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Optimization of Pressure Parboiling Conditions and Pre-Conditioned Moisture Content of Brown Rice (Unpolished Rice) for Microwave Puffing and its Comparison with Hot Sand Bed Puffing

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Abstract

Brown rice puffing (unpolished rice) gives a more nutritious product compared to traditional puffed polished rice and reduces the cost of the product significantly, even though, the rice bran layer resists expansion during puffing. In the present study, brown rice was puffed in a microwave and hot-sand-bed after its pressure parboiling. Pressure parboiling parameters, steam pressure (196, 294, 392, 490 kPa) and steaming time (5, 10, 15 min), along with pre-conditioned moisture content (8, 10, 12 % wb) were studied and optimized for puffing characteristics (puffing percentage, expansion ratio, whiteness index, bulk density and hardness). All the experiments were carried out using a full factorial design. Statistical analysis showed there was a significant effect of processing variables on puffing characteristics. Optimized steam pressure, steaming time and pre-conditioned moisture content for microwave puffing were found to be 303.6 kPa, 14.25 min and 11.6% (wb) respectively, and for hot-sand-bed puffing to be 260.7 kPa, 15 min and 8% (wb) respectively.

Keywords: Brown rice puffing; Cereal puffing; Microwave puffing; Hot-sand-bed puffing; Pressure parboiling

1 Introduction

In the modern lifestyle, consumers are looking for nutritious products that are easily accessible and conveniently prepared. Bulk handling and storage of puffed rice is inconvenient for vendors and families who prefer fresh puffed rice. During long periods of storage, puffed rice losses its crispiness, which is one of the most important sensory qualities of any puffed product, due to moisture absorption. Thus, microwave puffing of pre-packed, pre-conditioned brown rice provides a good option for obtaining a nutritious, hygienic and convenient product to meet the needs of this group of consumers on the go.

Puffed rice is a popular and traditional snack food in Southeast Asia. It is produced from parboiled polished rice by roasting it on an agitated hot-sand-bed at around 250 ^{o}C for 10 to 12 s. Other commercial puffing processes for rice are hot air puffing, gun puffing and oil puffing. However, microwave puffing is a recent development in this aspect (Maisont & Narkrugsa, 2009).

Puffed unpolished rice is receiving more interest than puffed polished parboiled rice because of the excellent health beneficial properties of the bran

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Nomenclature

P	Steam pressure, kPa	ER	Expansion ratio
T	Steaming time, min	WI	Whiteness index
M	Pre-conditioned brown rice moisture content, % (wb)	BD	Bulk density, kg/m^3
		Hd	Hardness, N
PP	Puffing percentage	D	Desirability

(Mir, Bosco, Shah, & Mir, 2016; Swarnakar, Srivastav, & Das, 2019). Rice bran is a good source of vitamins, minerals, fibre, protein and fat (Gul, Yousuf, Singh, Singh, & Wani, 2015). Polishing removes most of these nutrients and the extent of removal is proportional to the degree of polishing (Lamberts et al., 2007). It is pertinent to mention that, polishing is the most energy intensive step in the whole rice milling operation (Mohapatra & Bal, 2007), utilising approximately 43% of the total energy input (Ekasilp, Soponronnarit, & Therdyothin, 1995). Thus, puffing of brown rice could be economically beneficial if the quality of the product meets both the nutritional and organoleptic requirements of the consumers. Earlier efforts to puff brown rice using hot air were not satisfactory in terms of product quality (Chandrasekhar, 1989). With this present background, production of puffed rice from parboiled brown rice with acceptable quality attributes is a challenge.

The expansion ratio is a highly important quality parameter for any puffed product. Earlier studies show that expansion increased with an increase in the degree of gelatinization of native starch but decreased with starch retrogradation (Chinnaswamy & Bhattacharya, 1986; Mahanta & Bhattacharya, 2010). Pressure parboiling can gelatinise rice up to the centre, with less retrogradation (Ali & Bhattacharya, 1982). In the normal parboiling process, paddy is soaked for a few hours in water at 70 ^{o}C followed by steaming with moderate pressure steam (Bhattacharya, 2011). It requires a long time to complete the process. In the pressure parboiling process, partially soaked paddy is treated with pressurised steam (Ali & Bhattacharya, 1982). Compared to the normal parboiling process, pressure parboiling is a relatively quick process (Agidi, Dauda, & Igbeka, 2008; Roy et al., 2008).

In this study, puffing of pressure parboiled brown rice using microwave energy was chosen for convenience and hygienic reasons. The product quality was compared with that of the conventional process using hot-sand-bed puffing. Chandrasekhar (1989) has reported that about 0.6 % (w/w) of sand particles adhere on the surface of puffed rice in hot sand bed puffing. Several studies have been reported on microwave puffing of cereals (Maisont & Narkrugsa, 2009; Mishra, Joshi, Mohapatra, & Babu, 2015; Swarnakar, Kalpana Devi, & K. Das, 2014). Thus, present work aimed to investigate the effect and optimize the parboiling pressure, time and preconditioned moisture content of brown rice for its puffing in the microwave oven and comparision with hot-sand-bed puffing methods.

2 Materials and Methods

2.1 Rice variety

A high amylose content rice variety (IR 1010) was chosen for this study. Mahanta and Bhattacharya (2010) suggested that a high amylose content rice variety was a better option for puffing. Paddy was procured from the local market in Kharagpur, West Bengal, India. This IR 1010

is a slender rice variety, with a length to breadth ratio of 3.4 ± 0.2 (International Rice Research Institute, 2013), and its amylose content was estimated as 25.1 ± 0.4%.

2.2 Pressure parboiling of paddy

Parboiling of unhusked raw rice was carried out using steam pressure ranging between 196 and 490 kPa. A pressure vessel used in this parboiling process was fabricated. Detailed features of this vessel are shown in Fig. 1. About 2 kg cleaned paddy was placed inside the perforated vessel (2) and it was allowed to soak in normal tap water *in situ* for 7 min at ambient temperature and fully submerged conditions. After draining the water, the vessel was then closed. Steam from a high-pressure steam generator was allowed to pass through the perforated pipe (8). The desired steam pressure inside the vessel was regulated by operating the vent valves (10). Steaming was continued for the desired duration of time. After the steaming operation, the parboiled paddy was taken out and spread on a tray. Then the tray was put inside a hot air oven at 80 ^{o}C for drying of the paddy. The dried parboiled paddy was dehusked using a laboratory twin rubber roll dehusker (Model THU35A, Satake Engineering, Japan) equipped with an aspiration system for separation of the husk from brown rice. Broken kernels were separated from the whole brown rice using a laboratory rice grader (Burrows, Illinois, USA).

2.3 Preconditioning of parboiled brown rice

Pre-conditioning of the brown rice is a vital step. In this step, the desired level of salt (in water solution) was allowed to diffuse slowly into the rice kernels. About 200 g pressure parboiled brown rice was mixed with the 40 ml salt solution and it was kept for 1 h at room temperature for equilibration. The final salt content in the rice was maintained at around 3.5 % (w/w) as described by an earlier study (Minati & Das, 2011). The salt-infused brown rice was dried in a laboratory fluidized bed dryer (Lab model, Basic Technology, India) at 70 ^{o}C with an air flow rate around 3 m/s (Das, 2013) till the desired moisture content was obtained. The moisture content of the pre-conditioned brown rice was measured using a standard hot air oven method (Official methods of analysis. Association of Official Analytical Chemists, 1990). This process of heating and moisture regulation is called pre-conditioning of rice. The pre-conditioned brown rice was packed in an airtight container and kept for subsequent experiments.

2.4 Puffing of preconditioned brown rice

Puffing of pre-conditioned brown rice was carried out using a domestic microwave oven (Model: M197DL, SAMSUNG, India) and hot-sand-bed roasting for comparison. In microwave puffing, about 10 g pre-conditioned brown rice was packed in a paper envelope (245×100 mm) and sealed with adhesive tape. The packet was placed at the center of the turn-table of a 28-liter capacity domestic microwave oven. It was set for 35 s heating at its maximum output power level of 1000 W.

Hot-sand-bed puffing was carried out in batches using a hemispherical round bottom metal vessel containing coarse grade sand. The sand was heated at the bottom of vessel and grain was put into it with continuous agitation for uniform distribution of heat and proper heat transfer. Generally, the ratio of sand to rice was about 10:1. The grains were dropped on to the sand-bed when its temperature reached around 210-240 ^{o}C (Smita, 2008). After the completion of puffing, the puffed grains were removed from the sand bed, and a fresh batch of grain was dropped and puffed similarly. A small number of fine sand particles normally remained loosely adhered at the surface of the puffed rice. These sand particles were separated using a sieve and returned to the vessel.

2.5 Puffing characteristic of puffed brown rice

The puffing percentage (PP) and the expansion ratio (ER) were determined by using eqs. 1 and 2, respectively (Minati & Das, 2011). Puffing

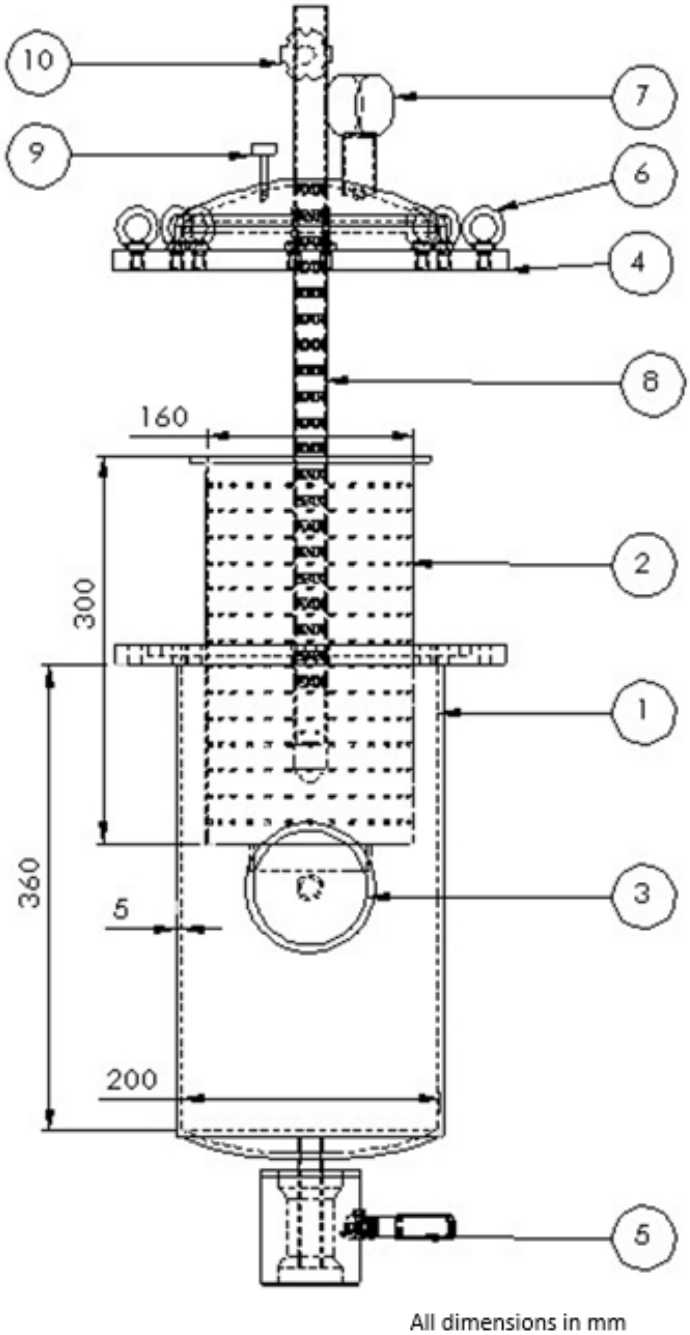

Figure 1: Front views of the pressure parboiling system - (1) Outer shell (2) perforated paddy container for holding paddy (3) dial thermometer (4) closer ring (5) steam condensate outlet (6) lid tightening bolt (7) pressure gauge (8) perforated pipe for steam distribution in paddy bed (9) safety valve (10) steam inlet pipe with vent valve

percentage is the ratio of the number of puffed rice (NP) to the total number of rice initially taken (N_i). Thus, mathematically it is

$$PP = \frac{N_p}{N_i} \times 100 \quad (1)$$

The expansion ratio is the ratio of the volume of the puffed rice to the volume of the initial rice. In order to obtain the volume of puffed rice, a sand displacement method was used as described by Minati and Das (2011). Puffed rice was poured into a graduated glass measuring cylinder. The voids in that bed were filled with clean (all organic materials removed by acid and alkali treatment) and completely dry fine sand with tapping, and the total volume was measured (V_t). This was followed by separation of sand and the puffed rice by a shaking-screen. After separating the sand from the puffed rice, the volume of sand was measured again. This is a void volume (V_v). The difference (V_t - V_v) is the volume of the puffed rice. The volume of rice before puffing (V_i) was measured by the same methodology. Thus, mathematically, ER is expressed as

$$ER = \frac{V_t - V_v}{V_i} \quad (2)$$

The quality of the puffed brown rice, viz., colour, bulk density (BD) and hardness (Hd) were measured as follows. Colour was measured in the L, a and b Hunter colour coordinate system using a Croma Meter (CR-400, Konica Minolta, Japan). Whiteness index (WI), a derived parameter, was estimated using eq. 3 following the methodology described by (Hsu, Chen, Weng, & Tseng, 2003). Three readings were taken and then averaged.

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (3)$$

Bulk density of puffed brown rice samples was estimated from the weight and corresponding bulk volume of the puffed rice. A known weight of puffed rice was placed in a graduated measuring cylinder, and after gently tapping ten times on the counter top, the volume was noted (Mariotti, Alamprese, Pagani, & Lucisano, 2006). The mean of three measurements was recorded.
Hardness of the puffed rice samples was measured using a texture analyser (TA-XT2i, Stable Microsystem, UK), with a probe diameter of 25 mm, 25 kg load cell and 10 mm/s cross head speed (Nath, Chattopadhyay, & Majumdar, 2007). The mean value of six readings for each of the samples was recorded.

2.6 Experimental design and optimization

The experiments were designed according to full factorial design on four levels of parboiling steam pressure (196, 294, 392, 490 kPa), three levels of steaming time (5, 10, 15 min) and three levels of pre-conditioned brown rice moisture content (8, 10, 12 % wb). Table 1 shows the details of these independent variables along with dependent variables. In order to visualize the effect of independent parameters (X) on the dependent parameters (Y) the following eq. 4 was used.

$$Y = b_0 + b_1X_1 + ... + b_{12}X_1X_2 + b_13X_1X_3 + ... + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 ... + \xi \quad (4)$$

Where b_o (constant term) b_1, b_2, ... (linear effect) b_{12}, b_{13},... (interaction effect) b_{11}, b_{22},... (quadratic effect) are the coefficients and ξ is the random error associated with them.

Statistical analysis:

The significance of all the terms in the regression equation was evaluated by analysis of variance (ANOVA). The adequacy of a regression equation was checked by the R^2 (coefficient of determination), adjusted R^2, predicted R^2, model p-value, adequacy precision value and coefficient of variation (CV%) (Giri & Prasad, 2007; Mohapatra & Bal, 2007). Regression equations of the dependent variables were obtained after rejecting the non-significant terms at the 95% confidence level ($p < 0.05$). Response surfaces between dependent and independent variables were produced after keeping the third independent variable at its centre level.
An ANOVA table of each regression equation coefficients was also generated by Design Expert software (Design Expert, version 7. 0. 0, Stat-Ease INC., 2009, USA) to determine the effect on the dependent variables. The significance of

Table 1: Independent and dependent variables used in puffing of brown rice

Independent variables (X)	Levels	Dependent variables (Y)
Steam pressure (P), kPa (gauge pressure)	196, 294, 392, 490	Puffing percentage (PP) (for microwave puffing only), Expansion ratio (ER), Whiteness index (WI), Bulk density (BD), kg/m3, and Hardness (Hd), N
Steaming time (T), min	5, 10, 15	
Pre-conditioned brown rice moisture content (M), % wb	8, 10, 12	

all the terms of independent variables in the regression equation was judged statistically at 95% confidence interval ($p < 0.05$).

Optimization:

Numerical optimization was carried out for optimizing processing variables in software (Design Expert, version 7. 0. 0, Stat-Ease INC., 2009, USA). Optimum condition was selected on the basis of higher overall desirability (D) value (eq. 5). D is a function of the individual desirability (d_i) of each variable that varies between 0 and 1 (Montgomery, 2017).

$$D = [d_1(y_1) \times d_2(y_2) ... \times d_k(y_k)]^{(1/k)} \quad (5)$$

In this equation, k denotes the number of variables. All independent variables were kept in the range while dependent variables PP, ER and WI were maximized and those of BD and Hd were minimized.

3 Results and Discussion

3.1 Model accuracy

Regression models obtained for responses in the microwave and hot-sand-bed puffing are shown in Table 3. Positive and negative terms of the models explain the positive and negative effect of processing variables on quality characteristics, respectively. Quadratic terms of processing variables showing its effect on quality characteristics are curvilinear. The factors that were insignificant ($p > 0.05$) were excluded from the models without affecting the models' hierarchy. It is inferred from Table 3 that all the regression models were found to be statistically significant at the 99.99% confidence level. The coefficient of variation of all the models except BD and Hd were found closer to 10 which indicates a good fit of the models (Giri & Prasad, 2007). All the models showed good fits with more than four adequate precision values, reasonable R^2 value and difference less than two between adjusted R^2 and predicted R^2. Thus, these models can be used for prediction of quality characteristics of puffed brown rice in microwave and hot-sand-bed puffing.

3.2 Puffing characteristic of pressure parboiled puffed brown rice

Puffing percentage

The effect of steaming pressure, time and preconditioned brown rice moisture content on PP during microwave heating is shown in Fig. 2. Parboiling at 196 kPa for 5 min was not found to be suitable for brown rice puffing. A white belly or ungelatinized core was observed in the brown rice which was due to the incomplete gelatinization of rice starch as mentioned by Ali and Bhattacharya (1982). Gelatinisation is the primary criteria for puffing of rice as it seals the cracks in grains and hardens the rice kernel which acts like a pressure vessel during puffing (van der Sman & Bows, 2017). In ungelatinised rice, during the puffing process in presence of moisture, rice starch undergoes a gelatinisation process and reduces the amount of moisture to create a proper vapour pressure for expansion (Gulati & Datta, 2016). Thus, increasing the pressure and

time up to 400 kPa and 12 min, respectively, increases the extent of gelatinisation which in turn has a positive effect on PP. Further increases in both of these variables leads to an increase in starch retrogradation and a negative impact on puffing (Chinnaswamy & Bhattacharya, 1986). This may be due to the fact that after 400 kPa and 12 min of rice steaming there was an adverse effect on PP (Fig. 2 a). Pre-conditioned brown rice moisture content has a positive but less significant ($p < 0.05$) effect on PP as compared to the other processing variables (Fig. 2b and Table 2). The F value of pre-conditioned moisture content's linear term was least among all the terms of processing variables.

Brown rice produced at 196 kPa with 5 min steaming did not puff in a hot-sand-bed (0% PP) as observed in microwave puffing. Brown rice produced at a higher steaming pressure and time than 196 kPa with 5 min showed 100% puffing in the hot-sand-bed. The hot-sand-bed provides a preheated medium for puffing of rice, which might help to generate sudden puffing vapour pressure inside the grain. This process helps with the puffing of rice. Sharma and Gujral (2011) also reported, sand bed roasting of barley showed a greater puffing index than with microwave roasting. Joshi, Mohapatra, Joshi, and Sutar (2014) also observed similar results during the puffing of rice in a preheated microwave oven at a different power level. Thus, the rapid or preheated medium in grain increases the magnitude of puffing.

Expansion ratio

The effect of processing variables on ER in microwave and hot-sand-bed puffing showed similar trends as depicted in Fig. 3. The expansion ratio of puffed brown rice gradually increased with the increasing processing variables as the linear terms of processing variables were found significant ($p < 0.01$) (Table 2). Parboiling pressure had a significantly higher effect ($p < 0.001$) on ER of microwave puffed brown rice than hot-sand-bed puffed brown rice. On the other hand, steaming time showed more effect ($p < 0.001$) on ER of hot-sand-bed puffed brown rice. These results for hot-sand-bed puffing agree with the findings of Chinnaswamy and Bhattacharya (1986) and Mahanta and Bhattacharya (2010). Moreover, the interaction terms of steaming pressure and time were found to have the most significant effect, as evident by the highest F values for both the puffing methods (Table 2). Gelatinisation increases gradually with steaming pressure and time (Ali & Bhattacharya, 1982), which increases the ER of puffed brown rice. Gelatinization of starch is a primary criterion for the puffing as previously mentioned and reported in several studies (Chandrasekhar & Chattopadhyay, 1991; Chinnaswamy & Bhattacharya, 1986; Mahanta & Bhattacharya, 2010; Moraru & Kokini, 2006). Excessive increase in the parboiling pressure was shown to have an adverse effect on the expansion of puffed brown rice (Fig. 3). The ER decreased in hot-sand-bed puffing beyond 400 kPa steam pressure whereas it showed a constant trend in the case of microwave puffing. Thus, the response surface of the ER with the processing variable was curvilinear (Fig. 3) and it followed a quadratic relationship (Table 3). The probable reason could be retrogradation of starch as previously discussed. In hot-sand-bed puffing, the effect of the quadratic term of steam pressure was greater in comparison to its linear term (Table 2). Chandrasekhar and Chattopadhyay (1991) also observed similar results with severe parboiling conditions during hot air puffing of rice. The ER of hot-sand-bed puffed brown rice was found to be less than the microwave puffed brown rice for 5 min steaming. However, the effect of steaming time on ER in hot-sand-bed puffing was more than in microwave puffing. This might be as a result of the preheated sand bed temperature helping in the expansion. Due to the preheated puffing medium, pre-conditioned brown rice moisture content was more effective in the hot-sand-bed puffing. The pre-conditioned moisture content of brown rice had an adverse effect on ER. Its effect was less than steaming time and pressure (Table 2). An increase in the moisture content decreases the glass transition temperature. These conditions produce less vapor pressure inside the rice kernel because the soft or rubbery stage of rice occurs at low temperature (Shimoni, Dirks, & Labuza, 2002) which is an important stage of the rice puffing process. Simultaneously, a high moisture content requires more energy to produce a high vapour pressure

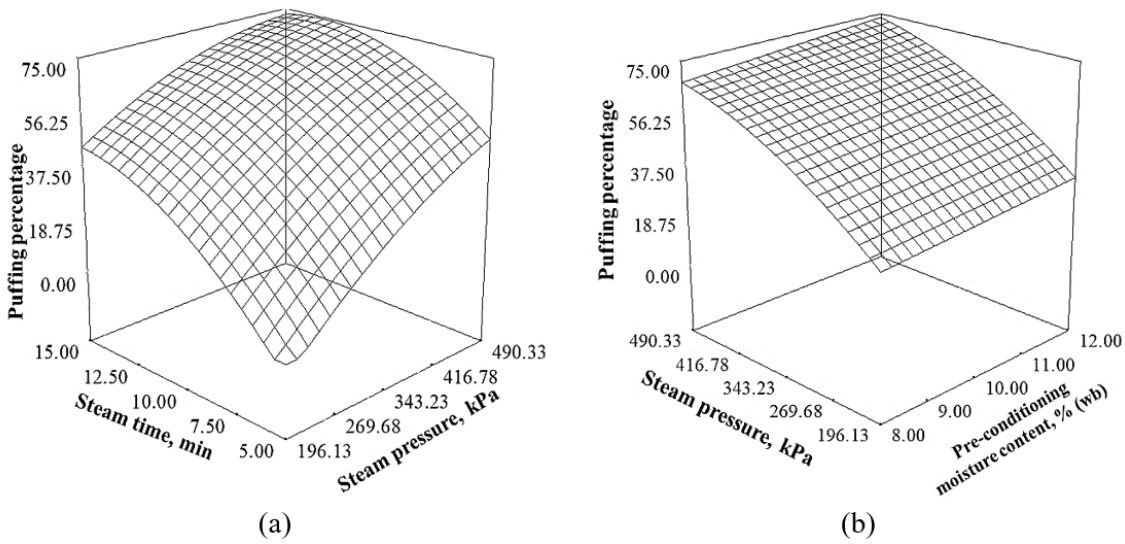

Figure 2: Puffing percentage of puffed brown rice with microwave heating under varied levels of steam pressure and steam time, and pre-conditioned brown rice moisture content

Table 2: ANOVA for the variables of linear, quadratic and interaction terms on each response

Variable/ Factor	F - value								
	PP	ER		WI		BD		Hd	
	MW	MW	HSB	MW	HSB	MW	HSB	MW	HSB
Model	57.48***	49.28***	53.72***	20.42***	7.82***	7.52***	21.27***	12.20***	13.60***
P	227.57***	104.95***	29.53***	47.10***	31.77***	16.58***	52.66***	40.06***	38.99***
T	236.52***	69.75***	230.22***	11.03**	0.54^{ns}	13.89***	48.21***	25.87***	29.18***
M	6.10*	9.72**	15.62***	3.13^{ns}	0.74^{ns}	0.37^{ns}	11.63**	0.94^{ns}	0.13^{ns}
P×T	14.13***	191.99***	122.21***	-	11.47**	21.55***	38.57***	30.41***	35.16***
P×M	0.026^{ns}	0.093^{ns}	3.119×10-3^{ns}	-	0.94^{ns}	0.2^{ns}	0.30^{ns}	1.54^{ns}	0.042^{ns}
T×M	0.025^{ns}	1.32^{ns}	2.33^{ns}	-	1.45^{ns}	0.14^{ns}	0.14^{ns}	0.60^{ns}	0.35
P^2	6.81*	60.28***	81.82***	-	-	11.33**	31.19***	6.67*	8.70**
T^2	24.12***	4.99**	1.25^{ns}	-	-	3.62^{ns}	8.76**	2.66^{ns}	8.57**
M^2	2.07^{ns}	0.49^{ns}	0.49^{ns}	-	-	0.014^{ns}	0.020^{ns}	1.05^{ns}	1.3^{ns}

*** significant at 0.001 level, ** significant at 0.01 level, * Significant at 0.05 level, ^{ns} insignificant
MW – Microwave puffing, HSB – Hot-sand-bed puffing
P – Parboiling steam pressure (kPa), T – Parboiling steaming time (min), M – Pre-conditioned brown rice moisture content (%, wb)
PP – Puffing percentage, ER – Expansion ratio, WI – Whiteness index, BD – Bulk density (kg/m^3), Hd – Hardness (N)

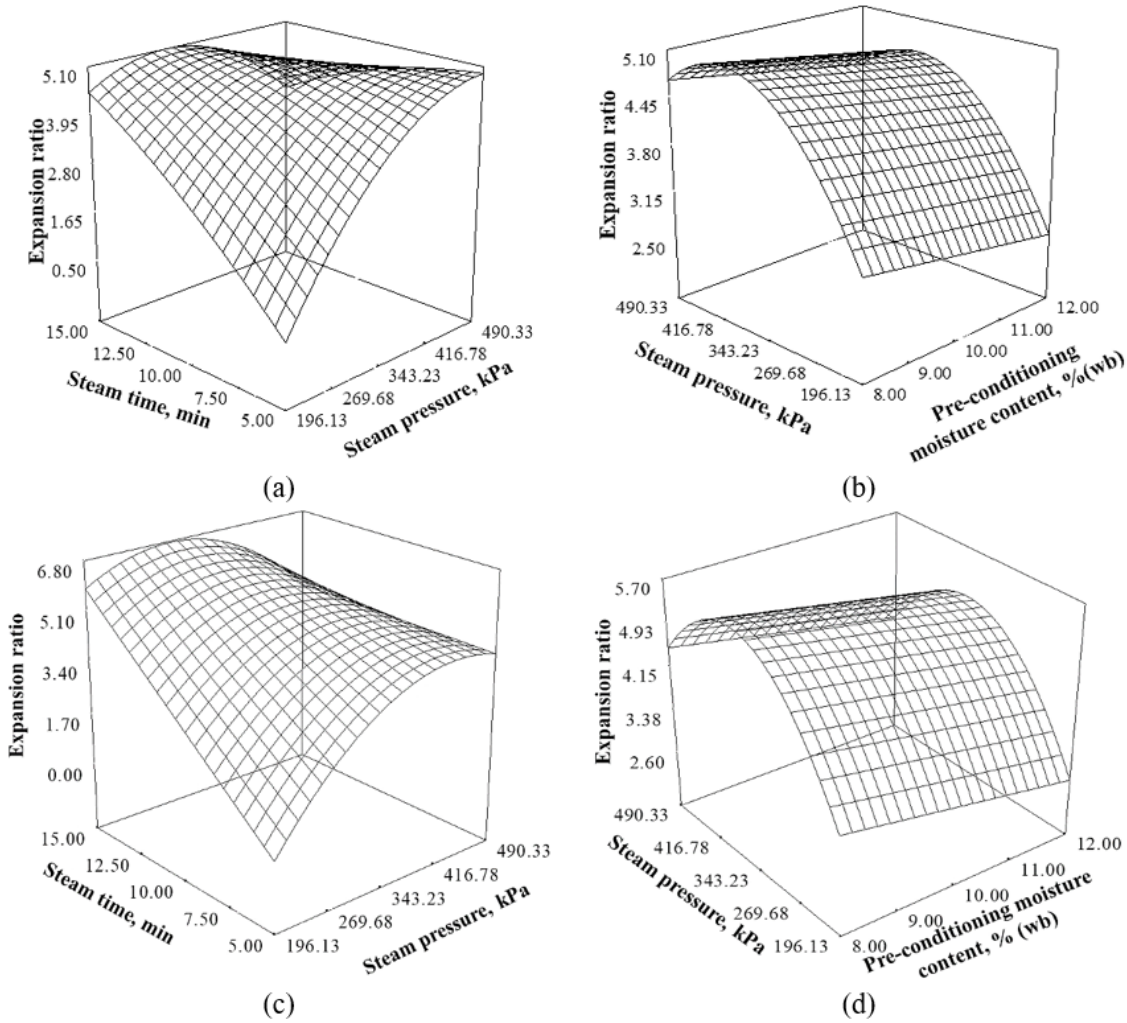

Figure 3: Expansion ratio of puffed brown rice under varied levels of steam pressure, time and pre-conditioned brown rice moisture content; (a & b) microwave puffing, (c & d) hot-sand-bed puffing

(Gulati & Datta, 2016). Thus, an increase in pre-conditioned moisture content decreases the ER of puffed brown rice.

Whiteness Index

Whiteness index of puffed brown rice was observed to decrease linearly with increasing steam pressure ($p < 0.001$) for both microwave and hot-sand-bed puffing (Fig. 4 a and c). Steaming time was observed to have a negative effect on WI in both puffing methods (Fig. 4 a and c) but it was significant ($p < 0.001$) in case of microwave puffing only (Table 2). However, WI was observed to be independent of pre-conditioned brown rice moisture content ($p > 0.05$) (Table 2) in both the puffing methods. Steam pressure was found to have more effect on WI of puffed brown rice than steaming time as indicated by F - values, which were 47.10 and 31.77 for microwave and hot-sand-bed puffing, respectively (Table 2). Parboiling steam pressure has more influence on the discoloration of parboiled rice than steaming time (Bhattacharya, 1996). The parboiling process increases the diffusion of water-soluble husk pigments in rice kernels and simultaneously the Maillard reaction which results in browning of rice kernels as reported by several researchers (Ali & Bhattacharya, 1982; Bhattacharya, 2011; Lamberts, Brijs, Mohamed, Verhelst, & Delcour, 2006; Lamberts, Rombouts, Brijs, Gebruers, & Delcour, 2008). These reactions during the parboiling process were responsible for the low WI of puffed brown rice at higher steaming pressure and time. Chinnaswamy and Bhattacharya (1986) and (Chandrasekhar, 1989) also observed discoloration of puffed rice prepared from high parboiling steaming pressure and time. Thus, the whiteness of puffed brown rice depends on the parboiling conditions as observed in this study.

Bulk density

Figure 5 represents the effects of parboiling steam pressure, steaming time and pre-conditioned brown rice moisture content on the BD of microwave and hot-sand-bed puffed brown rice. Parboiling at 196 kPa for 5 min steaming produces higher BD than the other parboiling conditions as observed by the poor expansion of puffed brown rice. Bulk density of puffed brown rice decreased as parboiling steam pressure ($p < 0.001$) increased from 196 kPa to 350 kPa. The BD showed a decreasing trend for microwave puffed brown rice with steaming time ($p < 0.001$) (Fig. 5 a). On the other hand, in hot-sand-bed puffing after 10 min of parboiling BD there was an increasing trend ($p < 0.01$) (Fig. 5 c). Thus, the behaviour of BD with processing variables was curvilinear (Fig. 5) and followed a quadratic order relationship (Table 3). Parboiling steam pressure was observed to have the most significant effect on BD of puffed brown rice of all the processing parameters (Table 2). Pre-conditioned brown rice moisture content was observed to have a significant ($p < 0.01$) effect on BD only in hot-sand-bed puffing and showed a positive relationship (Table 3). From the F - values (Table 2) it was also observed that all the processing parameters showed more effect in hot-sand-bed puffing than in microwave puffing. These changes may be due to the high expansion in puffed brown rice at above-mentioned parboiling conditions.

Hardness

The effect of parboiling steaming pressure, time and pre-conditioned moisture content of brown rice on puffed brown rice Hd is shown in Fig. 6. Hardness of puffed brown rice decreased significantly ($p < 0.001$) as steam pressure increases to 350 kPa in both puffing methods. Steaming time significantly ($p < 0.001$) reduced the Hd of puffed brown rice but after 10 min it was increased ($p < 0.01$) in the hot-sand-bed puffing. At lower steaming pressure and time, the Hd of puffed brown rice was related to less expansion. The influence of pre-conditioned brown rice moisture content was insignificant ($p > 0.05$) for Hd of puffed brown rice. The effect of moisture content was less on the expansion of puffed brown rice (Table 2) which may influence the Hd of the puffed grain. Chandrasekhar (1989) and Maisont and Narkrugsa (2010) also observed greater Hd in less expanded puffed rice. Less expanded puffed rice produces small air cells which give compactness to the grain and increases the Hd.

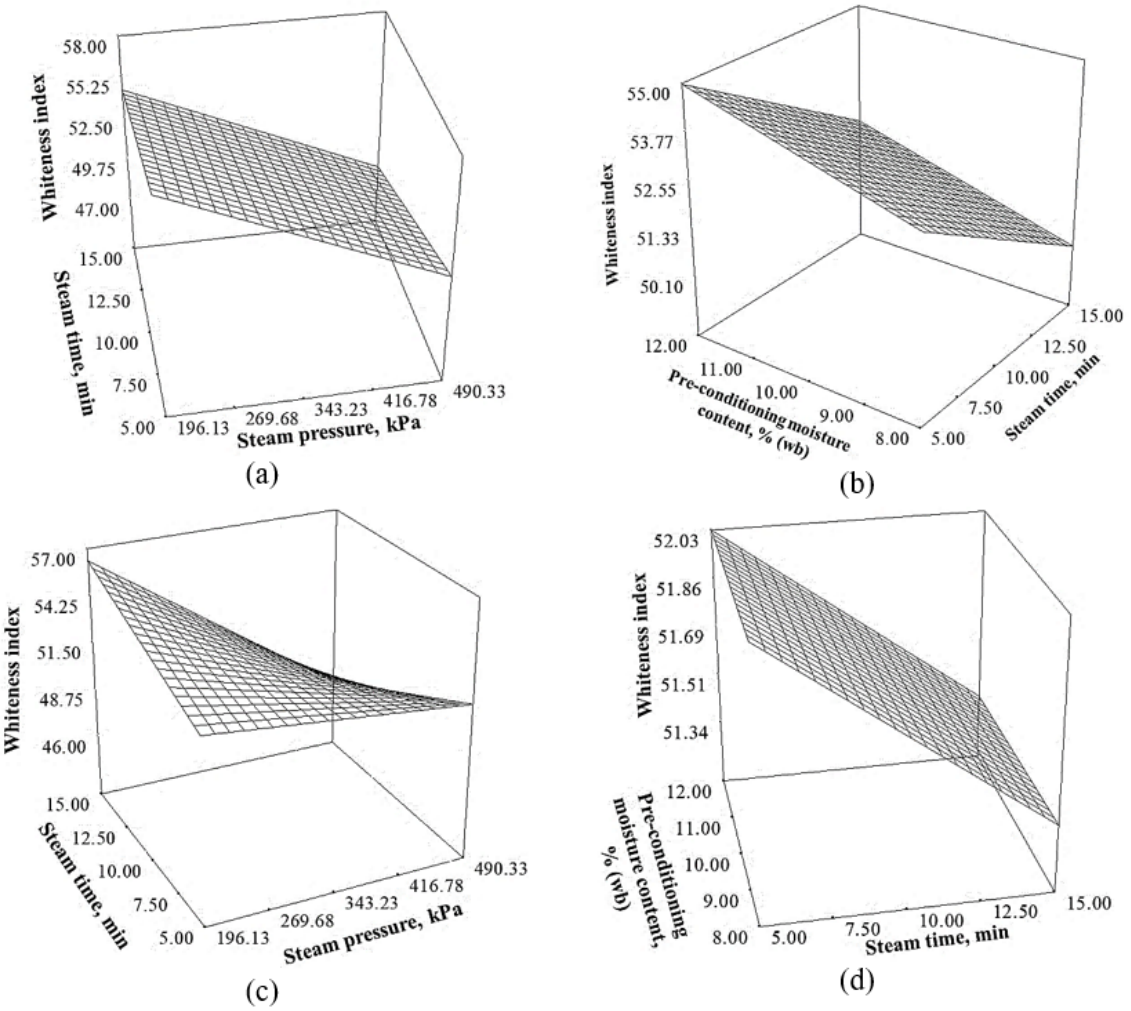

Figure 4: Whiteness index of puffed brown rice under varied levels of steam pressure, time, and pre-conditioned brown rice moisture content; (a & b) microwave puffing, (c & d) hot-sand-bed puffing

Table 3: Regression equations of quality characteristics of puffed brown rice with independent variables

Puffing method	Equation	R^2	Predicted R^2	Adjusted R^2	Adequate precision	CV%
MW	$PP=-145.108+0.406\times P+14.904\times T+1.504\times M-0.00835\times P\times T-0.00027\times^2-0.414\times T^2$	0.948	0.922	0.937	32.316	12.74
	$ER=-10.7738+0.058\times P+1.001\times T-0.116\times M-0.00188\times P\times T-0.000049\times P^2-0.011\times T^2$	0.940	0.904	0.928	32.039	9.15
	$WI=64.060-0.024\times P-0.315\times T$	0.623	0.543	0.600	14.856	4.58
	$BD=1350.336-4.567\times P-53.607\times T+0.124\times P\times T+0.0042\times P^2$	0.676	0.539	0.634	13.411	32.68
	$Hd=174.633-0.548\times P-8.208\times T+0.0184\times P\times T+0.0000403\times P^2$	0.7586	0.629	0.727	15.381	45.57
HSB	$ER=-12.664+0.074\times P+0.964\times T-0.191\times M-0.0019\times P\times T-0.0000744\times P^2$	0.941	0.920	0.931	38.336	11.09
	$WI=49.217+0.009\times P+0.915\times T-0.00286\times P\times T$	0.576	0.431	0.537	13.101	4.41
	$BD=751.364-2.297\times P-43.718\times T+7.948\times M+0.053\times P\times T+0.002\times P^2+0.955\times T^2$	0.878	0.813	0.813	22.751	11.80
	$Hd=90.965-0.227\times P-6.146\times T+0.00751\times P\times T+0.000174\times P^2+0.140\times T^2$	0.812	0.711	0.781	16.801	19.92

PP – Puffing percentage, ER – Expansion ratio, WI – Whiteness index, BD – Bulk density (kg/m^3), Hd – Hardness (N)
P – Parboiling steam pressure (kPa), T – Parboiling steaming time (min), M – Pre-conditioned brown rice moisture content (%, wb)
MW – Microwave puffing, HSB – Hot-sand-bed puffing

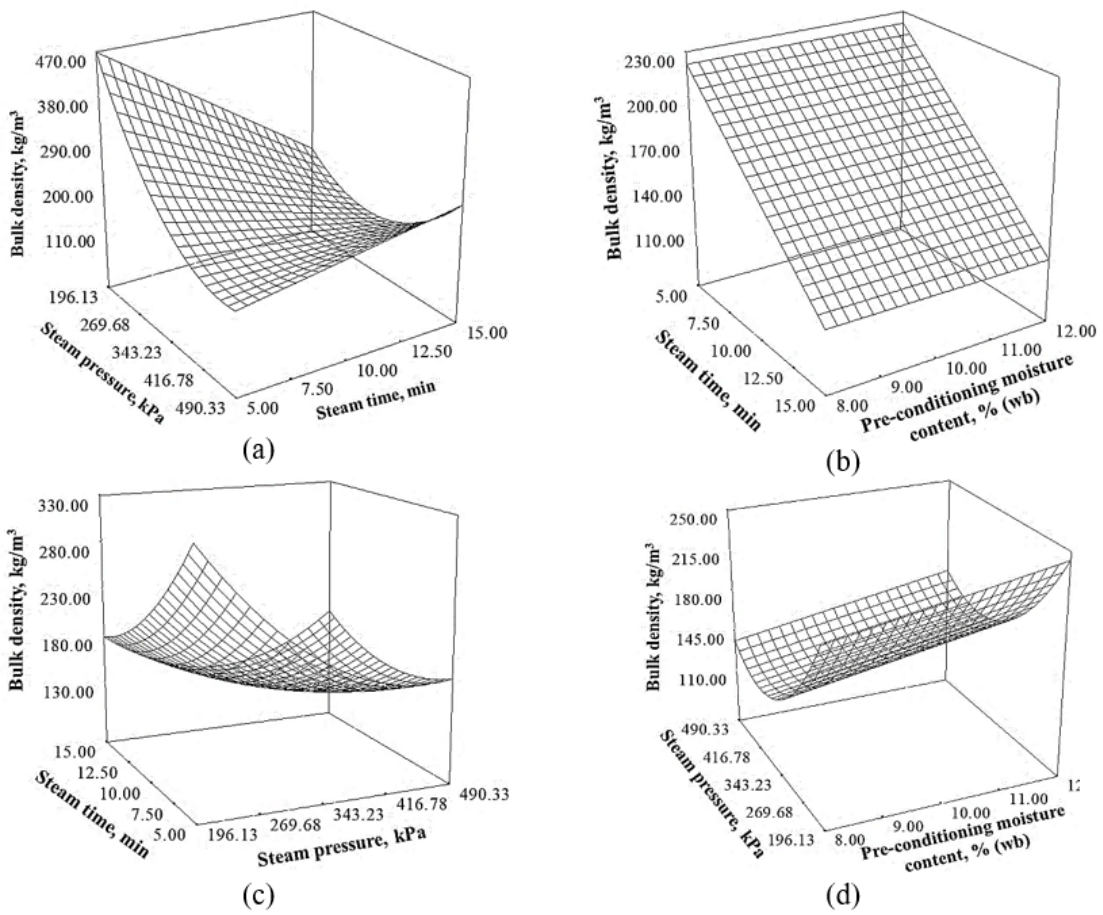

Figure 5: Bulk density of puffed brown rice under varied levels of steam pressure, time and pre-conditioned brown rice moisture content; (a & b) microwave puffing, (c & d) hot-sand-bed puffing

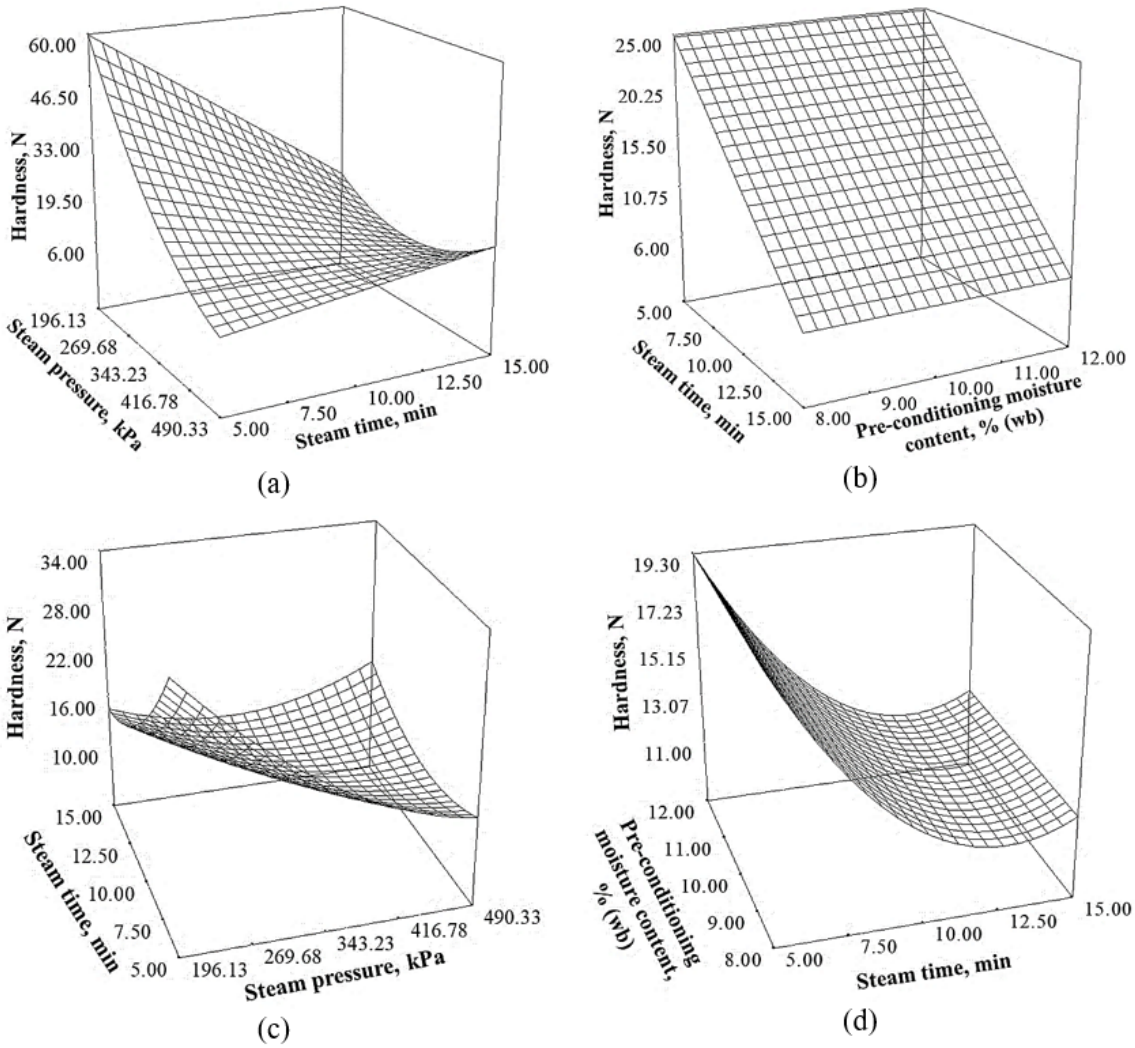

Figure 6: Hardness of puffed brown rice under varied levels of steaming pressure, time and pre-conditioned brown rice moisture content; (a & b) microwave puffing, (c & d) hot-sand-bed puffing

Table 4: Validation of optimized puffing conditions

Puffing method	Dependent variable	Predicted values	Actual values ± SD	Mean difference	p - value (2 tailed)
MW	PP	62.78	61.79 ± 0.78	0.99	0.091^{ns}
	ER	4.84	4.62 ± 0 .30	0.22	0.287^{ns}
	WI	52.16	52.85 ± 2.68	0.68	0.680^{ns}
	BD	124.09	146.4 ± 12.08	22.27	0.033*
	Hd	8.466	10.42 ± 0.40	1.95	0.001***
HSB	ER	6.45	6.055 ± 0.10	0.39	0.001***
	WI	55.93	56.550 ± 1.18	0.61	0.410^{ns}
	BD	131.83	157.636 ± 5.01	25.80	0.001***
	Hd	12.52	12.933 ± 0.77	0.41	0.371^{ns}

*** significant at 0.001 level, ** significant at 0.01 level, * Significant at 0.05 level, ^{ns} insignificant
PP – Puffing percentage, ER – Expansion ratio, WI – Whiteness index, BD – Bulk density (kg/m3), Hd – Hardness (N)
MW – Microwave puffing, HSB – Hot-sand-bed puffing
SD – Standard deviation

3.3 Optimization

Based on the highest D value, optimum conditions were selected. The ER and PP are essential quality characteristics for the puffed rice (Joshi et al., 2014). Emphasis was placed on ER and PP. Importance in the software system varied from 1 (+) to 5 (+++++) which represent least and most important, respectively. During the optimization of PP and ER, importance was set at four (software system for optimization) and WI, BD and Hd set on an importance of three or at default in the software for optimization. During the optimization PP, ER and WI were targeted at the maximum, and Hd and BD at the minimum. Optimum conditions obtained for microwave puffing were parboiling steam pressure of 303.6 kPa, steaming time of 14.25 min and pre-conditioned moisture content of 11.6% (wb). Corresponding optimum values of these parameters were 260.7 kPa, 15 min and 8% (wb), respectively for brown rice puffing in the hot-sand-bed method.
Validation of these responses (mean of three) is presented in Table 4. The t-test was carried out for comparison between these responses to predict responses. The null hypothesis was that there will be no significant difference between the predicted and the experimental values. The Significant difference was found for BD ($p < 0.05$) in both the puffing methods, Hd ($p < 0.001$) for microwave puffing and ER ($p < 0.001$) for hot-sand-bed puffing. The mean difference was found to be satisfactory for all the dependent variables during validation (Table 4).

4 Conclusions

In this study, the effect of parboiling steaming pressure, time and pre-conditioned moisture content of brown rice was studied and optimized to achieve the highest quality puffed brown rice in the microwave and hot-sand-bed methods. The optimized parboiling steaming pressure, time and pre-conditioned moisture content for microwave were 303 kPa, 14.25 min and 11.6 %, respectively and these levels for hot-sand-bed puffing were 260.7 kPa, 15 min and 8%, respectively. Puffing characteristics of brown rice followed a quadratic relationship with processing parameters in both the puffing methods but WI follows a linear relationship. Parboiling steaming pressure and time were the only parameters affecting WI and Hd of puffed brown rice. More than 350 kPa steaming pressure adversely affected the quality of the puffed brown rice.
Heating mediums used for puffing were found to have a substantial effect on the quality of the puffed brown rice. Optimum expansion of puffed brown rice in both puffing methods was obtained at 8% moisture level of the pre-conditioned brown rice. Although the brown rice puffing quality in the hot-sand-bed method was slightly better than in a microwave method, the microwave puffing process can replace hot-sand-bed puffing to obtain a hygienic, fresh and uniform quality product.

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A Numerical Model for Studying the Thermal Denaturation-Aggregation of Whey Proteins under Continuous Thermal Processing

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Abstract

A computational fluid dynamics model was designed to study the problem of thermal processing of a liquid food product containing whey proteins within a heat exchanger consisting of heating, holding and cooling tubular sections. This physical problem is associated with strong coupling between the phenomena of fluid flow, heat transfer, and thermal denaturation-aggregation of whey proteins. Our primary objective was to investigate the two-way coupling between these phenomena within the heat exchanger. This was carried out by analyzing the model predictions of velocity, temperature and product properties at both axial and radial directions. Attention was focussed on the whey proteins present in a cream cheese formulation. The thermal denaturation-aggregation kinetics was supposed to follow that of the beta-lacto-globulin, which plays a major role in fouling when milk derivatives are submitted to thermal processing in heat exchangers. Model predictions demonstrated that the apparent viscosity of the liquid product exhibited a complex behavior along the processing unit: in addition to its dependence on local temperature, it was affected by the local degree of denaturation of whey proteins – and hence on the product history previous to this position in the heat exchanger. The numerical model was structured into a sequence of computational domains; its versatility was illustrated by changing the length of the holding section and then assessing the impact on the final degree of denaturation of the whey proteins present in the liquid product.

Keywords: Computational Fluid Dynamics (CFD); Thermal denaturation-aggregation; Whey proteins; Beta-lacto-globulin; Heat exchanger

1 Introduction

Heat treatment of foods need to be optimized to promote beneficial effects and to counteract undesired effects (van Boekel et al., 2010). The advantages of using continuous processing units for fluid foods, instead of batch processing, are the increase in production rate, the reduction in energy consumption and the improvement on both sensorial and nutritional attributes of the product (Ramaswamy, Abdelrahim, Simpson, & Smith, 1995). Looking for reproducible manufacturing of fluid foods, it is necessary to understand a) the mechanisms driving the physicochemi-

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cal characteristics of ingredients, and b) the interactions occurring between ingredients under thermo-mechanical treatment. In other words, it is necessary to know how the liquid product is *transformed* along the processing pathway. The thermal continuous processing of liquid food products is very often conducted by employing heat exchangers whose basic elements are the heater, the holder and the cooler. The liquid product is first exposed to heating up to a target temperature, whose value depends on the changes desired in the physicochemical structure of ingredients present in the liquid product: denaturation-aggregation of whey proteins, swelling of starch granules, etc. Along the holder, the liquid product is supposed to flow at the target temperature under thermally-insulated conditions in order to progressively reach the target state of transformation. Finally, the liquid product is exposed to quick cooling in order to stop the changes affecting ingredients as well as the interactions among them. In summary, the final structure of the liquid product can depend on a number of factors, since the concentration of raw ingredients up to the operating conditions that are chosen in running the processing unit.

Modelling can reduce the amount of experimentation which is required in designing food products, processes and equipments; further, physics-based modelling provides a level of insight that is usually not possible experimentally (Datta, 2008). There are problems in which the evolution of the liquid has negligible impact on the phenomena of heat transfer in fluids. Indeed, in the case of the thermal inactivation of microorganisms and enzymes in food liquids, it seems sufficient to estimate the minimal thermal inactivation, which can be computed from the minimum residence time obtained after assuming plug-flow (Aguiar & Gut, 2014). The study of liquid products becomes very challenging from the modelling perspective when there is two-way coupling between phenomena of fluid flow and heat transfer in the processing unit. Such a coupling is relevant in the dairy industry, because it is associated with the formation of deposits of transformed product onto the heat transfer surfaces (fouling which can reduce heat transfer and add resistance to fluid flow (Goode, Asteriadou, Robbins, & Fryer, 2013; Khaldi et al., 2018; Li, Singh, & Lee, 2004). In such a coupled problem, thermal denaturation-aggregation of proteins can affect product rheology, which in turn drives the velocity field through the processing unit. On the other hand, fluid flow influences the temperature field and hence the denaturation-aggregation kinetics rate. Such a situation is clearly more complex that the problem of heat transfer in non-Newtonian fluids whose transport properties depend on temperature only (Chhabra, 1999, see for instance the review by).

Over the past 150 years, some 80 particular solutions have been found for the system of non-linear partial differential equations which describe the conservation of mass, momentum and energy for viscous flows. Each of these solutions satisfy the complete equations for some special geometry; as expected, almost all the known particular solutions are appropriate for incompressible Newtonian flow with constant transport properties (White & Corfield, 2006). Much effort has been devoted over decades to the theoretical study of temperature profiles under forced-convection inside heat-conducting tubular sections, including non-Newtonian flows (Bird, Stewart, & Lightfoot, 2007). Solutions have been reached a) by restricting the attention on the thermal entrance region with a fully developed laminar velocity profile, and b) by neglecting either the heat generation by viscous dissipation or the axial heat conduction in the fluid (Luna, Méndez, & Treviño, 2002). The non-linear and coupled nature of the conservation equations preclude the possibility of analytical results; numerical solutions are sought, even for as simple a situation as that of laminar flow in a circular tube (Chhabra, 1999). Numerical modelling is clearly required to solve problems related to liquid products whose transport properties evolve with thermal denaturation-aggregation of whey proteins.

A numerical model was implemented to solve the coupled problem of fluid flow, heat transfer, and thermal denaturation-aggregation of whey proteins under realistic conditions. The present study discusses the evolution of the cream cheese formulation of Coutouly, Riaublanc, Axelos, and Gaucher (2014) inside the different tubular sections of a processing pilot unit. Our primary

objective was to investigate the two-way coupling between those phenomena; in addition, the model was employed as a tool for virtual experiments, predicting the final transformation state of the liquid product under conditions which were not considered in the implementation of the model.

2 Coupled physical problem

Steady-state conditions were considered, on one hand because their industrial interest, on the other hand because all the experimental data available to this study were obtained under such conditions. Assuming incompressible fluid, the conservation equations for mass, momentum and energy are expressed as (Bird et al., 2007):

$$\vec{\nabla}.\vec{u} = 0 \tag{1}$$

$$\rho(\vec{u}.\vec{\nabla})\vec{u} = \vec{\nabla}.(-p\vec{I} + \eta(\vec{\nabla}\vec{u} + (\vec{\nabla}\vec{u})^T)) \tag{2}$$

$$\rho Cp(\vec{u}.\vec{\nabla})T = \vec{\nabla}.(\lambda\vec{\nabla}T) \tag{3}$$

where $\vec{u}$ is the velocity vector (magnitude in m/s), p the pressure (Pa), and T the temperature (K); ρ is the liquid product density (kg.m^{-3}), η its apparent viscosity (Pa.s), Cp its specific heat capacity (J.kg^{-1}.K^{-1}), and λ its thermal conductivity (W.m^{-1}.K^{-1}).

Beta-lacto-globulin is the major whey protein of mature bovine milk (Swaisgood, 1995). In the context of thermal processing of milk derivatives in heat exchangers, the beta-lacto-globulin plays a major role in the deposition mechanism for fouling at temperatures between 75 and 110 ^{o}C (Lalande, Tissier, & Corrieu, 1985). Hence, the thermal denaturation-aggregation behaviour exhibited by the beta-lacto-globulin was a good candidate for the behaviour followed by the whey proteins present in the liquid product studied. The thermal denaturation-aggregation of beta-lacto-globulin includes: a) the dissociation of native dimers, when the temperature increases above 40 ^{o}C; b) the unfolding of native monomers and the formation of a thermally-induced molten globule state, when the temperature increases above 60 ^{o}C; and c) depending on temperature and shear rate conditions, the occurrence of irreversible intra-molecular interactions allowing the formation of aggregates. As a result, the concentration of native proteins remaining in the liquid product, C (kg.m^{-3}), progressively decreases along the thermal treatment. Such a tendency has been represented through the reaction kinetics:

$$\frac{dC}{dt} = -k_m C^m \tag{4}$$

where k_m is the kinetics rate (s^{-1}.(kg.m^{-3})$^{-m+1}$). The reaction order m expresses the complexity of underlying mechanisms; in the case of the denaturation of beta-lacto-globulin, it usually takes values between 1 and 2, more commonly 1.5 (Tolkach & Kulozik, 2007).

Looking for the solution of the coupled problem of fluid flow, heat transfer and thermal denaturation-aggregation of whey proteins under stationary conditions, equation (4) can be written as a convection-reaction-diffusion equation:

$$\vec{u} \cdot \vec{\nabla}C = -k_m C^m + \vec{\nabla} \cdot (d_S \vec{\nabla}C) \tag{5}$$

where d_S is the diffusion coefficient (m^2.s^{-1}) for the whey proteins in the liquid product.

Actually, the temperature dependence of the kinetics rate k_m for denaturation-aggregation of beta-lacto-globulin exhibits a transition between two thermal regimes near 90 ^{o}C (Lyster, 1970). On one hand, at temperatures lower than that transition, the partial unfolding of monomers is slower than their aggregation, and the denaturation-aggregation kinetics becomes limited by the unfolding sub-reaction; on the other hand, at temperatures higher than that transition, the aggregation of thermally-induced molten globules is slower than their formation, and the denaturation-aggregation kinetics becomes limited by the aggregation process (Tolkach & Kulozik, 2007). Modelling the double regime for the temperature dependence of k_m with the help of few parameters constitutes a challenging task (Petit, Herbig, Moreau, & Delaplace, 2011).

The approach proposed by Tolkach and Kulozik (2007) is hereafter assumed for representing the temperature dependence of the denaturation-aggregation of beta-lacto-globulin. Following those authors, the kinetics rate k_m in equation

(4) is expressed as

$$k_m = \alpha^m k_{agg} \tag{6}$$

where α is the degree of unfolding, defined as the mass fraction of unfolded proteins in the liquid product, and calculated from the kinetics rates for unfolding and aggregation:

$$\alpha = e^{\left(\frac{ln(k_{unf})-ln(k_{agg})}{m}\right)} \tag{7}$$

Both kinetics rates are, in turn, expressed through Arrhenius equations:

$$ln(k_{unf}) = ln(k^0_{unf}) - E_{A,unf}/RT$$

$$ln(k_{agg}) = ln(k^0_{agg}) - E_{A,agg}/RT \tag{8}$$

where k^0_{unf} and k^0_{agg} are the frequency factors ($s^{-1}.(kg.m^{-3})^{-m+1}$) for unfolding and aggregation, $E_{A,unf}$ and $E_{A,agg}$ the corresponding energies of activation ($J.mol^{-1}$), and R the universal gas constant ($J.mol^{-1}.K^{-1}$). The following values are considered: $k^0_{unf} = 98.9$, $k^0_{agg} = 21.7$, $E_{A,unf} = 313.9 \cdot 10^3$ $J.mol^{-1}$, and $E_{A,agg} = 80.8 \cdot 10^3$ $J.mol^{-1}$ (Petit et al., 2011; Tolkach & Kulozik, 2007).

3 Experimental work

The liquid food product of interest is the cream cheese model studied by Coutouly et al. (2014). The product had 40 % w/w dry matter, including 33.1 % of fat, 3.5 % of caseins, 0.5 % of whey proteins, 2 % of lactose, and 0.8 % of sodium chloride.

The experimental setup was an Armfield FT174X ultra high temperature laboratory scale plant (Armfield Ltd., Ringwood, United Kingdom). Here the attention is focused on the heater, the holder, and the cooler elements of that processing unit (see Figure 1). The heater and the cooler were both constituted of a sequence of eight tubular sections, successively separated by 180° short curved sections (bends); the holding section was a helical configuration of large curvature compared to the tube diameter. All the sections and tubes have a radius of 4 mm; the heating and cooling sections are 38 cm long; the liquid product's pathway has a length of about 4.26 m in the holding section, and about 16 cm in the bends. The thermal evolution of the liquid product was studied by running the heat exchanger under stationary conditions. Upstream to the heater, the liquid product was pre-heated up to about 40 ^{o}C. Along the heater, the product was submitted to indirect heating from the condensation of water vapour on the outer surface of the heating sections, and warmed up to the prescribed holding temperature. Along the cooler, the product was subjected to counter-flow cooling, reaching a temperature of about 40 ^{o}C at the heat exchanger outlet. The product temperature was recorded at selected positions of the heat exchanger (Figure 1). Heat treatments associated with increasing severity were considered; the weakest was associated with a holding temperature of 72 ^{o}C and to a holding time of 20 s, whereas the strongest was associated with a holding temperature of 94 ^{o}C and to a holding time of 40 s. After each treatment, the transformation state of the liquid product was characterized by its denaturation ratio:

$$\delta_{outlet} = 1 - C_{outlet}/C_{inlet} \tag{9}$$

where C_{outlet} and C_{inlet} indicate the concentration of native proteins in the product at the heat exchanger inlet and at its outlet, respectively. The concentration of native proteins was obtained from measurements of nitrogen content, by assuming that all the denaturated-aggregated whey proteins were insoluble at pH 4.6. After selected heat treatments, the rheological behavior of the liquid product was characterized under decreasing shear rate at 30 and 50 ^{o}C, using a temperature-controlled rotational rheometer with double Couette geometry. Coutouly et al. (2014) provided additional information about this liquid product and the experimental methods employed in studying it.

Table 1 summarizes the available experimental results. The same initial sample of liquid product was submitted to three different heat treatments (experiments #1, #2 and #3); two other samples were submitted to the strongest heat treatment only (experiments #4 and #5). Measurements of apparent viscosity of the liquid product after experiments #1, #2 and #3 are employed in representing its rheological behavior (see 3.1); temperature and product transformation data obtained from these three experiments were em-

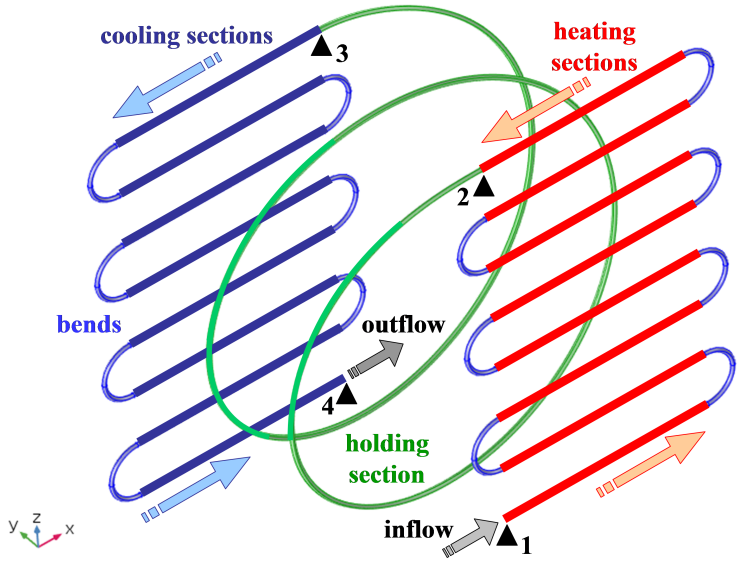

Figure 1: Tri-dimensional schematic representations of the heat exchanger under consideration. Heating sections are indicated in red, the holding section in green, cooling sections in dark blue, and bends in light blue; the heat exchanger's inlet and outlet are indicated. Positions 1 to 4 indicate where temperature measurements were conducted: at the inlet of the first heating section ($T_{heater,inlet}$), at the outlet of the last heating section ($T_{heater,outlet}$), at the inlet of the first cooling section ($T_{cooler,inlet}$), and at the outlet of the last cooling section ($T_{cooler,outlet}$), respectively.

ployed to identify the heat transfer coefficients required in the thermal boundary condition at the wall of heating and cooling sections (see 4.4). Finally, temperature and product transformation data obtained from experiments #4 and #5 were employed to assess the consistency of the numerical model 4.4.

3.1 Rheological parameters

Figure 2 displays the available measurements of apparent viscosity of the liquid product. It became shear-thinning as the thermal denaturation-aggregation of whey proteins progressed; for a given shear rate, the apparent viscosity increased with the transformation state; for given shear rate and transformation state, the apparent viscosity decreased with the temperature.

The apparent viscosity of the liquid product is hereafter described through the following approximations:

$$\eta\{\dot{\gamma},\delta,T\} = K\{\delta,T\}\dot{\gamma}^{n\{\delta\}-1}$$

$$K\{\delta,T\} = a_1 exp(a_2/(RT))exp(a_3\delta)$$

$$n\{\delta\} = 1 - a_4\delta \qquad (10)$$

where K is the consistency coefficient (Pa.s^n), n the flow behaviour index, and $\dot{\gamma}$ the shear rate (s^{-1}). Equations (10) express the mean dependence of the apparent viscosity with the shear rate, denaturation ratio and temperature, while requiring few parameters. Best fit values for the parameters required by equations (10) were estimated after minimizing the sum of squared deviations between the predictions and experimental values of the product apparent viscosity: $a_1 = 3.66\ 10^{-7}$ s^{n-1}, $a_2 = 2.59\ 10^4$ J.mol^{-1}, $a_3 = 3.68$ and $a_4 = 2.99\ 10^{-1}$. Lines in Figure 2 indicate predictions of the apparent viscosity corresponding to the three thermal treatments; the averaged relative error between

Table 1: Experimental results available to this study

Experiment	#1	#2	#3	#4	#5
heat treatment	Weak	Medium	Strong	Strong	Strong
holding temperature and holding time	72 ^{o}C, 20 s	80 ^{o}C, 30 s	94 ^{o}C, 40 s	94 ^{o}C, 40 s	94 ^{o}C, 40 s
volume flow rate (L/h)	37.4	25.5	18.1	18.0	18.0
condensation vapor temperature (^{o}C), T_{vapor}	92.0	95.7	108.4	108.4	108.1
temperature at heater inlet (^{o}C), $T_{heater,inlet}$	46.6	44.5	43.2	41.7	42.6
temperature at heater outlet (= at holder inlet) (^{o}C),	71.8	79.8	93.8	93.8	93.8
temperature at cooler inlet (= at holder outlet) (^{o}C),	71.5	78.8	91.4	91.1	89.0
temperature at cooler outlet (^{o}C), $T_{cooler,outlet}$	42.6	43.0	39.6	44.2	45.3
initial conc. of native whey proteins (kg/m3), $C_{heater,inlet}$	6.51	6.51	6.51	6.55	6.04
initial conc. of native whey proteins (mol/m3)	0.355	0.355	0.355	0.357	0.330
initial conc. of native whey proteins (%)	16.6 %	16.6 %	16.6 %	16.7 %	15.4 %
denaturation ratio at cooler outlet	6.0%	17.0%	62.0%	64.9%	61.0%
Reynolds number at heater inlet	260	166	113	111	108

predictions and experimental values is about 9 %.

The physical problem is two-way coupled. On one hand, the velocity field resulting from the solution of equations (1) and (2) drives heat and mass transfer, i.e. the temperature and concentration fields; in addition, the temperature field resulting from the solution of equation (3) drives the thermal denaturation-aggregation kinetics which is considered for estimating the concentration of native proteins (equations 4 and 5). On the other hand, both the temperature and the denaturation ratio of whey proteins affect the values assumed by the apparent viscosity of the liquid product (equations 10), which is a key variable in predicting the velocity field (equation 2).

The influence of thermal denaturation-aggregation on the apparent viscosity is far more complex than the double role played by the denaturation ratio on equations (10). The concentration of native whey proteins decreases with the time, and the rate of such a decrease depends on the temperature (see Section 2); hence the local value of the denaturation ratio at a given position of the heat exchanger depends on the whole thermal and kinetics history experienced, since the inlet of the first heating section, by the fluid particles that are running at the position of the interest. A similar situation has been discussed in studying the coupled physical problem which involves fluid flow, heat transfer and swelling of starch granules in aqueous suspension inside a heat exchanger; the thermal history of fluid particles in the processing unit is implicitly taken into account when the physical problem is solved under stationary conditions (Plana-Fattori, Chantoiseau, Doursat, & Flick, 2013).

4 Numerical model

The liquid product is hereafter characterized by the density 980 kg.m^{-3} (from measurements) and by the apparent viscosity approximated through equations (10). Because the large fraction of water in the liquid product (60 % in mass), both the specific heat capacity and the thermal conductivity are assumed to follow the behavior of pure water. The last line in Table 1 shows the Reynolds number at the heat exchanger inlet, i.e. where its highest value can be found for this liquid product and these operating conditions in the processing unit considered; the liquid product flows under laminar conditions.

The computational strategy can be summarized as follows. In the numerical model, the processing unit is represented by a sequence of two-dimensional, axi-symmetric computational domains. Displays A, B and C in Figure 3 illustrate such a representation for the first heating section. Figure 4 presents all the 31 domains that are required for representing the heating exchanger: eight heating sections separated by seven bends, one holding section, and eight cooling sections

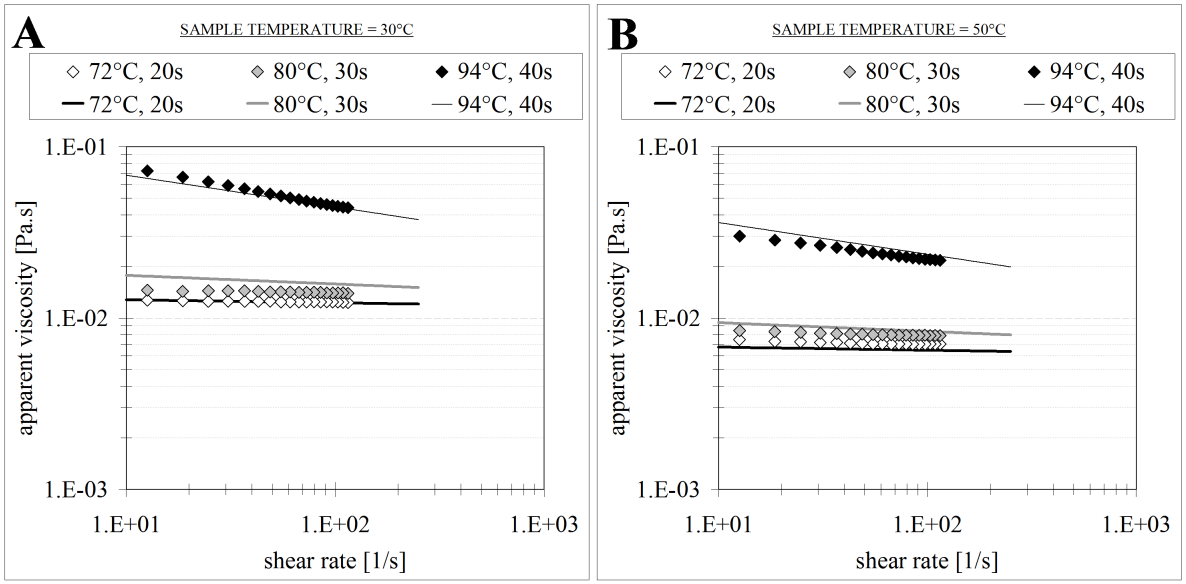

Figure 2: Apparent viscosity of the liquid product at 30 ^{o}C (display A) and 50 ^{o}C (display B), associated with the heat treatments conducted during experiments #1, #2 and #3. Treatments are characterized by the holding temperature and the holding residence time. Triangles indicate measurements, and lines indicate predictions provided by equations (10) for the apparent viscosity as a function of the shear rate and denaturation ratio.

separated by seven bends. The coupled physical problem is solved successively for each domain, by assuming the boundary conditions described in 4.1. The mathematical methods employed for solving the problem are summarized in 4.2; the application of the finite-elements method requires the geometrical subdivision of computational domains, which is explained in 4.3. The final step of the model implementation involves the identification of heat transfer coefficients required by the thermal boundary conditions at the heating and cooling walls, as discussed in 4.4.

4.1 Setting the boundary conditions

A summary of the boundary conditions taken into account is provided in Table 2. Here they are described in further detail.

Inlet: Besides the first heating section, the radial profiles of velocity, temperature and concentration of native whey proteins profiles are assumed to follow model predictions at the outlet of the previous domain. At the inlet of the first heating section (i.e. the heat exchanger inlet), the flow is assumed to be fully-developed; moreover, the temperature and the concentration of native whey proteins follow the measured values $T_{heater,inlet}$ and $C_{heater,inlet}$, respectively (see Table 2).

Outlet: The product is assumed to flow normally to the boundary under no viscous stress; heat conduction and mass diffusion are both neglected.

Wall: The velocity field is assumed to vanish. Inward heat fluxes $h_{heating}$(T$_{vapor}$-T) and $h_{cooling}$(T$_{water}$-T) are applied in heating and cooling sections, respectively; $h_{heating}$ and $h_{cooling}$ are heating and the cooling transfer coefficients, T_{vapor} is the condensation vapor temperature, and T_{water} = 5 ^{o}C. In the holding section, the product is

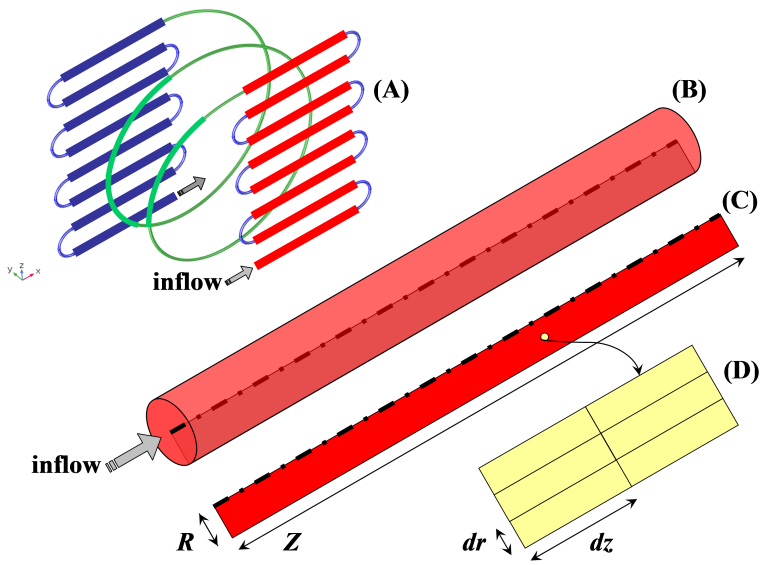

Figure 3: Schematic representation of the heat exchanger and of the strategy adopted for meshing the computational domains. Display A summarizes the heat exchanger shown in Figure 1. Display B is a close-up view of the first heating section; the slice is one axi-symmetric two-dimensional (2D) domain, and the dashed line is the axis of symmetry. Display C presents this 2D domain; its width $R = 4$ mm is the radius of all the tubular sections, while its length Z depends on the section under consideration. Display D illustrates the mesh built around a given position in the first heating section. Features are not in scale.

exposed to uniform inward heat flux estimated from the section's global energy budget, by employing the measurements of temperature at its inlet $T_{heater,outlet}$) and its outlet ($T_{cooler,inlet}$). The product is supposed to flow under thermally-insulated conditions in the bends.

Curved sections can play a mixing role, firstly in reducing the heterogeneity of temperature and composition, secondly in modifying the residence time distribution of fluid parcels (Ndoye, Erabit, Alvarez, & Flick, 2012; Vashisth, Kumar, & Nigam, 2008). The effectiveness of such a role depends on the geometrical features of the curved section, as well as on the relative importance of mechanisms due to molecular diffusion and to secondary flow (Kumar, Aggarwal, & Nigam, 2006). We compared two extreme scenarios. In both scenarios, the thermal evolution of the liquid product inside the bends was predicted by the numerical model according to the boundary conditions specified above; in both scenarios, the radial distributions $u_z\{\mathrm{r}\}$, $\{\mathrm{Tr}\}$, and $C\{\mathrm{r}\}$ for axial velocity, temperature, and concentration of native proteins predicted at the bend outlet were employed to build the boundary conditions at the inlet of the following heating or cooling section.

no mixing: the model predictions of $u_z\{\mathrm{r}\}$, $T\{\mathrm{r}\}$, and $C\{\mathrm{r}\}$ at the bend outlet were applied, with no changes, as boundary conditions at the inlet of the following section; and

full mixing: firstly, the model predictions of $u_z\{\mathrm{r}\}$, $T\{\mathrm{r}\}$, and $C\{\mathrm{r}\}$ at the bend outlet were employed for computing the mass-weighted (bulk) values $\tilde{T}$ and $\tilde{C}$ for temperature and concentrative of native proteins; secondly, these bulk values were applied as

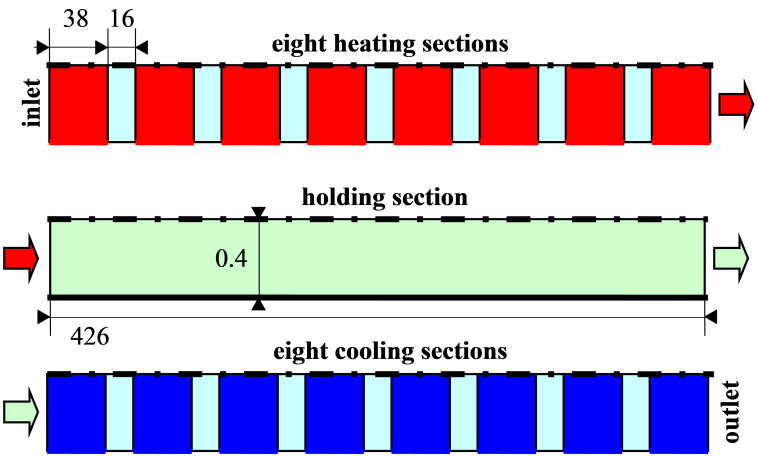

Figure 4: Two-dimensional axi-symmetrical schematic representations of the heat exchanger under consideration. Heating sections are indicated in red, the holding section in green, cooling sections in dark blue, and bends in light blue; the heat exchanger's inlet and outlet are indicated. Dashed lines indicate the axis of symmetry, and red and dark blue lines indicate heating and cooling walls; heating and cooling sections are geometrically identical; and all the dimensions are indicated in centimeters (features not in scale).

boundary conditions at the inlet of the following section; finally, the axial velocity at the inlet of following section was expressed as (Brodkey, Lee, & Chase, 1961):

$$u_z\{r\} = \overline{u}_z \left(\frac{3\tilde{n}+1}{\tilde{n}+1}\right)\left(1-\left(\frac{r}{R}\right)^{\tilde{n}+1/\tilde{n}}\right) \quad (11)$$

where $\tilde{n}$ is the flow behavior index given through equation (10) after replacing δ by $\tilde{\delta}_{bend,outlet}$, where $\tilde{\delta}_{bend,outlet} = 1$ - $\tilde{C}_{bend,outlet}$ / $C_{heater,intlet}$.

4.2 Solving the problem

The finite-element method was employed for solving the coupled physical problem constituted by the governing equations (1, 2, 3, and 5), applied to the liquid product of interest and to the computational domains displayed in Figure 3, while respecting the boundary conditions summarized in Table 2. The finite-element method for solving problems in engineering and mathematical physics had been developed decades ago . To solve the problem, this method subdivides the whole domain of interest into smaller, simpler parts that are called finite elements. In the finite-element method, instead of solving the problem for the entire body in one operation, the equations are formulated for each finite element and combine them to obtain the solution of the whole body (Pepper, Kassab, & Divo, 2014).
The finite-element method was employed as implemented in the simulation package COMSOL Multiphysics software (version 5.2.0.220; COMSOL, Inc., Burlington, Massachusetts) (Zimmerman, 2006). Phenomena of fluid flow, heat transfer and thermal denaturation-aggregation were represented with the help of three COMSOL Multiphysics interfaces: "Laminar Flow" (for equations 1 and 2), "Heat Transfer in Fluids" (equation 3), and "Transport of Diluted Species" (equation 5). In addition, the COMSOL Mul-

Table 2: Summary of boundary conditions assumed for the radial u_r and axial u_z components of the velocity, for the temperature T and for the concentration of native proteins C.

	fluid flow	heat transfer	transformation
Inlet (+)	velocity: $u_r = 0, u_z \{r\}$	temperature: $T\{r\}$	concentration: $C\{r\}$
Outlet	null pressure, no viscous stress	convective flux: $\partial T/\partial z = 0$	convective flux: $\partial C/\partial z = 0$
Axis	symmetry: $u_r = 0$, $(\partial u_z)/\partial r = 0$	symmetry: $\partial T/\partial r = 0$	symmetry: $\partial C/\partial r = 0$
Wall	no slipping: $u_r = u_z = 0$	inward heat flux	no diffusion:$\partial C/\partial r = 0$

(+) Excepting the inlet of the first heating section, where the temperature and the concentration of native proteins follow the measured values $T_{heater,inlet}$ and $C_{heater,inlet}$, and the axial velocity follows the fully-developed parabolic profile associated with the volume flow rate (see Table 1).

tiphysics "LiveLink for MATLAB" was used to drive the resolution of the problem over the sequence of computational domains.
Discretization of equations (1, 2, 3, and 5) considered second-order Lagrange finite elements for the velocity components, the temperature and the concentration of native proteins, and first-order elements for the pressure. The linear system obtained after discretization and linearization of governing equations was solved by applying the Multifrontal Massively Parallel Sparse Direct Solver (MUMPS) (Amestoy, Duff, L'Excellent, & Koster, 2001) with a relative tolerance smaller than 10^{-5}.

4.3 Meshing the domains

The resolution of governing equations through the finite-element method requires the subdivision of the computational domain into a number of small, non-overlapping cells (the mesh elements). The geometry of the domains suggested the adoption of a structured mesh, constituted of rectangular cells, characterized by dimensions dr and dz in the radial (r) and in the axial direction (Z), respectively. Indicating by R the domain's width and Z its length, the total number of mesh cells in a given domain can be written as $nr \times nz$, where $nr = \frac{R}{dr}$ and $nz = \frac{Z}{dz}$ are the numbers of cells in the radial and in the axial direction, respectively. We choose cells whose length is four times their width $\frac{dz}{dr} = 4$, as a compromise for representing gradients along both directions. Display D in Figure 3 illustrates these concepts, around a given position in the first heating section.
A mesh with relatively high resolution was adopted, corresponding to $nr = 2^7 = 128$. This is the finest resolution allowed by our computer resources (192-Gb RAM) for solving the coupled problem in the holding section (Z=4.26 m). Adopting such a resolution, about 13 million cells were required for meshing all the 31 computational domains which represent the heat exchanger. The influence of the mesh resolution is discussed in Subsection 5.2.

4.4 Identifying the heat transfer coefficients

The last step in implementing the numerical model was the identification of heat transfer coefficients $h_{heating}$ and $h_{cooling}$, which are required in the specification of the thermal boundary conditions at the walls of the heating and cooling sections (see Table 2). Such a task was performed by comparing measured temperatures from experiments #1, #2 and #3 and corresponding model predictions. Firstly, $h_{heating}$ was identified by minimizing the departure between the bulk value of temperature predicted at the outlet of the last heating section and the corresponding experimental value $T_{heater,outlet}$); secondly, $h_{cooling}$ was identified by minimizing the departure between the bulk value of temperature predicted at the outlet of the last cooling section and the corresponding experimental value ($T_{cooler,outlet}$).
The representation of coupled phenomena under the scenarios "no mixing" and "full mixing" in

the bends required different values for the heat transfer coefficient values at heating and cooling walls:

full mixing: $h_{heating}$ = 1025 W.m^{-2}.K^{-1} and $h_{cooling}$ = 475 W.m^{-2}.K^{-1}; and

no mixing: $h_{heating}$ = 9000 W.m^{-2}.K^{-1} and $h_{cooling}$ = 1030 W.m^{-2}.K^{-1}.

The denaturation ratio of whey proteins at the heat exchanger outlet was 6.0%, 17.0% and 62.0% respectively for experiments #1, #2 and #3 (see Table 1). These observations can be compared with the corresponding mass-weighted values $\tilde{\delta}_{cooler,outlet}$=1-$\frac{\tilde{C}_{cooler,outlet}}{C_{heater,inlet}}$ that were predicted at the heat exchanger outlet after applying the numerical model to the operating conditions of these three experiments. Indeed, after assuming "full mixing" at the bends' outlet, model predictions provided $\tilde{\delta}$ of about 0.4%, 3.4% and 55.4%, say -5.6%, -13.6% and -6.6% with respect to the experimental value; after assuming "no mixing" at the bends' outlet, model predictions provided $\tilde{\delta}$ of about 3.0% (-3.0%), 9.8% (-7.2%) and 51.4% (-10.6%), respectively for experiments #1, #2 and #3. Two results emerge from these tests.

- On one hand, the numerical model consistently underestimates the denaturation ratio of whey proteins as observed at the heat exchanger outlet. In assuming that the thermal denaturation- aggregation behavior exhibited by all the whey proteins in the liquid product is described through the approach proposed by Tolkach and Kulozik (2007) for beta-lacto-globulin in suspension, we have neglected the influence of other whey proteins on the overall denaturation ratio of the product, as well as the influence of other constituents of the product.
- On the other hand, the influence of the bends' mixing effectiveness on model predictions seems to be secondary in the case study here considered. Hereafter, in applying the numerical model to the study of the liquid product under thermal processing, only the scenario "full mixing" at the bends' outlet was considered.

These comparisons considered observations and model predictions corresponding to the operating conditions of experiments #1, #2 and #3; their datasets contributed to the implementation of the numerical model, either in the estimation of rheological parameters (see equations 10) or in the identification of heat transfer coefficients.
Two final simulations were carried out to assess the reliability of the numerical model, by considering observations which did not contribute to its implementation. The denaturation ratio of whey proteins at the heat exchanger outlet was 64.9% and 61.0% respectively for experiments #4 and #5 (see Table 1); after assuming "full mixing" at the bends' outlet, model predictions provided $\tilde{\delta}$ of about 54.7% (-10.2%) and 50.7% (-10.3%), respectively. In other words, in the case of the strongest heat treatment, a similar level of agreement between model predictions and observations is reached either for the experiment that contributed to the implementation of the numerical model (#3) or for those that did not (#4 and #5).

5 Results

5.1 Coupled phenomena throughout the heat exchanger

One major advantage of physics-based numerical modelling is the assessment of distributions of velocity, temperature and other variables at scales beyond current experimental capabilities. Figures 5-11 display model predictions obtained under the operating conditions of experiment #3, say the strongest heat treatment here considered (holding temperature 94 ^{o}C, holding time 40 s); the coupling between phenomena of fluid flow, heat transfer and thermal denaturation-aggregation are expected to be the most significant.
Figure 5 shows the temperature. In the heating sections, the temperature increases more slowly at the axis of symmetry because the liquid product is warmed from the wall; in the cooling sections, the temperature decreases more slowly at the axis of symmetry because the liquid product is cooled from the wall. In the

holding section, whose walls are assumed to be thermally-insulated and whose outlet and inlet are at about the same temperature (Table 1), the radial variations of temperature progressively vanish. In the bends, the product flows under thermally-insulated conditions; in bends situated after a heating section (cooling section), convective transfer promotes heating (cooling) from the vicinity of the wall towards the axis of symmetry. Figure 6 shows the denaturation ratio. The highest value occurs at the wall at the holding section outlet, due to the longer residence times at the wall. At the scale of the whole heat exchanger, the scenario "full mixing" accelerates the thermal denaturation-aggregation at the axis of symmetry while slowing it down near the heating walls. Product transformation is virtually halted after the second cooling section.

Figure 7 presents the flow behavior index n At the heat exchanger inlet, the liquid product was assumed to exhibit a Newtonian behavior due to the occurrence of native proteins only ($\delta = 0$) Along the liquid product pathway, the behavior index was assumed to decrease linearly with the denaturation ratio (equation 10); hence they exhibit similar axial and radial variations, being that the lowest value of δ occurs at the wall of the holding section outlet.

Figure 8 shows the consistency coefficient K ; it was assumed to increase exponentially with the denaturation ratio while decreasing with the temperature (equation 10). The influence of the thermal denaturation-aggregation of whey proteins is dominant, excepting: near the walls of the first heating sections (under weak thermal denaturation-aggregation) and also near the walls of the last cooling sections (under almost constant denaturation ratio).

Figure 9 exhibits the apparent viscosity η; it was assumed to follow a power-law representation, with local values depending on temperature, shear rate, and denaturation ratio. Its spatial distribution is complex even under nearly isothermal conditions, as those prevailing near the holding section outlet: on one hand it increases near the axis of symmetry, under vanishing shear rate values (Figure 11); on the other hand, it increases in the vicinity of the wall too, under relatively high denaturation ratio values (see Figure 6).

Model predictions of velocity magnitude and shear rate are presented in Figures 10 and 11, respectively. At the heat exchanger inlet, the flow was assumed to be fully-developed; at the inlet of a section succeeding a bend, the velocity field was assumed to follow the developed profile whose flow behaviour index corresponds to the bulk value of the denaturation ratio at the bend outlet (see equation 11). Along the first heating sections, the velocity magnitude decreases at the axis , whereas it increases in the vicinity of the wall; such a result can be related to the apparent viscosity values at the wall, which decreases as a consequence of progressive warming under low product transformation. On the contrary, along the remaining heating sections, the velocity magnitude increases at the axis and decreases near the wall as a consequence of product transformation and increase in apparent viscosity near the wall: fluid parcels travelling in the vicinity of the wall are slowed down, while those running at the axis undergo acceleration. As shown in Figure 11, this second pattern allows progressively high shear rate values along a tongue-like region which separates the vicinity of the heating wall and the region near the axis. Such a tongue-like region can also be recognized in the cooling sections, where the highest values of apparent viscosity occur in the vicinity of the cooling wall and also near the axis of symmetry.

5.2 Influence of mesh resolution

The above results were obtained after adopting $nr = 2^7 = 128$ rectangular cells in the radial direction when subdividing all the computational domains (see 4.2). The influence of mesh resolution was assessed by coarsening that subdivision, firstly by adopting $nr = 2^6 = 64$ and later $nr = 2^5 = 32$ cells. Model convergence for these two meshes required the diffusion coefficients $d_S = 2 \cdot 10^{-8}$ and later $d_S = 10^{-7}$ m^2.s^{-1} in equation (5), respectively. Results were obtained after assuming "full mixing" at the bends' outlet.

Figure 12 compares model predictions of the denaturation ratio at the outlet of the last heating section, where the radial variations in temperature and degree of denaturation are the highest

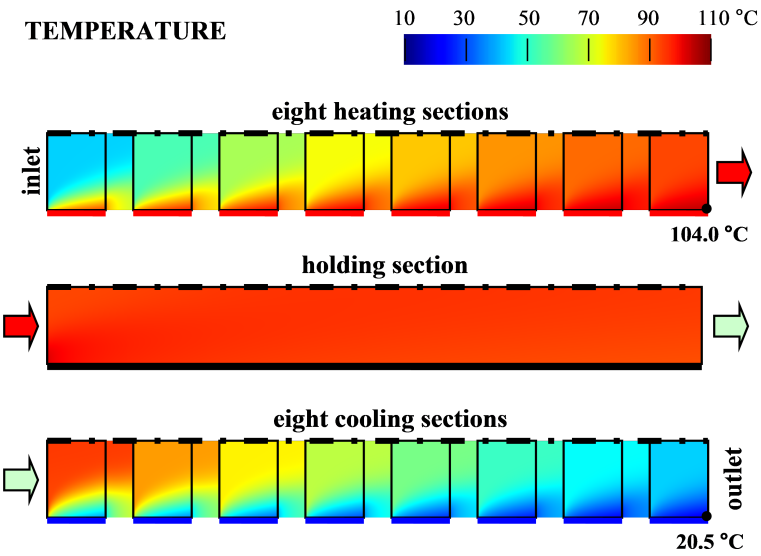

Figure 5: Numerical model predictions for the liquid temperature throughout the heat exchanger, considering the operating conditions of experiment #3, assuming "full mixing" at the bends' outlet. The lowest and the highest values are also indicated.

(see Figures 5 and 6); model predictions correspond to the operating conditions of experiment #3 (holding temperature 94 ^{o}C, holding time 40 s). Display A shows results obtained after adopting $nr = 128$, while display B presents the differences between results from a given mesh and those shown in display A. Differences are negative (i.e. thermal denaturation-aggregation is underestimated) in the vicinity of the heating wall; they become stronger with the coarsening of the mesh, and this can be explained by the progressively-poorer representation of the gradients of state variables. Further, differences are slightly positive across a wide region near the axis of symmetry, due to additional diffusion of transformed product from the heating wall. Taking as reference the denaturation ratio value of about 50% predicted by the model at the wall after adopting $nr = 128$, the impact of adopting firstly $nr = 64$ and later 32 can reach -5.4% and later -14.9%, respectively.
The overall influence of the mesh resolution can be assessed in terms of the mass-weighted value of the concentration of remaining native proteins in the liquid product at the heat exchanger outlet, $\tilde{C}_{cooler,outlet}$ or its interpretation $\tilde{\delta}_{cooler,outlet}$ = 1 - $\tilde{C}_{cooler,outlet}$/$C_{cooler,outlet}$ in denaturation ratio units. After assuming 32, then 64, and finally 128 rectangles in the radial direction, model predictions of $\tilde{\delta}_{cooler,outlet}$ were respectively 59.4%, 56.7% and 55.4% in the case of experiment #3. Differences between results associated with two successive meshes decrease as the latter become finer, suggesting a convergence-like behavior. We argue that a further leap in mesh resolution (from $nr = 2^7 = 128$ to $= 2^8 = 256$) would allow closer results, i.e. the differences between successive predictions of the outlet bulk denaturation ratio would be smaller than 1% in denaturation ratio units.

5.3 The numerical model as a tool for experiments

One advantage of physics-based models is their predictive capability, which enables the analysis of "what if" scenarios (Datta, 2008).
In the numerical model, the holder element of

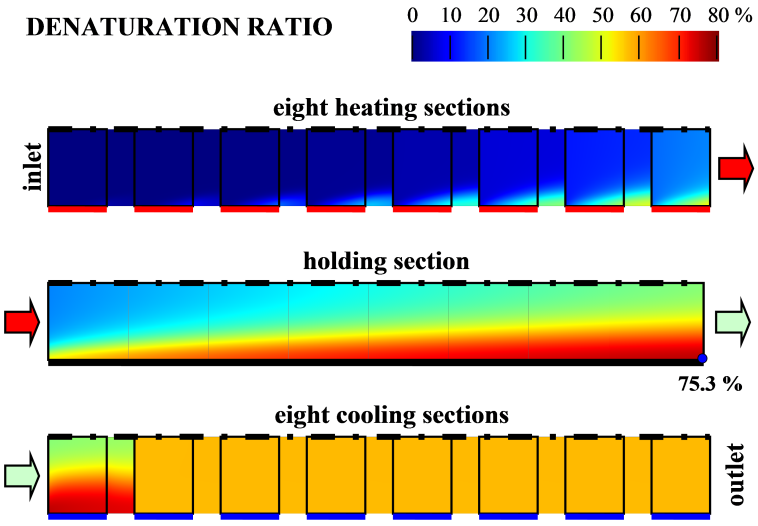

Figure 6: As in Fig. 5 but for the denaturation ratio. The highest value is also indicated; vanishing values occur along the heat exchanger inlet.

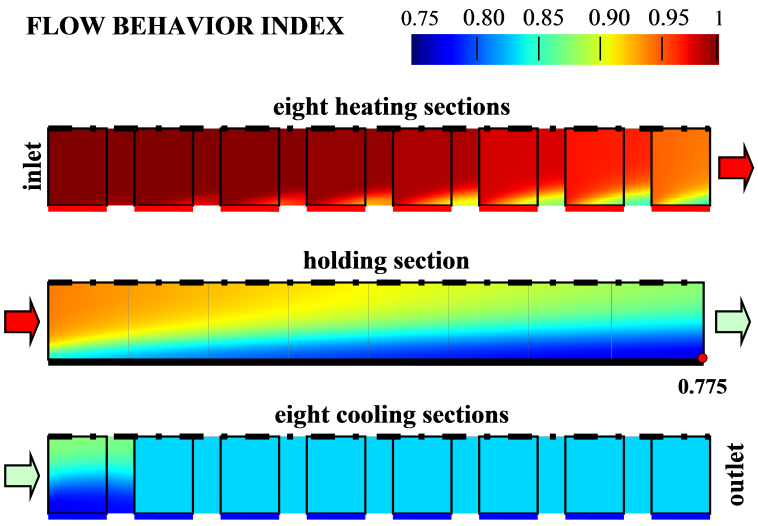

Figure 7: As in Fig. 5 but for the flow behavior index. The lowest value is also indicated; the behavior index equals the unity along the heat exchanger inlet.

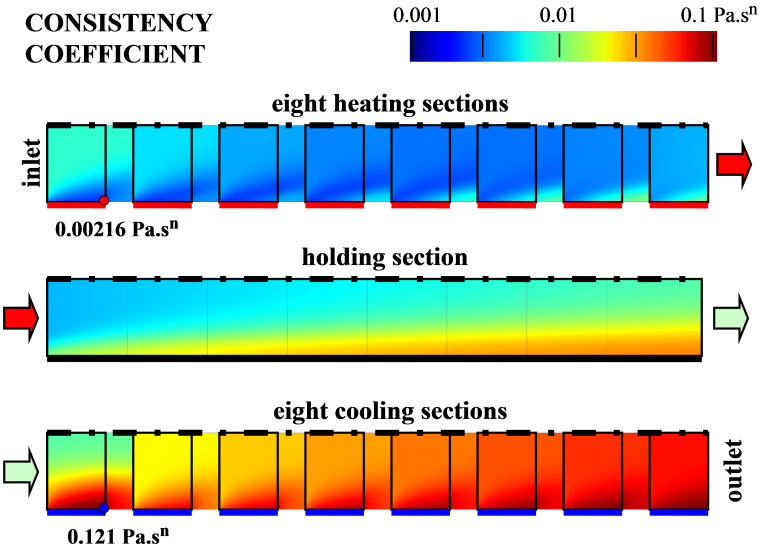

Figure 8: As in Fig. 5 but for the consistency coefficient

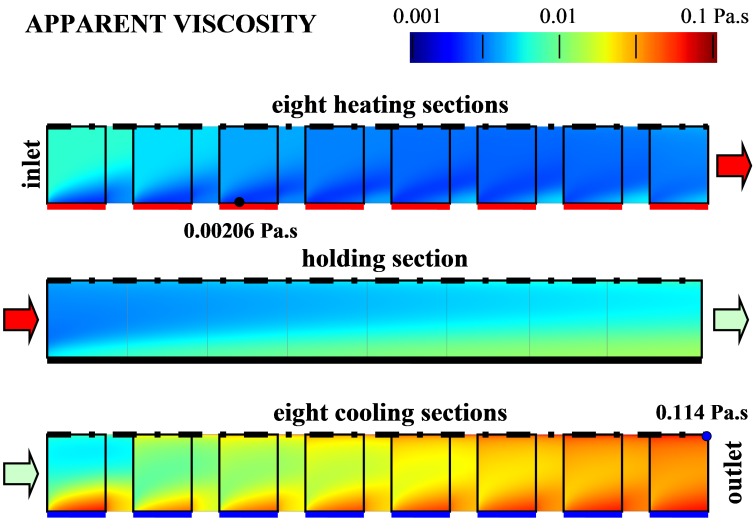

Figure 9: As in Fig. 5 but for the apparent viscosity

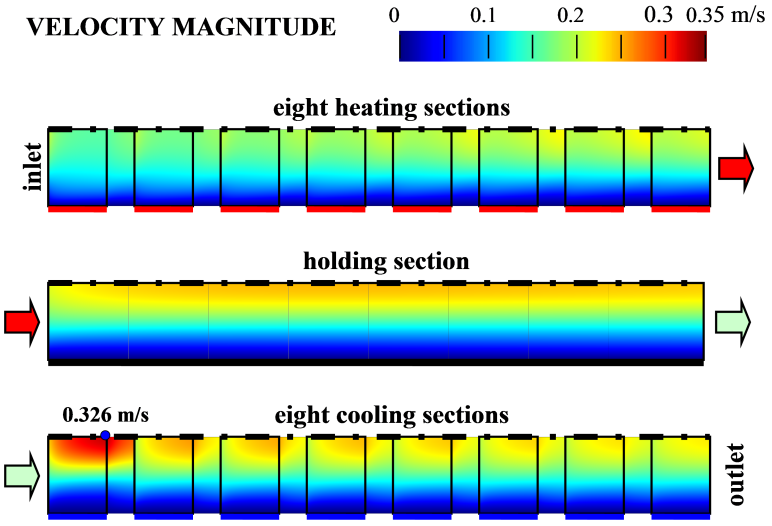

Figure 10: As in Fig. 5 but for the velocity magnitude. The highest value is also indicated; vanishing values occur along the walls

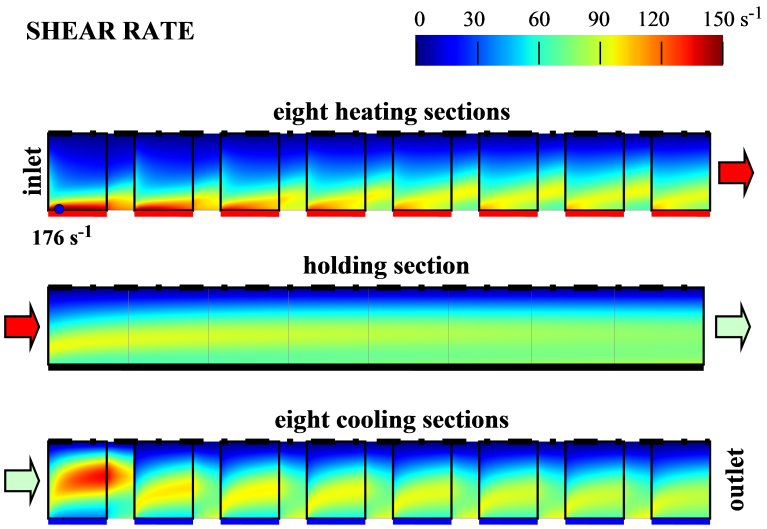

Figure 11: As in Fig. 5 but for the shear rate. The highest value is also indicated; vanishing values occur along the axis of symmetry.

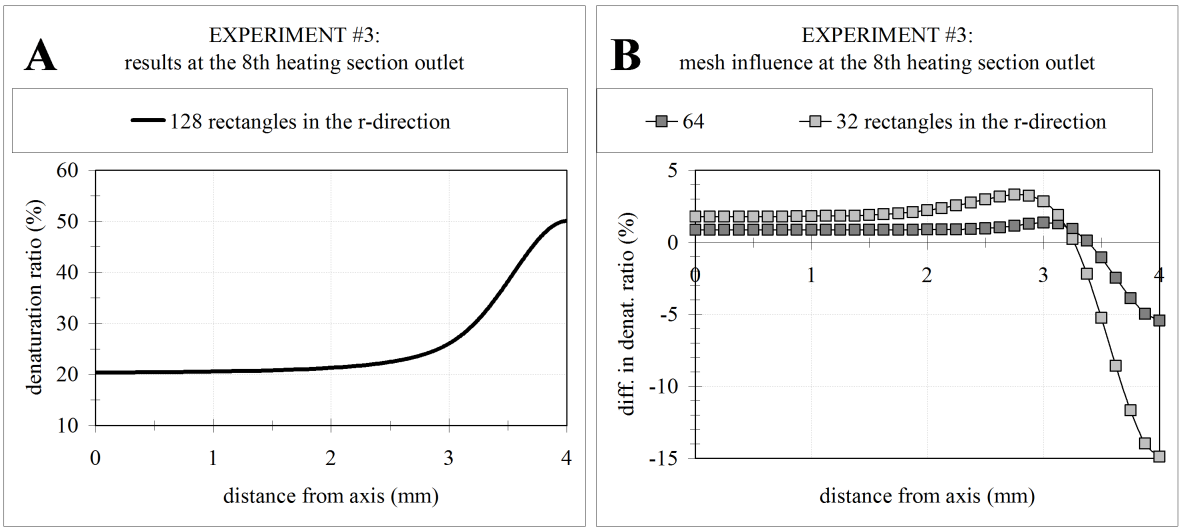

Figure 12: Denaturation ratio of the liquid product after the strongest heat treatment of the experiment #3 as predicted by the numerical model at the outlet of the last heating section: A) results obtained after assuming a mesh consisting of 128 identical rectangles in the radial direction; B) difference between results obtained after adopting successively 64 and 32 rectangles in the radial direction, and results shown in display A.

the pilot scale unit is represented as a cylindrical tube with length $L_{hold} = 4.26$ m. Model predictions provided the values $\tilde{\delta}_{heater,outlet} = 23.7\%$ at the outlet of the last heating section, $\tilde{\delta}_{holder,outlet} = 54.3\%$ at the outlet of the holding section, and $\tilde{\delta}_{cooler,outlet} = 55.4\%$ at the heat exchanger outlet. The bulk contributions due to the heating, holding and cooling sections are hence 23.7%, 54.3% - 23.7% = 30.6%, and 55.4% – 54.3% = 1.1% in denaturation ratio units. As suggested in Figure 6, the contribution due to the cooling sections is nearly negligible.

Two simple exercises were conducted to assess the impact of changes in the length of the holding section on model predictions. Operating conditions of experiment #3 (holding temperature 94 ^{o}C, holding time 40 s) and the scenario "full mixing" at the bends's outlet were taken into account. No changes, other than the length of the holding section, were operated in the numerical model.

After reducing the length of the holding section to 50 % of its reference value, model predictions provided the values $\tilde{\delta}_{holder,outlet} = 43.2\%$ and $\tilde{\delta}_{cooler,outlet} = 43.8\%$; the contribution due to the holding and cooling sections decreased to 43.2% - 23.7% = 19.5% and 43.8% - 43.2% = 0.6% in denaturation ratio units. Inversely, after increasing the length of the holding section to 150 % of its reference value, model predictions provided the values $\tilde{\delta}_{holder,outlet} = 62.8\%$ and $\tilde{\delta}_{cooler,outlet} = 63.6\%$; the contribution due to the holding and cooling sections increased to 62.8% - 23.7% = 39.1% and 63.6% - 62.8% = 0.8% in denaturation ratio units.

The denaturation ratio $\tilde{\delta}_{holder,outlet}$ increases with the length of the holding section because the latter drives the residence times of fluid parcels at temperatures associated with significant thermal denaturation-aggregation (see Figures 5 and 6). The consequences of changing the length of the holding section are quite small on the impact due to the cooling sections on the final value $\tilde{\delta}_{cooler,outlet}$ at the heat exchanger outlet.

The numerical model was implemented for solving a direct problem: assuming a given combination of geometry and boundary conditions, the model predicts the final state of the liquid prod-

uct of interest at the heat exchanger outlet. The exercises above demonstrate that the same numerical model can be employed as a tool for identifying possible solutions for the corresponding inverse problem; for instance, process designers might look for selected geometry configurations allowing the prescribed final state of the liquid product.

6 Summary and future work

This study represents the logical continuation of previous efforts (Chantoiseau, Plana-Fattori, Doursat, & Flick, 2012) in modelling the thermal processing of a liquid product containing whey proteins. In that contribution, the coupled physical model neglected the influence of the regime transition near 90 ^{o}C for the temperature dependence of the thermal denaturation-aggregation kinetics rate, as well as the progressively shear-thinning rheological behavior of the liquid product along its transformation history; both issues were effectively taken into account here. Further, the development of a liquid product of industrial interest (the cream cheese formulation of Coutouly et al. (2014)) was here discussed inside the tubular sections that represent the processing pilot unit. Experimental data from those authors allowed us to identify the external heat transfer coefficients required in the thermal boundary conditions to be applied at the walls of heating and cooling sections as represented in the 2D axi-symmetric numerical model. About the latter issue, the identification of heat transfer coefficients represented a key step in closing the problem: $h_{heating}$ and $h_{cooling}$ were estimated by comparing model predictions and observations, and therefore their values implicitly include the impact of approximations performed in implementing the numerical model. For instance, because its relatively high mass fraction in the liquid product (60%), pure water was considered a first candidate for describing the thermal properties required for solving the coupled physical problem.

In the case of the strongest heat treatment, the difference between model predictions and measurements of denaturation ratio of whey proteins at the heat exchanger outlet ranged from -10% to -11% in denaturation ratio units. This systematic behaviour occurs for experiment #3, which helped us to implement the model through the identification of rheological parameters and heat transfer coefficients, as well as for experiments #4 and #5, which did not contribute to the model implementation. Such a bias may be associated with the key assumption considered in implementing the numerical model: the strict validity of the Tolkach and Kulozik (2007)'s approach for describing the thermal denaturation-aggregation behavior of the whey proteins present in the cream cheese formulation of Coutouly et al. (2014). This assumption implies that the occurrence of other whey proteins in the liquid product (like lacto-albumins) were ignored, as well as the influence of other constituents which are present in the liquid product (like fat and caseins). A first conclusion of this study is that the Tolkach and Kulozik (2007)'s approach can be included in a numerical model for solving the coupled physical problem under consideration, allowing consistent results. The next logical steps about this issue should be: a) the test of this numerical model with experimental data from the thermal continuous processing of simpler liquid products (for instance, no other whey protein than beta-lacto-globulin), and b) the analysis of model predictions obtained after replacing that approach by a similar formalism able to consider more complex constitution of the liquid product.

Relatively powerful computational resources were required to obtain numerical solutions of the coupled physical problem; each run required about 12 hours duration of exclusive use of one 192-Gb RAM computer. The adoption of a high-resolution mesh (128 rectangles in the radial direction, over a distance of 4 mm) allowed a fine description of the variations experienced by key variables. To the author's knowledge, no previous study has displayed the 2D axi-symmetrical distributions of the apparent viscosity for a non-Newtonian liquid product, identifying the axial and radial variations of the consistency coefficient and the flow behavior index along the tubular sections which constitute a thermal processing unit. For instance, along the holding section, the denaturation ratio decreases from the wall, and the shear-thinning behaviour become less

pronounced towards the axis of symmetry. An important result from this study is that, superimposed to the progressive thermal denaturation-aggregation of the whey proteins in the liquid product since the heat exchanger inlet, radial dependence was predicted for all the variables under consideration, including transport properties. Such dependence might be anticipated in building the coupled physical problem (Section 2); model predictions revealed, further, how much this dependence can evolve along a given tubular section, and how much it can vary from a tubular section to another.

The final concluding remark concerns the flexibility of the numerical model here implemented. The problem is solved successively for the sequence of computational domains, while translating model predictions at a given outlet into boundary conditions at the following inlet. It is relatively easy to insert or to remove tubular sections before producing new predictions, demonstrating the potential of the numerical model as a design tool. Attention should be paid to the comparison of selected future scenarios with experimental data; more than to validate the numerical model, these comparisons could drive the attention of modelers to issues of future research.

The development of a numerical model as the one here described depends on the availability of a robust experimental dataset, firstly for representing the rheological behavior of the liquid product, secondly for identifying parameters for the relevant thermal boundary conditions. It is strongly recommended that the datasets should include a larger selection of heat treatments, as well as a larger selection of temperatures for measurement of the liquid product viscosity after every heat treatment.

Future efforts should be devoted to the detailed representation of aggregation processes, including interactions between whey proteins and the major other species contained in the liquid product.

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The Effect of *in vitro* Enzyme Digestion on Antioxidant and Anticholinesterase Potential of Tomato (*Lycopersicum esculentum*) Fruit and Two Commercially Processed Tomato Pastes

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Abstract

Tomato is a horticultural crop of interest, that is widely consumed fresh or as processed products. The present investigation was to evaluate the antioxidant indices (total phenolic content, flavonoid content, ferric reducing antioxidant power, radical scavenging activities, inhibitory action against lipid oxidation) and anti-cholinesterase action (acetylcholinesterase and butyrylcholinesterase) of tomato fruits (ripe and unripe) and pastes (*paste 2* and *paste 1*) after simulated gastrointestinal digestion. The total phenolic content (mg/g GAE) of the *in vitro* digested tomato fruits and pastes showed higher values (ripe tomato: 61.08; tomato paste1: 56.02; tomato paste 2: 60.36; unripe tomato: 38.97) than the ethanolic extracts, with digested ripe tomato ranking higher. Similar results were also obtained for total flavonoid content, ferric reducing antioxidant power, and the radical scavenging activities (DPPH*, $ABTS^{\cdot+}$, NO*, OH*), with the *in vitro* digested samples ranking high. The ability of the enzyme digested and ethanolic extracts of tomato fruits and pastes to inhibit iron and sodium nitroprusside induced lipid oxidation in rat's liver and brain homogenate increased in a concentration dependent manner, with the enzyme digested tomato fruits and pastes ranking high. Similarly, the ability of the *in vitro* digested tomato fruit and pastes to enhance activities of the antioxidant enzymes (GPx, GSH, SOD and Catalase) and to inhibit the formation of cholinesterases ranked high. The result of this investigation showed that the studied tomato fruit and pastes possess antioxidant and anti-cholinesterase activities that would be bio-available after the gastrointestinal digestion and by implication could be harnessed as functional food.

Keywords: *in vitro* Digestion; Antioxidant activities; Anticholinesterase Potential; Tomato fruit; Commercially- processed Tomato pastes

1 Introduction

Antioxidants are compounds that help to inhibit many oxidation reactions caused by free radicals, such as superoxide, peroxyl radicals, hydroxyl radicals, nitric oxide and lipid peroxyl, which process, prevents or delays damage to the cells and tissues (Birben, Sahiner, Sackesen, Erzurum, & Kalayci, 2012; Kong & Lin, 2010).Their

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mechanisms of action include scavenging of reactive oxygen and nitrogen free radical species; decreasing the localized oxygen concentration, and therefore reducing molecular oxygen's oxidation potential; metabolizing lipid peroxides to non-radical products; and chelating metal ions to prevent the generation of free radicals (Barzegar, 2012; Madhuri, Qairunnisa, Suresh, Kondam, & Chandrasekhar, 2014).

A number of studies have established that some fruits, grains and vegetables have antioxidant capacity; this has been attributed principally to their polyphenol and flavonoid contents (Oboh & Ademosun, 2011; Omoba, Dada, & Salawu, 2015; Saidu & Garba, 2011; Salawu et al., 2016; Salawu, Bester, & Duodu, 2014; Yafang, Gan, & Jinsong, 2011). Regular intake of antioxidant-containing foods can reduce the risk of many chronic diseases, such as cardiovascular diseases, heart diseases, diabetes, obesity and certain cancers, and improve the endothelial function and reduce blood pressure (Pellegrino, 2016; Zhang et al., 2015). Phenolic compounds have been reported by many researchers to be present in cereals, fruit and grain crops (Salawu et al., 2014; Shahidi & Chandrasekara, 2013).

Tomatoes are a concentrated source of phenolic compounds, such as flavonoids and hydroxycinnamic acid derivatives; containing 98% of the total flavonols in tomato skin as conjugated forms of quercetin and kaempferol (C., F., H., & Didier, 2011; Hossain, Strezov, Chan, & Nelson, 2010; Skrovankova, Sumczynski, Mlcek, Jurikova, & Sochor, 2015). The high content of these compounds in tomato has gained interest due to their apparent multiple biological effects, including free-radical scavenging, metal chelation, inhibition of cellular proliferation, and modulation of enzymatic activity and signal transduction pathways (Vallverdu-Queralt et al., 2011). Among the most prominent phytochemicals in tomatoes are the carotenoids, of which lycopene is most abundant in the ripened fruit, accounting for approximately 80-90% of the total pigments (Violeta, Trandafir, & Ionica, 2013). Aside lycopene, tomatoes also contain α-, β-, γ-, δ-carotene, zeaxanthin and lutein and also neurosporene, phytoene, and phytofluene C. et al. (2011). Lycopene has the capacity to prevent free radical damage to cells caused by reactive oxygen species. Studies have shown that it reduces the susceptibility of lymphocyte DNA to oxidative damage, inactivates H_2O_2 and NO and protects cells from NO induced membrane damage and cell death (Lobo, Patil, Phatak, & Chandra, 2010; Uttara, Singh, Zamboni, & Mahajan, 2009). Moreover, lycopene is also the most efficient singlet oxygen quencher with a capacity found to be more than twice of β-carotene (Shi, Dai, Kakuda, Mittal, & Xue, 2008). On the other hand, β-carotene is important due to its pro-vitamin A activity. Apart from carotenoids, tomato is also a source of ascorbic acid, which is an effective scavenger of superoxide, hydrogen peroxide, singlet oxygen and other free radicals (Yafang et al., 2011).

The cholinergic hypothesis of Alzhemier's disease (AD) holds that the degeneration of neurons in the basal forebrain, the associated loss of cholinergic neurotransmission in the cerebral cortex and hippocampus contribute significantly to cognitive deterioration in AD (Craig, Hong, & McDonald, 2011).The loss of cholinergic neurons in AD leads to a reduction in the synthesis of the neurotransmitter, acetylcholine (ACh), which has been associated with cognitive functions. This hypothesis has prompted the search for ways to increase ACh in AD patients. There is a need to inhibit the activity of cholinesterases (ChE) to increase the concentration of ACh needed for cognitive functions. Extracts from some plants such as *Lavandula viridis*, and some Nigeria green leafy vegetables have been documented to have ChE inhibitory activities (Costa, Gonçalves, Valentão, Andrade, & Romano, 2013; Oboh & Ademosun, 2011).

Antioxidants have to be present in some amount in the specific tissue or organ of plant foods to elicit their potential biological properties (Kasote, Katyare, Hegde, & Bae, 2015). However, the release of antioxidants from complex food materials during digestion will determine their actual biological properties. Thus, the biological extraction of antioxidants within the digestive system might be different from the extracts obtained using organic solvents. There have been reports that antioxidant activity from the chemical extracts of the food material might misjudge the actual antioxidant capacity in the digestive tract (Bhatt & Patel, 2015). *In vitro* digestion

method measures the bioavailability of the nutrient, which is the amount of the nutrient liberated from the food material during gastrointestinal digestion, which is, in turn, available for absorption in the body (Chandrasekara & Shahidi, 2012). Therefore, it can be used to evaluate a large number of food systems, which would be costly to analyze for different parameters using human or animal models.
Therefore, the objective of this work is to evaluate the antioxidant and anticholinesterase action of a Nigerian tomato fruit (Early girl) and two different commercially processed tomato paste after a simulated human gastrointestinal digestion.

2 Materials and Methods

2.1 Chemicals

Follin-Ciocalteu's reagent, gallic acid, sodium carbonate, iron (iii) chloride, potassium ferricyanide, trichloroacetic acid, aluminium chloride, potassium acetate, 2,2- diphenyl-1-picrylhydrazyl radical and 6-hydroxy-2, 5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), sodium nitroprusside, sulphanilamide, N-(1-Naphthyl-ethyl-diamine-dihydrchloride), orhophosphoric acid, sodium azide, adrenalin, GHS, xanthine oxidase, xanthine, acetylcholine iodide, butyrylcholine iodide were obtained from Sigma chemical company, USA. All other chemicals were obtained from standard chemical suppliers and were of analytical grade, while the water used was glass distilled.

Sample Collection

Tomato fruit, 'Early girl' (ripe and unripe) and two different types of processed tomato paste (Paste 1 and Paste 2) were bought from Shasha market in Akure, Nigeria. The fresh tomato fruit (ripe and unripe) were identified and authenticated in the Department of Crop, Soil and Pest Management, of the Federal University of Technology, Akure, Nigeria.

2.2 Sample Treatments and Preparation

The fresh tomatoes were washed and blended into paste. Both the blended and the commercially processed tomatoes were freeze-dried at the Central Laboratory of Obafemi Awolowo University, Ile-Ife, Nigeria. The freeze-dried samples (ripe tomato fruit, unripe tomato fruit, Paste 1 tomato, Paste 2 Tomato) were divided in to two groups each; the first group was extracted with ethanol, while the second group was subjected to *in vitro* enzyme (α- amylase, pespsin, pancreatin) digestion.

Preparation of Ethanolic Extract

The extraction steps were carried out by soaking 2 g of each of the lyophilized sample in 40ml of 96% ethanol for 24 hours, after which the supernatant was filtered with filter paper No.42 and stored in an amber bottle. This process was repeated by the addition of another 40ml of 96% ethanol to the residue for another 24 hours and the supernatant pulled together. The filtrate was stored at 4^oC.

Preparation of Brain and Liver Homogenates for Lipid Peroxidation Assay

The rats were anesthetized with chloroform and then sacrificed. The cerebral tissue (whole brain) and liver were rapidly isolated, weighed and placed on ice. The tissues were subsequently homogenized in 0.1 M Tris-HCl pH 7.4 with about ten up and ten down strokes at approximately 1,200 rpm in a Teflon–glass homogenizer. The homogenate was centrifuged for 10 minutes at 3,000 g to yield a pellet that was discarded and the low-speed supernatant was kept for lipid peroxidation assay, acetylcholinesterase (AChE) and butrylcholineesterase (BuChE) inhibition assay.

2.3 *In vitro* Enzymatic Digestion

The *in vitro* digestion using sequential enzymatic steps is based on a slightly modified method reported by Ross, Gutierrez-Botero, and Van Am-

burgh (2013). Two gram of each freeze-dried tomato sample was weighed and dissolved in 40 mL of distilled water. 300 μL of α-amylase (32.5 mg of α- amylase was dissolved in 25 mL of 1 mM calcium chloride at pH 7) was added to the tubes. The tubes were incubated in a shaking water bath set at 37°C for 10 minutes and at 80 strokes/minute. After 10 minutes, the pH was adjusted to 2 using concentrated HCl. After 30 minutes of incubation in a shaking water bath set at 37 ^{o}C, 2 mg pepsin, which was dissolved in 1 ML of 0.05 M HCl, was added to the tube. The tubes were then incubated in a shaking water bath set at 37 ^{o}C for 10 minutes and at 80 strokes/minute.
After further 20 minutes of shaking the tubes, the pH was adjusted to 6 using NaOH. Then, 10 mL of pancreatin (3 g of pancreatin was dissolved in 20 mL distilled water) was added, and the tubes were incubated in a shaking water bath set at 37 ^{o}C for 20 minutes. The pH was adjusted finally to 7.5 using NaOH (simulating the pH conditions in the small intestine). Then, the tubes were incubated for 10 minutes in a shaking water bath set at 37 ^{o}C. The digested sample was incubated at 100 ^{o}C for 4 minutes to inactivate the enzymes, and the digested sample was then centrifuged for 60 minutes at 3,200 g, and then, the soluble fraction was kept in the refrigerator for antioxidant, anticholinesterase and lipid peroxidation analyses. The insoluble fraction was discarded. An undigested control was also prepared using the same scheme of *in vitro* digestion but without the enzymes.

2.4 Antioxidant Indices

A modified Folin–Ciocalteu method (Berker, Olgun, Ozyurt, Demirata, & Apak, 2013) and a method reported by Meda, Lamien, Romito, Millogo, and Nacoulma (2005) were used to measure the total phenolic content (TPC) and total flavonoid content (TFC), respectively. Centrifuged ethanol extracts and enzyme digest were reacted with Folin–Ciocalteu phenol reagent and sodium carbonate (20 per cent, w/v for 2 hours) and absorbance was read at 760 nm. Gallic acid was used as a standard and the TPC expressed as mg of gallic acid equivalent (GAE) per gram. Similarly, the centrifuged ethanol extracts and enzyme digests were reacted with 0.5 mL ethanol, 50 μL of 10 per cent $AlCl_3$, 50 μL of 1 mol L^{-1} potassium acetate and 1.4 mL water and allowed to incubate at room temperature for 30 minutes. Thereafter, the absorbance of each reaction mixture was subsequently measured at 415 nm. The TFC was calculated using quercetin as a standard by making use of a seven-point standard curve (0-100 μg/mL). The ferric-reducing properties of the ethanolic extracts and enzyme digests were determined using the method of Oyaizu (1986), by reacting 1 mL ethanol extracts and enzyme digest with 1 mL of 200 mM sodium phosphate buffer (pH 6.6) and 1 mL of 1 percent potassium ferricyanide. The mixture was incubated at 50 ^{o}C for 20 minutes, and then, 1 mL of 10 percent trichloroacetic acid was added. This mixture was centrifuged at 353 x g for 10 minutes. The supernatant (2 mL) was mixed with an equal volume of water and 0.4 mL of 0.1 percent ferric chloride. The absorbance was measured at 700 nm. The ferric-reducing antioxidant power was expressed as milligram of ascorbic acid equivalent/gram of the sample. Radical scavenging antioxidant activity of the ethanolic extracts and enzyme digests were determined using the 2, 2–azino-bis-3-thylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging assay according to Awika, Rooney, Wu, Prior, and Cisneros-Zevallos (2003); 1, 1 diphenyl-2-picrylhdrazyl (DPPH) radical scavenging assay according to the method of Brand-Williams, Cuvelier, and Berset (1995); Hydroxyl radical scavenging assay by Halliwell and Gutteridge (1999) and nitric oxide radical scavenging assay by Griess reaction using the method of Sangameswaran, Balakrishnan, Deshraj, and Jayakar (2009). Trolox was used as a standard for ABTS and DPPH antiradical assay, and the values reported as μmol trolox equivalent anti-oxidant capacity per gram (μmol TE/g). Ascorbic acid was used as the standard for nitric oxide radical assay and the values were expressed as percentage inhibition.

2.5 Lipid Peroxidation Assay

The ability of the ethanolic extract and the *in vitro*-digested samples to inhibit lipid peroxida-

tion was assessed using a modified method presented in the study by Ohkawa, Ohishi, and Yagi (1979). The homogenates obtained (100 μL) from white rat's liver and brain were incubated with (or without for the blank); 50 μL of freshly prepared 0.071 mM $FeSO_4$, 30 μL 100 mM Tris-HCl (pH 7.4) and ethanol extract/*in vitro*-digested samples (0-100 μL), together with an appropriate volume of deionized water, to give a total volume of 300 μL were then incubated at 37 ^{o}C for 1 hour. The color reaction was carried out by adding 300 μL of 8.1 percent w/v sodium dodecyl sulphate, 500 μL of 0.15 percent v/v acetic acid solution (pH 3.4) and 500 μL of 0.6 percent w/v thiobarbithuric acid. The absorbance was read after cooling the tubes at a wavelength of 532 nm. As control, the homogenate was peroxidized with 0.071 mM $FeSO_4$ without the *in vitro* enzyme-digested samples or ethanolic extracts. A blank containing other reagents except $FeSO_4$, homogenate and the extracts was also prepared.

2.6 Acetylcholine and Butrylcholine Esterase Inhibitory Activity Assay

AChE and BuChE inhibitory activity were measured by the spectrophotometric method developed by Lin, Liu, Lin, and Wu (2004) with slight modifications, using acetylcholine iodide and butrylcholine iodide as substrates, respectively. The rate of production of thiocholine is determined by the continuous reaction of the thiol with 5, 5-dithiobis-2-nitrobenzoate (DTNB) ion to produce the yellow anion of 5-thio-2-nitrobenzoic acid. Briefly, 1 mL of 10 mM DTNB dissolved in 10 mM sodium phosphate buffer (pH 7.0) was added to 0.6 mL of distilled water. The brain homogenate, acting as the enzyme source (0.1 mL) and the digested sample/ethanol extract (0.1 mL) was then added to the mixture and incubated for 2 minutes at 25 ^{o}C before 0.2 mL of 8mM acetylcholine iodide (substrate) was added. The absorbance of the mixture was read at 412 nm at intervals of 30 seconds for 5 minutes immediately after the substrate was added. For the control, 0.1 mL of brain homogenate (enzyme source) was added to 1mL of 10 mM DTNB dissolved in 10 mM sodium phosphate buffer (pH 7.0) and 0.7 mL of distilled water. The mixture was incubated at 25 ^{o}C for 2 minutes before 0.2 mL of 8 mM of acetylcholine iodide was added and the absorbance was taken immediately. The distilled water (0.1 mL) and 10 mM DTNB (1 mL) were used as blank. The procedure was repeated using 8 mM butrylcholine iodide as the substrate. The results were expressed in μmol min^{-1}mg protein^{-1} using a molar extinction coefficient of 13.6 x10^3 M^{-1}cm^{-1}.

2.7 Statistical Analysis

All the analyses were run in triplicates. Results were then computed using Microsoft Excel software (Microsoft Corporation, Redmond, WA) and followed by one-way Anova Tukey Multiple Range Test (TMRT) to compare the means that showed significant variation by using graph pad for windows. The significance level was set at $p<0.05$.

3 Results and Discussions

3.1 Results

Antioxidant Indices

The result of the total phenolic content (TPC), is as presented on Table 1.The results revealed a higher TPC (mg GAE/g sample) for all *in vitro* enzyme-digested tomato samples (ripe tomato: 61.08; processed tomato *paste 2*: 60.36; processed tomato *paste 1*: 56.02; unripe tomato: 38.97) than the ethanolic extracts (ripe tomato: 35.59; processed tomato *paste 2*: 30.28; processed tomato *paste 1*: 29.99; unripe tomato: 14.91). Thus, the maximum amount of total phenolic compounds was released during *in vitro* digestion process because of the activity of the enzymes (α-amylase, pepsin and pancreatin) of the gastrointestinal tract. The result further showed that the TPC of *in vitro* digested ripe tomato (61.08 mg GAE g^{-1} sample) was higher than *in vitro* digested unripe sample (38.97 mg GAE g^{-1} sample) and that TPC of ripe unprocessed tomato was higher than the processed tomato (both *paste 2* and *paste 1*).

The total flavonoid content (mg quercetin equivalent/g of sample) of ethanolic extracts and *in vitro* enzyme digested samples of tomato fruits (ripe and unripe) and commercially processed tomato *paste 2* and *paste 1* is as shown in Table 1. The result also showed that the *in vitro* enzyme digested samples have higher total flavonoid content (ripe tomato: 50.86; processed tomato *paste 2*: 43.95; processed tomato *paste 1*: 29.47; unripe tomato: 25.06) than the ethanolic extracts (ripe tomato: 21.25; processed tomato *paste 2*: 18.06; processed tomato *paste 1*:10.62; unripe tomato: 10.01). The result also revealed that processed tomato *paste 2* has higher TFC compared to *paste 1*.

Similarly, the result of ferric reducing antioxidant power (mg ascorbic acid equivalent/g of sample) of ethanolic extracts and, *in vitro* enzyme digested samples of tomato fruits (ripe and unripe) and commercially processed tomato *paste 2* and *paste 1* (Table 1) , revealed that the digested samples have higher reducing power (ripe tomato: 19.89; processed tomato *paste 2*: 18.70; processed tomato *paste 1*: 12.86; unripe tomato:10.36) than the ethanolic extracts (ripe tomato: 11.98; tomato *paste 2*: 11.40; tomato *paste 1*: 7.39; unripe tomato: 1.70). Similar to what was observed in the total phenolic and flavonoid estimation, *in vitro* digested ripe tomato showed higher reducing power than digested unripe tomato. In addition, higher reducing power was recorded in ripe tomato compared to the processed tomato in both ethanolic extracts and digested samples.

The result of the radical scavenging potentials of the *in vitro* enzyme digested tomato samples displayed a higher radical scavenging activities (DPPH*, $ABST^{\cdot+}$, NO* and $^{\cdot}OH$*) compared to the ethanolic extracts. In addition, radical scavenging activities ($DPPH^{\cdot}$, $ABTS^{\cdot+}$, $NO^{\cdot}$ and $^{\cdot}OH$) was higher in digested ripe tomato than in digested processed tomato, while processed tomato *paste 2* revealed higher radical scavenging activities than tomato *paste 1*.

Antioxidant Enzyme Assay

In this current study, the ethanolic extracts and *in vitro* digested samples revealed enhanced activities of the antioxidant enzymes (GPx, GSH, SOD and CAT) in a manner similar to the trends observed in the phenolic content estimation and radical scavenging activities 3. The activities of the enzymes were enhanced to a higher degree after simulated *in vitro* digestion of both the tomato fruit and commercially processed tomato product. The digested ripe tomato fruit reveal higher activities of the antioxidant enzyme compared to processed tomato paste and unripe tomato fruit. Tomato *paste 2* also showed higher enhanced activities of the enzymes than tomato *paste 1*.

Lipid Peroxidation Assay

Both the ethanolic extracts and *in vitro* digested samples of tomato (fruit and paste) showed potential for inhibition of lipid peroxidation 2. Similarly, *in vitro* digested samples revealed higher inhibitory action against lipid oxidation compared to ethanolic extracts. Higher inhibitory action was also recorded for digested ripe tomato while the least inhibition action was observed in unripe tomato. The result also showed that processed tomato *paste 2* has higher inhibition action than tomato *paste 1*.

Acetylcholine and Butrylcholine Esterase Inhibitory Activity Assay

The result obtained from the cholinesterase (AChE and BuChE) inhibitory action of tomato fruit and paste revealed that *in vitro* enzyme digested and ethanolic extracts of tomato fruit and paste samples possessed appreciable potential of inhibiting AChE and BuChE activity (Figures 1 and 2). The *in vitro* digested samples displayed higher inhibitory action compared to the ethanolic extracts. The highest % inhibitory action against AChE and BuChE activity was recorded for digested ripe tomato followed by tomato *paste 2*, while the least % inhibition was observed in unripe tomato. Furthermore, processed tomato *paste 2* tomato had higher inhibition potential than processed tomato *paste 1*.

3.2 Discussion

The observed higher phenolic content in the *in vitro* digested tomato samples is in agreement

Table 1: Antioxidant Indices of tomato fruit (ripe and unripe) and paste (*paste 2* and *paste 1*)

Samples	TPC (mg GAE/g)	TFC (mg QE/g)	FRAP (mg AAE/g)	DPPH (μmol TE/g)	ABTS (μmol TE/g)	NO (% Inhibition)	OH (% Inhibition)
RT	35.59±0.09^{d}	21.25±1.01^{c}	11.98±0.01^{d}	50.03±0.40^{d}	89.86±0.00^{c}	58.67±0.60^{c}	18.11±0.90^{d}
UT	14.91±0.05^{a}	10.01±0.35^{a}	01.70±0.01^{a}	34.14±0.20^{a}	83.40±0.40^{a}	48.36±0.60^{a}	0.47±0.18^{a}
P1	29.99±0.03^{b}	10.62±0.62^{a}	07.40±0.00^{b}	37.04±0.35^{b}	87.64±0.60^{b}	49.24±0.11^{a}	10.57±2.09c
P2	30.28±0.05^{c}	18.06±0.30^{b}	11.40±0.21^{d}	42.13±0.12^{c}	89.38±0.60^{c}	53.48±0.00^{b}	10.60±0.09^{c}
DRT	61.08±0.30^{fg}	50.86±0.41^{g}	19.89±0.00^{g}	71.90±0.63^{g}	95.49±0.14^{e}	69.06±0.51^{f}	35.71±0.18^{g}
DUT	38.97±0.55^{e}	25.06±0.00^{d}	10.36±0.00^{c}	50.75±0.81^{d}	89.86±0.14^{c}	60.75±0.17^{d}	06.99±0.73^{b}
DP1	56.02±0.05^{f}	29.47±0.66^{e}	12.86±0.02^{e}	63.34±0.37^{e}	93.75±0.00^{d}	64.67±0.37^{e}	26.87±0.73^{e}
DP2	60.36±0.35^{fg}	43.95±0.66^{f}	18.70±0.01^{f}	65.06±0.27^{f}	94.86±0.60^{e}	67.55±0.06^{f}	32.43±0.91^{f}

Values represent mean± standard deviation of triplicate experiments. Values with different superscripts in the same column differ significantly ($p<0.05$). Abbreviations: RT= Ripe Tomatoes; UT= Unripe Tomatoes; P1= Paste 1 Tomatoes; P2= Paste 2 Tomatoes; DRT= Digested Ripe Tomatoes; DUT= Digested Unripe Tomatoes; DPT= Digested Paste 2 Tomatoes; DGT= Digested Paste 1 Tomatoes; GAE=Gallic Acid Equivalent; QE=Quercetin Equivalent; AAE=Ascorbic Acid Equivalent; TE=Trolox equivalents.

Table 2: IC50 values of ethanolic extract and *in vitro* digested tomato fruit and paste on iron and sodium nitropuside induced lipid oxidation (mg/g)

Samples	Fe^{2+} induced		SNP induced	
	Brain	Liver	Brain	Liver
RT	108.3±0.02^{d}	70.00±0.29^{d}	108.0±1.01^{d}	60.91±0.43^{b}
UT	183.2±0.44^{h}	119.6±1.34^{h}	183.2±0.19^{h}	125.3±1.63^{g}
P1	174.6±0.16^{g}	78.42±0.34^{f}	124.6±0.51^{g}	80.49±1.56^{e}
P2	161.3±0.08^{f}	72.47±1.02^{e}	116.2±1.56^{e}	65.05±0.00^{c}
DRT	69.51±0.09^{a}	53.21±0.45^{a}	79.51±0.00^{a}	52.01±0.04^{a}
DUT	126.1±1.02^{e}	95.09±0.69^{g}	122.1±0.97^{f}	86.54±1.02^{f}
DP1	83.13±0.06^{c}	66.19±0.75^{c}	83.13±0.04^{b}	76.81±0.56^{d}
DP2	76.42±0.05^{b}	63.73±1.34^{b}	94.42±0.68^{c}	61.01±1.01^{b}

Values represent mean± standard deviation of triplicate experiments. Values with different superscripts in the same column differ significantly ($p<0.05$). Abbreviation: RT= Ripe Tomatoes; UT= Unripe Tomatoes; P1= Paste 1 Tomatoes; P2= Paste 2 Tomatoes; DRT= Digested Ripe Tomatoes; DUT= Digested Unripe Tomatoes; DPT= Digested Paste 2 Tomatoes; DGT= Digested Paste 1 Tomatoes

Table 3: Effect of ethanolic extract and *in vitro* digested samples of tomato fruit and paste on antioxidant enzyme activities

Samples	GSH (U/mL)	GPX (U/mL)	SOD (U/mL)	CATALASE (U/mL)
RT	8.54 ± 0.65^{c}	14.6 ± 0.18^{e}	3.14 ± 0.02^{c}	2.49 ± 0.09^{c}
UT	5.39 ± 0.26^{a}	1.68 ± 0.11^{a}	0.68 ± 0.13^{a}	1.10 ± 0.02^{a}
P1	6.43 ± 0.16^{b}	2.83 ± 0.9^{b}	1.64 ± 0.03^{b}	1.57 ± 0.11^{b}
P2	6.63 ± 0.08^{b}	8.38 ± 0.06^{d}	3.12 ± 0.04^{c}	2.15 ± 0.47^{b}
DRT	10.68 ± 0.50^{e}	34.3 ± 0.02^{h}	6.04 ± 0.02^{e}	5.15 ± 0.14^{d}
DUT	5.78 ± 0.65^{a}	6.62 ± 1.98^{c}	1.46 ± 0.10^{b}	1.11 ± 0.01^{a}
DP1	9.24 ± 0.17^{d}	17.30 ± 0.75^{f}	3.27 ± 0.00^{c}	2.61 ± 0.23^{b}
DP2	9.94 ± 0.13^{d}	22.87 ± 0.08^{g}	4.06 ± 0.02^{d}	2.67 ± 0.25^{b}

Values represent mean± standard deviation of triplicate experiments. Values with different superscripts in the same column differ significantly ($p<0.05$) Abbreviation: RT= Ripe Tomatoes; UT= Unripe Tomatoes; P1= Paste 1 Tomatoes; P2= Paste 2 Tomatoes; DRT= Digested Tomatoes; DUT= Digested Unripe Tomatoes; DP2= Digested Paste 2 Ripe Tomatoes; DP1= Digested Paste 1 Tomatoes; GSH= Glutathion; PX=Glutathion peroxidase; SOD=Superoxide dismutase.

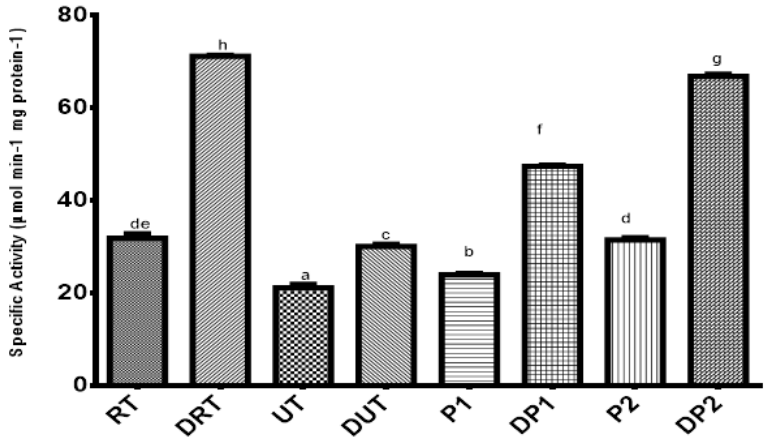

Figure 1: Acetylcholinesterase Inhibitory activity of *in vitro* digested tomato fruit and paste. Values are given as mean ± SE of independent experiments performed in triplicate. Bars with different letters are significantly different ($p< 0.05$) by Tukey Test. Abbreviation: RT= Ripe Tomatoes; UT= Unripe Tomatoes; P1= Paste 1 Tomatoes; P2= Paste 2 Tomatoes; DRT= Digested Ripe Tomatoes; DUT= Digested Unripe Tomatoes; DPT= Digested Paste 2 Tomatoes; DGT= Digested Paste 1 Tomatoes

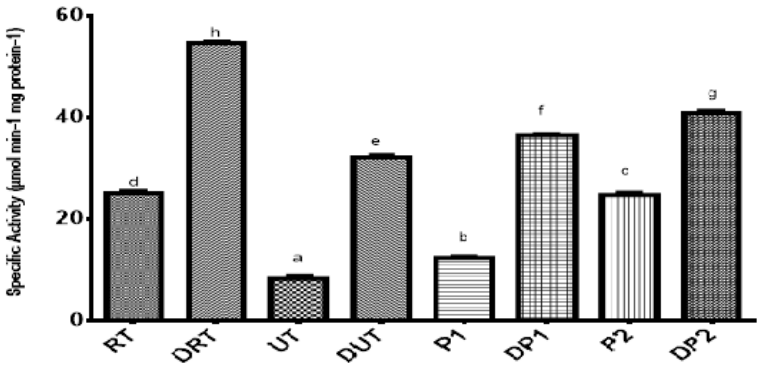

Figure 2: Butyrylcholinesterase Inhibitory of *in vitro* digested tomato fruit and paste. Values are given as mean ± SE of independent experiments performed in triplicate. Bars with different letters are significantly different ($p < 0.05$) by Tukey Test. Abbreviation: RT= Ripe Tomatoes; UT= Unripe Tomatoes; P1= Paste 1 Tomatoes; P2= Paste 2 Tomatoes; DRT= Digested Ripe Tomatoes; DUT= Digested Unripe Tomatoes; DPT= Digested Paste 2 Tomatoes; DGT= Digested Paste 1 Tomatoes

with a previous report of Tagliazucchi, Verzelloni, Bertolini, and Conte (2010), which suggested that digestion might be a determinant factor in the release of nutritionally relevant compounds from the food matrix. The result further showed that the TPC of *in vitro* digested ripe tomato (61.08 mg GAE g^{-1} sample) was higher than that of the digested unripe sample (38.97 mg TAE g^{-1} sample). The observed higher TPC in enzyme digested ripe tomato may be associated with a considerable accumulation of lycopene in ripped tomato, which is due to the enhanced enzymatic activity of phytoene synthase I that causes a massive production of lycopene precursors in ripening tomato fruits (Fraser, Enfissi, & Bramley, 2009). The observed higher TPC of ripe unprocessed tomato compared with the processed tomato (both *paste 2* and *paste 1*), may be ascribed to the different processing methods. This is in correlation with the work of Anese, Mirolo, Beraldo, and Lippe (2013), where it was reported that non-thermal processing seems to have an adverse effect on bioaccessibility of lycopene. For example, the loss of cell integrity observed with increasing ultrasonication time was accompanied by a decrease in lycopene bio-accessibility.

The observed high flavonoid content of the *in vitro* digested tomato fruit and pastes compared to the ethanolic extract is in agreement with the report of Salawu, Ajiboye, Akindahunsi, and Boligon (2017), where higher flavonoid content were recorded for the *in vitro* digested white and yellow bitter yams compared to their raw counterparts. This observation might be due to the breakdown of the insoluble fiber matrix of both bitter yam varieties thereby making its flavonoids more accessible for further breakdown by the enzymes of gastro intestinal tract. Ripe digested sample have a higher TFC than the digested processed samples. Ranilla, Genovese, and Lajolo (2007) mentioned that cooking time, temperature, soaking and draining can significantly reduce antioxidant activity of plants. This is also in agreement with the report of Fawole and Opara (2013), it was suggested that naturally occurring flavonoid could be obviously lost during processing and storage, which could affect the overall antioxidant and nutritional value after post-harvest treatment. The observed high flavonoid content in tomato *paste 2* compared to *paste 1* might be due to the varietal differences in the tomato fruits used in the production of tomato *paste 1* and tomato *paste 2* and the processing methods. Macheix, Fleuriet, and Billot (1990) suggested that genetic control is the primary factor in determining phenols in fruits and vegetables, their level may be affected by environmental condi-

tions, such as light, temperature and processing methods.

The ferric reducing antioxidant power (FRAP) of a compound may serve as an important indicator of its potential antioxidants activity (Liu et al., 2013). The observed higher FRAP in the *in vitro* enzyme digested samples is in agreement with the work of Saura-Calixto and Goni (2006), who reported that *in vitro* enzyme digested plant yield a higher reducing power that than organic extracts. The observed high reducing power in ripe tomato for both ethanolic extracts and *in vitro* digested samples compared to commercially processed tomato pastes may be attributed to various processing steps of the tomato paste. This is in agreement with the report of Szydlowska-Czerniak, Trokowski, Karlovits, and Szlyk (2011), where it was reported that ferric reducing antioxidant power was decreased by 41% during thermal processing of palm oil.

Most plant foods are rich sources of free radical scavenging molecules and other metabolites, which are rich in antioxidant activity (Nimse & Pal, 2015). The observed high radical scavenging activities ($DPPH^{\cdot}$, $ABST^{\cdot +}$, $NO^{\cdot}$ and $^{\cdot}OH$)the *in vitro* enzyme digested tomato samples compared to the ethanolic extracts is in agreement with the work of Hachibamba, Dykes, Awika, Minnaar, and Duodu (2013), where it was found that the TPC and radical scavenging properties of cowpea was increased with simulated *in vitro* enzyme digestion. The observed high radical scavenging activities in digested ripe tomato fruits compared to the commercially processed tomato pastes is likely to be as a result of heat processing to attain final solid level. Longer processing times, required to achieve the desired final solids levels, may be associated with increased losses. Other studies have also shown thermal processing to decrease significantly the TPC, anthocyanin content, and antioxidant activity (Hiemori, Koh, & Mitchell, 2009).

It has been found that a substantial link exists between free radicals and more than sixty different health conditions, including the aging process, cancer, diabetes, Alzheimer's disease, strokes, heart attacks and atherosclerosis. By reducing exposure to free radicals and increasing the intake of antioxidant enzyme rich foods or antioxidant enzyme supplements, our body's potential to reducing the risk of free radical related health problems is made more palpable (Worthington Enzyme Manual, 2009). The enhanced antioxidant enzyme parameters in the presence of the ethanolic extracts and *in vitro* digested tomato samples in a manner similar to the trends observed in the phenolic content estimate and radical scavenging assays is in agreement Jalili, Ilkhanipour, Heydari, Farshid, and Salehi (2007), and Ergüder and Durak (2006). These authors, confirmed that administration of lycopene at 100, 200 and 300 mg/kg doses decreased the level of oxidant parameters (MDA) and significantly increased blood and gastric antioxidant parameters (SOD, CAT and GSH-Px), and suggested that lycopene may reduce oxidative injury of gastric cancer rats partly through stimulating antioxidant enzyme activities. Antioxidant enzymes are, therefore, critical for maintaining optimal cellular and systemic health and well-being.

Both the ethanolic extracts and *in vitro* digested samples of tomato (fruit and paste) showed potential of inhibiting lipid oxidation. The result correlate with the work of Luo and Wu (2011), where it was reported that lycopene treatment in rats, with induced gastric cancer significantly ameliorated an increased MDA level at the end of the experiment. Since flavonoid represent the major component of the total phenolic content in tomato (Toor & Savage, 2005), hence, the flavonoids, lycopene and other phenolic compounds present in tomato fruit and paste may be responsible for their ability to inhibit lipid peroxidation.

In recent years, the search for inhibitors of cholinesterases has grown in interest, since these enzymes are associated with Alzheimer's disease, senile dementia, ataxia, myasthenia gravis, and Parkinson's disease among others (Mukherjee, Kumar, Mal, & Houghton, 2007; Vinholes et al., 2011). Plant alkaloids are best known for inhibiting cholinesterase enzymes, however, recent reports have indicated new classes of cholinesterase inhibiting phytochemicals such as coumarins, flavonols, terpenoids, and especially monoterpenes that are relevant antioxidant phytochemicals (Katalinic, Bosak, & Kovarik, 2014; Szwajgier, 2014). Hence, the observed anticholinesterase action in both ethanolic extracts

and *in vitro* enzyme digest of the studied tomato samples could be ascribed to the constituent phytochemicals in tomato fruits and commercially processed tomato pastes.

4 Conclusion

The result of this investigation showed that the studied tomato fruit and pastes possess a substantial level of antioxidant and anticholinesterase activities after the simulated human gastrointestinal digestion, with the ripe tomato fruit ranking high. This by implication suggest that the health promoting phytoconstituents of the tomato fruit and pastes would be readily bio-available after passing through the gastrointestinal digestive tract and therefore could be harnessed as functional food.

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Evaluation of the Effectiveness of Cereal Bran Extract for Sunflower Oil Stability during Frying

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Abstract

This study evaluated the effectiveness of black rice, millet and barley bran extracts against oxidative degradation of sunflower oil in frying, by determining the total antioxidant activity, total phenolic content, free fatty acid content, conjugated diene content and total polar content. It was reported that the total phenolic content rice bran was approximately three times higher than that of the millet bran extracts and five times higher than the results for barley bran extracts. The total antioxidant activity results for barley bran and rice bran extract (40.95 ± 0.07and 40.87 ± 0.04 Trolox equivalent μmol/g of bran, respectively) were two times higher than that of millet bran extract (17.16 ± 0.34 Trolox equivalent μmol/g of bran). The results of the effectiveness of the cereal bran extracts were significantly different ($p<0.05$). The free fatty acid content of the rice bran and propyl gallate enriched oil samples showed better results (2.02 ± 0.01% and 1.62 ± 0.00%) than millet, barley and control enriched oil samples (3.43 ± 0.01%, 3.13 ± 0.01% and 6.13 ± 0.01% respectively). In the same vein, conjugated diene content results from all the enriched oil samples indicated that the rice bran enriched oil sample had the least amount of secondary oxidized products compared to the other enriched oil samples. It can be concluded that rice bran extract can be used for frying without discarding or replenishing the oil.

Keywords: Bran extracts; Antioxidant; Oil Stability; Propyl gallate

1 Introduction

Hydrolysis, oxidation and polymerization are the most prominent chemical reactions that occur in the deep-fat frying process and the resulting products from these reactions adversely affect the flavor, quality of oil, the nutritional composition and safety of the fried food (Sumnu & Sahin, 2008). Free fatty acids, aldehyde, ketone, triglyceride, monoglyceride, cyclic and epoxy compounds, trans-isomers, dimer and oligomer are the primary resulting organic compounds from the series of chemical reactions mentioned above that do affect the safety and quality of the frying oil (Choe & Min, 2007).

Over the years, different oxidative-controlling methods have been used, such as nitrogen and carbon dioxide blanketing, vacuum frying, and the use of genetically modified oil. Addition of antioxidants has been developed to reduce the extent to which oxidative degradation reactions occur in oil during frying (Aladedunye, Matthäus & Przybylski, 2011), however, it was discovered that the addition of an antioxidant was both effective against oxidation in oil and cost efficient. Butylated hydroxy-

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Nomenclature

FFA	Free Fatty Acid	PG	Propyl gallate
TEAC	Trolox equivalent antioxidant capacity	RBE	Rice bran extracts
TPC	Total phenolic content	MBE	Millet bran extracts
TPM	Total polar material	BBE	Barley bran extracts

toluene (BHT) and butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ), 2-tert-butyl-4-methylphenol (TBMP), and propyl gallate (PG) are common examples of the antioxidants used in the vegetable oil industry (Pokorny, Yanishlieva & Gordon, 2001). Although, the application of these synthetic antioxidants to vegetable oils during frying had been effective at 200 ppm Codex Alimentarius standard over the years, there is a growing concern about safety due to some toxicological reports (Okubo, Yokoyama, Kano & Kano, 2003). This contributed to a consumer-initiated trend called "clean labeling" which simply demands that the majority of food additives should be replaced with ones from natural sources. Taghvaei and Jafari (2015) reviewed all the available research from 1997 to 2007 on application and stability of natural antioxidants (plant and animal sources) in edible oil in comparison with synthetic. They pointed out that besides the high effectiveness of the natural antioxidant against degradation in edible oils, sourcing for their raw materials are quite cheaper than the synthetic ones. A market survey conducted over the span of nine years (2000-2009) on the €15 million European antioxidant market, estimated 35 per cent compound growth rate for natural antioxidants (Thorat et al., 2013). This subsequently led to the genesis of sourcing for cheap natural source of antioxidants which are as effective as their synthetic counterparts.

Rehman (2006) has reported that by-products from fruit and vegetable processing had been the major source of natural antioxidants used in ensuring oil stability during storage and food processing. Nonetheless, contrary to popular opinion, epidemiological studies and literature have established that cereal grains have high phenolic antioxidant distribution in their outer layers, as much as fruit and vegetable by-products (Masisi, Beta & Moghadasian, 2016). Madhujith and Shahidi (2009) compared the antioxidant properties of different fractions of barley varieties using the Trolox equivalent antioxidant capacity (TEAC) assay on phenolic extracts. The results for TEAC showed that outermost layer of the barley grains for the varieties exhibited higher antioxidant capacity range (9.11-69.30μmol of Trolox equiv/g of defatted material) than the innermost fraction (0.45-7.85μmol of Trolox equiv/g of defatted material). (Huang & Lai, 2016) also reported that red and black rice bran showed a similar trend of high range of antioxidant capacity (6.69-14.55, 18.94-49.99 mg Trolox equiv/g of defatted material) using DDPH antioxidant assay. However, there are limited reports on the application of these cereal brans as natural antioxidants to improve oil stability during frying.

The aim of this study is to compare the effectiveness of three cereal bran extracts (black rice, millet and barley) as natural sources of antioxidants with a synthetic antioxidant (propyl gallate) against oil degradation during frying.

2 Materials and Methods

2.1 Samples and chemicals used

Whole grain black rice, millet and barley were kindly supplied by Healthy food suppliers West

Sussex, UK. Sunflower vegetable oil was obtained from a local Sainsbury store, Greater London, UK. All chemicals purchased were analytical grade (Fischer scientific and Sigma-aldrich)

2.2 Preparation of the bran

The barley, millet and rice bran were obtained following the procedure described by Arab, Alemzadeh and Maghsoudi (2011) with minor adjustments. A Kenwood grain mill (A941PK005/P, Havant UK) was used to mill the barley, millet and rice wholegrain, respectively and a 2.5-inch fine plastic mesh strainer was used to sieve the bran from the resulting flour. All brans were stored in a sealed plastic container immediately, except for the rice bran which was subjected to stabilization. The stabilization was carried out as described by Arab et al. (2011). The rice bran was then cooled at room temperature overnight and stored at 4°C until further analyses.

2.3 Extraction of phenolic extracts from the grain bran

The extraction was done according to the procedure described by Lai, Li, Lu and Chen (2009). Five grams of barley, millet and stabilized rice bran were extracted with 20ml (ratio 1:4) each of three different solvents, namely methanol, ethanol and ethyl acetate in an electric orbital shaker (55rpm; Stuart S5105, UK) at room temperature for (3) three hours. This was followed by filtration of the extract through 250mm of Fisher's filter paper and the residue was re-extracted twice, then the filtrate was dried under vacuum using a rotary evaporator (Stuart RE301P, UK) at 50°C. The percentage extract yield was calculated using the equation (1) below:

$$EY = \frac{(W_{after\ drying} - W_{before\ drying})}{Actual\ weight\ of\ sample} \times 100 \quad (1)$$

Where EY = Extraction Yield (%) W = weight of the round bottom flask.

2.4 Dermination of total phenolic content in the methanolic bran extracts

The total phenolic content of the bran extracts was determined using the Folin-Ciocalteu reagent method as described by Singleton and A. Jr. Rossi (1965). Results were calculated as Gallic acid equivalents (mg/g of bran) in triplicate.

2.5 Determination of total antioxidant capacity of the methanolic extracts by Trolox Equivalent Antioxidant Capacity (TEAC) assay

Antioxidant capacity of the bran extracts was assayed based on the decolourisation 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical cation ($ABTS^{\cdot+}$) as described by Re et al. (1999) with major adjustments in the preparation of radical cation stock and working solution. The radical cation ($ABTS^{\cdot+}$) stock solution was produced by reacting 50ml of 7mM aqueous solution ABTS (in distilled water) with 50ml of 2.45mM potassium persulphate ($K_2S_2O_8$) in the dark at room temperature for 16 hours in ratio (1:1).The working solutions were then prepared daily by diluting 1ml of the stock solution with 43ml of the extracting solvent (Methanol) to give 0.70 ± 0.05 absorbance at 734nm using a UV Visible Spectrophotometer (JK-UVS-752N,Jinke Scientific, Shanghai).
The standard calibration curve was prepared using a concentration range of 0 - 250μM from 2.5Mm of trolox stock solution. This major adjustment was verified for consistency by taking the absorbance of 0,100 and 200 μM in triplicate before the taking the measurement for the sample. 1.5ml of ABTS radical solution was mixed with 100μl of extract sample or standard solution and measured at 734nm after 6 minutes. The observed absorbance for the sample or the standard were expressed as percentage inhibition in triplicates using equation (2) below:

$$Inhibition\ \% = \frac{(A_{blank} - A_{extracts})}{A_{blank}} \times 100 \quad (2)$$

Where A_{blank}= Absorbance observed for blank after 6 minutes
$A_{extracts}$= Absorbance observed for extracts after 6 minutes

The standard curves were constructed using the inhibition % as a function of trolox concentration and the results for the sample extracts were expressed as Trolox equivalents in terms of μmolTE/g of the sample.

2.6 Enrichment of the sunflower oil with the synthetic and natural antioxidant

200ppm of propyl gallate was measured according to the codex regulation on the maximum amount of synthetic antioxidant required in edible oil. 1000ppm each of dried rice, barley and MBE were carefully measured and added to the 100ml of the sunflower oil in three different Duran bottles. Also, the control sample oil was prepared with no antioxidant addition. The resulting enriched vegetable oils were homogenized for even distribution of the added extracts by using a high-performance homogenizer (Ultra Turexx, T 25.IKA USA) at 7000rpm for 7min and the resulting stock oils were added to 900ml of the frying oil in the fryer. 100ml of each enriched oil sample and control sample were measured and stored at -4°C until further analyses.

2.7 Frying procedure

To compare the effectiveness of the added antioxidant extracts in the vegetable oil during frying, a standard black-eyed pea paste was made by mixing black eyed pea flour with a 2:1 ratio in water:100g of black eyed-pea flour with 50g of water. 25g of the resulting mixture was rolled into balls and two of balls were fried in each enriched oil sample using a deep fat fryer (Tefal FR333040 fryer, J.C Campbell electrics, Northern Ireland). The oils were heated to 175 ± 2°C, then black eyed pea balls were fried once for 9 minutes and oil was cooled for 6 minutes. 100ml of each oil sample was taken immediately after the cooling of the oil. The frying and cooling processes were repeated five (5) times throughout the frying process. Sample oils were collected after each process (15, 30, 45, 60 and 75 minutes) and stored at -4°C until further analyses.

2.8 Chemical analysis of the oil

The free fatty acid content of the oil samples taken during frying was measured. This is a titrimetric method that was done according to the official procedure described by AOCS Ca 5a-40 (1998). The free fatty acid content of the oil samples was expressed in percentage. Also, the conjugated diene and total polar content of the oil samples taken during frying were measured. These were carried out according to the standard method described by AOCS Ti la-64 (1998) and Xu (2000) respectively. Both oil stability parameters were measured in percentage

2.9 Data analysis

All data were expressed in mean ± standard deviation and chemical characteristics analyses were subjected to one- way ANOVA (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp) and Post hoc test (Tukey HSD) at significance 95% confidence level $p<0.05$)

3 Results and Discussion

3.1 Extraction of phenolic extracts from the grain bran

The methanol extraction (Table 1) proved more efficient, showing an extract yield (%) of about two times higher than the ethanol and ethyl acetate extractions, respectively. Millet bran gave the highest range of extract yield among the three extraction solvents whilst barley bran seems to have the lowest yield results.
The better extract yield shown by methanol can be attributed to high polarity of the solvent (Polarity: methanol > ethanol > ethyl acetate) and the functional group of the polar compounds present in the bran. The extract yields for this study were not as high as the range of results (13.2-20.16% and 7.85-16.3%) reported by Arab

et al. (2011) and Lai et al. (2009). However, the results pattern for each extraction solvent were similar, that is; methanol gave the highest yield extracts from all three brans.

3.2 Determination of total phenolic content in the methanolic bran extracts

The total phenolic content for the rice, millet and BBE reported in Table 2 showed that the result for rice bran is approximately three times higher than that of the MBE and five times higher than the result for BBE.
The better results recorded by the rice bran was due to the high level of pigment (proanthocyanidin and anthocyanin) in the bran compared to the other bran extracts. The main rationale behind the low output could be attributed to the solid-solvent extraction method used for the bran which is effective only for the free phenolic compounds in the bran, which are usually in low amount compared to the conjugated and insoluble bound phenolic compounds that might be present in the bran (Madhujith & Shahidi, 2009). The TPC results for rice bran from this study was significantly higher than the range results (35 ± 0.01 - 7.14 ± 0.60 mg GAE/g) reported by Moongngarm, Daomukda and Khumpika (2012) for varieties of black rice bran.

3.3 Determination of total antioxidant capacity of the methanolic extracts by Trolox Equivalent Antioxidant Capacity (TEAC) Assay

The results described in Table 3 showed that the antioxidant activity for barley bran and rice bran extract are two times higher than that of millet bran extract (17.16 ± 0.34 Trolox equivalent μmol/g of bran). Moreover, the barley bran extract (40.95 ± 0.07 Trolox equivalent μmol/g of bran) showed the best antioxidant activity, higher than the RBE (40.87 ± 0.04 Trolox equivalent μmol/g of bran).
The high antioxidant activity recorded for BBE was consistent within the range of TEAC results (9.11- 69.30μmol of Trolox equiv/g of defatted material) reported by Madhujith, Izydorczyk and Shahidi (2006) for the outermost layer (bran) for two varieties of barley.

3.4 Determination of the free fatty acid content of the frying oil samples

The results for the free fatty acid content in the oil samples during frying (Table 4) showed that all the free fatty acid content for the enriched oil samples and the control at all frying times except at 0, 15, 30 minutes were all significantly different from each other. It is further explained that, at 0 minutes, the Rice Bran extract enriched oil samples were not significantly different from the PG enriched oil sample, and at 15minutes and 30 minutes Barley bran extract enriched, and Millet bran extract enriched, and control oil samples followed the same trend, respectively. Overall results after 75minutes of frying showed that the PG enriched oil had a free fatty acid content (1.62 ± 0.00%) approximately four times lower than that of the control sample (6.13 ± 0.01%), almost two times lower than the FFA in both MBE and BBE (3.43 ± 0.01 and 3.13 ± 0.01%) enriched oil and 0.4% lower than the FFA found in RBE enriched oil samples. Free fatty acids in oil during frying are products of hydrolysis that occur as soon the food materials are immersed in the oil at high temperature. The amount of these compounds in oil is directly proportional to the rate of oxidation that occurred at the propagation step (formation of hydroxyl peroxide radical from the oil molecule) in the degradation of oil molecules by increasing the rate at which atmospheric oxygen (initiator of the propagation step) from the headspace dissolve in the oil (Choe & Min, 2007).
As expected, the FFA will increase with the frying time, but the legal maximum percentage of the FFA should not exceed 2% otherwise the oil should be replaced. Farahmandfar, Asnaashari and Sayyad (2015) reported a range of results slightly like the results for this present study. The FFA content from this present study showed that the RBE enriched oil samples are effective for frying food materials for 1hour 15minutes

Table 1: Extraction yield (%) of three different solvents on the rice, millet and barley bran

Sample	Methanol	Ethanol	Ethyl-acetate
Rice	4.2 ± 0.05	2.8 ± 0.03	2.8 ± 0.12
Millet	11.4 ± 0.21	4.8 ± 0.01	4.4 ± 0.11
Barley	3.0 ±0.15	1.6 ± 0.13	1.4 ± 0.02

Note: All results are reported in mean ± standard deviation

Table 2: The total phenolic distribution in the various cereal extracts

Extracts	Total phenolic content (mg Gallic acid/g of bran)
Rice bran	18.58 ± 0.71
Millet bran	6.94 ± 0.23
Barley bran	3.96 ± 0.12

Note: All results are reported in mean ± standard deviation

Table 3: Antioxidant activities of the cereal bran extracts

Extracts	Trolox equivalent μmol/g of bran
Rice bran	40.87± 0.04
Millet bran	17.16 ± 0.34
Barley bran	40.95 ± 0.07

Note: All results are reported in mean ± standard deviation

compared with the other extract enriched oils without replacing the oil, but not as effective as the synthetic antioxidant (PG) enriched oil.

3.5 Conjugated diene content of the frying oil samples

The conjugated diene content in oil is a reliable indicator to monitor the oxidative deterioration in oil during frying as it measures the primary oxidation products (conjugated diene/trienes) in the oil samples. These are secondary oxidation products of the degradation of oil that cause off-color and odour that affect the quality characteristics of the oil. The conjugated diene content (%) reported in Table 5 showed that all the enriched oil samples and the control at all frying times except at 0 minutes are significantly different from each other. The result further explained at 0 minutes, BBE and MBE are not significantly different from each other. The overall conjugated diene content results for all oil samples after 1 hour 15 minutes showed that the control oil sample had the highest results (70.00 ± 0.16 %) while the results for RBE enriched oil was approximately two times lower than that of BBE enriched oil sample and about 17.49% on average lower than that of MBE and PG enriched oil samples, respectively.

The implication of the abovementioned results means that the RBE was effective as a natural antioxidant, forming a stable compound with the lipid hydro-peroxide radical, thus preventing rapid degradation of the compounds to secondary oxidation products. This can potentially be attributed to γ oryzanol, which has been identified as the major antioxidative phenolic compound in black rice bran (Shin et al., 2017). Chen et al. (2016) reported the effectiveness of γ oryzanol against degradation of primary and secondary oxidation products, which would otherwise result in off-colour and odour. There is no

Table 4: Free fatty acid content for the oil samples at various frying times

Frying Time (minutes)	Control	RBE	MBE	BBE	PG
0	0.49 ± 0.01^a	0.22 ± 0.14^b	0.32 ± 0.01^c	0.60 ± 0.00^d	0.23 ± 0.21^b
15	0.92 ± 0.01^a	0.40 ± 0.00^b	1.23 ± 0.14^c	1.22 ± 0.14^c	0.62 ± 0.14^d
30	1.86 ± 0.01^a	1.31 ± 0.14^b	1.93 ± 0.01^c	1.83 ± 0.01^a	0.92 ± 0.01^d
45	3.77 ± 0.22^a	1.61 ± 0.01^b	2.33 ± 0.02^c	2.22 ± 0.01^c	1.23 ± 0.01^b
60	5.11 ± 0.01^a	1.91 ± 0.00^b	2.92 ± 0.01^c	2.31 ± 0.01^d	1.42 ± 0.01^e
75	6.13 ± 0.01^a	2.02 ± 0.01^b	3.43 ± 0.01^c	3.13 ± 0.01^d	1.62 ± 0.00^e

Results are expressed as mean ± standard deviation.

Means followed by different superscripts show a significant difference ($p < 0.05$) between the values in rows for each frying time.

RBE: Rice bran Extract, MBE: Millet bran extracts, BBE: Barley bran extracts, PG: Propyl gallate.

Table 5: Conjugated diene content for the oil samples at various frying times

Frying Time (minutes)	Control	RBE	MBE	BBE	PG
0	39.37 ± 0.21^a	17.13 ± 0.03^b	25.71 ± 0.63^c	26.31 ± 0.30^c	36.56 ± 0.41^d
15	48.22 ± 0.15^a	23.19 ± 0.06^b	25.81 ± 0.63^c	34.45 ± 0.17^d	38.95 ± 0.12^e
30	52.29 ± 0.14^a	27.71 ± 0.56^b	37.68 ± 0.12^c	51.77 ± 0.21^d	43.94 ± 0.63^e
45	63.41 ± 0.04^a	31.01 ± 0.17^b	36.32 ± 0.04^c	56.98 ± 0.08^d	47.96 ± 0.04^e
60	68.25 ± 0.03^a	32.14 ± 0.24^b	50.03 ± 0.36^c	58.98 ± 0.06^d	50.97 ± 0.02^e
75	70.00 ± 0.16^a	34.28 ± 0.18^b	51.17 ± 0.29^c	63.14 ± 0.00^d	52.36 ± 0.08^e

Results are expressed as mean ± standard deviation.

Means followed by different superscripts show a significant difference ($p < 0.05$) between the values in rows for each frying time.

RBE: Rice bran Extract, MBE: Millet bran extracts, BBE: Barley bran extracts, PG: Propyl gallate.

legal maximum percentage for conjugated diene in used oil samples, but the high value recorded for this present study was an indication of a high degree of degradation in oil samples since the presence of conjugated diene in the oil primarily means oxidation has occurred. Different researchers have attributed an increase in conjugated diene with frying time, temperature, the unsaturation level of the fatty acid composition of the oil (Aydeniz & Yilmaz, 2012).These further explained the high value recorded for the oil characterization carried out in this present study because the value increased with the frying time and the polyunsaturated fatty acids (PUFAs) of the sunflower oil used for this experiment was 69g per100g of oil.

3.6 Total polar material content for the frying oil samples

The total polar material content in oil is the sum of non-volatile polymerized compounds usually by-products of hydrolysis, oxidation, condensation and all other complex reactions that occur during degradation of oil during frying (Tompkins & Perkins, 2000). This is also an imperative, monitoring extent of oxidative degradation, reusability and quality of oil (Aydeniz & Yilmaz,

Table 6: Total polar material content for the frying oil samples

Frying Time (minutes)	Control	RBE	MBE	BBE	PG
0	2.72 ± 0.02^a	6.29 ± 0.09^b	6.34 ± 0.01^b	5.48 ± 0.01^c	2.98 ± 0.01^d
15	2.75 ± 0.03^a	6.17 ± 0.04^b	5.96 ± 0.02^c	5.39 ± 0.05^d	3.04 ± 0.05^e
30	2.84 ± 0.04^a	5.69 ± 0.06^b	5.36 ± 0.04^c	5.18 ± 0.11^d	3.04 ± 0.05^e
45	2.88 ± 0.01^a	5.55 ± 0.08^b	4.92 ± 0.00^c	5.12 ± 0.02^d	3.21 ± 0.05^e
60	2.94 ± 0.01^a	5.38 ± 0.04^b	4.68 ± 0.01^c	4.99 ± 0.00^d	3.17 ± 0.01^e
75	3.03 ± 0.03^a	5.08 ± 0.10^b	4.57 ± 0.04^c	4.76 ± 0.00^c	3.21 ± 0.02^d

Results are expressed as mean ± standard deviation.

Means followed by different superscripts show a significant difference ($p < 0.05$) between the values in rows for each frying time.

RBE: Rice bran Extract, MBE: Millet bran extracts, BBE: Barley bran extracts, PG: Propyl gallate.

2016). The TPM results for this study reported in Table 6 explained that the values were all significantly different from each other across the frying time except for 0 and 75 minutes. The overall results showed that control samples contained TPM values lower than the synthetic (PG) and other extract enriched oils while the RBE enriched oil had the highest content of polar compounds (5.08 ± 0.10 %). However, none of the extract enriched oil samples exceeded the maximum legal limit of 25% (Xu, 2000). The general low amount of polar material for all samples was consistent with the results reported by Karakaya and Simsek (2011) using the same frying time intervals for four different varieties of oil and recording an average TPM of approximately 5%.

It is interesting to note that, the total polar material for RBE, MBE and BBE enriched oil samples were high at 0 minutes and tended to decrease as the frying time increased. This phenomenon can be explained by the high pigmented nature of oil when the extracts were added and, as the frying time proceeded, there was a gradual reduction in the colour of the oil samples. Since the TPM value for the oil samples was measured using spectrophotometry methods, the observed absorbance for each oil sample decreases as the frying time increases. This similar decrease in TPM was also reported by Lee, Lee, Lee, Park and Choe (2002) where they evaluated the effect of different proportions (5%, 15% and 25%) of spinach powder on degradation of soybean oil at 160°C for 24hours.

4 Conclusions

From this study, the methanolic extraction gave the highest extract yield from the three solvents; Methanolic extracts of rice bran contained the highest distribution of phenolic compounds while methanolic extracts of barley bran exhibited the better antioxidant activity, slightly higher than that of RBE.

The application of these methanolic extracts to sunflower oil samples during frying showed that RBE was more effective than the other extract brans and as effective as the synthetic antioxidant (propyl gallate) against degradation of oil during frying.

Summarily, RBE can be used as a potential natural antioxidant source to fry black-eyed pea balls for 1-hour 15minutes without replacing or replenishing the oil and with around 90% of its (rice bran) world production been used as animal feedstock and landfills, it would be a potential economical source and represent sustainable usage as a natural antioxidant.

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Textural, Rheological and Sensory Properties of Spreadable Processed Goat Cheese

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Abstract

The aim of this work was to study the influence of the ripening degree of natural goat cheese on texture, rheological and sensory properties of processed cheese products. Processed cheeses were formulated using goat cheeses with 10, 20 and 40 days of ripening. We obtained four different formulations by varying the proportions of these cheeses in each formulation. The variation in major α, β and para-κ casein fractions, rheological properties and the texture of samples were determined, and a sensorial evaluation was carried out. Cheeses from Formulation 2 (50% cheese ripened for 10 days, 25% cheese ripened for 20 days and 25% cheese ripened for 40 days) had greater values of α and β–caseins, which is related to a greater content of intact casein resulting from a cheese with short ripening time. Hardness, adhesiveness and complex modulus (G*) decreased as the degree of ripening of the natural cheese (raw material) increased. Formulation 2 presented a G* value similar to that of the commercial processed cow cheese and the greatest firmness. Formulation 2 presented the characteristics we aimed to obtain, described as spreadable, slightly acid and salty cheese.

Keywords: Casein; Texture; Sensory characterization; Goat cheese; Complex modulus

1 Introduction

Processed cheese is elaborated by blending natural cheese of different ages and degrees of maturity in the presence of emulsifying salts and other dairy and non-dairy ingredients, followed by heating and continuous mixing to form a homogeneous product with an extended shelf life (Guinee, Carić, & Kalab, 2004). Different parameters are modified in the production of processed cheese, affecting the rheological behavior of the molten mass during processing and the texture of the final product. These parameters are ripening of natural cheese (i.e. the degree of proteolysis), pH of cheese melt, type and concentration of emulsifying salts, processing and storage conditions (processing temperature, speed of agitation, duration of heating, rate of cooling and temperature of storage), dry matter content, fat content, presence and concentration of ions (especially calcium, sodium and potassium) and use of hydrocolloids (Cernikova et al., 2008; Piska & Stetina, 2004).

The characteristics of natural cheese utilized to manufacture processed cheese have a major influence on processed cheese characteristics; therefore, appropriate selection of natural cheese is critical in order to achieve a pro-

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cessed cheese with the desired chemical and functional characteristics. The natural cheese used in a processed cheese formulation is generally selected on the basis of type, flavor, maturity, consistency, texture and pH (Piska & Stetina, 2004). Kapoor, Metzger, Biswas, and Muthukummarappan (2007) highlighted the importance of total intact casein in the quality of processed cheese. Once again, the major ingredient that contributes to the intact casein in a processed cheese formula is the type and age of the natural cheese used in the formula.

Regarding processed cheese, several studies have been carried out on sensory characterization in relation to processing factors and chemical composition. Their effects on structure, texture and rheological properties have been studied in order to improve knowledge and obtain acceptable products (Cernikova et al., 2008; Piska & Stetina, 2004). In these studies, descriptive sensory analysis was used for characterization of processed cheeses. New methods for sensory product characterization continue to be developed. Novel techniques are based on the evaluation of individual attributes, among which are intensity scales, check-all-that-apply questions or CATA, flash profiling and paired comparisons (Varela & Ares, 2012). It has been shown that results from CATA questions used with consumers are very similar to those obtained from trained panels (Bruzzone, Ares, & Giménez, 2012).

The chemical composition of goat milk and the content of each component make it possible to obtain cheeses with sensorial characteristics distinct from those obtained from cow milk. Semi-hard goat cheese is whiter than cheese made from cow milk, which presents an intense yellow color (Galván, 2007). Goat cheeses are usually soft and wet, and have high initial fermentative flavor, with predominant goat milk and lactic aroma. They present a plastic structure, are slightly firm, have adherence, a microstructure formed by small particles, high solubility and moisture sensation in mouth. They have an acid and slightly salty taste, characteristic aftertaste and medium to long term persistence.

Many studies about processed cheeses manufactured with cow milk describe rheological characteristics, textural and sensory parameters such as spreadability, firmness, softness, creaminess, taste and flavour (Cernikova et al., 2008; da Silva, de Souza Ferreira, Bruschi, Britten, & Matumoto-Pintro, 2016; Dimitreli & Thomareis, 2008; Hanaei, Cuvelier, & Sieffermann, 2015; Salek, Cernikova, Maderova, Lapcik, & Bunka, 2016). These kinds of cheese present a spreadable texture, brilliant white to yellow color, and flavor that depends on the ripened cheeses and the ingredients used for manufacture. They show a smooth and closed surface when cutting, and some small bubbles could appear as a result of the filling stage.

The dependence of texture parameters (measured within the deformation area), and rheological and sensory properties of the processed goat cheese on the maturity of cheese (raw material) have not been described in the literature. In addition, processed goat cheese is not currently on the market in Northwestern Argentina, where the study was conducted. The aim of this work was to determine how the ripening degree of the natural goat cheese (raw material) influences the textural, rheological and sensory properties of processed cheese products.

2 Materials and Methods

2.1 Processed cheese manufacturing

Different samples of processed cheeses were obtained by using goat cheeses ripened for 10, 20 and 40 days and made from pasteurized milk in the traditional way; cream; water; and 2.8 (g/100g) emulsifying salts JOHA S10 2.5 (g/100g) and JOHA HBS 0.3 (g/100g); sodium phosphate and polyphosphate salts. The composition of the ingredients and the processed cheese are shown in Table 1. The ingredients were smelted in an electric heater in combination with an omnimixer at 85 ^{o}C for 1 min (total melting time was 9 min at 1358 g). The hot melt was poured into cylindrical polyethylene containers (35 mm in diameter and 50 mm in height). Finally, the samples were cooled to 7 ^{o}C.

During the manufacturing process, we varied the proportion of cheese (raw material), with different ripening times, to obtain the different formulations shown in Table 2.

Table 1: Composition of processed goat cheese and its ingredients

	Dry Matter (DM) g/100g	Fat g/100g DM
Processed goat cheese	37	53
Goat cheese ripened		
10 days	58	46.5
20 days	62	48
40 days	70	50
Cream	46.8	42

Table 2: Proportions of ripened cheeses in each formulation of processed cheese

Formulation	goats cheese proportions		
	10 days	20 days	30 days
1	1	1	1
2	2	1	1
3	1	2	1
4	1	1	2

The resulting processed cheeses were analyzed using three replicates of each assay and every test was performed in triplicate. No processed goat cheese was available on the market, and therefore our results were compared to a commercial reference made solely from natural cow cheeses. The composition of the commercial reference was similar to that of our samples (35 dry matter g/100g, 52 fat g/100g in dry matter). This commercial processed cheese was purchased in the local market and selected from three different lots.

2.2 Casein fractions of processed cheeses (PC)

The variation in major fractions of α, β and para-κ casein was studied using HPLC ion exchange, following the methodology described by Veloso, Teixeira, and Ferreira (2002). The HPLC equipment consisted of a Spectra SYSTEM - Thermo Electron chromatograph, equipped with a P2000 pump and a Rheodyne Injector with a 20 μl loop. A Spectra SYSTEM UV 6000LP PDA, variable-wavelength ultraviolet detector was used. ChromQuest 4.1 SP2 software controlled the solvent gradient, data acquisition and data processing of the equipment. The column was a reversed-phase Phenomenex C18 (5 μm, 300 Å, 250×4.6 I.D.). Gradient elution was carried out with a mixture of two solvents. Solvent A consisted of 0.1% trifluoroacetic acid (TFA) in water and solvent B was acetonitrile–water–TFA (95:5:0.1, v/v). Proteins were eluted with a series of linear gradients increasing the proportion of solvent A, from 29% to 100% over 40 min. The flow rate was 1 ml/min, the column temperature was 46± 0.1 ^{o}C and the eluate was monitored at 280 nm.

2.3 Texture analysis

The texture was determined using an InstronBluehill® texturometer. The tests were carried out at 6 ± 2 ^{o}C (the sample measurement was performed immediately after removing them from a refrigerator where they were stored) after two days of storage according to the methodology described by Piska and Stetina (2004) and Weiserova et al. (2011). The parameters used in the tests were

double penetration of 30 mm into the samples, speed penetration of 1 mm/s, bristle of 10 N, penetrometer diameter of 12 mm and cylinder diameter of 36 mm at 10 ^{o}C (Lemes et al., 2016). According to the force deformation curve describing the dependence of the force needed (N) on time (s), the following textural parameters were determined: hardness, cohesiveness and adhesiveness (Weiserova et al., 2011). Each variant was measured six times.

Rheological measurements

The processed cheese samples were characterized in terms of their rheological properties. Rheological measurements were made using an AR-G2 rheometer (TA Instruments; New Castle, DE, USA) with parallel plate geometry (40 mm diameter, 2 mm gap). Temperature (21 ^{o}C) was controlled with a water bath (Julabo ACW100, Julabo Labortechnik; Seelbach, Germany) associated with the rheometer. The linear viscoelastic region was determined by an amplitude sweep test, while the frequency sweep mode was used to evaluate the viscoelastic properties of model samples. The storage (G') and loss (G") moduli were measured in the 0.1-100.0 Hz frequency range. The loss tangent (tan δ) and complex modulus (G*) for the reference frequency 1 Hz were calculated according to Eq. (1) and (2) (Gunasekaran & Ak, 2000):

$$tan\delta = \frac{G''}{G'} \quad (1)$$

$$G^* = \sqrt{G'^2 + G''^2} \quad (2)$$

The value of 1 Hz for reference frequency was recommended in the literature (Bennett et al., 2006; Piska & Stetina, 2004).

2.4 Sensory evaluation of processed cheeses

The participants in this study (n = 100, 23–68 years old, 62% female) were regular consumers of goat cheese but non-regular consumers of processed cheeses. They were recruited in cafeterias and public areas of the University of Jujuy, Jujuy Province, Argentina.

The consumers received a single CATA question featuring 24 attributes, which had been previously selected from the available literature (Hanaei et al., 2015) and from the results of an informal tasting session conducted by researchers of the university. The instruction given to participants was 'Please, check all that applies to the processed cheese you taste'. The 24 selected attributes were randomized between products and across consumers. The following terms were used: *thick, smooth goat milk flavor, strange taste, soft flavor, creamy, very salty, homogeneous, firm, slightly sour, not creamy enough, strong aroma of goat milk, soft texture, aftertaste, pleasant appearance, liquid, very sour, not salty enough, lumpy, fluid, aroma of goat milk, intense goat milk taste, salty, spreadable and heterogeneous*. Consumers were asked to try the cheeses individually and spread them on a toast, in the way spreadable cheeses are usually consumed. They evaluated the cheeses' general acceptance using a nine-point hedonic scale, with 1 being the worst and 9 the best quality. Consumers were finally asked if they would buy the product.

The attributes were randomized within each modality between products and across consumers. The frequency of use of each sensory attribute was determined by counting the number of consumers that used that term to describe each sample. Cochran's Q test was carried out to identify significant differences among samples for each of the descriptors included on the CATA question. The frequency (contingency) tables from each study were analyzed using Correspondence Analysis (CA). Bi-dimensional maps representing samples and descriptors were obtained. The maps that corresponded to all the consumers who participated in the test were used as a reference for evaluating the stability of product spaces.

The authors declare that, in Argentina, approval of an Ethics Committee is not needed for Sensorial Analysis. Nevertheless, these analyses were carried out following international tenets and informed consents were obtained.

2.5 Statistical analysis

All the formulations were prepared in triplicate. Minitab 16.0 statistical software (Minitab Inc.,

State College, PA, USA) was used for analyzing the experimental data. One-way analysis of variance was used to determine significant differences between means, with the level of significance (p) set at 0.05. Tukey's HSD test at 5% significance level was used as the multiple comparison tests on all main effect means. All statistical analyses of sensory evaluation were performed using XLStat 2009 (Addinsoft, Paris, France) and R language (R Development Core Team, 2007) using FactoMineR (Le, Josse, & Husson, 2008).

3 Results and Discussions

3.1 Casein fractions of PC

The casein fractions obtained in each cheese spread formulation are presented in Figure 1. The contents of α-casein in the formulations studied were ordered from highest to lowest: Formulation 2, Formulation 1, Formulation 3 and Formulation 4. This decreasing level in the contents of α-casein is due to the slow casein hydrolysis throughout 30 days of ripening (Burgos, 2016). By contrast, greater intact casein was present in cheeses with 10 days of ripening, resulting in the major casein content of Formulation 2.

Guinee and O'Callaghan (2013) studied the effects of protein and fat content on the properties of a processed cheese by substituting the protein with fat while maintaining constant moisture and emulsifying salt levels. The firmness of the processed cheese increased markedly with increased protein levels. Greater protein content in the formulation allows higher casein-casein interaction and stabilizes the cheese matrix (Hosseini-Parvar, Matia-Merino, & Golding, 2015). Dimitreli and Thomareis (2008) reported that proteins reinforce the strength of the three-dimensional matrix, leading to processed cheeses with more solid-like behavior.

The β-casein and para-κ-casein contents, in Formulations 1 and 4, which were lower than those in the other two formulations, showed significant differences between them. Both casein fractions got hydrolyzed as from day 30. Formulations 1 and 4 had a higher proportion of cheeses (raw material) ripened for 40 days, and therefore their hydrolyzation of casein fractions was higher.

The intact casein content of the cheese is inversely related to the age of the cheese. As a natural cheese is ripened, its intact casein content decreases (Kapoor & Metzger, 2008). This occurs because the enzymes and the residual starter or nonstarter lactic acid bacteria present in the cheese hydrolyze the proteins of natural cheese into peptides, thereby reducing the amount of casein that is still present in the intact (unhydrolyzed) form (Purna, Pollard, & Metzger, 2006).

The proportion of the different casein fractions in the cheeses produced different characteristics in the processed cheese matrix obtained with each formulation. Salek et al. (2016) indicated that the degree of casein proteolysis in the cheese applied during PC manufacture is a parameter that significantly influences its textural and viscoelastic properties (Brickley, Auty, Piraino, & McSweeney, 2007; Bunka et al., 2014; Piska & Stetina, 2004).

3.2 Texture Analysis

Table 3 show the results obtained for textural parameters: hardness, cohesiveness, springiness and adhesiveness.

The hardness of a matrix is used as an index of product strength while cohesiveness indicates the strength of internal bonding of the processed cheese (da Silva et al., 2016). Adhesiveness is the tendency of the processed cheese to resist separation from a material it contacts. High adhesiveness of processed cheese to the packaging material is one of the parameters limiting their consumption since consumers dislike products that are difficult to separate from the package (Hosseini-Parvar et al., 2015; Solowiej, Cheung, & Li-Chan, 2014); another parameter is the stickiness that occurs in the mouth as a sensory consequence.

We observed that cohesiveness was statistically similar in all studied samples. Hardness and adhesiveness increased in the following order: Formulations 4, 3, 1 and 2, followed by the commercial processed cow cheese (CPCC). Springiness of formulations 1, 2, 3 and CPCC was similar. Formulation 4 was elaborated with the largest pro-

portion of long-ripened cheese (40 days of ripening) and presented lower values of textural parameters than those of processed cheeses with the largest proportion of short-ripened cheeses, except for cohesiveness. This behavior was due to the higher content of hydrolyzed caseins in these cheeses.

We can relate the increase in the proportion of ripened cheese to the decrease in hardness and adhesiveness in the processed cheeses. Similar conclusions were drawn by Piska and Stetina (2004) when they studied cow processed cheese formulated with soft, semi-hard and hard cheeses with different ripening times, and by Hladka et al. (2014) who obtained processed cheeses using Edam cheese with different ripening times.

Brickley et al. (2007) studied the relationship between cheddar cheese ripening, with the emphasis being on proteolytic breakdown, and the resultant textural changes in PC manufactured from cheddar cheese. Using multivariate data analysis, they concluded that the concentration of intact αs1-CN in cheddar cheese was strongly correlated with the decrease in hardness, fracturability, springiness, adhesiveness and G' in the corresponding PC samples. Flowability increased in the PC samples and it was correlated with the production of free amino acids in cheddar cheese as well as development of the protein content of the pH 4.6 soluble fraction.

Besides, the influence of different maturity degrees of natural cheese was associated with different ternary mixtures of emulsifying salts affecting PC texture. The effect of composition of different ternary mixtures of the individual sodium salts of phosphates (especially disodium hydrogenphosphate, tetrasodium diphosphate, and sodium salt of polyphosphate) has been described by Bunka et al. (2014), Weiserova et al. (2011) and Salek et al. (2016). The higher the amount of emulsifying salts, the higher the hardness and cohesiveness of the processed cheeses was. Changing concentration of the emulsifying salts and pH adjustment to the optimal range (pH in the range of 5.69-5.84) only affected the absolute values of textural parameters of the processed cheeses (Bunka et al., 2014).

Moreover, values of the studied texture parameters for each formulation were lower than those of the commercial reference, probably because of the use of hydrocolloids by the industry to achieve a stable texture. Macku, Bunka, Voldanova, and Pavlinek (2009) stated that hydrocolloid incorporation can cause changes in product structure and texture (Bennett et al., 2006). These hydrocolloids (so-called stabilizing, gelling or thickening agents) can improve texture and consistency of food by water binding, gel creation or viscosity enhancing. Commercially important hydrocolloids which can be used in dairy industry are carrageenan, locust bean gum, xanthan, modified starches and pectin (Bennett et al., 2006).

The textural characteristics of Formulation 2 cheeses were similar to those of the commercial processed cow cheese used as a reference. This formulation produced a product with the desired texture, even without hydrocolloid or starch incorporation. The greater α and β casein contents could explain this effect, which allowed the formation of a firmer gel than that of the other formulations.

3.3 Rheological Properties

Table 4 shows complex modulus G* (Pa) and the loss tangent (tanδ) of all formulations and commercial processed cow cheese, at a frequency of 1 Hz.

Formulation 2 samples presented significant differences in G* and showed similar results to those of the commercial processed cow cheese, with a 95% significance level. This modulus describes the total resistance of the cheese matrix, considering the deformation behavior of the samples as elastic solids (Dimitreli & Thomareis, 2008). As shown in Table 4, Formulation 2 samples presented the greatest resistance. The behavior of the four studied formulations could be explained by their high α- and β-casein content, which allows the formation of a firm casein gel lubricated with fat, producing viscoelastic properties similar to those of the reference.

The higher protein content in the formulation allows more casein-casein interaction and stabilizes the cheese matrix (Hosseini-Parvar et al., 2015). Dimitreli and Thomareis (2008) reported that proteins reinforce the strength of the three-dimensional matrix, leading to processed cheeses

Table 3: Textural parameters in goat formulations and commercial processed cow cheese (CPCC)

	Formulation				CPCC
	1	2	3	4	5
Hardness (N)	$0.47\pm0.07^{(b)}$	$0.9\pm0.1^{(c)}$	$0.4\pm0.1^{(b)}$	$0.22\pm0.02^{(a)}$	$2.2\pm0.2^{(d)}$
Adhesiveness (N.s)	$6.2\pm0.1^{(b)}$	$14.2\pm0.1^{(c)}$	$5.3\pm0.9^{(ab)}$	$2.5\pm0.8^{(a)}$	$32.9\pm0.9^{(d)}$
Cohesiveness	$0.83\pm0.09^{(a)}$	$0.94\pm0.08^{(a)}$	$0.82\pm0.08^{(a)}$	$0.87\pm0.08^{(a)}$	$0.83\pm0.03^{(a)}$

^{abc}Means (n = 6) within the same line and parameter followed by different superscript are significantly different ($P < 0.05$).

with more solid-like behavior. The higher values of the gel strength may be explained by more intensive interactions occurring in the cheese samples, such as hydrogen bonds, hydrophobic interactions between caseins and fat or calcium-intervened electrostatic bonds among caseins, leading to the formation of a "denser" (more intensive) network structure (Salek et al., 2016).

Guinee (2016) stated that increasing the protein content of cheese results in significant increases in storage modulus, firmness (force required to attain a given deformation) and fracture stress of the unheated cheese. This is confirmed by the positive correlations between the content of intact casein and fracture stress and firmness of Cheddar processed cheeses.

Cunha, Grimaldi, Alcantara, and Viotto (2013) indicated that the high values of G' were due to the combined effects of a low degree of casein dissociation and a low percentage of soluble calcium/total calcium, which resulted in a more elastic protein network. These authors argued that increasing pH increases the negative charge of the protein molecules, causing their repulsion and expansion. In processed cheese, ionic repulsion in the pH range 5.7 to 6.0, instead of totally dispersing proteins, enhances different types of interactions, such as noncovalent bonds (hydrogen bonds, hydrophobic and electrostatic interactions), thus increasing elasticity. These changes resulted in an increase in casein hydration and in the formation of a more open reactive structure with higher water-binding capability and better emulsifying properties.

The tanδ gave a clear indication of whether elastic or viscous properties of processed cheese predominated (Table 4). The larger the tanδ value, the more the cheese flowed. The loss tangent values showed a predominantly viscoelastic behavior (tan $\delta > 1$ or G" > G') (Dimitreli & Thomareis, 2008) for samples formulated over the whole range of frequency tested. In our study, all tested formulations exhibited characteristics typical of a weak viscoelastic gel and presented values different from those of the commercial reference, which had a higher viscous component (0.6). The PC of formulation 4 had lower tan δ values (0.4) and were weaker gels due to the higher content of ripened cheese.

According to Dimitreli and Thomareis (2008), when the protein content is increased, the elastic and viscous moduli and complex modulus are increased and the loss tangent is increased, indicating a more liquid-like behavior of the samples. This agrees with the results of Joshi, Jhala, Muthukumarappan, Acharya, and Mistry (2004), who reported that proteins are responsible for increased values of the elastic and viscous module in processed cheese samples. Increasing concentration of caseins in the cheese matrix increases the intra- and inter-strand linkages. The matrix displays greater elasticity and it is more difficult to deform. The unfolded protein molecules approach each other by attractive forces, and water and fat globules are trapped into the matrix. Thus, after cooling, the role of proteins in the texture of the final product dominates over that of water and fat, resulting in products with increased viscoelastic properties and a more solid-like behavior.

Hosseini-Parvar et al. (2015) showed that tanδ (G"/G') may be a useful indicator of processed cheese meltability. The tanδ values increased with the increase in the protein concentration

while heating the samples up to 85 ^{o}C. Protein-protein interactions, at this temperature, increased the viscous behavior.

3.4 Sensory evaluation of PC

Q – Cochran Test results are shown in Table 5. Significant differences were found in the frequency at which 11 out of the 24 terms of the CATA question were used to describe the texture of the samples. This result suggests that the consumers were able to perceive differences in the sensorial characteristics of the evaluated processed cheese.
When we analyzed the frequency of responses, we found that the most widely used terms were *thick, smooth goat milk flavor, homogenous, salty, smooth goat milk taste, creamy, pleasant appearance, spreadable, slightly sour and soft texture*. Only four of these descriptors were significant, namely *thick, salty, spreadable and slightly sour*. These attributes describe the desired product.
Analyzing results presented in Table 5 and considering the significant parameters and the maximum values for each sample, we can state that the attributes which best describe the processed cheeses were as follows:

F1 intense goat milk taste

F2 spreadable, slightly sour, intense goat milk taste

F3 lumpy

F4 not creamy enough, very sour, fluid, salty.

CPCC 5 thick, firm, not too salty.

Figure 2 presents the first two dimensions of Correspondence Analysis of frequency data, where the relationship between the significant terms obtained with the Q - Cochran test for all the studied formulations can be seen.
As shown in the sensorial map, the first and second dimensions of the CA accounted for 90.78% of the variance of the experimental data, representing 68.8% of F1 and 21.98% of F2. We can distinguish three groups with different characteristics, as follows:

GI: Formulations 1 and 4, characterized by intense goat milk taste, and as not creamy enough, very sour, fluid.

GII: Formulations 2 and 3, characterized as spreadable, slightly sour, salty, lumpy.

GIII: Commercial processed cow cheese (5), characterized as thick, firm, not salty enough. According to these results, the commercial processed cow cheese is different from the others, mainly because of its texture. We found that consumers were able to perceive the firmness of this product, which resulted from the presence of carrageenan.

Formulations 1 (5.7±0.3), 2 (6.2±0.2), 3 (6.0±0.6) and the commercial reference (6.3±0.4) presented similar acceptance levels, while formulation 4 (5.1±0.1) had the least acceptance due to its low creamy content, fluid texture and acid taste, attributes that were not desired.
Thirty percent of the consumers perceived differences when the cheeses were tasted with toast. This perception was similar between formulations, probably due to the masking of the cheese flavor by the toast.
Seventy percent of the consumers stated their intention of buying cheeses with Formulations 2, 3 and the commercial reference, confirming that formulations 2 and 3 presented acceptable sensorial characteristics and that they were similar to the commercial reference. Fifty-eight percent of the consumers expressed their intention of buying Formulation 1, and 38% of the consumers said they would buy Formulation 4. Sensorial evaluation of cheeses with each formulation and of the commercial reference showed differences between samples, both in the terms that describe them and in their acceptability, which is related to the consumer's intention to buy the product.
Formulation 2 showed spreadable texture, brilliant white color and the characteristic flavor of goat cheese. These attributes were desired for the finished product, which was verified with the Q - Cochran test and the results of the Correspondence Analysis.

Table 4: Complex modulus (G*) and tanδ of processed cheese for all formulations (1-4) and commercial processed cow cheese (5)

Formulation	G*(Pa)	Tanδ
1	1498±380$^{(b)}$	0.50±0.03$^{(b)}$
2	2822±208$^{(c)}$	0.51±0.04$^{(b)}$
3	1390±316$^{(b)}$	0.51±0.02$^{(b)}$
4	872±231$^{(a)}$	0.46±0.05$^{(a)}$
5	2701±306$^{(c)}$	0.61±0.03$^{(c)}$

^{abc}Means (n = 6) within the same column and parameter followed by different superscript are significantly different (P < 0.05).

Table 5: Results of the CATA question. Frequency at which each term was used to describe each processed cheese for all formulations (1-4) and commercial processed cow cheese CPCC (5)

Term	Formulations			CPCC	
	1	2	3	4	5
Thick *	26	38	32	18	64
Soft flavor ^{ns}	36	38	44	48	46
Homogeneous ^{ns}	32	38	28	32	44
Not creamy enough *	18	8	8	28	18
Aftertaste ^{ns}	18	12	14	18	8
Very sour *	24	14	8	34	4
Fluid *	14	4	6	28	0
Salty *	36	42	44	48	12
Smooth goat milk flavor ^{ns}	34	44	48	32	36
Creamy ^{ns}	44	58	48	30	50
Firm *	8	26	28	2	56
Strong aroma of goat milk ^{ns}	4	14	8	14	12
Pleasant appearance ^{ns}	44	54	52	36	52
Not salty enough *	14	8	18	14	32
Aroma of goat milk ^{ns}	22	16	20	22	12
Spreadable *	40	52	30	22	40
Strange taste ^{ns}	4	12	8	18	12
Very salty ^{ns}	20	12	10	24	6
Slightly sour *	36	44	38	40	16
Soft texture ^{ns}	46	34	28	32	34
Liquid ^{ns}	2	2	0	20	0
Lumpy *	10	16	28	8	0
Intense goat milk taste *	20	20	6	16	6
Heterogeneous ^{ns}	6	6	8	2	0

* indicates significant differences at P < 0.05, ^{ns} whereas ns indicates no significant differences (P > 0.05) according to Q Cochran's test.

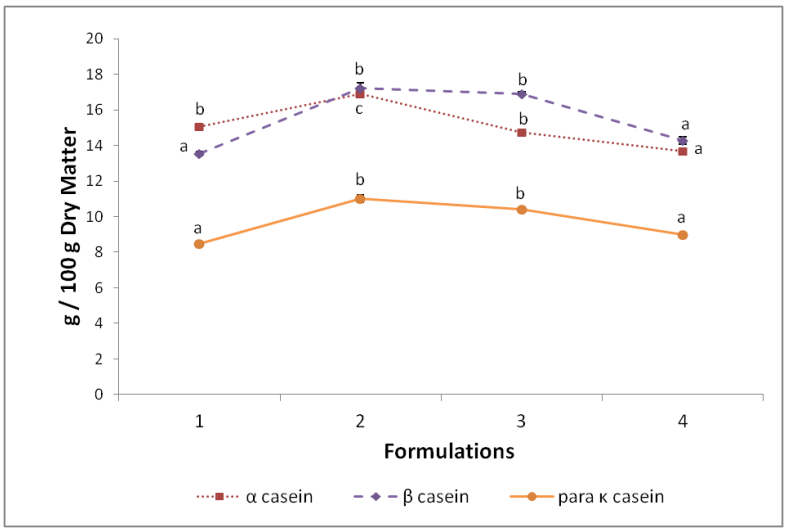

Figure 1: α-casein■, β-casein♦ and *para*-κ-casein• in formulations of processed cheese. The points with the different letters are significantly different ($P < 0.05$).

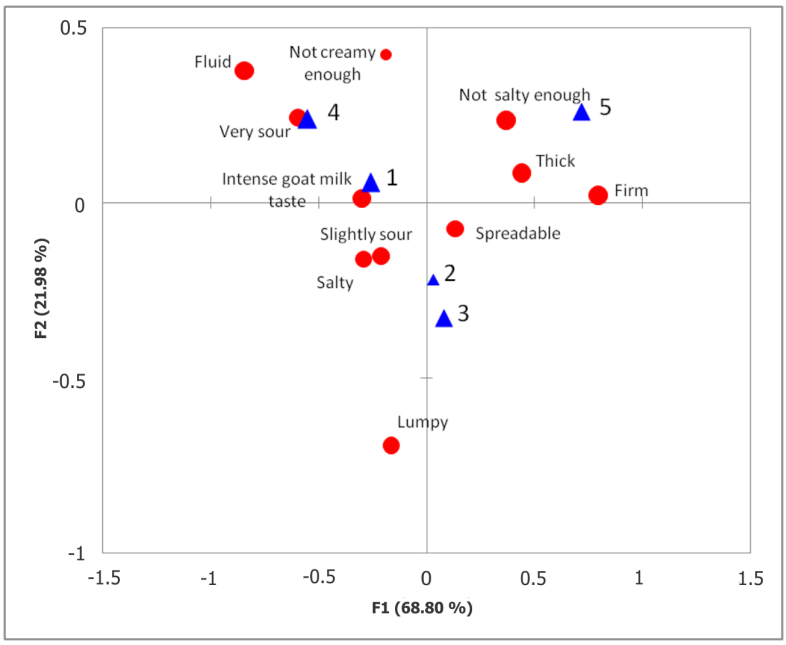

Figure 2: Sensorial map of significant terms. Representation of the formulations and reference processed cheese▲ and the terms• of Check-All-That-Apply (CATA) question in the first two dimensions of the multiple factor analysis of CATA counts.

The quality of a product and its acceptability are directly related to the quality of the raw material. Raynal-Ljutovac, Lagriffoul, Paccard, Guillet, and Chilliard (2008) argued that the characteristics of the milk itself have an impact on goat cheese characteristics but the cheese making steps may change the nutritional and sensorial characteristics of the final product. The processed goat cheeses obtained represent a high quality product that adds value to the goat milk sector.

The level of acceptance obtained represents a very important result considering that the sensorial evaluation was performed by non-habitual consumers of processed cheeses. This acceptance is an indicator that local producers could introduce such a product into the local market.

4 Conclusion

Hardness and adhesiveness in the PC samples decreased as the proportion of ripened cheeses used in the formulation increased, which is associated with intact casein content. All spreadable processed goat cheese formulations appeared as a gel, as shown by the values of the storage and loss moduli (G'>G"). Formulation 2, made from 50% cheese ripened for 10 days, 25% cheese ripened for 20 days, and 25% cheese ripened for 40 days, presented a complex modus (G*) similar to that of the reference. The resulting high firmness of the gel in Formulation 2 was due to the higher content of α- and β- caseins as a result of the greater proportion of young goat cheese, which has higher intact casein content. Consequently, increasing the proportion of goat cheese having intact casein in the formulation can increase the complex modulus of the spreadable processed goat cheese. Formulation 2 and the commercial processed cow cheese showed similar acceptance by consumers, validating texture results.

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Improvement of Microbiological Quality of Hen Egg Powder Using Gamma Irradiation

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Abstract

Eggs and their products such as desserts, confectioneries, bakery mixes, mayonnaise and many convenience foods have been implicated in food-borne disease outbreaks due to microorganism contamination. The effect of gamma irradiation on the presence of microorganisms in egg powder was investigated. Egg powder samples were exposed to several doses of irradiation: 0, 5, 10 and 15 kGy and stored for up to 12 months at ambient temperature (25 ^{o}C). Results indicated that the total viable count (TVC) (5.56 $\log_{10}$ cfu g^{-1}), total coliform counts (TCC) (6.46 $\log_{10}$ cfu g^{-1}) and mold and yeast counts (MYC) (9.12 $\log_{10}$ cfu g) in un-irradiated (control) samples of egg powder were higher than the maximum limits (4.88, 2.00 and 1.70 $\log_{10}$ cfu g^{-1}, respectively). Application of the higher doses (10 and 15 kGy) decreased the TVC, TCC and MYC of the egg powder samples to less than 1 $\log_{10}$ cfu g^{-1} and the counts remained almost constant during storage for 12 months. D_{10} values for *Escherichia coli* and *Salmonella* typhimurium were 0.714 and 0.278 kGy, respectively. Gamma irradiation treatment could be chosen on the basis of preliminary microbiological tests including TVC, TCC and MYC and help improve the hygienic quality by killing and reducing the microorganisms that might be present inside of egg powder to meet national and international standards.

Keywords: Egg powder; Gamma irradiation; Total viable count; Total coliform count; Mold and yeast count

1 Introduction

Egg is one of the most versatile and near perfect foods in nature, and its essential components form a balanced diet (Akpinar-Bayizit, Ozcan, Yilmaz-Ersan, & Gurbuz, 2010; Ndife, Udobi, & Amaechi, 2010).
Microbial contamination of eggs is a well-established phenomenon and has an important economic implication to the poultry industry (Farag et al., 2012; Wong & Kitts, 2002). Eggs become infected through a process of either transmission, or with moist faces contaminated with *Salmonella*. Following traversing of the eggshell, the associated membrane of the egg becomes permeable to *Salmonella* and other pathogens (Dadashi, Kiani, Rahimi, & Mousavi, 2017; Holt et al., 2011; Jaffer & Nazal, 2013; Nemeth et al., 2011).
Safety of the internal compartments of eggs, in the new alternative poultry production systems, could be microbiologically altered (Holt et al., 2011; Jaffer & Nazal, 2013). Consequently, researchers emphasized the need to rapidly remove any microbial contamination in order to reduce the risk of microbial penetration into the egg contents (Al-Bachir & Zeinou, 2006; Farag et al., 2012; Tan, Kanyarat, & Easa, 2012). Recently,

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the use of shelled egg in food production, as a raw material, has been reduced with the technological developments around the world food industry, and egg products such as frozen egg, and pasteurized liquid egg products have gained popularity (Koc et al., 2011; Ndife et al., 2010). Industrially, dehydrated eggs have many advantages over fresh ones, like inhibition of microorganism development, easier handing and a significant extension in their shelf life (de Jesus et al., 2013; Kumaravel et al., 2011). Drying is a technique used for preserving liquid food, by converting it to a powder form. The whole egg powder obtained by the processes employed, including all methods of drying, was subjected to different studies (Asghar & Abbas, 2012; Schuck et al., 2009).

With growing concerns about food safety, the use of irradiation has been well accepted as one of the best methods for the production of safe meat and poultry (Al-Bachir, 2010; Al-Bachir & Zeinou, 2006). Food irradiation is a non-thermal method, or used as an additional food safety tool, that serves as a compliment to other food safety technologies (Kim et al., 2016). Irradiation of egg and egg products has been used experimentally, as an alternative to heat pasteurization to eliminate *Salmonella*, which is a naturally occurring pathogen in eggs that causes a serious infection (Al-Bachir & Zeinou, 2006; Farag et al., 2012; Kim et al., 2016). Most research on microbial inactivation has tried to determine the proper irradiation dosage to pasteurize egg products. Since 2000, the food and drug administration (FDA), approved the use of up to 3 kGy ionizing radiation dose to reduce the level of *Salmonella* in eggs (Shahin, Swailam, & Abou Zeid, 2006).

The quality evaluation of food products is critical to improve the processing conditions for getting better quality products. Therefore, the aim of the present study was to use gamma irradiation to enhance safety of egg powders, and to analyze the microbial load of an egg powder after irradiation.

2 Materials and Methods

Fresh good quality eggs were obtained from Sidnaia poultry farms, in Damascus Syria. The eggs were candled to confirm their freshness and were cleaned by dusting, washing to remove dirt and other undesirable materials, to avoid any contamination and allowed to dry. They were carefully de-shelled and whole egg liquid obtained in a graduated cylinder. Whole egg liquid was mixed in a blender (WARING commercial blender model 32BL80 made in U.S.A.) for 1-2 min, liquid egg was spread thinly (0.5 – 1.0 mm thickness) on a tray, and oven dried at 60 ^{o}C for 48 hours in a laboratory oven (MEMMERT model 600) to constant mass and allowed to cool. Egg flakes were scooped, milled and sieved with 60 mm mash before being weighed. The egg powders were packed into different plastic films for further investigation.

2.1 Irradiation treatment

Egg powder were irradiated with doses of 0, 5, 10 and 15 kGy, at room temperature, using a gamma source ^{60}CO (ROBO, Russia) with a dose rate of 7.775 kGy h^{-1}. The absorbed dose was monitored by alcoholic chlorobenzene dosimeter (Al-Bachir, 2010). The irradiated and control samples of egg powders were stored for 12 months at ambient temperature (18-25 ^{o}C) under relative humidity (RH) of 50-70%.

2.2 Microbiological evaluation

Standard plate count method was employed to enumerate the microbial load in terms of colony forming units (cfu g^{-1}) in control and irradiated samples after 0, 6 and12 months of storage. Three replicates from each treatment were used, and 10 g of powdered egg samples was homogenized with serial dilutions were prepared according to standard methods (AOAC, 2010). Total viable count (TVC) was determined on diagnostic plate count agar (PCA) (Oxoid, CM 325, UK). Samples were incubated at 30 ^{o}C for 48 hr. Total coliform count (TCC) was determined on a Violet Red Bile Agar (VRBA) (Oxoid, CM 485, UK) at 37 ^{o}C for 48 h. Total mold and yeast (TMY) was done on Dichloran Rose-Bengal Chloramphenicol Agar (DRBC) (Merck, 1.00466, Germany) after incubation at 25 ^{o}C for 5 days. The colony count was reported as colony

forming units per gram of egg powder samples (cfu g^{-1}). Microbial counts were transformed to $\log_{10}$ cfu g^{-1}. To determine the survival curves before irradiation, eggs were artificially inoculated by thoroughly mixing them with a suspension of *Salmonella*. The suspension was prepared by mixing a culture colony of *Salmonella* with pure peptone water media. The *Salmonella* count in the prepared suspension was 10^7 per ml. Before inoculation, eggs were sterilized using gamma irradiation (25 kGy). The ratio of inoculation was 1 ml suspension to 9 ml eggs. The survival curve was estimated from irradiation doses of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 kGy. The survival level of *Salmonella* was determined by plate counting on Xylose Lysine Desoxycholate Agar (XLD) after 2 days of incubation at 37 ^{o}C. Similarly, the survival level of *E. coli* was determined by plate counting on Eosin Methylene Blue Agar (EMBA) (Oxoid, CM 69, UK), after 2 days of incubation at 37 ^{o}C. D_{10} value was calculated using the Cricketgraph (Cricket Software, Maluern, PA, USA) computer package.

2.3 Water activity determination

Water activity was determined using reference solutions (Al-Bachir, 2010).To determine the range capacity and calibration sensitivity of the measurement, the water activity of twelve saturated salt solutions was measured at 20 ^{o}C. Saturation equilibrium of solutions was checked after storage for 2 hr at 25 ^{o}C prior to measurement.

2.4 Statistical analysis

Four treatment doses (0, 5, 10 and 15 kGy), and three storage periods (0, 6 and 12 months) were distributed in a completely randomized design with three replicates for each treatment. Data were subjected to the analysis of variance test (ANOVA) using the SUPERANOVA computer package (Abacus Concepts Inc, Berkeley, CA, USA; 1998). The p value of less than 0.05 was considered statistically significant.

3 Results and Discussions

3.1 Microbiological qualities of egg powder

The extent of contamination by microorganisms in egg powder products was determined. As shown in (Table 1), the mean total viable count (TVC), total coliform counts (TCC) and mold and yeast count (MYC) for the control sample of egg powder were 5.56, 2.62 and 2.33 $\log_{10}$ cfu g^{-1}, respectively. Coliforms are defined as rod shaped gram-negative non-spore forming bacteria. They are a commonly used indicator of sanitary quality of foods. During storage at room temperature, the microorganisms in egg powder products increased gradually reaching, at the end of the storage period (12 months), 9.12, 3.04 and 4.03 $\log_{10}$ cfu g^{-1} for TVC, TCC and MYC respectively, indicating a high contamination percentage of these products. Microbiological population of used egg powder was found to be comparatively high, which was not in accordance with the national and international standards for egg powder that include less than 7.5 X 10^4 cfu g^{-1} in TVC, less than 100 cfu g^{-1} in TCC, less than 50 cfu g^{-1} in MYC (CODEX, 2007; SASMO, 2007). Although MYC contaminated egg powder products were relatively lower than TVC, their counts were also above the safety limits (Table 1). Our previous studies also indicated that the microbial contamination level was above the international and national limits in commercially available dried powder food products found in local markets (Al-Bachir, 2007; Al-Bachir & Al-Adawi, 2015; Al-Bachir, 2017). TVC is indicative of the populations of contaminated microorganisms, and act as an index of hygienic quality. On farms where eggs are produced, the sources of bacterial contaminants have been shown to be surrounding environment, as well as the chickens (Shahin et al., 2006). Microorganisms on the surface of the shell are able to pass through the pores of the shell to contaminate the interior of the egg (Foley & Lynne, 2008; Gantois et al., 2009; Jaffer & Nazal, 2013).
The results of the current research indicated that, *Salmonella* spp and *E. coli* were detected in fresh egg (Table 2). In the case of *Salmonella*

spp and *E. coli* in samples of fresh egg did not meet local and international standards of zero tolerance for food for human nutrition including eggs, absent in *Salmonella* spp, and negative in *Staphylococcus aureus* (CODEX, 2007; SASMO, 2007). The presence of *Salmonella* and *E. coli* in fresh egg samples, as well as in egg powder, may be the result of contamination from the environment and personal or from the raw materials used for preparation. *E. coli* in 10 g egg powder was detected, while *Salmonella* spp. were absent in 25 g egg powder, as demanded in our legislation (Table 2).

Much attention has been given to the role chicken eggs play in the transmission of bacteria, such as *Salmonella* to human populations (Shahin et al., 2006). The TCC and *E. coli* detected in egg powder samples was an indication of contamination by fresh fecal matter. High TCC are usually associated with significant levels of enteric pathogens (Adu-Gyamfi, Torgby-Tetteh, & Appiah, 2012). This, according to FDA (2011), can cause cholera, bloody diarrhea, and kidney failure in people with weak immune systems. Shell eggs and egg-containing products are the most significant sources of *Salmonella* (Min, Nam, Jo, & Ahn, 2012). *Salmonella Enteritidis* and *Salmonella Typhimurium* are the most commonly isolated serotypes in human cases of Salmonellosis, and contaminated egg is a very important source of infection with *S. Enteritidis* for humans (Rakonjac et al., 2014).

3.2 Effect of gamma irradiation on microbiological qualities of egg powder

Application of 10 and 15 kGy doses was enough to decrease the TVC, TCC and MYC in the egg powder to the safety level (to less than 1 $\log_{10}$ cfu g^{-1}), and the counts remained almost constant during storage for 12 months as clearly observed in Table 1. The TVC was found to decrease with a 5 kGy dosage, while TCC and MYC values of irradiated egg powder samples was found to be absent in irradiated samples with the same dose (at 5 kGy). In the present study, the mode of gamma irradiation processing of the egg powder products with the doses of 10 and 15 kGy, were chosen on the basis of preliminary microbiological tests. Since both doses (10 kGy and 15 kGy) completely eliminated microorganisms from the egg powder products. This observation underscores the need for Good Manufacturing Practices (GMP) in production protocols to ensure products have low contamination, and acceptable hygienic quality, and would be in line with the national and international criteria for decontamination of dry foods (SASMO, 2010). This is to be expected, since irradiation is one of the few processes that eliminates disease-causing microorganisms from foods and guarantees high hygienic quality (Adu-Gyamfi et al., 2012). The results corroborate the findings of Aquino, Lui, and Correa (2017), who observed that for the complete elimination of microorganisms in egg powder, it was necessary to use doses around 10 kGy.

Egg disinfection has two purposes. One is to reduce the overall abundance of TVC and MYC that may affect egg respiration and survival. The other is to reduce or eliminate pathogens that may affect egg and fry survival, and compromise the health certification of a hatchery (Barnes, Bergmann, Stephenson, Gabel, & Cordes, 2005). Results of this study indicated that the current practices of gamma irradiation treatment achieve the first and second objectives. This is important for situations in which hatcheries receive eggs from wild sources, or from other hatcheries and processes.

Our results are consistent with literature data, since similar results were obtained in a study performed on dried products similar to egg powder. A previous study showed that gamma irradiation at a dose range of 10–20 kGy was sufficient to eliminate or reduce to an acceptable level, the microbiological contamination of licorice root powders (Al-Bachir & Al-Adawi, 2015), chamomile powder (Al-Bachir, 2017) and aniseed (Al-Bachir, 2007).

Farag et al. (2012) and Adhitia, Octaviani, Rissyelly, Basah, and Mun'im (2017) mentioned that, ionizing irradiation inactivates microorganisms directly by lethal damage of microbial DNA, therefore obstructing bacterial division, and indirectly by free radicals generated during water radiolysis that disintegrate microbial cell membranes.

Table 1: Effect of gamma irradiation and storage period on total viable count (TVC), total coliform count (TCC) and total mold and yeast count (TMY) of egg powder ($\log_{10}$ cfu g^{-1}).

Treatments	Control	5 kGy	10 kGy	15 kGy	P-level
Storage period (Months)		Total viable count (TVC) ($\log_{10}$ cfu g^{-1})			
0	0.05 ± 5.56^{aC}	0.05 ± 3.40^{bC}	>1	>1	0.0001
6	0.18 ± 6.46^{aB}	0.08 ± 3.83^{bB}	>1	>1	0.0001
12	0.29 ± 9.12^{aA}	0.06 ± 4.14^{bA}	>1	>1	0.0001
P-level	0.0001	0.0001			
		Total coliform count (TCC) ($\log_{10}$ cfu g^{-1})			
0	0.08 ± 2.62^{aC}	>1	>1	>1	0.0001
6	0.04 ± 2.84^{aB}	>1	>1	>1	0.0001
12	0.1 ± 3.040^{bA}	>1	>1	>1	0.0001
P-level	0.0001				
		Total mold and yeast count (TMY) ($\log_{10}$ cfu g^{-1})			
0	0.03 ± 2.33^{aC}	>1	>1	>1	0.0001
6	0.21 ± 3.49^{aA}	>1	>1	>1	0.0001
12	0.19 ± 4.03^{aB}	>1	>1	>1	0.0001
P-level	0.0015				

^{abc} Mean values in the same column not sharing a superscript are significantly different.
^{ABC} Mean values in the same row not sharing a superscript are significantly different.
NS: not significant.
* Significant at $p<0.05$.
** Significant at $p<0.01$.

Table 2: Effect of gamma irradiation on Escherichia coli and *Salmonella typhimurium* contaminating egg powder

Treatments	Fresh egg	Egg powder	Egg powder 5 kGy	Egg powder 10 kGy	Egg powder 15 kGy
Escherichia coli	D	D	ND	ND	ND
Salmonella typhimurium	D	ND	ND	ND	ND

D: Detected ND: not detected

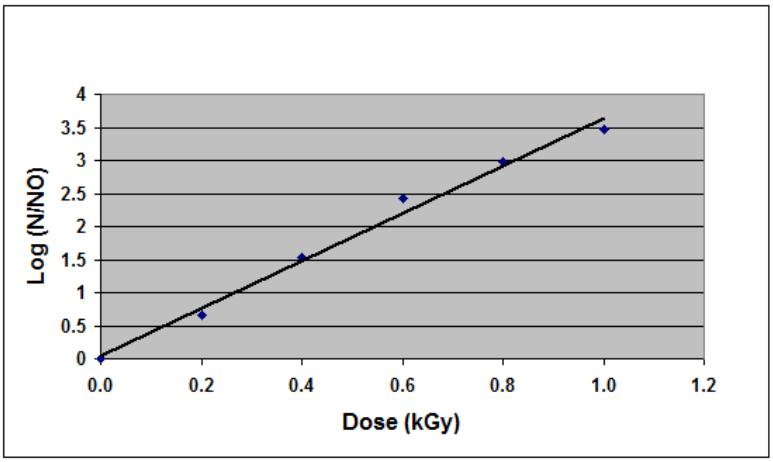

Figure 1: Behavior of *E. coli* inoculated egg powder samples subjected to gamma irradiation at 0, 0.2, 0.6, 0.8, 1.0 and 1.2 kGy (three replicates). (y=3.5929x+0.0488) (R^2=0.9885).

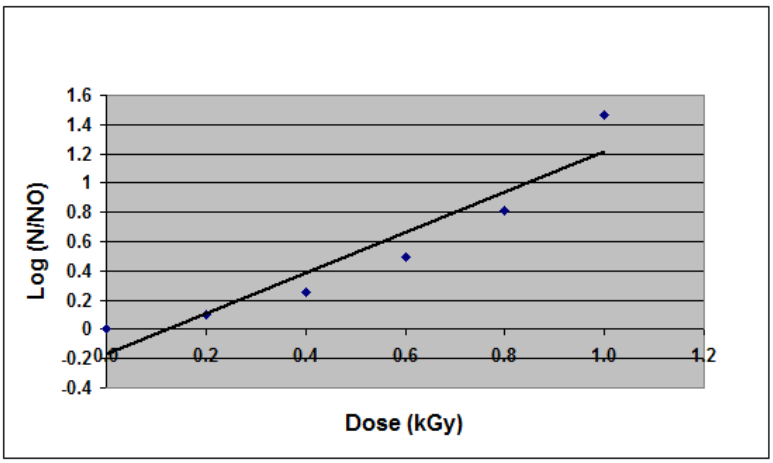

Figure 2: Behavior of *Salmonella* spp inoculated egg powder samples subjected to gamma irradiation at 0, 0.2, 0.6, 0.8, 1.0 and 1.2 kGy (three replicates). (y=1.3901x-0.1738), (R^2=0.8973).

The values of decimal reduction dose (D_{10} value) for *Salmonella* of the powdered egg is 0.714 kGy (Figure 1), while the D_{10} value for *E. coli.* of the powdered egg is 0.278 kGy (Figure 2). Results of the present study are in agreement with those obtained by Froehlich, Gombossy de Melo Franco, Destro, and Landgraf (2015) which indicated that irradiated powdered egg inoculated with *Salmonella* had D_{10} values which varied from 0.76 to 0.86 kGy. Kim et al. (2016) have reported that the D_{10} value of electron beam irradiated egg powder was 0.26 kGy for both *Salmonella typhimurium* and *E. coli.* D_{10} values of bacteria in food are affected by some factors including water activity. The relatively high D_{10} values for both *Salmonella* and *E. coli* in the present study may be due to the lower water content of the egg powder, since the water activity for used egg powder is 0.50 or 0.56% at 24 ^{o}C. Such a low water activity provides longer storage life, because the absolute limit for microbial growth is higher than 0.6 (Kumar, Gautam, Powar, & Sharma, 2010). Although, *Salmonella* spp cannot actively multiply in water below 0.92,they can survive for long periods in eggs (Froehlich et al., 2015). In preserving foods by drying, one seeks to lower the moisture content to a point where the activities of food spoilage and food-poisoning microorganisms are inhibited. Dried or Low Moisture Foods (LMF) are those that generally do not contain more than 25% moisture, and have a water activity (aw) between 0.00 and 0.60. Intermediate Moisture Foods (IMF) have a_w values of 0.60 to 0.85 (with moisture contents of 15 to 50%). They can be eaten without rehydration, but their shelf-life lasts for a relatively long period of time without refrigeration and they are considered microbiologically safe (Syamaladevi et al., 2016).

4 Conclusion

The results of this study demonstrated that the microbiological quality of an egg powder such as total viable counts (TVC), total coliform counts (TC) and mold and yeast counts (MYC) are significantly affected by gamma irradiation. An irradiation dose level of 10 kGy is a promising treatment for decontamination of dried hen egg powder products. The treatment is sufficient to eliminate or reduce TVC, TC and MYC and to maintain products of hygienic quality within safe levels as recommended by national and international food and health organizations either directly after irradiation or during storage.

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Various Factors Affect Product Properties in Apple Cider Production

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Abstract

Different parameters in cider processing were evaluated using different cultivars of Norwegian-grown table apples measuring the quality of cider. Seven different apple cultivars were mixed into four different apple juice mixtures. In this experiment, we evaluated the maturation of the apples along with commercial cider yeast and spontaneous alcoholic fermentation. Other parameters were fermentation temperature and filtration along with content of polyphenols, organic acids and volatile compounds that was analysed as an effect of the fermentation process. Succinic acid was the major organic acid in apples and ciders. The different apple juice mixtures did not reveal pyruvic and acetic acids but they appeared in relatively high amount in the ciders. The level of citric acid increased from apple to cider. Chlorogenic acid was the major polyphenolic compound found from 13-109 mg L^{-1} in the apple juice mixtures and between 27-200 mg L^{-1} in the ciders. The higher alcohol 3-methyl-1-butanol appeared in relatively large amounts in all the ciders (91-166 mg L^{-1}). The average content of acetaldehyde increased during the fermentation process, from apple juice mixtures 2.75 mg L^{-1} and 14.65 mg L^{-1} in the ciders. The content also increased for ethyl acetate with levels at 0.1 mg L^{-1} in the apple juice mixture and 20 mg L^{-1} in the cider. In the sensory evaluation experiment, the ciders produced from the apple cultivars Aroma, Gravenstein and Summerred got higher scores in fruitiness and complexity compared to the other apple juice mixtures.

Keywords: Cider; Apples; Alcoholic Fermentation; Polyphenols; Aromatic Compounds

1 Introduction

Apple (*Malus* sp.) originates from Central Asia and has been cultivated thousands of years mostly in cooler climates in Asia and Europe. In Scandinavia (nearly up to the Arctic Circle), apple has been known for more than 1000 years. The Nordic climate with long days and cool nights during summer gives a fresh acidic-sweet distinct aroma to the apples. In addition, the Nordic climate results in slow maturation of fruits in this region compared to apples grown in the South of Europe (Redalen, 1991).

Most of the apples in Norway are grown for fresh consumption, but there are long traditions for using apples in various dishes in addition to juice and cider making. Since the end of 1700 in the west coast area of Norway, Hardanger, cider was commercially produced. The cultivars used in this cider production are usually high in acids and low in polyphenols. Phenolic compounds in apples are found to be the most important contributors to cider qualities, such as complex taste and body, astringency and keepability (Alonso-

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Salces et al., 2001; Leforestier et al., 2015; Sanoner, Guyot, Marnet, Molle & Drilleau, 1999; Verdu et al., 2013). Previously, rowanberries were used as an ingredient in producing apple wine and cider due to lack of tannins in apples (Erken, 1932). Today cider producers are experimenting with mixing different dessert apples, Crab apples and cider apples along with hops to give cider more tannins and taste that is more complex. Importation of cider apple trees for cultivation in Norway is of great interest for apple growers, because cider apples have a higher content of polyphenols than dessert apples (Bamforth, 2004).

According to Tsao, Yang, Xie, Sockovie and Khanizadeh (2005), antioxidant activity is positively correlated with the total phenolic concentration in apples, whereas from in vitro-studies flavan-3ols/procyanidins were found to be the most important contributors to antioxidant activity in apples. In a study by Sanoner et al. (1999), they found the most important individual polyphenols in cider apples to be the procyanidin B2 and (-)-epicatechin, though the proportion of the polyphenol classes varied greatly among apple cultivars.

The selection of proper yeast is important for developing good sensory properties in the final product. The use of wild yeast fermentation (spontaneous fermentation) is less predictable but might give a product of more distinguished aromatic profile. However, there will always be a risk of microbiological spoilage and off-flavours. Traditional cider makers often add sulphite to prevent contamination when using wild yeast. Inoculating with selected yeast strains isolated from a cider of good quality might be a good alternative to wild yeast fermentation. Yeast strains of *Saccharomyces cerevisiae*, *S. bayanus* or *S. bayanus* var. *uvarum* will also produce ciders of good quality. The taste of a cider is a consequence of many different biochemical interactions that occur as the result of the selection of apples as a raw material and the multiple steps in the fermentation process. Cider styles vary between countries and regions. Ciders can be hazy or clear, still or carbonated, colourless to brownish, pasteurized or unpasteurized (Bamforth, 2004).

Today, there is an increased interest for renewing the old traditions of using apples as raw material for juice, cider and apple spirit. Of particular focus in Norway, is the use of apple cultivars with a higher content of polyphenolic compounds or the use of old cultivars or other ingredients for increasing the content of polyphenols in the product. New regulations in Norway (since 2015), allow farmers to produce and distribute wine and cider with alcohol content up to 22 % ABV directly from the farm. Four different juice mixtures from seven apple cultivars produced different ciders in this experiment. In order to evaluate the influence of various processing variables on cider quality fermentation methods, temperature and time, yeast strain, filtration and addition of hops were evaluated.

2 Materials and Methods

2.1 Apples

Seven apple cultivars were included in this experiment of product development using Norwegian grown apples for cider production. The apple cultivars used were Sunrise, Discovery, Aroma, Gravenstein, Summerred, Jonagold and Torstein. The apples were harvested in the fruit orchard at the Norwegian University of Life Sciences (NMBU) at Aas (10° 77' E, 59° 67' N) at harvest maturity stage in September and October 2015. All the apple cultivars were stored at +3 ^{o}C and 85 % RH in normal atmosphere before juicing. The cultivars were stored between 19 and 65 days before juicing, depending on the harvest date of each cultivar. The different ciders were processed and analysed at the Norwegian University of Life Sciences (NMBU), Ås, Norway, 2015. For details of the parameters, see Table 1.

2.2 Analyses measuring apple fruit quality

For evaluation of maturity stage and general quality on the various apple cultivars, ground colour, firmness, starch, titratable acidity and soluble solids were measured on five apples from each cultivar.

Analyses were evaluated in triplicate.

Colour

For evaluation of ground colour, a colour chart based on the cultivar Golden Delicious was used, ranging from 1–9, where 1 is green and 9 is yellow. The numbers indicate the degradation of chlorophyll in the apple skin, which is degrading during maturity, revealing the presence of carotenoids (yellow) when the apples are mature.

Firmness

A penetrometer (Fruit pressure tester FT 327, Italy) was used for measuring fruit firmness. Apples were peeled with a fruit peeler at three different places around the apple equator to remove the apple skin before measuring, resulting in 3 x 5 measurements per apple cultivar. Results are presented as kg/cm^2.

Starch

The starch content in the apples was tested, the apples were cut in half and the surfaces of one-half were soaked in potassium iodine for approximately 10 seconds. The apples were compared to a colour chart with a range from 1-9, where 1 indicates 100 % presence of starch degraded to 9 (no starch).

Acidity

Titratable acidity was measured using 10 mL of apple juice diluted with distilled water, using an automatic titrator (Titrator 716 DMS Titrano, Metrohm, Switzerland) using 0.1M NaOH and phenolftalein as indicator. Results are expressed as % TA. The calculation was based on the malic acid equivalent 67, and is regarded as synonymous to the % of malic acid in the sample.

Total Soluble solids

For total soluble solids (TSS) measurements, a digital refractometer (ATAGO, USA) was used for measurements. Results are expressed as % TSS in the juice and referred to as Brix degree (^{o}B).

2.3 Production of apple cider

Various processing parameters were included in the production of apple ciders.

Pressing of apple juice

All the apples were washed in cold water then crushed in a Speidel fruit mill (Speidel, Germany) and pressed in a 20 L Speidel hydro press (Speidel, Germany). Each press lasted for approximately 15 minutes. All the equipment was cleaned between crushing and pressing of each cultivar. The different apple juices were mixed according to information in Table 1.

Apple juice mixture and selection of apple cultivars

Cultivars Sunrise and Discovery are apples that mature early. They were pressed after 19 days of storage (apple juice blend A) and 62 days of storage (apple juice blend B). Cultivars Aroma, Gravenstein and Summerred are apples that mature later. They were pressed after 19 days of storage (apple juice blend C). The apple juice blend D contained the cultivars Aroma, Gravenstein, Summerred, Jonagold and Torstein and were pressed after 35 days of storage. The selection was done in order to include early and late cultivars in addition to storage time before pressing.

Fermentation and yeast addition

Either the apple juices were inoculated with cider yeast (M02 from Mangrove Jack´s – *Saccharomyces bayanus*) or not (spontaneous fermentation). Fermentation temperatures were 10 ^{o}C. For some batches, fermentation was started at 20 ^{o}C for 2 days and then continued at 10 ^{o}C. Fermenting vats were either 5 L or 30 L and experiments were done in triplicate. The ciders were fermented until dryness. Fermentation rate slowed down with time, and when no further reduction in % TSS was observed the end point was between 4 and 5 ^{o}Brix.

Sugar addition

Due to the relatively low levels of TSS in the apples, we chose to increase the sugar level by adding white table sugar to the apple juice mixtures, except cider style 6, to start the fermentation. From an average of 11.7 ^{o}Brix the level increased by adding approximately 18 g sugar L^{-1} to reach 13.5 ^{o}Brix (Table 1).

Addition of hops

Dessert apples are often low in tannin and astringency. In an attempt to add more aroma and body to the cider, hops were added. The hops, Amarillo and Cascade, were added in two different batches of cider in a quantity of 1 g L^{-1}, stored at 20 ^{o}C for 3 days before bottling and maturation.

Filtering

Some of the cider batches were filtered using a Colombo® 6-INOX system (Rover Pompe, Italy). Filters used were Rover 4: 10 μm and Rover 12: 1,5 μm. Filtering was applied as a process parameter to evaluate the effect on clarity and taste profile.

Carbonation and bottling

At the end of fermentation, the ciders were chilled down to 1-3 ^{o}C, extracted from the lees, bottled with addition of external CO_2, using beer gun equipment before capping and bottle pasteurization (66 ^{o}C for 30 minutes).

2.4 Chemical analyses

Polyphenols

Polyphenols were analysed according to Guyot et al., although we omitted the thiolysis step. Only the native polyphenols were analysed (Guyot, Marnet, Sanoner & Drilleau, 2001). Phenolic compounds were identified by HPLC on the basis of their retention time and their characteristic fragmentation pattern in comparison with available standards. The polyphenol standard solutions were (+)-catechin, (-)-epicatechin, procyanidin B1, procyanidin B2, phloretin, phloridzin, chlorogenic acid, caffeic acid, rutin and quercetin.

Total phenolic compounds (TP)

Total phenols were analysed according to the Folin Ciocalteu method modified as described by (Volden et al., 2008). Quantifications were obtained by reporting the absorbance at 765 nm to a calibration curve of gallic acid and are expressed as mg equiv. GAE 100 mL^{-1} of sample.

Nitrogen

Total nitrogen was analysed by the Kjeldahl method, according to IDF 2001, and expressed as mg N L^{-1}.

Free amino acids

Free amino acids were analysed by HPLC, based on a method by (Bütikofer & Ardö, 1999). The following standards were used for identification of the amino acids: L-aspartic acid, L-glutamic acid, L-asparagine, L-serine, L-glutamine, L-histidine, Glycin, L-threonin, L-citrulline, L-arginine, L-alanine, GABA, L-tyrosine, L-valin, L-metionin, L-norvalin, L-isoleucin, L-phenylalanin, L-tryptophane, L-leucin, L-ornitin and L-lysin. Only a limited number of amino acids are presented and expressed as mg L^{-1}.

Organic acids

Organic acids were analysed using HPLC as described by (Moe, Porcellato & Skeie, 2013). Organic acids for standard solutions were citric, pyruvic, succinic, lactic and acetic acids (all from Sigma). Malic acid was analysed in the apples by titration method but was not analysed in the ciders.

Volatile compounds

Volatile compounds were analysed using a headspace gas chromatography system (HSGC) according to (Gronnevik, Falstad & Narvhus, 2011). Peaks were externally identified and quantified using standard solutions of the following compounds: acetaldehyde, 2-butanone, ethyl

acetate, 2-methyl-1propanol, 2-methyl-butanal, 3-methyl-butanal, 3-methyl-1-butanol, 2-methyl-1-butanol, 2-methyl-1propanal, diacetyl, 1-butanol, 2-butanol, acetoin, iso-butylacetate, dimethylsulfide, acetone, 2.3pentadion. 2-hexanol, hexanal, isoamyl acetate, ethyl hexanoate, 3-carene, R-(+)limonene, ethyl heptanoate, ethyl octanoate, b-citronellol, ethyl nonanoate, ethyl decanoate, phenylethyl alcohol, ethanol, 1-propanol.

Sensory evaluation

A semi-trained sensory panel (20 judges) evaluated the drinking quality of the ciders. The panel evaluated haze, aroma, sweetness, acidity, bitterness, fruitiness, complexity and aftertaste using a scale from 1-10, where 1 indicated low level and 10 indicated high level of the individual property. For calibration of the judging panel, the commercially produced "Somersby dry apple cider" was used as a reference. The cider samples were served chilled (6 ^{o}C) in a quantity of 40 mL per sample.

Statistical analyses

Statistical analyses were performed with Minitab statistical software version 17 (Minitab Ltd., UK). One-way analyses of variance (ANOVA) were performed on the experimental data. For Principal Component Analyses (PCA), the statistical programme R was performed on the effect on cider quality.

3 Results and Discussions

The maturity of apples at the time of harvest might influence yield as well as chemical composition of the apple juice. If the apples need to be stored before pressing, time of storage and various storage conditions such as temperature, atmosphere, humidity and light will also affect the fruits (Børve & Vangdal, 2009). Apples harvested for this experiment were stored for 19-65 days, depending on cultivar and harvesting time, at +3 ^{o}C at 85 % RH and normal atmosphere until pressing. This was due to the differences in maturity time of the different cultivars in addition to evaluate the effect of apple storage on cider quality.

Apple firmness declined during storage while no significant effect appeared on the other physiological properties. The apple cultivars Torstein and Jonagold were firmest at the time of pressing with 9.3 and 8.5 kg/cm^2, respectively. For the less firm qualities, with firmness of 4-5 kg/cm^2, there were problems during pressing, with apple pulp packed up in the press. This problem was especially severe for the cultivars Sunrise and Summerred that had been stored for 59 and 42 days after harvest, respectively, before pressing.

The content of soluble solids varied between cultivars. We found the highest level in the cultivar Jonagold (13.9 ^{o}B) and the lowest in Summerred (10.9/10.5 ^{o}B) (Table 2). A high portion of the soluble solid content in apples is sugar, and cultivars grown in northern countries, usually have lower content of soluble solids and higher content of titratable acidity than cultivars grown further south. (Jolicoeur, 2013) indicated ideal acidity and specific gravity levels in apple juice for cider production to be 5-6.5 g L^{-1} (malic acid) and 1060-1075 (SG) (14.9-18.2 ^{o}B). Titratable acidity in our apples was between 0.57 and 0.84 %. A higher acidity level is crucial for the taste and freshness of the product and can be important to balance the sugar level. We found no effect of storage on the level of fermentable sugars and acidity in the apple cultivars in this experiment. Due to the relatively low levels of soluble solids in the apples, the sugar level was increased in most of the apple juice mixtures before the start of the fermentation process by adding white table sugar. The ciders fermented until dryness. Fermenting at 20 ^{o}C using M02 cider yeast finished within 10 days. When fermentation started at 20 ^{o}C for a couple of days and continued at 10 ^{o}C, the fermentation time increased to 13 days. Fermentation at lower temperatures resulted in longer fermentation times, 24-29 days when using M02 yeast and 39 and 56 days for the spontaneously fermented ciders (Table 1).

In apples, the content of nitrogen is affected by cultivar, soil, fertilization, climatic conditions and the age of the apple trees (Milosevic, Milosevic & Mladenovic, 2019; Planchon, Lateur, Dupont & Lognay, 2004). To prevent fermentation from stopping, a sufficiently high amount of nitrogen is necessary (Lea, 2015) . Lea recom-

mended a nitrogen level of approximately 100 mg N L^{-1}. If the nitrogen content is too high, fermentation might continue even after most of the fermentable sugars are used (Jolicoeur, 2013). Jolicoeur defined typical values for cider; 50 mg N L^{-1} is regarded as low and fermentation might be incomplete, 80-120 mg N L^{-1} is the range for most cider apple juices, 120-150 mg N L^{-1} is regarded as rich and 300 mg N L^{-1} is regarded to be high and unsuitable for cider production. Unstable yeast growth might lead to the development of undesirable aromatic components giving the cider an unpleasant taste. In order to get a smooth fermentation process, mixing of apple juices from different cultivars is possible to obtain a good mixture. By blending our apple juice, we obtained nitrogen content between 138-166 mg N L^{-1} (Table 3). Using Jolicoeur´s definition, our apple juice mixtures were all rich in nitrogen.
The most important amino acids, asparagine, glutamine and aspartic acid, accounted for 85-95 % of the total amino acids. L-asparagine accounted for about half of the amino acids. Content of amino acids decreased during fermentation (Table 3), indicating their importance as nutrients for the yeast. Alberti et al. (2016) also reported a significant decrease in the content of most amino acids during cider fermentation.

3.1 Organic acids

Citric and succinic acids were found in all apple juice mixtures while pyruvic, lactic and acetic acids were not detected (Table 3). The sum of citric and succinic acids was 1033 mg L^{-1} for apple juice blend A, 1178 mg L^{-1} for blend B, 1462 mg L^{-1} for blend C and 1616 mg L^{-1} for blend D. In the ciders, styles 1, 2 and 3, from blend A and B, together with style 4 from blend C, appeared more bitter and acidic and lower in sweetness than the other ciders (Figure 2).
After the fermentation process, pyruvic and acetic acids were present in all the cider samples. Content of succinic acid increased during fermentation for all the ciders except styles 2 and 6 which were spontaneously fermented. Lactic acid did not appear in the apple juice but was present in many of the ciders. In the malo-lactic fermentation, the yeast is able to degrade malic acid to ethanol, amyl alcohol, succinic acid, lactic acid and isobutanol with help from CO_2. The malo-lactic step is often desired when using dessert apples in cider production, due to their high acidity (Jolicoeur, 2013). The sensory evaluation of acidic taste did not correspond to measured acidity, indicating the importance of the malo-lactic transformation in giving a less acidic feeling.

3.2 Polyphenolic compounds

Content of phenolic compounds varied between the different apple juice mixtures (Table 4). The levels of the various polyphenols were lower than reported by other researchers (Kahle, Kraus & Richling, 2005; Wojdylo, Oszmianski & Laskowski, 2008). We also found a variation in the single components in the process from apple juice to cider. This is comparable to what was reported by (Laaksonen, Kuldjarv, Paalme, Virkki & Yang, 2017) but slightly different to results from (Ye, Yue & Yuan, 2014) who found decreases in most of the polyphenols in the apple juice to cider. Chlorogenic acid was the most abundant polyphenol in the apple juice mixtures, followed by procyanidin B2 and procyanidin B1, (+)-catechin and (-)-epicatechin. We found increases in caffeic acid during fermentation while for chlorogenic acid the results were more variable. This is in contrast to observations from Alberti et al. (Alberti et al., 2016) who observed decreases in both these components in ciders compared to apple juice. Quercetin and phloretin did not appear in the apple juices and rutin only in one sample. Phloretin appeared in most of the ciders. The highest level was observed in cider style 6. Quercetin and rutin were detected in all the ciders and in much higher quantity than the apple juice mixture they were made from, indicating a metabolization of rutin and quercetin during the fermentation process. We found a decrease in TP (Folin) from raw material to cider. For the single polyphenols no such change was observed (Table 4). On average, the TP content in apples and ciders from apple juice blend A and B was higher than in apples and ciders from blend C and D. In the sensory evaluation, cider styles 1, 2 and 3 from blend A and B were also evaluated to be more bitter than the

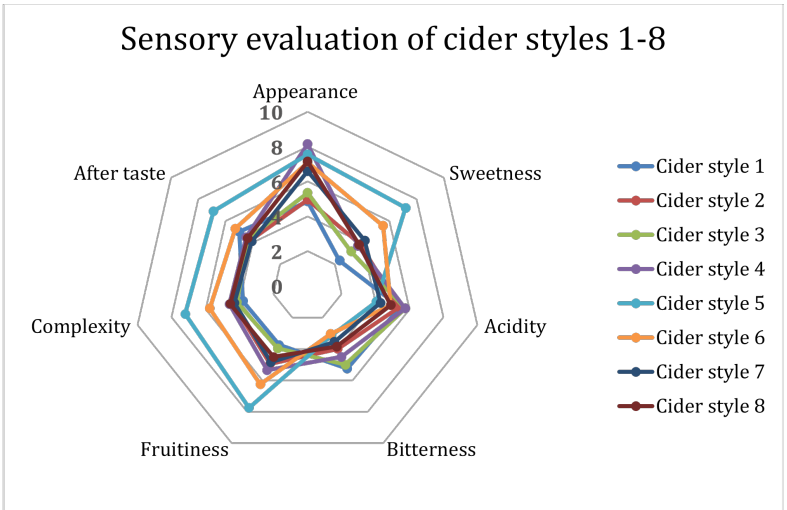

Figure 1: Sensory evaluation of cider styles 1-8. Sensory attributes were appearance, sweetness, acidity, bitterness, fruitiness, complexity and aftertaste

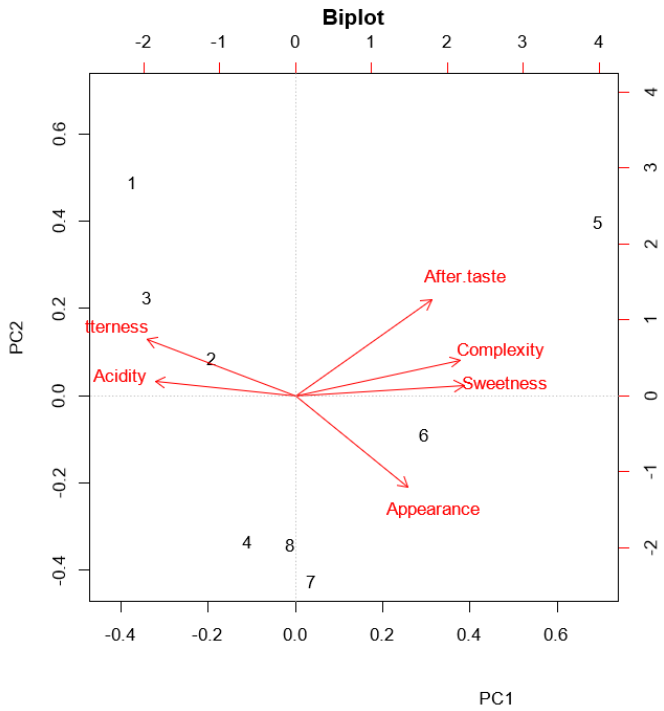

Figure 2: Biplot of cider styles 1-8 and sensory properties

Table 1: Parameters in cider processing

Cider style	Apple juice blend	Batch size (L)	Yeast type	Hop Hop	Soluble solids (^oB)	Temp (^oC)	Fermentation (days)	Filter Filter
1	A	5	M02		13.5*	20	10	-
2	A	30	Sp. ferm.		13.5*	10	56	4
3	B	5	M02		13.5*	10	24	4
4	C	5	M02		13.5*	20-10	13	-
5	C	30	M02	Am	13.5*	20-10	13	4
6	C	5	Sp. ferm.		10.6	10	39	4
7	C	5	M02		13.5*	10	24	12
8	D	30	M02	Cas	13.5*	10	29	4

Apple juice mixtures:
A: 'Sunrise' 50 % and 'Discovery' 50 %, stored 19 days before pressing
B: 'Sunrise' 50 % and 'Discovery' 50 %, stored 65 days before pressing
C: 'Aroma' 40 %. 'Gravenstein' 20 %. 'Summerred' 40 %, stored 19 days before pressing
D: 'Torstein' 15 %. 'Jonagold' 15 %. 'Aroma' 20 %. 'Summerred' 20 %. 'Gravenstein' 30 %, stored 35 days before pressing
*: adjustment of % TSS by sugar addition

For fermentation, various yeasts were added
M02 – Cider yeast (Mangrove Jack's. UK)
Spontaneous fermentation – no yeast addition

Hops: Am: Amarillo. Cas: Cascade

Table 2: Fruit quality at two maturity stages after storage of the apple cultivars Sunrise, Discovery, Aroma, Gravenstein, Summerred, Jonagold and Torstein at the time of juicing.

Apple cultivar	Firmness	Ground colour	Starch content	Soluble solids	Titratable acidity	Nitrogen	TP
	kg/cm^2 ± STD	1-9	1-10	^{o}B	% TA ± STD	mg N L^{-1} ± STD	GAE 100mL^{-1} ± STD
Sunrise ^{a}	7.26 ± 0.42	6.7	7.9	12.1	0.58	220 ± 6.1	35.3 ± 5.63
Sunrise ^{c}	5.37 ± 0.18	7.9	10.0	12.0	0.57 ± 0.02		27.1 ± 2.07
Discovery ^{a}	8.16 ± 0.33	8.0	10.0	11.8	0.82	65 ± 1.02	81.5 ± 0.13
Discovery ^{c}	6.07 ± 0.94	8.0	10.0	11.7	0.61 ± 0.05		101.7 ± 0.60
Summerred ^{a}	5.37 ± 0.33	7.3	9.7	10.9	0.82 ± 0.01	329 ± 18.2	15.0 ± 0.31
Summerred ^{b}	4.54 ± 0.11	7.4	10.0	10.5	0.77 ± 0.05		12.1 ± 0.15
Aroma ^{a}	6.19 ± 0.02	7.1	9.4	11.9	0.77 ± 0.01	125 ± 14.4	51.9 ± 0.94
Aroma ^{b}	5.68 ± 0.33	6.9	10.0	11.2	0.80 ± 0.01		11.2 ± 0.91
Gravenstein ^{a}	6.40 ± 0.03	6.2	9.9	11.5	0.68 ± 0.02	98 ± 12.8	49.2 ±2.15
Gravenstein ^{b}	5.12 ± 0.26	7.2	9.9	11.5	0.66 ± 0.04		44.8 ± 1.56
Jonagold ^{b}	9.31 ± 0.19	7.3	9.7	13.9	0.77 ± 0.04	91 ± 13.1	19.4 ± 0.47
Torstein ^{b}	8.54 ± 0.11	6.5	6.9	12.4	0.84 ± 0.02	212 ± 15.2	113.3 ± 1.25

^{abc} Days of storage of apples before pressing a: 19 days, b: 35 days, c: 62 days
TP: total phenols (Folin Ciocalteu)

Table 3: Content of organic acids, amino acids and total nitrogen from raw material to cider.

Apple juice blend	Cider style	Citric acid mg L^{-1}	Pyruvic acid mg L^{-1}	Succinic acid mg L^{-1}	Lactic acid mg L^{-1}	Acetic acid mg L^{-1}	Tot N mg N L^{-1}	L-asp acid mg L^{-1}	L-glut mg L^{-1}	L-asp mg L^{-1}
A		28	nd	1005	nd	nd	138±3.3	87.70	57.50	176.5
	1	37	75	1792	nd	145		nd	nd	nd
	2	120	70	1321	55	182		nd	nd	nd
B		47	nd	1131	nd	nd	149±2.7			
	3	60	60	1563	22	98				
C		56	nd	1370	nd	nd	151±15.1	141.00	49.90	370.7
	4	100	46	1831	nd	112		nd	0.44	nd
	5	88	75	1818	nd	137		0.27	0.73	0.16
	6	76	26	1233	24	89		nd	nd	nd
	7	77	38	1488	nd	208				
D		71	nd	1545	nd	nd	166±9.7	147.60	62.20	424.6
	8	108	49	2112	41	110		nd	nd	nd

Table 4: Content of polyphenols from raw material to cider

AB	C	CT	EC	B1	B2	CA	CAF	PLZ	XPL	QUE	RU	TOT	TP	
		mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	GAE mL^{-1}	100
A		2.8	4.4	8.1	18	109	1	0.1	nd	nd	0.3	143		60
	1	1.6	5.9	6.6	15	121	1.2	0.6	nd	nd	1.6	153		41
	2	1.5	4	5	12	87	1.1	0.3	0.1	0.03	0.9	112		23
B		4.1	1	9.3	15	89	1.7	0.2	nd	nd	nd	121		68
	3	4.7	1.4	nd	18	200	2.9	0.1	0.2	nd	6.9	234		38
C		1.4	2	8.1	4.3	28	0.1	0.2	nd	0.04	nd	44		26
	4	12	4.8	9	5	27	0.4	3.6	0.1	0.1	1.4	63		14
	5	18	5.3	6.5	4	28	0.5	5.8	0.1	nd	2.9	71		16
	6	8.3	5.7	4.1	4.8	28	0.6	4.1	0.6	0.01	2	58		9
D		3.3	4.4	2.7	4.9	13	0.1	0.1	nd	nd	nd	29		17
	8	2.1	2.0	6.0	7.2	19	0.3	1.0	0	nd	0.5	38		11

AB: apple juice blend: C: cider style: CT: (+)-catechin, EC: (-)-epicatechin, B1: Procyanidin B1, B2: Procyanidin B2, CA: Chlorogenic acid, CAF: Caffeic acid PLZ: Phloridzin, XPL: Phloretin, QUE: Quercetin, RU: Rutin, TOT: Sum Polyphenols, TP: Total Phenols – Folin Ciocalteu

other cider styles (Figure 2).

3.3 Volatile compounds

Esters are the dominant volatile compounds in ciders (Fan, Xu & Han, 2011) and are associated with the desirable taste of the product. Ethyl acetate is important for the sensory character in both wine and cider giving the product a fruity taste. Ethyl acetate originates from the apples and during the fermentation process. Ethyl acetate was the dominant ester in this experiment for ciders, though only registered in minor level in the apples. All the ciders contained substantial amounts of ethyl acetate and iso-amyl acetate that together with phenyl ethyl acetate, isobutyl acetate, ethyl hexanoate and hexyl octanoate are regarded to be the most important esters for fruitiness and for general cider quality (Xu, Fan & Qian, 2007). Butyl butyrate (fruity flavour) appeared in the apple juices and not in the ciders. We found no difference in formation of volatile compounds between ciders inoculated with M02 yeast and the spontaneously fermented ciders.

During the fermentation process of cider, considerable amounts of the higher alcohols: phenyl ethyl alcohol, 2-methyl-1-propanol, 1-propanol, 3-methyl-1-butanol and 2-methyl-1-butanol developed. These components are important for the fruity and characteristic cider taste of the product. 3-methyl-1-butanol and ethyl hexanoate (taste of ripe fruit) were not present in the apple juices, but found in relatively high amounts in the ciders (Table 5).

Acetaldehyde and diacethyl are important for the aromatic profile of fermented products (Berry &

Table 5: Content of volatile compounds from raw material to cider

Apple juice blend	Cider style	Acetald ehyde mg L^{-1}	Acet one mg L^{-1}	1propanol mg L^{-1}	Diacetyl mg L^{-1}	Ethyl acetate mg L^{-1}	2meth.1-prop mg L^{-1}	Buthyl butyrat e mg L^{-1}	3-methyl-1-butanol mg L^{-1}	2-methyl-1-butanol mg L^{-1}	Butyl acetate mg L^{-1}	Ethyl hexanoate mg L^{-1}	Phenyl ethyl alcohol mg/L
A		1.64	0.14	2.52	0.01	0.04	0.17	0.08	0	1.17	2.99	0	0.10
	1	11.62	0.32	7.89	0	14.59	14.86	nd	116.53	25.13	1.12	0.65	25.37
	2	11.68	0.27	5.81	0.01	12.15	21.88	nd	125.37	14.19	1.16	0.19	20.94
B		1.07	0.04	1.92	0	0.02	0.17	0.08	0	0.58	3.27	0	0
	3	26.31	0.30	7.37	0.02	17.57	12.07	nd	90.41	21.29	1.17	0.33	19.20
C		4.58	0.12	0.64	0.09	0.09	0.19	0.37	0	0.55	1.76	0	0.20
	4	6.53	0.30	11.93	0	21.96	30.98	nd	165.82	31.63	0.66	0.65	34.26
	5	26.65	0.57	13.33	0.08	22.90	26.41	nd	148.70	27.77	0.62	0.48	34.53
	6	11.76	0.32	8.87	0.00	38.86	17.93	nd	96.75	17.24	0.34	0.30	19.23
	7	8.90	0.22	5.83	0.02	21.44	22.01	nd	130.45	8.26	0.75	0.46	24.70
D		4.11	0.12	0.61	0.01	0.09	0.23	0.35	0	0.63	1.24	0	0.09
	8	19.98	0.32	10.20	0	19.82	10.01	nd	97.48	21.40	0.79	0.61	21.81

Slaughter, 2003). Low levels of acetaldehyde can give a fruit nice taste of green fruit, but high levels will give an unpleasant taste. Higher levels were present in cider styles 3, 5 and 8. Sensory evaluation of style 3 showed high scores for acidity and bitterness and low scores for fruitiness and sweetness. On the opposite side, style 5 (Amarillo hop added) got high scores for fruitiness, complexity, aftertaste and sweetness and style 8 (Cascade hop added) got relatively low scores on most attributes except appearance. We did not find any correlation between yeasts and the level of acetaldehyde in the ciders. Cider style 3 was slightly lower in higher alcohols than cider styles 1 and 2. These ciders were made from the same apple cultivars, but at different stages of maturity at the time of pressing. Although we did not find significant differences in chemical composition between the raw materials at different maturity, the levels most likely influenced the fermentation process and the formation of volatile compounds.
Generally, cider styles 4-7, all made from C apple juice mixture, tend to be higher in ethyl acetate, 2-methyl-1-propanol, 3-methyl-1-butanol and phenylethyl acetate than ciders from the other apple juice mixtures. These components are important for the fruity taste of the cider. These ciders also scored higher in the sensory evaluation of attributes like sweetness, fruitiness, complexity and aftertaste (Figure 1) and showing the same pattern in the PCA plot (Figure 2). Style 6 got higher scores for most of the attributes except bitterness compared to style 2. This indicates that apple juice mixture C was preferable for making cider in this experiment. Consequently, selection of apple cultivars is important for making a cider of good sensory properties.

3.4 Filtering and clarity

Filtering of the cider before bottling will also affect the appearance. Cider style 7 using filter number 12, became clearer, but lost some colour and taste attributes. Sensory evaluation showed that this cider got relatively low scores on most attributes except appearance. We found more haze in style 1 (apples stored a shorter time before pressing) and thus too many pectin substances in the juice. Bamforth (2004) recommended a maximum 2 % starch in the apples at the time of pressing, meaning that all of the pectin substances would be sufficiently degraded. On the other hand, overripe apples will provide low acidity, and give the cider a taste of "boiled apples". This corresponds with our findings that style 3 was clearer but contained some soluble solids that remained after filtration.
This cider was characterised to be less fresh and with a hint of boiled taste.

3.5 Sensory evaluation

In the sensory evaluation, ciders made from the apple juice mixtures A and B, styles 1-3, got lower scores in most attributes compared to ciders from the mixtures C or D. Styles 4-7 scored higher in attributes like fruity/flowery taste, complexity and aftertaste, criteria that usually are regarded as positive attributes for cider. We found a high correlation ($r=0.853$, $p<0.01$) between fruitiness and content of catechin in the ciders. Evaluation of cider made from the cultivars Sunrise and Discovery had low sweetness, being acidic with a strong sour aftertaste (Figure 1 and 2). Average TP content in ciders from A and B juice mixtures was 34 GAE 100 mL^{-1}, while for ciders from C and D juice mixtures the average was 12.6 GAE 100 mL^{-1}. TP content was positively correlated ($r=0.847$, $p<0.01$) to bitterness in ciders in the sensory evaluation. Cider style 5 was evaluated to be sweeter, less acidic and less bitter than the other ciders, while cider style 8 got much lower scores on these attributes (Figure 1 & 2). Selection of hops for the cider during processing is important for development of a good aromatic profile.

4 Conclusion

The most important factor for the impact on cider quality was the various mixtures of apple juice with different selection of apple cultivars and apple maturity. Fermentation temperature, hop addition and filtering also affected the product properties. The addition of hops was successful for one of the styles. Choosing a

proper hop variety that goes well with the taste profile of the cider is essential.
The ciders made from apple juice mixture C (apple cultivars Aroma, Gravenstein and Summerred) got superior sensory characteristics compared to cider mixtures A and B (cultivars Sunrise and Discovery) and D (cultivars Aroma, Gravenstein, Summerred, Jonagold and Torstein).
Ciders made from Sunrise and Discovery were higher in phenolic compounds as well as total phenols though they were ranked lower in sensory evaluation.

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Pequi Oil Microencapsulation by Complex Coacervation using Gelatin-Cashew Gum

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Abstract

New functional foods and beverages can be developed using bioactive compounds present in pequi oil. Complex coacervation is an encapsulation method used for preserving bioactive molecules, especially those that are hydrophobic or sensitive to high temperatures. The objective of this work was to produce and characterize pequi oil microparticles using cashew gum/gelatin matrix (CG/GE) through complex coacervation. Gum Arabic (GA) was also studied in comparison with CG. The coacervation process was performed without pequi oil to determine the ideal proportions of the matrix components, followed by the embedding of the oil in the microparticles for evaluation. Satisfactory microparticles were produced at pH 4.5 in the weight ratios of CG/GE = 2:1 and GA/GE = 1:3. Pequi oil release was greater in acidic pH, especially at pH 2 for the CG/GE matrix. The encapsulation efficiency for CG/GE and GA/GE was 72.53% (±4.80) and 82.77% (±6.09), respectively. The results showed that the CG/GE combination seemed very promising as an encapsulation matrix, especially for food applications involving pH values higher than 3.

Keywords: *Anacardium occidentale*; Coacervate; Encapsulation; Gelatin; *Caryocar coriaceum*; Polysaccharides

1 Introduction

Microencapsulation is often used in powder technology to describe the process of forming an amorphous polymeric coating around a core to control mass transfer and provide protection in the dry state from interactions with its environment, thereby minimizing changes in colour, aroma and flavour and enabling controlled release (Gouin, 2004).

The particles derived from encapsulation methods often display different physical shapes and structures that influence the release profile and storage stability of the core material (Aguilera,

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2005; Re, 2006). In the present study, microencapsulation was achieved through the complex coacervation method, which entails the preparation of a mixture of polyelectrolytes of opposite charges in an aqueous solution, resulting in separate layers; the dense layer is rich in polymers (coacervate) and the dilute phase is depleted in polymers (Black et al., 2014). This method is also recommended for the microencapsulation of hydrophobic materials and/or substances sensitive to high temperatures (Alvim & Ferreira Grosso, 2010).

Pequi tree oil is a seed oil with a peculiar odour and aroma that is distinct and noticeable. The consumption of this oil has attracted attention due to its potential health benefits. It is rich in oleic acid (Lopes et al., 2008) and widely used in foods because of its high content of vitamins (de Paula-Ju, H. Rocha, Donatti, Fadel-Picheth & Weffort-Santos, 2006) and carotenoids (Azevedo-Meleiro & Rodriguez-Amaya, 2004). The presence of these compounds provides skin protection by preventing free radical formation and, therefore, slowing down the aging process (Pianovski et al., 2008). Other benefits associated with the use of this oil include treatment for gastric ulcers (Quirino et al., 2009), anti-inflammatory property, and cutaneous wound-healing support (Batista et al., 2010). In view of its beneficial effects, it is highly desirable to preserve bioactive compounds in pequi oil through microencapsulation in order to develop functional food and drinks for the market.

Gums are among the biopolymers most commonly used as wall materials in microencapsulation methods (Jafari, Mahdavi-Khazaei & Hemmati-Kakhki, 2016; Khoshakhlagh, Koocheki, Mohebbi & Allafchian, 2017; Revuelta, Chacon Villalba, Navarro, Guida & Castro, 2016). Gum Arabic is widely used, despite its cost and occasional supply problems related to climatic, economic and political problems in the African region that produces it (Andrade, Carvalho & Takeiti, 2013). Complex coacervation with gum Arabic and gelatin has been extensively investigated (Anvari & Chung, 2016; Habibi, Keramat, Hojjatoleslamy & Tamjidi, 2017; Lv, Yang, Li, Zhang & Abbas, 2014); however, studies involving cashew gum-gelatin using this method are recent and relatively sparse in the literature (Comunian et al., 2016; de Souza et al., 2018; Gomez-Estaca, Comunian, Montero, Ferro-Furtado & Favaro-Trindade, 2016). Cashew gum is considered an alternative to gum Arabic, although it is not yet a commercial product (Rodrigues, 2004). Cashew gum is an anionic polysaccharide and has low viscosity in water. The negative charge on cashew gum in aqueous solution makes it possible to interact with positively charged polymers. Earlier studies in this laboratory have demonstrated cashew gum as an efficient encapsulating matrix (da Silva et al., 2018; de Oliveira, Paula & de Paula, 2014; Gomez-Estaca et al., 2016; Rodrigues & Grosso, 2008). In this work, pequi oil microparticles were produced using cashew gum/gelatin matrix through complex coacervation. Moreover, the particles were characterized by microscopy, encapsulation efficiency (superficial and total oil), yield and oil release, and particle size.

2 Materials and Methods

2.1 Materials

Cashew gum (CG) was collected from *Anacardium occidentale* L. plants from Embrapa Tropical Agroindustry Experimental Field in Ceará - Brazil (coordinates: 4°11'26.62" S and 38°29'50.78" W). Gum Arabic (GA) was purchased from JB Química Indústria e Comercio Ltda and the gelatin (GE) 225H type B was provided by Rousselot®. The pequi oil (*Caryocar coriaceum* Wittm.) was purchased from a local provider in Ceará, Brazil (coordinates: 07°18'19" S and 39°18'08" W). All reagents used were analytical grade.

2.2 Cashew Gum Isolation

The isolation of cashew tree polysaccharide exudate was carried out according to Torquato et al. (2004), with some modifications. A 500 g exudate sample was ground by a knife mill and solubilized in water in the proportion of 300:1 (g/L). After solubilization, the sample was filtered, centrifuged at 15,303 x g for 10 min at 25°C and precipitated in 1:3 ethanol (v/v) for 24

h at 10°C. The precipitate was dried in an air-circulating oven at 60°C for 24h and was ground afterwards. The moisture of the polysaccharide isolated was 12.58 ± 0.43 %.

2.3 Microparticles without pequi oil

Suspensions of CG 1% (w/v), GA 1% (w/v) and gelatin 1% (w/v) were prepared at pH 4, 4.5 and 5, respectively, for zeta potential analysis. From the data, the proportions of each polymer and the ideal pHs for the formation of the microparticles (CG/GE and GA/GE) were established in accordance with Prata and Grosso (2015).
The procedure used for the production of microparticles was as follows. The gelatin and gum Arabic solutions were prepared separately followed by homogenisation in an Ultra-Turrax homogeniser at 10,000 rpm for 3 min at room temperature. 100 mL of each suspension was homogenised in this way at room temperature. Then 400 mL of distilled water was added and homogenised in the Ultra-Turrax at 10,000 rpm for 3 min at room temperature. The pH was adjusted with hydrochloric acid (2M) to 4.0, 4.5 and 5.0, and the solutions were refrigerated (8 ± 2 ^{o}C) overnight for precipitation of the particles. Subsequently, excess water was eliminated and coacervate suspensions were obtained for the analyses.

Zeta potential analysis

Coacervate suspensions were lightly homogenised and the zeta potential was determined with a Zetasizer Nano ZS 3000 dynamic light scattering instrument (Malvern Instruments, UK), operating with a laser light at a wavelength of 633 nm.

Spectrophotometric analysis

The coacervate formation was indirectly analyzed by the reading of suspension absorbance using a spectrophotometer (Cary 50 Conc, Varian) before and after cooling (8 ± 2 ^{o}C) at the wavelength of 200 nm. Only the supernatant from each sample was used for the analysis (da Silva et al., 2018).

Coacervate yield

Coacervates were centrifuged at 15,303x g for 10 min at 25 ^{o}C and dried in a drying oven at 105 ^{o}C until constant weight was achieved. The yield was calculated through the relationship between the initial dry mass used and the final mass obtained from the formula $R = 100\ m_f\ /\ m_0$, where R is the percent yield, mf is the final dry mass and m_0 the initial mass (gum mass + gelatin mass) (Huang, Sun, Xiao & Yang, 2012).

2.4 Microparticles with pequi oil

Emulsions were prepared with pequi oil in gelatin dispersion at 10,000 rpm for 5 min at room temperature. The GA solution was then slowly added to the gelatin-stabilized emulsion to a final aqueous volume of 200 mL, using the same procedure adopted for evaluation of the coacervation process presented before without pequi oil (Section 2.3). Five levels of pequi oil were tested: 0.5g, 0.75g, 1g, 2g and 2.5g. Coacervates were characterised for their yield, oil release property, encapsulation efficiency, and by microscopy.

Characterisation by optical microscopy

The microscopic slides were previously sterilised with 70% ethyl alcohol. A drop of the coacervate suspension and a cover slip were placed on each slide. Optical micrographs were recorded on a Zeiss optical microscope coupled with a digital image acquisition system through a CCD camera.

Particle size determination

Microparticle size was determined with the use of the Malvern 3000 Zetasizer Nano ZS laser light scattering instrument (Malvern Instruments, UK). Precipitated coacervates were suspended in isopropyl alcohol in the proportion of 0.5 g to 25 mL of alcohol. The volume moment mean diameter $D_{4,3}$ was measured, which represents the diameter of a sphere with the same average volume in the sample and the Span which gives the information on the homogeneity of the

size distribution of the particles (Hosseini et al., 2013).

Yield analysis

The yield was calculated from the formula R = 100 m_f/m_0 as previously described (Section 2.3.3), in which R is the percent yield, m_f is the final dry mass and m_0 the initial mass (gum mass + gelatin mass + oil mass) (Huang et al., 2012).

Encapsulation efficiency

The suspensions of microcapsules were previously frozen in an ultra-freezer (Liotop FV500, Liobras) and put in the freeze dryer for 72 h (Liotop, model K1005) for determination of surface and total oil. The moisture content (%) was calculated on a wet-weight basis. The encapsulation efficiency (EE) was determined by Equation 1:

$$EE(\%) = \frac{TO - SO}{TO} \times 100 \qquad (1)$$

Where TO is the amount of the total oil and SO is the amount of the surface oil.

Determination of total oil (TO): Total oil extraction was performed according to the Bligh-Dyer method with modifications (Checci, 2003). Thus, 0.1 g of the freeze-dried microcapsules were weighed and resuspended in 10 mL chloroform, 20 mL methanol and 8 mL distilled water. The mixture was homogenized in a magnetic stirrer for 30 minutes; then, 10 mL chloroform and 10 mL of 1.5 % sodium sulphate solution were added and stirred for another 2 min. The material was transferred to a separating funnel and allowed to stand until complete phase separation. From the organic phase, approximately 15 mL was collected. About 1 g of sodium sulphate was added to the organic phase and then filtered. A 1 mL portion was transferred to a 10-mL volumetric flask, and made to volume with hexane. The amount of oil in the microparticles was calculated by using an appropriate calibration curve of free oil in hexane obtained on a spectrophotometer at 450 nm wavelength. Each batch of samples was measured in triplicate.

Determination of surface oil (SO): The amount of oil present on the surface of the freeze-dried microcapsules was evaluated spectrophotometrically, according to a method proposed by Higuita (2013) with modifications. About 0.1 g microcapsules was resuspended in 10 mL of hexane in a test tube and stirred on a vortex shaker for approximately 1 min. The amount of oil in the microparticles was calculated by using an appropriate calibration curve of free oil in hexane obtained on a spectrophotometer at 450 nm wavelength. Each batch of samples was measured in triplicate.

2.5 Oil release

Pequi oil release at different pH ranges was evaluated using the methodology described by Comunian et al. (2016) with some modifications. After microparticle formation, the pH was adjusted from 2.0 to 9.0 in 100 mL of coacervate suspension, which was kept under agitation with a magnetic stirrer for 2 min. Hexane was added in a ratio of 2:1 (v/v), agitated for 1 min, and centrifuged at 15.303x g for 10 min at 25°C . The organic phase containing the hexane was isolated and rotoevaporated for the determination of free pequi oil present. The amount of oil mass was measured in the pre-weighed boiling flask.

2.6 Statistical analysis

A completely randomized design was used in this work and the results represent the means of three replicates. The means were compared through Student's t-test. The statistical analysis was performed with Statistica 13.0 software.

3 Results and Discussions

3.1 Microparticles without pequi oil

In an acid medium, gelatin is positively charged (below the isoelectric point) and attracted to

the negatively charged cashew gum to form coacervate droplets. Thus, the proportions of the encapsulating matrices were determined based on an electrical equivalence study performed through an electrical charge analysis (zeta potential) of each biopolymer in solution at pH 4.0, 4.5 and 5.0. The zeta potential values of the polysaccharides and protein in the pH range at room temperature are shown in Table 1.

The optimum condition for coacervate formation is reached at a pH where the associated biopolymers are electrically equivalent in terms of opposing charges (Comunian et al., 2016; de Kruif, Weinbreck & de Vries, 2004; Schmitt & Turgeon, 2011). In order to reach this electrical neutrality, the proportions found for the CG/GE treatments were 2:1 at pH 4.5 and 1:1 at pH 5.0; for the GA/GE treatment, they were 1:4 at pH 4.0 and 1:5 at pH 4.5. However, in an attempt performed with 1:5 ratio at pH 4.5, the GA/GE suspension after refrigeration (8° C ± 2) formed a very viscous gel due to the high gelatin concentration, making it impossible to form the coacervate droplets. For this reason, the ratio was reduced to 1:3 at pH 4.5. Thus, the formation of the polyelectrolyte complex depends on the degree of polymer ionization, and, therefore, on the pH. In addition, the polymer stoichiometry, structural parameter (conformation and chain length), and other parameters such as ionic strength, temperature and nature of the reactants may also exert an influence on the complex formation and stability (Kim et al., 2016; Siow & Ong, 2013). Thus, although the electrical equivalence of the complexes has been deduced for the polymers separately, the zeta potential corresponding to each complex formed was also analyzed, as shown in Table 2.

G/GE, at the ratio of 2:1 and pH 4.5, showed a zeta potential value close to zero, demonstrating electrical equivalence between biopolymers that is conducive to coacervate formation. The combination of the two biopolymers in the proportion of 2.5:1 at pH 4.0 was previously tested by Comunian et al. (2016) to encapsulate Echium oil, although a prospective study of the best proportion at a different pH range has not yet been reported. At pH 4.5, GE acquires a positive charge and forms coacervates with anionic polysaccharides, as does GA. As the GE used is type B (acidic) and its isoelectric point lies in the range of 4.7 to 5.3, the pH selected to encapsulate pequi oil was in accordance with those reported in the literature (Azeredo, 2008).

The absorbance of the CG/GE and GA/GE suspensions before and after the refrigeration process (8 ± 2 ^{o}C) was monitored, so that the absorbance variation could be related to the coacervate precipitation. The results of the absorbance variation for the CG/GE and GA/GE treatments are shown in Figure 1.

A greater absorbance variation was found in the CG/GE complex at ratio 2:1 and pH 4.5, indicating a higher level of coacervate precipitation when compared to 1:1 at pH 5.0. For the GA/GE treatment, the ratio of 1:4 at pH 4.0 possibly presented a negative variation, indicating that, after refrigeration (8 ± 2 ^{o}C), the suspension became cloudier than before, probably because of the formation of a very dense gel. The negative absorbance variation value reflected the poor coacervate precipitation in this condition. However, for the ratio of 1:3 at pH 4.5, it displayed a considerable variation in absorbance and, consequently, more significant coacervate precipitation.

The conditions determined for each CG/GE and GA/GE complex were also evaluated regarding yield at a pH range. Yield is important because it is related to the production cost of the encapsulation process. The result found was in agreement with spectrophotometric and zeta potential data, where the treatments for 2:1 CG/GE at pH 4.5 and 1:3 GA/GE at pH 4.5 were found to be the most appropriate for coacervate formation (Figure 2).

3.2 Microparticles with pequi oil

Pequi oil microparticles were produced using five levels of oil (0.5 g, 0.75 g, 1 g, 2 g and 2.5 g) while adhering to the experimental settings of 2:1 CG/GE at pH 4.5 and 1:3 GA/GE at pH 4.5. Microparticles formed and precipitated were subjected to yield and particle size analysis by laser diffraction.

The yield based on dry-weight of the microparticles formed is shown in Figure 3. Through this analysis it was possible to establish a rela-

Table 1: Zeta potential values of cashew gum (CG), gum Arabic (GA) and gelatin (GE) in the pH range from 4.0 to 5 at room temperature.

	CG (1% w/v)	GA (1% w/v)	GE (1% w/v)
pH		Zeta potential (mV)	
4.0	3.11	-16.40	3.65
4.5	-2.09	-21.07	4.24
5.0	-1.55	-21.80	1.02

Table 2: Zeta potential values of coacervate suspensions without pequi oil at different pH values after cooling (8 ^{o}C) overnight for precipitation of the particles. The measurements were conducted at room temperature.

Treatment	Ratio (w/w) and pH	Zeta potential (mV)*
CG/GE	2:1 pH 4.5	-0.25 ± 0.47^{a}
	1:1 pH 5.0	-2.96 ± 0.70^{b}
GA/GE	1:4 pH 4.0	7.25 ± 2.33^{c}
	1:3 pH 4.5	1.34 ± 0.41^{d}

*All mean zeta potentials differed significantly (p≥0.05).

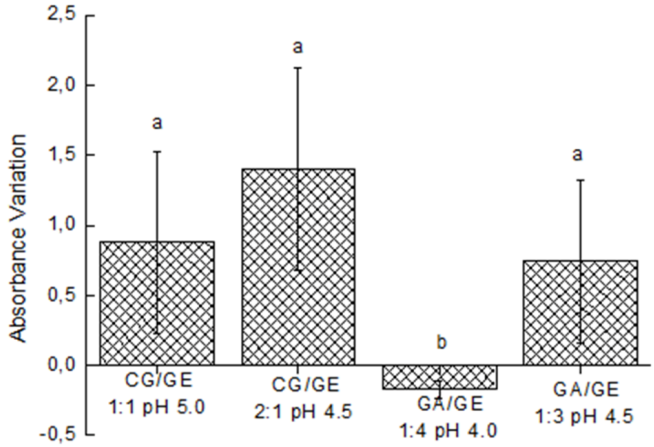

Figure 1: The variation of absorbance (at 200 nm) before and after coacervate refrigeration (8 ± 2 ^{o}C) with the use of cashew gum/gelatin (CG/GE) at 1:1 ratio/pH 5 and 2:1 ratio/pH 4.5, and gum Arabic/gelatin (GA/GE) at 1:4 ratio/pH 4 and 1:3 ratio/pH 4.5. Bars: standard deviations (n = 3)

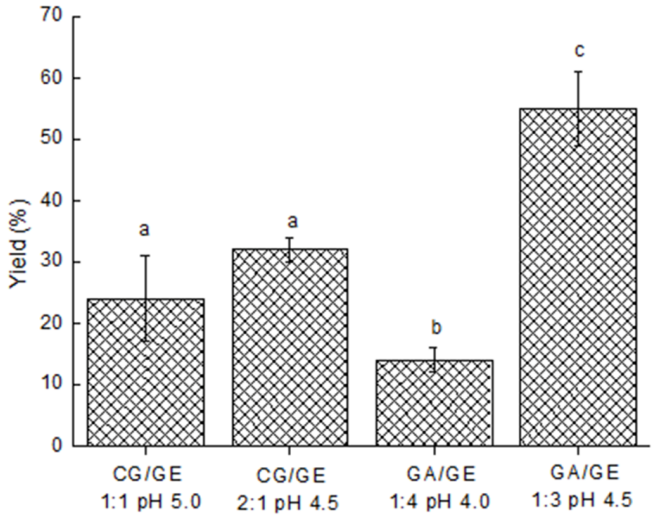

Figure 2: Coacervate yields of cashew gum/gelatin (CG/GE) at 1:1 ratio/pH 5.0 and 2:1 ratio/pH 4.5, and gum Arabic/gelatin (GA/GE) at 1:4 ratio/pH 4.0 and 1:3 ratio/pH 4.5 after drying in an oven at 105 ^{o}C. Bars: standard deviations (n = 3)

tionship between the matrix components and the core and coacervate formation, thereby avoiding material waste and ensuring optimal conditions for encapsulation.

Higher yields of CG/GE particles were found at the lower oil levels. According to de Conto, Grosso and Gonçalves (2013), the higher the wall material concentration and the lower the oil concentration, the higher the yield. However, GA/GE had a higher proportion of wall material in the coacervation process, and we obtained a higher yield when a larger quantity of oil was used. Therefore, in the ensuing work, the use of 1g and 2.5g of pequi oil was selected, respectively, for the treatments of 2:1 CG/GE at pH 4.5 and 1:3 GA/GE at pH 4.5. The 2:1 GC/GE and 1:3 GA/GE particles had a moisture content of 4.7 ± 0.50% and 5.54 ± 0.16%, respectively. These were the largest quantities of oil among those tested with each treatment that had good yield values. Yield results show that GA/GE matrix was more promising than CG/GE in pequi oil encapsulation. However, cashew gum is a reasonable alternative to gum Arabic especially for use in cashew-producing areas in the world.

The size distribution of the particles obtained by laser diffraction analysis displayed a unimodal distribution for the CG/GE and GA/GE treatments. The average particle size values are shown in Figure 4. The $D_{4,3}$ particles were micrometric in size, with GA/GE (4311.85 ± 1428.32 nm) smaller than CG/GE particles (8216.72 ± 1853.32 nm). The Span value found was 0.45 ± 0.04 and 0.23 ± 0.03 for GA/GE and CG/GE respectively, indicating a small dispersity of the particles. Optical microscopy analysis also confirmed the smaller size of GA/GE particles (Figure 5). It is known that the particle size distribution of emulsion droplets is affected by many factors relating to biopolymers and pH, e.g., the addition rate of the acidifying agent for anionic polysaccharides and the rate of formation of gelatin coacervates (Jyothi et al., 2010). In this work the greater amount of gelatin employed in the GA/GE complex probably promoted a better emulsification of the solution and resulted in smaller particles.

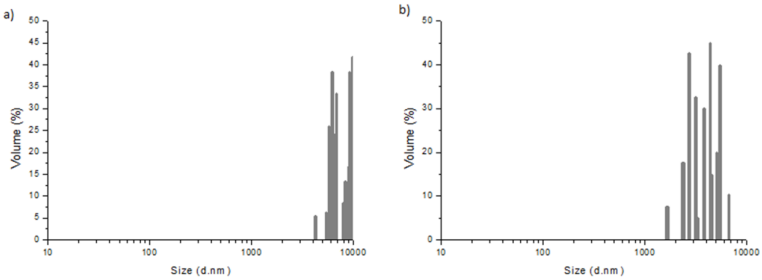

Figure 3: Microparticle yields with 0.5g, 0.75g, 1g, 2g and 2.5g of pequi oil in cashew gum/gelatin matrices (CG/GE) at ratio 2:1/pH 4.5 and gum Arabic/gelatin (GA/GE) at ratio 1:3/pH 4.5 after drying in an oven at 105 ^{o}C. Bars: standard deviations (n = 3)

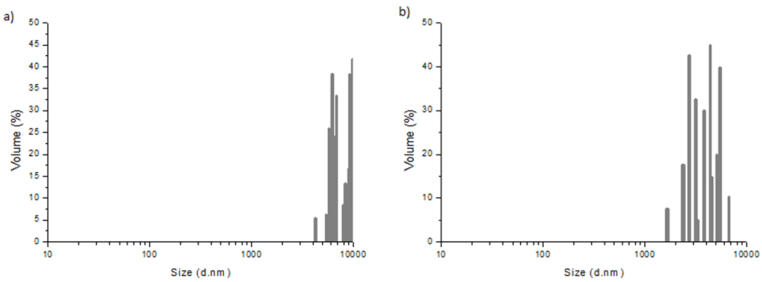

Figure 4: Size of microparticles of (a) cashew gum/gelatin (CG/GE) at ratio 2:1/pH 4.5 with 1g of pequi oil, and (b) gum Arabic/gelatin (GA/GE) at ratio 1:3/pH 4.5 with 2.5g of pequi oil. The measurements were conducted at room temperature.

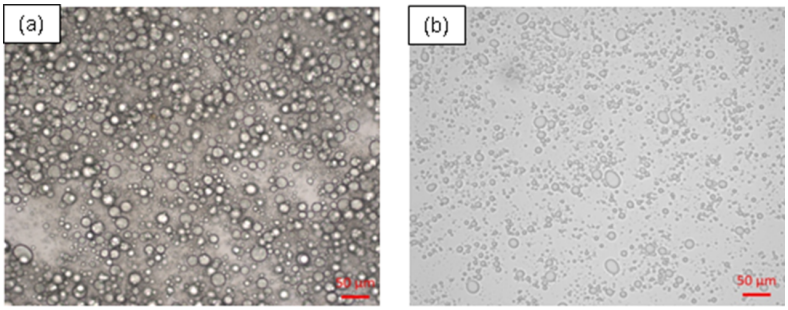

Figure 5: Images obtained by optical microscopy of (a) cashew gum/gelatin (CG/GE) microparticles with 1g of pequi oil, and (b) gum Arabic/gelatin (GA/GE) with 2.5g of pequi oil. The measurements were conducted at room temperature.

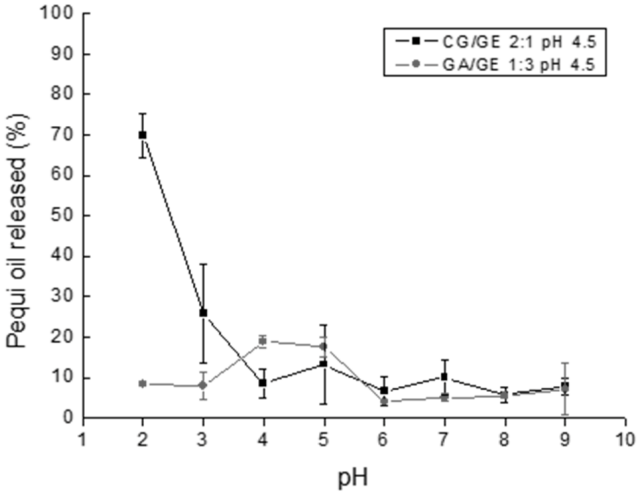

Figure 6: Pequi oil release from cashew gum/gelatin (CG/GE) microparticles at 2:1 ratio/pH 4.5 with 1g of pequi oil, and gum Arabic/gelatin (GA/GE) at 1:3 ratio/pH 4.5 with 2.5g of pequi oil, in the pH range of 2.0 to 9.0 at room temperature. Bars: standard deviations (n = 3)

Encapsulation efficiency

Encapsulation efficiency (EE) reflects the amount of oil trapped inside the particles. GC/GE and GA/GE showed EE of 72.53% (±4.80) of 82.77% (±6.09), respectively. Oil retention within the particles is affected by the encapsulation method and the nature of the biopolymers, as well as the particle wall thickness. Thus, a larger amount of wall material may favour oil retention and consequently optimize the encapsulation efficiency (Tang & Li, 2013). In this work, the experimental parameters for polysaccharide-protein complexation were experimentally determined that aimed at the best conditions for coacervate formation, and therefore showed different proportions that also resulted in statistically different EE ($p < 5\%$). Similar EE are found in literature using gum Arabic and gelatin for oil encapsulation, although there are different sources, suppliers and viscoelastic properties of gelatin. Liu, Low and T. Nickerson (2010) reported flaxseed oil EE of 84% using type-A gelatin from porcine skin and gum Arabic 1:1 at a constant total biopolymer concentration (2% w/v) and core-to-wall ratio (50:50). Gonçalves, Grosso, Rabelo, Hubinger and Prata (2018) worked with type-B gelatin from bovine skin and gum Arabic 1:1 for thyme essential oil encapsulation and found EE of 90%. Marfil, Paulo, Alvim and Nicoletti (2018) foundan EE of 83.5% for palm oil encapsulation using a 2% concentration of wall material (gelatin type-B from bovine skin and gum Arabic) and 42.8 : 57:2 ratio between core and wall material.

Oil release

The release of the core material is facilitated by certain conditions, such as pH alteration, mechanical stress, temperature, time and osmotic force, among others. This kind of study may be useful for future commercial applications in order to avoid unwanted release and thus contribute to preserving the integrity of the active compound. In this study we evaluated the oil release in the pH range from 2 to 9.

The 2:1 CG/GE treatment presented a high oil release with acidic pH, practically reaching a re-

lease of 70% at pH 2.0. This release decreased until pH reached 4.0, and then showed slight oscillations until pH reached 9.0. It can be affirmed that the pH range in which these microparticles released the most oil was between 2.0 and 3.0. This behaviour is visualized in Figure 6. Since the complex coacervation process is strongly influenced by pH variations, it is likely that in more acidic pH ranges, in which there should be a large CG/GE oil release, the electrostatic interactions that depend on the degree of biopolymer ionization have been undone, leading to a greater oil release. On the other hand, 1:3 GA/GE treatment exhibited low release in the pH range evaluated in this study (<20%). According to Shaddel et al. (2018) the best potential food items for enrichment purposes using gum Arabic and gelatin wall material and black raspberry core seem to be the ones with the pH values between 3.0 and 5.0. Siow and Ong (2013) evaluated garlic oil release from gelatin and gum Arabic particles and found about 90% release during incubation at 37 ^{o}C in pepsin solution (pH 2) for 3h. The same authors demonstrated that the oil release from microparticles is slower in cross-linked particles.

4 Conclusion

Pequi oil was successfully incorporated during the formation of CG/GE coacervates. Food applications for these microparticles involving a pH above 3 are recommended because of the greater oil release at acidic pH. It is clear that cashew gum has great potential as an encapsulation matrix, although it is possible to encapsulate a larger amount of pequi oil using GA/GE. Since cashew gum is a by-product of the cashew industry and currently has little commercial value, the possibility of using cashew gum for encapsulation should be a welcome development for the industry.

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Evaluation of Gum Arabic from *Acacia senegal* var *kerensis* and *Acacia senegal* var *senegal* as a Stabilizer in Low-fat Yoghurt

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Abstract

Gum arabic is a dried, gummy exudate obtained from the stems and branches of *Acacia senegal* and *Acacia seyal*. In Kenya, gum arabic comes from *Acacia senegal* var *kerensis* although its exploitation for commercial and industrial application is marginal. Therefore, the aim of this study was to characterize and determine the effect of the gum from *A. senegal* var *kerensis* on the quality characteristics of set low-fat yoghurt compared to gum arabic from *A. senegal* var *senegal*, with a view to increasing its utilization locally. Yoghurt was prepared containing gum arabic at four concentrations (0.2%, 0.4%, 0.6%, 0.8% gum w/v). Results showed that *A. senegal* var *kerensis* gum had higher molecular weight and gelling properties compared to *A. senegal* var *senegal* gum. In addition, *A. senegal* var *kerensis* gum was less susceptible to syneresis and showed a higher absolute viscosity compared to *A. senegal* var *senegal* gum at all concentration levels. Sensory evaluation revealed that addition of gum arabic significantly improved the body and the texture of the yoghurt. Therefore, *A. senegal* var *kerensis* gum is a better yoghurt stabilizer than gum arabic from *A. senegal* var *senegal*. An optimal gum concentration of 0.6% of *A. senegal* var *kerensis* gum in low-fat yoghurt is recommended from the results of this study.

Keywords: Gum arabic; Low-Fat Yoghurt; Stabilizer; Syneresis; Gum exudate

1 Introduction

Many health organizations consider the level of fat consumption to be too high. A recent World Health Organization (WHO) report recommended that the level of total fat intake should be between 15% and 30% of energy, of which saturated fatty acids should account for less than 10% since fat has been associated with an increased risk of obesity, arteriosclerosis, coronary heart disease, elevated blood pressure, tissue injury diseases associated with lipid oxidation and certain forms of cancer (Kaminarides, Stamou & Massouras, 2007). Thus, the goal of the food industry is to respond to consumer demand and to offer an increasing variety of low-fat choices, in which the attributes that consumers desire are not impaired. A reduction in fat content can be achieved by replacing it with several ingredients that provide the functionality of the missing fat. Hydrocolloids and carbohydrate-based fat replacers have been used safely as thickeners and stabilizers especially in dairy products, sauces and dressing formulations. Gum arabic (GA, E-Number 414) is an edible, dried, gummy

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exudate from the stem and branches of *A. senegal* and *Acacia seyal* that is rich in non-viscous soluble fiber (Williams & Phillips, 2009). It is defined by the Joint FAO/WHO Expert Committee for Food Additives (JECFA) as a dried exudate obtained from the stems and branches of *A. senegal* (L.) Willdenow or *Acacia seyal* (fam. *Leguminosae*) (FAO, 1999). Physically, it is a pale white to orange–brown solid which breaks with a glassy fracture. Chemically, gum arabic (GA) consists mainly of high molecular weight polysaccharide and their calcium, magnesium and potassium salts, which on hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid (FAO, 1999). The backbone is composed of 1, 3-linked β-D-galactopyranosyl units. The side chains are composed of two to five 1, 3-linked β-D-galactopyranosyl units, joined to the main chain by 1, 6-linkages (FAO, 1999). Gum arabic has wide industrial uses as an emulsifier, stabilizer and thickening agent mainly in the food industry. These properties have been exploited for their functionality in food systems including textural attributes and mouth feel. There are two forms available commercially, namely *A. senegal* var *senegal* and *A. senegal* var *kerensis*. Both are acceptable as food additives and conform to the specification now approved by the FAO Joint Expert Committee on Food Additives and the Codex Alimenarius Commission (FAO Food and Nutrition Paper 52 Add.7 1999). *A. senegal* var *senegal* gum (standard type), produced in Sudan and other gum-producing regions of Africa, for example Nigeria and Niger, is significantly different from *A. senegal* var karensis gum that is produced in Kenya. The *A. senegal* var *kerensis* gum has high specific rotation, high nitrogen content and a high molecular weight compared to the *A. senegal* var *senegal* gum (Al-Assaf, Phillips & Williams, 2005). There are few reports on the research that assessed the qualities of *A. senegal* var *kerensis* gum for its commercial and industrial application in yoghurt processing.

Yoghurt producers are motivated to market low-fat products with natural ingredients in order to capture a niche market that continues to grow. In addition, producers have added gum arabic as a prebiotic in yoghurt production (Niamah, Al-sahlany & Al-Manhel, 2016). However, research has shown that reduced fat yoghurt exhibits lower tension and firmness than full fat yoghurt. The partial or total removal of fat from yoghurt decreases the overall quality perceived by the consumer (Folkenberg & Martens, 2003). This is for two main reasons: a change in the texture of the product and a change in the retention of flavor compounds (Nongonierma, Springett, Le Quéré, Cayot & Voilley, 2006). The change in texture perception results from a modification of the structure of the gels (Kilcast & Clegg, 2002). Fat globules of homogenized milk are part of the gel network. To modify texture perception, fat substitutes or bodying agents are commonly added (Sandoval-Castilla, Lobato-Calleros, Aguirre-Mandujano & Vernon-Carter, 2004). Some of the additives that have been used include starch and skimmed milk powder. The need to consume low-fat foods has created increased consumer awareness and a dramatic increase in the supply of, and demand for, low-fat foods containing fibers. Gum arabic which is known to possess special emulsifying and stabilizing properties has not been evaluated vis-à-vis low-fat yoghurt stabilization. Thus, the aim of the present study was to determine its effect on the rheological properties of set low-fat yoghurt (EAS, 2006) with a view to increasing its utilization in Kenya.

2 Materials and Methods

2.1 Materials

Gum arabic from *A. senegal* var *kerensis* and *A. senegal* var *senegal* were obtained from Kenya Forestry Research Institute Laboratories (KEFRI) and used without further purification. Unpasteurized skimmed milk was obtained from a local supplier and used to make low-fat yoghurt the same day.

2.2 Yoghurt preparation

The skimmed milk (0.5% Fat) was heated to 85 ^{o}C for 20 min, stabilizer (0.2%, 0.4%, 0.6%, 0.8% gum w/v) was added and the mixture heated for a further 10 min at 85 ^{o}C. Yoghurt manufacture was adapted from the standard technique (Kosikowski, 2019). The mixture of milk and ad-

ded stabilizer was cooled to 45 ^{o}C and a *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (direct vat set culture (1 x 10^{10} cfu per gram)) (YF-L811, Chr. Hansen, Hamilton, New Zealand) was mixed into the milk and allowed to ferment for 3h at 42 ^{o}C in autoclaved glass jars. The warm yoghurt was then kept at 4 ^{o}C for cooling before the various analyses of physicochemical properties of yoghurt were performed after one day. Control yoghurt sample was manufactured following the same standard technique (Kosikowski, 2019) without the addition of a gum arabic.

2.3 Determination of the physicochemical and molecular characteristics of gum arabic

The physicochemical properties were obtained and molecular parameters of gum arabic measured using gel permeation chromatography online coupled with multi-angle laser light scattering system (GPC-MALLS). A Superose 6 10/300GL GPC column and a DAWN EOS multi-angle light scattering detector (Wyatt Technology Corporation, USA) were employed in the GPC-MALLS measurements at 25 ^{o}C. Aqueous sodium chloride solutions (0.2 M) were used both as a solvent and eluent. This technique is used to determine the molecular distribution of a polymeric system such as hydrogel of hydrocolloids including gum arabic (Al-Assaf et al., 2005; Montoro, de Fátima Medeiros & Alves, 2014). All chemicals used were of analytical grade and were obtained from BDH Chemicals (BDH Ltd, Poole, England) or Sigma Chemical Co. (St. Louis, Mo, USA) unless specified otherwise.

2.4 Analysis of physicochemical characteristics of yoghurt containing gum arabic

Chemical characterization

The following chemical analyses were carried out on the yoghurt, according to AOAC (2005): moisture (g/100 g w/w), ash (g/100 g w/w), total solids (g/100 g w/w), and fat (g/100 g w/w). All analyses were performed in triplicate.

Syneresis of yoghurt

The susceptibility of yoghurt to syneresis was determined using the method by Keogh and O'kennedy (1998). Centrifuge tubes containing 40 g of yoghurt were centrifuged at 222000 g for 10 min at 4 ^{o}C. The clear supernatant was poured off, weighed and expressed as percent weight relative to original weight of yoghurt.

pH value

The pH value of the yoghurt samples was measured at the end of the incubation time. Samples were vigorously stirred to break the formed gel and the pH was obtained using a pH meter (Orion 4 Star pH. ISE Benchtop, Thermo electric cooperation).

Acidity

Titratable acidity, expressed as percentage of lactic acid, was determined following FAO (1996) by mixing 10 g of yogurt with 20 mL of distilled water and titrating with 0.1N NaOH using phenolphthalein as indicator. Titratable acidity was then calculated as shown in equation 1:

$$TA = \frac{\frac{V_T}{1000} \cdot N_{NaOH} \cdot 90}{W_s} \cdot 100 \qquad (1)$$

Where TA is the tritatable acidity, V_T is the titer volume, N_{NaOH} is the normality of NaOH and W_s the weight of the sample.

Viscosity determination

Yoghurts were mixed with a hand blender at low speed for 15 s. This was to break the gel and to mimic the shaking or stirring by the consumer of the packed yoghurt. The apparent viscosity of the stirred yoghurt was measured with a Brookfield digital rotational viscometer (model DV-II+, Brookfield Engineering Laboratories Inc., Middleboro, MA) using a spindle 5 at 100 rpm in 150 mL of yogurt (Damian, 2013). The spindle rotated in the sample for 1 minute at 10 ^{o}C, the indicator stabilized, then the readings were taken.

Gel strength

The cylinder penetration test was performed using a Universal Testing Machine (Zwick Z2.5/TN1S, Zwick, Ulm, Germany) equipped with a 500 N force sensor (Guggisberg, Cuthbert-Steven, Piccinah, Buetikofer & Eberhard, 2009). An acrylic glass cylinder (h¼ 35 mm, Ø¼ 25.4 mm) was introduced vertically into the 150 g yoghurt cup with a constant speed of 30 mm min^{-1} for 40 mm. The software TESTXPERT (V10.1) was used to calculate the modulus of deformability (E modulus) using the secant of the values between 0.5 and 1.0 mm and the force at 35 mm (F (35 mm)). The penetration force was read directly from the machine. All yoghurts were measured at 10 ± 1 ^{o}C. The mean of two yoghurts from the same batch was calculated.

Rheological determination (Oscillatory test) of yoghurt

Rheological properties of yoghurt samples were investigated using a controlled stress rheometer (AR-550 TA Instruments, USA) as described by Karazhiyan et al. (2011). About 3.8 mL of sample were carefully placed in the measuring system and left to rest for about 10 minutes at 5 ^{o}C. Measurements were carried out on shear mode at 5 ^{o}C, using a cone and plate geometry. A shear rate sweep test was used with the shear rate ranging from 10^{-2} to 20^{-1} s. A frequency sweep test was also performed (with the frequency ranging from 1 to 10 Hz at a maximum strain of 4.06E-03, and amplitude of 1.42E-04). Because gels are viscoelastic materials, dynamic rheological tests to evaluate properties of gel systems are well suited for studying the characteristics of gels as well as gelation and melting (Walstra, Walstra, Wouters & Geurts, 2005). From dynamic rheological tests in the linear viscoelastic range, the storage modulus, G', and the loss modulus G", can be obtained. The G' value is a measure of the deformation energy stored in the sample during the shear process, representing the elastic behavior of a sample. In contrast, the G" value is a measure of the deformation energy used up in the sample during the shear and lost to the sample afterwards, representing the viscous behavior of a sample (Mezger, 2002). If the value G' is much greater than the G" value, the material will behave more like a solid; that is, the deformations will be essentially elastic to recoverable. However, if G" is much greater than G', the energy used to deform the material is dissipated viscously and the material behavior is liquid-like. These parameters represent the mouth feel from a consumer perspective.

Sensory evaluation of yoghurt containing gum arabic

Descriptive sensory analysis was performed following Meilgaard, Carr and Civille (1999) under normal light. The samples were placed in clear plastic cups. A panel consisting of seven semi-trained panelists was used for the evaluation. Three training sessions were held prior to testing using low-fat and full-fat yoghurt. In these sessions, the panelists were trained in the products and descriptors were chosen based on consensus among panelists, using low-fat products available on the market to cover a range of consistencies. A total of seven descriptors were used for the assessment of product appearance, texture, taste and overall acceptability. Test samples, identified by a three-digit code, were presented to the panelists in a randomized order immediately after being removed from the fridge (4 ^{o}C). Testing was conducted on duplicate samples, and each panelist was asked to assess them for each attribute on a nine-point scale.

2.5 Statistical analysis

The experiment was repeated twice (Trial 1 and Trial 2) in triplicate each time. Statistical analysis was performed using JMP Software. One-way analysis of variance (ANOVA) was done and mean comparison achieved using the Duncan's multiple range test at 95% confidence interval

(Sall, Stephens, Lehman & Loring, 2017).

3 Results and Discussions

3.1 Physicochemical and molecular characteristics of gum arabic

The results of the physicochemical and molecular testing of gum arabic are shown in table 1. The moisture content was 14.5% and 13.0% while the ash content was 3.6 % and 3.2 % for *A. senegal* var *kerensis* and *A. senegal* var *senegal* gum, respectively. The protein content of gum arabic from variety *kerensis* was higher than that of variety *Senegal*. In addition, both intrinsic and absolute viscosities were higher in the gum arabic from variety *kerensis*. This may be explained by the high molecular weight reported for variety *kerensis* compared to variety senegal. These results agree with Al-Assaf et al. (2005). According to these researchers, one of the major differences of the *A. senegal* var *kerensis* gum from Kenya is that it has high specific rotation, high nitrogen content and a high molecular weight compared to the *A. senegal* var *senegal* gum.

3.2 Moisture loss

The results of moisture loss in yoghurt containing gum arabic are shown in Tables 2 and 3. Moisture content was significantly reduced with addition of gum arabic from both varieties. Similar results were reported when gum arabic was added in *kobe*, a traditional fermented milk from Sudan (Hamad, Sulieman & Salih, 2013). In addition, Niamah et al. (2016) reported a slight decrease in moisture content of yoghurt when gum arabic was added up to a level of 1%. The moisture loss of the yoghurt stabilized with *A. senegal* var *kerensis* gum was significantly different ($P < 0.05$) compared to the yoghurt stabilized with *A. senegal* var *senegal* gum at all levels of gum concentration. Gum arabic from *A. senegal* var *kerensis* has been shown to retain higher moisture content in food products (Mwove, A. Gogo, N. Chikamai, Omwamba & M. Mahungu, 2016, 2018). This can be explained by the high protein content of *A. senegal* var *kerensis* gum which is much higher than that of *A. senegal* var *senegal* gum. Senthil, Ravi, Bhat and Seethalakshmi (2002), reported that protein has a high water-binding capacity.

The analysis of variance results of the physicochemical analysis of all the experimental yoghurts (1 day after preparation) are shown in Tables 2 and 3. The low-fat yoghurt stabilized with 0.8% *A. senegal* var *kerensis* gum had the highest total solids content while the control low-fat yoghurt had the lowest. Total solid increased as the level of gum arabic concentration increased. Mehanna, Ibrahim and El-Nawasany (2013), Obodoechi (2015) and Mahjoub (2016) reported an increase in total solids when gum arabic was added as a stabilizer in low-fat yoghurt. The ash content between yoghurt stabilized and the control was significantly different ($P < 0.05$). Similar results were observed when gum arabic was used in making Robe, a traditionally fermented milk product in Sudan (Hamad et al., 2013). In this research, addition of gum arabic at 5%, 7.5% and 10% significantly increased the ash content of the resulting product. It is evident that gum arabic did not affect the fat content. However, research involving higher levels of gum arabic, 1 – 4% have been found to reduce the fat content of yoghurt (Meso et al., 2013).

3.3 pH value and acidity

As shown in Tables 2 and 3, the pH and acidity values for the entire yoghurt samples did not show any significant difference from the control. The pH ranged from 4.32 to 4.41 and titratable acidity ranged from 1.12 to 1.38% lactic acid. No significant differences were noted between samples at different levels of both stabilizers. Results from this study indicate that the addition of gum arabic at different concentrations does not affect the pH or the titratable acidity of the low-fat yoghurts. Similar observations were reported when inulin, a plant extract was used (Guven, Yasar, Karaca & Hayaloglu, 2005) as a fat replacer. Other studies also reported that the pH of plain set yoghurt was not influenced by the incorporation of six different dietary fibers (Bayarri, Chulia & Costell, 2010).

Table 1: Physico-chemical characteristics of *A. senegal* var *kerensis* gum and *A. senegal* var *senegal* gum

Characteristic	*A. senegal var kerensis*	*A. senegal var senegal*	JECFA standards
Moisture content	14.5 %	13.0%	<15%
Ash content	3.6 %	3.2 %	<4%
Nitrogen content	0.68	0.38	-
Protein content (N x 6.63)	3.42	2.01	-
pH -1%	4.54	4.31	-
Viscosity- Intrinsic viscosity	27 ml/g	17.5 ml/g	-
Viscosity – Absolute viscosity	170 mPas	71.6 mPas	-
Optical rotation	-34.5	-28	-26 to -34
Gel determination	Moderate gel	Light gel	
Tannin Content	-	-	-
Equivalent weight	906	1150	-
Molecular weight	$1.19X10^6$	$5.99X10^5$	

Table 2: Physico-chemical properties of yoghurt stabilized with *A. senegal* var *kerensis* gum

	Trial 1*					Trial 2*				
	Control	0.2	0.4	0.6	0.8	Control	0.2	0.4	0.6	0.8
Moisture loss (%)	88.2a	84.6b	84.7b	84.0b	84.5b	87.6a	82.7b	82.4b	84.8ab	82.5b
Ash content (g/100g)	0.89d	2.14c	2.22bc	2.30b	2.41a	0.82e	2.14d	2.23c	2.34b	2.43a
Fat content (g/100g)	0.50b	0.53a	0.51ab	0.50b	0.51ab	0.51a	0.50a	0.54a	0.53a	0.53a
Total Solid (g/100g)	10.30e	11.68d	12.48c	14.87b	18.21a	10.50e	11.35d	12.56c	14.69b	17.67a
pH value	4.37a	4.33a	4.36a	4.37a	4.34a	4.38a	4.35a	4.33a	4.34a	4.35a
Acidity	1.16b	1.22a	1.22a	1.70b	1.23a	1.16a	1.12a	1.15a	1.14a	1.15a
Syneresis	68.0a	54.0b	50.0c	45.2d	42.0e	70.0a	54.7b	51.2c	48.2d	44.1e
Viscosity	870.0e	1351.6d	1381.7c	1455b	1526.7a	890.0e	1288.3d	1337.7c	1394.3b	1476.7a
Gel strength	125.5e	144.8d	154.1c	167.1b	187.7a	120.0e	131.9d	139.3c	145.2b	153.4a

a – e Means followed by the same letters are not significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$
*Means separation carried out separately for each trial.

Table 3: Physico-chemical properties of yoghurt stabilized with *A. senegal* var *kerensis* gum

	Trial 1*					Trial 2*				
	Control	0.2	0.4	0.6	0.8	Control	0.2	0.4	0.6	0.8
Moisture loss (% MC)	88.20a	87.60b	86.32b	87.45b	87.02b	87.45a	86.68b	85.42b	85.45b	85.67b
Ash content (g/100g)	0.89e	2.15d	2.24c	2.29b	2.38a	0.82d	1.18c	1.28b	1.32b	1.43a
Fat content (g/100g)	0.50a	0.54a	0.54a	0.50a	0.50a	0.50a	0.52a	0.55a	0.52a	0.52a
Total Solid (g/100g)	10.3d	10.2d	11.7c	13.5b	16.4a	10.5d	10.4d	12.0c	13.9b	17.6a
pH value	4.37a	4.32ab	4.32ab	4.33ab	4.30b	4.35a	4.39a	4.41a	4.37a	4.40a
Acidity	1.16a	1.15a	1.14a	1.17a	1.38a	1.12a	1.16a	1.16a	1.17a	1.34a
Syneresis	68.0a	56.3b	52.3c	49.7cd	47.0d	70.0a	56.3b	52.3bc	49.0cd	45.3d
Viscosity	870.0e	1176.7d	1208.3c	1231.7b	1258.3a	890.0d	1175.0c	1210.0b	1228.3ab	1251.7a
Gel strength	125.5e	127.1d	129.6c	133.2b	135.7a	120.0e	126.1d	129.0c	131.5b	135.0a

a – e Means followed by the same letters are not significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$
*Means separation carried out separately for each trial.

3.4 Syneresis index

The amount of syneresis in the control was significantly greater ($P < 0.05$) than the amount of syneresis in the treatments with both gum stabilizers used, as shown in Tables 2 and 3. The most important causes for syneresis in fermented products include the use of high temperatures for incubation, low solids content or inadequate storage temperatures (Lucey, 2001). Syneresis is for the most part due to a rearrangement of the network, leading to an increase in the number of particle-particle junctions. The network then tends to shrink, leading to whey separation (appearance of whey on the gel surface of set yoghurt). Although total solids were kept constant for both stabilizers, the yoghurt made from *A. senegal* var *kerensis* gum was less susceptible to syneresis and showed a significantly ($P < 0.05$) lower syneresis index compared to *A. senegal* var *senegal* gum at all concentration levels. The syneresis index for the gum-stabilized yoghurt decreased as the concentration level of the gum increased. This low syneresis in the *A. senegal* var *kerensis* gum-stabilized yoghurt can be attributed to the improved water holding capacity by the *A. senegal* var *kerensis* gum (Mwove et al., 2016, 2018). Enrichment of dry matter and / or of protein content are common means of avoiding whey separation in yoghurt (Tamime & Robinson, 1999). It has been shown that there is a relationship between the microstructure of yoghurt and firmness and susceptibility to syneresis. Yoghurts which have a denser structure and lower porosity exhibit more water retention capacity (Puvanenthiran, Williams & Augustin, 2002). It was reported (Staff, 1998) that low-fat yoghurts tend to have a higher degree of syneresis than high-fat yoghurts and this is the reason why stabilizers are added to low-fat yoghurt. The current work shows that the gum arabic from *A. senegal* var *kerensis* forms a better firm microstructure due to its high molecular weight than *A. senegal* var *senegal* gum as shown in reduction of syneresis. The stabilizers make the yoghurt less susceptible to rearrangements within its network, and consequently less susceptible to shrinkage and serum (whey) expulsion (Oh, Anema, Wong, Pinder & Hemar, 2007). Yoghurt is usually prepared from homogenized milk to improve stability. This process coats the increased surface of fat globules with casein, enabling the fat globules to participate as a copolymer with casein to strengthen the gel network and reduce syneresis (Keogh & O'kennedy, 1998). Therefore, it can be concluded that the gum arabic helped in forming protein-coated gum arabic spheres, which reinforced the gel structure by their association with casein micelles of the protein network.

3.5 Viscosity

The current result shows that there was a significant difference ($P < 0.05$) between the control and the yoghurt with added gum arabic (Tables 2 and 3). Significant differences ($P < 0.05$) were noted between samples from gum arabic *A. senegal* var *kerensis* at different levels of the gum concentration with viscosity increasing with increase in gum amounts for both. The higher absolute viscosity reported for low-fat yoghurt stabilized with *A. senegal* var *kerensis* gum than *A. senegal* var *senegal* gum is attributed to the higher molecular weight and gelling properties of the *A. senegal* var *kerensis* gum as compared to *A. senegal* var *senegal* gum. While studying the effect of guar gum and arabic gum on the physicochemical, sensory and flow behavior characteristics of frozen yoghurt, Rezaei, Khomeiri, Kashaninejad and Aalami (2011) found that increasing gum arabic in yoghurt increased the viscosity of resulting product. In addition, similar results were reported by Obodoechi (2015). Since yoghurt is usually prepared from homogenized milk to improve stability, this process coats the increased surface of fat globules with casein, enabling the fat globules to participate as a copolymer with casein to strengthen the gel network (Keogh & O'kennedy, 1998), hence increased viscosity. It has been previously reported that the protein network of low-fat yoghurt was less dense, more open, and with more void spaces than that of full-fat yoghurt. This is due to the smaller, fused casein micelle aggregates, probably due to lower number of fat globules acting as linking protein agents (Sandoval-Castilla et al., 2004). In the present study, the increase in viscosity suggests that the gum arabic participates as co-

polymer with casein. The *A. senegal* var *kerensis* gum is better at enhancing viscosity compared to *A. senegal* var *senegal* gum. The casein micelles in the low-fat yoghurt containing fat replacers form networks with differing structures depending on the chemical nature and functional properties of the fat replacers (Lucey, 2001).

3.6 Gel strength/ Firmness

The gel strength of gum arabic stabilized low-fat yoghurt was evaluated following a back-extrusion test performed on a universal testing machine (Houze, Cases, Colas & Cayot, 2005). The results for the samples containing gum arabic from *A. senegal* var *kerensis* and *A. senegal* var *senegal* gum are presented in Tables 2 and 3. The yoghurt samples stabilized with *A. senegal* var *kerensis* gum and *A. senegal* var *senegal* gum were significantly different ($P < 0.05$) from each other in terms of their gel firmness and also at different concentration level of the stabilizer. The gel strength of the yoghurt increased as the gum amount increased with the highest value record at the highest stabilizer concentration of 0.8% for both gums. This may be due to the increased levels of total solids, high molecular weight and gelling properties of gum arabic from Acacia senagal var *kerensis*, and also potential thermodynamic compatibility between casein and the gum arabic from *A. senegal* var *kerensis*. A high intrinsic viscosity or hydrodynamic molecular volume of the polysaccharide leads to smaller occupied volumes, which contribute to less exclusion of the polysaccharide in mixtures (Keogh & O'kennedy, 1998). This explains the difference in gel strength between *A. senegal* var *kerensis* gum and *A. senegal* var *senegal* gum. Thus, the aggregation of milk proteins, especially casein micelles decreases and consequently, phase separation is reduced. The potential electrostatic bonding between the hydroxyl groups of gum arabic and the positively charged regions on k-casein could have played a role in increasing the gel strength of the yoghurt (Guven et al., 2005). Similar results were reported on incorporation of either beta-glucan or inulin in yoghurt (Guven et al., 2005). The formulation resulted in an increase in product firmness and consistency in comparison with the control samples. The highest firmness and consistency of beta-glucan products was obtained from formulations containing a 2.5% addition level. The texture and the rheological results are in agreement with trends observed for yoghurt syneresis and increased gel strength (G' and firmness).

3.7 Rheological properties (Oscillatory test) of low-fat yoghurt

In the present study, storage (G') and loss (G") modulus values were determined and were found to be dependent on frequency at all concentrations studied (Figures 1- 4). Increasing the gum arabic concentration for both stabilizers up to 0.8% increased the value of both G" and G'. This is due to the increase in carboxylic cross-linking between the stabilizer and the casein micelles which play a dominant role in increasing the G' value of acid gels made from heated milk (Guven et al., 2005). Yoghurt enriched with *A. senegal* var *kerensis* gum at different gum arabic concentrations showed higher G' and G" values than control yoghurt. The same results were recorded for the *A. senegal* var *senegal* gum (Table 4). These values increased as the level of the stabilizer increased. Research has shown that heating milk to above 70 ^{o}C at natural pH predominantly promotes the unfolding of whey proteins and their complex formation with casein micelles involving β-casein (Guven et al., 2005). Gum arabic associates with casein micelles via the formation of intermolecular carboxylic bonds found in the AGP fraction. The binding of gum arabic to the micelle surface induces the formation of bridges between the casein particles and induces a network dominated by casein–AGP fraction interaction at pH 4.6. Gum arabic-arabinogalactan (AGP) fraction aggregates that associate with casein micelles help to crosslink casein particles and increase the number and strength of bonds between protein particles. This explains the rise of both G' and G" as the concentration of the gum is increased as shown in Table 4. The high G' and G" recorded for *A. senegal* var *kerensis* compared to *A. senegal* var *senegal* is due to the high molecular weight associated to the

Table 4: Physico-chemical properties of yoghurt stabilized with *A. senegal* var *kerensis* gum

		Control	0.2%	0.4%	0.6%	0.8%
A. senegal var kerensis	G'	99.92e	145.92d	191.92c	232.92b	262.92a
	G"	25.27e	26.27d	27.57c	28.57b	29.57a
A. senegal var senegal	G'	99.92e	139.79d	179.87c	221.80b	250.90a
	G"	25.27e	24.65cd	25.87bc	26.98ab	28.24a

a – e Means followed by the same letters are not significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$
*Means separation carried out separately for each trial.

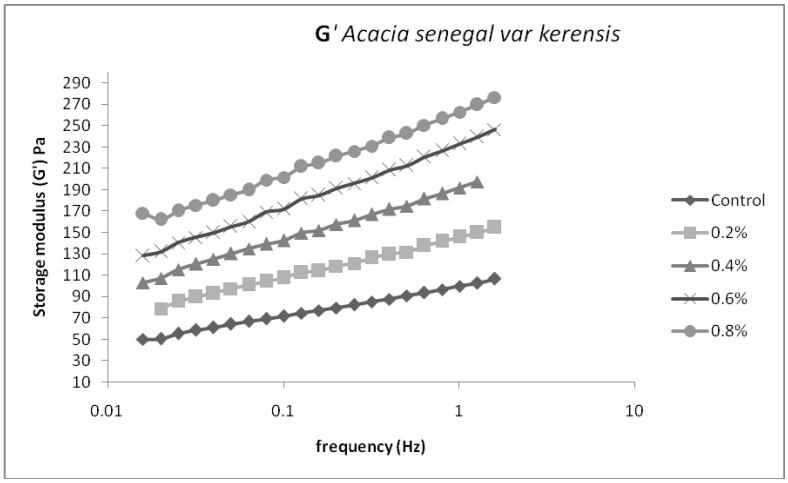

Figure 1: G' *A. senegal* var *kerensis* stabilized for low-fat yoghurt

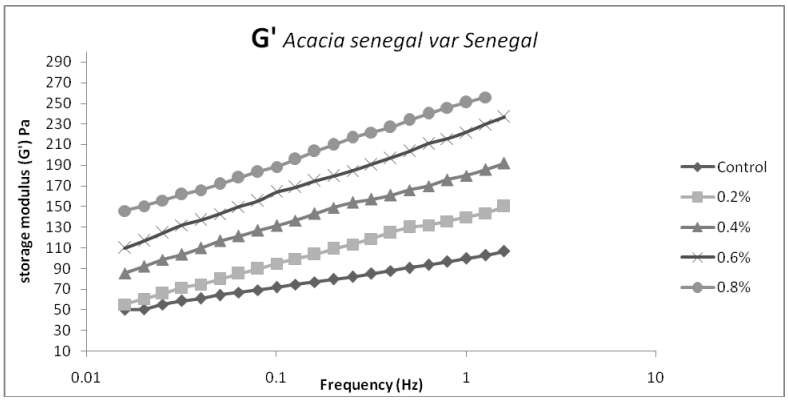

Figure 2: G' *A. senegal* var *senegal* stabilized for low-fat yoghurt

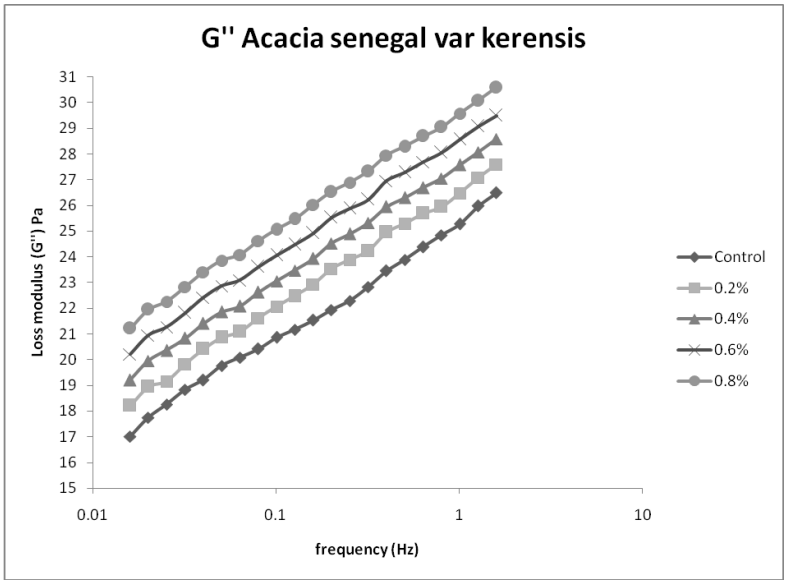

Figure 3: G" *A. senegal* var *kerensis* stabilized for low-fat yoghurt

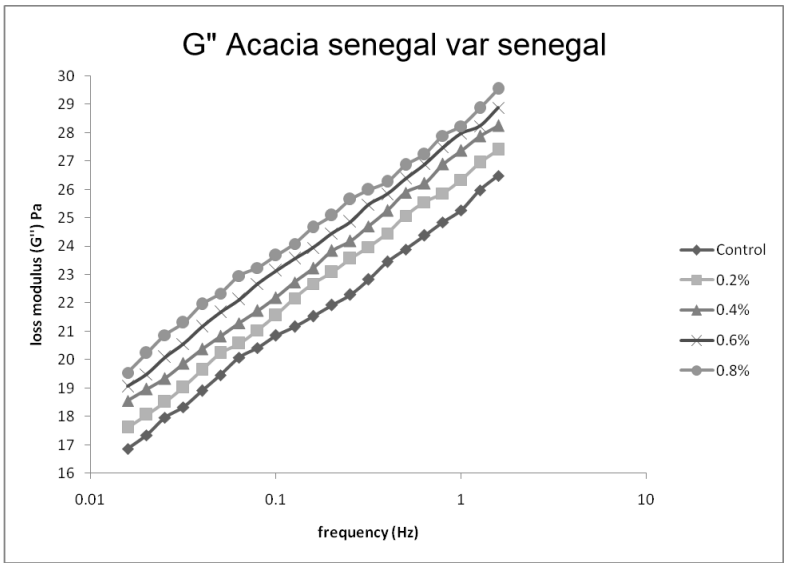

Figure 4: G" *A. senegal* var *senegal* stabilized for low-fat yoghurt

AGP content and the gelling property of the *A. senegal* var *kerensis* gum. High levels of hydrocolloids have been reported to increase whey protein self-aggregation (Mezger, 2002) and formation of a protein matrix with dominant whey protein aggregates. Most hydrocolloids are generally carboxylated or sulphated. Gum arabic carries carboxylic groups (Mezger, 2002) and is also an anionic polysaccharide, which can adsorb onto casein micelles during acidification. An adsorbing polymer, depending on its concentration, can lead to a colloidal system through the whole series of no influence – bridging – polymeric stabilization – depletion – destabilization (Syrbe, Bauer & Klostermeyer, 1998). If the amount of polymer is not large enough to completely cover the protein, a polysaccharide may be adsorbed onto more than one protein surface, thereby bridging two or more protein particles. However, flocculation becomes more and more effective up to about half of the saturation surface coverage (Mezger, 2002).

3.8 Sensory evaluation

Texture properties can often be assessed with instruments, but this is insufficient in characterizing the product. Many consumers use the sensory properties of foods to judge freshness and quality of a product (Kealy, 2006). Sensory properties including flavor, mouth feel and color can be evaluated by trained or untrained panelists (Kuenzel, Zandstra, El Deredy, Blanchette & Thomas, 2011). Consumer testing could provide the most meaningful and reliable information on the textural quality and acceptability of yoghurt (Jaworska, Szulinska, Wilk & Anuszewska, 2005). In the present study, panel testing procedures were carried out. Sensory analyses on appearance, texture, taste, body and overall acceptance of the *A. senegal* gum stabilized low- fat yoghurt as well as control samples were evaluated by 7 trained panelists using a 9-point hedonic scale (Kuenzel et al., 2011). Panelists were asked to score sample attributes from extremely like (9) to extremely dislike (1). Thus the highest numbers represented more desirable, and the lowest less desirable traits. The analysis of variance results are presented in Table 5 for *A. senegal* var *kerensis* and *A. senegal* var senegal. The control skim milk yoghurt had the lowest scores in all aspects except in appearance and taste. Both gum arabic from *A. senegal* var *kerensis* and *A. senegal* var *senegal* had no effect on the appearance/ color of the low-fat yoghurt as the gum content was increased. Gum arabic from the initial characterization was found to be tasteless and odorless thus it did not cause significant difference in the low-fat yoghurt. Similar results were reported by Akhtar and Dickinson (2007) and Yadav, Igartuburu, Yan and Nothnagel (2007) where gum arabic did not have any effect on taste and appearance of the beverage prepared.

Addition of gum arabic to skim milk yoghurt improved the texture and body of the yoghurt and the acceptability rating changed significantly ($P < 0.05$). Similar results were found when gum arabic was added to frozen yoghurt showing an increase in acceptability with increase in gum level up to a level of 0.5% (Rezaei et al., 2011). In addition, Moeenfard and Tehrani (2008) and Rezaei et al. (2011) reported an improvement in texture when stabilizers are used. However, Mahjoub (2016) reported a decrease in color, flavor, taste and overall acceptability when gum arabic and baobab were added up to 0.3% in yoghurt. The texture of the low-fat yoghurt increased as the level of concentration of gum arabic (both gums) increased. The results show that the panel preferred the yoghurt stabilized with *A. senegal* var *kerensis* gum to *A. senegal* var *senegal* gum. This was due to the high molecular weight and gelling property of *A. senegal* var *kerensis* gum leading to a better mouth feel. These results suggest that gel strength correlated with consumer acceptance. These findings are similar to earlier results suggesting a positive correlation between acceptance and gel strength of yoghurt (Frost & Janhoj, 2007).

The body of the yoghurt increased as the concentration of the gum arabic was increased (Table 5). These results correlate with the results from gel strength and rheological properties which showed that the G', and G" was highest in the 0.8% concentration level of gum arabic. Samples containing 0.4 and 0.6 % *A. senegal* var *kerensis* were also regarded as smooth. The low-fat yogurt containing 0.8% of *A. senegal* var *keren-*

Table 5: Sensory analysis results for low- fat yoghurt stabilized with *A. senegal* var *kerensis* and *A. senegal* var *senegal* gum

		Trial 1					Trial 2				
		Control	0.2	0.4	0.6	0.8	Control	0.2	0.4	0.6	0.8
A. senegal var kerensis	Appearance	7.5b	7.2b	7.2b	8.4a	8.4a	7.4b	7.2b	7.3b	8.3a	8.1a
	Texture	3.8d	5.5c	7.0b	8.0ab	8.8a	3.8d	5.5c	6.7b	8.2a	8.7a
	Taste	8.9a	7.0b	6.0c	7.0b	5.8d	8.9a	7.0b	6.0c	7.0b	5.8a
	Body	3.8d	5.5c	6.6b	8.2a	8.6a	3.8d	5.5c	7.0b	8.0ab	8.8a
	Overall acceptance	4.8c	6.0bc	6.6b	8.3a	6.6b	4.7c	5.8bc	6.8b	8.0a	6.5b
A. senegal var senegal	Appearance	7.5b	6.6b	7.2b	8.4a	8.4a	7.4b	7.2b	7.3b	8.3a	8.1a
	Texture	3.8d	5.0c	6.5b	7.0ab	7.4a	3.8d	4.8c	5.2b	6.8a	7.5a
	Taste	8.9a	6.8b	6.0c	7.0b	5.6d	8.9a	7.0b	6.2c	6.8b	5.6a
	Body	3.8d	4.0c	5.5b	6.0a	7.0a	3.8d	3.8c	5.6b	6.4ab	7.2a
	Overall acceptance	4.8c	5.0bc	6.0b	7.0a	6.0b	4.7c	5.2bc	5.8b	6.5a	6.0b

a – e Means followed by the same letters are not significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$

*Means separation carried out separately for each trial.

sis was said to have a slimy texture and some panelist described it as too smooth, which was not profound. Some authors have indicated that smoothness is a highly desirable sensory characteristic in food emulsions such as dairy products (Bayarri, Carbonell, Barrios & Costell, 2011). Smoothness of dairy products decreases due to increased average size of the fat globules by decreasing the average distance between them and increasing the variation in their size for full-fat yoghurt. Additionally, smoothness can be related to creaminess and thickness (which depends on the viscosity). Both proteins and polysaccharides contribute to the structural and textural properties of yoghurt. The expert panel indicated a preference for yoghurts containing 0.6 % *A. senegal* var *kerensis* after one day of storage.

4 Conclusion

Gum arabic from *A. senegal* var *kerensis* can be used as a stabilizer in low-fat yoghurt formulations and this increases consumer acceptability. The present study demonstrates that stabilization of low-fat yoghurt with *A. senegal* var *kerensis* improves the textural quality of set-style yoghurts. The study showed that *A. senegal* var *kerensis* gum imparts better rheological properties to low-fat yoghurt when used as a stabilizer compared to *A. senegal* var senegal. Gum arabic from *A. senegal* var *kerensis* can be used in low-fat yoghurt to prevent serum separation and to adjust the viscosity. When used at a sufficient level, stabilizers reduced serum separation and increased apparent viscosity. *A. senegal* var *kerensis* gum addition was found to be a better yoghurt stabilizer than gum arabic from *A. senegal* var senegal. The optimal gum concentration in low-fat yoghurt recommended from the results of this study is 0.6% of *A. senegal* var *kerensis* gum.

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Effect of Modified Atmosphere Packaging on Quality of Barhi Dates at Khalal Stage

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Abstract

Barhi Dates are an important food and often consumed and sold in the market during the stage Khalal, when the colour is yellow and their taste is sweet with the disappearance of their astringent taste. During the Khalal stage, these dates become physiologically mature with gives the sweet taste. For this reason, they are sold and consumed in a short period of time before these fruits turn into Rutab, a stage at which they lose that distinguishing characteristic. The high moisture, rapid ripening, and delays in transportation or improper storage conditions quickly result in Rutab stage. Thus The Khalal stage lasts for a short time until the fruits get ripe. In the present study, Barhi Khalals were packaged in air (control) and by two types of modified atmosphere packaging: MAP A (5% O_2+ 20% CO_2 and 75% N_2) and MAP B (40% O_2+ 20% CO_2 and 40% N_2). Afterwards, all samples were stored at 5^oC for 30 days. On days zero, 10, 20 and 30 of storage, the fruits were evaluated in terms of the changes in thequality indices ofweight loss, colour, Total Soluble Solids (TSS), and firmness of the fruits and sensory features. The results showed that the minimum weight loss was 0.45% in modified atmosphere packaging, especially with MAP A and the minimum increase in the TSS was 37.35 $Brix^o$ after 30 days of the storage. On the other hand, the results for firmness, colour, and sensory evaluation were better with control packaging.

Keywords: Dates; Barhi; Khalal; Packaging; Quality

1 Introduction

The palm (*Phoenix dactylifera L.*) is one of the most successful fruiting trees in several arid and semi-arid regions of the world. It is considered an important subsistence crop (Awad, 2007). Date fruits are the main source of staple food in arid and semi-arid regions of North Africa, Middle East and South-Asian countries. They have always played an important role in the economic and social lives of people of this area (Hasnaoui et al., 2010). Iran has an annual production of more than 1,000,000 tonnes (15% of total world production) and it is considered the second largest producer of dates in the world (Rastegar, Rahemi, Baghizadeh & Gholami, 2012). Dates are rich in certain nutrients and provide a good source of rapid energy, due to their high carbohydrate content (70–80%). Moreover, date fruits contain fat (0.20–0.50%), protein (2.30– 5.60%), dietary fibre (6.40–11.50%), minerals (0.10–916 mg/100 g dry weight), some vitamins (C, B1, B2, B3 and A) with very little or no starch (Al-Shahib & Marshall, 2003). The developmental stages of date fruit are designated by the Arabic terms Hababouk, Kimri, Bisir or Khalal, Rutab and Tamar that represent, respectively, the cell division, cell elongation or the immature green,

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Nomenclature

MAP	Modified Atmosphere Packaging	TSS	Total Soluble Solids

the mature firm full coloured, the soft brown and the hard raisin-like fruit (Awad, Al-Qurashi & Mohamed, 2011a, 2011b).

Dates can be consumed at three stages of their development mainly Khalal, Rutab and Tamar depend on cultivar characteristics, especially level of soluble tannins, climatological conditions and market demand (Glasner, Botes, Zaid & Emmens, 1999; Al-Qurashi & Awad, 2011). Barhi dates, a commercially important mid-season cultivar, are consumed at the mature full yellow coloured stage (Khalal) as a crispy apple-like fruit. However, after harvest and/or during storage, especially at ambient conditions, the fruits rapidly become softer and sweeter (the Rutab stage) and lose much of their market value (Al-Qurashi & Awad, 2011).

One of the primary technical challenges in marketing fresh Barhi fruits at the Khalal stage of maturity is the preservation of quality for the longest possible period after harvesting and during the marketing process. The proper packaging system should maintain the optimal storage, transport, and handling throughout the market chain for a specific commodity. Modified Atmosphere Packaging (MAP) has been beneficial in maintaining and extending the shelf life of several types of fresh produce. The MAP techniques rely on a modification of the atmosphere inside a package, achieved by the natural interplay between two processes: the respiration of the product and the transfer of gases through the package (Mahajan, Oliveira, Montanez & Frias, 2007).

Generally, there are very few studies about the packaging of Barhi dates at Khalal stage by modified atmosphere packaging technique, one such being that of Al-Eid et al. (2012). In another study, Baloch, Salem, Baloch and Baloch (2006) examined Dhakki dates equilibrated at water activities of 0.52, 0.58 and 0.75 stored at +10 ^{o}C for 4 months under a controlled atmosphere of nitrogen, oxygen and air. The study indicated that the darkening and titratable acidity had increased, whereas the pH declined gradually during the storage of the dates. The change in the quality of date fruit appeared to be a function of storage atmosphere and water activity. However, Alhamdan and Al-Helal (2008) reported that there was no commercial method available to preserve fruit at the Khalal stage of maturity beyond the few days provided by traditional refrigeration methods. The aim of this research was to study the effects of modified atmosphere packaging of Barhi dates at Khalal stage at 5°C on Total Soluble Solids (TSS), weight loss, colour, firmness and sensory properties after 10, 20, 30 days of storage and compare it to control packaging.

2 Materials and Methods

2.1 Sample Preparation

Barhi dates at Khalal stage were collected from a local farm in Alflahyh city in east-south of Iran. After fruits were transported to the laboratory of food packaging at the College of Agriculture, Ferdowsi University, the dates were washed with potable water, sorted, weighed, prepared, and packaged into 10 fruits per bag (20×30 cm) of three-layer polyethylene (LDPE), with a thickness of 80 microns. Two methods of packaging were used. The first method was control packaging by using plain air. The second method was by MAP, under two mixtures of three gases, MAP A (5% O_2 + 20% CO_2 and 75% N_2) and MAP B (40% O_2 + 20% CO_2 and 40% N_2) by introducing desired gas mixtures to the date samples and sealed using a Henkleman machine

(Model 200A, Henkleman Vacuum Systems, Hertogenbosch, Netherlands). After that, samples were stored in conventional refrigerator at 5°C and relative humidity of 80-85% for 10, 20, and 30 days. Five fruits were selected from each bag for assessment. The assessment included the Total Soluble Solids (TSS), weight loss, colour, firmness and sensory properties with five replicates of one cluster per treatment.

2.2 Weight loss

Weight loss was determined by weighing the content of the packages before and after the storage period using an electronic weighing balance (ML3002.E, Mettler Toledo, Switzerland). Weight loss was expressed as the percentage of loss of weight with respect to the initial weight.

2.3 Colour measurement

Colour measurement was carried out using a Hunterlab ColorFlex EZ Spetrophotometer (Model 45/0. Hunter Lab, Virginia, USA). Measured parameters included the degree of lightness (L), with L values ranging from 0 (black) to 100 (white); the 'a' value range from -100 (greenness) to + 100 (redness) and the 'b' values range from -100 (blueness) to + 100 (yellowness). These evaluations were conducted on three different date fruits in each package per three replicates. In addition, colour assessment was performed prior to treatment and on days 10, 20 and 30 of storage time (Ben Thabet et al., 2009).

2.4 Firmness

Firmness of Barhi Khalal dates was measured by using texture analyzer (Model RS-232, USA) with a cone weight of 102.3 g and a cone angle of 45°. Moreover, the firmness of the samples was expressed as the maximum compression force (N), which was required to rupture the arils. All the tests were conducted at room temperature (25°C) (Manolopoulou, Xanthopoulos, Douros & Lambrinos, 2010).

2.5 Total soluble solids (TSS)

TSS were measured as degrees Brix (%) in the date fruit juice using a refractometer (Atago,Tokyo, Japan), and adjusted with the border of zero between the dark- and light - coloured areas on the dates. Afterwards, two drops of the fruit juice were placed in a lens device, and the amount of TSS was determined (Association of Official Analytical Chemists, 1990).

2.6 Sensory evaluation

The sensory attributes of samples were evaluated at regular intervals in terms of colour, aroma, taste, appearance, texture and overall acceptability by a panel consisting of 10 trained evaluators using a five-point hedonic scale (5: excellent; 4: good; 3: acceptable; limit of marketability; 2: poor and 1: extremely poor) (Larmond, 1977). Samples were randomly drawn from each experimental block, coded and served to the panelists randomly.

Ethics and Consent

The research followed the tenets of the Declaration of Helsinki promulgated in 1964 and was approved by the institutional human experimentation committee or equivalent, and that informed consent was obtained.

Statistical analysis

All data were processed by analysis of variance as a one-factor general linear model procedure (ANOVA) using SPSS (IBM, New York, USA) statistical software. The treatment means were separated using the least significant difference method. Differences at $P \leq 0.05$ were considered as significant.

3 Results and Discussion

3.1 Weight loss

The weight loss throughout the storage time is demonstrated in Fig. 1. The weight loss of Barhi dates which were stored in natural air was 1.02%

after 10 d of the storage, while with MAP A (5%O_2), it was 0.15%, and with MAP B (40%O_2) it was 0.17%. It was observed that if the storage time was increased, the weight loss also increased to 3.52% in the samples which were stored in natural air. Generally, at all the storage times in both the natural air and modified atmosphere packaging, there was a significant reduction in the time that the weight losses remained within acceptable limits. As show in Figure 1, there was a relation between the storage via MAP and the least weight loss in the dates.

This was mainly attributed to the loss of the moisture under air packaging conditions, while MAP-treated dates were enclosed in packages that prevented the loss of excess water content (Al-Eid et al., 2012). In this regard, findings of Al-Yahia (1986) indicated that the weight reduction of Barhi date during the storage is probably due to the loss of water contents. Al-Redhaiman (2004) reported that the weight loss of Barhi dates is an inversely proportional relationship between CO_2 concentration at storing containers and the weight loss percent of the fruit. The highest percent of weight loss of the fruit was observed in control packaging in air, followed by low CO_2 concentrations (5% and 10%, respectively), whereas the lowest percentage of the fruit weight loss was recorded for the fruits that were packed in 20% CO_2 gas mixture.

3.2 Colour

The colour of Barhi dates is light yellow at the Khalal stage and that is usually a major factor for the consumer preference. At the end of the Khalal stage, the intensity of the distinctive colour is increased and the colour of the fruits changes to light brown during the Rutab stage. These changes occur since the enzyme invertase starts to slowly increase during ripening at the end of the Khalal stage and then increases sharply (Hasegawa & Smolensky, 1970; Hasegawa, Smolensky & Maier, 1972)

In this study the changes of the colour are shown in Table 1. On days 10, 20 and 30 of the storage, the control packaging and MAP wassignificantly less yellow and light (L and b) and the change of the colour was variable. On the other hand, packaging by MAP A (5% O_2) had the least significant impact on the colour of the fruits where it was noticeably lighter, when compared with packaging by natural air and MAP B (40% O_2), which changed from yellow/brown to dark brown as shown. As shown in L, b values in Table 1. In addition, a was higher in packaging with air and MAP B (40% O_2) than MAP A with 5% O_2. One of the reasons was the increasing concentration of nitrogen gas which was 75% with MAP A (5% O_2) (Kader & Ben-Yehoshua, 2000). Furthermore, increasing the concentration of CO_2 and decreasing O_2 play an important role in reducing the respiration rate of the fruits. Roy, Anantheswaran and Beelman (1995) observed that the activity of tyrosinase which is responsible for mushroom browning is dependent on O_2 concen¬tration. MAP induced higher L values and lowered the difference between ideal mushroom target and sample compared with those observed by mushrooms being stored in non-MAP (control packages). After 27 days of the storage, all MAP-treated dates had significantly higher b values than the control dates, suggesting that MAP dates were notably more yellow in appearance than the control dates. Finally, the results of this study indicated that MAP A had some potential in reducing the rate of the colour storage in the Barhi dates packed at the Khalal stage of maturity and stored at 5°C.

3.3 Firmness

The firmness of Barhi dates was significantly affected by the time period of the storage and packaging techniques during the storage (Figure 2). After 10, 20 and 30 days of the storage, firmness of all samples was observed to decrease so that the lowest values of MAP B (40% O_2) was 6.11 N after 30 days. On the other hand, the highest fruit firmness values were found in the control packaging at all storage days, because the non-permeable film of bags increased the water vapor inside the bags which caused a decrease in the firmness of the fruits.

This was clearly seen with MAP B (40% O_2) because of the increase in O_2 gas level. Dates at the Khalal stage are different from the rest because they do not ripen fully to reach the Rutap

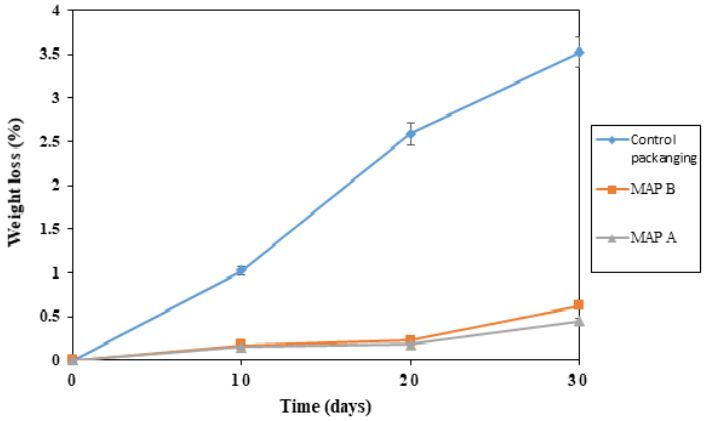

Figure 1: Weight loss for Barhi Khalal dates at 5°C over 10, 20, 30 days of storage

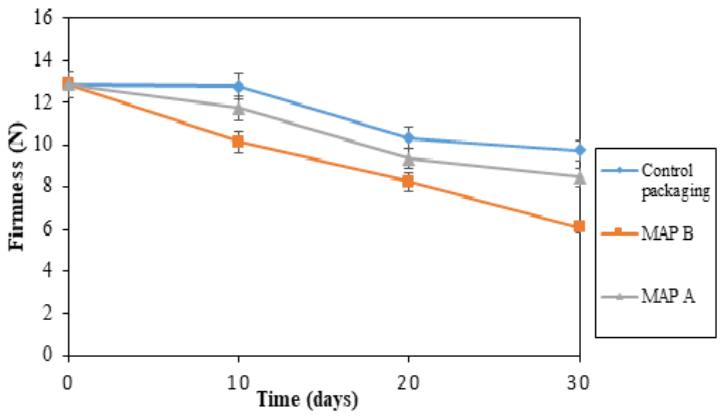

Figure 2: Effects of different atrmosphere packaging on the firmness of Barhi Khalal dates at 5°C over the 10, 20, 30 days of storage

and Tamar stages. So the high level of moisture leads to an increase in ripening Khalah dates. In addition, the ethylene gas produced by respiration of the fruits has an important role in accelerating the ripe of fruits (Jiang & Fu, 2000; Al-Redhaiman, 2004; Saltveit, 1999). Moreover, the data indicated that the firmness of fruits was closely associated with the ripening process during the storage period, as lower firmness of the fruit was observed in the advanced stage of ripening. Al-Jasim and Al-Delaimy (1972) reported that increasing pectin esterase activity during the Khalal stage of ripening dates leads to the breakdown of pectin or softening of the fruits. In this regard, the enzymatic activity of pectin (pectin esterase and polygalacturonate) were the most important factors involved in softening of the fruit firmness. It should be noted that each factor may lead to the maintenance of the fruit firmness so delaying enzymatic activity (Mortazavi, Arzani & Barzegar, 2007).

3.4 Total Soluble Solids (TSS)

The sugars of the date flesh mainly consist of fructose, glucose and sucrose. They are found

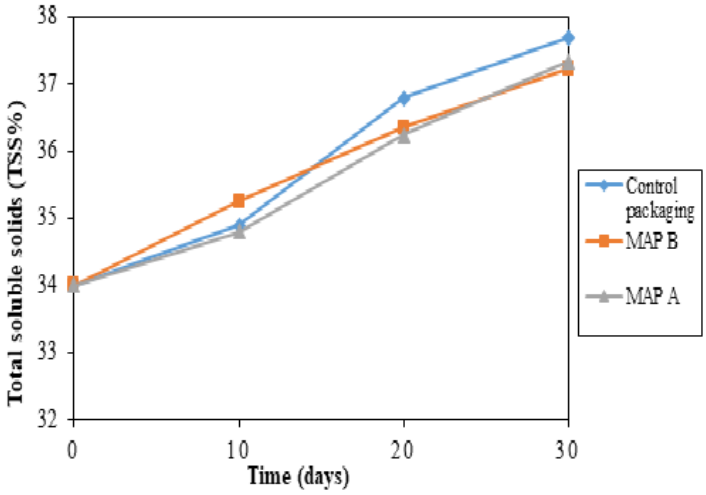

Figure 3: Effects of different atmosphere packag on TSS% of Barhi Khalal dates at 5°C over 10, 20, 30 days of storage

Table 1: Colour changes for Bafhi Khalal dates stored in natural air (control) and modified atmosphere packaging after 30 days

		Colour characteristics		
Treatment	Time	L	a	b
Natural air	10	49.95^a	4.98^c	39.93^a
MAP A	-	49.44^a	5.01^{ac}	39.37^{ah}
MAP B	-	47.87^b	5.23^{ac}	38.89^h
Natural air	20	43.90^h	5.23^{ac}	33.76^c
MAP A	-	42.78^c	5.29^a	32.74^{bc}
MAP B	-	40.41^d	5.66^b	29.88^f
Natural air	30	40.84^d	5.82^{bh}	32.64^b
MAP A	-	39.44^f	6.07^h	27.81^f
MAP B	-	37.54^e	6.40^g	30.41^g
Treatment		**	**	**
Time		**	**	**
Treatment × Time		NS	NS	**

Values (mean of three replicates) in the same column followed by the same letter were not significantly different by Duncan's test. NS, not significant.
*, P≤/0.05; **, P≤/0.01.
All dates were assessed in air at room temperature

Table 2: Effect of modified atmosphere and control packaging on sensory attributes of Barhi Khalals dates over 10, 20, 30 days of storage at 5^oC

		Colour characteristics		
Treatment	Time	L	a	b
Natural air	10	49.95^a	4.98^c	39.93^a
MAP A	-	49.44^a	5.01^{ac}	39.37^{ah}
MAP B	-	47.87^b	5.23^{ac}	38.89^h
Natural air	20	43.90^h	5.23^{ac}	33.76^c
MAP A	-	42.78^c	5.29^a	32.74^{bc}
MAP B	-	40.41^d	5.66^b	29.88^f
Natural air	30	40.84^d	5.82^{bh}	32.64^b
MAP A	-	39.44^f	6.07^h	27.81^f
MAP B	-	37.54^e	6.40^g	30.41^g
Treatment		**	**	**
Time		**	**	**
Treatment × Time		NS	NS	**

Values (mean of five replicates) in the same column followed by the same letter were not significantly
**, P≤/0.01, ** by Duncan's test. NS, not significant. *, P≤/0.05
All dates were assessed in air at room temperature

as predominant sugars of dates from the different cultivars at the maturation level differing in proportions between the cultivars (Rastegar et al., 2012). At the beginning of this study, TSS of the Barhi Khalal was 34° (Figure 3), while the total sugar content was observed to increase after 10, 20, 30 days of storage, which occurred in all the treatments that the various rates (Al-Redhaiman, 2004).
In this regard, the highest values were found in the control packaging for 10, 20 and 30 days, and the lowest values were found with MAP A (5% O_2). In general, the increased total sugar content associated with ripening of dates from the Khalal stage into the Rutab or Tamar stages will occur in the cells of the fruits when the moisture loss concentrates the sugar.
Bose (1985) reported that the control atmosphere (CA) with high CO_2 and low O_2 concentrations or high O_2 atmosphere had no significant influence on the TSS of apples and pears. Slow increases of the TSS of the control date may have been a result of the fresh dates being stored at the optimum storage temperature of 0^oC (Kader & Hussein, 2009). In studying the TSS range of Khalal dates, it was estimated to be at 30-45^oC Brix in three developmental stage, whilst it was 55-60° Brix at the Rutab stage, and 60-84° Brix at the Tamar stage.

3.5 Sensory Attributes

Sensory assessment of the samples was carried out on days 10, 20 and 30 of storage. Comparing MAP with natural air samples showed decreasing sensory scores in terms of overall acceptability for Barhi Khalal dates during the storage.
At the first 10 days, there was no significant difference ($p>0.05$) between MAP and control packaging. However there was a greater, more significant difference between MAP and control packaging on 20, 30 days of storage ($p\leq0.05$). In general MAP A had the best sensory properties compared with MAP B in the first 10 days, but there was no significant difference between MAP A and MAP B ($p>0.05$) in 20, 30 days of the storage (Table 2). The highest quality of Barhi dates was found in the samples packaged at 5^oC

with the control packaging on all days of the storage, especially for the first 10 days, followed by MAP A after 10 days of storage.

Off-odour development has been attributed to fermentative metabolism under anaerobic conditions. Burton, E. Frost and Nichols (1987) recommended that for the storage of the mushroom *Agaricus bisporus* the oxygen level inside the packages must not fall below 3-4% in order to avoid anaerobic respiration. In active modified atmosphere packages, off-odours were detected after 12 days of the storage, when the oxygen concentration fell below 5%. Thus, the results suggest that the O_2 concentration must remain above 5% for Barhi Khalal dates in order to avoid off-odour generation. On the other hand, the off-odour development was the process responsible for loss of quality of Khalal Barhi dates stored in the control packages, but mainly it was because the fruit moved from the Khalal stage into Rutab. In addition, MAP B had a high O_2 concentration with low sensory properties. This could be explained by assuming that Barhi Khalal deterioration could have been influenced by the high relative humidity inside the packages. The deterioration of the sensory attributes of some samples may have occured due to lactic acid bacteria or yeast growth, as the microbes were easily detected on account of giving bad flavours if present (Aidoo, Tester, Morrison & MacFarlane, 1996). This finding indicated that the use of MAP did not improve the sensory quality of the Barhi Khalals over time in the storage period compared with the control -packaged dates.

4 Conclusions

These results showed that the packaging of Barhi date fruits in the Khalal stage in polyethylene bags (80 microns) using natural air and modified atmosphere packaging at the temperature of 5^oC for 10, 20, 30 days storage, both MAP A (5% O_2 + 20% CO_2 and 75% N_2) and MAP B (40% O_2 + 20% CO_2 and 40% N_2) reduced the weight loss and the increase of TSS in the Barhi Khalal compared with the control but MAP was marginally better than MAP B. Neither MAP treatment was able to reduce deterioration, colour, firmness and sensory properties of the fruits over the storage period compared with the natural air packaging, though the loss of firmness was less under MAP A than MAP B.

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