but one of the 130 genera of the Annonaceae family thrives in the tropical region. Only the genus Asimina grows in the temperate climate zone (Callaway, 1993), specifically the USDA growing zone 5 (Pomper, Layne, & Peterson, 1999).

Pawpaws are climacteric fruits. Ethylene and respiratory climacteric peaks are clearly evident in the pawpaw within 3 days after harvest (Archbold & Pomper, 2003). Depending on the ripeness of the pawpaw when it is picked, the ultimate quality of the ripe fruit can be determined (McGrath & Karahadian, 1994b). Additionally, it has been found that the pawpaw will go from ripe to overripe in a matter of days at room temperature, however this process can be extended to 2-3 weeks while under refrigeration (Templeton, Marlette, Pomper, & Jones, 2003). Because of the limitations in their perishability, pawpaws are not commonly seen in processing or fresh markets. Ripening pawpaws have shown an increase in soluble solids concentration (exceeding 20%), softening

of the flesh, increased production of volatiles, and some genotypes have demonstrated a decline in green color (McGrath & Karahadian, 1994b). The volatiles produced during ripening are mostly ethyl and methyl esters (Shiota, 1991). Most researchers believe that color change cannot be accepted as a reliable indicator of pawpaw fruit ripeness because varying findings have been reported.

Research suggests that pawpaw fruit pulp has the potential to be added to various consumer goods to add increased nutritional benefits or flavor enhancement. The intense, tropical-fruit-like flavor makes it a potential source of natural fruit flavor (McGrath & Karahadian, 1994a). Sensory analysis on various pawpaw products at the 2nd Annual Pawpaw Field Day at Kentucky State University (KSU) in Frankfort in 1999, using 105 untrained sensory participants showed that acceptance of products varied by age and previous consumption of pawpaw. Younger participants (under 40) who had not had pawpaw before preferred sweeter products (cake, and ice cream) when compared to custard and juices (Templeton, et al., 2003). Previous researchers have utilized the pawpaw as a partial fat-reducing agent in muffins (Duffrin, Holben, & Bremner, 2001), and shortened cake (Wiese & Duffrin, 2003).

Consumers' decision to eat fruit depends on personal preference, nutritional properties, and environmental factors. Past studies have demonstrated that education level, gender, age group, and income level can affect frequency and amount of fruit intake. In addition, people with positive beliefs and attitudes towards healthy eating generally have an increased intake of fruits. Familiarity towards a product or fruit can also help or hinder a consumer's purchase (Kamphuis et al., 2006). Many studies mention that sensory characteristics, health considerations, and pleasure-seeking factors are the main reasons humans consume fruit and continue to consume fruit. In an assessment of consumer responses to an off-flavor in fruit juices, it was revealed that taste perception is a significant factor of level of consumption, and that consumption might discontinue if the first taste impression of the fruit is poor (Tuorila & Cardello, 2002).

From a sensory standpoint, the literature shows a wide range of tastes, aromas, and other descriptors being used to describe the pawpaw. The flesh has a smooth, custard-like texture (Pomper, Crabtree, Lowe, & Lehman, 2009) that has been described to be reminiscent of an avocado (Kral, 1960). The color of the pulp ranges from creamy white to bright yellow or even shades of orange in color (Pomper, et.al., 1999).

Past research has used untrained sensory panelists to conduct descriptive analysis on pawpaw pulp. A list of descriptors used to describe the fruit consisted of apple, banana, mango, melon, fresh, raw, and top note (Duffrin & Pomper, 2006). Most commonly, the flesh of the fresh fruit is described as having a tropical aroma and flavor that is often compared to a mixture of mango, banana and pineapple (Duffrin & Pomper, 2006). However, according to an untrained panel, frozen pawpaw has been described as tasting sour, bitter and resembling melon (Duffrin & Pomper, 2006). The similarity of aroma and tastes of the pawpaw to other tropical fruits may make it difficult to differentiate (Shiota, 1991). Poor-quality pawpaws have a mushy texture, lack sweetness, have an overly strong flavor, and bittersweet aftertaste, whereas high-qulity pawpaws that have a firm texture, a delicate blend of flavors, and no bitter aftertaste (Duffrin & Pomper, 2006).

Currently, no standard descriptive lexicon exists for pawpaw and many descriptors for pawpaw involve the use of other tropical fruits, which in themselves may have limited familiarity. The purpose of this study was twofold. Consumers were employed to characterize how well-known pawpaw fruit is and how much it is liked compared to more common tropical fruits (mango, papaya). Then, a descriptive sensory panel developed a descriptive lexicon for pawpaw and used it to monitor changes in pawpaw pulp during frozen storage.

2. Materials and Methods

2.1 Materials

All food ingredients were purchased from local retailers with the exception of pawpaws. Wild pawpaws from a single tree were donated for the study. This particular pawpaw tree has won two first-place awards and a second-place award in recent years at the "Best Pawpaw Contest" during the yearly Pawpaw Festival in Albany, Ohio, based on weight, appearance, skin surface, aroma, skin thickness, flavor, texture, aftertaste, and number of seeds. Frozen pawpaw pulp for panel training was purchased from a local pawpaw processor (Integration Acres, Inc., Albany, OH).

2.2 Sample preparation

The pawpaw pulp from each pawpaw was separated from the skins and seeds by hand. All of the pulp was pooled and divided into 100g portions. Once portioned, the pawpaw pulp was placed into randomly selected polyethylene/nylon 27.94-cm bags (FoodSaver, Jarden Corp., Rye, NY) with an oxygen transmission rate of 6.7 cc/m^2/24 h/23°C/0% RH. Once the bags were filled, they were either vacuum sealed (vacuum) or sealed without

attempting to remove air prior to sealing (air). Some of the bags were heat treated (cooked) in boiling water, until they reached an internal temperature of 75°C. The bags were transferred into frozen storage at -18 ºC. At two month intervals, pawpaw samples were transferred from -18 ºC to a freezer at -40ºC to maintain pawpaw quality until the day that sensory analysis was performed.

2.3 Sensory analysis

Sensory testing was performed using protocols approved by the Ohio University Institutional Review Board for the protection of human subjects. Ninety eight participants were recruited for the consumer study. After providing demographic data, each participant evaluated a randomly coded sample of pureed pawpaw pulp. Water was provided. Participants were asked, "Please taste the tropical fruit pulp. Identify as many tropical fruit flavors as you can and write them in the order of their intensity." Room was provided for five responses to this question. For the second set of samples, participants were presented with three cups of pureed fruit (mango, papaya, pawpaw) and asked, "Please taste the three tropical fruit samples on your tray. Rank them in the order of 1 (most liked), 2 (middle), and 3 (least liked). There are no ties. You must rank them 1, 2, 3. Then, guess what each of the tropical fruits are."

A descriptive sensory panel consisting of six trained members was used to identify and evaluate sixteen attributes in previously-frozen pawpaw pulp. After training, two of the panelists chose not to continue participation in the study, so the decision was made to continue the sampling only with the remaining four panelists. Prior to this study, the descriptive sensory panel was trained for 17 hours on recognition of basic tastes, oral perceptions including texture and mouthfeel, and on how to develop a sensory lexicon prior to sampling for research (Mah & Brannan, 2009). Approximately 26 additional hours were utilized to train the sensory panel on perception of flavors from the complex flavor profile of the pawpaw. Testing using complex solutions, as well as fresh and processed pawpaw pulp was utilized in the training of the panel. The panel used the results from the consumer study (Table 1) as well as aroma descriptors (McGrath & Karahadian, 1994b) and the "pawpaw flavor wheel" (Duffrin & Pomper, 2006) from previous studies as the basis for their development of a pawpaw lexicon. Descriptions and anchored references of the pawpaw sensory attributes are shown in Table 2. Room temperature samples (~30 g) were presented to panelists in small cups. Six randomized samples coded with a randomly generated three-digit number were tested one at a time at each sampling session. Panelists were supplied with a set of standards for each attribute that they could use throughout the tasting session, shown in Table 1, unlimited water and unsalted saltine crackers, and a ballot with a 15-cm line scale anchored with standards.

2.4 Color analysis

The CIE L*, a*, and b* values of thawed pawpaw pulp were measured using a Konica BC-10 (Konica Minolta Sensing Americas Inc., Ramsey, NJ). The meter was calibrated against a standard white plate before each use. The lightness (L*) and chromaticity coordinates (a*, b*) were calculated as the mean of three readings.

2.5 Texture analysis

Penetrometry tests using a Ta-XT2i Texture Analyzer (Texture Technologies Corp., Scarsdale NY/Stable Micro Systems, Godalming, Surrey, UK) were used to evaluate the texture of the pawpaw pulp by loading 5g of sample into a 18x63mm glass test tube and penetrating 60 mm at 5 mm/s with a TA-23 ½" Dia (13 mm)-¼ R probe. The grams of force required to compress the sample was recorded using Texture Expert software that controlled the texture analyzer.

2.6 Statistical analysis

A 7 x 2 x 2 full factorial design was constructed with three factors; month of storage (0, 2, 4, 6, 8, 10, 12), packaging condition (vacuum, air), and heat treatment (raw, cook). The complete design was not replicated due to the scarcity of sample. All statistical analysis was performed using the PASW Statistics 18 (IBM Corporation, Armonk, New York). Means for color and texture were generated from three observations. Means from descriptive sensory analysis for each patty were generated from the individual ratings of the four panelists. Analysis of Variance (ANOVA) was used to analyze differences between treatments and post-hoc means separation was achieved using Duncan's Multiple Range test. Exploratory factor analysis and theoretical fit was used to reduce the dimensionality of the data. Using Cronbach's Alpha, set at 5 percent, some of the 13 competency statements were categorized into a "factor," with varimax (orthogonal) rotations used to validate the factor analysis. Five factors were identified based on a Kaiser criterion of Eigenvalue greater than or equal to 1.0. Attributes with a variance greater than 0.6 were considered to be within a factor and the attribute was then loaded into the corresponding factor. Items below this variance were considered weakly correlated and eliminated, this in turn increased the reliability of the remaining items.

3. Results and Discussion

Pawpaw flavor is often referred to as a combination of banana, mango, papaya, and pineapple, however these flavor descriptors are often reported by tasters who are very familiar with tropical fruit in general and pawpaw in particular. In this study, a consumer sensory test utilized 98 subjects of varying degrees of familiarity with tropical fruit to assess familiarity of tropical flavors and to generate a pawpaw flavor lexicon. The demographics of the subjects in this study skew young and female (Table 3).

3.1 Tropical fruit flavors in pawpaw pulp

Table 1 shows consumers' identification of the "tropical" flavors when presented with a sample of pawpaw pulp. Of the 25 flavors in the pawpaw pulp identified by at least one consumer, banana and mango were identified as the most intense flavors by 77% of the consumers who made a choice and the second most intense flavors by 68%. Other flavors of note are citrus (orange, grapefruit, tangerine), papaya, and pineapple. The flavor "pawpaw" was identified only three times, once as the primary flavor, once as the third most intense flavor, and once as the fifth most intense flavor.

3.2 Preference of pawpaw compared to mango and papaya

However, a forced choice test in which consumers ranked their preference among mango, papaya, and pawpaw revealed that mango, papaya, and especially pawpaw flavors may not be very recognizable to consumers. As shown in Table 4, only one-third of consumers who selected mango as their favorite tropical fruit identified it correctly; only one-fourth of consumers who selected papaya as their favorite identified it correctly; and less than one in ten consumers who selected pawpaw as their favorite could identify it correctly. These results invite a different interpretation of Table 1, suggesting that consumers who identify tropical flavors, especially mango and papaya, may not actually be able to identify them.

With respect to preference, Table 3 shows that mango is preferred significantly compared to papaya and pawpaw overall and across most of the demographic classifications in this study. The data shown in Table 4 reinforces this conclusion as seven in ten consumers selected mango as their favorite, 4-5 fold higher than either papaya or pawpaw. Interestingly, identification of pawpaw seems to increase with increasing age, education, consumption of fruit, and consumption of tropical fruit (Table 3).

3.3 Descriptive sensory analysis of pawpaw pulp

Prior to this study, no thorough pawpaw lexicon for descriptive analysis of pawpaw had been generated. In this study, the trained descriptive panel began its lexicon development with three lists: 1) the twenty-five flavors identified by the consumer panel; 2) five aroma descriptors (fruity aroma, cut grass aroma, sweet aroma, melon-like aroma, and fermented aroma) identified by "a group familiar with pawpaw aroma characteristics" as cited by McGrath and Karahadian (1994b); and 3) eight flavor descriptors (apple, banana, mango, melon, citrus, estery, fresh, raw), three texture descriptors (viscosity, surface, body), and five appearance descriptors (viscosity, surface, body, color, intensity) generated by semi-trained students participating in a class project (Duffrin & Pomper, 2006). Using this as a basis, the panel developed and standardized the pawpaw lexicon that is described in Table 2.

The lexicon is composed of thirteen attributes including color, fermented odor, texture and two mouthfeel descriptors (body, astringency), two basic tastes (sweet, sour), five flavors (banana, melon, mango, papaya, tropical), and two aftertastes (bitter, rindy). A panelist effect was present. It has been reported that a panelist effect is not unusual in a descriptive analysis panel (N'Kouka, Klein, & Lee, 2004) and that it indicates that panelists may have been using the scales differently for evaluation even though they were anchored to standards. The panelist effect was identified early in training and much emphasis on intensity recognition of the standards in both group and individual settings was performed. Calibration by the individual judges based on reference means for each attribute was also attempted. None of these strategies alleviated the panelist effect from the final analysis.

The lexicon presented in Table 2 includes five attributes for which published sensory standards have been published (Meilgaard, Ceville, & Carr, 1999). The other attributes were standardized using foods available in the United States with comparable products likely to found in other countries, making the vocabulary universally applicable. The importance of universality of standards is underscored by the fact the recent Third International Pawpaw Conference (September 9-10, 2011) was truly international, with participants from Canada, the Netherlands, and Romania and an international update that included many more pawpaw-growing countries, especially in Europe.

3.3.1 Descriptive analysis of the main effect of month of frozen storage

As shown in Table 5, significant differences were observed during frozen storage of pawpaw pulp for the main effect of month of frozen storage as well as the two- and three-way interactions involving month of frozen storage for color (L*, a*, b*). Storage for 12 months produced pawpaw pulp that was measured to be significantly darker (i.e. higher L*), redder (i.e. more positive a*), and less yellow (i.e. less positive b*). Surprisingly, the differences that were observed via instrumental color analysis were not reflected in the sensory analysis, suggesting that the differences probably were slight enough not to be noticed. A discussion of factors that may affect discoloration during pawpaw storage occurs in section 3.3.2 of this paper. None of the sensory attributes were affected by frozen storage over the 12 month storage period.

3.3.2 Descriptive analysis of the main effects of packaging and heat treatment

With respect to the main effects of packaging and heat treatment, none of the sensory or color attributes were significantly affected by the presence or absence of air in the package or heat treatment before storage, except for the following exceptions: The samples that were exposed to air were more red (higher a*) and exhibited more body (i.e. thicker) than vacuum packaged pawpaw pulp. The samples that were not heat treated were more red (higher a*) and yellow (higher b*) than the heat treated samples. This difference was confirmed by the sensory panel who perceived the samples that were not heat treated to be significantly darker than the heat treated samples.

The two way interactions between packaging and heat treatment were significant for a*, suggesting that a relationship exists between the presence of oxygen and heat treatment. Most likely, this relationship involves the formation of colored pigments via the enzyme polyphenol oxidase, for which oxygen is required and heat would denature. Polyphenol oxidase has been shown to be present in pawpaw pulp (Fang, Wang, Xiong, & Pomper, 2007; Wang, Fang, Xiong, & Pomper, 2008) and likely is responsible for discoloration observed during refrigerated storage (Archbold, Koslanund, & Pomper, 2003). The discoloration of pawpaw pulp due to polyphenol oxidase has not been monitored in pawpaw pulp stored frozen.

3.4 Three way interactions of month of storage, packaging, and heat treatment

The three way interactions among month of storage, packaging, and heat treatment produced 28 distinct samples. Means for sensory and quality parameters of these samples are shown in Tables 6-8. Shown in Table 6, significant differences were observed for all three CIE color measurements (L*, a*, b*). There were few differences with respect to L*, however, it is worth noting that within the heat treated/vacuum stored sample set, the L* value significantly increased during the first four months of storage, from 53.8 to 68.9, then remained constant thereafter. The means of pawpaw pulp for the a* values were grouped into seventeen post hoc subsets. This very large number of subsets makes comparisons between and among samples difficult to interpret. Once again, the heat treated/vacuum stored sample set exhibited a clear pattern, with a* values decreasing during storage, from 7.0 at 0 month to 4.3 by 8 months of storage. During storage the cooked samples were often, but not always, significantly lower than their uncooked counterparts. The means of pawpaw pulp for the b* values were grouped into 9 post hoc subsets, but other than the fact that the lowest b* values were exhibited by the 12 month air samples, other interpretations of this data are difficult.

Descriptive sensory data for pawpaw flavor, odor, taste, aftertaste, and mouthfeel are shown in Tables 7 and 8. No significant differences were observed for any of the attributes. This is a surprising result since research from our laboratory has shown that phenolic and flavonoid compounds in pawpaw pulp are affected by frozen storage (Harris & Brannan, 2009). In spite of this, it appears likely that that the sensory attributes of pawpaw pulp are stable during frozen storage. Although there are no significant differences between any of the 28 three way interactions, the values are included in this paper because this is the first time that standardized descriptive sensory analysis of the pawpaw was undertaken, thus these values serve as a baseline for future research.

3.5 Principal components analysis of descriptive sensory data

The raw survey results were subjected to factor analysis and theoretical fit using Varimax rotations (Figure 1). The data was reduced in dimension from 14 sensory descriptors to three factors. These three factors accounted for more than 65% of the variance. Of the six sweet or "fruity" attributes in the original set, melon, banana, mango, and tropical were loaded into one of the three factors, while sweet and papaya were not. All of the factors contained at least one negative sensory attribute. The first factor, PC1, was composed melon, banana, and astringent. Banana was identified by consumers in Table 1 as the most intense flavor of the pawpaw, and astringency is inversely related to the ripening of banana. PC2 is composed of the competing descriptors of fermented odor and mango. Mango was identified by consumers in Table 1 as the second most intense flavor in

pawpaw pulp, although Table 4 shows that only 33% consumers can correctly identify it. Fermented odor is known to exist in pawpaw (Goodrich, Zjhra, Ley, & Raguso, 2006) and is generally considered a defect. PC3 is composed of sour aftertaste and tropical flavor. The tropical flavor descriptor was not necessarily a positive flavor descriptor in the sense that the standard reference for the attribute was a piece of canned papaya, whereas sourness is an important component of the sweet-acid balance of fruits. Overall, a reduction in the number of attributes via factor analysis may be beneficial for three reasons: 1) a large number of attributes (13) were developed for the pawpaw lexicon, making sensory analysis challenging; 2) a significant panelist effect was observed throughout the duration of training and sampling despite a concerted attempt to minimize it; and 3) fewer attributes will allow more straightforward elucidation of clusters of related terms.

4. Conclusion

This paper provides details of a comprehensive analysis of the sensory and quality of pawpaw pulp from a consumer and descriptive sensory perspective. The development of a defined, standardized pawpaw sensory lexicon is an important step in the evolution of pawpaw research which will allow scientific comparison between and among the 80+ known varieties. However, the consumer research yielded results that show that in spite of a standardized lexicon for the pawpaw, many consumers find it challenging to describe tropical fruits in general, even common ones like mango and papaya. This paper suggests that frozen storage may be a viable preservation option for pawpaw pulp and validates a commercial approach that is already being utilized by an entrepreneurial pawpaw processor (Integration Acres, Albany, OH). Future research should focus on validating and refining the pawpaw lexicon.

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Table 1. Consumers (n=98) free choice identification of tropical fruit flavors when presented pawpaw pulp in the order of their perceived intensity

Identified as Most Intense Flavor	Identified as 2nd Most Intense Flavor	Identified as 3rd Most Intense Flavor	Identified as 4th Most Intense Flavor
Banana (40)	Mango (24)	Mango (10)	Papaya (4)
Mango (24)	Banana (13)	Pineapple (9)	Mango (4)
Orange (7)	Papaya (8)	Orange (8)	Tangerine (3)
Papaya (7)	Orange (7)	Banana (7)	Guava (2)
Pineapple (5)	Grapefruit (2)	Papaya (4)	Pineapple (2)
Did not identify (15)	Did not identify (44)	Did not identify (60)	Did not identify (83)

Number in parenthesis indicates number of consumers who identified the flavor.

Table 2. Description and anchored references of sensory attributes generated by descriptive analysis of pawpaw pulp

Attribute	Description	References	Position (cm) [1]
Color	Color of the top surface of the pulp, detected prior to mixing[2]	Printed gradient color scale from: yellow, RGB values 255, 221, 0 brown, RGB values 106, 60, 0	 1 14
Fermented Odor	The degree of fermented odor, described as a complex combination of cloyingly sweet, fruity, and musty[2]	Overripe pawpaw pulp that had badly browned	4
Body	Mouthfeel sensation associated with the firmness, cohesiveness, and denseness of the pulp when compressed between the tongue and palate.[2]	Applesauce, unsweetened (Great Value brand) Creamed Wheat Cereal (Malt O Meal, prepared according to package, cooled to room temp.)	1 14
Sweet	The amount of sweet taste detected from the sample as it is being chewed before being swallowed or expectorated.[3]	Applesauce, unsweetened (Great Value brand)	4
Sour	The amount of sour taste detected from the sample as it is being chewed before being swallowed or expectorated.[3]	Applesauce, unsweetened (Great Value brand)	5
Banana	The amount of banana flavor detected from the sample as it is being chewed before being swallowed or expectorated.[3]	Banana pudding (Kroger brand, prepared according to package)	7.5
Melon	The amount of melon flavor detected from the sample as it is being chewed before being swallowed or expectorated.[2]	Honeydew melon, fresh, ½-1 inch cube	8
Mango	The amount of mango flavor detected from the sample as it is being chewed before being swallowed or expectorated.[2]	Mango (Del Monte brand, in light syrup)	8
Papaya	The amount of papaya flavor detected from the sample as it is being chewed before being swallowed or expectorated.[2]	Papaya, fresh, ½-1 inch strip	7
Tropical	The amount of tropical flavor detected from the sample as it is being chewed before being swallowed or expectorated.[2]	Papaya chunk (from Dole Tropical Fruit mix)	9.5
Bitter Aftertaste	The amount of bitter aftertaste detected from the sample after it is chewed and swallowed or expectorated.[3]	Black tea (Rose brand, steeped for 1 hour in hot water)	8
Rindy Aftertaste	The amount of rindy aftertaste detected from the sample after it is chewed and swallowed or expectorated.[2]	Orange rind, served with flesh and rind	9.5
Astringent	The amount of astringency on the tongue and in the mouth detected from the sample after it is chewed and swallowed or expectorated.[3]	Black tea (Rose brand, steeped for 1 hour in hot water)	6.5

[1] Position on 15-cm line scale.

[2] Generated by descriptive analysis panel.

[3] Adapted from Meilgaard et al. (1999).

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Table 3. Participant demographics, mean rankings for three tropical fruit purees from a three-way forced choice consumer ranking test, and percentage of participants who correctly identified pawpaw

	n	279 Papaya	852 Pawpaw	452 Mango	Correct Pawpaw Identification
Participant Demographics Overall	98	2.3 a	2.3 a	1.4 b	9%
Gender					
Male	26 (27%)	2.0 b	2.5 a	1.5 c	11%
Female	72 (73%)	2.4 a	2.2 a	1.4 b	8%
Age					
18-21	48 (49%)	2.2 a	2.4 b	1.4 b	4%
Older than 21	50 (51%)	2.4 a	2.2 a	1.5 b	14%
Education					
High School	4 (4%)	1.8 a,b	2.8 b	1.5 b	0%
Some College	60 (61%)	2.3 a	2.3 a	1.4 b	3%
Bachelor's	20 (20%)	2.2 a	2.5 a	1.4 b	10%
Master's or Ph.D.	14 (14%)	2.4 a	2.0 a,b	1.6 b	36%
Servings of Fruit Consumed per Day					
Less than 5	86 (88%)	2.3 a	2.3 a	1.4 b	8%
5 or more	12 (12%)	2.3 a	2.4 a	1.2 b	17%
Frequency of Consumption of Tropical Fruit per Week					
None	15 (15%)	2.5 a	2.2 a	1.3 b	7%
1-3	67 (68%)	2.3 a	2.3 a	1.4 b	7%
More than 4	16 (16%)	2.2 a,b	2.3 a	1.5 b	19%

Table 4. Frequency of fruit puree selected as the favorite (i.e ranked 1st) compared to the other two fruits in a three-way forced choice consumer ranking test. Correct Identification refers to the percentage of consumers who correctly identified the fruit puree that they ranked as favorite

Fruit	Favorite	Correct Identification
Mango	70%	33%
Pawpaw	16%	9%
Papaya	14%	26%

Table 5. P-values for the main effects of month of storage [0, 2, 4, 6, 8, 10, 12), packaging condition (vacuum, air), and heat treatment (raw, cooked), 2-way interactions, and 3-way interactions on sensory and quality attributes of pawpaw pulp

	Main Effects			2-way Interactions			3-way
	Month of Storage (MONTH)	Packaging Condition (PACK)	Heat Treatment (HEAT)	MONTH X PACK	MONTH X HEAT	PACK X HEAT	MONTH X PACK X HEAT
Color (sensory)	N.S.	N.S.	<0.001	N.S.	N.S.	N.S.	N.S.
L*	0.011	N.S.	N.S.	0.001	<0.001	N.S.	0.017
a*	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
b*	0.001	N.S.	<0.001	0.001	<0.001	N.S.	0.038
Body (sensory)	N.S.	0.031	N.S.	N.S.	N.S.	N.S.	N.S.
Fermented odor	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Banana flavor	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Melon flavor	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Mango flavor	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Papaya flavor	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Tropical flavor	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Sweet taste	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Sour taste	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Bitter aftertaste	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Rindy aftertaste	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Astringent	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S. = Not significant at $p<0.05$.

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Table 6. Mean values ± standard deviations of sensory color (n=4), sensory body (n=4), and CIE L*, a*, and b* values (n=6) for pawpaw pulp stored frozen raw or heat treated (Cook), and in the absence (vac) or presence (air) of air in the package. Different superscripts within a column denote significant differences at $P < 0.05$

Month	Treatment	Color		L*		a*		b*		Body	
0	Raw vac	6.3	± 1.8	67.9^{a}	± 0.1	5.4^{lmnop}	± 0.6	30.0^{efgh}	± 1.8	5.9	± 1.6
	Raw air	5.6	± 2.0	67.5^{ab}	± 0.4	7.0^{c}	± 0.2	33.2^{abcdea}	± 0.8	5.9	± 1.1
	Cook vac	5.6	± 0.6	53.8^{d}	± 1.4	4.9^{opq}	± 0.7	33.6^{abcd}	± 1.2	7.5	± 2.1
	Cook air	5.6	± 1.8	65.5^{abc}	± 0.6	5.5^{jklmn}	± 0.2	30.3^{defgh}	± 1.1	8.1	± 1.8
2	Raw vac	5.3	± 0.8	63.4^{abc}	± 1.8	6.6^{defg}	± 0.1	34.5^{ab}	± 1.1	7.0	± 2.6
	Raw air	6.5	± 1.0	64.8^{abc}	± 1.2	7.5^{bd}	± 0.5	32.4^{bcdef}	± 2.3	6.8	± 3.4
	Cook vac	5.8	± 0.9	65.4^{abc}	± 0.7	5.2^{mnop}	± 0.4	32.3^{bcdef}	± 1.5	7.4	± 1.4
	Cook air	3.9	± 0.8	64.2^{abc}	± 0.3	5.1^{nop}	± 0.5	30.6^{cdefgh}	± 1.6	8.0	± 3.0
4	Raw vac	5.9	± 1.2	66.1^{ab}	± 0.3	6.9^{de}	± 0.1	34.6^{ab}	± 2.6	4.7	± 1.7
	Raw air	6.1	± 0.7	67.6^{ab}	± 1.5	7.7^{bd}	± 0.4	35.9^{a}	± 0.1	7.7	± 2.2
	Cook vac	5.9	± 1.0	68.9^{a}	± 1.6	5.2^{mnop}	± 0.2	29.6^{fgh}	± 0.7	6.2	± 2.5
	Cook air	5.7	± 1.5	63.2^{abc}	± 0.5	6.1^{fghij}	± 0.1	28.0^{hi}	± 1.8	6.1	± 1.9
6	Raw vac	5.9	± 1.7	65.3^{abc}	± 0.6	7.6^{bd}	± 0.5	34.7^{ab}	± 0.6	8.3	± 2.5
	Raw air	6.8	± 1.7	62.1^{abc}	± 0.3	6.7^{def}	± 0.5	32.3^{bcdef}	± 3.1	6.8	± 2.8
	Cook vac	5.5	± 0.9	65.6^{abc}	± 0.4	5.9^{hijkl}	± 0.5	30.5^{cdefgh}	± 2.6	5.5	± 1.2
	Cook air	6.0	± 1.3	53.7^{d}	± 14.7	5.5^{klmno}	± 0.3	31.4^{bcdefg}	± 1.3	7.0	± 2.4
8	Raw vac	6.4	± 1.8	63.2^{abc}	± 1.2	6.5^{defgh}	± 0.2	33.9^{abc}	± 1.1	6.0	± 1.7
	Raw air	6.1	± 0.6	64.1^{abc}	± 0.9	7.8^{bd}	± 0.5	36.2^{a}	± 1.2	8.5	± 2.5
	Cook vac	5.0	± 0.7	62.0^{abc}	± 0.7	4.3^{q}	± 0.2	28.0^{hi}	± 2.6	5.9	± 1.6
	Cook air	4.8	± 0.4	64.1^{abc}	± 1.1	5.8^{ijklm}	± 0.4	28.3^{ghi}	± 2.5	7.1	± 2.6
10	Raw vac	6.0	± 1.0	60.7^{bc}	± 0.3	6.0^{hijkl}	± 0.2	33.5^{abcd}	± 1.3	5.5	± 2.8
	Raw air	6.5	± 0.9	61.8^{abc}	± 0.8	7.8^{bd}	± 0.5	32.5^{bcdef}	± 5.1	6.9	± 3.2
	Cook vac	5.2	± 1.5	63.4^{abc}	± 0.3	4.8^{pq}	± 0.4	30.1^{defgh}	± 1.0	7.4	± 1.6
	Cook air	4.5	± 0.5	64.3^{abc}	± 0.6	6.1^{fghij}	± 0.2	29.9^{efgh}	± 2.7	7.8	± 2.1
12	Raw vac	6.5	± 1.5	62.2^{abc}	± 1.9	6.3^{efghi}	± 0.6	32.3^{bcdef}	± 2.9	6.0	± 1.7
	Raw air	7.3	± 0.9	58.4^{cd}	± 0.7	9.6^{a}	± 0.4	27.5^{hi}	± 2.6	9.1	± 3.5
	Cook vac	5.0	± 1.9	63.7^{abc}	± 0.4	5.2^{mnop}	± 0.1	31.6^{bcdefg}	± 0.3	5.9	± 1.0
	Cook air	5.8	± 1.9	60.6^{bc}	± 1.3	6.1^{ghijk}	± 0.4	26.1^{i}	± 1.0	6.1	± 2.0

Table 7. Mean values ± standard deviations of descriptive sensory flavor attributes (n=4) for pawpaw pulp stored frozen raw or heat treated (Cook), and in the absence (vac) or presence (air) of air in the package

Month	Treatment	Banana		Melon		Mango		Papaya		Tropical	
0	Raw vac	5.1	± 1.1	4.4	± 2.1	5.0	± 2.0	4.3	± 1.4	2.2	± 1.1
	Raw air	5.1	± 1.8	4.8	± 0.8	5.6	± 1.2	4.8	± 1.3	2.2	± 1.7
	Cook vac	6.5	± 2.0	4.1	± 1.8	4.6	± 2.1	4.5	± 1.8	2.1	± 1.4
	Cook air	5.0	± 1.5	4.6	± 1.3	4.5	± 2.0	5.1	± 1.3	2.1	± 1.2
2	Raw vac	5.3	± 1.4	4.3	± 1.5	4.9	± 2.1	4.4	± 1.5	1.6	± 1.0
	Raw air	5.1	± 1.7	4.5	± 0.9	5.6	± 1.4	5.0	± 1.1	2.7	± 1.2
	Cook vac	5.9	± 0.9	4.3	± 1.9	4.5	± 1.9	4.7	± 1.1	1.9	± 1.2
	Cook air	6.0	± 0.9	5.1	± 1.6	5.0	± 1.9	5.0	± 0.3	2.6	± 1.9
4	Raw vac	5.5	± 1.1	4.3	± 1.9	4.1	± 2.0	4.4	± 1.6	2.0	± 1.0
	Raw air	5.9	± 0.9	4.6	± 1.1	4.8	± 1.9	5.1	± 1.1	2.0	± 1.2
	Cook vac	4.8	± 1.5	5.1	± 1.4	4.7	± 2.0	4.7	± 1.1	2.1	± 1.5
	Cook air	4.9	± 1.4	4.7	± 1.3	4.7	± 1.9	5.1	± 0.8	2.2	± 1.5
6	Raw vac	5.5	± 1.3	4.9	± 1.5	4.5	± 1.8	5.2	± 0.5	3.7	± 2.4
	Raw air	5.6	± 0.8	4.2	± 1.6	4.7	± 2.0	4.7	± 1.5	1.9	± 0.9
	Cook vac	4.5	± 1.6	4.8	± 0.9	4.2	± 1.8	4.9	± 0.9	2.1	± 0.4
	Cook air	5.5	± 0.6	4.4	± 1.4	4.8	± 2.2	4.2	± 1.9	3.5	± 2.5
8	Raw vac	5.1	± 1.0	4.9	± 0.8	4.4	± 1.7	4.3	± 1.7	2.1	± 0.9
	Raw air	5.3	± 1.1	4.9	± 1.7	5.0	± 1.6	4.7	± 1.2	2.3	± 0.9
	Cook vac	6.0	± 0.5	4.2	± 1.1	4.8	± 1.9	4.8	± 0.6	2.1	± 1.2
	Cook air	4.7	± 0.9	3.9	± 1.4	4.8	± 1.4	4.2	± 1.0	2.5	± 1.1
10	Raw vac	5.0	± 0.8	4.3	± 1.5	4.5	± 2.0	4.4	± 1.2	2.0	± 1.0
	Raw air	5.4	± 1.2	4.7	± 1.8	4.4	± 1.9	4.4	± 1.5	3.5	± 2.4
	Cook vac	5.0	± 0.8	4.0	± 1.5	4.7	± 2.2	4.9	± 0.9	1.8	± 1.3
	Cook air	5.6	± 1.0	4.7	± 1.3	5.0	± 1.3	4.5	± 0.4	2.4	± 0.8
12	Raw vac	4.7	± 1.6	3.9	± 1.5	4.7	± 0.9	4.6	± 0.7	2.6	± 1.1
	Raw air	5.5	± 1.1	4.7	± 1.2	4.6	± 1.3	4.4	± 0.5	2.1	± 1.2
	Cook vac	5.1	± 1.5	4.4	± 1.8	5.0	± 1.2	4.8	± 0.9	1.9	± 1.1
	Cook air	4.8	± 1.4	4.2	± 1.5	5.2	± 0.9	4.9	± 1.1	2.7	± 1.2

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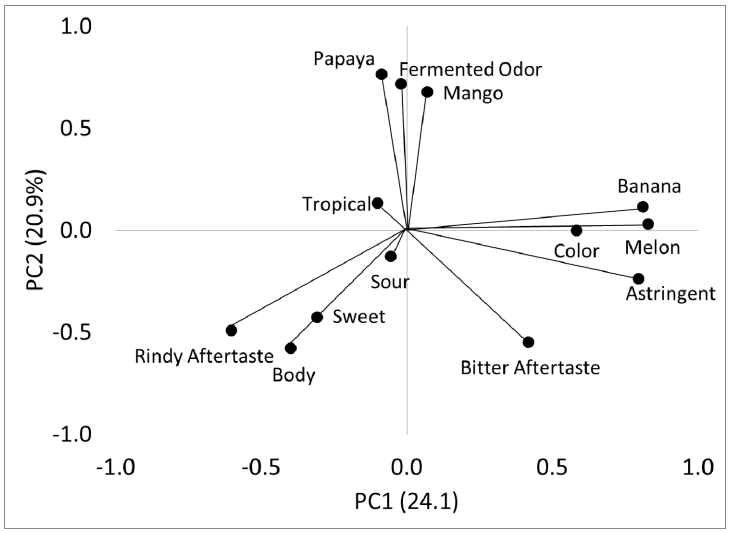
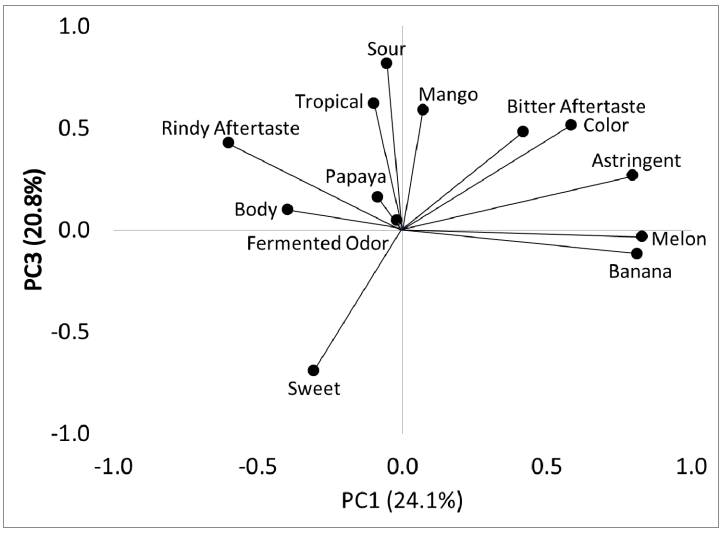
Table 8. Mean values ± standard deviations of descriptive sensory attributes (n=4) for pawpaw pulp stored frozen raw or heat treated (Cook), and in the absence (vac) or presence (air) of air in the package

Month	Treatment	Fermented Odor		Sweet Taste		Sour Taste		Bitter Aftertaste		Rindy Aftertaste		Astringent	
0	Raw vac	1.2	± 0.4	5.2	± 3.2	3.2	± 1.3	4.8	± 0.5	2.7	± 1.6	3.4	± 0.8
	Raw air	0.7	± 0.4	5.1	± 0.5	3.3	± 0.6	5.4	± 0.8	2.6	± 1.5	3.4	± 0.7
	Cook vac	1.5	± 0.7	5.2	± 1.4	3.3	± 2.3	5.4	± 1.9	2.9	± 1.5	3.7	± 0.5
	Cook air	1.1	± 0.7	4.6	± 1.9	2.8	± 1.1	4.3	± 0.7	2.3	± 1.0	2.7	± 1.2
2	Raw vac	1.5	± 0.9	4.9	± 0.5	3.0	± 1.0	5.7	± 1.1	2.9	± 1.9	4.0	± 1.0
	Raw air	1.3	± 0.8	4.6	± 1.6	2.8	± 0.5	5.9	± 0.4	3.2	± 1.8	4.0	± 0.4
	Cook vac	1.0	± 0.7	4.9	± 0.9	3.6	± 1.3	5.8	± 0.6	2.8	± 1.8	3.4	± 1.1
	Cook air	1.2	± 0.8	5.3	± 1.4	3.5	± 2.9	5.0	± 0.4	3.4	± 1.8	3.6	± 1.4
4	Raw vac	1.6	± 0.9	5.3	± 0.8	2.9	± 1.8	4.9	± 1.3	2.1	± 1.3	3.3	± 0.7
	Raw air	1.3	± 0.5	5.6	± 1.1	3.6	± 1.3	5.3	± 0.8	3.1	± 1.9	3.6	± 1.0
	Cook vac	1.1	± 0.9	4.6	± 1.6	3.2	± 1.0	4.6	± 0.9	2.2	± 1.8	2.9	± 1.0
	Cook air	1.0	± 0.4	4.4	± 0.8	3.1	± 1.7	6.0	± 1.3	2.9	± 2.1	3.7	± 0.9
6	Raw vac	1.0	± 0.9	4.9	± 1.2	3.4	± 2.2	4.7	± 1.0	2.6	± 1.5	3.0	± 1.2
	Raw air	1.3	± 0.4	4.8	± 2.4	4.0	± 2.5	5.5	± 2.5	2.9	± 1.7	4.0	± 2.1
	Cook vac	1.1	± 0.8	4.9	± 0.5	3.0	± 1.5	5.3	± 1.7	2.4	± 1.3	3.8	± 1.3
	Cook air	1.2	± 1.1	4.6	± 1.1	3.7	± 1.2	5.2	± 0.7	2.9	± 1.7	3.1	± 0.8
8	Raw vac	0.9	± 0.8	5.8	± 2.6	2.7	± 1.7	5.5	± 0.8	2.9	± 1.9	3.5	± 1.1
	Raw air	1.0	± 0.6	5.7	± 1.5	3.1	± 1.7	5.4	± 1.7	2.4	± 1.7	3.6	± 0.9
	Cook vac	1.5	± 1.1	4.5	± 1.1	2.9	± 1.0	5.9	± 0.3	3.1	± 1.8	3.5	± 0.8
	Cook air	0.9	± 0.6	5.0	± 1.5	3.0	± 1.0	5.3	± 2.2	2.4	± 1.4	3.6	± 1.6
10	Raw vac	1.0	± 0.2	5.1	± 0.8	2.4	± 0.6	5.6	± 2.0	3.3	± 1.6	3.7	± 0.8
	Raw air	1.4	± 1.5	4.9	± 2.8	3.3	± 1.4	5.6	± 1.2	2.4	± 1.8	3.9	± 1.1
	Cook vac	1.1	± 0.8	4.3	± 2.2	2.3	± 0.7	4.8	± 1.4	2.6	± 1.5	3.3	± 0.8
	Cook air	1.5	± 1.0	4.5	± 2.1	2.8	± 0.8	5.4	± 0.6	3.2	± 1.8	3.4	± 1.1
12	Raw vac	1.1	± 0.5	4.6	± 1.4	3.1	± 0.9	5.5	± 0.7	2.7	± 1.8	3.7	± 0.9
	Raw air	1.4	± 0.9	4.2	± 1.8	2.4	± 0.9	4.6	± 0.5	3.1	± 1.7	3.7	± 0.5
	Cook vac	0.7	± 0.4	5.3	± 2.8	2.3	± 1.2	4.6	± 1.1	2.3	± 1.0	3.6	± 0.9
	Cook air	0.9	± 0.8	4.2	± 1.2	3.8	± 1.0	6.0	± 1.5	2.8	± 1.9	4.4	± 0.8





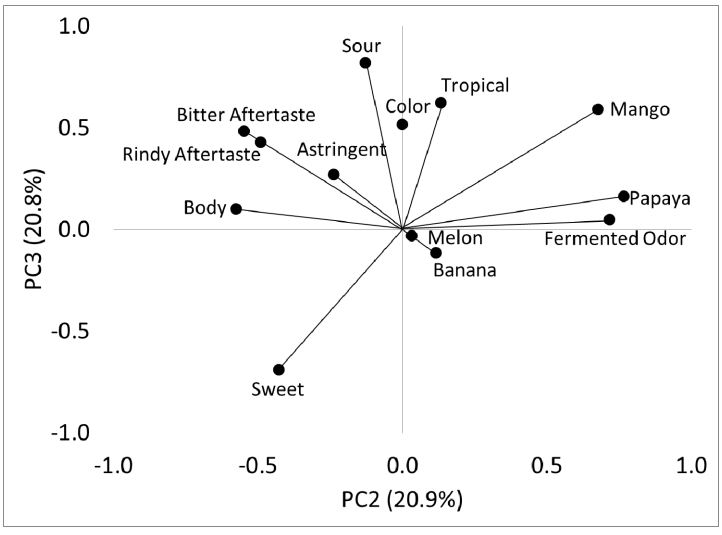


Figure 1. Principal component analysis showing correlations between factor loadings and the three factors for descriptive sensory analysis of pawpaw pulp. The percent of variance explained by each factor is shown in parenthesis

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Seasonal Influence and Heavy Metal Analysis in Marine Shrimp (*Penaeus spp.*) Sold in Trinidad, West Indies

Sophia Balfour (Corresponding author)
Department of Food Production, Faculty of Science and Agriculture
University of the West Indies, St. Augustine
Republic of Trinidad and Tobago, West Indies
Tel: 1-868-662- 2002 Ext. 83989 E-mail: sophia_balfour@yahoo.com

Neela Badrie
Department of Food Production, Faculty of Science and Agriculture
University of the West Indies, St. Augustine
Republic of Trinidad and Tobago, West Indies
E-mail: nbadrie@yahoo.com

Ivan Chang Yen
Department of Chemistry, Faculty of Science and Agriculture
University of the West Indies, St. Augustine
Republic of Trinidad and Tobago, West Indies
E-mail: ichangyen@sta.uwi.tt

Luctchminarine Chatergoon
Department of Chemistry, Faculty of Science and Agriculture
University of the West Indies, St. Augustine
Republic of Trinidad and Tobago, West Indies

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Abstract

Shrimp is a popular seafood in Trinidad and Tobago which is mainly caught in the coastal environs and sold mostly for domestic consumption. The objectives of the research were to determine if chemical contaminants copper, zinc, cadmium, chromium, nickel and mercury in the marine shrimp (*Penaeus spp.*) met international and local food safety standards and; to investigate any location by season interactions. Determination of all heavy metals was by flame atomic absorption spectroscopy except for mercury. Validation of methods was done using Certified Reference Materials namely dogfish liver and fish protein. The heavy metal findings in the marine shrimp were well below the United States Food and Drug Administration, Canada's Food Inspection Agency and, Trinidad and Tobago's admissible limits for human consumption. Significant differences were observed only for copper by location and season interaction and; zinc by location and season respectively.

Keywords: Heavy metal, International limit, Marine shrimp, Food safety

1. Introduction

Crustaceans and seafoods can be potential sources of toxic metals (Carbonell et al., 1998). While information is known worldwide on the implications of mercury, the general consensus from a regional awareness workshop on

mercury pollution held in Port of Spain, Trinidad from 18-21 January 2005 found that there were gaps in the data for the Caribbean islands in relation to mercury levels found in air, land, soil, fish and shellfish (UNEP, 2005).

It is also noticeably evident from two studies in Trinidad that land species crustaceans found near coastal villages and mollusks exceeded heavy metal permissible limits for human consumption (Singh, 1988 and and Rojas de Astudillo, 2002). The zinc (Zn) and copper (Cu) levels in two species of land crab (*Cardisoma guanhumi* and *Ucides cordatus*) collected at Guayamare, Mayaro and Cacandee during July 1985, 1986 and 1988 respectively were above the permissible limits for human consumption (Singh, 1988). The Zn levels in *C. guanhumi* crab ranged from (62.94 – 85.78) μg g^{-1} wet weight (wt.) and in *U. cordatus* (64.82 – 67.16) μg g^{-1} wet wt., both exceeding Zn's local permissible limit of 50 μg g^{-1} wet wt. Similarly, the Cu level in *C. guanhumi* ranged from 22.84 to 26.78 μg g^{-1} wet wt., exceeding Cu's maximum permissible limit (MPL) of 20 μg g^{-1} wet wt. In the second study Rojas de Astudillo (2002) reported that Zn and Cu levels in oysters (*Crassostrea spp*.) from Gulf of Paria sampled in November 1999 also exceeded local MPL (Cu = 20 μg g^{-1} wet wt.; Zn = 50 μg g^{-1} wet wt.) for human consumption. Similarly, Zn levels in the oysters collected at Chacachacare, Chaguaramas, Caroni, La Brea and Cedros ranged from 138.47 to 540.13μg g^{-1}, while the oysters sampled at Chaguaramas, La Brea and Cedros had Cu in the range 28.13 to 52.10 μg g^{-1} wet wt., many in excess of local MPL. Additionally, mussels (*Perna viridis*) sampled in November 1999 from the Caroni Swamp showed high levels of Zn at 89.23 μg g^{-1} wet wt., in excess of the MPL.

Several countries have developed maximum acceptable concentrations for commercialization and consumption of crustacean and seafood (Carbonell et al., 1998) such as the United States, Canada and also Trinidad and Tobago, a developing country. The safety of consuming shrimp in Trinidad and Tobago is unknown. There exist no data in the literature to provide evidence for the levels of the heavy metal in Trinidad and Tobago's shrimp as well as for seasonal variations. The objectives of the study were to determine: (1) if Cu, Zn, cadmium (Cd), chromium (Cr), nickel (Ni) and mercury (Hg) were present in Trinidad's marine shrimp (*Penaeus spp*.), at levels which met international and local food safety standards for human consumption as summarized in Table 1, and for which relevant action may be necessary and; (2) to evaluate any location (geographical) by season interactions as well as location (geographical) and seasonal variations respectively of heavy metals Cu, Zn, Cd, Cr, Ni and Hg in Trinidad's marine shrimp.

2. Materials and Methods

2.1 Source of shrimp, sampling protocol and collection

Shrimp (*Penaeus spp.*) were purchased at four wholesale and retail fish depots along the western coastline of Trinidad at Orange Valley, Otaheiti and Sealots, Port of Spain and Claxton Bay, as well as from three road side vendors in the Tunapuna region for the period January to December 2009.

Shrimp samples 8.5–11.5cm in length were purchased seasonally, four times per year from January to February, March to April, July to August and September to October in each of the five (5) selected areas from three vendors respectively on site. The total number of samples examined for this research was sixty (60): thirty (30) samples (or 2 batches each of 15 shrimp samples) in the dry season from January to May 2009 and another thirty (30) samples (or 2 batches each of 15 shrimp samples) in the rainy season from June to December 2009.

Two pounds (800g) of shrimp samples obtained from each vendor were placed into sterile bags and transported to the Food Production - Microbiology laboratory at The University of the West Indies within 2 hours of purchase in an ice cooler to maintain a temperature of approximately 4°C; and then frozen at -20°C until it was time to analyse for Cu, Zn, Cd, Ni, Cr and Hg.

2.2 Preparation of shrimps for heavy metal analysis

Approximately one pound (about 400 grams) of each sampled shrimp was de-headed, de-shelled and de-veined. The shrimp tissues were then rinsed with distilled water and well drained. The shrimp were then macerated at high speed for 2 minutes using a Waring Blender (Model 31BL92, Connecticut, U.S.A). The macerated shrimp tissues were then frozen in polythene bags at -20^{o}C and heavy metal analyses were conducted within one year of purchase.

All glassware used for heavy metal and mercury analyses were washed with detergent, rinsed in distilled water, pre-soaked in 5% nitric acid for 24h, rinsed with de-ionised water and allowed to air-dry before use.

2.3 Determination of Cu, Zn, Cd, Cr and Ni in shrimps (Penaeus spp.)

A slightly modified version of the method used by Rojas de Astudillo (2002) was carried out in this investigation. Triplicate 3g aliquots of shrimp tissue macerates were weighted into boiling tubes. Concentrated analytical grade

nitric acid (10mL) was added to directly to each sample and vortexed to ensure that all the macerated shrimp tissues were fully submerged in the acid. The samples were covered with glass plates in the fume hood and allowed to predigest overnight at room temperature. Reagent blanks were processed simultaneously in triplicate. The samples were refluxed on a heating block at 130-135 ^{o}C for 6 hours. After cooling for 20 minutes, 5mL deionised water was added to dilute each sample which was then filtered into a 25mL volumetric flask, using Whatman 542 filters, and made up volume with deionised water rinses of the residues. Cu, Zn, Cd, Cr and Ni in the samples were determined by flame atomic absorption spectroscopy (FAAS) using a Varian Model SpectraAA-880 (Australia). A deuterium background correction was used for all metals except chromium. In-date commercial metal calibration standards (BDH, Poole, U.K.) were used to prepare fresh multi-element calibration standards each time that the samples were analysed.

Optimisation of the method was conducted using of 3g duplicates of macerated shrimp tissue being pre-digested overnight in 10mL volume of nitric acid digesting at130-135 oC, since previous studies done by Rojas de Astudillo (2002) showed that the 10mL volume of nitric acid had provided optimal recoveries for the metals in shellfish. Optimization of the heavy metal method consisted of varying the reflux time at 4, 5, 6, 7 and 8 hours for obtaining the highest recoveries for Cu, Zn and Cd in the shrimp samples.

Cu and Zn were chosen for optimizing the method, as shellfish tend to have high levels of Cu and Zn, based on reports in published literature (Van den Broek, 1979; Singh, 1988; Guns et al., 1999; Rojas de Astudillo, 2002 & Liang et al., 2004).

2.4 Evaluation of plastic storage bags for possible heavy metal contamination

Since sealed polyethylene bags were used to store the macerated shrimp samples for extended periods before heavy metal analyses were conducted, it was necessary to determine whether these bags were possible sources of contamination of the samples.

Three bags from each box of bags were randomly sampled. Into each was measured 100mL of 0.1M nitric acid, the bags were closed and left to stand at room temperature for 1 hour. The leaching solutions from the sealed bags were analyzed by FAAS, using the nitric acid solution as a blank. The metal levels in the sealed storage bags were negligible (Cd and Cr: 0.00 μg mL^{-1}; Ni: 0.07 μg mL^{-1}; Cu 0.01 μg mL^{-1} and Zn: 0.02 μg mL^{-1}). The surface area of each the Ziploc bag was approximately 250cm^{2}.

2.5 Determination of mercury in shrimp samples (Penaeus spp.)

A slightly modified version of the method outlined by Rojas de Astudillo (2002) was used in this investigation. Three replicates of 3g aliquots of shrimp tissue macerates were weighted into boiling tubes. Then, 10mL concentrated analytical grade nitric acid was added to each macerate, and left to pre-digest overnight hours at room temperature in a fume hood, while covered with glass plates to avoid aerial contamination.

The boiling tubes were then placed on a heating block at 130-135 ^{o}C for 3 hours. Concentrated sulphuric acid (2.5 mL) and concentrated hydrochloric acid (1.0 mL) were then added slowly to each sample, which were then allowed to reflux for 3 more hours. After cooling for 20 minutes, potassium manganate (VII) solution (5% m/V) was added drop-wise to each sample until a first permanent pink coloration was observed. Hydroxlamine solution (10% m/V) was immediately added to destroy the excess potassium manganate (VII). The digest was diluted with 5mL deionised water, then filtered into 25mL volumetric flasks, through Whatman 542 filters, and made up volume with deionised water rinses of the residues. Triplicate reagent blanks were processed simultaneously.

Hg in shrimp was determined by cold vapour atomic absorption on the Varian Model SpectraAA-880 (Australia). In-date commercial metal calibration standards (BDH, Poole, U.K.) were used to prepare fresh calibration standards each time that the samples were analysed.

2.6 Validation of methods for Cu, Zn, Cd, Cr, Ni and Hg

The methods were validated using Certified Reference Materials (CRM) namely dogfish liver (DOLT-2) and (DOLT-4) respectively and fish muscle tissues (DORM-3) from the National Research Council of Canada with recoveries that were consistent and ranged from (91.83 to 111.89%), with the exception of DORM-3and DOLT-4 for nickel, for which percent recoveries were slightly higher (116.95 - 117.33%) as shown in Table 2. Three replicates of each CRM were subsequently analyzed with each batch of shrimp samples to monitor and control the quality of analyses.

2.7 Statistical analyses

Statistical analyses were carried out using Statistical Package for Social Sciences (SPSS) version 17 software. One-way Analysis of Variance (ANOVA) was used to determine significant variations in the reflux time for the

absorbencies of Cu, Zn and Cd at a 5% level of significance. Case summaries provided mean recoveries and standard errors for the CRM. Additionally, Univariate Analysis of Variance was used to determine significant differences in the interaction of location by seasons as well as significant variations of season and location respectively for each of the metal investigated in this research.

3. Results

3.1 Optimization of reflux time for Cu, Zn and Cd

One-way ANOVA showed that the absorbencies of Cu (P = 0.43), Zn (P = 0.36) and Cd (P = 0.17) did not vary significantly, in relation to the reflux times of 4, 5, 6, 7 and 8 hours respectively.

3.2 Heavy metal findings in shrimp sampled in Trinidad in 2009

3.2.1 Heavy metal findings in shrimp from Trinidad in relation to the international and local permitted levels for human consumption of foods

The shrimp sampled in Trinidad in 2009 had metal levels well below admissible limits for human consumption according to international and local standards (US FDA, 1993; T&T Food and Drug Regulation, 2007 & CFIA, 2009) as shown in Table 1. Additionally, the shrimp sampled from Claxton Bay in 2009 had Ni concentration below its limit of quantitation (LOQ) (Table 1).

3.2.2 Effect of location, season and location*season interaction of the shrimp sampled in Trinidad in 2009

Only Cu showed significant effects on the location by season interaction (P = 0.03) as shown in Table 3, with the dry season displaying higher trends of Cu concentrations in the shrimp samples purchased from Orange Valley followed by Otaheiti, Port of Spain, Tunapuna and Claxton Bay relative to the wet season.

Similarly, only Zn showed significant location (P = 0.00) and seasonal (P = 0.02) differences respectively. The Zn concentrations were highest in shrimp sampled at Tunapuna followed by Otaheiti, Port of Spain, Orange Valley and Claxton Bay respectively (Table 3). Additionally, the dry season showed an overall higher level of Zn in the shrimp samples relative to the wet season (Table 3).

Cd, Cr, Ni and Hg concentrations in the shrimp sampled in Trinidad in 2009 did not vary significantly (P > 0.05) by location, season or location by season interaction.

4. Discussion of Heavy Metal in Shrimp

A sample extraction reflux time of 6 hours was used for all shrimp samples in this project, to ensure the complete decomposition of all the organic Cu, Zn, Cd, Cr, Ni and Hg into their respective inorganic forms.

The CRM namely, DOLT-4, DORM-3 and DOLT-2 validated the methods for the metals of interest to this project as shown in Table 2, with recoveries ranging from (Cu: 107.29 – 111.89%; Zn: 98.28 – 102.65%; Cd: 95.53 – 95.77%; Cr: 91.83 – 96.70% and Hg: 86.67 – 98.60%). This showed the method to be accurate and precise for the determination of the selected heavy metals in shrimp.

In addition, the CRM recoveries for the metals Cu, Zn, Cd, Cr and Hg in this research agreed well with those found in the literature (Tu et al., 2008; Maanan, 2008 & Cui et al., 2011). However the nickel recoveries in DORM-3 and DOLT-4 were slightly higher and ranged from (116.95 -117.33%) which could have been attributed to possible matrix interferences (Sundberg, 1973). Nevertheless, the results of analysis were not adjusted for these recoveries for nickel.

The metal concentrations of the *Penaeus* shrimp species sampled in 2009 in Trinidad were well below the maximum admissible limits for human consumption according to international and local standards (US FDA 1993; CFIA 2011; and T&T Food and Drug Regulation 2007) as shown in Table 1, which suggested that they were safe to consume; although the Gulf of Paria's coastline is considered to be heavily polluted given that it is one of the most industralised areas in the region (Dhoray & Teelucksingh, 2007).

The metal levels found in the local Penaeid shrimp may be attributed to their short lifespan of approximately 1 to 2 years, as well as the constant movement of the shrimp during their life cycle (Kuruvilla, 2001), thus minimizing the shrimp's ability to accumulate the metals in their muscle tissue.

Cd, Cr, Cu, Zn and Hg findings in the local shrimp (*Penaeus* spp.) were lower than those reported in various shrimp species from India, China, Egypt and Turkey (Guhathakurta & Kaviraj, 2000; Soliman, 2006; Yılmaz & Yilmaz, 2007; Tag El-Din et al., 2009 & Wu & Yang, 2011), some of which exceeded the maximum admissible limits for human consumption when compared to international and local standards (US FDA, 1993; T&T Food and Drug Regulation, 2007 & CFIA, 2011).

Location by season interaction varied significantly (P = 0.03) for Cu concentrations in the shrimp samples with Orange Valley having the highest concentration of 3.82 μg g^{-1} wet wt. in the dry season, followed by Otaheiti, Port of Spain, Tunapuna and Claxton Bay at 3.39, 3.28, 2.86 and 1.86 μg g^{-1} wet wt. versus 2.38, 1.99, 2.19, 1.66 and 1.95 μg g^{-1} in the wet season (Table 3). This may have resulted from the run-offs of Cu-based agricultural pesticides mostly in the dry season when crops and rice are commonly cultivated in Trinidad. These run-offs travel through streams and rivers and, ultimately into the Gulf of Paria where shrimp are short-lived, harvested and then brought to vending sites for purchase. Additionally, high Cu concentration in the shrimp may also be associated to the essential role of the metal in crustaceans for the metabolic functioning of proteins such as haemocyanin (White & Rainbow, 1982).

The Zn concentration in the shrimp showed a significant seasonal difference (P = 0.02) with the dry season having higher concentration of 10.15 μg g^{-1} wet wt. versus 9.71 μg g^{-1} wet wt. in the wet season (Table 3), which may be attributed to the coastal habitats being affected by anthropogenic metal contamination and possibly widespread faecal pollution (Nunez-Nogueira and Rainbow, 2005; Bachoon et al., 2010**).**

The significantly higher concentrations of Cu and Zn from Trinidad's shrimp in dry season were consistent with the previous study done by Rojas de Astudillo (2002), except Cu and Zn concentrations in that study were found to be above permissible limits for human consumption in other types of shellfish, namely, oysters and mussels from Trinidad and Venezuela.

Also, the shrimp sampled at Tunapuna had the highest Zn concentration of 10.61 μg g^{-1} wet wt., followed by Otaheiti, Port of Spain, Orange Valley and Claxton Bay at 10.43, 10.05, 10.02 and 8.99 μg g^{-1} wet wt. respectively which suggested that continuous care must be taken to monitor the metal levels in marine shrimp especially if they exceed international and local permissible limits for human consumption.

The metals levels in the Ziploc storage bags were negligible, which suggested that these storage bags were not responsible for any heavy metal contamination of the sampled shrimp.

5. Conclusion

The marine shrimp (Penaeus spp.) in Trinidad were safe to consume since the metal (Cu, Zn, Cd, Cr, Ni and Hg) concentrations were well below local and international admissible limits for human consumption. Some vending sites (locations) had higher levels of metal contamination mainly in the dry season which may have been due to anthropogenic metal contamination along the coastline or further in-land.

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Table 1. Maximum permitted levels of trace metals in foods (crustacean and sea foods) in Trinidad and Tobago for human consumption in relation to the metal findings in shrimp sampled in 2009

Heavy metal contaminant	Cd[1]	Cr[1]	Ni[1]	Hg[2]	Cu[3]	Zn[3]
International and local action level/ μg g^{-1} wet wt.	3	12	70	0.5	20	50
Location (Sample Sites)	Mean ± Standard Error for each metal in the shrimp samples/μg g^{-1} wet wt.					
Orange Valley	0.10 ± 0.03	0.18 ± 0.04	1.48 ± 1.39	0.08 ± 0.00	3.10 ± 0.15	10.02 ± 0.20
Port of Spain	0.11 ± 0.02	0.11 ± 0.06	5.52 ± 1.36	0.06 ± 0.01	2.73 ± 0.15	10.05 ± 0.20
Otaheiti	0.13 ± 0.02	0.13 ± 0.04	1.76 ± 1.75	0.07 ± 0.01	2.69 ± 0.15	10.43 ± 0.20
Claxton Bay	0.11 ± 0.03	0.13 ± 0.03	< 0.64*	0.06 ± 0.01	1.77 ± 0.15	8.99 ± 0.20
Tunapuna	0.14 ± 0.02	0.10 ± 0.10	5.74 ± 1.36	0.07 ± 0.00	2.41 ± 0.15	10.16 ± 0.20
Sources: [1] US FDA 1993; [2] CFIA 2011; [3] T&T Food and Drug Regulation 2007						

* Metal level in shrimp was < LOQ (μg g^{-1} wet wt.).

Table 2. CRM mean recoveries (%) ± standard error

CRM	Cu	Zn	Cd	Cr	Ni	Hg
DOLT-4	107.29 ± 2.40	98.50 ± 0.62	95.53 ± 0.60	96.70 ± 5.77	117.33 ± 14.16	86.67 ± 2.66
DORM-3	107.48 ± 2.63	98.28 ± 1.44	95.77 ± 2.06	91.83 ± 5.00	116.95 ± 12.63	98.60 ± 12.16
DOLT-2	111.89 ± 3.77	102.65 ± 2.06				92.27 ± 4.88

Table 3. Significant effect of location by season interaction for Cu in shrimp and; location and seasonal differences of Zn

Cu: location by season interaction showing significant difference, P-value = 0.03			**Zn**	
Location	Wet Season: Mean ± Standard Error (μg/g) wet wt.	Dry Season: Mean ± Standard Error (μg/g) wet wt.	Location differences of Zn: Mean ± Standard Error (μg/g) wet wt.; (P -value = 0.00)	Seasonal differences of Zn: Mean ± Standard Error (μg/g) wet wt.; (P-value = 0.02)
Orange Valley	2.38 ± 0.21	3.82 ± 0.21	10.02 ± 0.20	Wet: 9.71± 0.13
Port of Spain	2.19 ± 0.21	3.28 ± 0.21	10.05 ± 0.20	Dry: 10.15± 0.13
Otaheiti	1.99 ± 0.21	3.39 ± 0.21	10.43 ± 0.20	
Claxton Bay	1.66 ± 0.21	1.87 ± 0.21	8.99 ± 0.20	
Tunapuna	1.95 ± 0.21	2.86 ± 0.21	10.61 ± 0.20	

Study of the *Micrococcaceae* and *Staphylococcaceae* throughout the Manufacture of Dry-Cured Lacón (a Spanish Traditional Meat Product) Made without or with Additives

José M. Lorenzo, María C. García Fontán, María Gómez, Sonia Fonseca,
Inmaculada Franco & Javier Carballo (corresponding author)
Área de Tecnología de los Alimentos, Facultad de Ciencias de Ourense
Universidad de Vigo, Ourense 32004, Spain
Tel.: 34-988-387-052 E-mail: carbatec@uvigo.es

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Abstract

Micrococcaceae and *Staphylococcaceae* were enumerated (on SPC agar + 7.5% NaCl) in samples from the surface and the interior of pieces of dry-cured lacón (a Spanish traditional meat product), at different stages of the manufacturing process, and from six different batches (three made without and three with additives -glucose, sodium nitrite, sodium nitrate, sodium ascorbate, and sodium citrate-). The use of additives did not affect the counts or evolution of this microbial group.

For four batches (two without and two with additives), a total of 335 strains were isolated and identified by classical methods.

Staphylococcus xylosus was the most abundant and constant species throughout manufacture of the batches made without and with additives. Other species of staphylococci were isolated, including: *Staph. equorum*, *Staph. sciuri*, *Staph. gallinarum*, *Staph. cohnii*, *Staph. intermedius, Staph. capitis*, *Staph. epidermidis*, *Staph. simulans* and *Staph. warneri*. Species of *Micrococcus* or *Kocuria* isolated in very low proportions, included *Kocuria varians*, *K. rosea*, *Micrococcus lylae* and *M. luteus*.

Keywords: Dry-cured lacón, Meat products, *Micrococcaceae*, *Staphylococcaceae*, Additives

1. Introduction

Dry-cured lacón is a traditional raw-cured meat product made in NW Spain, from the foreleg of the pig cut at the shoulder blade-humerus joint, following manufacturing processes similar to those used in the production of dry-cured ham. In the autonomous region of Galicia (NW Spain), the product is recognised as a Geographically Protected Identity (G.P.I.) (Official Journal of the European Communities, 2001).

Previous studies on dry-cured lacón have mainly concerned biochemical and sensory characterization of the final product (Marra et al., 1999; Veiga et al., 2003), as well as the biochemical changes that take place during the manufacturing process (Lorenzo et al., 2003; Lorenzo et al., 2008a; Lorenzo et al., 2008b). However, very few studies have explored the microbiological characteristics and refer only to the counts of some microbial groups at the end of the different stages of manufacture (Vilar et al., 2000). There is no information about the presence of microbial groups of technological interest or about the species that occur throughout the entire manufacturing process. This restricts the manufacturer's ability to control these microorganisms with the aim of improving the quality of the final product. Moreover, the product is traditionally manufactured with only coarse salt, and no other additives. Recently, manufacturers have begun to use some common additives with the aim of improving the appearance and quality of the final product (to develop the typical colour of cured meats and inhibit growth

of moulds on the surface). The effect of these additives on the microbial populations present throughout the manufacturing process is at present unknown.

The importance of the microorganisms belonging to the *Micrococcaceae* and *Staphylococcaceae* families in the biochemical processes that take place during the ripening of raw-cured meat products has been demonstrated by several authors (Liepe, 1983; Hammes, 1986; Hinrichsen and Pedersen, 1995), who emphasized the contribution of these microorganisms to the development of the colour and flavour of the products. The main functions of these microorganisms in cured meat products are: (i) production of catalase, (ii) production of nitrite reductase, (iii) reduction of nitrate, (iv) production of proteases, peptidades and lipases, which intervene in the degradative processes that take place throughout maturation of the products and are responsible for the production of the taste and aroma compounds that characterize the flavour of the final products. After lactic acid bacteria, these microorganisms are the most often used as starter cultures in the meat industry. However, strains belonging to this microbial group can develop undesirable actions such as production of biogenic amines or enterotoxins dangerous to consumers.

The aims of the present work, which forms part of a wider study on the microbiological and biochemical changes that take place during the manufacture of dry-cured lacón, were to quantify the *Micrococcaceae* and *Staphylococcaceae* on the surface and in the interior of this meat product at different stages of the manufacturing process, to identify the species present, and also to study the effect of the use of some additives on the presence and behaviour of this microbial group. In future studies, the technological and safety properties of the isolated strains will be investigated in detail in order to determine the role of these species in the manufacture of lacón, with the final purpose of using these strains as starter cultures.

2. Materials and Methods

2.1 Samples

Six batches of dry-cured lacón were manufactured in three different pork meat industries. Each batch comprised 9 pieces of lacón (from Landrace x Large White pigs) each weighing approximately 4 kg. Raw pieces from three batches, one per industry, were salted with an excess of coarse salt, to form piles with alternate layers comprising of pieces or salt. The pieces remained in the pile for four days (one day per kg of weight). The temperature of the salting room was between 2 and 5 ºC, and the relative humidity between 80 and 90%. After the salting stage, the pieces were removed from the pile, brushed, washed and transferred to a post-salting room where they remained for 14 days at 2-5 ºC and approximately 85-90% relative humidity. After the post-salting stage, the pieces were transferred to a room at 12 ºC and 74-78% relative humidity, where drying-ripening took place over the course of 84 days. Prior to the salting process, the pieces from the other three batches (one per industry) were each rubbed with a mixture of additives comprising glucose (2 g/kg), sodium nitrite (E_{250}) (125 mg/kg), sodium nitrate (E_{251}) (175 mg/kg), sodium ascorbate (E_{301}) (500 mg/kg), and sodium citrate (E_{331}) (100 mg/kg). In these batches, salting, post-salting and drying-ripening were carried out under the same conditions as in the batches manufactured without additives.

In each batch, samples were taken from fresh pieces, after the end of the salting stage, after 7 and 14 days of post-salting, and after 7, 14, 28, 56, and 84 days of drying-ripening. Each sample consisted of one whole piece of lacón. Samples were transported to the laboratory at <4 ºC and analysed on arrival.

2.2 Microbiological analysis

In each lacón piece, samples from the surface and interior were obtained for analysis. Surface samples were taken by aseptic removal of slices (surface area 100 cm^2 and about 2 mm in thickness) following the method of Fliss et al. (1991). Samples from the interior were taken with the aid of a sterile metallic rod, which was introduced into the musculature at five different points, after aseptic and consecutive removal of three 2 mm thick slices from the surface. Twenty-five grams from the surface and 25 grams from the interior of each piece of lacón were homogenized, in each case with 100 mL sterile 0.1% peptone (Oxoid, Unipath Ltd., Basingstoke, UK) water containing 0.85% NaCl and 1% Tween 80 as emulsifier, at 40-45 ºC for 2 min in a Stomacher 400 Lab Blender (Seward Medical, London, UK), to provide a 1/5 dilution. Successive tenfold dilutions were prepared by mixing 10 mL of the previous dilution with 90 mL 0.1% sterile peptone water.

In each sample, the *Micrococcaceae* and *Staphylococcaceae* were enumerated on Standard Plate Count Agar (Oxoid) + 7.5 NaCl, after incubation at 30 ºC for 48 h.

From each sample, 1 mL of each dilution was inoculated in duplicate on plates and mixed before solidification. After incubation, the number of colonies on plates with 30-300 colonies, were counted.

2.3 Isolation and identification of strains

For four batches (two made without and two made with additives), 10 colonies were taken at random from SPC agar + 7.5% NaCl plates, from each sampling point corresponding to each batch (five from the surface sample and five from the interior sample) with the aid of a Harrison disk (Harrigan and Mc Cance, 1976). For some sampling points it was not possible to collect this number of colonies because of lack of growth or insufficient growth on the plates. Ninety strains were isolated from the surface and 80 from the interior of the pieces in the batches made without additives, and 90 strains were isolated from the surface and 75 from the interior of the pieces in the batches made with additives. The isolates were purified by 4 alternate subcultures on BHI agar and in BHI broth (Oxoid). The purified strains were then maintained at –80 ºC with 20% glycerol as a cryoprotective agent.

Gram-positive, catalase-positive cocci grouped in pairs, tetrads or irregular clusters were identified following the methods and criteria described by Schleifer and Kloos (1975a, 1975b), Schleifer (1986) and Kloos et al. (1992). The following tests were carried out on each isolate: oxidation and fermentation of glucose and mannitol; assimilation of glycerol in aerobic conditions; growth in furazolidone agar and growth in lysostaphin agar. The aerobic cocci that were resistant to furazolidone and lysostaphin and incapable of assimilating glycerol were considered to be *Micrococcaceae*; the facultatively anaerobic cocci, incapable of growth in furazolidone agar and lysostaphin agar and able to assimilate glycerol were considered to be *Staphylococcaceae*. In order to identify the strains to species level, the following tests were carried out: colony pigmentation on BHI agar; growth in 7.5, 10 and 15% NaCl; characteristics of growth in Baird-Parker agar; aesculin hydrolysis; growth at 10 ºC; acetoin production; nitrate reduction; presence of oxidase, alkaline phosphatase, urease, arginine dihydrolase, ornithine decarboxylase, ß-galactosidase, arginine arylamidase, pyrrolidonyl arylamidase, ß-glucuronidase; novobiocin resistance and acid production from N-acetyl glucosamine, L-arabinose, D-cellobiose, D-fructose, glucose, glycerol, lactose, maltose, D-mannitol, D-mannose, raffinose, D-ribose, sucrose, D-trehalose and D-turanose.

Identity of the strains was confirmed using the system API 32 STAPH (bioMérieux, Marcy-l'Etoile, France).

2.4 Biochemical analysis

Total solid, NaCl and nitrate contents, and pH and a_w values were determined using the methods cited by Lorenzo et al. (2003). All chemical determinations were carried out in duplicate in each sample.

2.5 Statistical analysis

In order to detect any significant differences between the different sampling points during ripening of the batches from the same manufacturing process (without or with additives), and between the two types of processes at each sampling time, analyses of variance (ANOVA) were performed, with a confidence interval of 95% (P<0.05). Means were compared by the least squares difference (LSD) test, with the Statistica© 5.1 computer programme for Windows (Statsoft Inc, 1996, Tulsa, OK, USA).

3. Results and Discussion

3.1 Microbial counts throughout the manufacturing process

Table 1 shows the changes in the counts in SPC agar + 7.5% NaCl corresponding to the surface and the interior of the pieces, in the batches made without and with additives. This table also shows the counts of total aerobic mesophilic microflora on SPC agar (Oxoid), already published (Lorenzo et al., 2010), with the only purpose to show and to appreciate the contribution of the *Micrococcaceae* and *Staphylococcaceae* to the total aerobic mesophilic microflora in this meat product.

High counts of total aerobic mesophilic microflora and *Micrococcaceae* and *Staphylococcaceae* were observed, both at the surface and in the interior of the fresh pieces, which appears to be associated with appreciable contamination of the pieces and an important degree of microbial multiplication in the quartering rooms and in the manufacturing industries.

The counts of the *Micrococcaceae* and *Staphylococcaceae* at the surface of the fresh pieces were similar to those observed by Silla et al. (1989) and of the order of 0.5 $\log_{10}$ units higher than those reported by Huerta et al. (1988) for fresh ham pieces. The counts of this microbial group in the interior of the fresh pieces were around one $\log_{10}$ unit lower than those observed by Huerta et al. (1988) in raw pieces used in the manufacture of raw-cured ham.

After salting, the counts of the *Micrococcaceae* and *Staphylococcaceae* at the surface showed a small decrease, sometimes even imperceptible. The decrease in numbers was more intense in the interior of the pieces.

The *Micrococcaceae* and *Staphylococcaceae* increased significantly (P<0.05) during the post-salting stage and during the first days of the drying-ripening stage, with maximum counts reached after 7 days of drying-ripening (around 9 $\log_{10}$ CFU/g in the surface and 3-4 $\log_{10}$ CFU/g in the interior of the pieces); the microbial counts then remained stable until the end of the manufacturing process.

An increase in the counts of the salt tolerant microflora during the post-salting and the first days of the drying-ripening stage has also been observed by several authors during the manufacture of other raw-cured meat products made from whole pieces such as ham (Huerta et al., 1988; Silla et al., 1989; Carrascosa et al., 1992) and Spanish cecina (García et al., 1995).

Due to the intensity of the salting process in this meat product, and to the high salt concentrations reached both at the surface and in the interior of the pieces immediately after the post-salting stage (see Table 2), the counts of the *Micrococcaceae* and *Staphylococcaceae* from the end of the post-salting stage (14 days of post-salting) were almost the same as those of the total aerobic mesophilic microflora, as a consequence of the selective action exercised by the high NaCl contents. This effect has previously been observed in other meat products subjected to salting followed by a drying-ripening process (Giolitti et al., 1971; Graham and Blumer, 1971; Van der Riet, 1982; Carrascosa et al., 1988; Huerta et al., 1988; Silla et al., 1989; Rodríguez et al., 1994; García et al., 1995).

At each stage of manufacture, comparison of the average counts of the *Micrococcaceae* and *Staphylococcaceae* corresponding to the surface and the interior of the pieces of the batches made without additives with those obtained in the pieces of the batches made with additives, revealed that the use of additives had little effect on the growth of this microbial group.

3.2 Species isolated throughout the manufacturing process

Of the 90 strains isolated from the surface of the pieces from the batches made without additives, 52 were identified as *Staphylococcus xylosus*, 5 as *Staph. equorum*, 1as *Staph. warneri*, 1 as *Staph. epidermidis*, 7 as *Staph. intermedius*, 2 as *Staph. capitis*, 2 as *Staph. sciuri*, 1 as *Staph. gallinarum*, 2 as *Kocuria varians*, 1 as *M. lylae*, 11 did not belong to the *Micrococcaceae* or *Staphylococcaceae* families and 5 strains were lost in the course of the purification process.

The distribution in the sampling points of the species isolated from the surface of the pieces during the manufacture of the lacón batches made without additives is shown in Table 3.

Of the 80 strains isolated from the interior of the pieces from the batches made without additives, 56 were identified as *Staphylococcus xylosus*, 3 as *Staph. equorum*, 2 as *Staph. simulans*, 1 as *Staph. intermedius*, 1 as *Staph. capitis*, 1 as *Staph. cohnni*, 1 as *Staph. gallinarum*, 2 as *Kocuria varians*, 1 as *K. rosea*, 11 did not belong to the *Micrococcaceae* or *Staphylococcaceae* families and one strain was lost during the course of the purification process.

The distribution in the sampling points of the species isolated from the interior of the pieces during the manufacture of the lacón batches made without additives is also shown in Table 3.

Of the 90 strains isolated from the surface of the pieces from the batches made with additives, 56 were identifed as *Stahylococcus xylosus*, 4 as *Staph. equorum*, 2 as *Staph. warneri*, 3 as *Staph. epidermidis*, 4 as *Staph. intermedius*, 5 as *Staph. capitis*, 3 as *Staph. sciuri*, 1 as *Micrococcus luteus*, 1 as *K. varians*, 1 as *K. rosea*, 6 strains did not belong to the *Micrococcaceae* or *Staphylococcaceae* families and 4 strains were lost during the course of the purification process.

The distribution in the sampling points of the species isolated from the surface of the pieces during the manufacture of the lacón batches made with additives is shown in Table 4.

Of the 75 strains isolated from the interior of the pieces from the batches made with additives, 51 were identified as *Staphylococcus xylosus*, 2 as *Staph. equorum*, 1 as *Staph. simulans*, 5 as *Staph. intermedius*, 1 as *Staph. capitis*, 1 as *Staph. cohnni*, 1 as *Staph. sciuri*, 1 as *Kocuria varians*, 9 strains did not belong to the *Micrococcaceae* or *Staphylococcaceae* families and 3 strains were lost during the course of the purification process.

The distribution in the sampling points of the species isolated from the interior of the pieces during the manufacture of the lacón batches made with additives is also shown in Table 4.

Of the 335 strains isolated in the present study during the manufacture of the four lacón batches, 322 strains were identified (13 were lost during the purification process). Of these 322 strains, 285 were identified as *Micrococcaceae* (genera *Micrococcus* and *Kocuria*) or *Staphylococcaceae* (genus *Staphylococcus*) (88% of the

strains subjected to identification), which reflects the high selectivity of the SPC agar + 7.5% of NaCl medium for this microbial group.

In the present study, many more isolates were identified as *Staphylococcaceae* (genus *Staphylococcus*) than as *Micrococcaceae* (genera *Micrococcus* and *Kocuria*). The *Staphylococcus* strains comprised 95.9% of the strains identified from the surface of the pieces made without additives, 95.6% of the strains identified from the interior of the pieces made without additives, 96.25% of the strains identified from the surface of the pieces made with additives, and 98.4% of the strains identified from the interior of the pieces made with additives. The *Micrococcus*/*Kocuria* only comprised 4.0%, 4.4%, 3.75% and 1.6% of the strains identified, respectively.

The predominance of the isolates of the genus *Staphylococcus* over those of the genera *Micrococcus* or *Kocuria* is a common finding in studies of characterization of the microflora of meat products (Graham and Blumer, 1971; Von Rheinbaben and Seipp, 1986; Molina et al., 1989; Comi et al., 1992; Kotzekidou, 1992; Delarras et al., 1994; Rodríguez et al., 1994; García et al., 1995; Rodríguez Jovita, 1997; Papamanoli et al., 2002; Martín et al., 2006). This same phenomenon has been observed by Delarras et al. (1994) in different raw foods of animal origin.

The prevalence of the genus *Staphylococcus* may be at least partly due to the greater resistance to high concentrations of salt that the staphylococci show, in addition to their wider distribution in nature. The *Staphylococcus* grow in the presence of salt contents of up to 15%, while the *Micrococcus* cannot tolerate concentrations of more than approximately 10% (Seager et al., 1986; Campanini et al., 1987; Kotzekidou, 1992). The best adaptation of the *Staphylococcus* to the low values of redox potential that become established as consequence of the drying of the product may affect this phenomenon (Kotzekidou, 1992). The percentage of *Staphylococcus* and of *Micrococcus* in particular products varies depending on the salt content and on the values of a_w and Eh; variations in pH do not appear to affect this percentage.

In general the same species of *Staphylococcus* and of *Micrococcus*/*Kocuria* were isolated from the batches made without and with additives.

Staphylococus xylosus was the most abundant and most consistent species throughout the manufacture of the batches made without and with additives, and prevailed as much in the surface as in the interior of the pieces. This species comprised 73% of the strains of staphylococci isolated from the surface of the pieces in the batches made without additives, 86% of those isolated from the interior of the pieces in the batches made without additives, 73% of those isolated from the surface of the pieces in the batches made with additives, and 82% of those isolated from the interior of the pieces in the batches made with additives. This species has also been identified as the most abundant species of *Staphylococcus* in other raw-cured meat products made from whole pieces (Von Reinbaben and Seipp, 1986; Molina et al., 1989; Carrascosa and Cornejo, 1991; Cornejo and Carrascosa, 1991; Rodríguez et al., 1994) and in fermented sausages (Seager et al., 1986; Coppola et al., 2000; Cocolin et al., 2001; Papamanoli et al., 2002; Rantsiou et al., 2005; Martín et al., 2006; García Fontán et al., 2007b; Martín et al., 2007). This species is, in fact, one of the most resistant to unfavourable environmental conditions (McMeekin et al., 1987; Chandler and McMeekin, 1989). *Staphylococcus xylosus* is also one of the most genetically variable microbial species, which may lead to the existence of diverse biotypes with different phenotypic characteristics (Kloos, 1980); this phenomenon may be due to the capacity of this species to adapt to different environmental conditions (Rodríguez Jovita, 1997). Some authors have proposed preparing a starter culture with this species in order to ensure good development of the process for ham, as well as for other raw-cured meat products (Hammes et al., 1985; Lücke and Hechelmann, 1987).

Among the novobiocin-resistant species of staphylococci, *Staph. equorum* was the next most abundant, although present in much smaller proportion than *Staph. xylosus*. This species was isolated equally from the batches made without additives and from those made with additives, and from the surface and the interior of the pieces. *Staphylococcus equorum* was the main species of staphylococci isolated from Spanish cecina (García et al., 1995) and has also been isolated by different authors in other raw-cured meat products made from whole pieces (Cornejo and Carrascosa, 1991; Kotzekidou, 1992; Rodríguez et al., 1994; Rodríguez Jovita, 1997), and from fermented sausages (Cantoni and Pizzo, 1980; Simonetti and Cantoni, 1983; Comi et al., 1986; Seager et al., 1986; García Fontán et al., 2007b). *Staphylococus equorum* is, along with *Staph. xylosus*, one of the most abundant species in fresh meat (Schleifer et al., 1984; Kloos, 1990).

Staphylococcus sciuri was isolated in very low numbers and was not isolated from the samples of the interior of the pieces in the batches made without additives. This species is, like *Staph. xylosus*, one of the species of staphylococci that is isolated in highest proportions at different stages of the process of manufacture of ham (Von Rheinbaben and Seipp, 1986; Molina et al., 1989). This species has also been isolated from sausages (García Fontán et al., 2007a), but in a very low proportion.

Finally, among the novobiocin-resistant species of staphylococci, *Staph. gallinarum* and *Staph. cohnii* were isolated in very low proportions (only two strains of each species).

Among the species of staphylococci sensitive to novobiocin, the most abundant was *Staph. intermedius* (17 strains). This species was obviously much less abundant than *Staph. xylosus*, but it was isolated equally from the surface and from the interior of the pieces in the batches made without or with additives. *Staphylococcus intermedius* has so far not been identified in raw-cured meat products made from whole pieces, but it has been isolated from raw-cured sausages (Comi et al., 1992; García-Varona et al., 2000). The other novobiocin-sensitive species of staphylococci isolated in the present study were *Staph. capitis* (9 strains), *Staph. epidermidis* (4 strains), *Staph. simulans* (3 strains) and *Staph. warneri* (3 strains). The finding of these four species is consistent with the novobiocin-sensitive species of staphylococci isolated by other authors in other meat products (Delarras, 1980; Kloos et al., 1992; Martín et al., 2006). The proportions in which the latter authors isolated the species were also low and very similar to those found in the present study.

The salt used to cure the product is generally accepted to be the source of species of novobiocin-resistant staphylococci. In a study of isolates of *Staphylococcus* originating from marine salt and from salt previously used in the salting process of hams from white or Iberian pigs, Cordero and Zumalacárregui (2000) found *Staph. xylosus* and *Staph. equorum* to be the main species of staphylococci in the three types of salt. However these species may also originate from other types of contamination, since they are widespread in nature and comprise part of the normal flora of the skin of many farm animals; in fact, in recent studies (Leroy et al., 2006; Corbière Morot-Bizot et al., 2010) *Staph. equorum* was described as the most prevalent species of staphylococci in the environment of small units manufacturing traditional sausages. The presence of these species in the samples from the interior of the cured meat pieces is generally associated with the salt that penetrates the pieces. The microorganisms may therefore originate from the salt or may be present as superficial contaminants in the pieces.

The novobiocin-sensitive species originated from contamination from the skin of humans and the animals themselves (Schleifer, 1986; Comi et al., 1992). This microbial group has been isolated preferably from human skin (Kloos and Musselwhite, 1975; Kloos, 1990) and its presence in meat products has been related to handling of the pieces prior to the salting process. However, some of the novobiocin-sensitive species of staphylococci isolated in the present study (*Staph. intermedius*, *Staph. capitis*, *Staph. epidermidis* and *Staph. simulans*) were also isolated by Cordero and Zumalacárregui (2000) from salt, after hams were subjected to the salting process, although these authors suggest that the species may have originated from human sources. Judging from the identity of the species of staphylococci isolated from the lacón and from their proportions, it appears that salt is the main source of these microorganisms.

As already pointed out, microorganisms belonging to the *Micrococcaceae* family (genera *Micrococcus* and *Kocuria*) were isolated in very low proportions relative to those of the family *Staphylococcaceae*; the species isolated were: *Kocuria varians* (6 strains), *K. rosea* (2 strains), *Micrococcus lylae* (1 strain) and *M. luteus* (1 strain).

According to Schleifer et al. (1984), *Kocuria varians* is the predominant species of animal origin. Other authors have also isolated this species from other raw-cured meat products made from whole pieces (Van der Riet, 1982; Kotzekidou, 1992; García et al., 1995) and from fermented sausages (Papamanoli et al., 2002; Martín et al., 2006; García Fontán et al., 2007b), although also in very low proportions. This species is included among the starter cultures used in raw meat products (Lücke and Hechelmann, 1987).

Kocuria rosea has not been described in raw-cured meat products made from whole pieces, but it has been observed, although in very low proportions, in raw-cured sausages (Coppola et al., 1997) and in foods of animal origin (Delarras et al., 1994). It is present in soil and water (Schleifer, 1986), and its presence in dry-cured lacón may be associated with contamination of this origin.

One strain of *Micrococcus luteus* was isolated from the surface of one piece manufactured with additives. Isolation of *M. luteus* is not common in raw-cured meat products. This species dominates on the human skin (Kloos et al., 1974) and its presence in the lacón may be associated with handling of the pieces, although this species was also isolated by Cordero and Zumalacárregui (2000) from salt originating from the salting of hams from white pig.

Micrococcus lylae has been isolated by De la Rosa et al. (1990) in semi-preserved meat products, by García Fontán et al. (2007b) in fermented sausages, and by Cordero and Zumalacárregui (2000) from salt from the salting process of hams from white and Iberian pigs. Its origin also appears to be associated with handling of the pieces since this species is habitually present on human skin (Kloos et al., 1974).

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Table 1. Evolution of the counts (log CFU/g) in the different culture media on the surface and in the interior of the pieces during the manufacture of dry-cured lacón made without and with additives (data are the average ± standard deviation of three batches in each manufacture type)

			Fresh piece	After salting	Post-salting (days)		Drying-ripening (days)				
					7	14	7	14	28	56	84
Without additives	SPC agar	Surface	6.71±0.44^{ab}	5.93±0.76^{a}	5.56±0.44^{a}	7.51±0.96^{bc}	8.45±0.73^{cd}	9.29±0.17^{d}	9.27±0.27^{d}	9.13±0.31^{d}	9.26±0.33^{d}
		Interior	3.51±0.38^{acde}	0.57±0.99^{b}	1.26±1.10^{abc}	3.27±1.64^{abcde}	3.53±1.25^{cde}	2.99±2.64^{abcde}	4.19±1.14^{de}	1.59±1.39^{abcd}	4.40±0.90^{e}
	SPC agar + 7.5% NaCl	Surface	4.56±1.51^{a}	3.99±0.63^{a}	4.24±0.43^{a}	6.39±2.64^{b}	8.43±0.77^{c}	9.28±0.17^{c}	9.33±0.33^{c}	9.22±0.23^{c}	9.31±0.25^{c}
		Interior	1.91±1.06^{ab}	-	1.63±0.46^{ab*}	2.71±2.37^{b}	3.49±1.32^{b}	2.97±2.62^{b}	4.03±1.21^{b}	1.64±1.43^{a*}	3.60±1.35^{b}
With additives	SPC agar	Surface	7.04±1.75^{a}	5.60±0.94^{b}	5.47±0.49^{b}	6.55±1.47^{ab}	8.60±0.48^{c}	8.85±0.21^{c}	9.41±0.02^{c}	9.24±0.30^{c}	8.37±0.88^{c}
		Interior	2.88±2.75^{ab}	0.89±1.54^{a}	2.23±2.52^{ab}	2.89±2.63^{ab}	3.99±0.81^{b}	3.76±0.94^{b}	4.46±1.12^{b}	4.25±0.36^{b}	2.92±2.53^{ab}
	SPC agar + 7.5% NaCl	Surface	4.36±1.09^{ab}	3.88±0.95^{a}	4.12±0.73^{ab}	5.70±2.19^{b}	8.58±0.54^{c}	8.83±0.27^{c}	9.39±0.05^{c}	9.29±0.24^{c}	8.39±0.88^{c}
		Interior	1.43±1.65^{ab}	-	1.74±1.64^{abd*}	2.59±2.57^{bcd}	3.66±0.38^{bcd}	3.80±0.95^{bcd}	4.42±1.19^{cd}	4.16±0.31^{d*}	2.46±2.30^{abcd}

[a-e] Values in the same row (corresponding to the same culture media and location in the piece) not followed by a common letter differ significantly (P<0.05).

[*] Values which were significantly different (P<0.05) in that sampling point when batches made without additives were compared with those made with additives.

- = Absence in 0.2 g.

Table 2. Values of some physico-chemical parameters during the manufacture process of dry-cured lacón made without and with additives (average values ± standard deviations of three batches in each manufacture type)

			Fresh piece	After salting	Post-salting (days)		Drying-ripening (days)				
					7	14	7	14	28	56	84
Without additives	T. S.[1]	Surface	26.19±1.41^{a}	37.00±4.68^{b}	38.56±0.10^{b}	48.16±2.44^{c}	48.89±0.64^{c}	62.34±5.72^{d}	60.46±1.67^{d}	64.54±3.04^{de}	69.07±0.37^{e}
		Interior	29.66±6.23^{a}	36.54±5.72^{b}	36.95±5.16^{b}	40.08±3.44^{bcd}	39.43±2.08^{bc}	43.90±4.40^{cd}	46.03±2.92^{de}	51.09±2.97^{e}	57.98±2.81^{f}
	NaCl[2]	Surface	0.45±0.03^{a}	23.37±2.75^{b}	22.67±2.50^{b}	16.04±1.63^{c}	14.77±2.11^{cd}	12.43±1.82^{cd}	12.06±3.82^{cd}	12.20±2.97^{cd}	10.99±2.24^{d}
		Interior	0.47±0.02^{a}	9.27±1.82^{b}	13.14±1.70^{b}	13.34±2.00^{b}	14.33±1.02^{b}	12.76±3.38^{b}	14.26±3.79^{b}	13.88±4.19^{b}	13.06±4.90^{b}
	pH	Surface	6.08±0.02^{ac}	6.09±0.03^{ac}	6.28±0.21^{abcd}	6.48±0.07^{bd}	6.19±0.32^{cd}	6.55±0.15^{bd}	6.43±0.04^{d}	6.34±0.16^{abcd}	6.33±0.23^{abcd}
		Interior	6.36±0.27^{a}	6.17±0.22^{a}	6.22±0.06^{a}	6.24±0.21^{a}	6.16±0.16^{a}	6.34±0.24^{a}	6.25±0.07^{a}	6.25±0.09^{a}	6.40±0.22^{a}
	a_w	Surface	1.000±0.001^{a}	0.912±0.036^{b}	0.928±0.014^{b}	0.907±0.020^{b}	0.904±0.010^{bc}	0.894±0.021^{bc}	0.834±0.057^{cd}	0.836±0.069^{cd}	0.806±0.069^{d}
		Interior	0.997±0.003^{a}	0.968±0.003^{ab}	0.962±0.003^{ab}	0.951±0.015^{ab}	0.947±0.014^{bc}	0.944±0.016^{bc}	0.930±0.025^{bc}	0.900±0.059^{cd}	0.876±0.075^{d}
	Nitrate[3]	Surface	42.94±4.24^{acd}	92.35±4.24^{b*}	84.90±10.01^{bd*}	70.00±9.63^{bcd*}	58.24±13.87^{cd*}	61.76±16.60^{d*}	60.20±9.51^{c}	50.39±11.90^{c*}	53.14±3.40^{c*}
		Interior	37.45±7.19^{acd}	44.51±8.91^{ad}	35.88±10.26^{abcd*}	32.75±4.75^{abcd*}	37.06±4.24^{acd*}	33.53±3.11^{abcd*}	24.12±3.53^{bc*}	31.96±4.45^{cd*}	39.02±6.04^{d*}
With additives	T.S.[1]	Surface	26.71±1.98^{a}	39.32±1.12^{b}	41.59±0.78^{b}	51.59±7.88^{c}	53.25±3.19^{c}	63.82±3.84^{d}	64.18±2.86^{d}	64.75±3.36^{de}	69.83±3.50^{e}
		Interior	28.72±4.25^{a}	40.21±2.61^{b}	40.45±3.53^{b}	38.73±2.25^{b}	42.18±5.21^{bc}	44.06±4.74^{bc}	48.00±3.23^{cd}	50.83±3.50^{de}	54.98±2.86^{e}
	NaCl[2]	Surface	0.46±0.03^{a}	22.59±2.15^{b}	20.05±1.68^{bc}	17.45±4.78^{c}	16.14±4.24^{cd}	11.70±1.42^{def}	10.87±2.37^{ef}	12.74±3.57^{df}	11.64±3.39^{f}
		Interior	0.45±0.03^{a}	8.33±1.45^{b}	11.06±1.59^{b}	11.34±2.84^{b}	13.26±2.49^{b}	13.35±3.50^{b}	12.62±4.87^{b}	12.44±5.63^{b}	13.14±5.58^{b}
	pH	Surface	6.22±0.28^{abc}	6.06±0.16^{ac}	6.38±0.03^{bc}	6.49±0.01^{b}	6.12±0.33^{c}	6.34±0.14^{abc}	6.23±0.08^{abc}	6.44±0.10^{ab}	6.21±0.24^{abc}
		Interior	6.58±0.10^{a}	6.02±0.10^{bc}	6.32±0.21^{abc}	6.23±0.31^{bc}	6.20±0.19^{bc}	6.13±0.27^{bc}	6.26±0.07^{bc}	6.38±0.12^{c}	6.30±0.23^{abc}
	a_w	Surface	0.999±0.001^{a}	0.893±0.085^{b}	0.920±0.014^{b}	0.910±0.020^{b}	0.889±0.035^{b}	0.864±0.032^{bc}	0.856±0.032^{bcd}	0.800±0.059^{cd}	0.787±0.051^{d}
		Interior	0.996±0.001^{a}	0.966±0.011^{ab}	0.956±0.016^{abc}	0.953±0.024^{abc}	0.945±0.023^{ef}	0.940±0.022^{bc}	0.928±0.037^{bcd}	0.910±0.034^{cd}	0.885±0.032^{d}
	Nitrate[3]	Surface	51.96±10.67^{a}	124.12±9.19^{bc}	115.10±6.79^{bce}	131.57±30.23^{b}	124.12±18.93^{bc}	101.76±24.02^{cdef}	78.63±9.87^{def}	92.75±22.08^{ef}	87.25±16.53^{f}
		Interior	47.25±7.83^{a}	50.39±5.56^{a}	85.69±5.31^{b}	83.33±12.02^{b}	88.04±1.36^{b}	80.20±12.58^{bc}	76.27±8.83^{bc}	68.82±8.48^{c}	70.78±8.67^{c}

[1] Total Solids (Expressed as g/100 g); [2] Expressed as g/100 g of Total Solids; [3] Expressed as ppm

[a-f] Values in the same row (corresponding to the same parameter and location in the piece) not followed by a common letter differ significantly (P<0.05)

[*] Values which were significantly different (P<0.05) in that sampling point when compared the batches made without additives with those made with additives

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Table 3. Changes in the species isolated from SPC agar + 7.5% NaCl from the surface and the interior of the pieces during the manufacture of dry-cured lacón made without additives (two batches)

	Species	Fresh piece		After salting		Post-salting (days)				Drying-ripening (days)										Total	
						7		14		7		14		28		56		84			
		Nº of strains	%	Nº of strains	%	Nº of strains	%	Nº of strains	%	Nº of strains	%	Nº of strains	%	Nº of strains	%	Nº of strains	%	Nº of strains	%	Nº of strains	%
Surface	*Staph. xylosus*	2	20	1	10	10	100	6	60	7	70	7	70	5	50	8	80	6	60	52	57.78
	Staph. equorum	0	0	1	10	0	0	2	20	0	0	0	0	1	10	0	0	1	10	5	5.55
	Staph. warneri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10	0	0	1	1.11
	Staph. epidermidis	0	0	0	0	0	0	0	0	0	0	1	10	0	0	0	0	0	0	1	2.22
	Staph. intermedius	0	0	1	10	0	0	0	0	2	20	2	20	1	10	0	0	1	10	7	5.55
	Staph. capitis	0	0	0	0	0	0	0	0	1	10	0	0	0	0	0	0	1	10	2	2.22
	Staph. sciuri	0	0	2	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2.22
	Staph. gallinarum	0	0	1	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1.11
	Kocuria varians	1	10	0	0	0	0	0	0	0	0	0	0	1	10	0	0	0	0	2	2.22
	Micrococcus lylae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10	0	0	1	1.11
	No *Micrococcaceae* or *Staphylococcaceae*	7	70	2	20	0	0	2	20	0	0	0	0	0	0	0	0	0	0	11	12.22
	Isolates lost	0	0	2	20	0	0	0	0	0	0	0	0	2	20	0	0	1	10	5	5.55
	Total isolates	10	100	10	100	10	100	10	100	10	100	10	100	10	100	10	100	10	100	90	100
Interior	*Staph. xylosus*	2	20	0	0	6	60	6	60	7	70	9	90	10	100	10	100	6	60	56	70
	Staph. equorum	0	0	0	0	1	10	2	20	0	0	0	0	0	0	0	0	0	0	3	3.75
	Staph. simulans	1	10	0	0	0	0	0	0	1	10	0	0	0	0	0	0	0	0	2	2.5
	Staph. intermedius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10	1	1.25
	Staph. capitis	0	0	0	0	1	10	0	0	0	0	0	0	0	0	0	0	0	0	1	1.25
	Staph. cohnii	0	0	0	0	1	10	0	0	0	0	0	0	0	0	0	0	0	0	1	1.25
	Staph. gallinarum	0	0	0	0	1	10	0	0	0	0	0	0	0	0	0	0	0	0	1	1.25
	Kocuria varians	1	10	0	0	0	0	1	10	0	0	0	0	0	0	0	0	0	0	2	2.5
	Kocuria rosea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10	1	1.25
	No *Micrococcaceae* or *Staphylococcaceae*	6	60	0	0	0	0	1	10	1	10	1	10	0	0	0	0	2	20	11	13.75
	Isolates lost	0	0	0	0	0	0	0	0	1	10	0	0	0	0	0	0	0	0	1	1.25
	Total isolates	10	100	0	0	10	100	10	100	10	100	10	100	10	100	10	100	10	100	80	100

Table 4. Changes in the species isolated from SPC agar + 7.5% NaCl from the surface and the interior of the pieces during the manufacture of dry-cured lacón made with additives (two batches)

	Species	Fresh piece		After salting		Post-salting (days)				Drying-ripening (days)										Total	
						7		14		7		14		28		56		84			
		Nº of strains	%	Nº of strains	%	Nº of strains	%	Nº of strains	%	Nº of strains	%	Nº of strains	%	Nº of strains	%	Nº of strains	%	Nº of strains	%	Nº of strains	%
Surface	*Staph. xylosus*	1	10	7	70	6	60	9	90	7	70	3	30	6	60	9	90	8	80	56	62.23
	Staph. equorum	0	0	0	0	1	10	1	10	1	10	1	10	0	0	0	0	0	0	4	4.44
	Staph. warneri	0	0	0	0	1	10	0	0	1	10	0	0	0	0	0	0	0	0	2	2.22
	Staph. epidermidis	0	0	1	10	1	10	0	0	0	0	0	0	0	0	0	0	1	10	3	3.33
	Staph. intermedius	0	0	0	0	0	0	0	0	1	10	3	30	0	0	0	0	0	0	4	4.44
	Staph. capitis	1	10	0	0	0	0	0	0	0	0	3	30	1	10	0	0	0	0	5	5.55
	Staph. sciuri	1	10	1	10	0	0	0	0	0	0	0	0	0	0	0	0	1	10	3	3.33
	Micrococcus luteus	0	0	0	0	0	0	0	0	0	0	0	0	1	10	0	0	0	0	1	1.11
	Kocuria varians	1	10	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	1	1.11
	Kocuria rosea	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1.11
	No *Micrococcaceae* or *Staphylococcaceae*	4	40	1	10	0	0	0	0	0	0	0	0	1	10	0	0	0	0	6	6.66
	Isolates lost	2	20	0	0	1	10	0	0	0	0	0	0	0	0	1	10	0	0	4	4.44
	Total isolates	10	100	10	100	10	100	10	100	10	100	10	100	10	100	10	100	10	100	90	100
Interior	*Staph. xylosus*	0	0	0	0	5	50	8	80	8	80	7	70	9	90	8	80	6	60	51	68
	Staph. equorum	0	0	0	0	0	0	0	0	0	0	1	10	0	0	0	0	1	10	2	2.67
	Staph. simulans	0	0	0	0	0	0	0	0	0	0	0	0	1	10	0	0	0	0	1	1.33
	Staph. intermedius	0	0	0	0	2	20	1	10	0	0	1	10	0	0	1	10	0	0	5	6.67
	Staph. capitis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10	0	0	1	1.33
	Staph. cohnii	0	0	0	0	1	10	0	0	0	0	0	0	0	0	0	0	0	0	1	1.33
	Staph. sciuri	0	0	0	0	0	0	0	0	0	0	1	10	0	0	0	0	0	0	1	1.33
	Kocuria varians	0	0	0	0	1	10	0	0	0	0	0	0	0	0	0	0	0	0	1	1.33
	No *Micrococcaceae* or *Staphylococcaceae*	5	100	0	0	0	0	0	0	1	10	0	0	0	0	0	0	3	30	9	12
	Isolates lost	0	0	0	0	1	10	1	10	1	10	0	0	0	0	0	0	0	0	3	4
	Total isolates	5	100	0	0	10	100	10	100	10	100	10	100	10	100	10	100	10	100	75	100

Influence of Run Time and Aging on Fouling and Cleaning of Whey Protein Deposits on Heat Exchanger Surface

Adel Fickak & Emma Hatfield
Biotechnology and Food Engineering Group
Department of Chemical Engineering, Monash University
Clayton Campus, Victoria 3800, Melbourne, Australia

Xiaodong Chen
Biotechnology and Food Engineering Group
Department of Chemical Engineering, Monash University
Clayton Campus, Victoria 3800, Melbourne, Australia
&
Department of Chemical and Biochemical Engineering
College of Chemistry and Chemical Engineering, Xiamen University, P R China
E-mail: xdc@xmu.edu.cn & dong.chen@eng.monash.edu.au

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Abstract

In the cleaning operations of heat exchange surfaces in dairy processing plants, the effect of heating/run time (HT) and aging on the fouling/cleaning of heat exchangers is not well understood. Longer heating time of the same deposit is expected to result in the formation of stronger and perhaps more cleaning resistant deposit. In this study, the phenomenon of heating and aging on the formation and cleaning of dairy fouling is investigated using the heat induced whey protein gels (HIWPG) produced in laboratory. The processes were also investigated with the whey protein deposits formed in pilot-scale plant trails.

The HIWPGs were produced in tubular capsules for various heating (or run) times (60, 120, 240, 1440 and 2880 min respectively) and then dissolved in aqueous sodium hydroxide (0.5 wt %). The heating process here would have been of 'pure' aging. The dissolution rate was calculated based on the previously established UV Spectrophotometer analysis. The structure and texture of the gels were analysed using Scanning Electron Microscope (SEM) and texture analyser. In the pilot-scale plant study, whey protein fouling layers were generated by recirculating whey protein solution (6 wt %) for various heating periods (30, 60, and 90 min respectively). The deposit layers were then removed by recirculating aqueous sodium hydroxide (0.5 wt %) and the cleaning efficiency was monitored in the form of the recovery of heat transfer coefficient while both fluid electric conductivity and turbidity were recorded as indications of cleaning completion. It was found that increasing the HIWPG heating time significantly increased the gel hardness and dissolution time, implicating the difficulty in cleaning. Similarly to these results on gels, increasing the pilot-scale plant heating/run time increased the extent of fouling. The fouling layer formed next to the metal surface experienced the longest period of aging and the slope of the heat transfer coefficient increase seen at the final cleaning stage is related to this aging effect. The rate of cleaning for deposits formed initially on the metal surface is lower indicating a 'pure' aging effect of the deposit near surface.

Keywords: Cleaning-in-place (CIP), Fouling, Heat induced gels, Dissolution, Dairy processing, Deposit aging

1. Introduction

It is known that heating of dairy fluids is a normal operation for pasteurisation or varying viscosity purposes. Sterilisation and evaporation processes also incur heating. The heating/run time (HT) varies between different

products depending on the specifications. However, severe fouling is frequently associated with prolonging the HT. Longer HT means that the fouling layers on the heating surface will experience aging. Though often mentioned as being an important process, the aging process of the fouled dairy deposits is poorly understood in a quantitative manner. It was early documented by Epstein in 1983 as one of the principal mechanistic stages in the fouling process. However, more recent studies have shown that aging facilitates longer reaction time between the fouling layer matrixes (Liu et al., 2002) and affects the fouling process resulting in a strong and firm fouling layer that is difficult to clean particularly when the fouling layer is aged for a long period (Wilson, 2005).

In studying the fouling and cleaning behaviours of dairy fluids, researchers face numerous difficulties in generating a reproducible fouling deposit due to the variations found in milk systems. To overcome these difficulties, many researchers (e.g. Xin et al., 2002a; Xin et al., 2002b) found heat induced whey protein gels (HIWPG) as a suitable model material to study the fouling and cleaning behaviors of the proteinaceous milk fouling. They found HIWPG to have similar nature as type "A" milk fouling. Furthermore, HIWPG are easy to reproduce (shape, size and concentration, and chemical state) in the laboratory under well-controlled conditions (Xin et al., 2002a; Mercadé-Prieto et al., 2006).

In this study, the effect of heating time and aging on the formation and dissolution behaviours of HIWPG has been investigated. The effect of heating time and aging on the fouling and cleaning behaviours of a pilot scale heat exchanger has also been studied using whey protein solution to mirror the finding made in the HIWPG experiment. Whey protein solution was used as an accepted milk fouling model and has been studied for its heat induced chemical changes (Bird and Fryer, 1991; Tuladhar et al., 2002; Xin et al., 2004; Hooper et al., 2006).

2. Experimental

2.1 Materials

The whey protein concentrate (WPC 85) powder was obtained from a local supplier. The approximate composition of the WPC is given in Table 1. The cleaning reagent, 60 wt % sodium hydroxide solution (NaOH), was purchased from LabServ, Melbourne, Australia.

2.2 Methods

2.2.1 Preparation of HIWPG in the lab and dissolution experiments

The same sort of heat induced gels and the methodologies established previously (Xin et al., 2002a; Mercadé-Prieto et al., 2006; Fickak et al, 2011) were used in the current study. All gels were prepared in triplicate and the errors calculated. Briefly, we introduce the procedures as below.

An experimental apparatus (see Figure 1 drawn after Fickak et al, 2011) was employed to dissolve HIWPG in the laboratory. The apparatus consists of an analytic balance, a digital magnetic stirrer plate with a stirring speed controller, a digital controller of the water bath, a dissolution cell (a 600ml shock bottle and a capsule holder) and a peristaltic pump to recirculate the solutions through a UV spectroscope linked to a computer.

The HIWPGs were formed in 'capsule' shape using the method developed by Mercadé-Prieto and Chen (2006). HIWPGs were formed inside test tubes (inner diameter 12 mm; length 75 mm) using well mixed 17 wt % whey protein solutions (pH 6.4 ± 0.05). The capsules were filled with the whey solution, then covered with plastic stoppers and sealed with foil, and then held vertically in a water bath kept at 80 °C for different heating times ('pure aging') (60, 120, 240, 1440 and 2880 min respectively). After that and before the dissolution, the top 2 mm of the gel was removed with a spatula to ensure an even surface before each dissolution experiment (Mercadé-Prieto et al., 2008). The gels were kept at 4 °C overnight before use. The gel capsules were then dissolved in batch mode and the dissolution rate of each gel was calculated based on the measured increase of the dissolved protein concentration in the NaOH solution over time. The concentration of NaOH solution was verified by titration of an aliquot with HCl (0.01 N). As shown in Figure 1, the gel capsules were held vertically with stainless steel wire that was fixed to a hole at the top of the bottle lid and submerged inside a 600 ml test bottle containing 500 ml of NaOH (0.5 wt %) solution, which was placed in a water bath at 60 ± 1 °C. The solution was stirred at 200 rpm using a magnetic stirrer to ensure the reading of a well mixed solution. A small fraction of the agitated solution was circulated through the cell of a UV spectrometer (HP Agilent 8453 Spectrophotometer, model number: G1103A, Agilent Technologies, Melbourne, Australia) using a peristaltic pump. Using the method established by Mercadé-Prieto and Chen (2006) and also adopted by Fickak et al (2011), the absorbance of the dissolved proteins was continuously recorded at 20s intervals for at least 8000s at wavelength 280nm. The rate of dissolution was calculated by measuring the increase of the concentration of protein (calibrated again UV absorbance) in the NaOH solution over time.

2.2.2 Gel strength measurements

The hardness of HIWPGs is an indicator of the ease for removal during cleaning as shearing is involved in deposit removal in reality. The hardness may be affected by the heating time during the gelation process. Here a depth sensing indentation hardness test, was used which has been widely used for characterizing the consistency of fats and other materials (DeMan, 1983). The test was standardized by the American Oil Chemists Society (AOCS) method 'Cs 16-60' (DeMan, 1983). The test involves the penetration of a sample surface by a metal probe indenter with a known geometry (DeMan, 1969). A parameter referred to as the 'yield value' or 'hardness index' can be calculated by monitoring the penetration force of the probe and the time taken to achieve that penetration depth (DeMan, 1983; Narine and Alejandro, 2001). The sample gels were made in a 50 ml beaker using 10 ml of well mixed whey protein solutions (17 wt %) and held in a water bath at 80 °C for different heating times (60, 120, 240, 1440 and 2880 min respectively). The gels were kept at 4 °C overnight and brought to room temperature (23 ± 2°C) before being tested. The texture analyses were performed at room temperature (23 ± 2°C) using a EZ Graph texture analyser ("EZ Graph 100N", Shimadzu Scientific Instruments, Melbourne, Australia) equipped with a 100-N load cell, and a 12.8 mm diameter cylindrical probe. The test procedure was developed using Shimadzu Instruments texture tests guidelines. Each test was performed by allowing the probe at speed of 50 mm min^{-1} to penetrate 10 mm into the gel, and the force at 5 mm of penetration was taken as gel strength value.

2.2.3 Microstructure analysis

Images of the microstructure of the gel samples may be informative of the fouling formation and were obtained using Scanning Electron Microscope (SEM) (JEOL JSM-840A SEM, 1986, Japan), located at Monash University, Clayton Campus, Melbourne, Australia. Prior to image scanning, $2mm^{-2}$ gel samples were sliced and placed in a dry oven at 30 ^{0}C for 10 min. The gel samples were then sputtering coated (approximately 1mm) with gold palladium for charge dissipations. The samples were then viewed with the SEM operating at 15KV using back scattering electrons.

2.2.4 Pilot-scale fouling and cleaning-in-place (CIP) experiments

A pilot-scale plant test rig was designed, constructed to generate and remove whey protein fouling layers (refer to Figure 2, after Fiakak et al (2011)). This system has a 60 L reservoir was equipped with heating coils to preheat the sample solutions. The solution in the holding tank was stirred continuously by re-circulating through the bypass valves. A centrifugal pump was used to ensure an easy circulation of sample solutions. The flowrate in the heating section was kept constant during the fouling and cleaning processes and monitored through a flowrate meter (Model 257-133' from RS Components, Melbourne, Australia) installed at the inlet of the fouling section. The velocity in the heating section (see Table 2) was calculated using the flowrate and the heating section area.

As mentioned by Fickak et al (2011), the heating section consisted of a heater rod (diameter= 17mm, length =160mm) fixed inside a sealed glass tube reactor (ID= 80mm with length= 300mm) with the bottom inlet of ID=20mm. Three outlets (ID=20mm) were uniformly distributed (120^o apart) at the top. A turbidity meter (Model TB750G) and conductivity meter (Model DC402G) from YOKOGAWA Electric Corporation, Melbourne, Australia) were installed downstream of the fouling section to monitor the cleaning process. In the current study, the rig operating conditions were kept constant for each run (see Table 2).

2.2.5 Generation of the fouling layer

Similarly in Fickak et al (2011), whey proteins concentrate powder (WPC, 80 wt %) was reconstituted to protein concentration of 6 wt % (pH 6.4 ± 0.05) in 50 litres of RO water. The solution was then transferred to the holding tank and preheated to approximately 70 ± 0.5 ^{o}C and then held for 5 min before being pumped through the fouling section and back to the tank. The recirculation of the solution in the holding tank through the bypass valve results in a continuous stirring of the solution, this help to sustain the required tank temperature. At the start of the experiment, the heater rod surface temperature (the average of the temperature around the outside diameter of the heater rod upper, middle and bottom surface) was obtained at approximately 81 ± 1 ^{o}C by applying the heat flux described in Table 2 using a 5A variac autotransformer. The fouling layers were formed in 30, 60 and 90 min respectively. The fouling layer formed in each run was found to be reasonably evenly distributed along the heater rod surface. The small flow velocity (see Table 2) was employed to exaggerate the heat-to-foul effect as the removal force by the fluid became rather small.

2.2.6 Monitoring of fouling

The most common method for monitoring fouling and cleaning is to record the change in the heat transfer coefficient. A drop in the heat transfer coefficient simply indicates the formation of a fouling layer on the heater surface. The heat transfer coefficient was calculated using the following equation:

$$U \approx \frac{Q}{A(T_S - T_B)} \quad (1)$$

where U = overall heat transfer coefficient ($W.m^{-2}.K^{-1}$); Q = power input to the heater rod (W); A = heater rod surface area (m^{-2}); T_S = heater rod surface temperature (^oC) (The average of the temperature around the outside diameter of the heater rod upper, middle and bottom surface); T_B = bulk fluid temperature (^oC) measured in the fouling section.

2.2.7 Monitoring of the temperatures of the heater rod surface

As mentioned above the average temperature measurement of the heater rod surface was obtained by attaching three thermocouples around the outside diameter of the upper, middle and bottom surface of the heater rod. The bulk fluid temperature was measured through another thermocouple that was placed inside the bulk solution in the heating section of the transparent section leading to the heater rod. The inlet and outlet temperatures of the fouling section were monitored using thermocouples inserted at the inlet and outlet of the fouling section. The tank temperature was monitored using a thermocouple located at the centre of the tank. All measured temperatures (surface, bulk, inlet, outlet and tank) were logged to a computer.

2.2.8 Cleaning of the fouled surface

The fouled surface was cleaned using a three-stage cleaning method. In the first stage, after the fouling layer was formed the whey protein solution was drained and the system was rinsed with water at a velocity of about 0.104 $m.s^{-1}$, for approximately 10 min, until there were no protein traces left in the rinsing water. The rinsing efficiency was indicated using the turbidity meter by detecting the level originally present in the clean water stream. The rinsing process was stopped once the standard turbidity of drinking water (0.5 to 1NTU) was reached (USEPA, 2001).

In the second stage, similar to that described by Fickak et al (2012) in a study emphasizing the effect of protein concentration, the cleaning solution (50L of NaOH at 0.5 wt %) was used. During cleaning, the cleaning solution temperature was kept constant at 60 ± 0.5 ^oC. In the cleaning in place (CIP) process, first the cleaning solution was recirculated through the system. The heater rod surface temperature (approximately 66 ± 0.5 ^oC) was attained by applying the heat flux described in Table 2 using enclosed 5A variac autotransformer. The CIP process was monitored visually by observing the change in the heat transfer coefficient and by visually observing the complete removal of the fouling layer through the glass wall of the fouling section (see Figure 6b). An increase of the heat transfer coefficient corresponds to the removal of the fouling layer on the heater surface. The CIP solution was drained when the fouling layer was seen to be completely removed. The cleaning fluid velocity was set at 0.104 $m.s^{-1}$, which was the comfortable upper limit of the rig.

In the third stage, the system was continuously rinsed with water for approximately 10 minutes until there were no NaOH traces left in the rinsing water. The rinsing at this stage was monitored using the conductivity meter. The rinsing was stopped when the typical conductivity of drinking water (<500 μS/cm) (DeZuane, 1990) was reached. The turbidity and conductivity measurements were both used to indicate the efficiency of the cleaning process. After this, an observation of the heater rod was made for the cleanliness of the above processes.

3. Results and Discussion

3.1 Effect of heating time on HIWPG formation

The scanning electron microscope (SEM) images of the HIWPG deposits microstructure (see Figure 3) show that the HIWPG deposit formed at shorter heating time (30 min) contains small and perhaps undeveloped aggregates (see Figure 3a), while the HIWPG deposit formed at longer heating time (1440 min) contained more and larger aggregates (see Figure 3b).

The texture analysis (see Figure 4) shows that the HIWPG deposit formed at shorter heating time (60 min) required a penetration force of approximately 3N to penetrate through the gel, indicating a soft textured gel. In contrast, the HIWPG deposit formed at longer heating time (1440 min) required much higher force of approximately 25N to penetrate through the gel surface, indicating a firm textured gel.

These results can be explained as follows. Upon heating the whey protein solution forms a gel due to the reaction between the proteins molecules in the matrix forming many small monomers. These protein monomers contain a number of cysteine residues (Sawyer et al., 1985; Brownlow et al., 1997) and upon heating the free – SH groups of cysteine residues get oxidized and form disulfide bonds (Verheul and Roef, 1998; Galani and Apenten, 1999). These bonds are involved in cross-linking the protein monomers to form the aggregates (Galani and Apenten, 1999). However, increasing the heating time provides more reaction time between the protein matrixes, increasing the number of disulphide cross-links between the protein molecules. These protein molecules may aggregate more intensively to form a hard and rigid gel. The longer the heating time, the harder the gel becomes.

3.2 Effect of heating time on HIWPG dissolution

The dissolution experiments of HIWPG deposits obtained with different heating times (see Figure 5) show that HIWPG deposit heated for longer time (1440 min) had the lowest dissolution rate (2.23g m^{-2} s^{-1}). Another observation was that the dissolution rate of HIWPG deposit formed in 240 min or longer (1440 min) remain almost the same (0.24 and 0.23g m^{-2} s^{-1}) indicating the structure of the gels stabilized after 240 min.

As discussed earlier, longer heating time provides the time for greater extent of the reactions to happen, in particular for the thiol-disulfide exchange reactions (Livney et al., 2003; Jayat et al., 2004). The disulfide bridges are known to be a key factor that affects the gel strength (Hoffmann and Van Mil, 1999; Creamer et al., 2004) and the aggregation process through the polymerization of monomers to form large polymer chains. However, there is only limited availability of the bonds to be formed and hence excessively longer heating time may not be effective anymore. In the dissolution of large polymer chains, or gels, (Devotta et al., 1995; Narasimhan and Peppas, 1996) predicted that the large clusters or the large chains polymers are expected to disengage very slowly from the gel matrix into the solvent. Our results seem to be within the expectation based on previous works.

3.3 The effect of heating/run time on whey protein solution fouling

Figure 6a shows the examples of fouled and cleaned heater rod. The fouling layer formed is similar as type 'A' deposits described by (Lyster, 1965; Burton, 1968). It can be seen that longer heating time resulted in visibly more (denser) fouling (the thickness is not measured here). Figure 7 shows (a) the overall heat transfer coefficient profile during the fouling process; and as an example (b) the temperature difference (ΔT) between the heat exchange surface (average) and the bulk solution during the 90 min run. The decreasing overall heat transfer coefficient is an indication of the fouling layer formation on the heat transfer surface. The formation of fouling layer on the heat exchange surface leads the overall heat transfer coefficient to drop.

The fouling behaviour observed in the first 30 min was similar for all runs particularly for the 30 and 60 min runs. The overall heat transfer coefficient decreased continuously with increasing run time (see Figure 7) indicating the formations of more heat resistant fouling on the processing surface. Since the deposit chemical composition would not change during heating so the same deposit experiences 'aging' in essence. The possible explanation for this is that heating the whey protein solutions above 60°C, provokes complex changes of the molecular conformation and undergo denaturation (Aymard et al., 1999). Similarly as discussed in the HIWPG formation, this leads the protein molecules to exhibit hydrophobic areas on its surface (Cantor and Schimmel, 1980; Verheul and Roef, 1998; Galani and Apenten, 1999). The denaturation of whey proteins particularly β-lactoglobulin is considered by many e.g. Grijspeerdt et al. (2004), to be significant in most dairy fouling. The partially denatured proteins aggregate via the exposed covalent disulfide bonds (Galani and Apenten, 1999; Perez-gago and Krochta, 2001) and eventually precipitate on the heat exchange surface. The kinetics of aggregation and the aggregation rate are influenced by the heating conditions (Aymard et al., 1999). With increasing heating time, the formation of strong covalent disulfide bonds may become more extensive within the deposit. The aggregates grow in strength and size, while the fraction of native proteins decreases (Durand et al., 2002). These observations are, in principle, consistent with the observations of Liu et al (2002) on non-dairy product and Wilson (2005) on a general discussion who suggested that increasing the heating time results in increasing the yield and the deposit hardness due to the deposit structural changes. Regardless of the 'structural consolidation', the accumulation of deposit on the heat transfer surface should lead, in any case, to greater thermal resistance hence the overall heat transfer reduction shown Figure 7.

3.4 The effect of heating/run time during fouling on the subsequent cleaning

Figure 8 shows (a) the overall heat transfer coefficient profile during the cleaning in place (CIP) process; and as an example (b) the temperature difference (ΔT) between the heat exchange surface (average) and the bulk solution during the cleaning process of the fouling layer from 4wt % whey protein solution. The time course of

the recovery of the overall heat transfer coefficient is equivalent to the cleaning time required to remove the fouling layer from the heat transfer surface.

Figure 8 shows the recovery of the overall heat transfer coefficient (CIP, or removal time). CIP time of about 1400s was required to remove the deposits formed at the longest (90 min) heating/run time. A CIP time of about 500s and of about 1000s were needed to clean deposits formed at shorter heating/run times of 30 min and of 60 min respectively. The slopes of the first stage of the cleaning curve were not so different, which is an indication of the nature of the deposits that were formed at the final stages of fouling where the temperature at the fouling layer surface would be fairly close to the bulk solution temperature, and the fouling formed at this stage would be weak and more likely interacted via hydrogen bonds which is highly water soluble (Perez-gago and Krochta, 2001) making it easy to remove. The slopes of the cleaning curve in the final stages of the cleaning should indicate more accurately the effect of ageing as this should be the cleaning of the most inner layers of the deposits which would have 'consolidated' for the longest time. The results by comparison are quite dramatic. The slopes marked as $\alpha 1$, $\alpha 2$ and $\alpha 3$ in Figure 8 simply indicates that the aging is most pronounced at the inner layers and that the longer the aging time of the inner layers, the harder it is to remove the fouling. This mirrors the results of the gel strength tests and the gel dissolution experiments (the rate of the recovery of the overall heat transfer coefficient α values for the three different fouling periods are plotted against cleaning time; see Figure 5), described earlier.

4. Conclusions

The HIWPGs, obtained for different heating periods, show dissolution behaviour which mirrors well the cleaning behaviour of whey protein fouling layers formed in the pilot-scale rig as far as the trend is concerned. In the HIWPG experiment, longer heating time ('pure aging' behaviour) produced harder gel materials. The dissolution rate of HIWPG decreased with increasing heating/run (aging) time, and the rate stabilizes after 240 min of heating/aging.

In the pilot-scale experiment, two separate effects are inter-related: The heating/run time and the aging. The prolonged experiment created more fouling. The inner fouling layer experienced considerable ageing. The aging has caused significant reduction on the cleaning rate, this effect may be due to the further chemical bond formation or some kind of structural re-arrangements within the deposit which does not affect much the heat conduction, but clearly affect the difficulty of disentanglements (thus harder to remove). The exterior fouling layer shows similar cleaning rate regardless of run time.

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Nomenclature

Q = Power requirement (W)

A = Heating surface area (m-2)

T = Temperature (K)

α = Slope (J.m-2.K-1.s-2)

HT = Heating time (s)

Subscripts

S Heater surface

B Bulk

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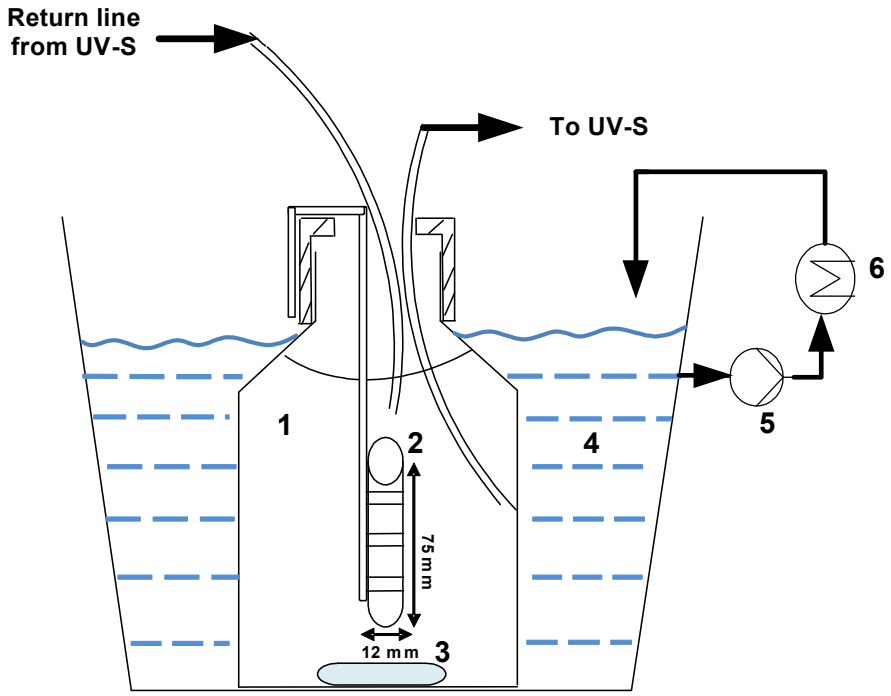
Table 1. Whey Protein Concentrate (WPC) powder composition

Component	**Content (wt %)**
Proteins	82.0
Fat	6.2
Moisture	3.5
Ash	8.3

Table 2. Pilot plant operation conditions during fouling and CIP processes

Operation variable	**Fouling**	**CIP**
Feed tank liquid volume	50 L	50 L
Feed tank temp	70 ± 0.5°C	60 ± 0.5°C
Velocity	0.001 $m.s^{-1}$	0.104 $m.s^{-1}$
Heat flux	6.560 $kW.m^{-2}$	1.098 $kW.m^{-2}$



1. Dissolution solvent (0.5 wt %) NaOH
2. Capsule containing heat induced whey protein gel
3. Magnetic stirrer
4. Water bath
5. Pump
6. Heater

Figure 1. Apparatus for the dissolution of heat induced when protein gels

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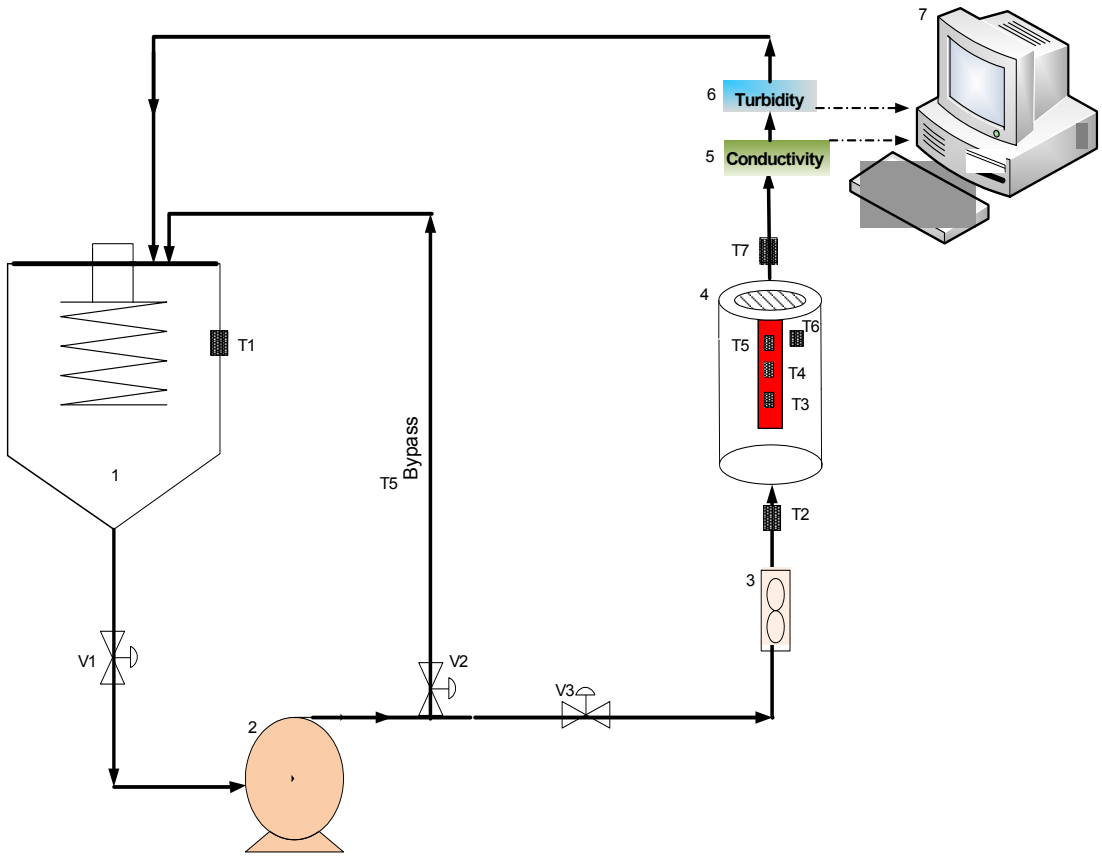

1 Tank including coiled heater
2 Centrifugal pump
3 Flow meter
4 Fouling section (include a heater rod)
5 Conductivity meter
6 Turbidity meter
7 Computer for temperature, turbidity and conductivity recording

TI-1: Tank temperature sensor
TI-2: Fouling section inlet temperature sensor
TI-3: Heater rod bottom surface temperature sensor
TI-4: Heater rod middle surface temperature sensor
TI-5: Heater rod top surface temperature sensor
TI-6: Bulk solution temperature sensor
TI-7: Fouling section outlet temperature sensor
((TI-3) + (TI-4) + (TI-5)) / 3: Surface temperature

Figure 2. Fouling and cleaning test system

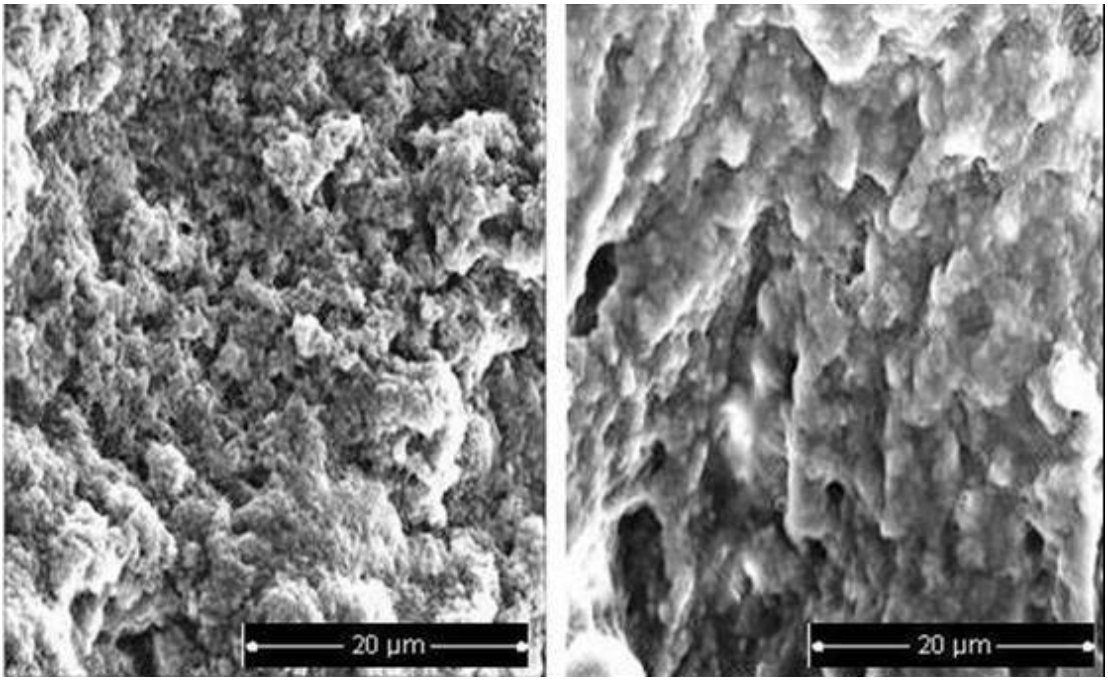

Figure 3. SEM images of the microstructure of the 17 wt % HIWPG formed at (a) 30 min heating time, and (b) 1440 min heating time

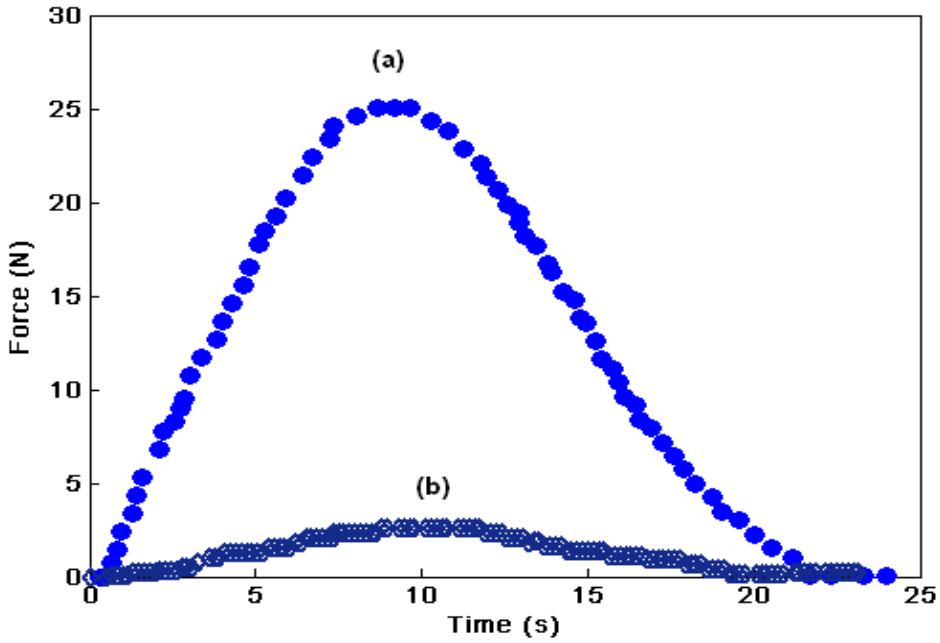

Figure 4. Texture analysis of (a) 17 wt % HIWPG obtained after 1440 min, and (b) 17 wt % HIWPG obtained after 60 min

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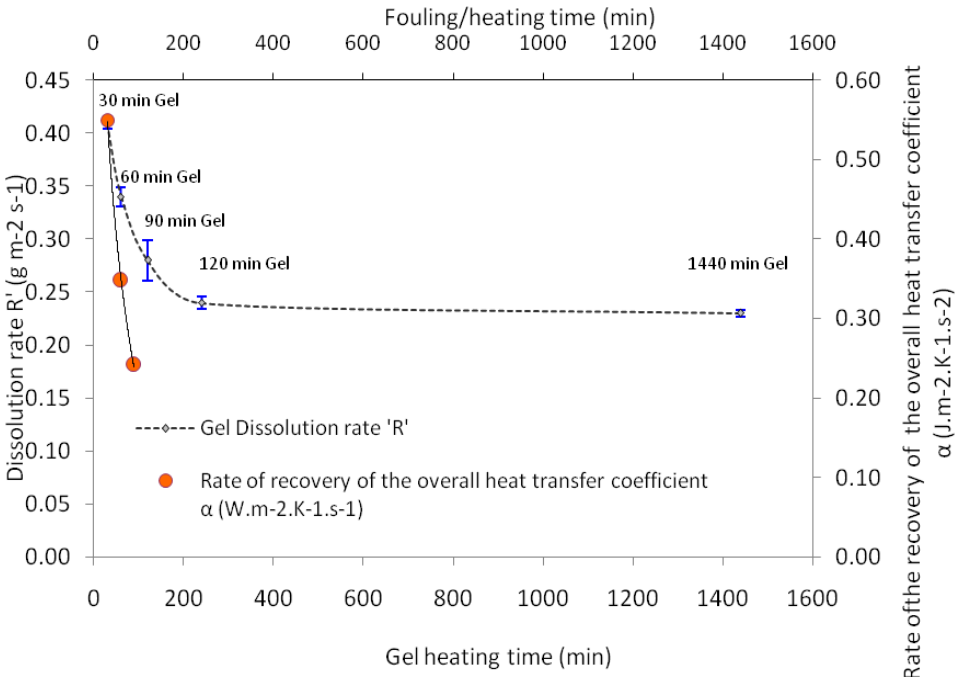
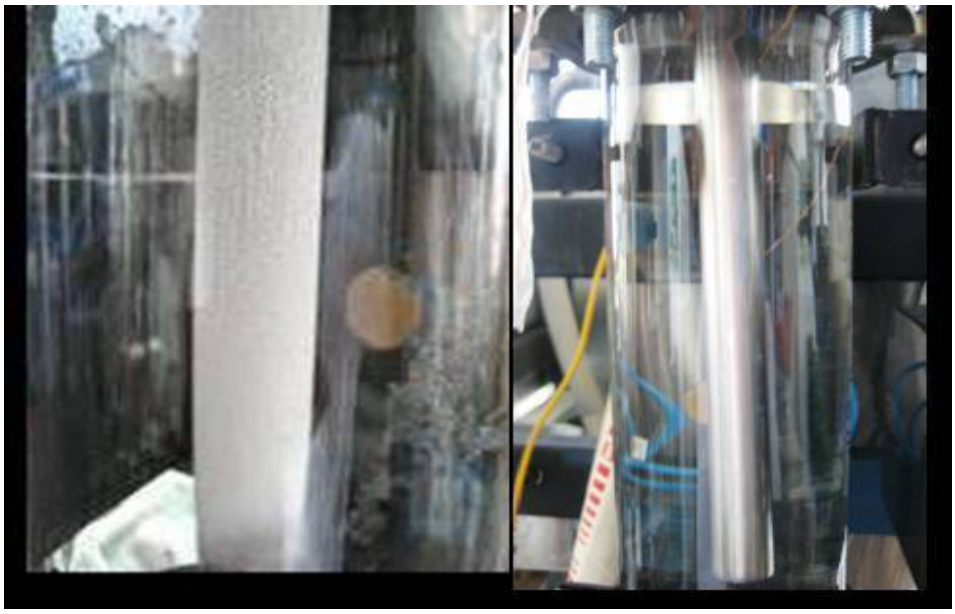


Figure 5. Effect of different heating time (60, 120, 240 and 1440 min) on the dissolution rate 'R' of 17 wt % HIWPG in 0.5 wt % NaOH at 60 ^{o}C, compared with the slopes 'α1, α2 and α3' from the CIP cleaning curves in Fig 11 corresponding to different fouling/heating times



(a) **(b)**

Figure 6. (a) Fouled rod heater with, 6 wt % whey protein solution; fouling layer formed in 90 min heating time before cleaning, and (b) after cleaning

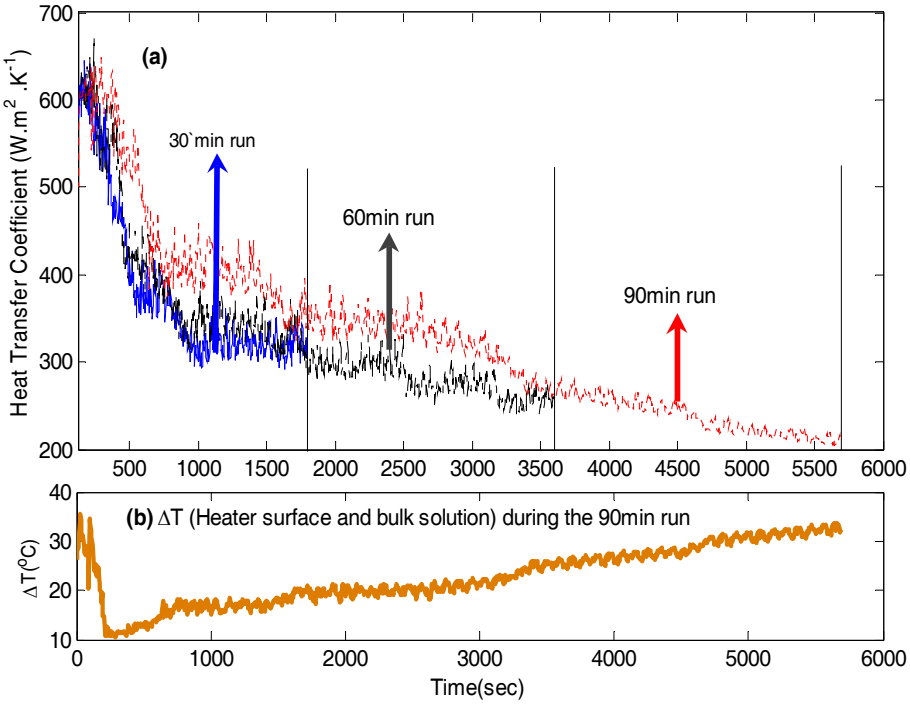


Figure 7. (a) Effect of run/heating time and aging on the fouling behaviour of whey protein solution, (b) Heater surface and bulk solution temperature difference during the 90 min fouling

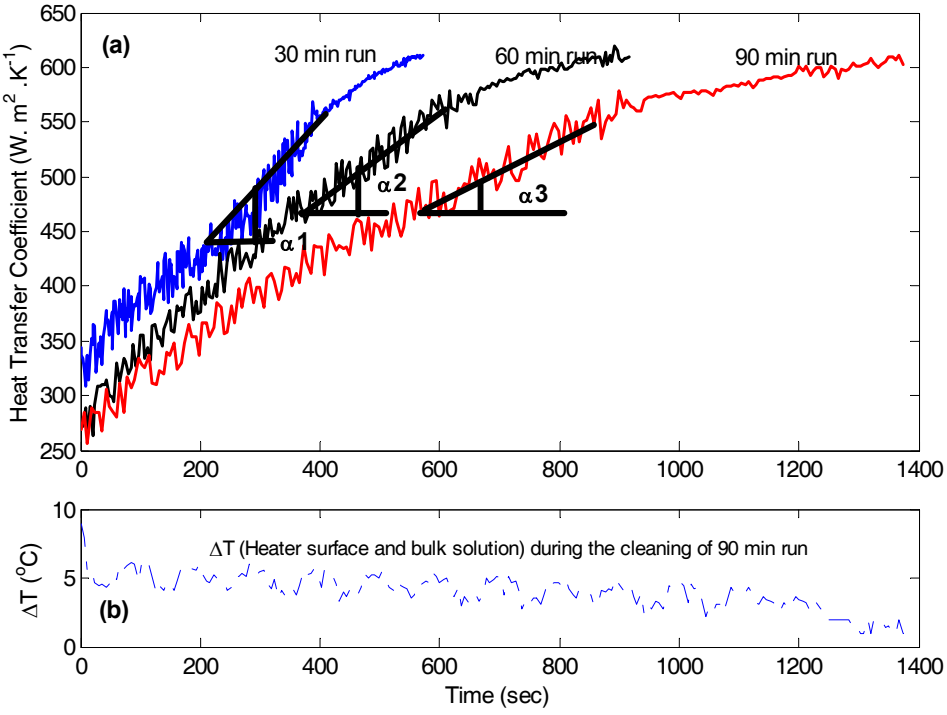


Figure 8. (a) Cleaning-in-place (CIP) behaviour of whey protein concentrates (WPC) solution fouled at various run times; α1, α2 & α3 show the significant slopes towards the finish of the cleaning run. (b) Heater surface and bulk solution temperature difference profile during the cleaning of the 90 min fouling

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The Evaluation of Frozen Strawberries Quality by Studying the Kinetics Change of the Antioxidants Activity

Elisaveta Sandulachi (Corresponding author)
Technical University of Moldova, 168, Stefan cel Mare Avenue
Chisinau, MD-2004, Republic of Moldova
Tel: 373-6850-4188 E-mail: luiza_sandulachi@yahoo.com

Tatarov Pavel
Technical University of Moldova, 168, Stefan cel Mare Avenue
Chisinau, MD-2004, Republic of Moldova
Tel: 373-2232-1946 E-mail: p_tatarov@yahoo.com

Diana Croitor
Technical University of Moldova, 168, Stefan cel Mare Avenue
Chisinau, MD-2004, Republic of Moldova
Tel: 373-7944-6171 E-mail: croitordiana88@yahoo.com

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Abstract

This paper presents the study results on quality assessment of frozen strawberries estimating the inetics change of antioxidant activity of fruit during storage.

Fresh strawberries purchased from the trading system were used for the research. The study was conducted over 3 years. A total of 60 batches of strawberries harvested in Moldova were analyzed , from each batch were estimated three samples.

It was appreciated the content of antioxidants: L-hidroascorbic acid, anthocyanins, total polyphenols and reducing activity of fruit. The precision of the characteristics determination for oxido-reducing state of fruit samples (K, mg AA/g SH) is obtained with the trust coefficient $P< 0.01$ or $P<0.05$, deviation error limit being ± (0.2 … 0.8) % from the average values of the K indicator. The correlation of the total hydrosoluble antioxidants content (ascorbic acid, anthocyans, polyphenols) and the oxido-reducing state indicator of the fruit (K) attests the values of the Pearson coefficient (R) within 0.88 … 1.0 and has a functional dependence. The validity of frozen fruit was demonstrated by determining the probability of quality modification of frozen strawberries.

Keywords: Frozen strawberries quality, Antioxidant activity, Oxido-reducing state, Hydrosoluble antioxidants, Pearson coefficient

1. Introduction

The quality of a product is given by macronutrients and micronutrients content. To assess the nutritional value of fruit products a special attention is paid to the content of bio antioxidants – biologically active substances. From the viewpoint of the scientists from different countries, the antioxidants play a protective role in preventing the cardiovascular diseases, diabetes, gastrointestinal diseases and have a positive role on lifetime, maintaining youth and reducing the oxidative stress (Ivan, 2007; Becker et al., 2004; Proteggente et al., 2002).

In general, the current scientific studies suggests that the colored fruits and vegetables are important sources of antioxidants, provide the necessary of vitamins, minerals and fibers for a proper health, protects against the

effects of aging and reduce the risk of cancer and the cardiovascular diseases (Olsson et al. 2006; Kiss, et al. 2005; Becker et al., 2004).

These new findings confirm and expand on the results of three studies published in 2003, 2005 and 2006, which show that strawberry extracts can reduce the growth rate of human lung and liver cancer cells, and mouse skin cancer cells, to substantial extents (Olsson et al., 2006; Ramos et al., 2005; Wang et al., 2005; Meyers et al., 2003).

Strawberries are delicious fruits, with a good smell and taste, nice appearance. That is why they are requested fresh. Their sensory properties are determined by the presence of phenol substances and anthocyanins. Strawberry are an excellent source of vitamin C and manganese. They are also a very good source of dietary fiber and iodine. Plus, strawberries are a good source of potassium, vitamin B2, vitamin B5, vitamin B6omega -3 fatty acids, vitamin K, magnesium, and copper (Ivan, 2007; Wang et al., 2005; Gherghii et al., 2001). Strawberry also contain an array of beneficial phytonutrients, including flavoinoids, anthocyanidins and ellagic acid (Wang et al., 2005; Sandulachi, 2005; Gherghii et al. 2001; Proteggente et al., 2002).

Srtawberries, like other berries, are famous in the phytonutrient world as a rich surce of *phenols.* In the strawwberry, these phenols are led by the *anthocyanins* (especially anthocyanin 2) and by the *ellagutannins.* The *anthocyanins* in strawberry not only provide its flush red color, they also serve as potent antioxidants that have repeatedly been shown to help protect cell structures in the body and to prevent oxygen damage in all of the body's organ systems. Strawberries unique *phenol* content makes them a heart-protective fruit, an anti-cancer fruit, and an anti-inflammatory fruit, all rolled into one (Wang et al., 2005; Proteggente et al., 2002).

The strawberry, like many other perishable fruits suffer changes in time. Strawberries are perishable and under the action of peroxidase enzymes that contribute to the appearance of brown compounds and the loss of smell, they support permanent changes of phenol substances (their oxidation) (Tatarov et al., 2010; Sandulachi et al., 2010; Croitor et al., 2010 ; Wang et al., 2005; Gherghii et al. 2001; Duckworth, 1975).

The results (Proteggente et al., 2002) indicate that fruits soch as strawberry are an excellent source of phenolics and vitamin C and therefore possess an extremely high antioxidant potential in fact, the anthocyanins, which are the major class of phenolics inthis type of fruits, generally have demonstrated high antioxidant activity in *vitro systems.*

During storage and processing, the content of these substances is reduced according to their involvement in the process of oxido -reduction. Their evolution depends on several factors, as: the chemical composition of the fruits, parameters of the technological process, storage conditions, etc (Tatarov et al., 2010; Banu et al., 2004; Banu et al., 2003; Mihalca et al., 1986).

We need to mark that the conservation through freezing is one of the most effective method of preserving the perishable fruits quality. Commonly, strawberries are preserved by freezing and stored frozen. In industrial conditions frozen strawberries are usually kept at the temperature of -18°C (Banu et al., 2004; Banu et al., 2003). At the same time, the congealed products in storage period suffer some modification of sensory characteristics and nutritional value (Sandulachi et al., 2006; Marfart, 1996; Mihalca, et al., 1986; Saguy et al., 1980).

2. Materials and Methods

2.1 Cnemicals

Folin-Ciocalteu phenol reagent, gallic acid monohydrate, glacial acetic acid, L-ascorbic acid, sodium carbonate was obtained from EM Science, alcohol, hydrochloric acid.

2.2 Fruits

For oficial examination were used autochthonous strawberry, acquired and trade network. There were evaluated a total of 60 batches of strawberries, harvested in R. Moldova during three years. Strawberry samples were frozen and kept at the temperature of minus 18°C for 10 months. Experimental studies have been performed for the following varieties: Victoria, Sega-Segana, Frumuşica. The study was conducted both for fresh and frozen strawberries. The estimation of the reducing state of frozen strawberries was carried out in samples stored 3, 6 to 10 months. It was evaluated the dependency between the reducing state of frozen strawberries on bio antioxidants content and the influence of the technological process on the quality of strawberries.

2.3 Sample freezing

The samples were congealed in „Ghiocel" freezer at the temperature of -18^{o}C. Before congelation preventive operations were made: sorting, washing, top water –drying. The samples were packed in polisterol bags or Al/bald with weight 30.0 – 50.0g. Before being hermetically closed, the samples were treated with N_2 for

reducing the O_2 strength. For freezing the samples it was performed the following scheme: reception of raw material → Washing → Inspection → Removing water from the surface (through vibrating and air blowing) → Weighting the samples (g) → Packaging, polystyrene packages, aluminium foil. Sample mass 30–50g → Vacuuming and nitrogen treatment (Pressure value P =1.5 atm., for 2-3 minutes) → Tightening the packages → Freezing at -18°C, freezer → Storage. Depositing the frozen samples (t = -18°C, for 3, 6 and 10 months). Nitrogen (N_2) was used to remove the air from the package, in order to have anaerobic conditions (prevents aerobic spoilage and oxidative degradation of bioantioxidants).

2.4 Thawing of samples

Rapid thawing (temperature of 98°C, for 1-2 minutes), for the samples in which was determined the content ascorbic acid, polyphenols and anthocyanins. Slow thowning (room temperature, for 30 minutes) for the samples in which was investigated the loss of juice during thawing. In fresh samples and in the congealed one, was estimated the antioxidant capacity, acid ascorbic through method potentiometric (GOST 24556-81, 1981); total pholyphenol content through Folin-Ciocalteu method (Snegiryova et al., 1976); total anthocyanins content through physical-chemical method (Snegiryova et al., 1976); and property of redox state, expressed with K-index, mg AA/ g HS (Macari et al., 2005).

2.5 Tested indicators

There were determined the following indicators in the samples: content of soluble substances, the active acidity, pH, the maturity and the content of bio antioxidants: ascorbic acid by potentiometric method, total polyphenols by Folin-Ciocalteau method, total anthocyanins by the standard physicochemical method. Also, it was assessed the oxido-reducing state of strawberries by potentiometric method, expressed by the oxido-reducing index (K) (Sandulachi, 2006; Sandulachi et al., 2006; Macari et al., 2005;). The interdependence of reducing state of frozen strawberries and the content of ascorbic acid, anthocyanins and total polyphenols were assessed by processing statistically the experimental data. (Sandulachi, 2006 “a”; “b”; Sandulachi et al., 2006; Гутер et al., 1970). Pearson correlation coefficient was determined by using Microsoft Excel program.

2.6 General principles of evaluation of redox state

Generally speaking, the process of oxidation and reduction is evaluated according to the rate of redox potential of a closed system, in our case - food product (Шульц et al., 1984;). The fixed value of redox potential is a sign of equilibrium state in all the redox couples of food. Redox couples represent multiple redox reactions of chemical compounds, including bioantioxidants from food. The principle of the elaborated method is based on the laws of chemical thermodymanics, by forming the equilibrium state between two separate systems-product and etalon system. Two electrochemical cells were used with aim. In each cell there were introduced two electrodes: platimum electrode and silver electrode. The silver electrodes were connected to each other, platinum electrodes were connected to the potentiometer. In one cell there was introduced a food sample for testing and in the other one-the etalon solution. According to Nernst equation the redox potential of etalon system-ascorbic acid and the redox potential of the tested food (Macari et al., 2005). The ratio:

$$\lg\left\{\frac{[\mathrm{AA}_{ox}]}{[\mathrm{AA}_{red}]} / \frac{\left[\sum P_{ox}\right]}{\left[\sum P_{red}\right]}\right\} = K \qquad (1)$$

Is a relation with constant value and it is expressed by the K coefficient. Relation (1) shows the ratio between ascorbic acid concentration and the concentration of the tested product in equilibrium state ($E_1 = E2$). The K coefficient is a constant index that depends on the values of standard potential of ascorbic acid and values of standard formal potential of the food product (Macari et al., 2005).

2.7 The experimental method for assessing the redox state

Before the determination, raw material was preventively thermally treated at +96°C during 2-3 minutes, then it was homogenized, 10-15g of sample was diluted in proportion of 1:1 using distillated water and was introduced in the measuring cell. In cell II was introduced buffer solution with *p*H of the analyzed product – the reference solution. After the thermo stating of sample at 20°C, electrodes of platinum and silver chloride are introduced in cells. We register the difference between the redox potentials of cells, and in cell II we dose the etalon solution (ascorbic acid) until there is equality between the potentials of cell I and II. The potentiometer shows E =0. The redox state of the tested products is expressed by the K coefficient as ascorbic acid equivalent mg per g product hydrosoluble solids (HS). The K coefficient is calculated using the following relation:

$$K = \frac{C_1 \times V_1 \times m_1}{m_2 \times C_2 \times m_3} \quad (2)$$

where: C_1 – concentration of the etalon solution, mg/ml;

C_2 – the mass fraction of hydrosoluble solids, g/g;

V_1 – the volume of the etalon solution of ascorbic acid that is equivalent to the redox potential of the tested system, ml;

m_1 – the mass of the analyzed sample after dilution, g;

m_2 – the mass of reference solution, g;

m_3 – the mass of the analyzed sample, g.

3. Discussion and Suggestions

Sensorial analyze of frozen strawberry appearance showed that the fruit color remained almost the same compared to the fresh fruits. We observed just a weak appearance of brown color. Analyzing the contents of L-hidroascorbic acid, pholyphenols and whole anthocyanins it was observed a diminution of these indexes on depositing way. Medium values are presented in Table 1. Higher shown index diminution was different: depending by the staple primary quality, staple procession previous freezing, keeping term and pack quality.

The antioxidants activity consists the biological value of the fruits. These indicators are directly proportional (Tatarov et al., 2005: Proteggente et al., 2002). The biological value of the product is higher with the increasing of the reducing activity. The basic index that characterize the reducing activity of the antioxidants is oxido-reducing condition (Tatarov et al.,2005; Sandulachi, 2006, "b"; Macari et al., 2005). As the chemical composition of strawberries, including content and antioxidant activity was very variable, the experimental data were analyzed by statistical methods, using the probability theory Sandulachi, 2006, "b"; Sandulachi, 2006, "c"; Sandulachi et al. 2006).

It was found that there is a slow degradation of antioxidants in the frozen strawberries in the first three months of storage. At a longer storage – the oxidative degradation of antioxidants is faster. The study reveals a decrease in: polyphenol content within 15.2 - 66.6%, anthocyanin content within 6.85 - 37.83%, and L-Hydroascorbic acid content 7.15 – 33.25%.

Therefore, it was found that during the storage of frozen strawberries there are changes in the content of antioxidants and their oxido-reducing. Reduction of these features is a function of shelf life and the freezing temperature. Contents diminution in pholyphenols after 10 months at frozen strawberry was medium estimated at 46.18 %, contents diminutions in anthocyanins was medium estimated at 23.50 %, but contents diminution in ascorbic acid was estimated at 24.72 % (Figure 1).

It was found that in congealed and stored strawberries at the temperature of -18^{o}C there is a slow antioxidant degradation in first months. At a longer depositing period (10 months, t=-18°C) biologically active substances degrade oxidative at an accelerated rate. The degree of degradations occurs in the following sequence: polyphenols, ascorbic acid, anthocyanins.the content of biologically active substances in dependency of raw material, conditions of storage, processing, freezing and storage (Sandulachi, 2006, "b", "c","d". Sandulachi et al.,2004).

Probably through the water contents in unfrozen decrease we can explain a very big diminution of plolyphenols, ascorbic acid and anthocyanins in investigation samples. The activity and enzymatic reaction speed almost touches the maximal values of water stratums in frozen strawberry. Probably this phenomenon leads at modification the chemical compounds, plus antioxidants and redox state decrease in food environment.

In the frozen products the enzymatic reactions are slower, but not completely blocked. In general, the enzyme activity in frozen strawberries is related to the presence of unfrozen water. At a temperature of minus 18°C the water content in frozen strawberries is about 89% of total water content. The unfrozen water will be 11%. At a temperature of minus 30°C the frozen water content will be 91% of the total water content and 9% of unfrozen water (Duckworth, 1975).

In this paper there are presented the results of the experimental researches of the oxido-reduction state of strawberries, the influence of the state of berries by the content in active state of the ascorbic acid, of anthocyanines, and total polyphenols during the period (for 3 years). It is also presented the statistical analysis of

the experimental data of the dependence of the oxido-reduction state of the strawberries on the biologically active substance of the berries: ascorbic acid, anthocyanines and total polyphenols. The correlation coefficient of the oxido-reduction state of the fruits and the content of the biologically active substances was estimated between 0.66 … 0.99 (Figure 2).

Experimental data obtained are related to bibliographic data. Also, it was evaluated the influence of the technological process on the quality of strawberries preserved by freezing. In industrial conditions, the frozen strawberries are stored at a temperature of minus 18°C. Several scientists researches shows that during storage there are nutritional values and sensory properties changes in the frozen strawberries and this generated loss of quality. At the same time, it is considered that one of the most effective methods of preserving the quality of the perishable fruits is by freezing. Level of quality of frozen strawberries was achieved by studying changes in the content of antioxidants and the reducing state of fruits during storage.

In general, the oxido-reducing state of strawberries reflects not only the total content of reducing substances, but also the reducing activity of the compounds with major activity. The oxido-reducing state of strawberries is influenced on the content of ascorbic acid, anthocyanins and polyphenols. The study showed that the oxido-reducing state is strongly influenced by the polyphenols content. The correlation cefficient between K and the polyphenols content was between 0.88 and 0.91. These results indicate that once again that polyphenols exibits the largest reducing activity (Sandulachi et al., 2006). The Pearson correlation varies within 0.9…0.99 (Figure 3).

Datelor experimentale şi prelucrarea statistică [Sandulachi] demonstrează că există o corelaţie semnificativă intre starea oxido-reducătoare şi conţinutul în bioantioxidanţi din căpşune. Cercetările efectuate demonstrează că în căpşune raportul intre conţinutul total de substanţe în stare redusă şi în stare oxidantă este deplasat în partea redusă. Conţinutul substanţelor reduse este mai mare decît cel al substanţelor oxidate. În general starea oxido-reducătoare a căpşunelor poate fi caracterizată ca stare redusă cu valorile indicelui K – 11.83 – 19.93 mg AA/g SH.

Analyzing the experimental data it was found that oxido-reducing condition of frozen strawberries is reduced exponentially. As shown Figure 4.

In accordance with the fundamental principles of the kinetics of chemical reactions the change rate of food quality can be expressed by a functional reaction depending on the chemical composition of food and the environmental parameters (Kennth et al., 2004; Saguy et al., 1980):

$$\frac{dQ}{d\tau} = \mathbf{F}\,(\mathbf{C_i}, \mathbf{E_j});\qquad (3)$$

where: $\frac{dQ}{d\tau}$ - the quality change rate of food;

Q - is the integrated indicator of food quality;

Ci – intrinsic factors, ie chemical composition of food factors (concentration of various chemical substances, water activity, enzymes, etc.);

Ej – extrinsic factors, ie environmental factors (temperature, relative humidity, radiation and other physical effects).

In this research the $\mathbf{C_i}$ represent the antioxidant content: of L-hydroascorbic acid, polyphenols and total anthocyanins. The quality of frozen strawberries was evaluated by changing the antioxidants activity. So, $\mathbf{C_i}$ indicate the changing of the strawberries activity (**K** index).

$\mathbf{E_j}$ indicator – external factor, represents the freezing and storing temperature (-18°C).

The integral quality of frozen strawberries **Q** was expressed by the reducing activity of the fruits antioxidants. The quality index was presented as follows:

$$\mathbf{Q = A_o}$$

$\mathbf{A_o}$ is the quality of frozen strawberries by reducing activity of antioxidants.

The quality of frozen strawberries ($\mathbf{A_o}$) was measured by biological value, meaning the summary activity of antioxidants (L-hydroascorbic acid, polyphenols and total anthocyanins) expressed by **K** index.

In general, the quality change rate of frozen strawberries $\mathbf{A_o}$ during storage at minus 18°C is presented by the relationship:

$$\frac{dQ}{d\tau} = \mathbf{F\ (\ K\ ,\ t\ (-18\ ^{o}C));} \quad (4)$$

The kinetics of the changing of the antioxidant activity – L-hydroascorbic acid, anthocyanins and total polyphenol content can be summarized as follows:

$$\frac{d(A_o)}{d\tau} = \mathbf{K_i\ [A_o^{I}]^{n1}[A_o^{II}]^{n2}[A_o^{III}]^{n3};} \quad (5)$$

where: **Ao** is the general value of antioxidant activity, expressed by K index.

$\mathbf{A_o^{I}, A_o^{II}, A_o^{III}}$ – accordingly the activity of L-hydroascorbic acid, anthocyanins and total polyphenols;

$\mathbf{K_i}$ - chemical reaction rate constant;

$\mathbf{n_1, n_2, n_3}$ - order of reaction.

In the case of complex systems containing antioxidants (5) the speed of reactions is irreversible.

In strawberries simultaneously presenting real systems, there is an interdependence between content and the antioxidant activity and the chemical composition and the physiological structure of plant tissue. The separation of antioxidants in strawberries will result in destruction of the structure and physic-chemical characteristics of strawberries. The research of the changing of antioxidants in strawberries was performed taking into account these properties. We studied the kinetics of changes in the antioxidant activity.

Equation (5) was simplified by using the integral activity of antioxidants – $\mathbf{A_o}$

$$-\frac{d(A_o)}{d\tau} = \mathbf{K_j\ [A_o]^{\alpha}} \quad (6)$$

where: **Ao** is the total antioxidant activity;

α - the order of the oxidation reaction of the antioxidants;

Ki - reaction rate constant (Pseudo constant because it is not known the order of the reaction – α).

First, it is necessary to determine the order of the reaction **α**. Through logarithming the (6) equation, we obtain:

$$\mathbf{\log\ [} \frac{d(A_o)}{d\tau} \mathbf{\ ] = -\log K_i + \alpha \log\ [A_o]} \quad (7)$$

The experimental data form a straight line in the following coordinates $\mathbf{\log\ [} \frac{d(A_o)}{d\tau} \mathbf{\ ] - \log\ [A_o]}$ (Figure 5).

The reaction order was calculated through statistical processing. Receiving the coefficient of correlation R=0.977, antioxidants oxidation reaction was of first degree.

Based on the results, the equation (6) can be summarized as follows:

$$-\frac{d(A_o)}{d\tau} = \mathbf{K_i\ (A_o)} \quad (8)$$

Equation (8) was used to determine the numerical value of the reaction rate constant – Kj

Integrating the equation (8) we obtain:

$$\mathbf{\tau\ K_1 = \log\ [A_{o1} / Ao]} \quad (9)$$

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Where $\mathbf{A_{o1}}$ is the initial activity of antioxidants Ao1 (τ=0)

$\mathbf{A_o}$ – the antioxidants activity during the time of preservation of strawberries (τ).

From the equation (9) was determined the reaction rate constant K1. Experimental data in semi logarithmic coordinates log (Ao) – τ (Figure 6) shows a straight line.

The angle α of the straight line (Figure 6) is numerically equal to the reaction rate constant Kj:

$$\mathbf{K_j = 0{,}1065\ ^1/\ months}$$

The change in antioxidants activity during storage of frozen strawberries is characterized by the equation:

$$\mathbf{\log (A_o) = - 0{,}1065 + \log (A_{o1})} \quad (10)$$

K1 coefficient was used for determining the change in the quality of frozen strawberries Ao during storage at a temperature of -18°C.

There were assessed the probability of quality loss during the storage of frozen strawberries.

Because the chemical composition of strawberries, including antioxidant content is extremely variable, it is impossible to assess the change of the quality using equation (10). Equation (10) reflects only the quality changes of strawberries with equal reaction rate constant Kj = 0.1065 1 / months. In fact, there can be purchased strawberries with different constant values of Kj and antioxidant activity Ao.

This divergence can be resolved using the probability theory. The problem is to determine the probability of quality loss Ao during storage at the temperature of -18°C, for a period of 0 ... τ months.

According to equation (10), the experimental data (Figure 5) shows that the evolution of the antioxidants in the examined frozen strawberries (respectively the quality Ao) is subjected to exponential equation:

$$\mathbf{Ao = A_{o1} * \exp (-0{,}1065\tau)} \quad (11)$$

For strawberries containing a variable antioxidants content and activity, the equation (11) can be represented in general:

$$\mathbf{A_o = A_{o1} \exp (-\lambda\tau)} \quad (12)$$

where λ is a parameter of the exponential equation with a constant value, λ = const.

According to the probability theory, for the exponential distribution the probability of distribution of density φ (τ) within the limits (0 ... τ) will be [9]:

$$\begin{cases} \mathbf{0}, & \\ \mathbf{A_0 \exp (-\lambda\tau)}, & \mathbf{\tau \geq 0} \end{cases} \quad (13)$$

The probability density function φ has the following property:

$$\int_{-\infty}^{+\infty} \varphi(\tau) d\tau = 1 \quad (14)$$

Using equation (14), the integral within [0, ... τ], according to the probability distribution function will be:

$$\int_{-\infty}^{+\infty} \varphi(\tau) d\tau = \int_{-\infty}^{+\infty} A_o e^{-\chi\tau} d\tau = - \frac{A_o}{\chi} \int_0^{\infty} = \frac{A_o}{\lambda}$$

From equation (14) follows:

$$\frac{A_o}{\lambda} = 1, \qquad A_o = \lambda, \qquad \Rightarrow \qquad A_o e^{-\lambda\tau} = \lambda e^{-\lambda\tau}$$

Probability density function F (τ) is given as (Гутер et al., 1970):

$$F(\tau) = \int_{-\infty}^{X} \varphi(\tau) d\tau \tag{15}$$

From (15) we get:

$$F(\tau) = \int_{-\infty}^{\tau} \varphi(\tau) d\tau = \int_{0}^{\tau} \lambda e^{-\lambda\tau} d\tau = -e^{-\lambda\tau} \int_{0}^{\tau} d\tau = \lambda e^{-\lambda\tau}$$

After calculations we obtain:

$$\mathbf{1 - A_0 \ exp(-\lambda\tau), \qquad \tau \geq 0} \qquad F(\tau) \tag{16}$$

The probability of changing of Ao in time is:

$$\mathbf{P(\tau) = 1 - F(\tau) = 1 - F(1 - e^{-\lambda\tau})} \tag{17}$$

where P (τ) is the quality probability of frozen strawberries

Equation (14) is used to calculate the quality probability of frozen product during storage.

$$P(0 < \tau < ß) = F(ß) - F(\alpha) = F(1 - e^{-\lambda\tau}) - F(1 - e^{-\lambda 0}) = 1 - e^{-\lambda\tau} - 1 + 1 = F(1 - e^{-\lambda\tau})$$

where τ is the storage time, months

$$\mathbf{P(\tau) = F(1 - e^{-\lambda\tau})} \tag{18}$$

The λ parameter characterizes the rate of quality change of the product. In these researches λ = K = 0,1065 ^{1}/ months. Physical meaning of λ is the speed of reducing activity change of antioxidants.

The corelation (18) reflects the quality level probability of frozen strawberries depending on storage duration (τ).

The quality losses Q of frozen strawberries are determined by the relationship:

$$\mathbf{P(\tau) = 1 - F(1 - e^{-\lambda\tau})} \tag{19}$$

The relationship (19) was used to calculate the probability of quality loss of frozen strawberries (Figure 7).

Figure 4 shows that the first 3 months there has been a slow decrease of quality as the probability of loss is up to 26%. Based on experimental data, it has been determined a slow decrease in antioxidant content and a slight reduction in the reducing state. After 7 to 12 months the probability of storage loss becomes more relevant, up to 50% ... 71%. Perhaps at a lower freezing temperature (- 25 ... - 30 ° C) quality losses of the strawberries were much smaller.

4. Conclusions

The oxido-reducing state of strawberries depends on the content of polyphenols, anthocyanins and ascorbic acid. The oxido-reducing state is strongly influenced by the content of polyphenols.

In frozen strawberries, during storage there are nutritional and sensory properties changes, resulting in loss of quality. Quality can be determined by analyzing the kinetics of the changing of the bio antioxidants activity.

It was found that the frozen strawberries stored at a temperature of -18°C in the first three months show a slow degradation of the biologically active substances. At a longer storage (10 months, t=-18°C) the rate of the degradation is accelerated.

Based on presented methodology is possible to predict the reliability of frozen fruits and also the quality term of maximum nutritional value and the reducing activity of antioxidants.

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Table 1. The modification of antioxidants in congealed strawberry during stor

The average walue of the annual sample	Antioxidants content, mg/100g			Redox state decrease K, mg AA/g HS	The correlation coefficient Pearson between K index and antioxidants (R)			
	A*	Ph*	L- AA*		A*; R_1	Ph*; R_2	AA*;R_3	The total antioxidants content , R_s
Fresh strawberries								
1	20.7 ± 0.53	290.5 ± 9.47	39.4 ± 0.24	16.3 ± 0.65	0.78	0.92	0.84	0.93
2	21.7 ± 0.39	157.0 ± 8.93	35.2 ± 0.37	15.0 ± 0.38	0.99	0.99	0.67	0.97
3	41.9 ± 0.47	251.87 ± 5.70	45.7 ± 0.30	13.7 ±0.25	0.88	0.99	0.96	0.99
Frozen strawberries 3 months								
1	16.7 ±0.13	165.44 ± 8.35	34.1 ± 0.35	3.21 ± 0.28	0.80	0.99	0.80	0.99
2	19.2 ± 0.54	96.5 ± 7.60	28.9 ± 0.42	3.5 ± 0.45	0.67	0.95	0.88	0.90
3	37.5 ± 0.32	230 ± 2.60	32.5 ± 0.34	8.10 ± 0.23	0.94	0.99	0.99	0.99
Frozen strawberries 10 months								
1	14.9 ± 0.43	96.9 ± 9.51	26.3 ± 0.44	1.23 ± 0.32	0.99	1.00	1.0	0.94
2	16.6 ± 0.65	84.5 ± 7.57	26.5 ± 0.18	2.7 ± 0.28	0.98	1.00	0.83	1.00
3	36.7 ± 0.14	164.5 ± 3.12	27.5 ± 0.15	7.72 ± 0.15	0.99	1.00	0.99	1.00

*where: A – Anthocyans, Ph - Total Polyphenols, AA - L-hydroascorbic acid.

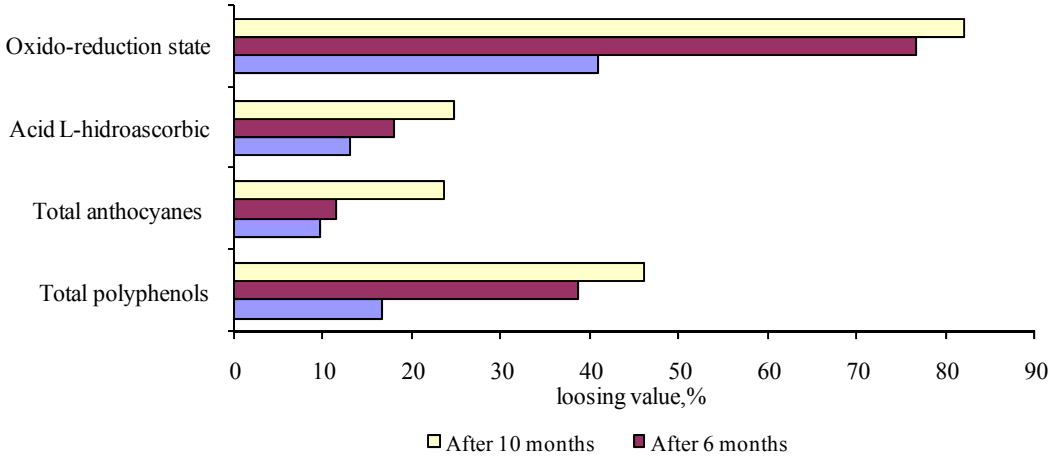

Figure 1. Phisical-chimical index degradation rate in frozen strawberries

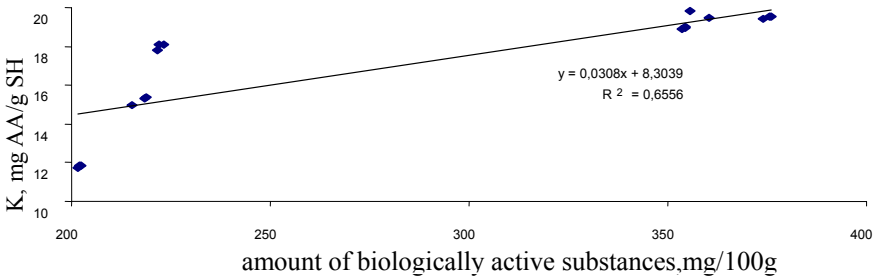

Figure 2. Kinetics of Antioxidant Activities

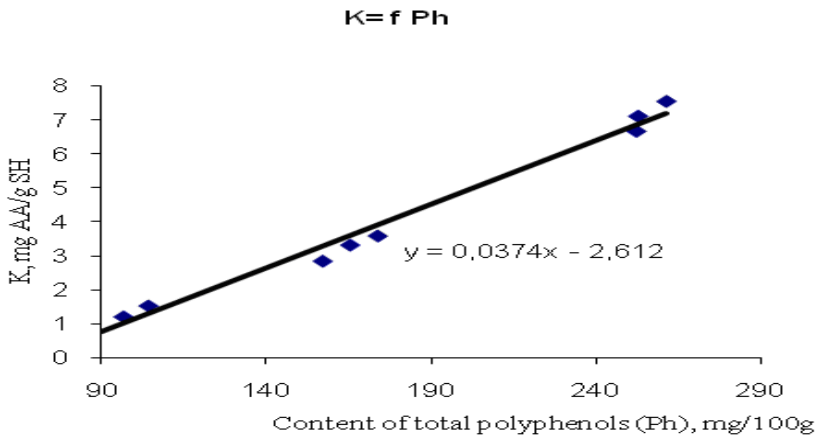

Figure 3. The correlation between K index and polyphenols content in frozen strawberries

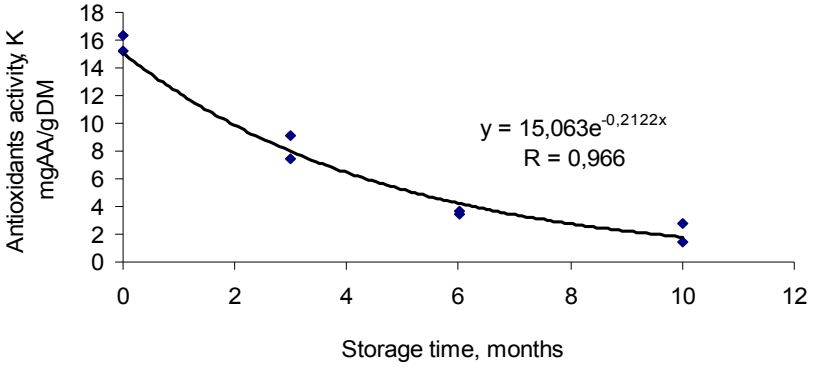

Figure 4. The evolution of antioxidants activity in frozen strawberries

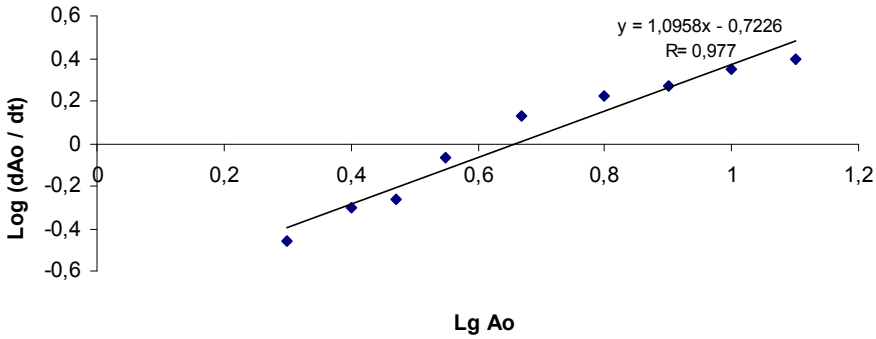

Figure 5. Kinetics of Antioxidant Activities

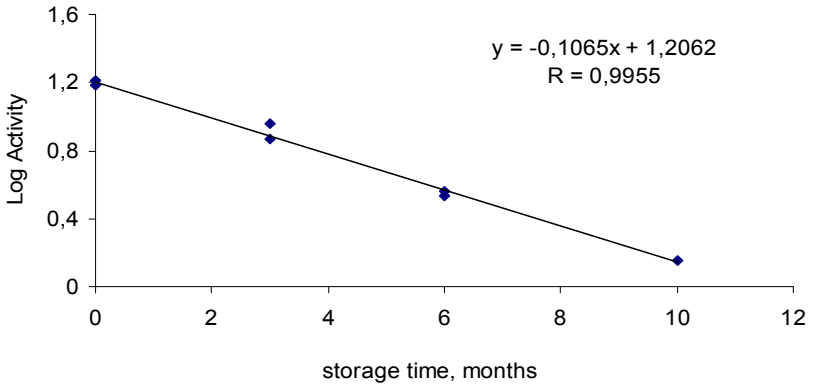

Figure 6. Changes in antioxidants activity during the storage of frozen strawberries

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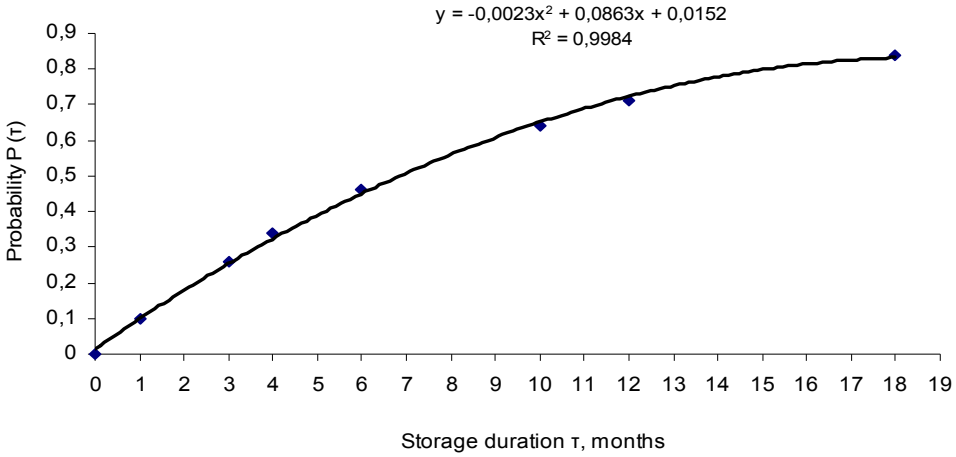

Figure 7. The probability of quality loss of frozen strawberries during storage

The Value of Women's Indigenous Knowledge in Food Processing and Preservation for Achieving Household Food Security in Rural Sudan

Fatma Osman Ibnouf
Development Studies and Research Institute (DSRI), University of Khartoum,
PO. box 321, Postal Code 11115, Khartoum, Sudan
E-mail: fatma_ibnouf@hotmail.com

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Abstract

Availability of food supply and access are crucial to achieve household food security. Using of indigenous knowledge in solving food shortage remains a powerful means of sustaining household food security. In rural Sudan women are often responsible for food processing and storage, collecting of water and firewood and for generating incomes for subsistence. The objective of this article is to shed light on women ability to manage available resources by using indigenous knowledge to secure food supplies for their household in rural Sudan. The paper argues that women have more roles and responsibilities in achieving household food security especially among the rural communities of Sudan, since it is woman who is responsible for food processing and preserving to increase its availability. Processing of vegetables, fruits, and milk, in times of abundance for times of scarcity using indigenous techniques such as drying or fermentation is exclusively the women's task in rural Sudan. Traditional/indigenous foods provide inexpensive, safe, nutritious foods throughout the whole year. These indigenous foods contribute to diversify the diet of rural people in normal times and are crucial to their survival during times of food shortage. Thus, these indigenous foods become basis of the survival of the rural community. Trial and error experiments contribute to develop many indigenous techniques and practices for processing and preserving foods at rural community level. Indigenous methods and solutions applied by women to sustain household food supplies are culturally acceptable, economically practicable, and more appropriate for the local environment and conditions than modern techniques and solutions suggested by scientific experts. The article gives a brief description of some indigenous foods from various rural areas of Sudan. Rural women have an important role to play in using and preserving this valuable indigenous knowledge. These local-level experts manage to achieve sustainable food security at household levels, with practical, efficient and economic solutions. Thus, one effective means to achieve household food security in rural Sudan is by learning from the women's indigenous knowledge and inherent capabilities. The value of women's indigenous knowledge needs to be better recognized and supported within policy.

KeyWords: Indigenous Knowledge, Women, Food Security, Rural Sudan

1. Introduction

The economy of rural Sudan has been centered on subsistence agricultural crop production and livestock rearing. Women represent the majority of the rural population (up to 70% according to censuses), especially where migration and male flee or death due to conflicts have left them as head of households. Women are extensively involved in agricultural activities which are the dominant livelihood feature of rural areas. Sudan's climate limits the production of food for only one rainy season (June to October). Food security at the household level refers to the ability of food providers to secure adequate food at all times to meet the dietary requirements and cultural preferences of their household members (Ibnouf, 2011). Indigenous knowledge represents valuable source of local solutions to the food insecurity in terms of accessibility by the rural population, particularly during seasonal food shortage or major stress periods such as droughts. As the managers of available live-based local resources and managers knowledgeable about local resources and environment, rural women are best placed to ensure sustainable food supplies and hence achievement of household food security. During seasonal food gaps, rural people are entirely dependent on the preserved foods as the only food source until the onset of harvest later in the

year. Rural women possess an enormous amount of indigenous knowledge about food processing, preserving and other important survival skills. However, there is a concern that agricultural policy fall short of contributing to sustain food security, strengthening people-centred, respect the rights of all culture and wisdom of indigenous peoples and local communities.

In this paper, there is interchange in usage of the terms traditional and indigenous knowledge. Indigenous knowledge is knowledge that is unique to a given culture or society (Grenier, 1998). For the purpose of this paper indigenous knowledge includes local knowledge, skills known, and practiced in food processing, preservation, and storage activities which rural women derived from their direct interaction with the local environment. Such indigenous knowledge and its associated skills and practices are developed over ages, and passed down through the generations and became entrenched in people's cultures. New knowledge is regularly added to women's indigenous knowledge through innovation and through transfer of external knowledge to adapt to the changeable conditions. The indigenous foods produced through the use of these traditional methods and practices are sustainable means for achieving household food security in rural Sudan. The potential for indigenous knowledge to contribute to the achievement of household food security is tremendous. Indigenous knowledge is a resource widely ignored by development planners and policymakers. Hence, the paper objects to highlight how sustainable food security can be achieved in the vagaries climate situation of rural Sudan.

The methodology of this article depends on a survey of literature review and extensive use of practical examples to provide a comprehensive overview of indigenous knowledge used by rural women in processing and preserving of food in rural Sudan for household survival. In this context, the researcher utilized the data gathered through a number of her field studies throughout rural Sudan about the extent and nature of rural women's roles in food production and household food security. Direct observations of women engaged in food processing and preservation and grains storage activities. Most of indigenous food processing and preparation methods are well known to the researcher who is sometimes involved directly or indirectly in these food activities. Information was also obtained from documentary sources and relevant supportive literature such as books, journals, articles related to this study particularly a series of articles published by Prof. Hamid Dirar.

There has been little in the way of research that examines the contribution of indigenous knowledge to sustain household food supplies in rural Sudan. These knowledge gaps may sharply reduce the chances of achieving success in food security programs in the adversely affected region by environmental uncertainties, socio-economic problems, and conflicts. The use of the indigenous technique and practices in processing and preserving food products is also largely limited by lack of their documentations. A lot can also be learned from rural women's existing indigenous knowledge and its associated methods and practices for processing and preservation food products. More recognition of the role of indigenous knowledge in achieving household food security will give rural women a sense of the value and importance of traditional resources. These may assist to build resilience food security strategy for rural Sudan. The efficiency of the national food security policies can be judged by their ability to ensure food accessibility by the entire population in all regions of the Sudan, particularly the vulnerable population in rural areas.

2. Background to Rural Sudan

A clear understanding of the role of indigenous knowledge in the food security in rural Sudan entails a brief description of rural Sudan. Sudan is considered a very rich and diverse in land potential but equally so with ecological risks (Abdelgalil & Cohen, 2001). Rural Sudan is already showing visible climate changes and further climate change is envisaged. Sudan is the single most arid county in Africa, thirty-one per cent of the territory is hyper-arid, and 63% are drylands susceptible to desertification and where 82% of the populations live (Ayoub, 1998). Most rural Sudan, like the rest of Africa's semi–arid and arid zone, suffered from the increasing frequency and severity of drought from the 1980s to date. The agricultural production in most Sudan and in turn food security depends upon the natural resources base. An observable danger is degradation of the natural resource base (the livelihood-based resources) and an increase in desertification. Despite technological advances such as improved crop varieties and irrigation systems, weather and climate are still key factors in agricultural productivity (Parry, *et al.* 1999). It follows, then, that the food security in rural areas is usually affected by the environment and natural resources. Most of the agriculture farming systems in rural Sudan is rain-fed, approximately 90 percent of the Sudan's cultivated areas depend exclusively on rainfall and the traditional rain–fed sector supports 70% of rural population (Federal Ministry of Environment and Physical Development 2003, p. 40–41). Crop production is thus highly vulnerable to the climate hazards. Thus climate threatens the agro-pastoral and pastoral livelihood systems of arid and semi–arid rural people in several ways. The rural Sudan had experienced and is expected to experience marked reductions in agricultural productivity in the future as a result of recurrent droughts, desertification and other environmental hazards. The consequence of armed war and

tribal conflicts in western Sudan region has also significantly impacted the country food security. A massive destruction of the natural resources has resulted as a direct consequence of these tribal conflicts and wars. Drought and civil conflicts led to increase the rural-urban migration, particularly of males from the subsistence farms to the urban areas.

In general, rural people are small-scale farmers and herders in the traditional rain-fed farming and livestock sectors. They usually practice subsistence agriculture, and their livelihoods are based on crop cultivation, herding and fishing, where available. Smallholder farmers are hindered by the limited size of their landholdings and low rates of productivity. Due to the lack of rainfall and domestic water supplies, for most farmers the growing season is short and crop failures are frequent. People living in rural areas have been or continue to be affected by drought and conflict – particularly in Darfur and the south Kordofan. A study indicates that food shortage is quite common before the rainy season until the harvest when food stocks are low, as indicated by 88% of surveyed sample in rural areas of northern Kordofan state (Ibnouf, 2008). However, over generations rural women have learned how to cope with these seasonal shortages by developed and adopted traditional foods based on their indigenous knowledge.

Patterns of adaptation behavioural responses in relation to an environment and socio-economic crises vary greatly across variety of contexts. Although there are many traditional adaptations used in rural Sudan, as well in Africa, however the use of indigenous knowledge is considered by virtue of its effectiveness (Orindi & Murray, 2005; Ibnouf, 2008). Traditional adaptation strategies based on indigenous knowledge have played vital role in the lives of the arid and semi-arid communities in virtually every part of Africa (ibid). The indigenous knowledge is a critical safety valve that has created and developed out of necessity. Indigenous knowledge represents a valuable cultural capital and a concrete resource. Most of rural people in Sudan depend on indigenous knowledge in agriculture operations as well as in solving food shortage (Ali, 1997; Ibnouf, 2008). Rural people use their own knowledge of indigenous foods and agriculture provides better prospects for long-term sustainability than imposing solutions from outside. Both men and women may develop mechanisms to reduce risks or reverse processes of the specific conditions that take place in their local environment. However, it is often the rural women who produce, manage and market most of the food for their families and societies, and who work directly with natural resources (FAO, 2003 cited in Mwangi & Dohrn, 2008, p. 240). It has also been stressed that rural women play a major role in actions to safeguard the environment, and therewith their communities' livelihoods and survival (Dankelman, 2002). Through using of indigenous knowledge in food processing and preservation women in rural Sudan, more than men, are usually seeking to find effective ways to ensure sustainability of the household food security. The preparation and storage of fermented foods by women are strongly dictated by the ecology of a hostile environment of drought, desertification and recurrent food shortage (Dirar, 1994).

2.1 Importance of indigenous knowledge as life saving resources in rural Sudan

Based on the above background, there is a need to learn about and to pay due attention to indigenous knowledge base of the rural communities. The resources available on arid and semiarid rural Sudan are cultivable lands, natural vegetation, and domestic animals, in addition to people indigenous knowledge. The indigenous knowledge enable people in rural Sudan, as well in Africa, survive the stressful conditions. This collective knowledge is critical to the survival and future wellbeing of local communities, and especially, of indigenous peoples as they try to maintain their livelihoods under difficult environmental conditions (Parrotta & Agnolett, 2007). Indigenous knowledge provides the basis for problem-solving strategies for household food security in rural Sudan. Due to recurrent drought as a consequence of declining of rainfall and increase of dry season role of indigenous knowledge in sustain household food supplies is expanding in rural survival in rural arid and semi–arid areas. According to Elsiddig (2006) indigenous knowledge aims at management of the natural environment and is used to adapt to the requirements of the local conditions, allowing survival of the people in the western Sudan. In sub-Saharan Africa in general people have also traditionally utilized indigenous knowledge and skills, most often locally developed and handed down in the course of centuries (Oniang'o, *et al.* 2004). In surveys conducted in rural western Sudan, 80.4 percent of those surveyed confirmed that indigenous processed and preserved food products represent essential sources of foods and this is not limited to times of food shortage (Ibnouf, 2008). Processing and preserving of food products are greatly increases the value of perishable food stuffs by making them available for longer periods of time (Osunbitan, *et al.* 2000).

The traditional life cycle of rural people is a continuous process of acquiring, experiencing, possessing and sharing of traditional knowledge. Rural women tend to look for the most cost effective alternatives sources of foods for household survival. Traditional food processing and preservation activities constitute a crucial body of indigenous knowledge handed down from one generation to another. Social networks have still played crucial

roles in indigenous knowledge transmission. The rural women have their own network through which they constantly gather information about new indigenous varieties.

Women in rural Sudan possess substantial indigenous knowledge about food production, processing, and preserving. Indigenous knowledge is that embedded in women daily practices to secure food consumption for their household members. Dried and fermented foods together with the seeds and fruits that can be gathered from the wild have saved lives especially those of children in the past and in the present in times of shortage in Sudan (Dirar, 1993).

Through understanding of indigenous knowledge, there is potential for sustaining food security in an environment of climate change and socio-economic problems. Many studied revealed that the traditional knowledge developed by women of processed and preserved foodstuffs has played an important role in coping with periods of food scarcity and famine in rural Sudan (Ali, 1997; Elsiddig, 2006, Ibnouf, 2008). Locally available agricultural and animal raw materials as well as wild products were processed into food products at relatively low cost resulting in food with higher nutritive value compared to the raw materials, had better taste, were culturally appropriate and acceptable, and had a longer shelf-life (Ibnouf, 2011). This clearly illustrates that rural women are quite capable of undertaking their own informal experiments and may arrive at practical solutions not originally anticipated by scientists and extensionists.

3. Indigenous Knowledge and Practices in Food Processing and Preservation

Besides being ethnically diverse, Sudan is one of the wealthiest countries in Africa in terms of cultural diversity. Culture plays a fundamental role in people eating habits. The types of food eaten by people are also determined by foods availability, affordability and what people are accustomed to eating. So, indigenous foods are plentiful and varied in rural Sudan. The indigenous knowledge and its associated skills is the result of centuries of adaptation to difficult food shortage crises. Indigenous food processing and storage operations are predominantly managed by women in rural Sudan. Rural women have been and will continue to be the inventor and preserver of the indigenous food techniques.

Rural women are usually using a diversity of simple and traditional food processing techniques to make a variety of traditional food products. These traditional food processing techniques are simple and low-cost and aim at long storage of foods. The technologies are based on indigenous knowledge and are adaptable to the culture of the people and to the environment. These processing techniques help in preventing growth of the micro–organisms that cause foods to decay and foods can be kept at ambient temperatures for long periods. Most of indigenous food processing and preparation methods are well known to most households in rural and urban areas alike. However, rural women are continuously experimenting, adapting and modifying traditional methods and techniques and practices for processing and preserving foods. Most indigenous processing methods are applied to foods to achieve preservation.

3.1 Indigenous food processing and preservation techniques

Sun drying – dehydration – is the one of the indigenous methods of food processing and preservation. Sun-drying traditional technique is usually done in three main ways. One method is to immerse fresh products e.g. vegetables in salted boiling water for a few minutes and then dry them under the sun for about three days. These are then stored in a safe, dry place. This method is also used to dry edible insects such as locusts and caterpillars. Another method is to directly spread the food under the sun. Food crops like sorghum, pearl millet, beans and groundnuts are usually kept drying under the sun before storage in traditional underground store or pits (locally called matmorah – can be of different shape and size) to increase their shelf life. Other food is first salted if there is danger of decaying during the drying process, as is the case with meats and tomatoes and afterwards stored in dry place at room temperature. Dried-tomatoes are then soaked in warm water to be turned into tomatoes sauce. Meat from slain animal sheep, goats, cow, and camel is first cut in long pieces, salted, smeared with powdered coriander, and dried for about a week to give a product called “shermout”. In rural areas of western Sudan women tend to dry the layer of fat around the stomach that is called locally miriss and can be stored for a year. The large intestine may be cleaned and stuffed with fat and hung to dry for days as a type of sausage. The clean small intestines may also first be sun dried together with strips of the lungs, heart, kidneys, and liver and then all pounded together and mixed with some potash and molded into a fist-sized ball and allowed to slowly ferment and dry, to give twini-digla (Dirar, 1993). Women are key innovators, developing new ways to secure food supplies. Women in the rural western Sudan have developed a new food source from watermelon (*Citrullus lanatus*) by drying and then grinding watermelon seeds and using these dried seeds to make a porridge (called locally bajbaji), dried watermelon seeds can be preserved for years (Ibnouf, 2011).

Fermentation is one of the oldest methods of food processing to make naturally fermented and cultured foods worldwide. Fermented foods and beverages are estimated to make up approximately 1/3 of the human diet (Van Hylckama Vlieg, *et al.* 2011). As the sun-dried food can be stored indefinitely without deterioration, the fermentation processes represent a food preservation technique particularly well suited to the climate and conditions of arid and semi-arid areas. Fermentation is an important food processing technology usually developed by women in most Africa and Asian countries. However, the Sudanese women are more innovative using their indigenous knowledge of food fermentation as basis for further experimentation. For instance, Sudanese women invented unique fermented products which are white and red abreh/hulu-mur, a non-alcoholic fermented sorghum refreshing soft drinks for Ramadan (the holy month of fasting). While red abreh/hulu-mur is made of fermented sorghum dough prepared with equal amounts of flours from malted and un-malted sorghum, the white abreh is made from un-malted fermented sorghum dough. In indigenous fermentation, the starter consists of small amount of previously fermented materials to accelerate the fermentation process. The raw materials from which fermented foods are prepared include the better-known products such as sorghum, pearl millet, milk, fish, and meat, wild plants and also a number of unorthodox raw materials are fermented, bones, hides, skins, hooves, gall bladder, fat, intestines, caterpillars, locusts, honey, and cow urine (Dirar, 1994). Fermentation enhances the nutritional quality of foods and contributes to food safety particularly under conditions where refrigeration or other foods processing facilities are not available (Motarjemi, 2002) such as in rural Sudan. Some indigenous preparations, involving food fermentation, are quite elaborate and may take up to two weeks to complete. For example in rural western Sudan, a favourite choice to the meat substitute is kawal. Kawal is derived from the two-week long fermentation of the pounded green leaves of the *Cassia obtusifolia* legume. In the same region, the oil seedcake remaining after oil extraction from sesame seed (*Sesamum orientale*) is fermented for a week to make another meat substitute, called locally sigda.

Combination of two or three indigenous food processing techniques is common practices, i.e. fermentation and drying (Kawal) or salting, fermentation, and drying “shermout”, germination, fermentation and drying “abreh/hulu-mur”, etc. Processing of some products by women entails a complicated multi-step process, especialy those involving double methods of fermentation and sun-drying, are rather complicated and may take up to two weeks to complete. Fermented products are usually stored for a long period regardless of the weather conditions.

In southern Sudan, duma (a type of alcoholic drink) is made by fermenting diluted honey. The duma-making process is unique to southern Sudan, it is very fast, taking less than 12 hours; organisms which tolerate heat are involved; and the key link in the process is a special starter culture called iyal-duma (seeds of duma) (Dirar, 1993). The starter is originally raised from the roots of certain trees through a painstaking enrichment technique; it is then made into a paste consisting of an aggregate of a capsulated bacterium and two kinds of yeast (ibid). If washed thoroughly with water and sun-dried after each use, iyal-duma can be kept for years without losing its capacity to immediately start up fermentation when diluted honey is added (ibid).

The most widespread indigenous dairy products of Sudan are roob, kush-kush cheese and mish (spiced traditional yoghurt) which are usually produced during the rainy season and can be stored for use for the whole year. To prepare roob, fresh milk is left overnight for the souring process with some previous roob usually added as starter to accelerate the fermentation process. Another method to prepare roob is by putting sour milk in a skin bag and that is then churned and shacked to produce butter and roob. During this process small quantities of cold water are added once in a while to speed up the butter production. The butter is then removed by hand to a separate pot and heated to give traditional gee called locally samin which can be stored for several months. The remaining sour milk after butter removal is roob. Another traditional fermented milk product is mish, made by inoculating fresh milk with a small quantity of soured roob then spices like black cumin, fenugreek, garlic and black pepper are added and allowed for lengthening fermentation. Mish can be preserved and consumed for long time after it has been made. Nomadic women in Sudan make a type of a grainy indigenous cheese called kush-kush. To make kush-kush, women put sour milk into a skin bag that is fastened to the saddle of a camel to allow sour milk to continue the souring process until the curd is separated from the whey, and then the curd is collected and sundried to give kush-kush and is usually eaten with sorghum porridge.

Indigenous fermented and dried products are used to prepare a variety of sauces and stews. Different indigenous dishes are made of fermented-dried meat or vegetables or sour milk, with onions, oil, tomato puree, salted and spiced and served with porridge or kisra. Animal fat and offal are incubated in clay pots and left to ferment until foul, then pounded to soft paste and used as such (boiled with beans, or okra, onions, spices to make a sauce). In some rural areas of Sudan thick strips of fat-bearing meat are hung on a rope indoors and left to go through

fermentation and then allowed to dry slowly to make another type of shermout and is made into a stew that is eaten with acida (stiff porridge) or kisra (unleavened bread).

Fresh bones may be fermented in a number of ways, the large bones, with pieces of attached meat and tendons, may simply be thrown on a thatched roof to ferment slowly for weeks or even months to give the product called adum; the meshy ball bone endings of the ball and socket joints may be pounded fresh and fermented into a paste called doddery*; t*he vertebrae of the backbone may be chopped into smaller pieces that are sun dried, pounded with stones, mixed with a little water and salt, molded into a ball, and allowed to ferment and dry to give kaidu-digla (Dirar, 1993).

Sour milk "roob" substitutes are made from oil-bearing seeds in a manner analogous to the use of soybeans to give dairy product analogs, roob-heb is made from the seeds of the watermelon and roob-fui is made from peanuts (Dirar, 1993). In either case the seeds are pounded into a paste that is allowed to undergo a souring fermentation, when mixed with water and turned into sauce the product has the color (off white) and taste (sour) of the sour milk sauce called muiah-roob (ibid). A related product is um-zummatah*,* obtained by the souring fermentation of watermelon juice (Dirar, 1993).

The traditional commonly practiced fish preservation techniques are produced by fermentation with salting and drying, fermentation and drying without salting or fermentation with salting but without drying. Fessiekh and maluha/terkeen are types of wet-salted fermented fishery products and they are common in northern Sudan. Some of other fish products include kejeik (hard fermented sun-dried fish) popular as food fish for people in southern Sudan, mindeshi (minced small fish paste, fermented, and may be dried later) used by the people of western Sudan. Most of fermented fish is usually made from the whole small fishes and stored in an airtight container or dry place and it is soaked in lukewarm water before being prepared into various types of delicious foods.

Collecting and processing wild foods such as fruits, nuts, caterpillars, and some birds is also an important way of acquiring food for people who lives in rural areas. In Sudan, processed and preserved wild food products help in some cases to ensure a year round food supply (Hamid, 2006). Kawal a strong-smelling product derived from a two-week long fermentation of the pounded green leaves of the wild legume *Cassia obtusifolia* and can be preserved and consumed years after they have been made. The collection and preservation of forest and wild food products such as *Cassia obtusiflora* and *Sonchus* spp. is exclusively women's responsibility. These wild products are freely available in rural Sudan and represent important alternative food source during food shortage and famine. During the famine in Bahr el Ghazal, Southern Sudan in 1998, wild foods contributed more than any other food sources, including relief food, in saving the lives of large numbers of famine victims because of its unique characteristics of being easily available and affordable by all (Guvele, *et al.* 2003).

Women are contributing to increase food safety for households by locally-made traditional pesticides. The traditional pesticides such as citrus lemon leaves and neem (Indian Lilac; *Azadirachta indica*) are usually used for controlling pests that damage vegetable plants or attack the stored food grains (Ibnouf, 2008).

3.2 Examples of indigenous foods

This paper pays a closer attention to special category of indigenous food products that are developed by women in rural Sudan. The traditional food products by using indigenous knowledge have been a matter of survival for people in rural Sudan. There are substantial different types of indigenous processed foods in Sudan, which prepared from a wide range of substrates.

Sorghum and pearl millet have been an important part of Sudan's food culture for most rural populations. A very common soft drink that is associated of Ramadan is a unique drink called red abreh or 'hulu- mur literally means sweet and sour'. This is a slightly sweetened, refreshing drink made of thin flakes of fermented malted and un-malted sorghum flour. Preparations for making it start a month before the coming of Ramadan. The half patch of sorghum grain is moistened with water and allowed to germinate for six days – the germinated grain called 'zereah' which subsequently sun-dried and grinded. The other half of un-germinated sorghum grain is milled into fine flour and cooked directly into slightly thinner porridge and then malt flour is added to the porridge while is still hot then the mixture dough is left to ferment naturally in a dry place at room temperature for 24 to 36 hours – note that no kind of starter is used. Nine grounded spices add to dough to improve flavor, such as ginger, cinnamon, cardamom, pepper in addition to the liquid additives e.g. extracts of red hibiscus and tamarind and also date. The resulting red-colored sweet-sour fermented dough is then diluted with water to consistency of batter. About 50 ml of batter is spread on hot ceramic pan for baking. Finally baked-sheets are left to dry in the shade for two days. The dry flakes may be stored for more than two years and used when desired. The baked-sheets crumble into flakes that are dispersed in water and allowed to soak for about an hour and then

strained before sweetening and served cold. It is refreshing drink usually used to quench thirst after a long day of fasting (see Figure 1). The flake remains, after soaking and straining, is usually used as an animal feed.

Another type of abreh is white abreh which is also nonalcoholic drink with a slightly sour taste. White abreh is made using white variety of sorghum flour which is mixed with water and subsequently are left to ferment for three to five days before straining to remove the bran, adding the spices, and baking like kisra into very thin flakes and then dried for few hours and can be stored for the whole year. It is sweetened to taste and drunk without prior straining, which is also usually served during Ramadan (see Figure 2). Unlike hulu-mur, no malting is involved and the product is not strained.

Kisra bread is prepared from sorghum or pearl millet flour or their mixtures (depending on the region and production area). The fermented dough is prepared traditionally by mixing a small amount of the previously fermented dough to the mixture of flour and water to act as a starter, and then the dough is left to ferment overnight. The fermented dough is then baked on a hot steel plate (150-160°C) in a process known as 'aowasa' and is a unique Sudanese art (El-Tinay *et al.* 1985). Dry piece of palm leaf (like a thin ruler) is used to swiftly spread small amount of the fermented dough into a very thin layer using quick, smooth sideways movements. The kisra is ready in about 30-40 seconds and ready to be peeled off (see Figure 3). Acida preparation: amount of stiff sorghum dough or pearl millet or their mixtures (prepared in the same manner as in kisra fermentation) is added to the boiling water with continuous stirring. Stirring continues until the dough is well cooked to stiff porridge (see Figure 4). Both kisra and acida are usually used on the daily basis and can be served with stews/sauce or with water and condiments or just eaten on its own as a whole meal. The indigenous stew/sauces are very diverse and can be made from cooked dry okra, curdled milk, fermented meat or vegetables are usually poured over kisra or acida and it is eaten with fingers.

Fessiekh is a wet-salted fermented fish (usually stored in an airtight container) is used as staple food and is widely produced and consumed in northern Sudan. It is used in the preparation of a common traditional dish called also fessiekh. The main components of the fessiekh stew, beside salted fermented fish are dried onions, tomatoes sauce, spices and peanut butter and usually served with traditional thick, soft, medium-size pancake bread made of corn and called locally groasah (see Figure 5).

The essential role of indigenous foods in the life of people in rural Sudan is reflected in their daily use as the above traditional dishes showed. Rural women are usually depended on resource-household level to make these traditional forms of food. The indigenous foods are 'the way of life' of the people in rural areas, and are considered 'the food of survival'. Rural women manage their available resources over the course of a year to meet their household food needs and to bridge food gaps and to sustain household food supplies. Women's indigenous knowledge for making of traditional forms of food is usually playing a vital role in survival of household in normal times as well in crisis situations.

4. The Nutritional Value of Various Indigenous Foods

One of the most common causes for dietary deficiencies and food insecurity appears to be the decreasing diversity of traditional diets. The article discusses the effects of indigenous food processing methods on food products nutrients. Indigenous foods are an important contribution to balancing the diet. Using of indigenous knowledge in food processing and preservation e.g., fermenting and malting may contribute to increase of the nutritional potential. Indigenous processed food products contribute to promote nutrient content and diversity and hence improving health of rural populations.

Through the years rural women have used very effective indigenous methods and techniques of food preservation. The products can be preserved for years and thus such products can be regarded as survival and nutritional foods. In food processing and preservation the scientific knowledge of women is clearly demonstrated in identification of the variety, and in innovation of ways to preserve the food for a long time without loss of nutrition as well as taste. Indigenous foods have the nutritional equivalent of introduced vegetables and cereals and in some cases are superior to them. Processing techniques such as soaking, germination and fermentation have been found to reduce significantly the levels of phytates and tannins by exogenous and endogenous enzymes formed during processing (Nuha, *et al.* 2010). A number of studies confirmed the importance of fermentation as a simple technique for improving both the nutritional and functional properties of traditional staple food grains and animal products (see Belton & Taylor, 2004). The beneficial effects associated with fermented foods include reduced loss of raw materials, reduced cooking time, improvement of protein quality and carbohydrate digestibility, increased shelf life and microbiological safety of a food and improved bioavailability of micronutrients, general improvement in the texture, taste, aroma and elimination of toxic and anti-nutritional factors (Iwuoha & Eke, 1996; Mensah, 1997; Caplice & Fitzgerald, 1999; Motarjemi, 2002).

Cereals, legumes, and tubers that are used for the production of fermented foods may contain significant amounts of anti-nutritional or toxic components such as phytates, tannins, cyanogenic glycosides, oxalates, saponins, lectins, and inhibitors of enzymes such as alpha-amylase, trypsin, and chymotrypsin (Reddy & Pierson, 1994). The latter authors state that these substances reduce the nutritional value of foods by interfering with the mineral bioavailability and digestibility of proteins and carbohydrates. In natural or pure mixed-culture fermentation of plant foods by yeasts, molds, and bacteria, anti-nutritional components (e.g. phytate in whole wheat breads) can be reduced by up to 50%; toxic components, such as lectins in fermented foods made from beans, can be reduced up to 95% (ibid). Indigenous fermented milk products have been evaluated by a number of Sudanese scholars. The processing of roob and mish, using spices like black cumin, fenugreek, garlic and other known spices, those spices were proved to have significant effect as preservative (El-Zubeir, *et al.* 2005).

A considerable portion of the nutritional needs of rural people is usually meet through traditional fermented foods. Traditional fermented foods consist of a wide range of products sourced from cereals, fruits, legumes, meat, fish, milk and wild foods. These traditional fermented foods are of great importance because they provide and preserve vast quantities of nutritious foods in a wide diversity of flavors which improve the value of foods. In rural Sudan, particularly in Kordofan and Darfur regions of western Sudan popular substitutes for the meat are indigenous fermented foods kawal, sigd, and furundu. All these products are dried after fermentation in the form of hard, irregular, small balls and may be kept for years. Leaves of *Cassia obtusifolia* (Sicklepod), widely grown in western Sudan, are fermented and sundried to produce a traditional food product, called locally kawal. Kawal is produced by solid state fermentation of readily available plant material of little or no economic value *Cassia obtusifolia* which, though unpalatable and toxic in its natural state, contains protein rich in sulfur amino acids (Dirar, 1984). Fermentation was found to cause significant changes in *Cassia obtusifolia* leaves major nutrients; fat content increased from 3.50 to 4.50 %, protein content from 24.81 to 35.13 %, ash content from 13.67 to 18.00 %, and fiber content decreased from 13.04 to 12.90 % (Dirar, 1984; Nuha, *et al.* 2010). The anti-nutritional factors (tannin, phytate and total poly-phenols) of the *Cassia obtusifolia* leaves were significantly ($P \leq 0.05$) decreased after fermentation and cooking (Nuha, et al. 2010). Furundu, a similar meat substitute, is prepared by cooking the seeds of hibiscus (*Hibiscus sabdariffa*) or red sorrel and then fermenting for more than a week. The results of the study conducted by Yagoub & Mohammed (2008) indicate that furundu raised from the cooked hibiscus seed have considerable amounts of essential amino acids and minerals and the HCl-extractability of minerals were improved by furundu preparation. The latter authors show that the protein content of the hibiscus seed was 32.28%, fermentation of the cooked hibiscus seed (furundu) significantly ($p< 0.05$) increased the level of the protein to 33.03% (see Table 1).

In Sudan, sorghum and pearl millet are staple foods and form a major dietary component for a large part of the rural population. The diets of rural Sudan populations are basically traditional fermented cereals (sorghum and pearl millet) based. Fermenting sorghum and pearl millet preserve and give a sour flavor which is well-accepted and particularly enjoyed. The process upgrades the taste and nutritive value of these crops. It raises the levels and bio-availability of proteins, vitamins and minerals. The chemical characteristics of fermented dough and baked kisra (traditional flatbread) and acida (stiff porridge) have been examined by many Sudanese researchers (El-Tinay *et al.* 1985; Abdelgadir, *et al*. 2005). The chemical composition of sorghum and pearl millet before and after fermentation and the nutrient contents of the traditional fermented food items kisra and acida were shown in Table 2. The crude fiber and protein were reported to have been increased by fermentation.

In preparation of hulu-mur, germination of grains caused significant increases ($p<0.01$) of zinc (90%), lead (65%) and molybdenum (58%) (Mahgoub, *et al.* 1999). The latter authors pointed out that addition of spices to hulu-mur dough caused significant increases ($p<0.01$) of strontium (80%), calcium (60%) and iron (35%). Baking of hulu-mur did not cause any significant loss ($p<0.01$) in the contents of minerals (ibid).

Fermentation also helps protecting food from harmful microorganisms and reduces the risk of pathogenic diarrhea, a leading cause of infant death in rural Sudan. Microbial analysis of the sorghum before and after fermentation revealed that coli-form bacteria '*E. coli'* counts exceeded 2.400 unites per gram (cfu/g) in the raw sorghum flour but the counts were very low in the fermented dough (Yagoub, *et al.* 2009). Latter authors point-out that *Salmonella* detected in the different sorghum varieties disappeared in the fermented dough after 24 hours fermentation. This might be due to the reduction of pH and accumulation of organic acids in the fermented sorghum flour, to production of certain microbial byproducts which eliminated these pathogens (ibid).

5. Lessons Learned from Women Indigenous Knowledge in Food Processing and Preservation

This paper attempts to focus on women's roles in the household survival in rural Sudan by considering how their indigenous knowledge leads to sustain household food supplies. Women more than men are often generated a

great deal of indigenous knowledge in terms of dealing with different situations. Indigenous knowledge and practices in food processing and preserving can be regarded as the optimum method of dealing with practical circumstances surrounding survival of households in rural Sudan. The lesson learnt from the analyses presented in this paper, however, is that the indigenous knowledge and practices are fundamental to the endurance of rural women as they struggling to achieve household food security in rural areas of Sudan. Rural women's active involvement in diversify of food sources and efforts to develop alternative sources for household food supplies constitutes an essential part of achieving household food security.

This review indicates that rural women are more likely than men to use available resources and skills to sustain the living and further improve the nutritional status of their family. The traditional knowledge methods using in processing and preserving food products are regularly modernized and adapted since it becomes part of rural livelihood systems. Efficient utilization of local resources and efforts play a key role in enhancing rural people capacity to adapt different climate and socio-economic stressful conditions.

Women in most rural Sudan offer a number of traditional food products that are favourable to the food supply, quality and safety. These food items contribute to improving food access and availability to meet household needs in undesirable climate and socio-economic conditions. Locally available agricultural, raw material is processed into food products at relatively low cost resulting in food with a higher nutritive value compared to the raw material, a better taste and a longer shelf-life (cf. Van de Sande, 1997). Given the importance of indigenous knowledge that is used for food processing and preservation, it may therefore play a critical role in ensuring food security for the rural Sudan. Thus it will ensure the availability and utilization of these indigenous food sources for resource-poor rural communities. Therefore, indigenous knowledge and practices in food processing and preservation provides some valuable lessons to policymakers. Although insufficient attention has been given to use of the indigenous knowledge to sustain household food supplies, however integrating the indigenous knowledge within the mainstream development intervention may facilitate the culturally acceptable and desirable practicing. Therefore, interventions agreeable by both local people and stakeholders can be identified since they will possibly more successfully than other interventions which may jeopardize the indigenous people living systems. When indigenous techniques are improved, dissemination is usually faster, more widespread and cheaper because it fits better into local environmental and socio-economic niches (Reijntjes, *et al.* 1994 cited in Van Dijk, 1997, p. 17).

The indigenous food processing technologies are infrequently documented, evaluated and/or incorporated in the modern food chain. The potential disappearance of most of indigenous knowledge could have a negative effect on rural people who have developed and make a living through them. Therefore, paying the needed attention to indigenous knowledge will contribute to promote the cultural dimension of rural development. Unfortunately, so far there are no development strategies that seek to encourage rural women to use the indigenous knowledge to achieve household food security.

6. Concluding Remarks and Policy Recommendations

In conclusion, this study argued that women in rural Sudan tend to use available locally resources effectively in achieving household food security, and since women are responsible for food processing and food preserving, they contribute more to household survival. This paper argues that women in rural Sudan employ a variety of methods based on indigenous knowledge in processing and preserving of agricultural and animal raw materials and wild food products to acquire alternative food sources and to relieve the stress of seasonal food scarcity particularly during shortage of rainfall or drought and thus achievement household food security. Based on the result of this study in rural Sudan, different indigenous techniques from fermentation to sun-drying are viewed as unique techniques to preserve foods for years. Although indigenous knowledge about foods is usually invisible in conventional economic analysis, however indigenous foods have been a matter of survival for people in rural Sudan. So, it is necessary to look at indigenous knowledge as a way of building the capacity of rural women and give them larger extent to utilize their potential, since their using of indigenous knowledge in food processing and preserving is a very practical mechanism. Therefore, instead of transferring alien approaches and plans, it seems meaningful, socially as well as technically, to upgrade indigenous knowledge for achieving rural food security.

Given the vital role that indigenous knowledge plays in securing household food supplies, the continued undervaluing such knowledge is prejudice to rural women and is inimical to development of rural areas. Therefore, the most critical need in rural areas is to foster women that can promote sound adaptive strategies in the context of sustainable rural development objectives. So, some policy recommendations are made as follow:

Support of rural women's indigenous knowledge may contribute to achieve of household food security in rural areas as it does allow for a variety of options. Promote sustainable development that is culturally appropriate to traditional societies requires the enhancing of the indigenous knowledge which allow the survival and renewal of culture, and is important generator of innovation.

In the designing of food security strategies and policies, studying rural women's indigenous knowledge can be a rewarding experience. Only when policy makers in Sudan accurately understand rural peoples' indigenous knowledge and its socio-cultural values that support survival of these people under stressful conditions of food shortage can they enact policies that will realistically achieve sustainable food security.

Construct new food habit and change the ways of living are often undesirable by indigenous people. Include of indigenous knowledge in any programs construction assist to increase people confidence on themselves and their ability to have their own solutions for food insecurity problems. Incorporating of indigenous knowledge into food security intervention in any rural areas development programs can provide valuable input for alternative resources.

Considering the current environment obstacles and future threatens, conservation of indigenous knowledge should be closely evaluated, as it is often the result of long experiences. There is the risk that the erosion of this indigenous knowledge in favour of modern knowledge could leave many traditional rural communities with neither traditional nor modern preparations to combat food crises. Indigenous knowledge is usually orally transmitted and infrequently recorded. In Africa, AS WELL IN Sudan, indigenous knowledge is stored in peoples' memories and activities and is expressed in stories, proverbs, cultural values, beliefs, and local language (Grenier, 1998). One of the highest ranking priority activities should be the development of recording systems, to record the useful indigenous knowledge about food before they are completely lost.

There is a need for a systematic effort to inventory and analysis of the role of indigenous foods in alleviating food insecurity and understand its working in the context of rural communities.

Sustain household food supplies through traditional food that is based on women indigenous knowledge in rural Sudan could be enhanced and supported to function as food security national policy frameworks.

There is a need for developing agricultural and nutritional training programs which address ways of preserving indigenous knowledge for sustainable food security.

It is hope that this paper will encourage further, much needed interdisciplinary research on topics related to the potential future roles of indigenous knowledge that governed and guided indigenous foods practices, embedded deeply in the socioeconomic and cultural fabric of Sudan rural communities, in promoting more sustainable food security for people in this era of climate change. Further research into the nutritional values of the indigenous food may eventually enhance a change of attitude and the possible future propagation of those indigenous foods. The findings of such analysis should be disseminated through publications and extension workers to benefit the population at large. These will help in identifying and documenting what already exists. It is therefore important to have relevant studies undertaken and indigenous knowledge documented in order to help counter rural food insecurity.

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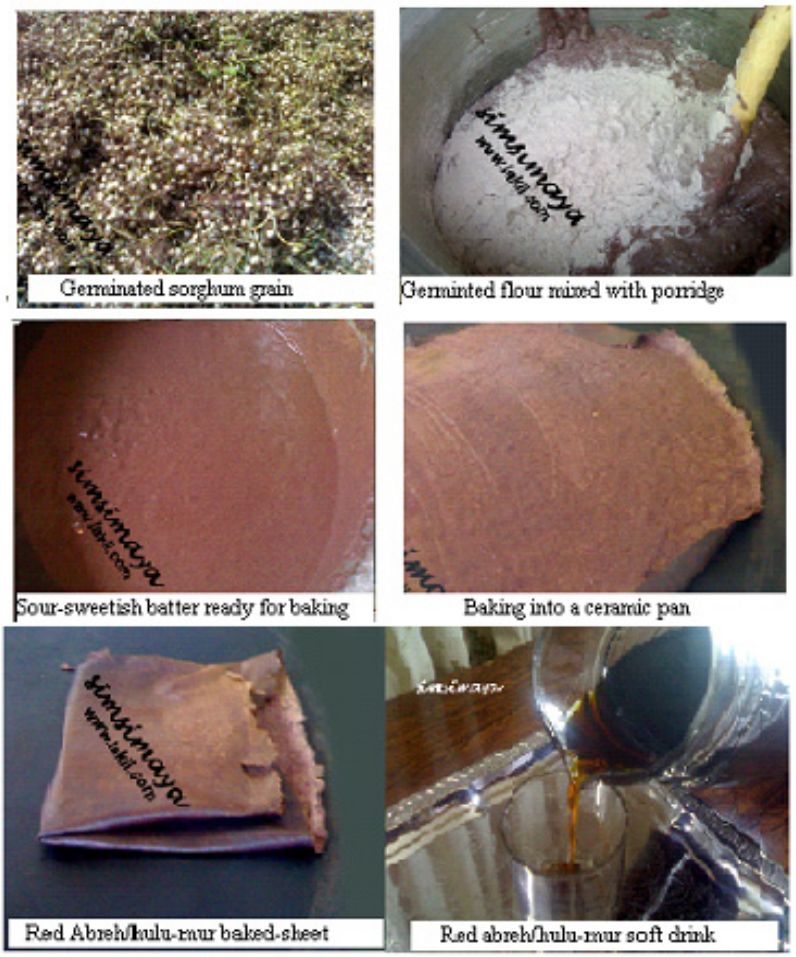

Figure 1. The indigenous technique of making red abreh/hulu-mur

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Figure 2. The indigenous technique of making white Abreh

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Figure 3. Making of kisra (traditional flatbread): Kisra is a type of bread made from fermented sorghum or pearl millet flour

Figure 4. Making of acida (stiff porridge): acida is stiff porridge made from fermented sorghum or millet flour

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Figure 5. Preparation of traditional fessiekh stew. Fessiekh is a type of wet-salted fermented fishery product

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Table 1. Amino acids composition (g/100 g protein) and protein content (%) of hibiscus seed and furundu product

Composition	Hibiscus seed	Furundu product
Leucine*	7.99	7.73
Isoleucine*	4.24	4.13
Threonine*	3.34	3.42
Methionine*	1.11	0.93
Lysine*	4.84	4.95
Total	32.28b	33.03a
Protein	(0.03)	(0.10)

*Some of essential amino acids.

Adapted from Yagoub & Mohammed, 2008, p. 354.

Table 2. Analysis of raw and fermented materials in sorghum and pearl millet (% per/100 g)

Item	Moisture	Carbohydrate	Protein	Fat	Crude fiber
Sorghum	6.13	74.43	12.95	3.88	1.00
Pearl millet	6.33	70.22	13.35	6.08	1.43
Sorghum kisra	4.50	77.60	13.69	1.14	1.43
Sorghum acida	4.50	47.10	15.43	1.91	1.81
Pearl millet kisra	5.00	72.28	15.72	3.36	1.36
Pearl millet accida	5.80	27.73	15.46	2.43	1.27

Adapted from Abdelgadir, *et al.* 2005, p. 215.

Stability and Physical Properties of Emulsions Prepared with and without Soy Proteins

Allaoua Achouri, Youness Zamani & Joyce Irene Boye
Agriculture and Agri-Food Canada, Food Research and Development Centre,
3600 Casavant Boulevard West, St. Hyacinthe (Quebec) J2S 8E3, Canada
Tel: 1-450-768-3232 E-mail: joyce.boye@agr.gc.ca

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Abstract

The ability of emulsifiers (gum arabic, starch and soy protein isolate (SPI)) and texture modifiers (gum tragacanth and carrageenan) alone and as a mixture with SPI to stabilize oil-in-water emulsions at high oil concentrations was studied. Parameters investigated included droplet size distribution, color (ΔE), viscosity and creaming stability. At 10% (w/v) emulsifier to oil ratio, emulsions stabilized with gum arabic, starch and SPI had average oil droplet sizes lower than 1μm. Increasing the oil content by two-fold (emulsifier/oil ratio of 10:20), increased the average droplet size of the gum arabic and SPI emulsions to 1.5 and 4.2 μm, respectively, while that of the starch emulsion remained unchanged. At 5% oil concentration, gum tragacanth and carrageenan stabilized emulsions yielded two average droplet sizes of 0.5 μm (52.7%) and 1.8 μm (46.5%), and 0.5 μm (48.8%) and 1.7 μm (46.6%), respectively. Increasing the oil concentration to 10% increased the particle size with fairly similar proportions. Gum arabic stabilized emulsions had lower ΔE than starch and SPI (except at 20% oil content). ΔE of carrageenan and tragacanth emulsion decreased significantly when used as a mixture with SPI. Droplet size strongly affected color. All concentrated emulsions exhibited shear-thinning behavior irrespective of oil, emulsifier type and concentration. SPI, followed by starch and gum arabic emulsions showed good stabilities over the 15 days storage period. Gum tragacanth and carrageenan emulsion stabilities were only improved when combined with SPI, particularly at 5% oil concentration.

Keywords: Gums, Emulsions droplet size distribution, Color, Viscosity, Stability

1. Introduction

Emulsions form the basis of a wide variety of natural and manufactured materials used in the food, pharmaceutical and cosmetic industries (Becher 1985 and 1988; Schramm, 1992; Lin and Mei, 2000; McClements, 2005). Existing and new ingredients are regularly incorporated into food systems to improve their rheological, physicochemical and nutritional properties. These ingredients, however, may sometimes slowly degrade and lose their activity, undergo oxidation, react with components present in the food system which may limit their bio-availability, or change the colour or taste of a product (Schrooyen et al., 2001), making it necessary that they be stabilized.

One of the major concerns for emulsions is keeping the emulsion droplets uniformly distributed during storage and consumption (Chanamai and McClements, 2002). This has led the food industry and many researchers to investigate the ability of hydrocolloids and proteins to stabilize emulsion droplets against creaming, flocculation and coalescence, depending on their intended application. Previous studies have also shown that the stability of oil-in-water (O/W) emulsions depends on both the type and concentration of ingredients contained in the emulsion as well as processing and storage conditions (McClements, 1999). Concentrated emulsions are a unique class of O/W emulsion in that they can be consumed in highly diluted form (low viscosity fluids such as milk and fruit juice beverages) or in their original concentrated form (such as in creams, margarine or butter). Thus, the emulsion must have a significant degree of stability in both the concentrated and diluted forms (Tan, 1990).

At present, gum arabic is one of the most widely used biopolymers in foods and beverages (Randall et al., 1988; Williams et al., 1990; Garti and Reichman, 1993). Its arabino galacto protein (AGP) component is responsible for its unequalled emulsifying properties, including the ability to form stable emulsion over a wide pH range and

in the presence of electrolytes (Nussinovitch, 1997; Islam et al., 1997; Buffo et al., 2001). Nevertheless, there have been many efforts to find alternative sources of natural emulsifiers, because gum arabic is very expensive and its supply and quality can be somewhat erratic (Chanamai and McClements, 2002). Starches are now widely used in dairy-based food products because of their good emulsifying properties, and have been identified as promising replacements for gum arabic (Trubiano, 1995). Other alternative gums including tragacanth (Imeson, 1992) and λ-carrageenan (Huang et al., 2001) have been reported as effective thickeners and stabilizing agents (Stephen 1990). Proteins, including whey and soy are emulsifiers that can alter both emulsion droplet charge and interfacial membrane thickness in oil-in-water emulsions (Kim et al., 1996; Keogh and O'Kennedy, 1999; Kulmyrzaev et al., 2000; Singh and Ye 2000) and form stable films against oxidation (Djordjevic et al., 2008; McClements, 2004). Although the aforementioned molecules have been extensively studied and widely used as active ingredients in milk-based products (ice cream, milk beverages, yogurt and milk puddings), very little is found in the literature about their ability to stabilize concentrated emulsions containing higher oil concentration (> 10%). In addition, factors that could contribute to the emulsion stabilization/destabilization such as, the type of hydrocolloid, the possible combination of hydrocolloid with protein, and the concentration of oil to be dispersed have not been reported.

There is growing interest in the formulation of value-added products containing health ingredients (i.e., Omega-3, vitamins and minerals, etc.,) for the functional food market. Such emulsion concentrates can be useful as feedstock emulsions for other suspension emulsions as well as constituting a stable oil-in-water emulsion delivery. Therefore, understanding and monitoring the factors that influence the stability and shelf-life of prepared emulsion matrices is critical for their continued success in the market place.

The present work was aimed at (1) evaluating the ability of emulsifiers, protein and texture modifiers alone and as a mixture with soy proteins to prepare emulsion concentrates, and (2) investigating the characteristics of the emulsion concentrates (i.e., droplet size distribution, color and viscosity of the dispersed oil-in-water emulsions) and studying their stability over time.

2. Materials and Methods

2.1 Materials

Starch was obtained from the National Starch and Chemical Co. (Boucherville, Qc, Canada). Gum arabic, gum tragacanth and λ-carrageenan were kindly donated by Frutarom Inc. (NJ, USA) and refined vegetable oil was purchased from the local market. Soy protein isolate (PRO-FAM 873) was purchased from ADM Co., USA (protein content 90%, fat 1%, ash 4%, and moisture 6%). Cow's milk (3.25% fat) was purchased from the local supermarket and used as a reference for color measurements. All solutions were prepared using distilled water, and all measurements were taken at 25°C unless otherwise stated.

2.2 Preparation of solutions

In general, concentrated oil-in-water emulsions are prepared at emulsifier-to-oil ratio of 1:1 or 1:2 (Lee & Tadros, 1982). For this study, dispersed solutions of the emulsifiers (gum arabic and starch, at 10% w/v); protein (SPI, at 10% w/v), texture modifiers (gum tragacanth and carrageenan at 0.025% and 0.1% w/v, respectively) and SPI-tragacanth (10%+0.025% w/v) and SPI-carrageenan (10%+0.1% w/v) mixtures were prepared in distilled water and allowed to hydrate overnight. The pH of these solutions were adjusted to 6.5 ± 0.1 before use. Gum tragacanth and carrageenan solutions were prepared at very low concentrations (0.025 and 0.1% w/v, respectively) due to their high viscosities and their low permissible levels of utilization in the food industry.

2.3 Preparation of emulsion concentrates

Table 1 reports the ratios used for the preparation of the different emulsion concentrates. 10% and 20% oil-in-water emulsions were prepared using gum arabic, starch and SPI. Emulsion concentrates with tragacanth and carrageenan alone and in mixture with SPI were prepared at 5% and 10% oil-in-water emulsions. Coarse emulsions were first prepared using the rotor system Polytron PT 10/35 (Kinematica AG, Switzerland) for 1 minute, then homogenization of the coarse emulsions was achieved with a pressure valve homogenizer (Emulsifex-C5 Avestin, Don Reid Drive, Ontario, Canada) for 60 s at a pressure of 200 bar (3000 psi) and were analyzed for stability, viscosity and color.

2.4 Quickscan measurements

Stability of the emulsions was monitored using a Coulter Quickscan^{TM} turbidity analyzer (Fullerton, USA) following the method described by Chanamai and McClements (2000). Emulsions (5 mL) were placed into flat-bottomed cylindrical glass tubes (100 mm height, 10-mm internal diameter) and introduced into the Quickscan machine. At t=0, the instrument measures the back scattering and/or transmission of monochromatic

light (λ = 850 nm) from the sample (as a function of its height) by scanning vertically along the cylinder sample cell. Two kinds of light scattering/transmission modifications were detected and plotted by the Coulter Quickscan. The test tubes were then stored at 4 °C and the stability of the emulsions was monitored over time (during a 15 days storage period). For curve interpretation, the decreases or increases of particular areas of the graphs represent particle migration to the top or bottom of the sample indicating creaming or sedimentation. Second, decreases in the overall back scatter values along the length of the graph indicate particle or aggregate size variation indicating coalescence or flocculation. The back-scattering profile at t=0 was considered as the reference to analyze the stability of the system.

2.5 Droplet size distribution and Zeta-Potential

Emulsion droplet size distribution was determined by the laser light-scattering method using a Zetasizer 4 Malvern (Malvern Instruments, Malvern, UK). The instrument uses photon correlation spectroscopy (PCS) to measure particle size in constant random thermal or Brownian motion. This motion causes the intensity of the light scattered from the particles to vary with time. Large particles move slowly than small ones, so that the rate of fluctuation of the light scattered from them is also slower. PCS uses the rate of change of these light fluctuations to determine the size distribution of the particles scattering light. The particle diameter range and number of photon counts per second [kilo Count per second (kCps)] were evaluated at room temperature when the volume fraction of oil in the diluted emulsion was about 1:4000 for all the cases. The emulsions were analyzed right after preparation in triplicate and results were expressed in percentage of volume of sample occupied by particles of a similar size class. The zeta-potential was determined by measuring the direction and velocity that the droplets moved in the applied electric field.

2.6 Color measurements

The color of the prepared emulsion concentrates was measured following the method of Chantrapornchai et al. (1998), on an instrumental colorimeter (Labscan II, Hunter Associates Laboratory, Reston, VA), which was calibrated using a white color standard tile with tristimulus values: X = 78.38 , Y = 83.21, and Z = 87.41 (Standard No. LS-13593, Hunter Associated Laboratory). A fixed amount of emulsion sample was poured into the measurement cell and measured. The instrument reports the color of the samples in terms of the *L, a, b* color space system, where, *L* represents the lightness, *a* and *b* are color coordinates: *L*, 0 (black) to 100 (white); *a*, – 80 (green) to 100 (red); *b*, – 80 (blue) to 70 (yellow) (Francis and Clydesdale, 1975). The data is also represented in terms of Chroma (C) and ΔE which is a measure of the difference in optical properties between a sample and a reference emulsion (milk):

$C = (a^2 + b^2)^{1/2}$ and $\Delta E = ((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)^{½}$, where ΔL, Δa and Δb are the differences in the specified tristimulus coordinate between the sample and the cow's milk (3.25% fat) used as reference.

2.7 Rheological measurements

Viscosity measurements were performed following the procedure described by Anton et al. (2001), using a rheometer (AR 1000-N, TA Instrument, Newcastle, DE), with the following cone geometry: acrylic 2° cone angle, 6.0 cm diameter, 46 μm truncation. The temperature was maintained at 22 °C in all the experiments. The torque vs. angular velocity data, were converted first to shear stress vs. shear rate, and to apparent viscosity vs. shear rate, using TA software "Rheology Advantage Data Analysis" (TA Instrument, Newcastle, DE). The apparent viscosity was determined from the equation: $\sigma = K \gamma^n$ where, σ is sheer stress (Pa), γ is the shear rate (sec^{-1}), K is the consistency index, and n is the flow behaviour index.

2.8 Statistical analysis

All experiments were conducted in triplicate and reported as means ± standard deviations. Statistical analyses were performed using *t*-test with a confidence interval of 95%.

3. Results and Discussion

3.1 Droplet size distribution and zeta potential of prepared emulsion concentrates

3.1.1 Emulsifiers *versus* SPI

The oil droplet size distribution of the prepared emulsions is presented in Table 2. Emulsion concentrates stabilized with gum arabic and soy protein isolate (SPI) (with emulsifier/oil ratio of 10:10) both showed an average droplet size of 0.7 μm. Increasing the oil content by two-fold (emulsifier/oil ratio 10:20) resulted in a significant ($p < 0.05$) increase in the average droplet particle size for gum arabic (1.45 μm) and SPI (4.17 μm) emulsions. These large particles are probably the result of droplet aggregation (flocculation). Similar increases in particle size have been reported at neutral pH in hydrocarbon oil-in-water emulsions stabilized by

β-lactoglobulin (Kim et al., 2002). Protein surface denaturation increases the surface hydrophobicity of emulsion droplets, which increases the hydrophobic attraction between droplets (Fang and Dalgleish, 1997). The average oil droplet size for the starch-stabilized emulsion was smaller (0.4 μm and 0.45 μm at 10 and 20% oil concentrations, respectively). These results suggest that starch was more effective at producing small droplets during homogenization than gum arabic and SPI at both oil concentrations.

Measurements of zeta potential (the electrical charge of the droplets), along with particle size, can be used to predict the stability of fat emulsions. Theoretically, a high negative zeta potential prevents aggregation of the emulsion droplets and increases stability through electrostatic repulsion. The zeta potential values of starch-stabilized emulsions at different oil ratios were lower (less negative) than the zeta potentials of gum arabic and SPI. Increasing the oil content to 20% had no noticeable effect on the zeta potential of these emulsions.

3.1.2 Texture modifiers

Gum tragacanth and carrageenan stabilized emulsion concentrates prepared with 10% oil content resulted in average droplet sizes of 0.7 μm and 4.4 μm for gum tragacanth, and 0.5 μm and 2 μm for carrageenan. Each droplet size represented ~ 50% of the volume of the emulsion sample. These emulsions, however, showed a thicker consistency (mayonnaise-like consistency). Reducing the oil content by half (to 5%) and maintaining the same hydrocolloid concentration, resulted in a more fluid emulsion with significantly ($p < 0.05$) lower droplet sizes; 0.5 μm and 1.8 μm for gum tragacanth, and 0.5 μm and 1.7 μm for carrageenan. The proportions of the particle distribution did not change.

Due to their anionic nature, gum tragacanth and carrageenan emulsions showed higher negative zeta potentials; however, for some reason these emulsions were very unstable as revealed by their Quickscan graphs (Figure 3).

3.1.3 SPI-texture modifiers mixtures

When mixed with soy protein isolate, the SPI-gum tragacanth stabilized emulsion with 5% oil content gave lower average droplet sizes, while SPI-carrageenan had larger average droplet sizes at both emulsifier/oil ratios. This trend was expected because, the SPI-carrageenan complex showed a higher tendency to self-aggregate (visual observation). These aggregates could have been emulsion droplets held together by electrostatic carrageenan bridges (formation of carrageenan-protein complexes) (Dickinson and Stainsby, 1988), or due to droplet aggregation (flocculation or coalescence).

When mixed with soy protein isolate, the zeta potential of the droplets of SPI-tragacanth and SPI-carrageenan-stabilized emulsion concentrates decreased markedly (became less negative) and was almost similar to the droplet charge of emulsion stabilized with SPI alone. This result suggests that λ-carrageenan molecules may have been only weakly bound with soy protein molecule at this pH (6.5) of the emulsion concentrates. Ledward (1994) and Ward-Smith et al. (1994) reported that strength of the electrostatic interactions between proteins and polysaccharides is dependent on the sign, number, and distribution of ionizable groups on the molecules at specific pH values.

3.2 Stability analysis of prepared emulsion concentrates

Quickscan analysis allows for the evaluation of various types of instability phenomena, normally due to differences in density between the dispersed and the continuous phases. This includes creaming, sedimentation and particle size variation, which could be reversible (flocculation) or irreversible (coalescence). Figure 1 shows an example of a typical Quickscan profile showing different instability phenomenon which allows a macroscopic visualization of the stability of concentrated dispersions (Bru et al., 2004).

3.2.1 Stability of emulsion concentrates prepared with emulsifiers

Stabilities of the emulsion concentrates prepared with the different emulsifiers (gum arabic, starch, SPI) are presented in Figure 2. The results indicated that the SPI emulsion concentrates (at both 10% and 20% oil concentrations) showed the highest stability as evidenced by the stability of its backscattering flux during storage. Starch, on the other hand, showed a slight decrease in backscattering across the height of the sample which is characteristic of a flocculation phenomenon (i.e., particle aggregation) and likely due to an increase of the size of the particles,. At 20% oil concentration, the emulsion stability improved except for a noticeable decrease of the backscattering level at the top of the sample, indicative of creaming. For gum arabic, the backscattering flux decreased at the bottom of the sample tube due to a decrease in particle concentration (clarification) whereas it increased at the top of the sample due to an increase of the concentration of the dispersed phase (creaming).

3.2.2 Stability of emulsion concentrates prepared with texture modifiers

As shown in Figure 3, concentrated emulsions stabilized with carrageenan (0.1 %) and gum tragacanth (0.0125 %) alone at 10 % oil content were very unstable and resulted in phase separation. From the backscattering and/or transmission fluxes of tragacanth and carrageenan concentrated emulsions, it was observed that the level of backscattering decreased across the height of the sample tube due to an increase of the size of the particles, which is characteristic of coalescence. Carrageenan concentrated emulsions also exhibited creaming phenomena at the top of the sample as evidenced by an increase in the peak thickness of the backscattered light. Garti and Reichman (1993) in an earlier review reported that not all hydrocolloids act as good emulsifiers. Gum arabic and some proteins (e.g., β-lactoglobulin, soy proteins) are good emulsifiers, whereas gum tragacanth, carrageenan, xanthan gum and galactomannans have limited surface activity and are more effective as stabilizers (Dickinson and Stainsby, 1988).

3.2.3 Stability of emulsion concentrates prepared using a mixture of SPI and other texture modifiers

Using a combination of SPI with either gum tragacanth or carrageenan improved the stability of the emulsions concentrates at 10% oil concentration (Figure 3). Reducing the oil content in the concentrated emulsions to 5 % increased their stabilities. This is in good agreement with the findings of Gu et al. (2004, 2005a,b) who reported that oil-in-water emulsions prepared with β-lactoglobulin-ι-carrageenan had higher stability than emulsions stabilized with either β-lactoglobulin or ι-carrageenan solutions alone. They explained that in these systems a two-component interfacial membrane is produced, leading to the formation of a two-layered interface surrounding the droplets. Emulsions stabilized by two-layered membranes reportedly have better stability against environmental stresses (such as thermal processing, freeze-thaw cycling, lipid oxidation, and high ionic strengths) than those stabilized by single-layered membranes (McClements, 2004).

In general, visual inspection of the samples showed that concentrated emulsions prepared using gum arabic, starch and SPI were stable compared to tragacanth and carrageenan over the 15 days of storage (Figure 4).

3.3 Color and viscosity analyses

3.3.1 Color and viscosity of emulsion concentrates prepared with emulsifiers

The results of color measurements (*L, a, b* tristimulus values; color intensity, chroma (*C*); and color difference relative to reference (*ΔE*)) of the emulsion concentrates are presented in Table 3. Emulsion concentrates stabilized with gum arabic (emulsifier/oil ratio of 10:10) had lower *ΔE* value than for the starch and SPI samples. A large decrease in the *ΔE* of SPI-stabilized emulsion (i.e., from 13.06 to 4.98) was observed when the oil concentration was increased from 10 to 20%. As the average droplet size increased from 0.7 μm to 4.17 μm for SPI emulsion (Table 2), the *L*, *a* and *b* values decreased resulting in the lowering of the *ΔE* value. In their earlier work, Chantrapornchai et al. (1998, 2001) reported that as the droplet size increases, the scattering efficiency of the droplets decreases, which causes a reduction in lightness (lower *L* value) and an enhancement of color. However, increasing oil concentration may sometimes negatively affect emulsion color (by increasing the *ΔE* value) if the two other tristimulus coordinates (*a* and *b*) do not decrease with the increase in the particle size; this was observed with gum arabic and starch where the chroma value (C) increased from 12.4 to 16.1 and from 12.7 to 14.8, respectively. In fact, absorption of the light wave by the emulsion is largely responsible for 'chromaticness' (blueness, greenness, redness, etc.) which is strongly related to *a* and *b* values (McClements, 1998).

Viscosity measurements of the emulsion concentrates are presented in Table 3. The emulsion concentrates stabilized with gum arabic, starch and SPI at both oil concentrations (10 and 20 %) exhibited a decrease in viscosity with increasing shear rate, reflecting the pseudoplastic (shear thinning) nature of these emulsions. SPI showed higher viscosity than gum arabic and starch. Increasing the oil concentration to 20% resulted in an increase in viscosity, probably due to the increase in particle as reported previously (Table 2). Results reported by Chanamai and McClements (2002) suggest that the rheology of concentrated emulsions (viscosity) is fairly dependent on droplet concentration, size and shear rate, as well as the electrostatic repulsion between droplets.

3.3.2 Color and viscosity of emulsion concentrates prepared with texture modifiers

Gum tragacanth and carrageenan stabilized emulsions showed higher *ΔE* values at 10% oil concentration. Their poor emulsifying properties resulted in oil droplet coalescence, oil phase separation at the surface of the emulsion, which increased the tristimulus values (*a* and *b*) and, consequently, the *ΔE* and Chroma values. Decreasing the oil content to 5% decreased the *ΔE* values but did not improve their stabilities. The viscosities of these emulsion concentrates were also higher due to the thickening effect of gum tragacanth and carrageenan. Interestingly, when these two hydrocolloids were each used separately in combination with soy protein isolate,

the SPI/gum tragacanth and SPI/carrageenan stabilized emulsions resulted in the lowest ΔE value and, consequently, the whitest emulsion color. Gu et al. (2005a,b), have reported that, in addition to color, emulsions formed by combining protein and polysaccharides are highly stable and resistant to a broad range of environmental stresses (such as pH, ionic strength, and thermal processing).

4. Conclusion

The stability of oil-in-water emulsions was strongly influenced by the droplet size, charge and concentration. Oil droplet size lower than 1μm and higher zeta potential was required for improved stability. Under the studied conditions, concentrated emulsions prepared using the three emulsifiers; SPI, starch and gum arabic (with emulsifier/oil ratios of 10:10) were the most stable over the 15 days storage period. Concentrated emulsions stabilized with texture modifiers (gum tragacanth and carrageenan) showed several instabilities including clarification, particle size variation and creaming. Combination of SPI with either gum tragacanth or carrageenan improved the stability and color of the concentrated emulsions, particularly at 5% oil concentration. Overall the results indicated that emulsions stabilized by SPI and starch had better stability and could be promising replacements for gum arabic in food application due to their stable composition, availability and relatively low price. Many beverages are made from concentrated emulsions, which are diluted in aqueous solutions in order to simplify production processes and costs. Thus, the results of this study could prove useful in helping improve our understanding of how colloidal instabilities arise in emulsions and how they could be controlled in order to improve the quality of finished products.

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Table 1. Composition of prepared emulsion concentrates

	Batch No.1		Batch No.2	
	Emulsifier (%)	Oil (%)	Emulsifier (%)	Oil (%)
Emulsifiers (gums and protein)				
Gum Arabic	10	10	10	20
Starch	10	10	10	20
Soy protein isolate (SPI)	10	10	10	20
Texture modifiers				
Carrageenan	0.1	5	0.10	10
Tragacanth	0.025	5	0.025	10
Protein and texture modifier mixtures				
SPI + Carrageenan	10 + 0.1	5	10 + 0.10	10
SPI + Tragacanth	10 + 0.025	5	10 + 0.025	10

Table 2. Droplet size distribution and zeta potential of prepared emulsions concentrates

Emulsifier/ Oil ratio (%)	Zeta potential	Droplet size distribution (μm)*
Emulsifiers (gums and protein)		
Gum Arabic		
10:10	– 16.9 ± 0.23	0.70 (100%) a
10:20	– 18.5 ± 0.56	1.45 (100%) b
Starch		
10:10	– 10.3 ± 0.1	0.40 (90.3%); 0.77 (9.7%) a
10:20	– 12.1 ± 0.45	0.45 (100%) a
Soy protein isolate (SPI)		
10:10	– 16.1 ± 0.25	0.1 (15.6%); 0.71 (84.4%) a
10:20	– 16.1 ± 0.15	0.08 (9.9%); 0.49 (26%); 4.17 (64%) b
Texture modifiers		
Tragacanth		
0.025:5	– 33.4 ± 0.51	0.08 (0.9%); 0.5 (52.7%); 1.8 (46.5%) a
0.025:10	– 36.4 ± 0.31	0.7 (47.5%); 4.4 (52.5%) b
Carrageenan		
0.1:5	– 37.3 ± 0.12	0.09 (0.5%); 0.5 (48.8%); 1.7 (46.6%); 4.7 (4.1%) a
0.1:10	– 39.9 ± 0.42	0.07 (3.7%); 0.5 (50.8%); 2.0 (45.5%) b
Protein and texture modifier mixtures		
SPI + Tragacanth		
10:0.025:5	– 15.9 ± 0.49	0.11 (12.9%); 0.6 (87.1%) a
10:0.025:10	– 15.4 ± 0.35	0.09 (8.7%); 0.6 (63.9%); 1.7 (10.8%); 3.5 (16.6%) b
SPI + Carrageenan		
10:0.1:5	– 20.4 ± 0.65	0.7 (2.1%); 0.5 (15.5%); 2.1 (35%); 6.1 (43.4%) a
10:0.1:10	– 15.1 ± 0.55	0.58 (1.9%); 3.5 (95.9%); 21.3 (2.2%) b

* Droplet size distribution percentage represents percentage of sample volume occupied by particles of similar average size. Zeta potential values are mean and standard deviation of triplicate measurements. The letters (a and b) within the row indicate significant ($p < 0.05$) difference for the same hydrocolloid at different oil ratios.

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Table 3. Color and viscosity measurements of prepared emulsion concentrates

Emulsifier/Oil ratio (%)	Color values					Viscosity (Pa.s)
	L	*a*	*b*	*ΔE*	Chroma	
Cow milk (3.25%)	77.87	– 1.45	6.66			
Emulsifiers (gums and protein)						
Gum Arabic						
10:10	84.97	– 0.21	12.36	9.19 ± 0.003	12.36 ± 0.04	0.63 ± 0.0034
10:20	85.02	– 0.21	16.10	11.87 ± 0.07	16.02 ± 0.14	0.74 ± 0.0029
Starch						
10:10	90.80	– 1.53	12.63	14.24 ± 0.01	12.72 ± 0.04	0.88 ± 0.0031
10:20	93.10	– 1.28	14.71	17.22 ± 0.02	14.76 ± 0.13	0.95 ± 0.0028
Soy Isolate (SPI)						
10:10	89.53	0.52	12.20	13.06 ± 0.03	12.21 ± 0.04	1.02 ± 0.0025
10:20	77.17	0.20	11.30	4.98 ± 0.09	11.30 ± 0.09	1.47 ± 0.0020
Texture modifiers						
Tragacanth						
0.025:5	92.29	– 2.23	15.34	12.15 ± 0.08	15.50 ± 0.01	0.90 ± 0.0051
0.025:10	84.31	– 2.67	16.79	16.85 ± 0.03	17.00 ± 0.03	1.07 ± 0.0049
Carrageenan						
0.1:5	88.33	– 1.93	9.14	10.76 ± 0.14	9.34 ± 0.14	1.07 ± 0.0048
0.1:10	79.16	– 2.49	19.87	13.31 ± 0.12	20.03 ± 0.13	2.28 ± 0.013
Protein and texture modifier mixtures						
SPI + Tragacanth						
10:0.025:5	81.97	0.67	10.38	5.93 ± 0.04	10.40 ± 0.01	ND
10:0.025:10	84.13	0.17	9.79	7.19 ± 0.05	9.79 ± 0.09	ND
SPI+ Carrageenan						
10:0.1:5	80.30	0.65	11.12	5.50 ± 0.005	11.14 ± 0.02	ND
10:0.1:10	ND	ND	ND	ND	ND	ND

The L, a, b values are tristimulus values. ''L'' denotes lightness-to-darkness from 100 to 0 units, respectively. ''a'' represents redness (+ a) to greenness (– a), and ''b'' represents yellowness (+ b) to blueness (– b). ND: not determined (the emulsion was very thick and could not be homogenized). Values are means and standard deviation of triplicate measurements.

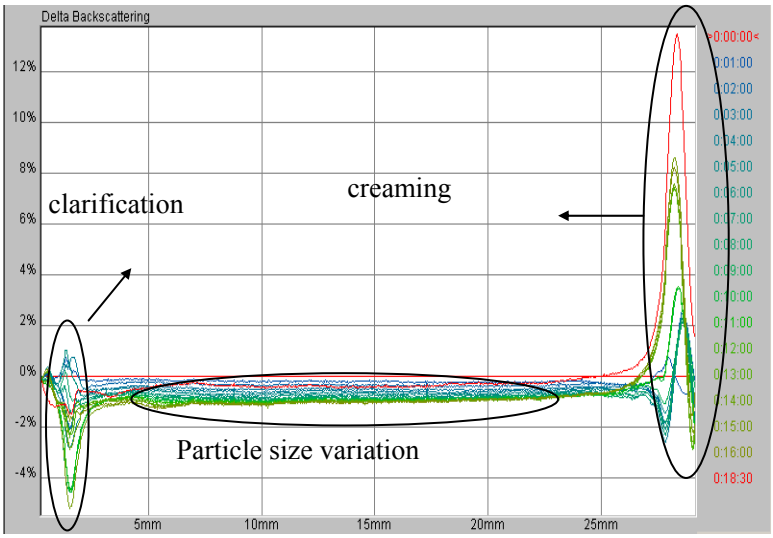

Figure 1. Typical instability phenomenon encountered in emulsions (reproduced with permission from Bru et al., 2004)

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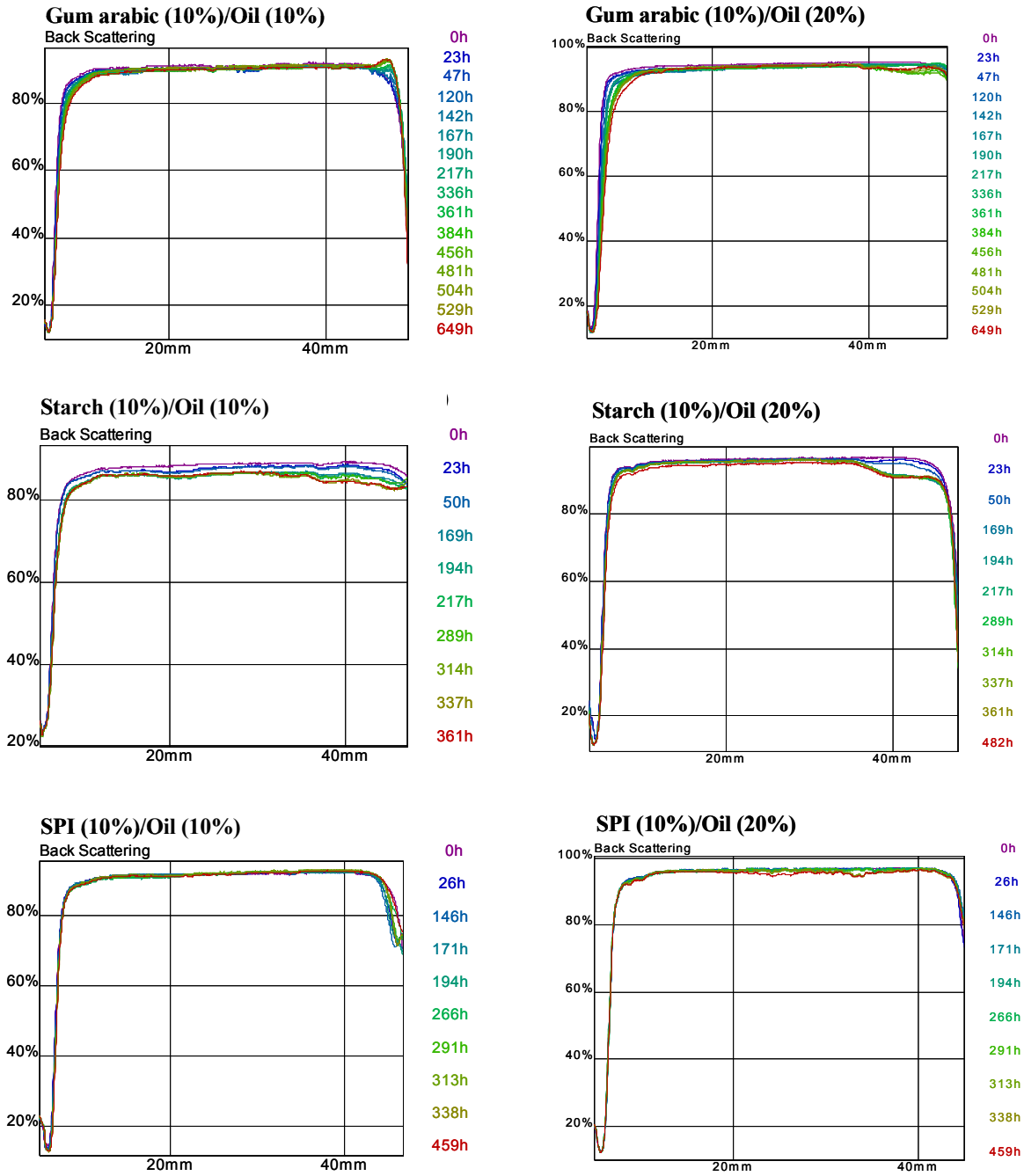

Figure 2. Backscattering profiles of concentrated emulsions stabilized with gum arabic, starch and SPI at emulsifier/oil ratios of 10:10 and 10:20% % (w/v)

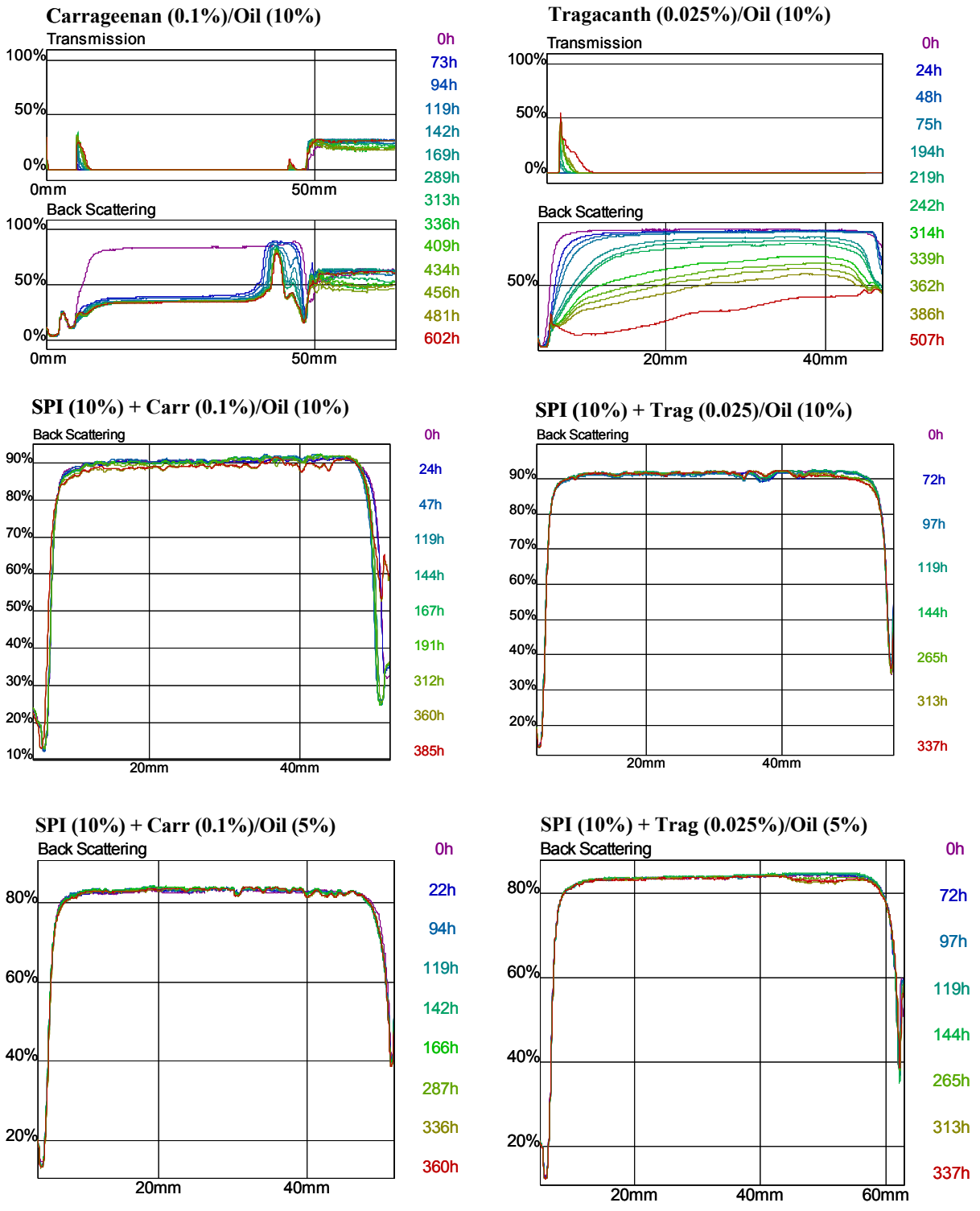

Figure 3. Backscattering profiles of concentrated emulsions stabilized with tragacanth and carrageenan alone and as a mixture with SPI at both 5 and 10% oil concentration

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Figure 4. Picture showing the physical stability of the prepared emulsion concentrates

Prebiotic Activity and Bioactive Compounds of the Enzymatically Depolymerized Thailand-Grown Mangosteen Aril

Pranee Anprung (Corresponding author)
Department of Food Technology, Faculty of Science
Chulalongkorn University, Bangkok 10330, Thailand
Tel: 66-022-185-530 E-mail: pranee.a@chula.ac.th

Sasina Sangthawan
Program in Biotechnology, Faculty of Science
Chulalongkorn University, Bangkok 10330, Thailand
Tel: 66-023-803-032 E-mail: froggy11346@hotmail.com

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Abstract

The aim of this research was to investigate the effect of pectinase on prebiotic activity and bioactive compounds of enzymatically depolymerized Thailand-grown mangosteen aril. It was found that after varying enzyme concentration in 0-3.0% (v/w), mangosteen aril treated with enzyme concentration of 2.5% (v/w) and hydrolyzed for 0, 1, 2, 4 and 6 h can be hydrolyzed at 5 different levels of reducing sugar. They were 18, 25, 31, 40 and 45 mg glucose/g fresh weight. Hydrolysis of mangosteen aril at 6 h resulted in the highest release of bioactive compounds with the antioxidant activity value measured by DPPH assay (EC_{50}) of 3.05 µg dw/µg DPPH and FRAP value of 45.53 µM TE/g dw. Total phenolic content was 16.00 mg GAE/g dw, total flavonoid content was 3.08 mg CE/g dw, and soluble dietary fiber was increased to 16.56g/100g dw. Moreover, enzymatically depolymerized mangosteen can increase prebiotic activity score of *L. acidophilus* and *B. lactis* to 0.21 and 0.17, respectively.

Keywords: Mangosteen, Pectinase, Antioxidant, Phenolic, Fiber, Prebiotic

1. Introduction

Garcinia mangostana Linn., mangosteen is one of the most praised tropical fruits and belongs to the family *Clusiaceae* (syn. *Guttiferae*). The major producing countries are found in Southeast Asia, namely Malaysia, Philippines, Indonesia and Thailand. About 85% of the total production of four countries is in Thailand. Mangosteen cultivation in Thailand is limited to the South and the East due to climatic condition. Commercial orchards have been well established in Surat Thani province in the South, and Chanthaburi and Rayong provinces in the East (Morton, 1987; Osman, 2006). The edible aril is white, soft, juicy, sweet and slightly sour taste (Zadernowski, 2009; Yu *et al*., 2007; Palapol *et al*., 2009). It can be stored only for short time period, mainly consumed fresh and can also be canned, frozen or processed into juice, jam, preserve, syrup and candy (Morton, 1987; Osman, 2006). Moreover, the mangosteen aril contains many bioactive compounds such as phenolic, flavonoid and other antioxidants which are our first line of defense against free radical damage, and are critical for maintaining optimum health and well-being (Percival, 1996). Total dietary fiber found in this fruit can be divided into insoluble and soluble dietary fiber (pectin). Furthermore, it also contains many vitamins and minerals including vitamin A, vitamin C, thiamine, riboflavin, niacin, calcium, phosphorous, and iron (Osman, 2006). Plant cell wall consists of cellulose, hemicelluloses and pectin, which is barrier for the release of the intracellular substances. In addition, there are many phenolic hydroxyl groups existing in flavonoids which can combine with cellulose, hemicelluloses and pectin as complexes due to hydrogen bonding interactions (Fu *et al*., 2008).

These bioactive compounds can be extracted by many methods. One example is the extraction with solvents such as ethanol, methanol, hexane, and acetone. Nevertheless, this method dissociates the carotenoid pigments from the proteins and causes water insolubility and ease of oxidation (Cinar, 2005.a). While releasing of the carotenoid by enzyme extraction from orange peel, sweet potato and carrot, these pigments remain in their natural state binding with proteins. This bond structure prevents pigment oxidation and also affects colour stability (Cinar, 2005.b). There are several studies on enzymatic extraction to increase the extractability of bioactive compounds. These were enhancement of anthocyanins and other phenolics in black currant juice (Landbo and Meyer, 2004), enrichment of antioxidant phenols from pomace for the production of puree-enriched cloudy apple juices (Oszmiański *et al.,* 2011), increasing of polyphenol and polysaccharide composition of red wines (Ducasse *et al*., 2010), improvement of the quality of black tea extracts (Chandini *et al*., 2011), increasing of the phenolic content of virgin olive oil (Najafian *et al*., 2009), enzyme-assisted extraction of antioxidants from vegetal matrixes (Pinelo and Meyer, 2008), and increasing of lycopene from tomato tissues (Choudhari and Ananthanarayan, 2007). Additionally, there were also increasing of carotenoid, antioxidant activity, soluble dietary fiber and volatile substances from bael fruit hydrolysate, and flesh and placenta of Sunlady cantaloupe (Charoensiddhi and Anprung, 2010; Wuttisit and Anprung, 2011). These studies represent the benefits of enzyme hydrolysis.

There are many enzymes that increase the extractability of bioactive compounds such as cellulase, beta-glucosidase, pectinase (Fu *et al*., 2008; Cinar, 2005.b). Pectinase enzyme was used in this study because it helps to release bioactive compounds from plant cell wall. The Pectinex®Ultra SP-L, pectinase enzyme, a liquid commercial enzymatic preparation from *Aspergillus aculeatus*, is currently using as a pectinolytic and cellulolytic preparation in fruit juice processing (Ghazi *et al*., 2007). So, this research was to investigate the effect of pectinase on prebiotic activity and bioactive compounds of enzymatically depolymerized Thailand-grown mangosteen aril, and expected that the enzymatically depolymerized mangosteen aril would be effectively used as sources of antioxidant, dietary fiber, and prebiotic. Moreover, this study is an alternative processing way to add the value of the mangosteen which is one of the traditional economic fruits (Thailand had the highest mangosteen production, so the mangosteen is vital to the economy of the country).

2. Materials and Methods

2.1 Raw material preparation

Mangosteen, *Garcinia mangostana* Linn. was purchased from a mangosteen orchard (Rayong province, Thailand) and was harvested at 13th week after full blooming. The fruits of the weight between 55-65 g were incubated at 30±2 ºC for 8 days to be fully ripe. Then, the peels were removed from the fruits. Prior to enzyme hydrolysis, the mangosteen aril browning was controlled by steam blanching until it reached 85ºC at the center and holding for 3 min, then it was blended for 3 min until a homogenized fruit aril was obtained.

2.2 Enzymatic hydrolysis

The homogenized and anti-browning controlled mangosteen aril (200 g) was hydrolysed by Pectinex®Ultra SP-L (Novozymes Switzerland AG, Dittengen, Switzerland) which had enzymatic activity of 10,292 PGU/ml and produced from *Aspergillus aculeatus* and controlled the reaction temperature at 30±2°C. The enzyme concentrations were varied into 7 levels (0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0% v/w) and hydrolysis times were varied into 9 levels (0, 0.5, 1.0, 1.5, 2, 3, 4, 5, and 6 h). The hydrolysis reaction was stopped by heating at 100±5ºC for 5 min. Then, hydrolysis efficiency was determined by the amount of reducing sugar as glucose using the method of Nelson-Somogyi (Nelson, 1944). All chemicals and solvents used in this study were analytical grade, purchased from Sigma Chemical Co., Ltd (St. Louis, MO. USA) and Sigma Aldrich Co., Ltd (Steinheim, Germany). All analyses were performed in triplicate.

2.3 Determination of antioxidant activities

2.3.1 Sample preparation

Preparation of sample was modified from the method of Velioglu *et al*. (1998). Mangosteen hydrolysate was extracted with 95% ethanol, kept in the dark at 25°C for 4.5 h and stored at -20ºC until further analysis.

2.3.2 Antioxidant activities

The antioxidant activities of sample extract were evaluated from DPPH and FRAP assays. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was followed by the method of Maisuthisakul *et al*. (2007). A series of extract concentrations with different ratios of extract to methanol were prepared. Then 4.9 ml of each dilution was mixed with 100µl of 5mM DPPH in methanol and were placed in the dark for 30 min. The absorbance of three samples, the diluted sample with the addition of DPPH solution (A_1), the diluted sample without the addition of DPPH solution (A_S), and DPPH solution (A_0) were read at 517 nm

using a spectrophotometer with methanol as a blank. The decreased content of DPPH radical scavenging activity in each concentration of sample extract could be calculated by the formula as shown:

DPPH radical scavenging activity (%) = $[A_0 - (A_1 - A_S)]/ A_0 \times 100$

The percentage of DPPH radical-scavenging activity was plotted against the sample extract concentration (µg/ml) to determine the amount of extract necessary to decrease DPPH radical concentration by 50% (called EC_{50}). The unit of EC_{50} was later converted to µg/ µg DPPH as follow:

EC_{50} (µg/ µg DPPH)= [(µg extract/ml)/(µg DPPH/ml)]

Ferric reducing antioxidant power (FRAP) assay was modified from the method of Benzie and Strain (1996). The sample extracts (50 µl) were allowed to react with 950 µl of FRAP solution (FRAP solution consist of 25 ml of acetate buffer, 2.5 ml of Ferric chloride solution and 2.5ml of TPTZ solution) and were placed in the dark for 4 min. The standard curve was linear between 100 and 700 µM trolox. Results were expressed in µM trolox equivalent (TE)/ g dw).

2.4 Determination of total phenolic content

Sample was likewise prepared as the determination of antioxidant activity. Total phenolic content was evaluated by the method of Waterhouse (2005). The standard curve was linear between 50-500 mg gallic acid/l. The results were expressed as mg gallic equivalent (GAE)/ g dw.

2.5 Determination of total flavonoid

Sample was likewise prepared as the determination of antioxidant activity. Total flavonoid content was evaluated by the method of Zhishen et al. (1999). The standard curve was linear between 20-100 mg catechin/l. The results were expressed as mg catechin equivalent (CE)/ g dw.

2.6 Determination of dietary fiber

Total, soluble and insoluble dietary fibers were analyzed according to AOAC method (AOAC1995).

2.7 Determination of prebiotic activity

Prebiotic activity assay was modified from Huebner *et al*. (2007). *Lactobacillus acidophilus* La5, *Bifidobacterium lactis* Bb12 (Christian Hansen, Denmark) and *Escherichia coli* ATCC 25922 (Culture Collection Unit, Chulalongkorn hospital, Thailand) were used in this study. *L. acidophilus* La5 and *B. lactis* Bb12 were prepared by streaking onto MRS agar and MRS agar supplement with 0.05% L-cystein HCl (decrease of oxidation-reduction potential), respectively and streaking onto Tryptic soy agar (TSA) for *E. coli* ATCC 25922. Then, incubated at 37ºC for 24-48 h under aerobic condition for *L. acidophilus* La5 and *E. coli* ATCC 25922, anaerobic condition in anaerobic jar with anaerogen gas pack (Mitsubishi) for *B. lactis* Bb12. After that, one colony from each plate was transferred into 10 ml of MRS broth for *L. acidophilus* La5, MRS broth supplement with 0.05% L-cystein HCl for *B. lactis* Bb12, and Tryptic soy broth (TSB) for *E. coli* ATCC 25922 and incubated overnight. For *L. acidophilus* La5, an additional transfer of 1% (v/v) was transferred to MRS broth with 1% w/v glucose or 1% w/v samples. For *B. lactis* Bb12, an additional transfer of 1% (v/v) was transferred to MRS broth supplement with 0.05% L-cystein HCl and 1% w/v glucose or 1% w/v samples. For *E. coli* ATCC 25922, an additional transfer of 1% (v/v) was transferred to minimal medium broth with 1% w/v glucose or 1% w/v samples and incubated overnight.

The prebiotic activity score was determined using the following equation:

Prebiotic activity score =

$$\left[\frac{(\text{probiotic}\log\frac{\text{cfu}}{\text{ml}}\text{ on the prebiotic at 24 hr} - \text{probiotic}\log\frac{\text{cfu}}{\text{ml}}\text{ on the prebiotic at 0 hr})}{(\text{probiotic}\log\frac{\text{cfu}}{\text{ml}}\text{ on glucose at 24 hr} - \text{probiotic}\log\frac{\text{cfu}}{\text{ml}}\text{ on glucose at 0 hr})}\right] -$$

$$\left[\frac{(\text{enteric}\log\frac{\text{cfu}}{\text{ml}}\text{ on the prebiotic at 24 hr} - \text{enteric}\log\frac{\text{cfu}}{\text{ml}}\text{ on the prebiotic at 0 hr})}{(\text{enteric}\log\frac{\text{cfu}}{\text{ml}}\text{ on glucose at 24 hr} - \text{enteric}\log\frac{\text{cfu}}{\text{ml}}\text{ on glucose at 0 hr})}\right]$$

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2.8 Statistical analysis

Results of experiment were presented as mean ± standard deviation of three independent determinations. All statistical analyses were performed with SPSS version 11.5. One- way analysis of variance (ANOVA) by Duncan's new multiple range test was used to compare the mean values. Differences were considered to be significant at $p<0.05$.

3. Results and Discussion

3.1 Effect of enzyme treatment on reducing sugars

It was found that the more increasing the hydrolysis time and the enzyme concentration, the significantly higher the level of reducing sugar ($p\leq0.05$), as shown in Figure 1. It could be explained that pectinase can hydrolyze polysaccharide by cleaving the α-1,4-glycosidic bond of galacturonic acid, so the reducing group was produced in depolymerized mangosteen aril (Wuttisit and Anprung, 2011; Chareonsiddhi and Anprung, 2010). However, the obtained reducing sugar was not significantly different ($p>0.05$) between using the enzyme concentration of 3.0% and 2.5% (v/w). Using 2.5% (v/w) of enzyme concentration can be resulted in all range of low to high reducing sugar levels of approximately 5 levels (18, 25, 31, 40 and 45 mg glucose/g fw) and hydrolyzed for 0, 1, 2, 4 and 6 h. These conditions were selected for the study of bioactive compounds, soluble dietary fiber and prebiotic activity score of enzymatically depolymerized mangosteen aril.

3.2 Antioxidant activity

It can be seen from Table 1 that the antioxidant activity value of depolymerized mangosteen was significantly increased ($p\leq0.05$) due to the increasing of hydrolysis time. At hydrolysis time over 4 h, it had a minimum of DPPH value in form of EC_{50}. The antioxidant activity was highest when EC_{50} value was lowest. On the other hand, FRAP value or the antioxidant activity was highest. Basically, these results indicate that the enzyme can hydrolyze and degrade plant cell wall components. So the intracellular contents, especially antioxidant substances, were easily released (Chen *et al.*, 2011; Choudhari and Ananthanarayan, 2007). The bael fruit pulp treated with enzyme concentration of 2.5% (v/w) and hydrolyzed at 6 h gave the highest antioxidant activities up to 18.85 µg /µg DPPH (Chareonsiddhi and Anprung, 2010). Antioxidant activities in the flesh and placenta of cantaloupe hydrolysate detected by DPPH method were 1.57, 0.66 µg /µg DPPH, respectively higher than the undegraded samples.(Wuttisit and Anprung, 2011). Increasing degree of hydrolysis for pectin in mango pulp had effect on antioxidant activity. The depolymerized mango pulp with reducing sugar content of 95 mg glucose/g fw (DP 95) has antioxidant activity in form of FRAP value as 28.2µM TE/g dw (Kewalee and Anprung, 2010). It has a report that enzymatic extraction can give higher yield of antioxidant activity than the solvent extraction (Wang *et al*., 2011).

3.3 Total phenolic and total flavonoid content

The hydrolysis time at 6 h had given the highest total phenolic and flavonoid contents of 16 mg GAE/ g dw and 3.08 mg CE/g dw, respectively as shown in Table 1. The experiment was related with the different literature; for example, total phenolic and flavonoid contents were increased after enzymatic hydrolysis in black current juice (Landbo and Meyer, 2004), olive oil (Najafian *et al*., 2009), ginkgo leaves (Chen *et al*., 2011), and cactus pear (Ayoub *et al*., 2011). Total phenolic contents in the flesh and placenta of cantaloupe hydrolysate were 14.95, 39.72 mg GAE/100 g, respectively higher than the undegraded samples. And total flavonoid contents in the flesh and placenta of cantaloupe hydrolysate were 2.25, 12.24 mg CE/100g, respectively higher than the undegraded samples. (Wuttisit and Anprung, 2011). Total phenolic content in flesh and peel of red dragon fruit hydrolysate were 1,049.18, 561.76 mg GAE/100 g, respectively higher than the undegraded samples, and total flavonoid content in the flesh and peel of red dragon fruit hydrolysate were 1,310.10, 220.28 mg CE/100 g, respectively higher than the undegraded samples. (Sornyotha and Anprung, 2011) It can be explained that pectinase does not only degrade plant cell wall, but also improves releasing of intracellular contents as bioactive compounds such as phenolic and flavonoid. Moreover, it was found that enzymatic extraction improved phenol released from apple skin more than solvent extraction (Pinelo *et al*., 2008).

3.4 Dietary fiber

From the Table 2, total dietary fiber content in sample without hydrolysis (A1) was found not different from sample with hydrolysis (A2) ($p>0.05$). On the other hand, the soluble dietary fiber of A2 was higher than A1 (16.56, 12.91 g/100g dw), respectively. These results were caused by Pectinex Ultra SP-L which is complex enzyme that can be divided into 3 groups: depolymerase, pectinesterase and protopectinase. Depolymerase can cleave the α-1,4-glycosidic bonds of pectic substances, pectinesterase can catalytically deesterify the methyl ester linkages of pectic substances to release acidic pectins and methanol. In addition, protopectinase can

hydrolyze insoluble protopectin to get more highly soluble pectin, therefore these enzymes are able to produce soluble dietary fiber. It was related with the experiments in fully ripe fresh Thai bael fruit degraded by enzyme that the soluble dietary fiber of bael fruit hydrolysate (4.58g/100gfw) was higher than nonenzyme treated sample (3.51g/100gfw) (Chareonsiddhi and Anprung, 2010). And the soluble dietary fiber in flesh and peel of cantaloupe hydrolysate (0.76, 0.52 g/100gfw, respectively) were higher than undegraded enzyme (0.65, 0.32 g/100gfw, respectively) (Wuttisit and Anprung, 2011).

3.5 Prebiotic activity score

Lactobacilli and Bifidobacterium strains were chosen to test the prebiotic activity score because they are used in dairy foods and have good potential probiotic properties. From the determination of prebiotic activity of A1 (sample without hydrolysis) compared with A2 (sample with hydrolysis) and inulin, the amount of bacterial cell grown in media with mangosteen aril samples (A1 and A2), inulin and glucose for 24 h can be found (shown in Table 3). The amount of L. acidophilus La5 and B. lactis Bb12 grown on media with inulin and media with A2 were significantly higher ($p \leq 0.05$) than ones grown on media with A1 and media with glucose. On the other hand, the amount of E.coli ATCC 25922 (representative of Enteric bacteria) grown on glucose (no prebiotic) was significantly higher ($p \leq 0.05$) than other media with prebiotic. Moreover, prebiotic activity scores were derived from cell density values as shown in Figure 2. It was found that the highest prebiotic activity scores wer L. acidophilus La5 and B. lactis Bb12 grown on media with inulin (0.41, 0.38, respectively), followed by L. acidophilus La5 and B. lactis Bb12 grown on media with A2 (0.21, 0.17, respectively), and the lowest scores were L. acidophilus La5 and B. lactis Bb12 grown on media with A1 (0.16, 0.12, respectively). From these results, it can be concluded that the different bacterial strain affected to the different prebiotic activity score owing to the differences in the metabolic capacity of related strains apparently existed, and utilization of prebiotics by these related bacteria requires the presence of specific hydrolysis and transport systems for the particular prebiotic (Hubner et al., 2007). Moreover, prebiotic activity score of B. lactis Bb12 was lower than L.acidophilus La5, this may be caused that the different cell density value between prebiotics, and glucose of B. lactis Bb12 was less than L.acidophilus La5 affected to the prebiotic activity score of B. lactis Bb12 lower than L.acidophilus La5. When compared the prebiotic activity score which used depolymerized mangosteen aril as prebiotic with other studies which used bael fruit hydrolyzate as prebiotic, it was found that probiotic bacteria were higher grown on depolymerized mangosteen aril than bael fruit hydrolyzate. This may be caused that the depolymerized mangosteen aril was hydrolyzed to smaller molecules than bael fruit hydrolyzate (Charoensiddhi and Anprung, 2010) that appropriated for supporting the growth of probiotics.

4. Conclusion

The Pectinex®Ultra SP-L had a potential to release the bioactive compounds from plant cell wall and affected to changes in prebiotic activity and amount of bioactive compounds from enzymatically depolymerized mangosteen. Results show the significant increasing in amount of antioxidant activity, total phenolic, total flavonoid, soluble dietary fiber as well as promoting the growth of probiotics. It could be said that enzymatically depolymerized mangosteen was effective to be used as sources of antioxidant, dietary fiber, and prebiotic. Moreover, the depolymerized mangosteen aril as prebiotic give the highest activity scores among the studied Thailand-grown fruits. Regarding to a slimy characteristic of mangosteen hydrolysate and the results of bioactive compound from this study, it could be possible to further study about rheological profile of enzymatically depolymerized mangosteen aril for using as a valuably functional stabilizer in the emulsion system.

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Table 1. Effect of enzyme hydrolysis on bioactive compounds of depolymerized mangosteen aril

Bioactive compounds	Hydrolysis time (h)				
	0	1	2	4	6
Antioxidant activities					
DPPH (EC_{50}, μg dw[1]/μgDPPH)	11.53±0.13[a]	6.37±0.06[b]	5.70±0.11[b,c]	4.17±0.09[c,d]	3.05±0.04[d]
FRAP (μM TE[2]/g dw)	34.4±0.53[d]	35.67±0.81[d]	37.53±0.50[c]	42.60±1.24[b]	45.53±0.70[a]
Total phenolic (mg GAE[3] /g dw)	9.03±0.50[e]	10.57±0.31[d]	12.53±0.35[c]	14.57±0.70[b]	16.00±0.82[a]
Total flavonoid (mg CE[4]/g dw)	1.17±0.08[d]	1.68±0.14[c]	2.35±0.32[b]	2.66±0.17[b]	3.08±0.15[a]

Each value represented a mean±standard deviation.
Means with the different letter in the row are significantly difference at $p \leq 0.05$.
[1]dw= dry weight, [2]TE=Trolox equivalents, [3]GAE=gallic acid equivalents , [4]CE=catechin equivalents.

Table 2. Effect of enzyme hydrolysis on total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) of depolymerized mangosteen aril

Total dietary fiber	A1[2]	A2[3]
(g/100g dw[1])		
Total dietary fiber	25.15±0.50[ns]	25.60±0.21[ns]
Soluble dietary fiber	12.91±0.13[b]	16.56±0.10[a]
Insoluble dietary fiber	12.24±0.37[a]	9.04±0.11[b]

Each value represented a mean±standard deviation.
Means with the different letter in the row are significantly difference at p≤0.05.
[1]dw = dry weight basis, [2]A1 = Sample with no hydrolysis, [3]A2 = Sample with hydrolysis.

Table 3. Effect of enzyme hydrolysis on the increasing of bacterial cell density between 0 and 24 h in different carbohydrates

Bacterial culture	Cell density [$\log_{10}$(cfu/ml)]			
	Glucose	Inulin	A1[1]	A2[2]
L. acidophilus La5	2.02±0.04[c]	2.38±0.04[a]	2.22±0.03[b]	2.35±0.12[a]
B. lactis Bb12	2.14±0.06[b]	2.45±0.09[a]	2.27±0.03[b]	2.42±0.09[a]
E. coli ATCC 25922	2.11±0.02[a]	1.62±0.02[c]	1.98±0.03[b]	2.02±0.06[b]

Each value represented a mean±standard deviation.
Means with the different letter in the row are significantly difference at $p \leq 0.05$.
[1]A1 = Sample with no hydrolysis, [2]A2 = Sample with hydrolysis.

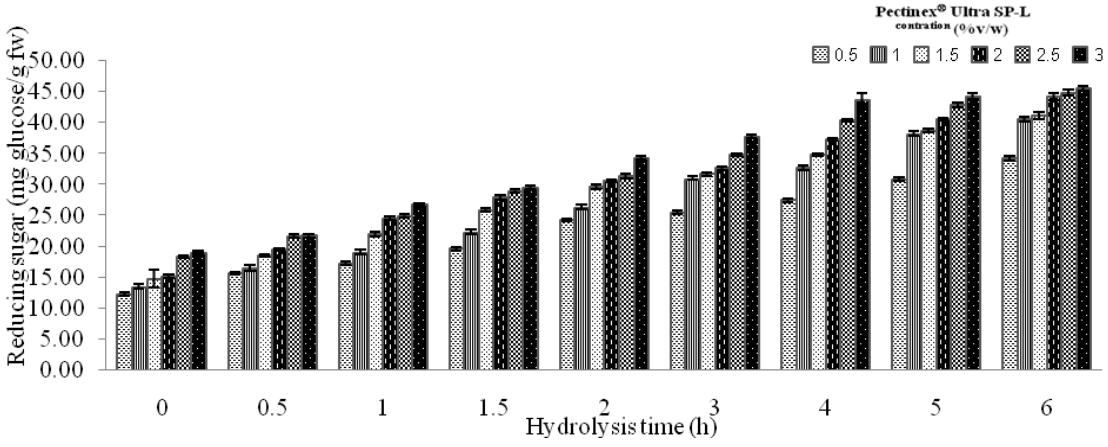

Figure 1. Changes in the reducing sugar content during enzyme hydrolysis

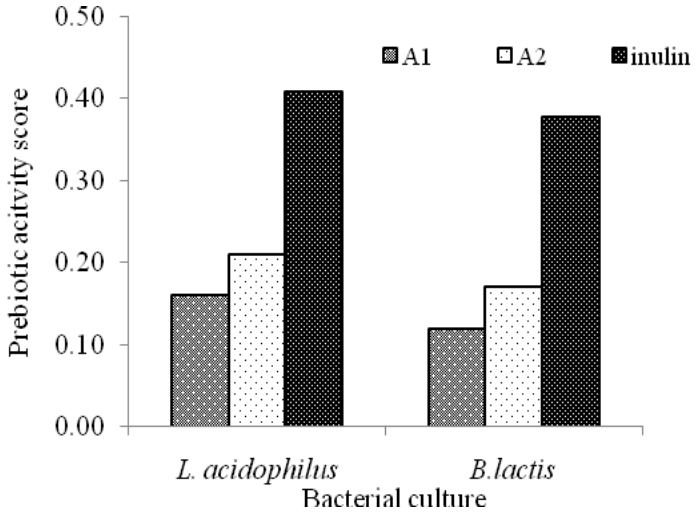

Figure 2. Prebiotic activity score of depolymerized mangosteen aril
A1 = Sample with no hydrolysis, A2 = Sample with hydrolysis

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Dietary Intake of Vitamins and Minerals in Adolescent Sprint Athletes: A Three Year Follow-up Study

Dirk Aerenhouts (Corresponding author)
Department of Human Biometry and Biomechanics
Vrije Universiteit Brussel, Pleinlaan 21050 Brussel, Belgium
Tel: 32-2629-2733E-mail: dirk.aerenhouts@vub.ac.be

Peter Deriemaeker
Department of Human BiometryandBiomechanics
Vrije Universiteit Brussel, Pleinlaan 21050 Brussel, Belgium
Tel: 32-2629-2731 E-mail: pderiema@vub.ac.be

Marcel Hebbelinck
Department of Human BiometryandBiomechanics
Vrije Universiteit Brussel, Pleinlaan 21050 Brussel, Belgium
Tel: 32-2629-2729 E-mail: mhebbel@vub.ac.be

Peter Clarys
Department of Human Biometry and Biomechanics
VrijeUniversiteitBrussel, Pleinlaan 21050 Brussel, Belgium
Tel: 32-2629-2739 E-mail: pclarys@vub.ac.be

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Abstract

This study aimed to assess micronutrient intake and supplement use in adolescent sprint athletes. Anthropometrics, micronutrient intake and supplement use of 60 adolescent sprint athletes were recorded every six months over a 3-year period. Age at start was 14.8 ± 1.6 years for the female and 14.7 ± 1.9 years for the male athletes. Over the 3 years, both girls and boys normally gained in body height and - weight. Consistent low intakes were observed for iron in girls, and for calcium, vitamin E and B2 in both sexes, whilst sodium intake exceeded the upper reference limit. Supplements were used without prescription and athletes with the better nutritional profile showed higher supplement use compared to those with poorer habits. General non-stringent advice for dietary improvement resulted in significantly favourable changes only for the consumption of wholegrain bread, vegetables, and soft drinks. Micronutrient intakes of adolescent sprint athletes are relatively stable and not always according the guidelines, regardless of supplement use. Repeated nutritional feedback induced only moderate improvements.

Keywords: Adolescents, Micronutrients, Supplements, Sprint athletes

1. Introduction

Pubertal growth and development increase the need for energy and nutrients. For adolescent athletes, extra care should be given to their diet, since the needs of their physical activities are added to their nutritional needs for

growth and development (Petrie, Stover, &Horswill, 2004).

Since literature on the nutritional profile in adolescent sprint athletes is scarce, there is a need for more research on the nutritional requirements of young athletes in specific disciplines (Meyer, O'Connor, & Shirrefs, 2007; Tipton, Jeukendrup, & Hespel, 2007). Dietary guidelines for athletes focus primarily on energy, macronutrient and fluid intake. Less attention goes to adequate intakes of micronutrients. Nonetheless, minerals, trace elements and vitamins facilitate the use of macronutrients for physiologic processes and they are involved in homeostasis, nerve conduction, muscle contraction, and some have antioxidant capacities (Lukaski, 2004; Kreider et al., 2010).

As is the case for energy and macronutrients, adolescent sprint athletes probably have additional needs for micronutrients as compared to their non-athletic peers. In adolescents involved in different sports, inadequate intakes for fluid, fibre, iron, calcium, potassium, magnesium, folate, vitamin A, D and E have been reported (Ziegler et al., 1998; Garrido, Webster, & Chamorro, 2007; Aerenhouts, Hebbelinck, Poortmans, & Clarys, 2008; de Sousa, Da Costa, Nogueira, & Vivaldi, 2008; Juzwiak, Amancio, Vitalle, Pinheiro, & Szejnfeld, 2008; Kabasakalis et al., 2009).

Supplementation of vitamins and minerals is very common amongst athletes in an attempt to maintain health, to optimize performance and recovery and to compensate for an imbalanced diet (Dorsch & Bell, 2005; Maughan, Depiesse, & Geyer, 2007). Athletic trainers appear to have the greatest influence on nutritional and supplementation practices of athletes (Burns, Schiller, Merrick, & Wolf, 2004; Nieper, 2005). However, improper dietary habits and supplement use is often observed in combination. In most of the research on dietary habits of athletes it is concluded that proper monitoring of the diet is highly recommended and that the individual athlete should receive adequate nutritional information. Consumption of micronutrient-rich foods such as wholegrain foods, fruit and vegetables is very important (Lukaski, 2004). In general, for maintaining and optimizing health and performance, adolescent athletes should be advised to consume a well-balanced diet containing a variety of foods in sufficient amounts to meet energy demands (Meyer et al., 2007; Kreider et al., 2010). Dietary supplements should only be advised when a food-based solution is not available (Maughan et al., 2007; Kreider et al., 2010).

It was the aim of the present study to estimate intakes of micronutrients and supplements in adolescent sprint athletes over a period of 3 years. In addition, the study protocol allows an evaluation of dietary habits between seasons and throughout a 3-year period during adolescence.

2. Methods

2.1 Participants

Based on the Flemish Athletics League rankings, 120 sprint athletes aged 12 to 18 years were selected and invited to participate in a 3-year follow-up study on sprint start performance, physical parameters and nutrition. Due to logistical limitations, only 60 of the 76 responders were retained (29 girls and 31 boys, age 14.8 ± 1.6 years and 14.7 ± 1.9 years respectively). Participating athletes and their parents were given detailed information about the study. They were asked to give their written informed consent, in accordance with requirements of the university's ethical committee.

2.2 Food intake

A 7-day food record was administered in both the spring and autumn of 2006, 2007 and 2008. The participants were clearly instructed to maintain their normal eating patterns and to report all foods as accurately as possible by time of the day, portion size, preparation and composition of foods. For portion sizes they were asked to weigh the items. When this was impossible, household measures were used to provide an estimate of the portion size (Health council, 2009). The participants were also asked to weigh themselves before breakfast of day 1 when they started recording as well as after day 7. Within 2 weeks of completion, the record was checked by the investigator in the presence of the athlete and at least one of his or her parents. At that moment, extra information was obtained where necessary. Analysis of the food records was done by the same investigator using the Becel nutrition software program BINS 3.0.1, based on the Belgian (NUBEL 2004) and Dutch (NEVO 2001) food composition databanks. Since the Recommended Dietary Intake (RDI) for micronutrients is gender and age specific, micronutrient intakes are represented as a percentage of this RDI ((intake/RDI) x 100). Due to incompleteness of the food data banks used, only a selection of micronutrients (sodium, potassium, calcium, phosphorus, iron, magnesium, retinol equivalents (RE), and vitamins E, B1, B2, B12 and C) are discussed in the present study. Vitamin A (retinol) and beta-carotene intakes were used in the calculation of retinol equivalents (RE (μg) = retinol (μg) + beta-carotene/12 (μg)). Dietary supplements were taken into account after dietary

analysis. Supplements were defined as products delivering extra micronutrients or amino acids, creatine or having an alkalizing effect. Sports drinks, energy bars and homeopathic products were not considered as a supplement. Results and non-stringent advice for improvement of the diet regarding fluid intake, wholegrain instead of refined foods, fruit and vegetable consumption were fed back to each individual by e-mail.

2.3 Anthropometry

During the same visit when handing over the food records, anthropometric data were collected. Standing height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Weight was measured with the TANITA-TBF 410 weighing scale to 100 g, and body composition was estimated by underwater weighing densitometry using the formula of Siri (1961). Basal energy expenditure (BEE) was calculated using the Institute of Medicine equation (2005). A ratio of 1.1 between total energy intake and BEE was used as a tool for detecting underreporting (Goldberg et al., 1991). Criteria for not including records for analysis were underreporting and reporting less than 7 days.

2.4 Statistical analysis

Statistical analysis was performed with SPSS 17.0. The Kolmogorov-Smirnov test was used to test for normal distribution of the data. A repeated-measures ANOVA followed by a paired t –test with Bonferroni correction were applied to compare nutrient and food intakes between occasions. In the case of non-parametric data, the Kruskal-Wallis test and Mann-Whitney U test were applied. Descriptive statistics on physical characteristics and number of participants using food supplements was done on all accepted data per occasion. Micronutrient intakes of girls and boys were compared to the recommendations of the Belgian Health Council (2009) with a Student's t-test. The significance level was set at $p < 0.05$.

3. Results

The total numbers of records and rejected records on each occasion, as well as age and anthropometric data have been published elsewhere (Aerenhouts, Deriemaeker, Hebbelinck, & Clarys, 2011). Self-measured body weight at start of the week of recording was not statistically different from on the day after, except in boys on occasion 5 (66.9 ± 8.1 kg vs. 66.6 ± 8.1 kg, $p = 0.024$).

Intakes of vitamins and minerals as a percentage of the RDI are presented in figures 1 and 2, respectively. Table 1 shows the percentage of athletes reaching the RDI, as well as the ratio: number of supplementing athletes / number of these athletes who already reached the RDI through their diet.

On the first occasion, one male subject took an essential amino acid supplement and on the final occasion another one supplemented with creatine. On all other occasions only vitamin and mineral supplements, often in combination, were taken. The number of girls and boys taking supplements was comparable. For both sexes per occasion, 15%, 34%, 35%, 28%, 27% and 15% of the athletes supplemented one or more micronutrients. As shown in Table 1, athletes taking supplements had in many cases already sufficient intake through the diet alone (for example vitamin C and magnesium) whilst athletes with dietary shortages often did not take supplements (for example iron in girls and calcium). One girl supplemented micronutrients on prescription after being diagnosed with low iron blood concentration (occasions 2, 3, 4, 5).

(Figure 1)

(Figure 2)

(Table 1)

Consumption of bread, fruit and vegetables is illustrated in Aerenhouts et al. (2011). The quantity of white bread consumed by both girls and boys did not change over the 3 years, as did the quantity of wholegrain bread for boys only. Girls ate more wholegrain bread on occasions 4, 5, and 6 than on occasion 1 ($p = 0.001$, $p = 0.010$, and $p = 0.003$) and more on occasion 4 compared with occasions 2 and 3 ($p = 0.012$ and $p = 0.002$).

In both sexes, no significant change in fruit consumption was observed and there was no difference between girls and boys. The reference dietary intake for fruit of 250 g per day was achieved by 13%, 25%, 36%, 28%, 32%, and 28% of girls, and by 29%, 19%, 15%, 20%, 31%, and 27% of boys on occasions 1–6. Mean daily vegetable consumption was similar between girls and boys during the study, but vegetable intake significantly increased during the study period for boys only. In boys, more vegetables were consumed on occasions 3, 5, and 6 than on occasion 1 ($p = 0.004$, $p < 0.001$, and $p = .001$), as well as on occasion 5 compared with occasion 2 ($p = 0.003$). None of the girls reached the reference intake for vegetables of 300 g per day on occasion 1–4 and only one on occasions 5 and 6, while none of the boys reached the reference dietary intake on occasions 1 and 4, only one boy on occasions 3, 5, and 6, and two boys on occasion 2.

4. Discussion

This follow-up study provided a clear picture of adolescent's dietary habits with respect to micronutrient and supplement intake in combination with their physical development over a 3-year period. Since the first aim of this study was the estimation of dietary habits among these athletes we deliberately did not intervene directly in the diet, but we did provide limited and non-stringent nutritional feedback and information to the athletes and their parents.

Due to a lack of specific reference data, micronutrient intakes of the athletes in this study were compared to the age and gender dependent RDI for non-athletic adolescents (Health council, 2009). Although the self-measured body weights at start and at the end of the recording weeks were stable, observed shortages should be interpreted with caution since dietary underreporting is commonly observed, also in highly motivated athletes (Burke, 2001). True nutrient shortages can only be revealed by using biomarkers (Jenab, Slimani, Bictash, Ferrari, & Bingham, 2009), which were not used in this study.

High standard deviation scores of food and micronutrient intakes indicate a high variability in intake of certain micronutrient-rich foods among these athletes. However, mean intake values over the 3-year period show a stable dietary intake with only little improvement despite repeated, but non-stringent, dietary feedback. Girls had significant higher vitamin B1 intakes on occasion 4 and 5 as compared to the first occasion. This might be explained by the simultaneous change observed in wholegrain bread consumption since wholegrain foods are rich in vitamin B1. Sufficient intakes as compared to the RDI for Belgian adolescents were observed for RE, vitamins B1, B12 and C and for potassium, phosphorus, magnesium and in boys only, iron. Only a limited number of girls and boys reached the RDI values for vitamin B2, vitamin E, calcium, and in girls only, iron. Dietary shortages of these micronutrients can have consequences for both health and performance (Lukaski, 2004; Kreider et al., 2010). Severe vitamin E deficiency increases oxidative stress in the muscle, causing degradation and inflammatory processes leading to muscle dystrophy. Vitamin B2 is involved in glucose metabolism during exercise and a shortage can therefore have an immediate impact on the performance level of these athletes. Calcium is in first place necessary for bone acquisition and the needs for calcium reach maximal levels during puberty. Considering the physical activities characterized by high impacts these athletes are involved in, optimal bone density can be expected if combined with an adequate calcium intake (Vicente-Rodriguez et al., 2008). Low iron intakes in girls are a matter of concern, since low iron stores is one of the most prevalent nutrient deficiencies observed among female athletes. Iron deficiency can impair muscle function and limit work capacity.

High intakes of sodium were observed. However, these athletes may have elevated sodium needs because of elevated sweat losses.

Dietary shortages of vitamin B2, vitamin E, calcium and iron were earlier observed in other studies on children and adolescent athletes. On the other hand, most sprint athletes in this study had sufficient intakes for vitamin A, magnesium and potassium which is in contrast with other reports (Ziegler et al., 1998; Aerenhouts et al., 2007; Garrido et al., 2007; de Sousa et al., 2008; Juzwiak et al., 2008; Kabasakalis et al., 2009).

Supplement use was observed in athletes regardless their dietary intake. Micronutrient intakes that where below the RDI as well as micronutrient intakes that reached the RDI were supplemented by a comparable number of athletes. Apparently, athletes with concern about sufficient micronutrient intakes who took dietary supplements met already the RDI through the diet alone. On the other hand, otherathletes with dietary shortages were not aware of this deficiency or its possible consequence since these were not taking any supplement. This can indicate for subgroups within this sample of athletes with higher and on the other hand lower concern for sufficient nutrient intakes. Higher supplement use in more health conscious people was earlier observed (Stang, Story, Harnack, & Neumark-Sztainer, 2000; Rock, 2007; Mullie et al., 2009). In the present study, supplements were taken without consulting a physician or dietician, with one exception. This is a matter of concern since overuse of micronutrients can be potentially harmful for health (Maughan et al., 2007; Rock, 2007; Kreider et al., 2010). A review by Bjelakovic, Nikolova, Gluud, Simonetti, & Gluud (2007) pointed out that overuse of beta-carotene and vitamin A and E, singly or combined, significantly increases mortality. Still, compared to other findings on supplement use by track and field athletes, relatively few (<40%) athletes in this study took supplements. Around 80% of the sprint athletes competing at international championships from 2005 to 2007 reported to use supplements (Maughan et al., 2007). The definition of supplements (including energy bars and sports drinks or not) as well as a different performance level and age could explain this difference.

The important role of athletic coaches, more than dieticians and physicians, in providing nutritional information has been shown (Burns et al., 2004, Nieper, 2004). Therefore, there is an urge for well-educated coaches in

collaboration with dieticians and doctors in order to provide an adequate nutritional support for athletes. The use of biomarkers should provide the decisive answer in whether advising supplements or not.

Shortages of micronutrients may be related to the low consumption of wholegrain foods and cereals, fruits and vegetables. These foods are highly necessary in an athlete's diet since they contain indispensAble fibre, micronutrients and antioxidants. This information was shared with the athletes. Although there was a trend towards a higher fruit intake, there were no significant changes throughout the study period. For vegetables, significant but insufficient improvements were observed since practically all participants remained far below the recommended 300g/day on all occasions. A higher consumption of fruit and vegetables will be crucial also after the athletics career to maintain health at older ages. Indeed, it has been suggested that these foods help prevent cancer, cardio-vascular and metabolic diseases (Craig & Mangels, 2009).

No seasonal influences on intakes of selected foods and micronutrients were observed. A positive but rather modest evolution over the 3 years was observed only for consumption of vegetables and wholegrain bread. Other studies on dietary habits of adolescents (Löwik et al., 1994; Alexy, Sichert-Hellert, & Kersting, 2002) show that dietary changes occur gradually. These changes are mainly influenced by commercial advertising, mass communication and the food industry itself (Nicklas, Webber, Srinivasan, & Berenson, 1993). Therefore, observed changes in the diet of these sprint athletes may also be attributed to other sources.

This study shows that the micronutrient intake of adolescent sprint athletes is relatively stable. A higher consumption of micronutrient-dense foods offers a dietary based solution to reach the RDI for nutrients with shortages. Dietary supplementation should be done with more care and only after acquiring qualified advice. It appears to be difficult to improve dietary habits solely based on providing nutritional information.

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Table 1. Percentage of girls and boys reaching the RDI for micronutrients, followed by the ratio:number of participants supplementing/ number of participants supplementing with dietary shortage

	Spring '06	Autumn '06	Spring '07	Autumn '07	Spring '08	Autumn '08
RE (µg) ♀	67%, 1/1	79%, 2/0	72%, 1/0	84%, 3/0	64%, 1/0	72%, 2/0
♂	62%, 3/1	88%, 4/0	65%, 8/1	72%, 5/1	73%, 3/0	95%, 2/0
vit E (mg) ♀	37%, 2/2	21%, 3/3	12%, 1/1	24%, 4/1	45%, 3/2	50%, 2/0
♂	42%, 3/2	50%, 5/4	46%, 8/5	48%, 5/3	58%, 3/2	36%, 2/2
vit B1 (mg) ♀	96%, 1/0	96%, 3/0	92%, 1/0	92%, 4/0	91%, 4/1	94%, 1/0
♂	83%, 3/1	87%, 5/1	88%, 8/1	92%, 5/0	88%, 3/0	91%, 2/0
vit B2 (mg)♀	71%, 1/0	62%, 3/1	72%, 1/0	76%, 4/1	77%, 4/2	72%, 1/0
♂	54%, 3/1	46%, 5/3	61%, 8/2	60%, 5/2	50%, 3/3	59%, 2/1
vit B12 (µg) ♀	100%, 2/0	96%, 4/0	88%, 2/1	92%, 3/0	95%, 4/0	94%, 1/0
♂	100%, 3/0	100%, 4/0	100%, 8/0	96%, 5/0	96%, 3/0	100%, 2/0
vit C (mg) ♀	83%, 2/0	83%, 6/0	80%, 4/1	80%, 4/0	86%, 3/1	83%, 2/2
♂	79%, 3/0	81%, 6/2	92%, 9/0	72%, 8/2	88%, 4/0	91%, 2/2
Na (mg) ♀	0%, 0/0	0%, 0/0	0%, 0/0	0%, 0/0	0%, 0/0	0%, 0/0
♂	0%, 0/0	0%, 0/0	0%, 0/0	0%, 0/0	0%, 0/0	0%, 0/0
K (mg) ♀	96%, 0/0	100%, 0/0	100%, 0/0	100%, 0/0	91%, 1/1	94%, 0/0
♂	100%, 0/0	96%, 1/1	100%, 3/0	100%, 0/0	100%, 0/0	100%, 1/0
Ca (mg) ♀	12%, 1/1	17%, 1/0	12%, 0/0	8%, 1/1	14%, 1/1	11%, 1/1
♂	12%, 0/0	23%, 3/3	35%, 6/4	24%, 2/2	27%, 2/2	9%, 2/2
P (mg) ♀	100%, 1/0	75%, 1/0	96%, 0/0	96%, 1/0	95%, 0/0	89%, 1/0
♂	100%, 0/0	77%, 1/1	100%, 1/0	96%, 2/0	100%, 2/0	100%, 1/0
Fe (mg) ♀	0%, 2/2	4%, 3/3	4%, 4/4	4%, 5/4	0%, 5/5	0%, 2/2
♂	67%, 1/0	77%, 4/1	81%, 6/1	72%, 5/2	77%, 3/1	82%, 2/0
Mg (mg)♀	79%, 1/0	75%, 3/0	72%, 2/0	84%, 4/0	86%, 3/0	78%, 1/0
♂	92%, 2/1	92%, 5/0	92%, 8/0	72%, 4/1	92%, 4/0	77%, 2/0

RE: Retinol Equivalent.

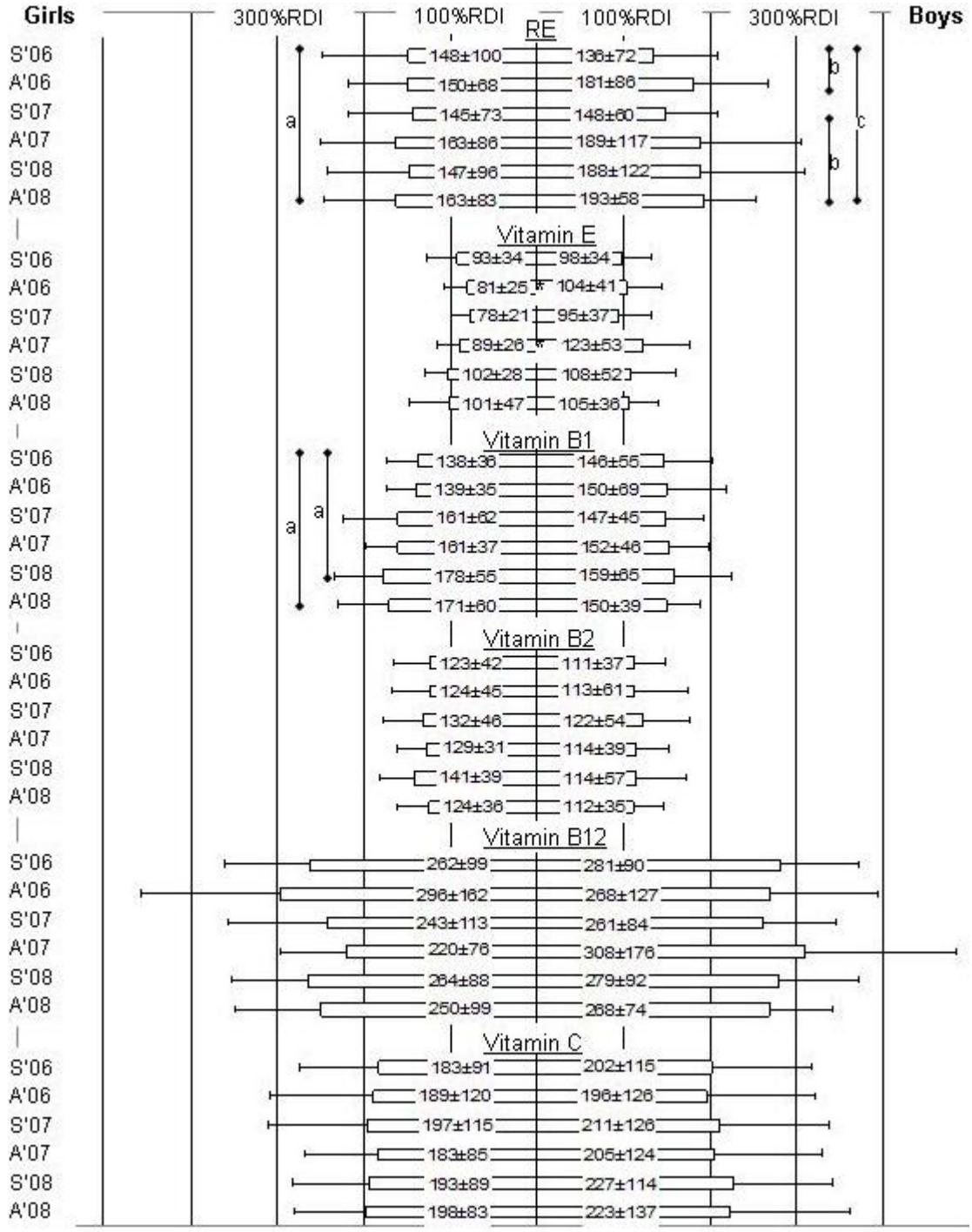

Figure1. Mean dietary vitamin intake as a percentage of the RDI (Health Council, 2009)

S: Spring period, A: Autumn period. RE: Retinol Equivalent. a, b, c: difference between occasions at $p < .05, .01, .001$, respectively. *: difference between girls and boys at $p < .05$.

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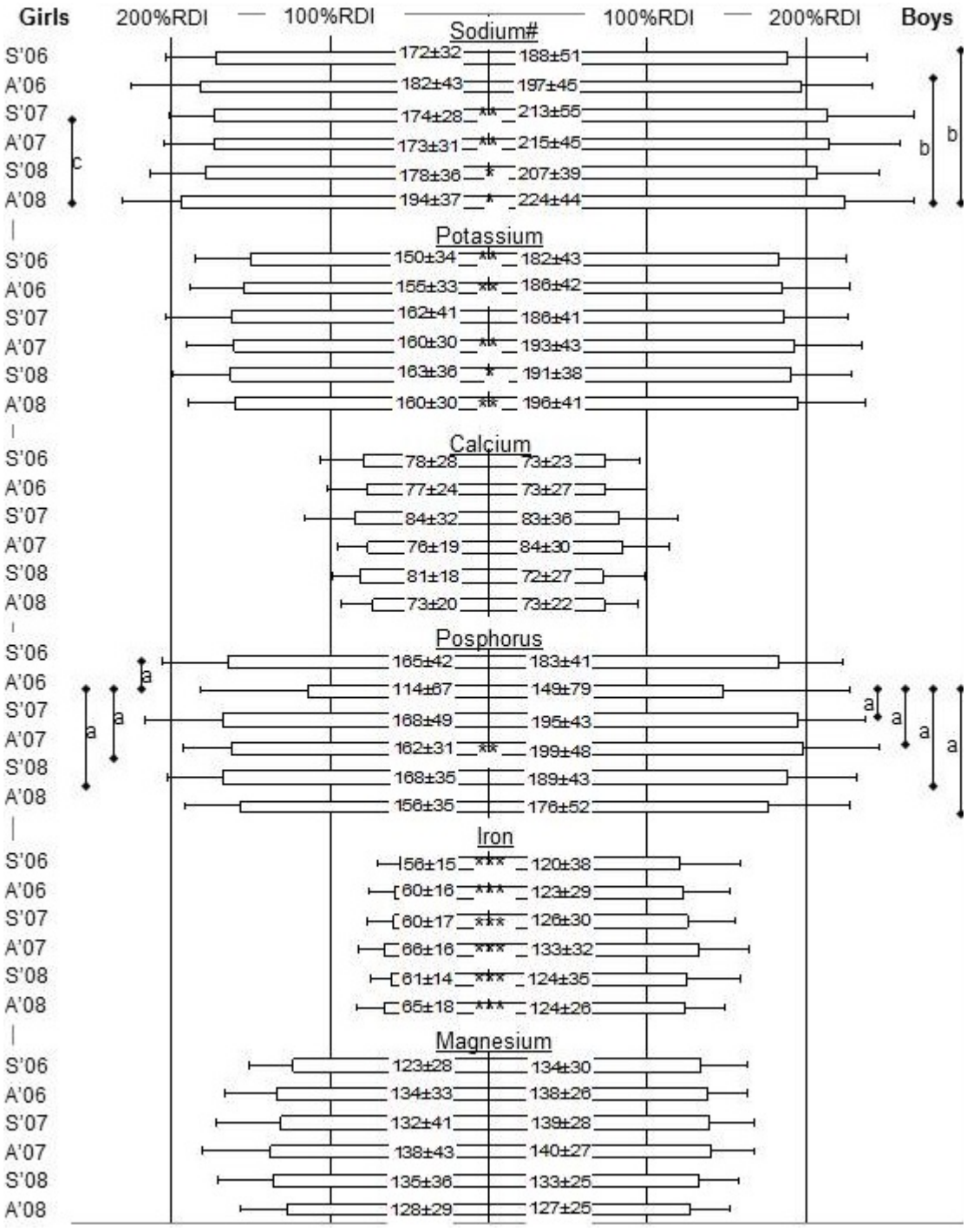

Figure 2. Mean dietary mineral intake as a percentage of the RDI (Health Council, 2009)

S: Spring period, A: Autumn period. #: 100% = upper RDI limit. a, b, c: difference between occasions at $p < .05, .01, .001$, respectively. *, **, ***: difference between girls and boys at $p < .05, .01, .001$, respectively.

Global Food Crisis: Public Capital Expenditure and Agricultural Output in Nigeria

Suleiman G. Purokayo (Corresponding author) & Aminu Umaru
Department of Economics, Federal University of Technology, Yola
E-mail: suleimangambiyo@ymail.com

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Abstract

The focus of this paper is to examine the effects of global food crisis on developing countries which most times are considered most vulnerable due to a number of factors ranging from conflicts in the region, to poverty and inequality, and factors that affect policies on agriculture. Issues concerning the use of food as alternative energy sources are still in its infant stages in Africa, and may not be relevant. The paper investigates the impact of capital expenditure on agriculture and credit to agricultural sector on the output of agriculture in Nigeria. Annual data covering 1990 – 2004 were used. Unit roots of the series were examined using Augmented Dickey – Fuller techniques. The overall results indicate that output of agriculture is positively related to capital expenditure on the sector but negatively related to the credit to agriculture. The paper therefore recommends improvements in government capital expenditure on agriculture. Credit policies and institutions should target beneficiaries (rural farmers).

Keywords: Agricultural output, Government policies, Access to credit, Capital expenditure

1. Introduction

Growing food shortfalls in the global economy had introduced high degree vulnerabilities for Africa and Asia. Global food crisis have become a worldwide phenomenon and its effects are felt all over the world. Food shortages have been attributed to various factors ranging from conflicts around the world, flooding, droughts in Africa, new uses of some crops for energy sources, the biofuels, and the growing needs for ethanol as a source of energy. Other reasons attributed are, environmental factors due to the changing climates around the globe, and growing fuel prices which oil speculators believe will hit $300 bench mark before the end of 2008. This affects transport systems, with chain effects on food prices.

Global food crisis has sparked riots around the world; even the developed economies had experienced such riots for example; the strike of heavy vehicles drivers around the world in June 2008 caused by increasing oil prices. In Africa, the situations have reached its high points with riots in most countries of Africa. A report by Peace and Freedom (2008) state how governments and organization react to the growing food crisis: "…….after Vietnam won the Philippine contract by under pricing the world's number one rice exporter, Thailand, a few things happened. The communist government of Vietnam said that the Philippines deal would not commence until at least June, in order to give the government time to distribute rice at home and to assess rice stocks and possible reserves." It also reported that in Bangladesh the army had to be called out to protect the areas where rotten rice is dumped. The poor were savaging and eating rice soaked and rotting.

The situation in Africa calls for urgent concerns due to high costs of foods in the continent. Africa seems to be shifting or detracted by this trend to earmarking huge amounts of the budgets for food and agricultural production rather than growing needs for provisions of much needed infrastructure – good roads, energy needs and recent focus of governments in Africa for empowering the poor and the desired level of growth of the economies ravaged by conflicts and increased poverty levels of the population.

Changes in the population and growth in the developing world has also raised concerns for the short falls in food supply. Population dynamics as defined by Turchin 2001, "the study of how and why population numbers change in time and space'. This is important because changes may bring about positives, in terms of raising a dynamic manpower for countries desiring to belong to the so called 'club' of developed and powerful nations – group of 8 industrialized nations, world nuclear power nations, and so on. The trends in population changes may bring about

socio-political problems, economic-related problems, relating to growth goals and other dynamic aspects. Abrupt and seemingly inexplicable changes in population numbers have fascinated and puzzled humanity from prehistoric times. This growth also defines the productivity of the population coupled with some auxiliary factors like standards of living, the environment and so on.

Food crisis also defines food security of nations. According to the Food and Agriculture Organization (FAO 2003) of the United Nations, "Food security refers to the availability of food and one's access to it. A household is considered food secure when its occupants do not live in hunger or fear of starvation. Debates around the world which seems to link current food crisis to growing need for alternative energy and current hikes in fuel prices has increased debates for food vs fuel. This is the dilemma regarding the risk of diverting farmland or crops for biofuels production in detriment of the food supply on a global scale. Biofuel production has increased in recent years. Some commodities like maize, sugar cane, or vegetable oil can be used either as food or to make biofuels. For example, since 2006, land that was also formerly used to grow other crops in the United States is now used to grow maize for biofuels, and a larger share of maize is destined to ethanol production, reaching 25% in 2007. With global demand for biofuels on the increase due to the oil price increases taking place since 2003, there is also fear of the potential destruction of natural habitats. Environmental groups have raised concerns about this trade-off for several years, but now the debate reached a global scale due to the 2007–2008 world food price crisis.

Global food crisis has called attention of world leaders on the urgency for a global partnership in fighting this crisis. According to report by Reuters of the Rome summit, it has been described as a global crisis pushing 100 million people into hunger, threatening to stoke social and political turmoil and set the fight against world poverty back by seven years. "Now, the food price crisis will be tackled by world leaders who meet in Rome to seek ways of reducing the suffering for the world's poorest people and ensure the Earth can produce more food to sustain an ever growing population. This also echoed the concerns of Jacques Diouf, the head of the United Nations Food and Agriculture Organization (FAO) who called the summit late last year before the full extent of the food price crisis was clear. Also, World Bank President Robert Zoellick underlined the urgency of the problem, announcing $1.2 billion in loans and grant financing for countries struggling with food and fuel costs. At the Rome food summit (June 15, 2008), the position paper was: "*The reasons for food insecurity are mainly to be found in social and economic factors such as poverty and inequality."* There are reports that suggest the way forward in the current food crisis. Many such suggestions have been advocated, also adding their voices to the World food summit in Rome, June, 2008. An outlook by the Wall Street Journal written by Bob Davies, suggest four ways, boast research on improving farms yields, invest more in irrigation and rural transportation, power African farms with solar and wind energy and produce biofuels with inedible vegetation, instead of corn.

2. Literature

Literature in agriculture and related fields are enormous. There is 'heavy traffic' in this field - studies done by International bodies/institutions like FAO, governments - DFID,USAID that have sponsored researches to assist and develop agricultural sectors in developing countries. Researchers are engaged in researches in various aspects of agricultural productivity. This study builds a simple model for examining how capital expenditure (public expenditure), access to credit effects on output and related fields. This is the contribution of this paper.

The conceptual basis for measuring agricultural output in Nigeria has all times being traced to structural changes of the Nigerian economy - the discovery of oil in the 1970s. Agriculture constitutes the springboard for the growth of the economy and the sources for foreign exchange budget before the advent of oil. According to Obadan, (1983), "agriculture accounted for 97.3 percent of the total share of export in Nigeria." .Nigeria's fortune in the new found wealth affected the agriculture sector from 1973. Continuous decline of agriculture despite huge investments in the sector – establishment of river Basins and rural development, Agricultural Development Programmes, ADP (funded jointly by the World Banks, and Federal, state Governments and the Agricultural Accelerated Programmes, NAFPP), and more than 20 Agricultural Research Institutions, seems to have compounded the rise in oil revenue. This was the beginning of Nigeria declining from a position food exporter to net importer of food.

The principal constraint to the growth of the agricultural sector is the fact that the structure and method of production have remained the same since independence more than four decades ago - because some minor innovations in agricultural investment were not sustained over the years. The United Nations Food and Agriculture Organization rate the productivity of Nigeria's farmland as low to medium— but with medium to good productivity if properly managed by the country. To be effective, and attain higher level of productivity and growth in the agricultural sector there is a need to identify the major factors that determine its growth. This study

will examine agricultural output and capital expenditure using a model to identify mainly credit availability and how capital expenditure had impacted on output.

An important fact in Nigerian Agriculture, and indeed Africa, is still the fact that most of the literature shows that agriculture growth in Africa remains fundamental to growth and poverty reduction on the continent. "A one percent increase in crop yield reduces the numbers living in under dollar one per day poverty by six and a quarter million, with *95* percent of these in Africa and Asia". (Thirtle, Lin and Piesse 2003). This statement is always a case in point due to some of the following:

a). The problems associated with the land use policies that had results to communal conflicts

b). The prevalence of HIV/AIDS that affected the labour force participated rate – (see Abdulazeez Abubarkar et al, 2008)**,** and many other literature on HIV/AIDS Scourge in Africa.

c). Conflict zones in Africa which has created an increase in refugees.

d).Climate related problems in Africa and Asia as opined by J.W. Knox, et al (2011).

The global food crisis had therefore made the existing situation (shortfalls experienced in the Africa and the rest of the world) grave from a point view of a cross-country data and reports. Some of the arguments are the fact that the growing trends in food situations shows the complex nature of the demand for and supply of food. Most literature had focused on Agriculture polices, arguments about alternative uses of foods, fuel propelled arguments for soaring food prices, Poverty and inequality arguments, which features in most literature and points to Africans and Asia situation. It defines vulnerability of these groups in most the development literature, it is generally used relative to poverty, and is defined as the *ex ante* risk that non-poor households will fall below the poverty line and poor households will remain poor (Tesliuc and Lindert, 2002 citing Holzmann, 2001). Defining vulnerability relative to both current and potential future poverty is important from a policy perspective, insofar as poverty is a stochastic phenomenon (Chaudhuri, 2003). In other words, even if poverty rates stay the same, the poor of today may not be the poor of tomorrow, and conversely, the non-poor of today may be the poor of tomorrow.

This study had made a survey of some related literature on the following which have important implications and are critical in aggravating food situations in many economies**:** (The authors have referred to these reports as basis for myriad problems which sometimes had formed literature and important references in food problem/crisis – for further research in salient aspects like the financial market conditions and commodity speculator activities who are sometimes encouraged to operate under corrupt regimes)

2.1 Agricultural policies

There are many reasons why the economy of Africa should grow sufficient food to feed it growing population. Firstly, most of Africa is rural with arable land, except the Sahel regions, coupled with short rainfalls most yields in Africa depended on rain fed. Secondly, bulk of the population in Africa practice some form of agriculture and thirdly, mostly governments in the regions of Africa commit large portions of their budget to the agricultural sector. Where did the Africans miss it?

Current global development policy, the Millennium Development Goals (MDGs), outlined the importance of the agricultural sector. Goal number one, Eradicate extreme poverty and hunger is a great commitment for the growth of the sector. According to World Food Programe (WFP), 75 million people need to be lifted out of poverty in Africa.

Problems have always been levels of commitments of policies in the sector over the years. In Nigeria for instance, the current developmental focus (the 7-point agenda of the regime) places Food security as the topmost points to achieve. Also, backed up by the NEEDs programme and other policies to increase output in the agricultural sector in Nigeria, the microfinance policies, the Agricultural Credit Guarantee Schemes other programmes that provide periodic large funds released in assisting the sector (especially during the Obasango regime in Nigeria), Rural Financing Projects that provide credit facilities for small farmers, development of existing Dams /river basins

(i) Anambra – Imo River Basin

(ii) Benin Owena River Basin

(iii) Chad River Basin

(iv) Hadeja-Jamaare River basin

(v) Cross River Basin

(vi) Lower Benue River Basin

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(vii) Lower Niger River Basin

(viii) Niger-Delta Basin

(ix) Ogun-Osun Basin

(x) Upper Benue River Basin

(xi) Upper Niger River Basin

(xii) Sokoto-Rima River Basin

The Development of the Dams is to encourage an all-year-round farming. Some of agricultural activities (irrigation) have been successful, but most of these Dam projects have remained big liabilities to government in terms of maintenance.

One of the greatest obstacles to increase output in the agricultural sector has been in infrastructure development and inputs (fertilizer) required , and since most farmers depend on rain fed crops, there is need for developing quick yielding crops especially in Northern parts of Nigeria and countries in the Sahel that experience low rainfall in the rainy season. Recent meeting with top managers of the country only took measures for the increase of food through imports in the short run. Long run measure is to cooperate between Agriculture and Water ministry. This is a wait and see situation in the current position of food crisis in Nigeria.

2.2 Food vs. biofuel arguments (Agflation)

The rise in food prices has generated a lot of debates overt who the villains are. But there are reports about the rising cost of oil which calls for alternative energy sources. This had led to demand for foods used as energy – biofuels and ethanol which are renewable fuels. According to IRIN's reports by the Assistant Director-General of the UN Food and Agriculture Organization (FAO).

(Figure 1)

"Various studies and think-tanks have come up with estimates of the impact of biofuel on food prices that range from 10 percent to 60 percent." From the graph shown above, rise in food prices is connected to rise in the use of food as source of energy (The so called Agflation). It shows a sharp increase in food prices as a result of increasing use of food for fuel. Supporters of this campaign blame corn-based ethanol and government fuel policies, including other related factors are responsible in hiking prices of food. Most supporters believe that the food –to-fuel policies led to expanded corn planting, which is responsible for changing land use around the world that threatens crucial ecosystem and at the same time, rising demand for corn set off global food price increase, and threatening nations in riots, deeper into poverty and other vices especially in poor countries.

For developing countries that have not experienced use of food for alternative energy sources, the current rise in fuel prices may force governments to a rethink of policies for devoting resources towards the production of foods use for fuel, especially maize and so on.

A report by IRIN (2008); Africa shows signs of increasing African governments considering alternative source of fuel in Congo. "We are working to develop a balance within agriculture in land use between land reserved for food cultivation and land reserved for biofuels," Congolese President Denis Sassou Nguesso said a few days earlier after returning from Rome where he took part in a high-level UN Food and Agriculture Organization (FAO) summit on food security, climate change and biofuels." "The Congo government has therefore set aside 8.2 million hectares of land". According the reports, Congo has serious food shortages.African governments should not be drawn into the production of food use for fuel because of the 850 million people in the world suffering from hunger; some 820 million people are living in the developing countries according FAO. All lands should be used to grow foods in Africa.

2.3 Fuel prices propelled factors

The rising fuel prices have affected the global economy, and especially the real sector. The phenomenon of rise in the price of oil (petroleum products) had propelled a rise in prices worldwide boosted energy profits, but crushed the economy. This trend had contributed to hikes in the prices of foods around the world.

Oil also provides most energy for mechanized food production and transport. Higher prices for liquid fuels from petroleum increase the demand for biofuels which may result in diverting some crops from food to energy. Even though per-capita petroleum consumption among the world's poorest people is very low, what petroleum the poor do consume is disproportionately in the form of fossil fuel inputs to the food they eat, especially to any food imported from industrial agriculture powerhouses such as the United States. People who were already living at a

subsistence level when oil was relatively cheap are extremely vulnerable when oil prices rise, and may simply lack the means to afford enough daily food calories to survive.

In the developing nations for instance, higher fuel prices are glaringly notice to have affects on transports of people and goods. Changes in prices are to a certain extent mostly tied to availability of the commodity (petrol). Periodically in Nigeria, petrol plays a big factor in price hikes. If transport fares increased it immediately have effects on the prices of foods around the markets in Nigeria, and especially in the northern part of Nigeria. Arguments are; not availability of any commodity, but prices increase excludes many from consuming a particular good or service.

2.4. Poverty and inequality arguments.

Poverty and inequality has been a big issue in the Millennium Development Goals (MDGs) for Africa and Asia. There are growing concerns that these regions are ranked to be surviving on less than a dollar. Incidence of poverty is high in these regions. There are many factors attributed to this phenomenon, It range from quality of government interventions in agricultural policies (in terms of effective monitoring of issues like farm input distribution, poverty related programmes and income distribution amongst other). That means tackling poverty and the power imbalances that underpin it.

The number of people in sub-Saharan Africa who subsist on less than a dollar a day has almost doubled since 1981, to 313 million people in 2001, representing 46 per cent of the population. Most of Africa's poor and undernourished people live in rural areas. Smallholders, nomadic pastoralists, and women are particularly vulnerable to hunger due to marginalization and neglect. The joint effort promised by African governments and donor governments to eradicate poverty must therefore deliver rural policies that involve and prioritize these vulnerable groups. Even small improvements in what they produce and earn will have a major impact in reducing hunger, as well as driving equitable growth. This should be determined country-by-country, through consultation between governments, civil society and donors, and agricultural producers themselves. However, a key ingredient must be proper investment in long-term rural development programmes and infrastructure, including support for organizations that represent the voices of marginalized groups. African governments have committed themselves to increase their spending on the rural sector to 10 per cent of their budgets. This should be backed by greater external assistance financed from the recent G8 commitments to increase development aid and debt relief.

An important lesson from the flawed market reforms introduced from the 1980s by the International Monetary Fund and World Bank, backed by the major donors, is that rural markets on their own cannot deliver food security. State action is also needed. There is growing evidence, for example, that government policies to stabilise prices, to provide cash transfers or targeted agricultural inputs, can be a more timely and cost-effective way (than emergency food aid) of ensuring food security.

The international community must act to stabilise the volatile commodity prices which create such hardship for African producers. The rich-country trading blocs must stop forcing open African markets for their own benefit, and end the dumping of their subsidized farm produce.

2.5 Current credit crunch arguments (financial market conditions)

Credit crisis has introduced liquidity crisis in financial institutions and the ability or inability to create credits for users. According to a report on the wall street journal (2008), "global surge in food and energy prices is being driven primarily by fundamental market conditions, rather than an investment bubble, say the majority of economists in the latest Wall Street Journal forecasting survey"

Credit conditions have consequences on borrowers. Even though economists are of the view that a full scale credit crunch does not exists, the effects has trickle down to most economies around the globe. The effects of this state of affairs have had impacts in big agro allied business, shipping lines and so on. Africa depended on some of these services offered by these institutions for imports and technical partnership. The effects is manifested in slowed imports of agricultural inputs, technology related aspects of agriculture. This ultimately has effects on food prices or availability.

2.6 Commodity speculators

Commodity speculators buy wide range of commodities ranging foods and energy and other aspects. The main objective of these speculators is to make profit over time. Commodity speculators apply various strategies in buying and selling in order to earn profit. Their strategies depend on existing business/legal environment within which they operate. In most advanced countries, in the United States for instance, the Wall street Journal (WSJ) made a wide range of survey of commodity speculators over the years. Here's how the speculators drive prices sky-high.

In Africa, the role of commodity speculators seems to aggravate food situations in most African economies. In Nigeria for instance, speculators seems to have 'government' blessings in their operations. Food meant for the rural poor is most times diverted, sometimes given to government officials who made contacts with local speculators; within a short time such commodities are displayed in local market stalls. The case of fertilizer supply is worst. Small farmers cannot afford the commodity because middlemen (speculators) had fixed higher prices. For instance, government price for fertilizer is N1500 for 2007-2008 farming seasons but the price in the market is N3500, since most soil has been used for many years, the yield is low without fertilizer. This has contributed to low agricultural output in most of rural Nigeria. This is also the state of food imports in Nigeria. Multinationals, which have been contracted to import fertilizer, also aggravate prices of agricultural inputs.

3. Data and Method of Analysis

The main focus of this paper is to examine Nigeria's experience over the years and trends (given data available) in agricultural sector, its effects on whether the country is food secure or food insecure. The data used for this study are basically time series covering 1990- 2004, that is fifteen (15) years which were sourced from Central Bank of Nigeria (CBN) Statistical bulletin.

3.1 Model specifications

Regression model (multiple regressions) is used to analyze the relationship between agricultural output and capital expenditure on agricultural sector and credit on agricultural sector over the period of 15 years (1990 – 2004) to determine the degree of vulnerability over the years. The model is therefore specified as follows:

$$LOGAGRIC = B_O + B_1LOGCEA + B_2LOGCAG + \mu$$

Where:

B_0 = Constant or intercept, B_1 and B_2 are the coefficients of the explanatory variables of the model.

LOGAGRIC = Log of agricultural output.

LOGCEA = Log of capital expenditure on agricultural sector.

LOGCAG = Log of credit on agricultural sector defined as public and private credit as well as community bank credit.

μ = white noise error term.

A priori expectation: $B_0>0$, $B_1>0$, $B_2>0$

Justification for the model and variables selected.

Agricultural sector is the major contributor of food in Nigeria hence the need for improvements

to boost its productivity. This can be achieved by increasing capital expenditure, which includes public investments in the sector – heavy farm machinery, ariel sprays of farmland against birds and insects for most of the northern region that grow grains (corn, g/corn, millet) as well increase credit, provided by the Central Bank and managed by Nigerian banks, made available to small holders and microfinance institutions for the sector which justifies the selection of the variables in the model.

3.2 Econometric Diagnostic Test:

Unit Root Test

This study used or adopted Augmented Dickey-Fuller (ADF) Technique to verify the unit root property of the series. Macroeconomic data are generally characterized by stochastic trend which can be removed by differencing. Unit root test is used to test whether the data is stationary or not.

4. Results and Discussions

Table 2 contains the multivariate regression results of the model. The results indicate that both the capital expenditure on agricultural sector and credit to agricultural sector are statistically significant (including the constant). Precisely, the coefficient of capital expenditure on agriculture (CEA) is found to be statistically significant at 1 percent level as indicated by its probability value 0.0000 and rightly signed (positive). This, therefore, implies that a unit percent increase in capital expenditure on agriculture raises the output of agriculture (AGRIC) by 16.6 percent. However the coefficient of the other explanatory variable, credit to agricultural sector (CAG), go contrary to the theoretical expectation and found to be negative. The coefficient of credit to agriculture is also found to be statistically significant at 1 percent level as indicated by its probability value 0.0000 though not rightly signed (negative). This, therefore, implies that a unit percent increase in credit to agricultural sector (CAG) decreases the output of agriculture (AGRIC) by 3.73 percent. The constant is also is statistically significant at 5

percent level as indicated by its probability value 0.0000 and rightly signed (positive). This also indicates that output of agriculture does not depend only on capital expenditure and credit to agricultural.

The R^2 0.88 (88%) implies that 88 percent of total variation in the output of agriculture is explained by regression equation. Coindentally, the goodness of fit of the regression remained high after adjusting for the degrees of freedom as indicated by the adjusted R^2 (R^2 = 0.86 or 86%). The F-statistic 43.5, which is a measure of the joint significance of the explanatory variables, and the explanatory power of R^2 is found to be statistically significant at 1 percent as indicated by the corresponding probability value (0.000003). The model is found not spurious which is indicated by the DW (2.6) greater than R^2 (0.88) and R^2 (0.86). Since DW (2.6) is high, there is autocorrelation between the disturbance terms.

The results of unit root test are contained in table 3-5. The results show that output of agriculture (LOGAGRIC) and capital expenditure on agriculture (LOGCEA) are both stationary at first difference (d(1)) and 5 percent significance level; while credit to agriculture is stationary at second difference (d(2)) and 5 percent significance.

5. Conclusions

The paper investigates the impact of both capital expenditure and credit to agricultural sector on the growth of the output of agriculture in Nigeria and how it relates to the global food crisis. In order to avoid spuriousity of the estimates, the unit roots of the series were verified using Augmented Dickey-Fuller (ADF) technique. The key findings include long-run positive impact of capital expenditure on the growth of agricultural produce while long-run negative impact of credit to agriculture on the output of agriculture. The paper concludes that the output of agriculture over the years under review is on a declining trend, increasing food insecurity in the country despite levels of capital expenditure. Increasing credit may not be necessary but targeting rural farmers who most times do not benefit from such facilities. This category provides 60 percent of all agricultural output in Nigeria, thus agricultural policies that benefit them may be a realistic solution.

6. Recommendations

The paper therefore, recommends improvement in government capital expenditure on agriculture, and increase incentive to agricultural sector. Increasing incentives to sector – main players and making sure that such incentive reaches the target beneficiaries (rural farmers). The establishment of more agricultural banks for farmers to access credit and enacting policies and programmes aimed at improving agricultural output are central to the realization of a food secure Nigeria.

Specifically, regional agricultural policies (due to different climatic conditions in the country) as suggested by OneWorld, where most of the underweight children are located in just six states in the North-Eastern Nigerian, which is semi-arid land and encouraging quick yielding variety of crops may reduce the vulnerability index in these regions.

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Table 1. Agriculture: GDP and Financial/Credit Flows in billion from 1990 -2004

Year	Total GDP	**Agric. GDP**	% Contribution to GDP	**Capital Expenditure on Agric (FGN)**	Credit to Agric	
					Public and Private	Community Bank
1990	257.87	35.80	13.9	1.60	6.93	-
1991	320.25	36.50	11.4	1.22	8.00	-
1992	544.33	37.30	6.9	0.94	11.00	-
1S993	691.61	37.80	5.5	2.18	25.00	-
1994	911.09	35.60	4.2	2.18	25.00	-
1995	1960.69	40.00	2.0	2.41	35.00	-
1996	2740.46	41.70	1.5	3.89	44.00	-
1997	2835.00	43.50	1.5	6.25	37.00	-
1998	2721.51	45.60	1.7	4.33	19.00	-
1999	3250.67	47.60	1.5	8.88	31.00	-
2000	4547.10	48.99	1.1	6.91	41.00	1.61
2001	5187.90	51.47	1.0	5.76	50.50	0.08
2002	5465.30	64.41	1.2	32.36	NA	0.39
2003	7191.10	68.02	0.9	8.57	NA	0.63
2004	8553.30	72.20	0.8	38.67	NA	0.48

Sources: CBN (2003), Cotemporary Economic policy issues, edited by Nnanna et al Pg198; CBN Annual Report 2004 pg 114 and 131, CBN statistics Bulletin Vol.2, 1999, pg 119; Esthiobo Samuel Shola (2008).

Table 2. Regression results using observations 1990-2004 (n = 15) Dependent variable: LOGAGRIC

Dependent Variable: LOGAGRIC

Method: Least Squares

Date: 08/26/11, Time: 20:24

Sample: 1990 2004

Included observations: 15

Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	1.589693	0.032118	49.49547	0.0000
LOGCEA	0.166187	0.026337	6.309984	0.0000
LOGCAG	-0.037299	0.016660	-2.238902	0.0449
R-squared	0.878718	Mean dependent var		1.661067
Adjusted R-squared	0.858505	S.D. dependent var		0.102762
S.E. of regression	0.038655	Akaike info criterion		-3.491432
Sum squared resid	0.017930	Schwarz criterion		-3.349822
Log likelihood	29.18574	Hannan-Quinn criter.		-3.492940
F-statistic	43.47162	Durbin-Watson stat		2.621608
Prob(F-statistic)	0.000003			

Source: Computer output.

Table 3. Unit Root Test for log of output of agriculture

Null Hypothesis: D(LOGAGRIC) has a unit root

Exogenous: Constant

Lag Length: 0 (Automatic - based on SIC, maxlag=3)

			t-Statistic	Prob.*
Augmented Dickey-Fuller test statistic			-3.409973	0.0306
Test critical values:	1% level		-4.057910	
	5% level		-3.119910	
	10% level		-2.701103	

Augmented Dickey-Fuller Test Equation

Dependent Variable: D(LOGAGRIC,2)

Method: Least Squares

Date: 08/26/11 Time: 20:33

Sample (adjusted): 1992 2004

Included observations: 13 after adjustments

Variable	Coefficient	Std. Error	t-Statistic	Prob.
D(LOGAGRIC(-1))	-1.018571	0.298704	-3.409973	0.0058
C	0.023183	0.010321	2.246217	0.0462
R-squared	0.513875	Mean dependent var		0.001346
Adjusted R-squared	0.469682	S.D. dependent var		0.040074
S.E. of regression	0.029183	Akaike info criterion		-4.089829
Sum squared resid	0.009368	Schwarz criterion		-4.002913
Log likelihood	28.58389	Hannan-Quinn criter.		-4.107694
F-statistic	11.62792	Durbin-Watson stat		2.020674
Prob(F-statistic)	0.005826			

Source: Computer output.

Table 4. Unit Root Test for log of capital expenditure on agriculture

Null Hypothesis: D(LOGCEA) has a unit root

Exogenous: Constant

Lag Length: 1 (Automatic - based on SIC, maxlag=3)

			t-Statistic	Prob.*
Augmented Dickey-Fuller test statistic			-6.756561	0.0002
Test critical values:	1% level		-4.121990	
	5% level		-3.144920	
	10% level		-2.713751	

Augmented Dickey-Fuller Test Equation

Dependent Variable: D(LOGCEA,2)

Method: Least Squares

Date: 08/27/11 Time: 04:17

Sample (adjusted): 1993 2004

Included observations: 12 after adjustments

Variable	Coefficient	Std. Error	t-Statistic	Prob.
D(LOGCEA(-1))	-2.996129	0.443440	-6.756561	0.0001
D(LOGCEA(-1),2)	0.710519	0.266317	2.667945	0.0257
C	0.302547	0.065381	4.627421	0.0012
R-squared	0.942026	Mean dependent var		0.063975
Adjusted R-squared	0.929143	S.D. dependent var		0.663256
S.E. of regression	0.176552	Akaike info criterion		-0.418086
Sum squared resid	0.280535	Schwarz criterion		-0.296859
Log likelihood	5.508513	Hannan-Quinn criter.		-0.462968
F-statistic	73.12127	Durbin-Watson stat		2.223971
Prob(F-statistic)	0.000003			

Source: Computer output.

Table 5. Unit Root Test for log of credit to agricultural sector

Null Hypothesis: D(LOGCAG,2) has a unit root

Exogenous: Constant

Lag Length: 2 (Automatic - based on SIC, maxlag=3)

			t-Statistic	Prob.*
Augmented Dickey-Fuller test statistic			-5.933900	0.0011
Test critical values:	1% level		-4.297073	
	5% level		-3.212696	
	10% level		-2.747676	

Augmented Dickey-Fuller Test Equation

Dependent Variable: D(LOGCAG,3)

Method: Least Squares

Date: 08/27/11 Time: 04:25

Sample (adjusted): 1995 2004

Included observations: 10 after adjustments

Variable	Coefficient	Std. Error	t-Statistic	Prob.
D(LOGCAG(-1),2)	-5.771409	0.972616	-5.933900	0.0010
D(LOGCAG(-1),3)	3.663451	0.852252	4.298555	0.0051
D(LOGCAG(-2),3)	2.558567	0.736959	3.471792	0.0133
C	-0.273876	0.189914	-1.442108	0.1994
R-squared	0.947097	Mean dependent var		0.003020
Adjusted R-squared	0.920646	S.D. dependent var		1.914821
S.E. of regression	0.539404	Akaike info criterion		1.892470
Sum squared resid	1.745739	Schwarz criterion		2.013504
Log likelihood	-5.462349	Hannan-Quinn criter.		1.759696
F-statistic	35.80505	Durbin-Watson stat		2.138163

Source: Computer output.

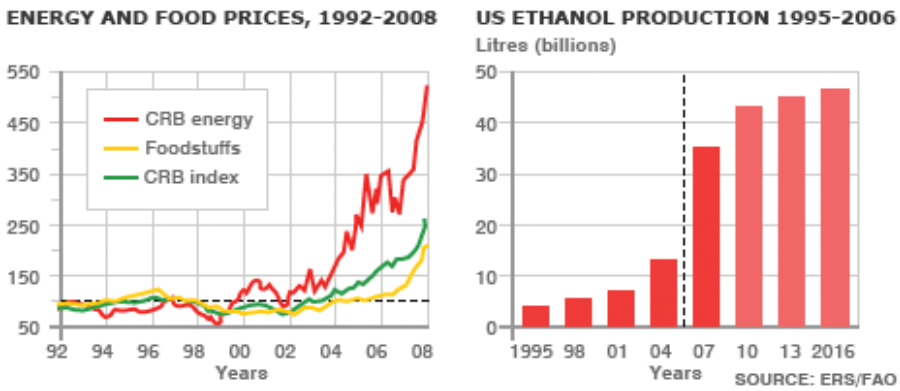

Figure 1. The Commodity Research Bureau and the FAO show a take off in energy prices, before food prices start to rise

Biological Evaluation of Wheat-Salty Extract, Milk-Wheat Solution and Fermented Soymilk for Treatment of Castor-Oil Induced Diarrhea in Rats

Ghada M. Khiralla (Corresponding author) & Safaa A. Salem
Division of Medical Foods
National Organization for Drug Control and Research (NODCAR)
6-7, AboHazem St, Pyramids, PO Box 29, Giza, Egypt
E-mail: gkhiralla@yahoo.com

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Abstract

Functional food or medicinal food is any healthy food claimed to have a health-promoting or disease-preventing property beyond the basic function of supplying nutrients. Three nutritional preparations including wheat powder salt solution (WPSS), milk-wheat solution (MWS), fermented milk (FM) and fermented soymilk (FSM) were evaluated for their anti-diarrheal activity by oral administration in model of Castor oil induced diarrhea in rats. Oral rehydration solution (ORS) was used as positive control. The fermented products were prepared using a mixture of *Lactobacillus acidophilus* ATCC 4356: and *Bifidobacterium bifidum* ATCC 700541 (1:1 v/v) to obtain a final level of 10^{7-8} CFU/ml after incubation at 37°C. Beside the gain body weight (BW), certain biochemical parameters such as total protein, albumin, globulin, urea, creatinine, alanine amino-transferase (ALT), aspartate amino-transferase (AST), sodium, potassium, magnesium, iron and phosphorus were determined.

According to follow the diarrheal symptoms including stool frequency, stool characteristics and BW, rats administrated with FSM were recovered from diarrhea (on the 3rd day) faster than other groups followed by those subjected with FM and CY. The ORS-positive control group rats were recovered on the 6th day, while diarrheal symptoms still appeared on the negative control rats (subjected with basal diet only; without ORS) with 16% death rate. Minerals, especially sodium, potassium, magnesium and phosphorus, were the most significant biochemical parameters for following recovery from diarrhea. The normal levels of these minerals were recovered in the blood serum at the end of experiment in rats administrated with the fermented products (FSM, FM and CY). Some renal functional parameters were suggested to follow diarrhea, but all studied liver functional parameters were not significantly recommended.

Keywords: Diarrhea, Wheat, Soymilk, *Lactobacillus acidophilus, Bifidobacterium bifidum*

1. Introduction

Diarrheal disease persists to be a main reason of morbidity and mortality among infants and young children in developing countries (Torun & Chew, 1991; Brown, 2003). Nutritional hazard factors for diarrhea can be grouped as anthropometric hazard factors, infant and child feeding practices and micronutrient status. Frequency rates and the duration and severity of illness are the main measures of morbidity resulting from diarrhea (Samadi, Chowdhury, Huq, & Shahid, 1985; Brown, 2003). After the widely use of glucose-based oral rehydration solution (ORS) recommended by the World Health Organization for treatment of diarrheal episodes, the mortality due to acute diarrhea has been decreased significantly (WHO,2004) .The ORS formulation has, however, no effect on decreasing the volume, frequency, and duration of diarrhea. This causes the necessity to search for other formulations of oral rehydration solution (Mahalanabis, 1985; Khiralla, Rasmy, El-Malky, & Ibrahim 2009). In a clinical trial, three different mixtures of home-available foods were evaluated for their anti-diarrheal effects in children. These mixtures were; i) a soy-protein-isolate ii) diet contained wheat flour, pea flour, carrot flour, sucrose,

and vegetable oil and iii) potato flour, dried whole milk, carrot flour, sucrose, and oil (Alarcon, Montoya, Perez, Dongo, Peerson, & Brown., 1991). They concluded that use of lactose free formulas were suitable for management of diarrhea. Also, the mothers perceived wheat flour, rice water and selected herbs as anti-diarrheal agents, while more than 70% of mothers decreased fluid intake during diarrhea episodes (Othero, Orago, Groenewegen, Kaseje, & Otengah, 2008).

Probiotics are beneficial live microorganisms that encourage growth and protection of beneficial bacteria in the bowel, while suppressing harmful bacteria. The overall result is a healthier digestive system (Fuller, 1989). In other words, the term 'probiotic' evolved from the food industry to describe 'live microbial food ingredients that are beneficial to health of the host', by improving its intestinal microbial balance (Twetman & Stecksn-Blicks, 2008). There are incremental effort focused on bacteriology of the gut leading to clinical observations claiming benefit through enhancement of 'gut health' and the prevention of diarrhea (Gionchetti, Rizzello, Venturi & Campieri. (2000); Parvez, Malik, Kang, & Kim. (2006); de Vrese & Marteau, 2007; Twetman & Stecksén-Blicks, 2008). The use of probiotics (e.g. lactobacilli and bifidobacteria) has been widely supported in foodstuffs such as fermented milk products. These products contained viable cultures and are used to support multiply of the microbial populations in the colon. To be effective, probiotics must be capable of being prepared in a viable manner and on large scale (e.g. for industrial purposes). During use and under storage, the probiotic should remain viable, stable, and be able to survive in the intestinal ecosystem. At the same time, the host animal should gain beneficially from harboring the probiotic. Some of these requirements may be difficult to attain (Rycroft, Rastall & Gibson (2001). A number of specific strains, including *Lactobacillus* GG, *L. reuteri*, *Sacchromyces boulardii*, *Bifidobacterium spp*., and others, have been shown to have significant benefit for diarrhea Pant, Graham., Alle, Harikul, Sabchareon ,Cuevas, & Hart. (1996); Saavedra, 2000; Benchimol & Mack 2004), travelers' diarrhea Hilton, Kolakawaki, Singer, & Smith. (1997) and diarrhea disease in young children caused by rotaviruses (Vanderhoof, 2000). The probiotic species that showed the most promise in treating diarrhea diseases in children include *L. reuteri*, *L. casei*, *Bif. bifidum* and *Streptococcus thermophilus* and *Sacch. boulardii* (Pant et al., 1996; Oberreuther-Moschner, Jahreis, Rechkemmer, & Pool-Zobel B. L. (2004); Tomas, Claudia Otero, Ocana, & Nader- Macias. (2004); de Vrese & Marteau, (2007). *L. bulgaricus*, *L. casei,* and *Bif. longum* were used for preparation of fermented soymilk products that were successfully applied for minimizing diarrhea symptoms in young rats Khiralla, Rasmy, El-Malky & Ibrahim (2009).

Based on the knowledge available in literature reviews, most cases of diarrhea in children are management in house oral rehydration solutions (ORS) beside some homemade blends. From view of healthy workers, there is need to develop and implement interactive communication strategies with mothers to address perceptions and misconceptions and facilitate positive change in the household practice on management of diarrhea among under-fives Othero, Orago., Groenewegen., Kaseje&, Otengah. (2008).Therefore, the aim of the present research is to study the anti-diarrheal activity of some formulas based on home-available and low-cost staple foods such as wheat flour salt solution, wheat-milk solution, fermented milk, commercial yogurt and fermented soymilk. The aim includes evaluation of the nutritional value of the therapeutic diets in rats through determination body weight gain, with the assessment of different biochemical parameters reflecting nutritional status.

2. Methods

2.1 Raw materials

Defatted soybean milk (Soybean, Glycine max) was obtained from Soybean Products Pilot Plant, Food Research and Technology Institute, Agriculture Research Center (ARC), Giza Egypt. Whole wheat, low fat cow milk and yoghurt were purchased from the local market, Cairo, Egypt.

2.2 Bacterial cultures

Pure cultures of *Lactobacillus acidophilus* ATCC 4356: and *Bifidobacterium bifidum* ATCC 700541 were obtained from Microbiological Resource Center Cairo (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. The Strains were maintained and grown on MRS agar (Difco, USA).

2.3 Inoculum preparation

Lactobacillus acidophilus ATCC 4356: and *Bifidobacterium bifidum* ATCC 700541 were separately grown on MRS broth (Difco, USA) at 37 °C for 24 h. 0.5% (w/v) L-cysteine-HCl (Sigma, USA) was added to decrease the redox potential of the medium. The cells were harvested by centrifugation (Sigma 3K12, 5000 xg, 10 min) and washed two times with sterilized distilled water. Cell pellets were reconstituted in sterilized soymilk or milk used for inoculation in further work.

2.4 Probiotic fermentation of soymilk and milk

Probiotic fermented milk and soymilk were prepared according to the method described by Wei, Chen, & Chen (2007). The milk or soymilk was sterilized by autoclaving at 121°C for 15min, and then inoculated with mixture of *Lactobacillus acidophilus,* and *Bifidobacterium bifidum* (1:1 v/v) to obtained a cell level of 10^3 CFU/ml. The inoculated milk and soymilk were fermented at 37 °C for 24 h (Wei et al., 2007). pH of milk and soymilk was measured before and after fermentation using a pH meter (Horiba Ltd., Kyoto, Japan). The pour plate method was applied for enumerating viable populations of bacteria in fermented products. MRS agar (Difco, USA) contained 0.5% (w/v) L-cysteine-HCl was used and the plates were incubated at 37°C for 24 h (Wei et al., 2007). The fermented probiotic products were stored at 4°C and utilized before 3 days.

2.5 Preparation of WPSS and MWS

Whole wheat grains were ground in a Junior Mill to pass through 60-mesh sieves. A fresh wheat powder salt solution (WPSS) was prepared by adding 30 g of wheat powder and 3.5 g salt to 250 ml distilled water. This mixture was boiled at 100 °C for 10 minutes with stirring, then the final volume was adjusted to 1 liter with distilled water. MWS was freshly prepared by adding 30 g wheat powder to 250 ml of water and boiled for 10 minutes. The final volume would be about 200 ml and then mixed with the low fat milk in a proportion of 1:2 (v/v). WPSS and MWS were stored at 4°C.

2.6 Experimental design and basal diet

Forty eight male albino rats (110-130 ±5g) were obtained from the farm of the National Organization for Drug Control and Research, Giza, Egypt. The formula of the basal diet used in the present study was as follows: 10% protein, 10% corn oil, 4% salt mixture, 1% vitamin mixture, 5% cellulose and 70% starch (AOAC, 1995). Under the ethics rules, this experiment was carried out in the animal house of National Organization for Drug Control and Research, Giza, Egypt. Animals were housed in separate stainless steel cages and raised in a well-ventilated room with 12-h light/dark cycle. Animals were given free access to food and water throughout the experimental period (7 days, according to René, Pouokam, Fonkoua, Penlap, & Biwole (2005). After adaptation period (7 days, in which rates fed on basal diet and free access to water), rats were divided into Seven groups (n=6). Diarrhea was induced by oral administration 1 ml of Castor oil. CN-group was the control negative that fed on basal diet and free access to water. In addition to PC-group (positive control) the other groups fed on basal diet and free access to water contained Oral Rehydration Solution (ORS, CID Co., Egypt). ORS was prepared according to the manufacturers' instructions. Every day rats were administrated with 2ml of wheat powder salt solution (WPSS-group), milk- wheat solution (MWS-group), fermented soymilk (FSM-group), fermented milk (FM-group), or commercial yoghurt (CY-group).

2.7 Diarrheal parameters

Body weight (BW) of the rats was recorded just before and after diarrheal induction, and then was followed on the 3rd and 7th day. Death rate was expressed as percentage from the initial number of rats in each group (n=6). Stool frequency was recorded daily before diarrheal induction and during 7 days following. Rats were observed daily for the appearance of any symptoms of discomfort that might be related to studied treatments as mentioned by René et al. (2005).

2.8 Biochemical parameters

Blood samples were collected three times during the experiment; before and after induction of diarrhea that were assayed and presented as initial and diarrheal biochemical parameters, respectively; and at the end of experimental period. Samples were collected into dry clean centrifuge tubes from the eye plexuses of animals by a fine capillary glass tubes and placed immediately on ice. Serum was separated after centrifugation for 10 min at 1500 xg and kept at –20 °C until analysis (Schermer, 1967). The normal levels of all tested parameters which used for discussing the obtained results were obtained from the site: http://www.bloodbook.com/ranges.html.

Creatinine and urea were determined as biochemical parameters of kidney functions according to the method described by Larsen (1972), and Patton , & Crouch (1977), respectively. Total protein and albumin were measured directly and globulin concentration was calculated by subtracting albumin from total protein according to the manufacturers' instructions which based on the methods of Gornall, Bardawill., & David. (1949)and Doumas, Watson & Biggs. (1971). respectively.

Alanine aminotransferase (ALT, EC 2.6.1.2) and aspartate aminotransferase (AST, EC 2.6.1.1), were determined as biochemical parameters of liver functions according to the methods mentioned Bergmeyer &Harder (1986).

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Minerals including Na+, K+, Mg++, iron and phosphorus were determined by the methods of Trinder (1951), Sunderman &Sunderman (1958), Grindler & Heth (1971), Dreux (1977), and El-Merzabani, El-Aaser, & Zakhary (1977) respectively.

3. Statistical Analysis

For each treatment, data from three independent replicate trials were pooled and the mean values and standard deviations were determined. Differences between samples were determined by Duncan‘s and were considered to be significant when $p \leq 0.05$ (Snedecor & Cochran, 1980)

4. Results and Discussion

4.1 Diarrhea symptoms and Changes in Body weight

During the adaptation period and before induction of diarrhea, rats presented normal feces described as solid, molded, brown or dark and rough feces. Diarrheal stools appeared longer than normal stools 24 h after diarrheal induction. Stools were also either soft or liquid. The frequency of diarrheal feces was recommended in previous work as a good diarrheal index (Khiralla et al., 2009). In the present study, all tested group except the negative control (NC-group) diarrheal symptoms were gradually reduced by extending the experiment period. According to the stool frequency (Table 1), quick recovery was noticed by rats in FSM-group on 3rd day followed by FM- and CY-group on the 4th day. FSM- and FM-groups were administrated, respectively, with soymilk and low fat milk fermented with mixture of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (1:1 v/v). Diarrheal symptoms were disappeared on the 5th day in MWS-group and on 6th day in the PC- and WPSS-group. However, diarrheal symptoms still to the end of experiment period in the NC-group, with death rate of 17%. In this respect, René et al., (2005) has been recognized that severe diarrhea sometimes followed by death.

In previous studies, oatmeal soup fermented with *Lactobacillus reuteri* was shown to prevent the development of acetic acid-induced colitis Fabia, Ar’Rajab, Johansson, Willen, Andersson, Molin, & Bengmark,. (1993)or methotrexate-induced colitis Mao, Nobaek, Kasravi, Adawi, Stenram, Molin, & Jeppsson. (1996). The decrease in stool frequency on the 3rd day in FSM-group and on 4th day in FM-group may have been due to the importance of the high number or the metabolic activity of probiotic bacteria in the intestinal tract. Moreover, Madsen, Cornish, Soper, Mc Kaigney, Jijon, Yachimec, Doyle., Jewell, & De Simone (2001) mentioned that probiotic bacteria are capable of exerting good effects on the host organism by improving the balance of intestinal flora. The mechanism of action appears to be through protective, trophic and anti-inflammatory effects on bowel mucosa Gionchetti, et al.,. (2000)and Petrof., Kojima., Ropeleski., Musch, Tao, De Simone&, Chang. (2004). Moreover, the FSM is distinguished from FM with the presence of some prebiotic components, such as dietary fibers (Préstamo, Rupérez., Espinosa-Martos., Villanueva, & Lasunción. (2007) that may promote probiotic bacteria to achieve its function in the intestinal Wei et al., (2007).

Body weight (BW) of tested rats was recorded just before and after diarrheal induction, and then was followed on the third and seventh day. Change percentages in BW were calculated based on the initial weight at beginning of the experiment and illustrated in Figure 1. Significant loss ($p<0.05$) of BW in all tested rat groups was observed after diarrheal induction (ADI) with Castor oil. Where, the loss percentage of BW in all groups was ranged from 10.5 ±1.7% to 17.9 ±1.5%. In NC-group, manifested loss in BW was recorded along the experimental period. At the end of the experiment, the loss percentage was reached to 30.2 ±1.6%. Moreover, on the 5th day death rate was 16 % in the NC-group. Although, loss of BW in PC-group continued during the experiment period, the loss percentage of BW significantly attenuated (Figure 1). ORS presented to the PC-group contained salts and sugars. This may led to reducing sharpness of BW loss, but did not reduce the period of diarrhea. In previous work Pant et al., (1996) stated the importance of using ORS to minimize, the risk of dehydration resulting from diarrhea. However, ORS neither shortens the duration of diarrhea nor provides any significant nutritional value. In the other groups that administrated with the functional preparations positive enhancements were recorded on the 3rd day (Figure 1). The rats of FSM-group got the highest gain weight on the 3rd and 7th day compared with the other groups.

4.2 Biochemical parameters

Liver and kidney functions in blood serum of rats before and after diarrheal induction and at the end of experimental period (on 7th day) were presented in Table 2. Total proteins level had significantly ($p<0.05$) reduced after diarrheal induction. This reduction continued until the end of the experiment in NC- PC- and WPSS-group rats. Total proteins returned to their level after diarrheal induction (just before beginning of the experiment) when rats were administrated with wheat milk solution (MWS-group), where it was 5.4 g/dl (Table 2). These results could be interrupted by those mentioned by Hayes (2007). He stated that, decreased serum

protein concentrations result from decrease protein synthesis or increase protein loss. He added also that, loss of albumin and globulin occurs with exudative lesions such as severe diarrhea.

In the other groups (FSM-, FM- and CY-groups) administrated with fermented preparations total protein level was significantly ($p<0.05$) enhanced at the end of experiment period. Similar trend was noticed in albumin and globulin levels. In addition to the FSM-, FM- and CY-groups, albumin level in the serum of MWS-group rats had returned to the normal level at the end of experiment (Table 2). In general, concentration of total proteins and albumin reached to the normal levels (6-8.4, and 3.5-5 g/dl, respectively) only in the rats administrated with the fermented preparations. (FSM-, FM- and CY-groups). The enhancement effect may be referred to use of mixture (1:1 v/v) of probiotic strains *Bif. bifidum* and *L. acidophilus*. The obtained results were in agreement with those observed previously by soymilk fermented with *L. casei*, *L. bulgaricus*, *Bif. longum*, and mixed culture (1:1:1 v/v) of these stains (Khiralla et al., 2009).

Creatinine levels in the blood serum of all tested groups were not significantly ($p>0.05$) affected and were in the range of the normal level (0.6-1.2 mg/dl). From the above-mentioned results, it can be concluded that, total proteins, albumin and urea could be used as a good indicators for studying the effect of diarrhea on the kidney functions in young rats. Concerning the liver functions in all tested groups, no significant ($p>0.05$) effect was obtained due to diarrheal induction or administration of probiotic soymilk preparations (Table2). This results were in agreement with the previous work (Khiralla, et al., 2009), in which some of kidney functions has been affected by diarrhea. This may be due to loss of body fluid. . Table 2: Liver and kidney functions in blood serum of rats administrated with functional probiotic and prebiotic formulas before and after diarrheal induction and at the end of experimental period (7 days)

4.3 Minerals

Sodium, potassium, magnesium, iron and phosphorus were determined in blood serum of rats before and after diarrheal induction and on the 7th day (Table 2). Significant ($p< 0.05$) decrease in all tested minerals was recorded just after diarrheal induction. These concentrations were at the level of hyponatremia (less than 135m. mol Na^+/l), hypokalemia (less than 3.5m. mol K /l), and hypomagnesemia (less than 1.7mg Mg^{++} /dl). Loss of these minerals were previously reported as a result of diarrheal induction Schweinfest, Spyropoulos, Henderson, Kim, Chapman., Barone, Worrell., Wang., & Soleimani. (2006)Although recovery from diarrheal symptoms in NC-group rats was noticed on the 7th day, blood chemistry showed hyponatremia and hypomagnesemia but not hypokalemia (Table 3). Similar trend was observed by rats of PC-group, but with mineral values near to the normal values. The serum sodium returned to the normal level (135-145m.mol) somewhat slowly in WPSS- and MWS-group, whereas, magnesium concentration stilled in the less than the normal level in blood serum (1.7-2.3mg Mg^{++} /dl). Remarkably, all tested minerals in the blood serum of rats administrated with fermented products returned to the normal levels on the 7th day (Table 3). These results indicated that the probiotic soymilk preparations may be having a positive effect on the microbial flora balance resulted in decreasing inflammatory bowel symptoms and electrolytes losing associated with diarrhea (Benchimol &Mack 2004; de Vrese & Marteau, 2007). This was demonstrated by accelerating recovery of Na^+ and K^+ levels in rats subjected with probiotic soymilk (Table 3). These minerals were the most important electrolytes, which involved in water balance, pH balance, membrane transport and electrical conduction in the muscle and nerve cells (Pizzaro, Posado, Sandi & Moran, 1991; Shah., Das., Kumar, Singh. & Bhandari, 2006).

5. Conclusion

The best results were observed with fermented preparations, especially fermented soymilk. In general, the obtained data indicated that, serum electrolytes especially sodium, potassium, magnesium and phosphorus should be gained and carefully monitored in patients with diarrhea (Irwin & Rippe 2008). Also, some biochemical parameters such as total proteins, albumin, globulins and urea in blood serum might be useful for follow the real recovery from diarrhea.

Home management of diarrhea is one of the key household practices targeted for strategy enhancement in the Community Integrated Management of Childhood Illness (C-IMCI). The obtained data provided useful information about some locally available, low-cost staple food mixtures offer a safe and nutritionally adequate substitute products for the dietary management. In addition to their nutritional values the products prepared in the present study, such as fermented soymilk and WSSP, represent alternative products especially for persons who have dairy allergies.

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Table 1. Stool frequency (number of feces per day: Nbr/day) rats administrated with probiotic and prebiotic formulas

Rat groups*	**Stool frequency (Nbr/ day)**								**Recovery day**	**Death rate %**
	0	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**		
NC	48-60	72-96	72-96	72-96	72-96	72-84	72-84	48-60	7	17
PC	48-60	72-96	72-96	72-96	72-84	72-84	48-60		6	-
WPSS	48-60	72-96	72-84	72-84	72-84	72-84	48-60	-	6	-
MWS	48-60	72-96	72-84	72-84	72-84	48-60	-	-	5	-
FSM	48-60	72-96	72-84	48-60	-	-	-	-	3	-
FM	48-60	72-96	72-84	72-84	48-60	-	-	-	4	-
CY	48-60	72-96	72-84	72-84	48-60	-	-	-	4	-

* Rat groups fed on basal diet, free access to ORS and administrated daily with 2ml of wheat powder salt solution (WPSS); milk wheat solution (MWS); fermented soymilk (FSM); fermented milk (FM); commercial yoghurt (CY). NC, negative control fed on basal diet and free access to water; PC, positive control fed on basal diet and free access to water contained. Fermented milk and fermented soymilk was prepared using mixed culture of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* (1:1 v/v).

Table 2. Liver and kidney functions in blood serum of rats administrated with functional probiotic and prebiotic formulas before and after diarrheal induction and at the end of experimental period (7 days)

Biochemical Parameters of blood serum*	Before diarrheal induction	After diarrheal induction	Rat groups#						
			NC	PC	WPSS	MWS	FSM	FM	CY
Kidney functions									
T. proteins (g/dl)	$6.3^{a}\pm0.27$	$5.4^{d}\pm0.27$	$4.9^{e}\pm0.17$	$4.9^{e}\pm0.17$	$4.9^{e}\pm0.17$	$5.4^{d}\pm0.13$	$6.1^{bc}\pm0.21$	$6.04^{c}\pm0.14$	$6.2^{b}\pm0.17$
Albumin (g/dl)	$3.5^{a}\pm0.14$	$3.0^{ab}\pm0.19$	$2.8^{c}\pm0.23$	$2.8^{c}\pm0.23$	$2.8^{c}\pm0.23$	$3.1^{a}\pm0.37$	$3.5^{a}\pm0.12$	$3.4^{a}\pm0.21$	$3.6^{a}\pm0.18$
Globulin (g/dl)	$2.8^{a}\pm0.35$	$2.4^{bc}\pm0.33$	$2.1^{c}\pm0.21$	$2.1^{c}\pm0.21$	$2.1^{c}\pm0.21$	$2.3^{bc}\pm0.43$	$2.6^{b}\pm0.26$	$2.64^{b}\pm0.26$	$2.6^{b}\pm0.31$
Urea (mg/dl)	$16.4^{c}\pm0.12$	$16^{c}\pm0.17$	$18.4^{a}\pm0.81$	$18.4^{a}\pm0.81$	$18.4^{a}\pm0.81$	$16.9^{b}\pm0.94$	$16.1^{c}\pm0.4$	$16.1^{c}\pm0.16$	$16.0^{c}\pm0.17$
Creatinine (mg/dl)	$0.82^{a}\pm0.02$	$0.85^{a}\pm0.04$	$0.84^{a}\pm0.05$	$0.84^{a}\pm0.05$	$0.84^{a}\pm0.05$	$0.82^{a}\pm0.05$	$0.83^{a}\pm0.03$	$0.82^{a}\pm0.02$	$0.83^{a}\pm0.01$
Liver functions									
ALT (µg/l)	$37.3^{a}\pm3.9$	$37.1^{a}\pm3.3$	$36.5^{a}\pm5.3$	$36.5^{a}\pm5.3$	$36.5^{a}\pm5.3$	$35.9^{a}\pm4.7$	$36.8^{a}\pm3.7$	$38.1^{a}\pm3.3$	$37.1^{a}\pm2.5$
AST (µg/l)	$42.7^{a}\pm4.8$	$40.0^{a}\pm4.6$	$40.7^{a}\pm4.2$	$40.7^{a}\pm4.2$	$40.7^{a}\pm4.2$	$41.8^{a}\pm2.9$	$44.2^{a}\pm3.7$	$40.3^{a}\pm4.6$	$41.2^{a}\pm3.8$

* Means ($n = 6$) ± SD in the same row with different letters are significantly different ($p<0.05$). # Rat groups fed on basal diet, free access to ORS and administrated daily with 2ml of wheat powder salt solution (WPSS); milk wheat solution (MWS); fermented soymilk (FSM); fermented milk (FM); commercial yoghurt (CY). NC, negative control fed on basal diet and free access to water; PC, positive control fed on basal diet and free access to water contained. Fermented milk and fermented soy milk was prepared using mixed culture of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* (1:1 v/v).

Table 3. Minerals in blood serum of rats administrated with functional probiotic and prebiotic formulas before and after diarrheal induction and at the end of experimental period (7 days)

Minerals *	Before diarrheal induction	After diarrheal induction	Rat groups#						
			NC	PC	WPSS	MWS	FSM	FM	CY
Na^{+} (mmol/l)	$148^{a}\pm3.5$	$116^{d}\pm2.4$	$125^{e}\pm3.1$	$132^{d}\pm2.1$	$138^{c}\pm3.2$	$136^{c}\pm2.5$	$145^{b}\pm2.2$	$142^{b}\pm3.1$	$143^{b}\pm2.6$
K^{+} (mmol/l)	$5.5^{a}\pm0.35$	$3.3^{d}\pm0.33$	$4.2^{c}\pm0.23$	$5.1^{b}\pm0.28$	$5.3^{b}\pm0.4$	$5.3^{a}\pm0.22$	$5.4^{a}\pm0.29$	$5.0^{b}\pm0.22$	$5.1^{b}\pm0.32$
Mg^{+2} (mg/dl)	$1.71^{a}\pm0.08$	$1.42^{c}\pm0.05$	$1.48^{d}\pm0.08$	$1.62^{c}\pm0.06$	$1.68^{a}\pm0.06$	$1.58^{cd}\pm0.06$	$1.79^{a}\pm0.09$	$1.76^{b}\pm0.05$	$1.74^{b}\pm0.04$
Iron (µg/dl)	$41.1^{a}\pm2.1$	$38.3^{c}\pm1.9$	$35.5^{d}\pm2.1$	$35.6^{d}\pm0.33$	$38.9^{c}\pm0.44$	$39.2^{bc}\pm0.49$	$40.8^{a}\pm0.48$	$40.2^{b}\pm0.38$	$40.1^{b}\pm0.51$
P (mg/ dl)	$6.42^{a}\pm0.62$	$4.76^{d}\pm0.81$	$4.51^{d}\pm0.61$	$5.12^{c}\pm0.58$	$5.85^{b}\pm0.55$	$5.37^{c}\pm0.61$	$6.51^{a}\pm0.62$	$6.33^{a}\pm0.62$	$5.92^{b}\pm0.54$

* Means (n = 6) ± SD in the same row with different letters are significantly different (p<0.05). # Rat groups fed on basal diet, free access to ORS and administrated daily with 2ml of wheat powder salt solution (WPSS); milk wheat solution (MWS); fermented soymilk (FSM); fermented milk (FM); commercial yoghurt (CY). NC, negative control fed on basal diet and free access to water; PC, positive control fed on basal diet and free access to water contained. Fermented milk and fermented soy milk was prepared using mixed culture of Bifidobacterium bifidum and Lactobacillus acidophilus (1:1 v/v).

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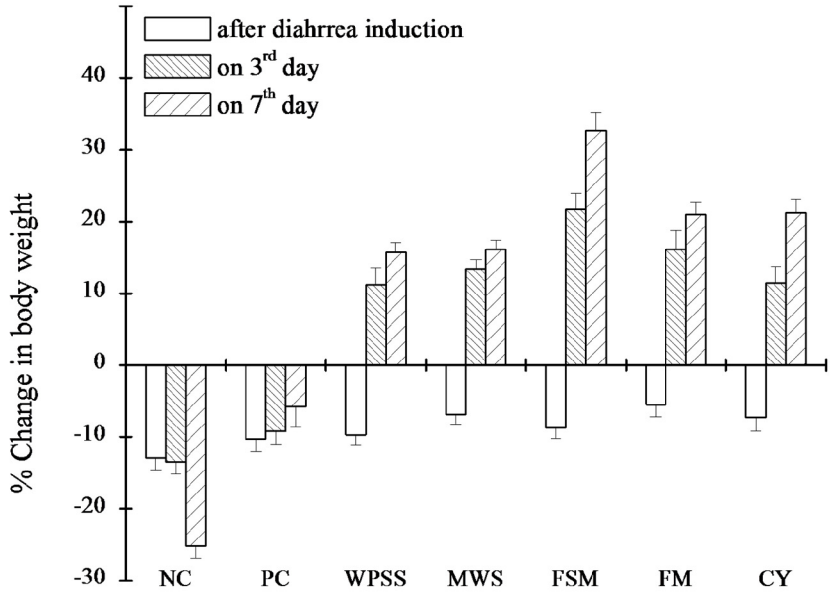

Figure 1. Changes of body weight of rats due to diarrheal induction and during 7-days experiment

Rat groups fed on basal diet, free access to ORS and administrated daily with 2ml of wheat powder salt solution (WPSS); milk wheat solution (MWS); fermented soymilk (FSM); fermented milk (FM); commercial yoghurt (CY). NC, negative control fed on basal diet and free access to water; PC, positive control fed on basal diet and free access to water contained. Fermented milk and fermented soy milk was prepared using mixed culture of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* (1:1 v/v).

Characterization of Queso Fresco during Storage at 4 and 10°C

Michael H. Tunick (Corresponding author), Diane L. Van Hekken, Susan K. Iandola & Peggy M. Tomasula
Dairy & Functional Foods Research Unit, Eastern Regional Research Center
Agricultural Research Service, U.S. Department of Agriculture
600 E. Mermaid Lane, Wyndmoor, PA 19038, USA
Tel: 1-215-233-6454 E-mail: michael.tunick@ars.usda.gov

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Abstract

Queso Fresco, a popular Hispanic cheese variety, was prepared and its chemical, rheological, textural, functional, and sensory aspects were evaluated during storage at 4 and 10°C to determine changes in quality. Decreases in lactose and pH levels were observed and attributed to activity by spoilage microorganisms. The appearance of volatile compounds derived from lipids indicated that lipolysis was taking place, and some proteolysis was also noted. Minor variations in texture profile, torsion, color, and melt analyses were seen throughout 8 wk of storage. No microstructural changes were observed. A consumer taste panel generally liked laboratory-made and two commercially-made cheeses, and could not distinguish one of the commercial samples from the laboratory sample. The results provide a basis for assessing the quality traits of Queso Fresco during storage.

Keywords: Queso Fresco, Rheology, Texture, Microstructure

1. Introduction

Queso Fresco, a rennet-set soft white cheese, is gaining popularity in the US. It is often eaten fresh, hence its name, but many consumers and retail outlets would like the option of refrigerating it for a few weeks. To do this, the characteristics of Queso Fresco have to be investigated over time at typical storage temperatures. Home refrigerators in the U.S. are usually maintained around 4°C, but a significant number are kept at 10°C, and the average in Europe is 6.7-7.0°C (Pouillot, Lubran, Cates, & Dennis, 2010). Warehouse storage and delicatessen dairy cases may also be above 4°C. Changes with time on textural properties previously reported for this variety compared 4°C storage for 1 and 8 d (Sandra, Stanford, & Meunier Goddick, 2004) and 4°C storage for 1 and 8 wk (Guo, Van Hekken, Tomasula, Shieh, & Tunick, 2011a). Earlier research on Queso Fresco in our laboratory compared rheology, texture, and microstructure of commercial samples made from raw and pasteurized milk (Tunick & Van Hekken, 2010). Queso Fresco made in our laboratory and containing 0-2.5% NaCl has been evaluated for color, melt, rheology, and microstructure (Guo et al., 2011a) and for microbial count and proteolysis (Guo, Van Hekken, Tomasula, Tunick, & Huo, 2011b). Volatile compound identification has not been reported for this variety.

Queso Fresco has a pH over 6.0 and is commonly milled before salting. It is characteristically a crumbly cheese (Hwang & Gunasekaran, 2001) and a non-melting cheese (Guo et al., 2011a). Sensory tests revealed that Hispanics and those familiar with Queso Fresco preferred samples with high NaCl and pH levels, and consumers who had little previous experience with Queso Fresco preferred low NaCl and pH levels (Clark, Warner, & Luedecke, 2001). Its acceptability relies in part on its color (bright white), texture, and structure (Van Hekken & Farkye, 2003). This paper describes the quality traits of Queso Fresco from starter-free, pasteurized, homogenized milk and stored for up to 8 wk at 4 and 10°C. The research was divided into two phases: in Phase I the main focus was on the functional properties of color and melt, and in Phase II the emphasis was on consumer sensory tests and effect of storage on protein profiles and volatile compounds.

2. Materials and Methods

2.1 Cheese preparation

Queso Fresco cheeses were manufactured in eight production runs in the Dairy & Functional Foods Research Unit laboratory. Locally-obtained milk was standardized to 3.5% fat and pasteurized at 72°C for 15 s. After

two-stage homogenization at 6.9 and 3.4 MPa, 180 kg of milk containing 180 g of added $CaCl_2$ was adjusted to 32°C in a stainless steel vat. The milk was coagulated with chymosin (14 mL Chy-Max, Chr. Hansen, Milwaukee, WI, USA; diluted in 200 mL water) and cooked at 39°C for 30 min. Approximately one-third of the whey was drained and the curd was salted in three applications at 1.1% NaCl (w/w). The rest of the whey was drained and the curds were cooled to 21°C, finely milled into small (< 1 cm) pieces with a grinder (Bosch universal kitchen machine, Robert Bosch Hausgeräte, Dillingen, Germany), and hand-packed into molds for storage overnight at 4°C. Cheeses were removed from the molds the next day, sliced into three blocks, and vacuum packaged. Samples were stored for up to 8 wk at 4 or 10°C. Phase I cheeses consisted of Queso Frescos from the first five production runs, and Phase II cheeses were from the last three runs.

2.2 Composition

The following compositional analyses were run in triplicate: moisture (forced-draft oven, AOAC Official Method 948.12; AOAC International 1998), protein (EA1112 nitrogen analyzer, CE Elantech, Lakewood, NJ, USA; nitrogen result multiplied by 6.38), ash (heating in muffle furnace at 550°C for 16 h; AOAC Official Method 935.42; AOAC International 1998), lactose (YSI 2700 biochemistry analyzer, YSI, Yellow Springs, OH, USA), and pH (Orion model 611, Orion Research Corp., Cambridge, MA, USA). The following were run in duplicate: fat (Babcock method; Kosikowski & Mistry, 1997), NaCl (chloride titrator strip, Hach Co., Loveland, CO, USA, AOAC Official Method 971.19; AOAC International 1998), and titratable acidity (titration, AOAC Official Method 920.124; AOAC International 1998). Ash and titratable acidity were assayed on Phase II cheeses only.

2.3 Protein profiles

Protein profiles of Phase II cheeses were obtained by SDS-PAGE using the procedure of Van Hekken, Tunick, Tomasula, Molina Corral, and Gardea (2007) and Tunick, Van Hekken, Call, Molina Corral, and Gardea (2007). The PhastSystem (American Pharmacia Corp., Piscataway, NJ, USA) was used to separate proteins on 20% homogeneous gels. Gels were stained with Coomassie blue and scanned with a model 375A Personal Densitometer SI (Molecular Dynamics, Sunnyvale, CA, USA). Protein distributions were calculated with ImageQuant software version 4.2 (Molecular Dynamics). Lanes were analyzed in duplicate with bands being identified as α_{s1}-, α_{s2}-, β-, and para-κ-caseins, and minor whey proteins. Casein fragments were grouped into molecular mass ranges of 22, 22-18.5, 18-15, and <14 kDa.

2.4 Volatile compounds

In a preliminary study, volatile compounds in Phase II cheeses were identified qualitatively by GC-MS. Samples were removed from a -80°C freezer and a 5-g portion was finely diced and sealed in a 20-mL glass vial. The vial was incubated at 60°C for 10 min, and a SPME fiber (50/30 μm DVB/Carboxen/PDMS Stableflex, Supelco, Bellefonte, PA, USA) absorbed volatiles from the headspace at 60°C for 30 min. The sample was desorbed for 5 min into a 30 m x 0.250 cm ID column (Agilent Technologies, Wilmington, DE, USA) inside a 7890A GC equipped with a 5975C MS detector (Agilent). The injector temperature was 250°C and the splitless flow was 1 mL min^{-1} ultrapure He. The column temperature was held at 40°C for 10 min, ramped at 5°C min^{-1} to 225°C, and held for 5 min.

2.5 Texture

Texture profile analysis (TPA) was performed as previously described (Tunick & Van Hekken, 2002). Four cylinders measuring 14 mm diameter and 14 mm height were cut from the samples and compressed twice by 75% in a Sintech 1/G universal testing machine (MTS Systems, Eden Prairie, MN, USA) operating at a crosshead speed of 100 mm min^{-1}.

2.6 Rheology

Torsion gelometry and SAOSA were conducted as previously described (Tunick & Van Hekken, 2002). Torsion analyses were performed using a torsion gelometer (Gel Consultants, Raleigh, NC, USA) operating at 2.5 rpm. Four plugs were bored from each sample, milled to a capstan shape, and twisted until fracture. SAOSA was performed using an AR-2000 rheometer (TA Instruments, New Castle, DE, USA) with parallel aluminum plates. Three disks measuring 25 mm in diameter and 4 mm thick were used. Strain sweeps were run to determine the linear viscoelastic range and frequency sweeps were then performed from 1 to 100 rad s^{-1} at 0.8% strain. Data for elastic or storage modulus (G′) and viscous or loss modulus (G″) were obtained; values at 10 rad s^{-1} (1.6 Hz) are reported.

2.7 Color and melt

Color and melt properties were analyzed on Phase I cheeses based on the procedure described by Olson, Van Hekken, Tunick, Soryal, and Zeng (2007) as modified by Guo et al. (2011a). Briefly, six disks (5 mm thick, 38 mm in diameter) were prepared from each sample and initial color values for L*, a*, and b* were measured using a Hunter Lab ColorQuest XE 2382 colorimetric spectrophotometer (Hunter Associates Laboratory, Reston, VA, USA). Four measurements for each disk were made. Three disks were heated at 232°C for 5 min or at 130°C for 30 min. After cooling, color measurements were collected again and used to calculate total color change (ΔE), hue angle, and color saturation or chroma.

Meltability was determined in triplicate using the Schreiber Melt Test as described by Kosikowski and Mistry (1997). The extent of melting was measured on the disks that were heated to 232°C for 5 min and used to collect color data.

2.8 Microscopy

The microstructure was examined with scanning electron microscopy (SEM) as previously described (Tunick, Van Hekken, Cooke, Smith, & Malin, 2000). Cubes measuring about 5 mm on a side were cut from the interior of the cheese and fixed in glutaraldehyde. They were then dehydrated, defatted in a graded series of ethanol solutions, freeze-fractured, critical-point dried, and imaged in a Quanta 200 field emission gun SEM (FEI Co., Hillsboro, OR, USA) operated in the high vacuum, secondary electron imaging mode.

2.9 Sensory analysis

Hedonic, difference, and ranking evaluations were conducted on Phase II cheeses to compare the flavor and overall acceptance of laboratory-made Queso Frescos to commercial samples. Three cheeses were tested by an untrained consumer taste panel in booths on the same day using protocols for a large group (over 50 people) established by Meilgaard, Civille, and Carr (1999). The laboratory cheeses were evaluated 8-10 d after manufacture, following testing by an independent certified laboratory to ensure their microbial safety. Two commercially available 5-kg wheels of Queso Fresco were obtained. One sample, designated C-1, was received directly from the manufacturer < 24 h after it was made, and within 24 h of the manufacture of the laboratory cheese. Sample C-2 was obtained from a local distributor and estimated from its sell-by date to be 14-21 d old. The morning of the taste panel, cheese were removed from their packaging, cut into 2.5 cm^3 cubes, placed in 3-oz (84 mL) coded, lidded soufflé cups, and allowed to warm to 20°C. Sixty-five panelists were obtained in-house and received unsalted crackers and distilled water to cleanse their palates between samples.

In hedonic tests, samples were graded on a nine-point scale where 1 was "dislike extremely" and 9 was "like extremely." Triangle tests were used as difference tests; panelists tasted three cheeses, two of which were the same, and asked to identify the different one. In ranking tests, panelists tasted all three samples and ranked them by preference. Results are reported as raw numbers in accordance with Meilgaard et al. (1999).

2.10 Statistics

Statistical analyses were performed by analysis of variance with mean separation using the LSD technique (SAS 2004). Differences are described as significant when $P < 0.05$.

3. Results and Discussion

3.1 Composition

The composition of all samples was similar to Queso Frescos containing 2.5% NaCl that were prepared in earlier studies (Guo et al., 2011a, 2011b). The protein and lactose content of the Phase II cheeses were lower than those of Phase I and the NaCl content was higher (Tables 1 and 2). The fat, protein, and ash did not vary significantly over the 8 wk. The NaCl values were virtually identical for 4 and 10°C at each storage time, and the values significantly decreased with aging. Although the moisture levels were unchanged in the 4°C samples, all of the cheeses exhibited loss of whey during storage, and it is probable that some of the NaCl was dissolved in the lost whey.

The pH decreased and the lactose content and titratable acidity increased in the 10°C samples during storage. These results indicate that microorganisms were breaking down lactose and generating lactic acid at 10°C, but that this activity was insignificant at 4°C. The pH was above 6.0 in all of the samples.

3.2 Protein profiles

SDS-PAGE of Phase II cheeses revealed that α_{s1}- and β-caseins decreased and casein fragments increased with storage (Table 3). Chymosin cleaves α_{s1}-, β-, and κ-caseins, and plasmin, an indigenous milk proteinase,

hydrolyses α_{s1}-, α_{s2}-, and β-caseins (McSweeney, 2004). Over the 8 wk, the α_{s1}-casein decreased by one-fourth in the 4°C cheeses and by one-third in the 10°C cheeses. The levels of β-casein during storage went down by two-fifths at 4°C and by a half at 10°C. Similar results were reported previously (Guo et al., 2011b). Since no starter culture was used, the increased breakdown of casein at 10°C was apparently due to spoilage microbes introduced by contamination during processing. In the earlier study on Queso Fresco, which used the same facilities and procedures, bacteria found in the cheeses were identified as typical spoilage organisms found in dairy products: *Pseudomonas mandeli, Pseudomonas putida, Paenibacillus polymyxa,* and *Microbacterium oxydan* (Guo et al., 2011b). *Microbacterium* spp. is highly proteolytic (Hantsis-Zacharov & Halpern, 2007), and may have hydrolyzed the α_{s1}- and β-caseins.

3.3 Volatile compounds

GC-MS of the Phase II cheeses showed that a number of volatile compounds were generated during storage. Nonalactone and the methyl ketones 2-heptanone and 2-nonanone were noted at each storage time, and δ-dodecalactone appeared at 4 and 8 wk. The 10°C samples tended to contain more of these. The alcohols 3-methyl-1-butanol, phenyl ethyl alcohol, and 2,3-butanediol were present at 8 wk, and they were detected in the 10°C samples after 4 wk. The aldehydes pentanal, hexanal, and heptanal were present in the cheeses at 1 and 4 wk only, and presumably degraded into smaller compounds over the final 4 wk. Phenylacetaldehyde and 3-methyl butanal were present after 4 and 8 wk in the 10°C cheeses and after 8 wk in the 4°C samples. Nonanal and decanal appeared in every sample at every storage time. Acetic acid was observed at 4 and 8 wk.

Many of the volatile fatty acids normally found in cheese were also detected. Octanoic, decanoic, and dodecanoic acids were found throughout. An ester of propionic acid was also observed throughout, and nonanoic acid was seen at 8 wk, but butanoic and hexanoic acids were not observed. These results indicate that lipolysis was occurring. The major products of lipolysis and fatty acid catabolism include methyl ketones and secondary alcohols from β-ketoacid breakdown, lactones from hydroxyacid degradation, aldehydes from unsaturated fatty acid cleavage, and free fatty acids (Le Quéré & Molimard, 2002). All of these classes of compounds appeared in the samples. The cheesemilk was pasteurized, which should have inactivated lipolytic enzymes that may have been present, and starter culture and lipase were not added, so the lipolysis may have arisen from either the chymosin or from spoilage microorganisms. Lipolytic activity has not been reported for the brand of chymosin used in this study, implying that spoilage microbes are responsible. *Pseudomonas* spp. are known to have lipolytic activity in dairy products (Hantsis-Zacharov & Halpern, 2007), and it is possible that these microorganisms were responsible for products from lipid degradation.

3.4 TPA

Hardness is the amount of force required to compress a specimen, and cohesiveness is the ratio of the force-time areas from the two compressions. Hardness values were between 12 and 15 N for all of the samples (Tables 4 and 5), which was slightly higher than the 9.4-10.7 N range observed in a previous study (Guo et al., 2011a) but was low compared with other cheeses. For example, hardness values for fresh Cheddar, Gouda, and Mozzarella are 47, 77, and 68 N, respectively (Tunick & Van Hekken, 2002). The cohesiveness was between 0.13 and 0.21, in contrast to 0.21, 0.28, and 0.41 for fresh Cheddar, Gouda, and Mozzarella. Queso Fresco is thus quite compressible and not very cohesive, providing evidence for a crumbly texture. Using different experimental conditions than ours, Hwang & Gunasekaran (2001) and Sandra et al. (2004) determined Queso Fresco to be a crumbly cheese by TPA, finding consistency with sensory results. Springiness is a measure of the rebound of the specimen after the first compression, and ranged from 5.0 to 8.4 mm. In comparison, the Cheddar, Gouda, and Mozzarella have springiness values of 8.5 to 10.0 mm, meaning that Queso Fresco does not recover from compression very well. Chewiness is calculated by multiplying hardness, springiness, and cohesiveness, and the values of 10-24 mJ were much lower than those of other fresh cheeses (Tunick & Van Hekken, 2002). The cohesiveness, springiness, and chewiness values were within the ranges observed previously (Guo et al., 2011a).

None of the 10°C cheeses exhibited significant changes in the TPA values during storage, but the cheeses stored at 4°C lost some springiness and chewiness between 1 and 4 wk. The changes in the 4°C samples may have been due to loss of water during the test. The results were analogous to compressing a sponge. After 1 wk at 4°C, the first TPA compression squeezed a small amount of water from the specimen, which was able to recover somewhat for the second compression like a wrung-out sponge. More water was squeezed out of the other samples, and, like a saturated sponge, the specimens recovered less. The hardness was unaffected by water loss since it only measures the force required for the initial compression. Except for the springiness and chewiness values for the 4°C samples, the TPA data did not show significant differences from 1 to 8 wk despite the proteolysis that was observed. The β-casein levels decreased by half, but it is α_{s1}-casein that provides structural

strength to the cheese matrix. The lesser degradation of α_{s1}-casein did not affect the hardness or cohesiveness to a significant extent.

3.5 Rheology

The torsion shear stress is a measure of the force required to twist a sample until fracture, the torsion shear strain indicates the angular deformation at fracture, and the torsion shear rigidity (shear stress divided by shear strain) is a measure of the sample brittleness. The values for shear stress for the Queso Fresco samples were between 9 and 13 kPa (Tables 4 and 5); the values for fresh Cheddar, Gouda, and Mozzarella are between 42 and 50 kPa (Tunick & Van Hekken, 2002), which shows that Queso Fresco fractures easily. The shear strain values of 0.69 and 0.87 were similar to that of fresh Cheddar (0.83), but much lower than Gouda (1.13) and Mozzarella (1.56). The shear rigidity values were between 11.2 and 17.3 kPa, lower than those of Cheddar, Gouda, and Mozzarella (52, 44, and 31 kPa, respectively). Queso Fresco therefore does not undergo much deformation before breaking, and is rather brittle. These results are consistent with the crumbly characteristics of a typical Queso Fresco.

The shear strain for all samples increased from 1 to 8 wk, which may be a reflection of wheying off affecting the ability to twist the specimen. The 10°C samples had significantly higher shear stress and shear rigidity values at 4 wk than at 1 or 8 wk, which may have been due to a combination of wheying off and lack of sample homogeneity. The torsion values were all within the ranges found previously (Guo et al., 2011a).

In SAOSA, G′ is a measure of the energy stored and recovered per oscillation and G″ is a measure of the energy dissipated and lost as heat per oscillation. The G′ values for all of the cheeses were between 11.6 and 17.4 kPa, and G″ values were from 3.2 to 4.5 kPa (Tables 4 and 5); the ranges found in an earlier study were 14.5-19.0 kPa for G′ and 4.1-5.3 for G″ (Guo et al., 2011a). In comparison, the G′ and G″ values for fresh Mozzarella cheese were 36 and 14 kPa, respectively, and the values for Cheddar and Gouda were higher (Tunick & Van Hekken, 2002). Relatively low values for G′ and G″ indicate that bonds between particles are being made and broken during the observation time, either spontaneously or from applied forces (Tunick, 2010). The SAOSA data indicate that the structure of Queso Fresco is granular and crumbly. The SAOSA results did not vary with storage temperature or time.

3.6 Color and melt

None of the Phase I samples melted, which is expected of this style of cheese. The high pH levels ensured that colloidal $CaPO_4$ would remain in the casein matrix, preventing melting.

The color properties changed significantly upon heating (Table 6). The change in a* values upon heating from positive (magenta) to negative (green) resulted in large changes in hue, which is based on the ratio of b* to a*. The largest decrease in whiteness (L*) was measured in samples heated at 130°C for 30 min (baking conditions) and resulted in ΔE values 2.5 times higher than those for samples heated at 232°C for 5 min (broiling conditions). Chroma values averaged 8.9 before heating, 14.6 after heating at 130°C, and 14.1 after 232°C heating. The corresponding values found in a previous study were 7.7, 15.6, and 12.0, respectively (Guo et al., 2011a). The only significant ΔE increase during storage was for cheese heated at 130°C for 30 min, where the values increased to 29.37 at wk 8. All other values were stable over 8 wk of storage at 4°C ($P > 0.05$).

3.7 Microstructure

In the SEM images, the round dark areas correspond to fat globules and the light areas correspond to the casein matrix (Figure 1). All specimens exhibited the same patterns of fat globules 0.5-2.0 μm in diameter, surrounded by a granular and rough-surfaced matrix. The microstructure was similar to that seen in an earlier study (Tunick & Van Hekken, 2010). The fused matrix commonly found in semi-hard and hard cheeses that have pH levels from 5.0 to 5.6 was not observed. The microstructure of the Queso Fresco did not change appreciably with storage at 4 or 10°C. If the protein matrix had degraded with time, which occurs with ripened cheeses, the images at 8 wk would have revealed a more open structure and possibly aggregation of fat globules. No microorganisms were observed in any of the images. The SEM results were indicative of cheeses that were not very cohesive and did not show structural damage during storage, which was consistent with the rheological results.

3.8 Sensory analysis

A total of 65 participants (33 males, 32 females) evaluated the Phase II cheeses. Six were 30 yr old and under, 19 were 31-50, and 40 were at least 50 yr old. All panelists reported they consume cheese; 51 eat cheese multiple times a week and 35 consume Hispanic cheese at least once a month. Based on the hedonic scale results, all three cheeses were generally liked (Figure 2). The laboratory samples had the highest number of “like extremely” but

also had the highest number of "dislike slightly;" the overall average was "like slightly." The two commercial cheeses were in the "like moderately" category with C-1 having the highest number of "like very much."

In triangle tests, panelists could correctly distinguish laboratory from C-1 cheeses ($P < 0.001$) but could not separate laboratory and C-2 cheeses ($P > 0.1$). In ranking, C-2 was the first choice (average 1.74 of 3) whereas C-1 and laboratory were at 2.11 and 2.14, respectively. This result is interesting since C-2 had the highest score for liking and in ranking but could not be separated from laboratory cheese in side-by-side comparisons. The most common comment on ranking cheeses differently was perceived salt content. The laboratory cheeses, at 2.5% NaCl, were saltier than the commercial Queso Fresco at 1.8% NaCl.

4. Conclusions

Queso Fresco manufactured with pasteurized milk and without starter culture was analyzed throughout 8 wk of storage at 4 and 10°C. The cheese was crumbly and did not melt, and cheeses made in the laboratory could not be distinguished from a commercial sample by a consumer panel. Proteolysis and lipolysis occurred during storage, as evidenced in changes in lactose, pH, protein profiles, and volatile compounds, but had little or no effect on texture, rheology, melting, or microstructure. Cheesemakers and consumers can therefore store Queso Fresco for at least 2 mo without significant degradation of quality.

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Table 1. Composition of Phase I Queso Fresco cheeses during storage at 4°C (means from five production runs ± standard deviation)

	Storage time (wk)		
	1	4	8
Moisture (%)	$56.4^{a} \pm 0.9$	$56.0^{a} \pm 1.1$	$55.0^{a} \pm 1.0$
Fat (%)	$21.9^{a} \pm 1.4$	$21.2^{a} \pm 1.1$	$22.0^{a} \pm 1.2$
Protein (%)	$17.8^{a} \pm 1.0$	$18.0^{a} \pm 0.7$	$18.0^{a} \pm 0.9$
NaCl (%)	$1.79^{a} \pm 0.29$	$1.70^{a} \pm 0.20$	$1.51^{a} \pm 0.06$
Lactose (%)	$3.12^{a} \pm 0.40$	$3.31^{a} \pm 0.17$	$3.01^{a} \pm 0.18$
pH	$6.39^{a} \pm 0.10$	$6.27^{ab} \pm 0.09$	$6.22^{b} \pm 0.04$

[ab]Values with different superscript letters within the same row are different ($P < 0.05$).

Table 2. Composition of Phase II Queso Fresco cheeses during storage at 4 and 10°C (means from three production runs ± standard deviation)

	Storage time (wk)		
	1	4	8
Moisture (%)			
4°C	$56.2^{ab} \pm 1.7$	$55.9^{ab} \pm 2.9$	$56.7^{ab} \pm 2.3$
10°C	$56.9^{a} \pm 1.5$	$55.2^{ab} \pm 1.9$	$54.9^{b} \pm 1.9$
Fat (%)			
4°C	$21.8^{a} \pm 0.9$	$21.0^{a} \pm 0.9$	$21.5^{a} \pm 1.0$
10°C	$22.1^{a} \pm 0.5$	$21.6^{a} \pm 1.7$	$22.1^{a} \pm 1.3$
Protein (%)			
4°C	$15.2^{ab} \pm 1.0$	$15.7^{a} \pm 1.5$	$15.1^{ab} \pm 1.1$
10°C	$14.7^{b} \pm 0.8$	$15.3^{ab} \pm 1.6$	$15.0^{ab} \pm 0.5$
NaCl (%)			
4°C	$2.48^{a} \pm 0.04$	$2.30^{ab} \pm 0.32$	$2.13^{b} \pm 0.29$
10°C	$2.48^{a} \pm 0.04$	$2.30^{ab} \pm 0.30$	$2.12^{b} \pm 0.30$
Ash (%)			
4°C	$3.15^{a} \pm 0.35$	$3.39^{a} \pm 0.13$	$3.30^{a} \pm 0.43$
10°C	$3.21^{a} \pm 0.42$	$3.26^{a} \pm 0.30$	$3.26^{a} \pm 0.39$
Lactose (%)			
4°C	$2.71^{a} \pm 0.17$	$2.58^{a} \pm 0.20$	$2.68^{a} \pm 0.15$
10°C	$2.73^{a} \pm 0.26$	$2.50^{ab} \pm 0.25$	$2.45^{b} \pm 0.38$
Titratable acidity			
4°C	$0.15^{a} \pm 0.02$	$0.17^{a} \pm 0.03$	$0.20^{ab} \pm 0.03$
10°C	$0.14^{a} \pm 0.01$	$0.18^{a} \pm 0.04$	$0.33^{b} \pm 0.17$
pH			
4°C	$6.34^{a} \pm 0.07$	$6.38^{a} \pm 0.16$	$6.32^{a} \pm 0.18$
10°C	$6.32^{a} \pm 0.10$	$6.28^{a} \pm 0.18$	$6.10^{b} \pm 0.30$

[ab]Values with different superscript letters within the same group are different ($P < 0.05$).

Table 3. Percentages of caseins and casein fragments in Phase II Queso Fresco cheeses during storage at 4 and 10°C (means from three production runs ± standard deviation)

	Storage time (wk)		
	1	4	8
Caseins	4°C storage		
α_{s2}	7.46 ± 3.05	6.39 ± 1.85	6.15 ± 1.85
α_{s1}	37.98 ± 6.45	33.67 ± 7.37	29.40 ± 7.11
β	32.35 ± 3.69	25.27 ± 3.35	19.39 ± 2.11
para-κ	10.35 ± 0.68	11.36 ± 0.98	11.44 ± 1.42
Fragments (kDa)			
22	0	6.67 ± 2.62	8.32 ± 2.48
22-18.5	4.10 ± 2.88	7.85 ± 3.31	11.55 ± 1.97
18-15	3.02 ± 2.22	3.24 ± 2.83	4.86 ± 2.85
< 14	2.64 ± 1.32	4.79 ± 1.87	7.63 ± 2.44
	10°C storage		
Caseins			
α_{s2}	7.00 ± 2.08	6.78 ± 1.49	6.06 ± 1.40
α_{s1}	36.09 ± 1.73	28.17 ± 4.14	23.05 ± 7.89
β	30.99 ± 0.55	19.08 ± 6.85	15.79 ± 1.58
para-κ	11.17 ± 0.83	12.71 ± 2.19	12.62 ± 1.37
Fragments (kDa)			
22	0	8.41 ± 3.22	12.00 ± 5.49
22-18.5	5.45 ± 1.86	9.82 ± 1.69	12.82 ± 2.06
18-15	3.51 ± 0.86	4.32 ± 2.80	4.74 ± 2.71
< 14	3.76 ± 0.67	9.40 ± 4.28	11.64 ± 2.20

Table 4. Texture profile analysis and rheology of Phase I Queso Fresco cheeses during storage at 4°C (means from five production runs ± standard deviation)

	Storage time (wk)		
	1	4	8
Hardness (N)	14.1^{a} ± 3.0	14.4^{a} ± 3.3	13.0^{a} ± 2.6
Springiness (mm)	7.97^{a} ± 1.21	6.73^{b} ± 1.43	6.81^{b} ± 1.33
Cohesiveness	0.20^{a} ± 0.04	0.19^{a} ± 0.03	0.20^{a} ± 0.05
Chewiness (mJ)	22.8^{a} ± 7.8	18.0^{a} ± 5.7	18.3^{a} ± 7.8
Shear stress (kPa)	10.42^{a} ± 4.25	10.91^{a} ± 2.77	9.62^{a} ± 2.08
Shear strain	0.69^{a} ± 0.06	0.82^{b} ± 0.08	0.84^{b} ± 0.10
Shear rigidity (kPa)	15.21^{a} ± 6.57	13.47^{a} ± 4.20	11.65^{a} ± 2.88
Elastic modulus (kPa)	17.39^{a} ± 6.32	16.80^{a} ± 5.48	16.78^{a} ± 5.79
Viscous modulus (kPa)	4.51^{a} ± 1.60	4.42^{a} ± 4.73	4.50^{a} ± 1.24

[ab]Values with different superscript letters within the same row are different ($P < 0.05$).

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Table 5. Texture profile analysis and rheology of Phase II Queso Fresco cheeses during storage at 4 and 10°C (means from three production runs ± standard deviation)

	Storage time (wk)		
	1	4	8
Hardness (N)			
4°C	13.3[a] ± 3.0	12.5[a] ± 4.0	14.1[a] ± 3.5
10°C	12.2[a] ± 2.7	13.0[a] ± 4.4	13.1[a] ± 2.1
Springiness (mm)			
4°C	8.35[a] ± 0.49	5.06[b] ± 0.35	5.61[b] ± 0.54
10°C	6.41[b] ± 1.54	5.94[b] ± 1.21	6.19[b] ± 1.78
Cohesiveness			
4°C	0.17[a] ± 0.04	0.15[a] ± 0.04	0.13[a] ± 0.06
10°C	0.19[a] ± 0.04	0.18[a] ± 0.04	0.17[a] ± 0.09
Chewiness (mJ)			
4°C	23.8[a] ± 4.0	10.1[b] ± 2.9	11.1[b] ± 5.6
10°C	15.3[b] ± 7.1	14.1[b] ± 7.3	13.9[b] ± 8.3
Shear stress (kPa)			
4°C	10.0[ab] ± 1.8	11.7[ac] ± 2.6	10.9[ab] ± 1.9
10°C	9.4[b] ± 2.1	13.0[c] ± 3.9	9.4[b] ± 3.0
Shear strain			
4°C	0.71[a] ± 0.09	0.81[ab] ± 0.08	0.83[b] ± 0.12
10°C	0.76[ab] ± 0.09	0.76[ab] ± 0.05	0.87[b] ± 0.22
Shear rigidity (kPa)			
4°C	14.5[ab] ± 3.9	14.9[ab] ± 4.2	13.7[bc] ± 4.4
10°C	12.9[bc] ± 4.1	17.3[a] ± 5.8	11.2[c] ± 4.0
Elastic modulus (kPa)			
4°C	11.66[a] ± 6.13	11.59[a] ± 4.58	13.58[a] ± 6.66
10°C	13.09[a] ± 6.60	16.27[a] ± 8.78	15.42[a] ± 6.09
Viscous modulus (kPa)			
4°C	3.19[a] ± 1.65	3.27[ab] ± 1.40	3.89[ab] ± 2.00
10°C	3.50[ab] ± 1.80	4.40[b] ± 2.29	4.28[ab] ± 1.70

[abc]Values with different superscript letters within the same group are different ($P < 0.05$).

Table 6. Color properties of Phase I Queso Fresco cheeses after 1 wk storage at 4°C (means from five production runs ± standard deviation)

Color properties[1]	Before heating		After heating	
			130°C for 30 min	232°C for 5 min
ΔE			26.89[a] ± 4.56	9.96[b] ± 2.29
Hue	86.12[a] ± 1.21		-82.31[b] ± 3.93	-86.02[b] ± 3.25
Chroma	8.88[b] ± 0.63		14.57[a] ± 1.96	14.08[a] ± 1.34
L*	92.48[a] ± 0.93		67.53[c] ± 5.88	84.04[b] ± 3.77
a*	0.607[a] ± 0.203		-1.81[b] ± 0.66	-1.01[b] ± 0.82
b*	8.87[b] ± 0.633		14.29[a] ± 1.95	14.09[a] ± 1.32

[1]ΔE = total color change, hue = hue angle, chroma = color saturation, L* = whiteness, a* = magenta/green, b* = yellow/blue.

[abc]Values with different superscript letters within the same row are different ($P < 0.05$).

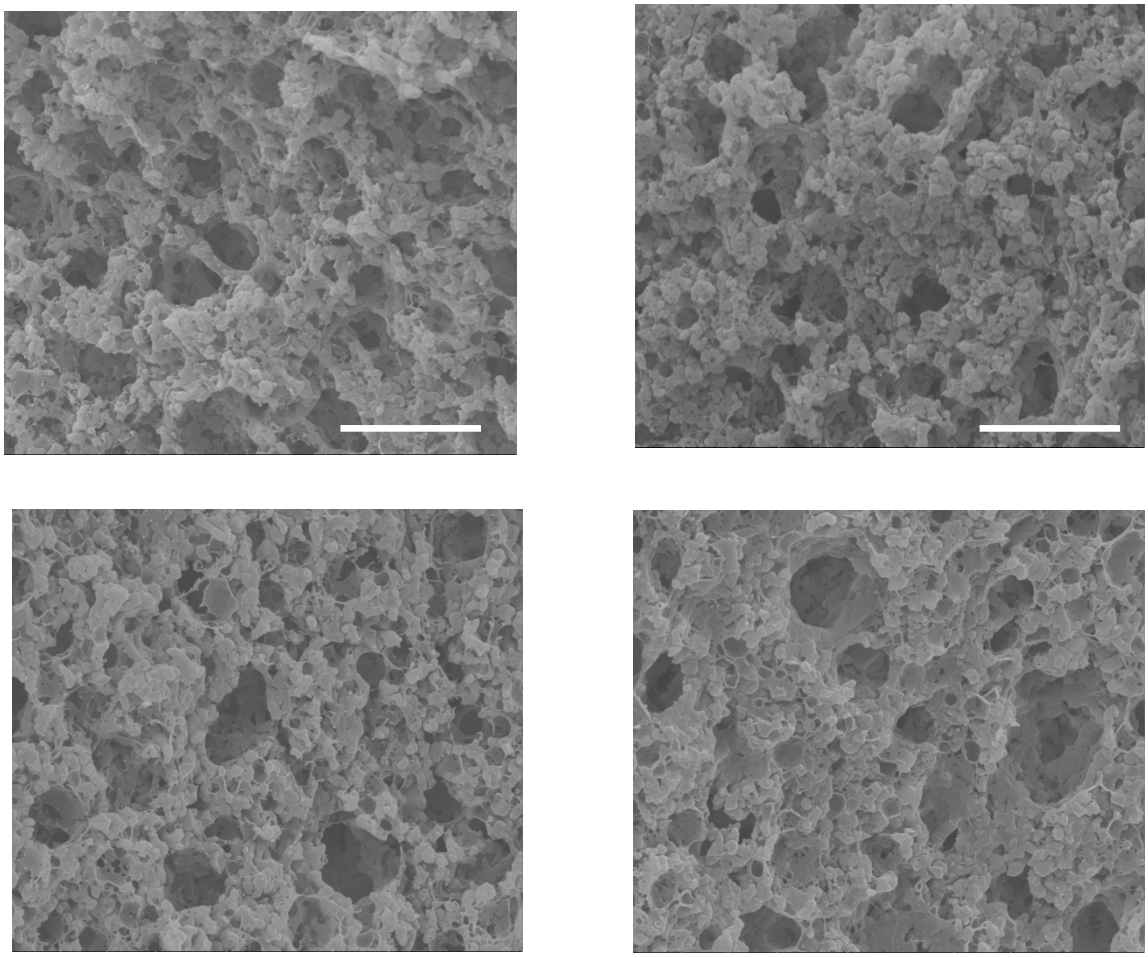

Figure 1. Scanning electron micrographs of Queso Fresco cheeses stored at 4 or 10°C for 1 or 8 wk

Upper left: 4°C, 1 wk. Upper right: 10°C, 1 wk. Lower left: 4°C, 8 wk. Lower right: 10°C, 8 wk. Bars at lower right of each micrograph measure 2.0 μm.

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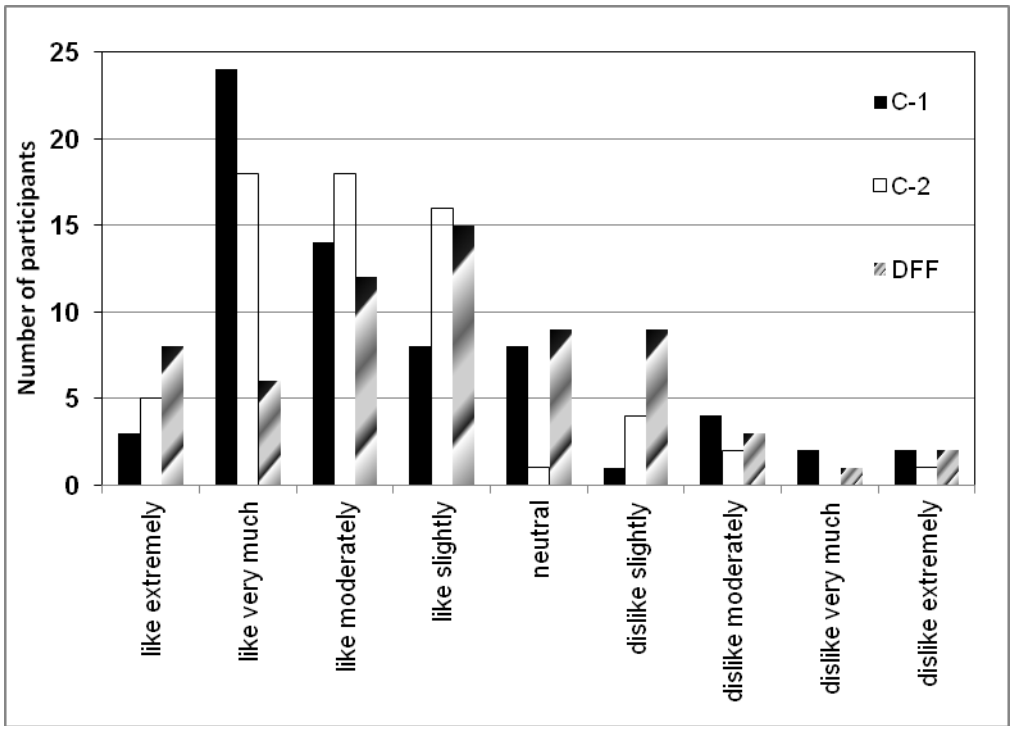

Figure 2. Hedonic scores for fresh Queso Fresco cheeses. C-1 and C-2 are two commercial samples and DFF was made in the Dairy & Functional Foods Research Unit laboratory

Determination of SpontaneousIgnition of SSSR and Fish Meal duringTransport and Storage

Naoharu Murasawa
Faculty of Risk and Crisis Management, Chiba Institute of Science 3
Shiomi-Cho, Choshi 288-0025, Japan
E-mail: rd10r01@cis.ac.jp

Hiroshi Koseki
National Research Institute of Fire and Disaster 4-35-3
Jindaiji-Higashimachi, Chofu 182-8508, Japan
Tel: 81-422-44-8331 E-mail: koseki@fri.go.jp

Yusaku Iwata
National Research Institute of Fire and Disaster 4-35-3
Jindaiji-Higashimachi, Chofu 182-8508, Japan
Tel: 81-422-44-8331 E-mail: iwata@fri.go.jp

Yasuhito Shibata
Fire Research Laboratory, Nagoya City Fire Bureau2280-12
Nagahazama, Shimoshidami, Moriyama-ku, Nagoya 463-0003, Japan
Tel: 81-52-736-2201 E-mail: 00kenkyu@fd.city.nagoya.lg.jp

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Abstract

Trends of wastes-recycle generated from the food industry have started to appear. There are some cases in which food wastes or products manufactured by recycling have generated heat at the low temperatures because of fermentation or oxidization, resulting in spontaneous ignition during transport or storage. In addition, the case in which depletion of oxygen in the storage area owing to fermentation and oxygen deprivation causing death of workers has been reported.In this study, Soy sauce squeezing residue produced during the brewing of soy sauce and Fish meal produced during the processing of Fish residue were considered. Hazard assessment tests focusing on heat analysis were conducted, aiming to acquire basic data for recurrence prevention.

Keywords: Soy sauce squeezing residue (SSSR), Fish meal, Fermentation, Spontaneous ignition, Thermal analysis

1. Introduction

Recently, there has been an increase in the trend to reduce waste generation as much as possible and carry out recycling and energy recovery to construct a recycling-oriented society (Hoshino, Iwata & Koseki, 2007; Shibata, Koseki & Shimizu). Even certain wastes that are difficult to recycle can be effectively used to the maximum by collecting heat energy generated by incineration in the form of electricity or steam. Examples of products manufactured by recycling wastes include wood chips manufactured from scrap wood (Li, Koseki & Momota, 2006), refuse-derived fuel (RDF) manufactured from household-generated waste (Fu, 2005; Li, 2008), refuse paper and plastic fuel (RPF) manufactured from waste paper and plastic and sludge fuel manufactured from

sewage sludge (Li, 2008). Existing studies have shown that fermentation may lead to heat generation and fire (Koseki, 2011).

Soy sauce and Fish play vital roles in the Japanese food culture. Sushi, which uses both soy sauce and Fish, is also a very popular Japanese food. Even in other countries, soy sauce and Fish are representative of the Japanese food culture. Soy sauce squeezing residue (SSSR) and Fish meal are waste products generated from their respective food industries. SSSR is nutritious and is used as feed in animal husbandry and as incinerator fuel. Fish meal is used as fertilizer and pet food. However, there have been cases in which SSSR and Fish meal have caught fire during transport or storage because of spontaneous ignition and oxygen deprivation in the storage area has led to death of workers. Fermentation is believed to be responsible for such hazards that involve the aforementioned wood chips or RDF.Because the composition of waste material is not fixed, simulation experiments are difficult and the investigation of the causes of hazards is even more difficult. When investigating the causes, the identification of the causes of and scenarios for heat generation in the early stages of fires, accidents and their processes is considerably difficult. In this study, various heat analysis methods were applied, and hazard assessment tests were conducted by focusing on SSSR and Fish meal to examine the causes and scenarios leading to spontaneous ignition and oxygen deprivation.

2. Experiments

2.1 Samples

A large amount of SSSRare produced when moromi (unrefined soy sauce) is compressed to extract raw soy sauce. When first produced, SSSR is plate shaped, but it is then finely ground for storage and processing (Figure 1). Fish meal refers to the crushed powder obtained by boiling residual Fish substances, removing water and oil from this residue by using a presser and then finally drying the remaining solid with hot air or steam (Figure 2).

To ascertain the effects of fermentation, samples were sterilized for seventeen hoursusing ethylene oxide gas (EOG). To ascertain the influence of fat and oil, samples were defatted for six hours using diethyl ether as solvent.

2.2 Thermo gravimetric differential thermal analysis

A thermo gravimetric differential thermal analysis (TG-DTA) system (RigakuThermoplus TG 8120) was used to study the overall thermal characteristics of the samples. An aluminium open container (0.05ml) was used as sample container. Figure 3 shows the schematic of TG-DTA. We studied the thermal characteristics of ~20 mg samples by heating them from room temperature to 600 °C at a rate of 2 K/min in air circulating at a speed of 150 ml/min.

2.3 Calorimetry

A Calvet calorimeter (Setaram C80, France) was used for additional thermal testing. The C80 (Figure 4) is a twin type highly sensitive heat-flux calorimeter. It can reduce the effects of evaporation of water contained in the sample by using a high-pressure closed vessel (8ml) and can make measurements from room temperature to 100 °C, which is a difficult temperature range to measure by TG-DTA. For these measurements, the temperature was increased at 0.1 K/min. A 1500 mg sample in a closed stainless steel vessel raised the temperature in the vessel from room temperature to 300 °C under a limited-air atmosphere.

2.4 Gas chromatography

To study gas emission during storage, an about 50 g sample was placed in a 1 L glass bottle that was sealed airtight and placed in a thermostat oven. The gas produced was collected (Figure 5) and measured by gas chromatography (Shimadzu, GC-14B) using standard gas (CO: 0.0500%, C_2H_6: 0.995%, H_2: 0.097%, CO_2: 0.996%, CH_4: 0.987%) for calibration and a thermal conductivity detector (TCD, 200 °C, sensitivity was 50 mA, carrier gas was Ar at 20 ml/min). The column temperature was ranged from 40 °C (6 min hold) to 80 °C (12 min hold) to 150 °C (10 min hold) and changed at a rate of 40 °C/min. Moreover, an air cylinder (O_2: 21 %,N_2: 79 %) was used as the standard gas for measurement of O_2 and N_2. For these measurements, we used a TCD (200 °C, 20 ml/min Ar carrier gas, 30 mA sensitivity) with the isothermal column temperature maintained at 30 °C.

3. Results and Discussion

3.1 Thermo gravimetric differential thermal analysis

The TG-DTA results with temperature increase rate of 2 K/min are shown in Figure 6, and a summary of the mass loss at 100 °C and the heat generation onset temperature is shown in Table 1. The heat generation onset temperature is the temperature at which the DTA curve shifted by 0.1 μV (0.01K) in the heat generation direction from the baseline where the DTA curve is constant. The horizontal axis shows the sample temperature and the

vertical axis shows the TG (weight change) and DTA (thermal behaviour). The bottom of the TG curve indicates weight decrease and the top indicates weight increase. For the DTA curve, the downward direction indicates an endothermic reaction and the upward direction indicates an exothermic reaction. The thermal decomposition of SSSR and Fish meal is divided into three phases.

Because of dehydration in the SSSR, the TG curve indicates a weight decrease from room temperature to 100 °C. These results show that about 26% of the SSSR water content was consumed during this period. Weight decrease and combustion due to decomposition of organic components was observed from 180 to 380 °C. During this phase, the weight decreased by about 70%. In the next-higher temperature range, we observed weight decrease and heat generation due to decomposition and combustion of carbide, until about 10% residue. From room temperature to 100 °C, dehydration in Fish meal led to about 7.8% mass loss.

A more moderate rate of mass loss was observed for Fish meal compared with that observed in SSSR, which resulted in a smaller overall heat-generation peak for Fish meal compared with SSSR. Thus, SSSR exhibited a more active heat-generation reaction. However, a larger amount of residue remained for Fish meal. In the DTA curve, the shift in the heat generation direction began at 172.9 °C for SSSR and around at 187.3 °C for Fish meal. Self-heat generation is assumed to begin at near these temperatures, and the fire occured.

3.2 Calorimetry

The results of increasing the temperature rate of SSSR and Fish meal to 0.1 K/min are shown in Figsure 7 and 8. In addition, the heat-generation-onset temperature and the total heat generated between room temperature and 100 °C is summarised in Tables 2 and 3. The heat-generation-onset temperature is defined as the temperature at which the heat generation direction given by the TG-DTA curve first starts to shift after beginning the measurement at room temperature. These results indicate that a more detailed thermal characterisation would result from using a closed pressure-resistant cell and increasing the sample volume than by TG-DTA measurements.

The heat-generation onset in unprocessed SSSR and Fish meal occurred at the temperatures below 30 °C. These results show that a fire is likely caused with spontaneous ignition when conditions are conducive to heat accumulation even if the materials were stored near room temperature. Furthermore, the likelihood of reaching the self-heat generation temperatures is high when the rate of heat generated by fermentation and oxidation exceeds the rate of heat externally released.

No heat generation was observed for EOG treated and sterilized SSSR and for SSSR defatted by diethyl ether between room temperature and 50 °C. For the defatted case, we attribute this result to the diminish of fermentation microorganisms during the defatting process.

In refard to EOG treated and sterilized Fish meal, the heat generation onset temperature was only slightly higher than that of untreated Fish meal. For defatted Fish meal, the heat generation onset temperature was higher than those of untreated and EOG-treated Fish meal. By comparing the results of SSSR and Fish meal at the beginning of the heat-generation period, we conclude that heat generation by SSSR is largely caused by microbial fermentation and heat generation by Fish meal, although influenced by fermentation, is affected more by fat oxidation. We attribute the heat generation observed from 50 to 100 °C to oxidation of residual fat that could not be defatted under the present conditions and to oxidation of other components.

3.3 Gas chromatography

The results of the C80 test indicate that, between room temperature and 50 °C, fermentation more strongly affects temperature increase in SSSR than in Fish meal. To further study the effect of gas generation and fermentation for storage at 50 °C, the sample storage temperature was changed from 5 to 50 °C, and the amount of gas generated was collected and measured. Since the water content is assumed to affect fermentation, a sample with 20% added distilled water was also measured.

Results for SSSR are shown in Table 4 and those for Fish meal are shown in Table 5. Figure 9–12show the oxygen concentration and carbon dioxide concentration against storage temperature based on the data of Tables 4 and 5. These results show that most of the gas generated from SSSR during storage was carbon dioxide. In addition, we found that Fish meal generates combustible gases, such as hydrogen and carbon monoxide, in addition to carbon dioxide.

Regarding SSSR, we found that carbon dioxide can be generated in amounts that can affect the human body (Wada, 2006), and the amount of carbon dioxide generated increased with the addition of moisture. Generation of a large amount of carbon dioxide was observed at temperatures below 25 °C, even when moisture was not added These results show that ventilation and the humidity level should be carefully monitored, even for storage

in a refrigerated space.

Regarding Fish meal, nearly no carbon dioxide was generated at or below 25 °C, even when moisture was added. However, above 25 °C, we found a rapid reduction of oxygen concentration and the generation of large amounts of carbon dioxide for samples with added moisture. For both SSSR and Fish meal, the amount of generated carbon dioxide increased with temperature between 5 and 30 °C but, at higher temperatures, the amount of carbon dioxide gradually decreased with increasing temperature. We attribute this result to more active fermentation in SSSR than in Fish meal, which is the same as for the C80 test. Moreover, we found that, when stored around 30 °C, SSSR and Fish meal primarily generated carbon dioxide, which reached doses lethal to humans.

4. Conclusion

1) We found that SSSR contained about 26% moisture, whereas Fish meal contained about 8%. Thermal decomposition of both SSSR and Fish meal occurred in three separate phases, but overall, SSSR exhibited a more active heat generation reaction. Moreover, the temperature at which start of self- heat generation was at about 173 °C in SSSR and at about187 °C in Fish meal. Fermentation and oxidation lead to an increase in temperature and, once the temperature reaches this level, self-heat generation begins, which can lead to fire due to spontaneous ignition.
2) The onset of heat generation was observed in both SSSR and Fish meal at 30 °C or below. Between room temperature and 50 °C, we found that heat generation in SSSR was more affected by fermentation, whereas heat generation in Fish meal was more influenced by fat oxidation. Thus, storage of these materials under conditions of high temperatures and humidity should be avoided, and storage bags should be sealed to avoid contact with the air to the extent possible.
3) Finally, we found that significant quantities of carbon dioxide were generated under sufficiently moist conditions, even for low-temperature storage. The largest amount of carbon dioxide was generated (and reached lethal levels) when the SSSR and Fish meal were stored in a closed environment at temperatures around 30 °C. To prevent accidents due to oxygen deficiency and to protect the health of the workers, we advise measuring the oxygen concentration of the storage area with an oximeter that can be read from outside the storage area. In this way, the safety of the storage area may be verified before entering.

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Table 1. Mass loss at 100 °C and heat-generation-onset temperature

Sample	Mass loss at 100 °C (%)	Heat generation onset temperature (℃)
SSSR	25.6	172.9
Fish meal	7.8	187.3

Table 2. Heat generation onset temperature and total heat generated for SSSR (room temperature to 100 °C)

Sample	Heat generation onset temperature (°C)	Total heat generation (J/g)
SSSR	27.8	12.9
EOG treated SSSR	53.8	10.8
Defatted SSSR	55.1	10.3

Table 3. Heat generation onset temperature and total heat generated for Fish meal (room temperature to 100 °C)

Sample	Heat generation onset temperature (°C)	Total heat generation (J/g)
Fish meal	41.2	13.2
EOG treated Fish meal	42.3	12.9
Defatted Fish meal	51.3	11.9

Table 4. GC results for SSSR (5 to 50 °C)

Sample name	Storage Period	Storage Temperature	GC Analysis Results (%)		
			O_2	N_2	CO_2
SSSR	10 days	5℃	18.57	77.39	1.25
SSSR + Distilled water 20%			12.33	77.78	5.83
SSSR		15℃	16.21	78.76	2.35
SSSR + Distilled water 20%			5.32	79.32	15.31
SSSR		25℃	1.71	79.38	16.08
SSSR + Distilled water 20%			1.66	79.12	18.19
SSSR		30℃	1.65	81.20	16.55
SSSR + Distilled water 20%			1.54	79.63	18.22
SSSR		40℃	2.13	79.20	15.31
SSSR + Distilled water 20%			0.89	80.28	16.43
SSSR		50℃	12.76	80.22	5.62
SSSR + Distilled water 20%			10.23	78.90	7.53

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Table 5. GC results for Fish meal (5 to 50 °C)

Sample name	Storage Period	Storage Temperature	GC Analysis Results (%)				
			O_2	N_2	H_2	CO	CO_2
Fish meal	10 days	5℃	20.42	76.67	-	-	0.04
Fish meal + Distilled water 20%			19.62	77.13	-	-	0.13
Fish meal		15℃	20.30	76.82	-	-	0.04
Fish meal + Distilled water 20%			18.65	77.46	-	-	0.57
Fish meal		25℃	20.11	77.42	0.0006	0.003	0.49
Fish meal + Distilled water 20%			3.95	83.31	0.0010	0.014	11.60
Fish meal		30℃	14.28	81.26	0.0025	0.050	0.85
Fish meal + Distilled water 20%			3.47	82.10	0.0058	0.0073	12.34
Fish meal		40℃	16.06	79.70	0.0029	0.0406	0.62
Fish meal + Distilled water 20%			5.67	80.52	0.0293	0.0714	10.55
Fish meal		50℃	17.21	79.22	0.0058	0.1491	0.54
Fish meal + Distilled water 20%			6.88	80.23	0.0069	0.2061	8.53

Figure 1. Soy sauce squeezing residue (SSSR)

Figure 2. Fish meal

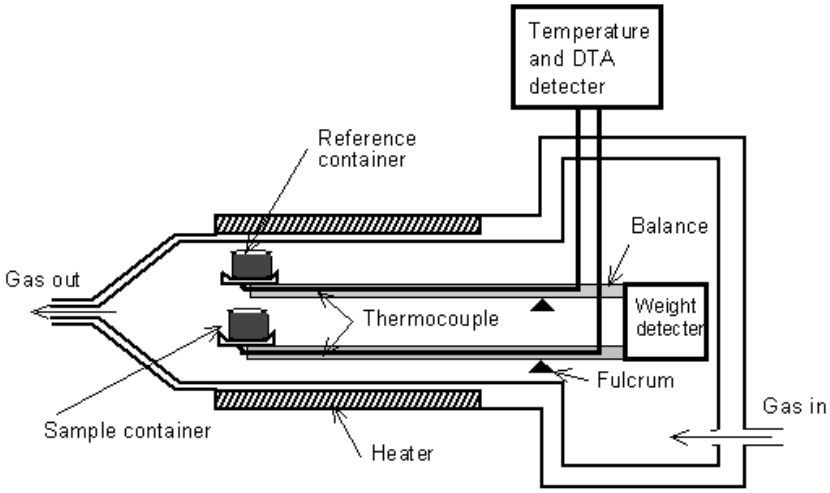
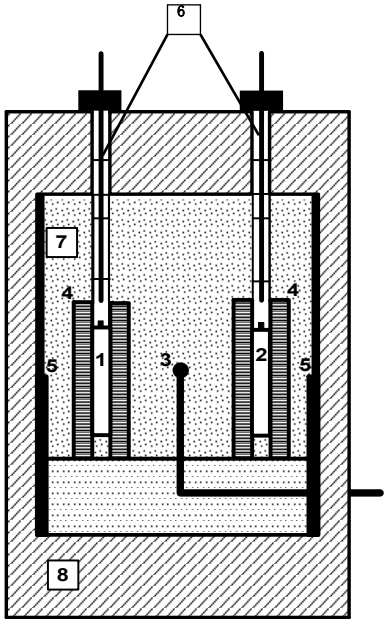


Figure 3. Schematic of TG-DTA



1: Reference vessel
2: Sample vessel
3: Thermostat
4: Heat-flow detector
5: Heaters
6: Insulating space
7: Calorimetric block
8: Insulating material

Figure 4. Schematic of C80

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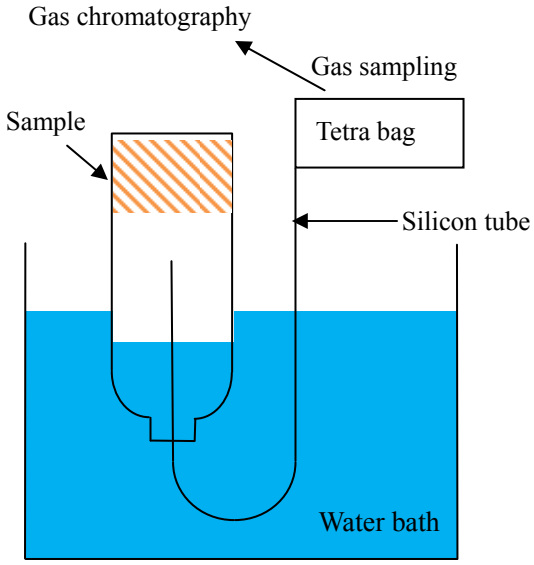

Figure 5. Schematic of gas chromatography measuring device

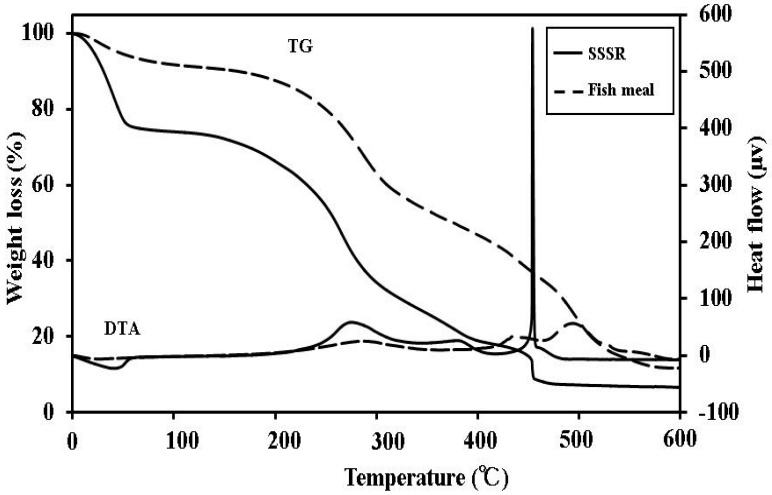

Figure 6. TG-DTA results for SSSR and Fish meal (2 K/min)

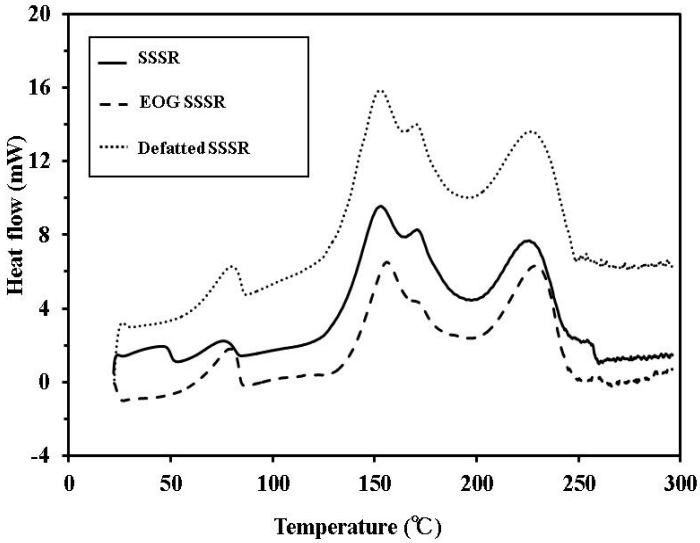

Figure 7. C80 results for SSSR (0.1 K/min)

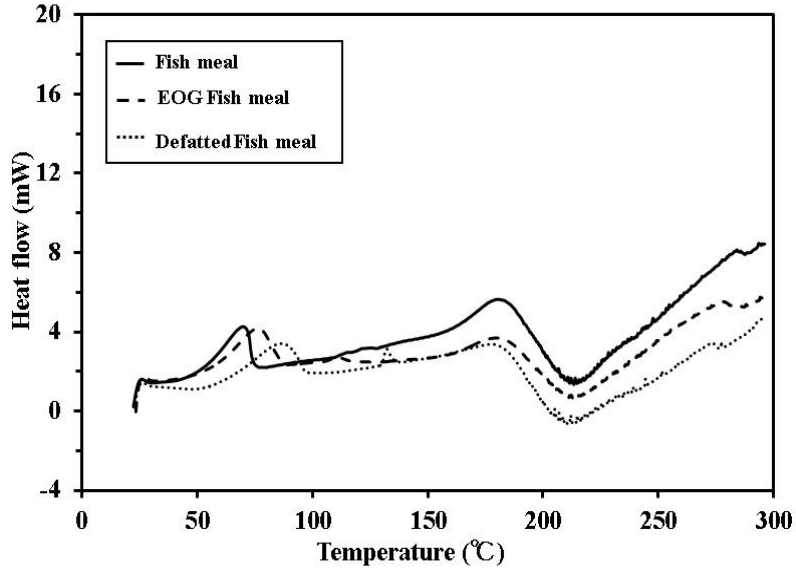

Figure 8. C80 results for Fish meal (0.1 K/min)

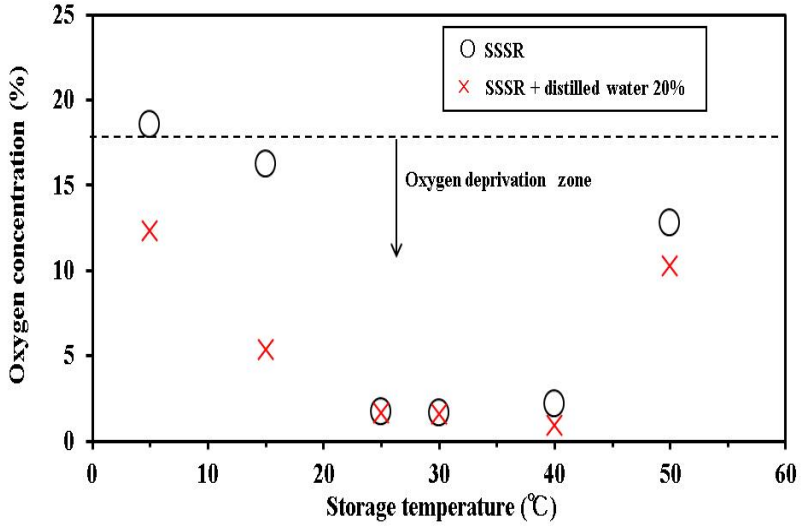

Figure 9.Correlation between storage temperature and oxygen concentration (SSSR)

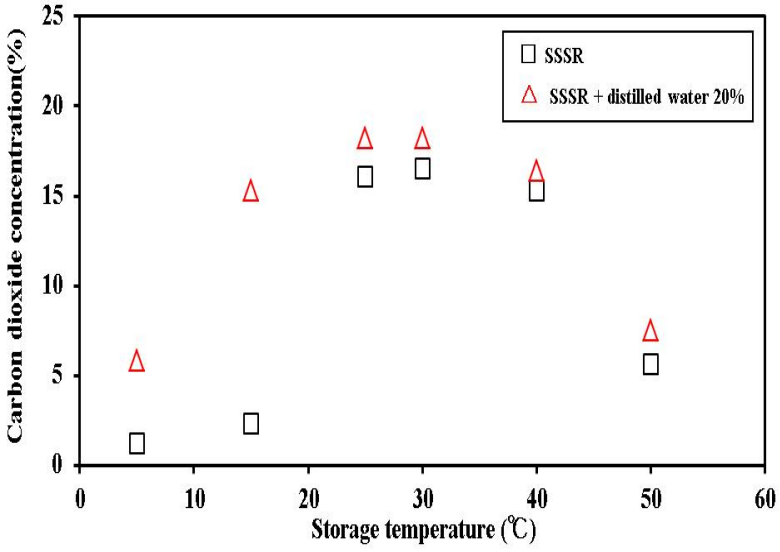

Figure 10. Correlation between storage temperature carbon dioxide concentration (SSSR)

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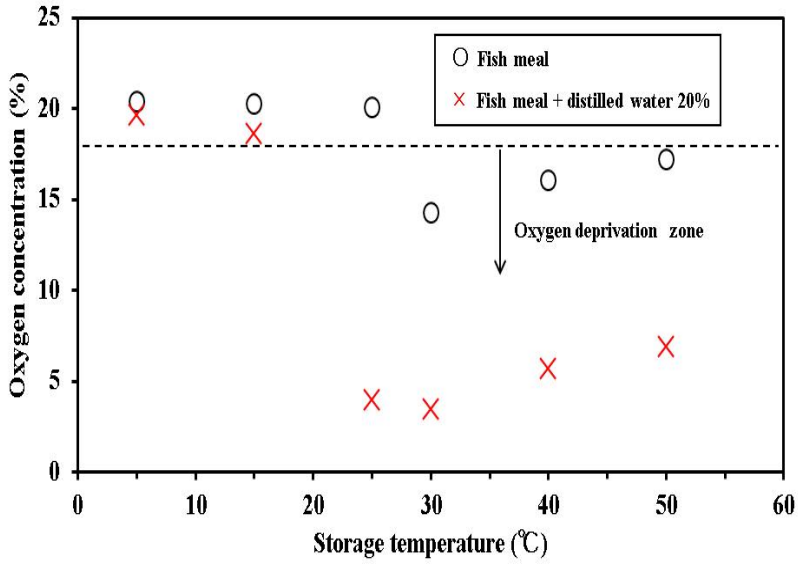

Figure 11. Correlation between storage temperature and oxygen concentration (Fish meal)

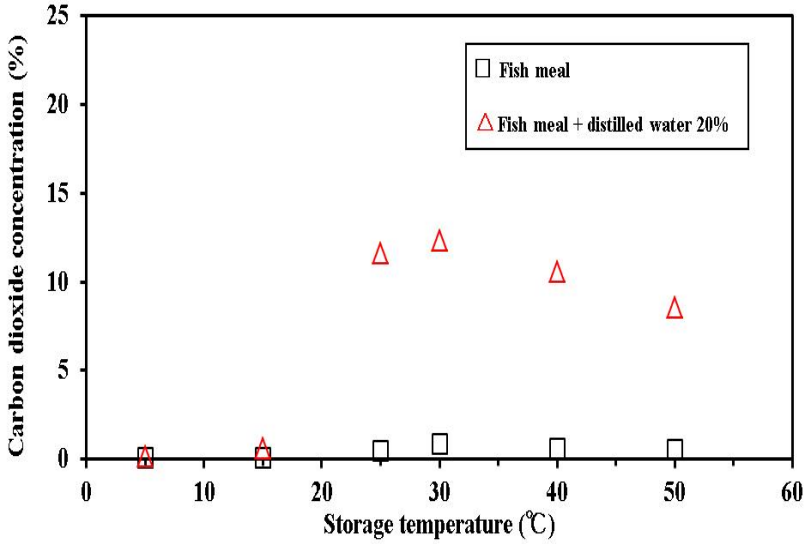

Figure 12. Correlation between storage temperature and oxygen concentration (Fish meal). and carbon dioxide concentration (Fish meal)

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Address 1120 Finch Avenue West, Suite 701-309, Toronto, ON., M3J 3H7, Canada
Telephone 1-416-642-2606
Fax 1-416-642-2608
E-mail jfr@ccsenet.org
Website www.ccsenet.org/jfr

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