

Changes in the Structure of Brined Cheese Modified with Basil Seed Gum Based on Protein-Polysaccharide Interactions

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ABSTRACT

Poor organoleptic and physical properties of Low Fat Cheese (LFC) suggest the use of some hydrocolloids in its production. In this study, the effect of addition of Basil Seed Gum (BSG) into the structure of low-fat white brined cheese was investigated. To obtain a good view about the protein and polysaccharide interactions in cheese, Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared (FTIR) spectroscopy were also used. The results indicated that the incorporation of BSG into the cheese matrix and the creation of new interactions caused some changes in the cheese properties. There was considerable slump in the hardness value of the cheese samples containing BSG throughout ripening. The addition of BSG in the cheese matrix weakened its microstructure due to a decrease in the electrostatic attraction between the macromolecules, which was mainly a result of high salt concentration. Thermal properties and FTIR spectra of cheese samples were altered with polysaccharide incorporation as well as the ripening period.

Keywords: Differential Scanning Calorimetry, Fourier Transform Infrared spectroscopy, Low fat cheese, Organoleptic properties.

INTRODUCTION

Iranian white cheese is a close-textured brined cheese, which is made of the milk of cow, sheep, or a combination of both. Because of the key role of fat in the flavor, texture, and appearance of food, it quickly becomes clear such that the development of low-fat products with qualities matching their full-fat counterparts is somehow difficult when one substitutes the fat with alternative ingredients (Romeih *et al.*, 2002). Several researchers have examined the optimization of Low-Fat Cheese (LFC) qualities through utilizing hydrocolloids as fat replacer (Hosseini *et al.*, 2014; Akın and Kirmaci, 2015).

Ocimum basilicum is a member of genus *Ocimum*. It is one of the peculiar plants in Iran, which is produced and utilized as a pharmaceutical plant in high quantity. The chemical composition of Basil Seed Gum (BSG) has been determined to be comprised of two major fractions; one acid-stable core glucomannan (43%) with glucose/manose ratio of 10:2, and a linked xylan (24.29%) including acidic side chains in the acid-soluble portion. In addition, it has a minor fragment of glucan (2.31%) (Mirhosseini and Amid, 2012). Incorporating the hydrocolloids into the cheese structure obviously changes the interactions between the proteins.

Several methods have been practiced so far for examining the interactions between

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proteins and polysaccharides in the cheese structure. The study of changes in the cheese microstructure during the aging provides important information about the modifications of casein during the ripening period (Aminifar *et al.*, 2010; Aminifar and Emam-Djomeh, 2014). Differential Scanning Calorimetry (DSC) is a potent technique for characterizing the energetic changes as well as mechanisms of temperature-induced conformational changes of biological macromolecules (Spink, 2008). Thermal analysis by DSC of cheese has been conducted by several researchers (Tunick *et al.*, 1989). Fourier Transform Infrared (FTIR) spectroscopy is a direct, reliable and quick technique that makes it possible to gain special information about various parameters mainly in the 4000- 400 cm^{-1} region since bands are related to vibrations of functional groups of the molecules. The related bands of proteins, fats, lactose and lactic acid are well known, and their role has been described in cheese (Martín-del-Campo *et al.*, 2007).

The objective of this study was to evaluate the qualitative attributes of the low-fat Iranian white cheese made with two concentrations of BSG during ripening.

MATERIALS AND METHODS

Milk Composition, Treatments, Cultures, Rennet, and Basil Seed Gum

The mean \pm SD of fat, moisture, protein, and pH of full-fat vs. low-fat milks used for cheese production were (3.13 ± 0.14 vs. $0.58\pm 0.11\%$), (87.78 ± 0.15 vs. $91.07\pm 0.19\%$), (3.07 ± 0.01 vs. $3.13\pm 0.01\%$), and (6.74 ± 0.14 vs. 6.73 ± 0.04), respectively. Based on our preliminary experiments, concentrations of BSG were selected. Four treatments were applied: (1) Control Full-Fat Cheese (FFC), (2) Control LFC without BSG (CLFC), (3) LFC with 0.0012 g of BSG kg^{-1} of cow milk (OB 0.12), and (4) LFC with 0.0048 g of BSG

kg^{-1} of cow milk (OB 0.48). One lyophilized direct-to-vat mesophilic mixed culture (FD-DVS FRC-65, Chr. Hansen's, Dairy Culture, Hoersholm, Denmark) containing *Lactococcus lactis* ssp. *cremoris* ($8 \log \text{ cfu mL}^{-1}$), *Lactococcus lactis* ssp. *lactis* ($7 \log \text{ cfu mL}^{-1}$), *Streptococcus thermophilus* ($7.6 \log \text{ cfu mL}^{-1}$), and *Lactobacillus delbrueckii* ssp. *bulgaricus* ($7.93 \log \text{ cfu/mL}$) was used as starter. Commercial bovine chymosin was purchased from Chr. Hansen Company, Denmark. The crude BSG was extracted from basil seeds purchased from the local market in Karaj, Iran, according to the procedure proposed by Razavi *et al.* (2009).

Preparation of Cheeses

The Iranian white brined cheese was made according to the method proposed by Rahimi *et al.* (2007) with some modifications. Raw skim milk ($< 0.25\%$ fat) was standardized with cream of a determined fat content to 3% fat for the FFC and to 0.5% fat for CLFC. One kg of the standardized milk (to 0.5% fat) was heated to 35°C , and then supplemented with two levels of powdered BSG and flash-pasteurized at 75°C for 15 seconds. The milk was then transformed to a cheese vat and mixed with 9 kg of 0.5% fat pasteurized milk. Agitation was gradually continued for 10 minutes for complete mixing. The milk was cooled down to 35°C in this period and then supplemented with 0.15 g of $\text{CaCl}_2 \text{ kg}^{-1}$ of milk. Before addition of rennet, the milk was held at 35°C for about 1 hour after inoculation of the culture for starter activity. The curd was cut crossways in cubes of 1.2 cm^3 (after approximately 45 min). Afterward, the curd was left to be settled for 3 to 5 minutes. Then, it was softly agitated at a gradually increasing rate for 10 minutes to prevent the fusion of freshly cut curd cubes and to make it easier for whey expulsion. The procedure was continued by draining of whey and pressing the transferred curd into molds for 0.5 hours (under pressure of 0.3 kPa) to

complete the draining procedure. After pressing, the curd was cut into blocks (5×5×2 cm). The blocks were situated in airtight plastic containers, and covered with a 12% brine solution. The storage time of sealed containers in a cold room at 5 to 6°C was 60 days. The FFC and CLFC were produced similarly, yet without adding BSG.

Chemical Analysis

The pH of the milks and cheese samples was determined with a digital pH meter (Jenway, 3510 pH meter, UK). The cheeses were analyzed for moisture content using AOAC (2005) method. The fat and protein content were determined by the Gerber (Ceirwyn, 1995) and Kjeldahl methods, respectively. Salt content was measured by the method previously described by Johnson and Olson, (1985). All measurements were carried out in triplicate.

Textural Properties

The texture profile was determined employing a texture analyzer (Testometric M350-10CT, Lancashire, UK). The cheese cubes (20×20×20 mm) adjusted to 12±5°C were compressed to 70% of their initial height by a plunger with a size of 40 mm in diameter and 60 mm min⁻¹ speed. The force needed to do this was considered as the hardness value. Adhesiveness (work necessary to overcome the attractive forces between food and probe surfaces) and springiness (degree to which a product returns to its original shape once it has been compressed) were also determined (Szczeniak, 1963).

Microstructure

Cheese samples were prepared for electron microscopy scanning according to Aminifar, *et al.* (2010). These pieces were attached to aluminum stubs by silver paint, dried to the

critical point, and covered with gold in a sputter-coater for 10 minutes (type SCD 005, Baltec Inc., Balzers, Switzerland). The samples were viewed in a scanning electron microscope (VEGA TESCAN-LMU, Czech Republic). The 1000× magnification was selected at 3.0 kV.

Image analysis software (Image J; National Institutes of Health, Bethesda, MD, USA) was used to analyze the SEM micrographs with the setting used in previous study (Aminifar and Emam-Djome, 2015).

Sensory Evaluation

An acceptance sensory panel assessed randomly the coded cheese samples. The trained taste panelists comprised of 60 members (28 males and 32 females) ranging in age from 25 to 45 years and they were requested to score the cheese samples on a 5-point Hedonic scale (1= Liked least, 5= Liked most). Sensory evaluation was carried out at day 60 of ripening.

FTIR Spectroscopic Analysis

The samples were examined in KBr discs according to ASTM E1252-98 (2013) standard. The technique involves the grinding of a solid sample, mixing it with KBr powder, and pressing the resulting mixture into a pellet or disk. The spectra were obtained with a FTIR (Bomem B-100 FT-IR spectrometer, Canada) spectrometer. Spectra of the samples in the region between 4000 cm⁻¹ and 400 cm⁻¹ were obtained with a resolution of 4 cm⁻¹ using 22 scans/sample.

DSC

Thermal properties of the cheese samples were studied by DSC. The measurements were carried out on DSC calorimeter (MettlerToledo, Star system, USA). An empty pan was used as a reference. The



samples (about 10 mg), previously weighted in aluminum pans, were analyzed at a heating rate of $2^{\circ}\text{C min}^{-1}$ from -10 to 150°C .

The denaturation Temperature (T_d) was determined from the maximum heat flow. The enthalpy of denaturation (ΔH) was calculated by the peak area in the endotherm (Yamasaki et al., 1990).

Statistical Analysis

All experiments were done in triplicate. An ANOVA was carried out using the SPSS statistical software version 21 (1989; IBM corp., USA) to determine the effects of treatment of all variables. Duncan's multiple comparison test was utilized as a guide for pair-wise comparisons of the treatment means and effect of time on the cheese samples during ripening. The significance level was scaled at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical Analysis

Physico-chemical characteristics of the cheese samples are illustrated in Table 1; as shown, when the fat decreases, the moisture increases significantly. This difference in the moisture content of CLFC and the FFC may be attributed to their protein content, i.e. a higher protein content of LFC may be related to the increased water binding capacity of the cheese matrix (Rahimi et al., 2007; Romei et al., 2002). The decrease of the curd moisture during ripening is caused by the increasing concentration of salt in the curd. Extent of salt transfer into curd takes place because of the difference of salt concentration between the curd and brine (Azarnia et al., 1997). Cheeses containing BSG had higher moisture content and were compared to the FFC and CLFC. Higher amounts of moisture content in cheeses containing BSG could be attributed to intrinsic qualities in bonding the free water in their structure (Razavi et al., 2012). This

hydrocolloid (i.e. BSG) incorporated as fat replacer was able to show some functional properties similar to those of fats by bonding water molecules in some foods like low-fat ice cream, low-fat mayonnaise and low-fat yoghurt (Afshar Nik, 2011; Razavi et al., 2012). It has been suggested that fat replacers prevent the shrinkage of the casein matrix (Koca and Metin, 2004) and then reduce the driving force involved in driving out the water from the curd particles (Nateghi et al., 2012).

There was a significant difference between the fat content of the samples containing BSG and CLFC and FFC on day 1 ($P < 0.05$). Aminifare et al. (2014) reported that reduction of the fat content in LFC containing Xanthan gum was due to its higher moisture content. So, lower fat content of cheeses containing BSG could be attributed to their higher moisture contents. In the present study, fat content of the cheese samples decreased significantly during ripening probably due to the lipolysis (Romei et al., 2002).

The protein content of the cheese samples on day 1 differed significantly. BSG had a significant effect on reducing the protein content of low fat cheese samples. A substantial portion of the rennet was lost in the whey during cheese making and, generally, only about 6% of the rennet added to cheese milk remained in the curd (Karami et al., 2009). The ratio of the residual rennet to casein was higher in high-moisture cheese than low-moisture cheese and so the rate of proteolysis will be increased. Rahimi et al. (2007) reported that with increasing concentration of tragacanth, proteolysis expanded due to an increase in the moisture content. A decrease in the protein content in white brined cheese is related to proteolysis and the following diffusion of amino acid to the brine (Khosrowshahi et al., 2006).

It was shown that the pH value of CLFC was slightly higher than that of the FFC throughout cheese ripening. Similar observations have been made by Romei et al. (2002) on low-fat white-brined cheese.

Addition of BSG into the LFC formulation had no significant effect on its pH value ($P < 0.05$). The pH values in CLFC and FFC decreased significantly during ripening. This decrease in the pH of brine cheese during aging is mainly due to the completion of lactose fermentation and the release of amino and free fatty acids following proteolysis and lipolysis (Azarnia *et al.*, 1997). In OB 0.12 and OB 0.48, the pH values did not alter significantly throughout ripening. Karamiet *al.* (2009) reported that the pH value of ultra-filtered Feta cheese did not change significantly during the ripening period.

The values of fat in dry matter of cheese samples are shown in Table 1. The increased moisture content of LFC induced a decrease

in the fat in dry matter. According to Rahimi *et al.* (2007), this parameter decreases during ripening.

Salt content in CLFC was higher than in FFC. Salt diffusion into the cheese texture was affected by the cheese moisture content (Geurtset *al.*, 1972). When the cheese is put in the brine, a dynamic two-way diffusion process is started as NaCl molecules move from the brine into the cheese; while water diffuses out through the cheese matrix. It decreases the moisture content and increases the salt content as it ripens (Azarnia *et al.*, 1997). At the day 1, there was no significant difference between the salt content of OB 0.12 and OB 0.48 ($P < 0.05$) probably owing to this fact that day 1 was the beginning of the dynamic reciprocal salt diffusion

Table 1. Changes in the physicochemical properties of cheese samples during the aging (mean \pm SD of three trials).^a

Physicochemical characteristics	Sample	Age (day)	
		1	60
Moisture	FFC	70.02 \pm 0.02 ^{d1}	68.92 \pm 0.03 ^{d2}
	CLFC	72.15 \pm 0.02 ^{c1}	71.19 \pm 0.06 ^{c1}
	OB 0.12	73.43 \pm 0.07 ^{b1}	75.87 \pm 0.08 ^{b1}
	OB 0.48	73.76 \pm 0.02 ^{a1}	76.62 \pm 0.12 ^{a2}
	FFC	11.44 \pm 0.02 ^{a1}	9.39 \pm 0.02 ^{a2}
Fat	CLFC	2.56 \pm 0.02 ^{c1}	1.88 \pm 0.04 ^{c2}
	OB 0.12	2.16 \pm 0.07 ^{b1}	1.33 \pm 0.04 ^{d2}
	OB 0.48	2.05 \pm 0.05 ^{b1}	1.89 \pm 0.06 ^{b2}
	FFC	9.07 \pm 0.8 ^{d1}	9.04 \pm 0.09 ^{d1}
Protein	CLFC	19.19 \pm 0.04 ^{a1}	15.19 \pm 0.1 ^{a2}
	OB 0.12	18.83 \pm 0.1 ^{c1}	12.77 \pm 0.08 ^{c2}
	OB 0.48	19.04 \pm 0.05 ^{b1}	14.62 \pm 0.07 ^{b2}
	FFC	6.50 \pm 0.03 ^{b1}	5.87 \pm 0.01 ^{c2}
pH	CLFC	6.93 \pm 0.02 ^{a1}	6.25 \pm 0.04 ^{b2}
	OB 0.12	6.87 \pm 0.04 ^{a1}	6.81 \pm 0.06 ^{a1}
	OB 0.48	6.83 \pm 0.07 ^{a1}	6.78 \pm 0.05 ^{a1}
	FFC	38.15 \pm 0.04 ^{a1}	30.21 \pm 0.09 ^{a2}
Fat in dry matter	CLFC	9.39 \pm 0.05 ^{b1}	6.52 \pm 0.08 ^{d2}
	OB 0.12	8.57 \pm 0.03 ^{c1}	6.75 \pm 0.07 ^{c1}
	OB 0.48	9.6 \pm 0.04 ^{b1}	9.19 \pm 0.09 ^{b2}
	FFC	4.95 \pm 0.05 ^{c1}	5.32 \pm 0.04 ^{d1}
Salt (%)	CLFC	6.03 \pm 0.07 ^{a1}	6.3 \pm 0.03 ^{c2}
	OB 0.12	5.23 \pm 0.04 ^{b1}	6.86 \pm 0.07 ^{a2}
	OB 0.48	5.17 \pm 0.8 ^{b1}	6.74 \pm 0.3 ^{b2}
	FFC		

^a: ^{a-d} Means within the same row with different superscripts differ ($P < 0.05$). ¹⁻² Means within the same column with different superscripts differ ($P < 0.05$).



process; however, after ripening period, OB 0.12 had more NaCl content. The higher amount of salt in cheeses containing BSG than CLFC and FFC may be attributed to their higher moisture contents (Aminifar *et al.*, 2015); because the diffusion of salt into the cheese texture is affected by cheese moisture content (Geurts *et al.*, 1972).

Textural Analysis

The effect of ripening time and polysaccharide addition on the hardness, adhesiveness, and springiness of brined cheeses is shown in Table 2. The hardness of CLFC was considerably higher than that of FFC. Fat globules act as fracture points in the cheese matrix and reduce its hardness (Sipahioglu *et al.*, 1999). This is in agreement with the results of Koca *et al.* (2004) and Akin and Kirmaci (2015), who reported that the low-fat control cheese was significantly harder than the full-fat control cheese owing to its high protein content. The ripening period caused an increment in the hardness value of CLFC and FFC due to fact that water leaves from the cheese texture as a result of the osmotic pressure of salt in the brine. Actually, the increase in their hardness depends on the decrease in their moisture content (Prasad and Alvarez,

1999). Such an increase in hardness is in agreement with the findings of Farahaniet *al.* (2014), who reported that the hardness value of Iraninan white brined cheese increases after the ripening time.

On the first day of ripening, there was a significant difference in the hardness of OB 0.48 and CLFC, while no such difference was observed between OB 0.12 and CLFC. Higher hardness value in OB 0.48 may be attributed to the interaction of BSG and rennet casein in the cheese matrix. Additionally, a probable web network created by BSG chains could strengthen the network formed by casein strands (Hosseini-Parvaret *al.*, 2015). It has been reported that BSG could create a firmer structure in model processed cheese and low-fat set yoghurt (Afshar Nik 2011; Hosseini-Parvar *et al.*, 2015). This is in agreement with the findings of Černíková *et al.* (2008), who reported that increasing carrageenan concentration in model processed cheese led to a denser network structure as a consequence of more intensive interactions between the carrageenan chains. There was a dramatic drop in the hardness value of cheeses containing BSG after aging, which could be attributed to their higher moisture content due to the water absorption properties of BSG (Johary *et al.*, 2015). Softer texture resulted in increased diffusion of salt into

Table 2. Texture profile analysis results of cheese samples during ripening. ^a

	Sample	Age (day)	
		1	60
Hardness (N)	FFC	4.57±0.45 ^{c1}	5.45±0.27 ^{b2}
	CLFC	8.23±0.51 ^{b1}	12.91±0.2 ^{a2}
	OB 0.12	6.81±0.8 ^{b1}	2.95±0.43 ^{c2}
	OB 0.48	10.46±1.28 ^{a1}	4.89±0.24 ^{c2}
Adhesiveness (N.s)	FFC	0.76±0.13 ^{d1}	0.36±0.06 ^{b2}
	CLFC	2.8±0.14 ^{a1}	0.91±0.08 ^{a2}
	OB 0.12	1.06±0.04 ^{c1}	0.66±0.02 ^{c2}
	OB 0.48	1.23±0.07 ^{b1}	0.51±0.03 ^{d2}
Springiness (mm)	FFC	0.75±0.12 ^{b1}	0.96±0.02 ^{a2}
	CLFC	0.98±0.01 ^{a1}	1±0.04 ^{a1}
	OB 0.12	1±0.02 ^{a1}	0.99±0.03 ^{a1}
	OB 0.48	0.97±0.01 ^{a1}	1±0.01 ^{a1}

^a: ^{a-d} Means within the same row with different superscripts differ ($P < 0.05$). ¹⁻² Means within the same column with different superscripts differ ($P < 0.05$).

the cheese texture. As ripening progressed, the salt content of the cheese was increased, which caused a change in the macromolecules' interaction. The net charge carried by the proteins and polysaccharides is reduced by interaction with the micro-ions, resulting in a decrease in the electrostatic attraction between the macromolecules at high salt concentrations. At high ionic strength, screening the charges of the proteins and polysaccharides also leads to a decrease in electrostatic interactions (Ye, 2008). According to Lucey *et al.* (2003), diffusion of salt into cheese changes the strength of the interactions taking place between the protein molecules by screening the charged groups (which decreases the electrostatic repulsion while lessening the number of plus-minus interactions of the protein molecules). The domination of the latter effect probably weakens the structural bonds of the protein matrix of cheese, which, in turn, causes less resistance against the applied stress (Khosrowshahi *et al.*, 2006).

CLFC was more adhesive than the rest of the treatments, which can be attributed to its higher protein content (Nateghiet *al.*, 2012). Cheeses containing BSG had lower adhesiveness than CLFC. Salvatore *et al.* (2014) reported that samples containing inulin were characterized by lower values of adhesiveness. FFC had lower springiness than the LFC samples. According to the literature (Tiwari *et al.*, 2010), fat reduction increases springiness of reduced fat cheeses. Springiness values of other treatments showed no significant differences. The same texture behavior in springiness was observed when other fat replacers were used in the low-fat cheeses (Nateghi *et al.*, 2012).

Microstructure

The SEM micrographs of FFC, CLFC and OB 0.12 as well as their 3D images after 1 and 60 days of ripening are shown in Figure 1. In the electron micrograph scanning of FFC, the protein matrix was open, with spaces occupied

by the fat globules (Rahimiet *al.*, 2007). CLFC contained fewer fat voids and larger stretches of uninterrupted protein matrix due to decreased fat content (Drake *et al.*, 1996). A reduction in fat content caused the protein matrix to be more compact. As observed in the SEM, the microstructure of OB 0.12 was compact in the first day of ripening; however, after aging time, it was weak. This result is in good agreement with the data obtained from hardness results. Water holding capacity of BSG and creation of new serum channels allows retention of the serum adjacent to the fat replacer particles. When such large serum channels are spread throughout the cheese matrix, it will be softer and more pliable owing to higher moisture (McMahon *et al.*, 1996). The hydrolysis of cheese protein network and the following diffusion of small peptides and free amino acids to the surrounding brine may clarify the microstructural changes observed during ripening (Khosrowshahi *et al.*, 2006).

Sensory Evaluation

Table 3 shows the scores of taste panelists for cheese treatments 60 days after storage. As expected, FFC received the highest score in all attributes. The reduction of fat content significantly affected the texture, appearance, flavor, and overall acceptability of Iranian white cheese. Rahimi *et al.* (2007) also reported that low-fat Iranian white cheese received lower flavor and texture scores than FFC. Cheeses with lower fat usually have a less distinct flavor than full-fat products, possibly due to the flavor dilution of the LFC because of excessive moisture retention (Sipahioglu *et al.*, 1999) and lack of some of the fat-soluble compounds, which contribute to the overall flavor. The fat in cheese carries much of the flavor, and when it decreases, the cheese flavor diminishes. In the present study, the cheese containing BSG received lower scores than other samples in terms of appearance and texture; flavor was lower as a result of higher saltiness and they had too smooth texture. Hence, their overall

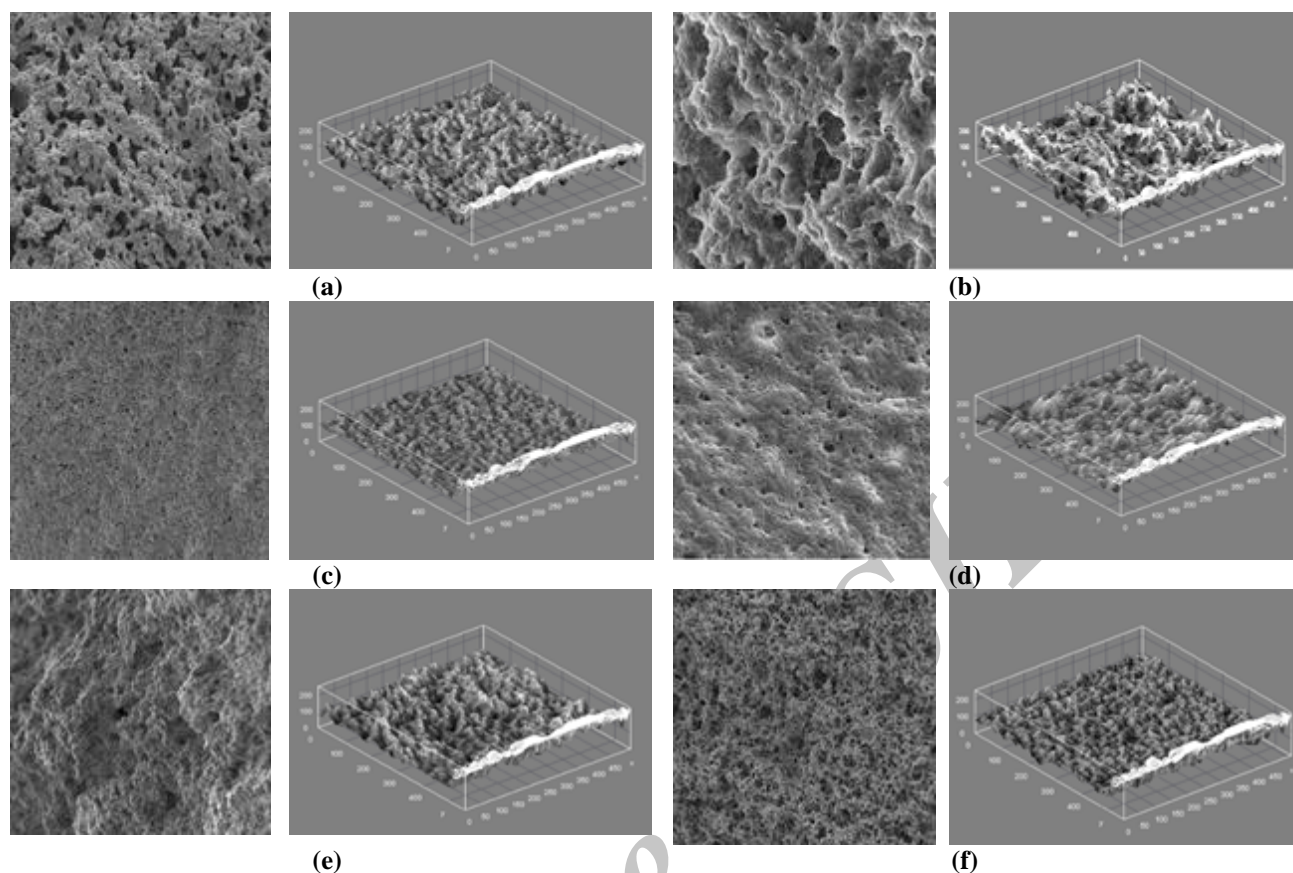


Figure 1. Microstructure and 3D images of SEM micrographs of the cheese samples: (a) FFC at day 1, (b) FFC at day 60, (c) CLFC at day 1, (d) CLFC at day 60, (e) OB 0.12 at day 1, and (f) OB 0.12 at day 60 of ripening period (1000 \times magnification levels). FFC: Full Fat Cheese; CLFC: Control Low Fat Cheese; OB 0.12, LFC with 0.0012 g of basil seed gum kg^{-1} of cow milk; OB 0.48, LFC with 0.0048 g of basil seed gum kg^{-1} of cow milk.

Table 3. Means \pm SD of sensory attributes of FFC, CLFC, OB 0.12 and OB 0.48.^a

Sample	Appearance	Texture	Flavor	Odor	Overall acceptance
FFC	4.84 \pm 0.25 ^a	4.36 \pm 0.14 ^a	4.61 \pm 0.13 ^a	4.18 \pm 0.24 ^a	4.4 \pm 0.26 ^a
CLFC	4.12 \pm 0.21 ^b	3.76 \pm 0.12 ^b	3.55 \pm 0.13 ^b	3.36 \pm 0.15 ^d	3.5 \pm 0.22 ^b
OB 0.12	3.09 \pm 0.19 ^c	3.06 \pm 0.1 ^c	2.71 \pm 0.17 ^c	3.6 \pm 0.2 ^c	2.81 \pm 0.14 ^c
OB 0.48	2.8 \pm 0.17 ^d	2.74 \pm 0.22 ^d	2.75 \pm 0.11 ^c	3.97 \pm 0.15 ^b	2.8 \pm 0.16 ^c

^a: ^{a-d} Means within the same column with different superscripts differ ($P < 0.05$).

acceptance was much lower than that of FFC as well as CLFC.

FTIR Spectroscopic Analysis

FTIR spectra of the cheese samples are presented in Figure 2. The band at 2,930 cm^{-1} owing to C-H content represents a good

reference for total carbohydrate content (Guerrero *et al.*, 2014). As can be seen in Figures 2 (b) and (d), this peak in OB 0.12 has stronger absorbance than CLFC in both days 1 and 60. In the first day of ripening, the FTIR spectrum of OB 0.12 showed a good separated absorption at 1,746 cm^{-1} (Figure 2-b). This peak is related to the carbonyl groups of various *R(CO)OR* (Chen

et al., 1998). Addition of BSG in the cheese matrix may result in the formation of ester groups due to the interaction of COO^- of uronic acids and the hydroxyl groups of some amino acids (e.g. Tyrosine and Threonine) in casein. This peak had higher absorbance after 60 days (Figure 2-c); an increase in the number of the carbonyl groups of $R(\text{CO})\text{OR}$ is caused by proteolysis and lipolysis during the ripening period (Chen *et al.*, 1998).

There is a very strong and broad band belonging to water, which is highly absorbed in the range between $3,700$ and $3,100\text{ cm}^{-1}$ (Chen *et al.*, 1998). An increase in this region is observed in OB 0.12 after 60 days of ripening time (Figure 2-c). It has been

reported that the intensity of the band around $3,600\text{--}3,300\text{ cm}^{-1}$ corresponding to the O-H stretching vibration, as well as those of the polar groups of proteins, are in close relation to the hydration process and to water content of the system (Lucia *et al.*, 2001). Thus, an increase in the moisture content of this cheese after ripening time could be confirmed by an increase in the peak area of water.

In spite of a decrease from 72.15 to 71.19% in the moisture content of CLFC sample after 60 days (Table 1), a higher intensity in its FTIR spectrum in $3,600\text{--}3,300\text{ cm}^{-1}$ region was observed (Figure 2-a). This could be related to the higher ratio of bound water to the free water. Proteolysis

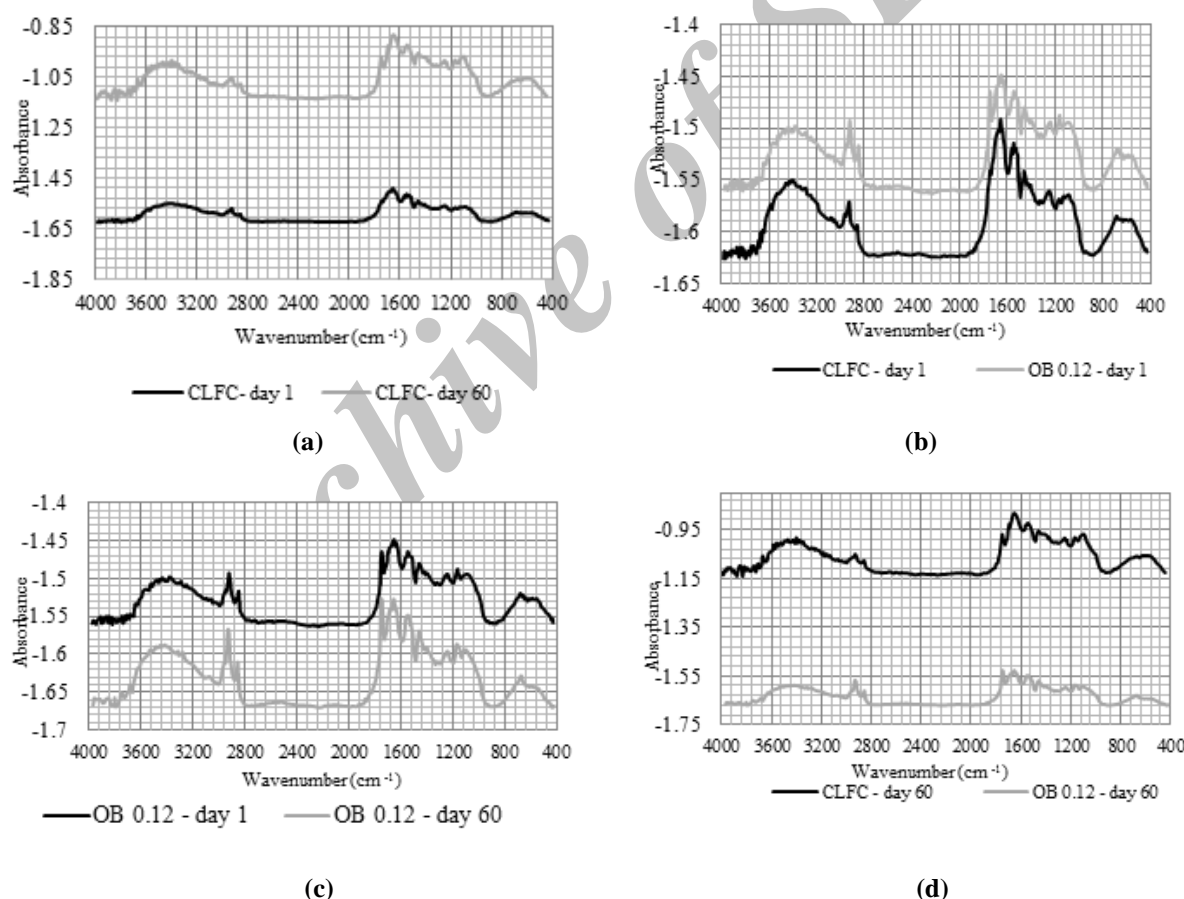


Figure 2. (a) FTIR spectra of CLFC at days 1 and 60 of ripening, (b) FTIR spectra of CLFC and OB 0.12 at the first day of ripening, (c) FTIR spectra of OB 0.12 at days 1 and 60 of ripening time, (d) FTIR spectra of CLFC and OB 0.12 at day 60 of ripening time. CLFC: Control Low Fat Cheese; OB 0.12, LFC with 0.0012 g of Basil seed gum kg^{-1} of cow milk.



could have important changes in the caseins' secondary structure, as well as the accumulation of the degradation products and their interactions with the water molecules existing in the system. Schlessner *et al.* (1992) found a relationship between water sorption and proteolysis in Camembert cheese at different water activity values. Actually, they reported that as the proteolysis increased, water sorption would increase, and the end of aging took place when the maximum amount of water was bound with the hydrophilic groups caused by proteolysis. Therefore, the higher intensity of the band around $3300\text{--}3,600\text{ cm}^{-1}$ of CLFC sample after 60 days could be correlated to the hydration process as well as the water sorption of degradation products (Lucia *et al.*, 2001).

According to Figure 2 (c), the peaks at the OB 0.12 FTIR spectrums after ripening were narrower than those in the first day. Generally, peak width indicates the uniformity of the samples, and the samples with wider peaks correspond to less uniform samples (Haque *et al.*, 2010). This uniformity after ripening time could be confirmed by Figure 1 (f).

Differential Scanning Calorimetry (DSC)

The denaturation Temperature (T_d) and enthalpy (ΔH) of the cheese samples were determined by DSC (Figure 3, Table 4). The T_d value of OB 0.12 was lower than that of CLFC at the first day of ripening. In the presence of polysaccharide, T_d of the low fat cheese was reduced. Imeson *et al.* (1977) studied the interactions of some anionic polysaccharides and proteins, and found similar results. On the 60th day, the DSC curve showed two peaks (Figure 3-a). According to Yamasaki *et al.* (1990), appearance of two peaks in the DSC curve could be attributed to the binding of NaCl. Farkas and Mohácsi-Farkas (1996) suggested that salt can increase T_d in milk proteins; thus, higher value of T_d in OB 0.12

after its ripening period could be related to the increase of its salt content.

While the bonds maintaining the protein-protein aggregates form both electrostatically and hydrophobically i.e. weakly endothermic, the protein-polysaccharide bonds form electrostatically i.e. virtually athermal (Imeson *et al.*, 1977). Thus, on the first day of ripening, the lower value of enthalpy of OB 0.12 in comparison with CLFC could be due to the electrostatic nature of the interactions (which are virtually athermal) between BSG and casein proteins. Moreover, it was reported that the protein-polysaccharide aggregations systems are less endothermally (or more exothermally) than the systems containing no polysaccharide (Imeson *et al.*, 1977). Since the interactions between cheese proteins and BSG occur electrostatically in nature (interaction of COO^- of uronic acids and the hydroxyl groups of some amino acids previously described in FTIR), they would virtually be considered athermal; hence, they could not increase the enthalpy in OB 0.12 as much as in CLFC.

Enthalpy of OB 0.12 in day 60 was higher compared to that of CLFC. Yamasaki *et al.* (1990) found that the value of ΔH in bovine serum albumin increased with the increase in its ionic strength at the same temperature circumstances i.e. $25\text{--}95^\circ\text{C}$. So, one reason for the higher enthalpy of OB 0.12 in comparison with CLFC in day 60 of ripening period could be its higher salt content.

The DSC curve of OB 0.12 in day 60 indicated a narrower peak compared to its curve in day 1 (Figure 3-a). The broader peaks in the mixture of protein and polysaccharide indicate the more heterogeneous nature of the blend (Spada *et al.*, 2015). So, the narrower peak in the DSC curve of OB 0.12 in day 60 could be related to its homogenous mixture.

CONCLUSIONS

The present study indicated that adding BSG into the milk with the aim of improving the properties of low-fat cheese caused a too smooth texture and salty taste,

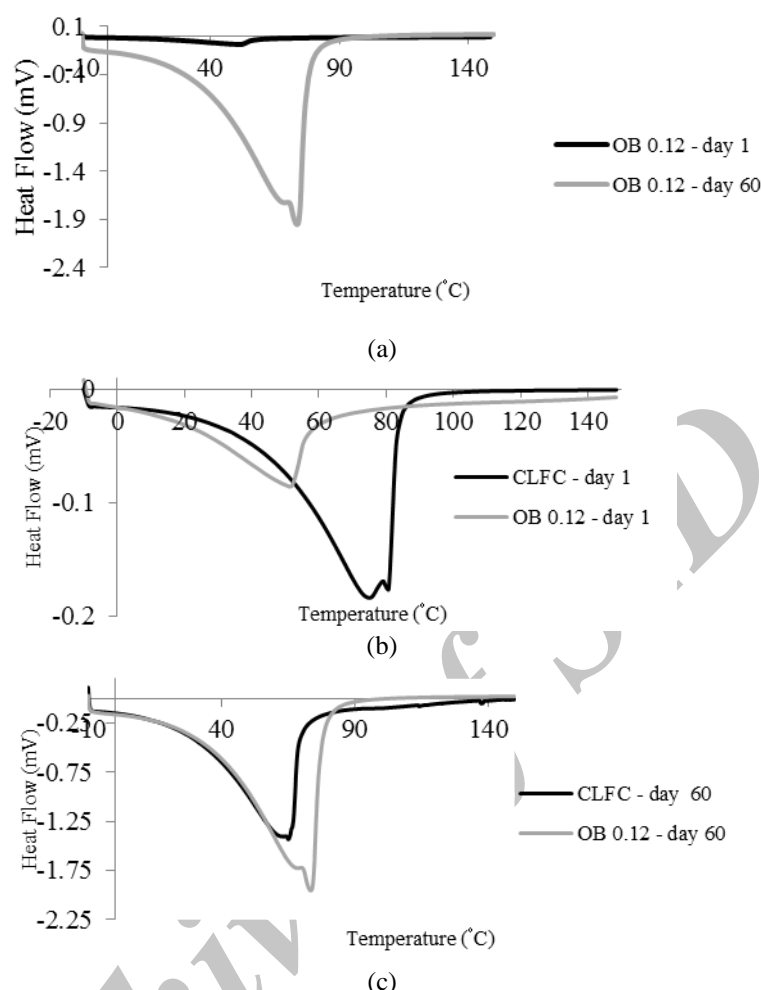


Figure 3. (a) DSC curves of OB 0.12 at days 1 and 60, (b) DSC curves of CLFC and OB 0.12 at day 1, (c) DSC curves of CLFC and OB 0.12 at day 60. CLFC: Control Low Fat Cheese; OB 0.12, LFC with 0.0012 g of Basil seed gum kg^{-1} of cow milk.

Table 4. Thermal properties of cheese samples during ripening.

Thermal properties	Ripening time	Treatment			
		FFC	CLFC	OB 0.12	OB 0.48
T_d (°C)	Day 1	-	74.97	51.25	-
	Day 60	-	65.05	73.50	-
(Delta H, J g^{-1})	Day 1	-	-1886	-449.72	-
	Day 60	-	-1056.12	-1485.15	-

which was observed in day 60. The hardness parameters indicated a firmer structure at the first day of ripening, and increase in softness of the product after 60 days. The weakness observed in the microstructure was also an indicator thereof. This gum could be used in those types of cheeses in which a soft texture is desirable. According to the FTIR spectra,

addition of BSG into the cheese matrix caused the formation of the ester group due to the interaction of COO^- of uronic acids and the hydroxyl groups of some amino acids in the casein protein. Thermal analysis offering some information about T_d , enthalpy and the shape of DSC curve (broadness) could be an appropriate method



for monitoring the interactions in the cheese matrix. A more homogenous structure for OB 0.12 at day 60 of ripening time can be confirmed by its SEM micrograph, 3D image, FTIR spectra, and DSC curve.

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بررسی تغییرات ساختاری پنیر آب نمکی اصلاح شده با صمغ دانه ریحان بر اساس برهم کنش های پروتئین - پلی ساکارید

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چکیده

ویژگی های ارگانولپتیکی و فیزیکی ضعیف پنیر کم چرب، استفاده از برخی هیدروکلوئیدها را برای فراهم کردن کیفیت دلخواه این نوع پنیر پیشنهاد می دهد. در این مطالعه، اثر افزودن صمغ دانه ریحان به ساختار پنیر آب نمکی کم چرب مورد بررسی قرار گرفت. برای دستیابی به اطلاعات کافی در مورد برهم کنش های پروتئین - پلی ساکارید در پنیر، گرماسنجی روبشی افتراقی و طیف بینی تبدیل فوریه مادون قرمز نیز استفاده گردید. نتایج نشان دادند که قرار گیری صمغ دانه ریحان در ماتریکس پنیر و تشکیل برهم کنش های جدید باعث ایجاد تغییراتی در ویژگی های پنیر شد. افت زیادی در میزان سختی پنیرهای حاوی صمغ دانه ریحان در طول رسیدن رخ داد. افزودن صمغ دانه ریحان در ماتریکس پنیر، ریزساختار آن را به دلیل کاهش جذب الکترواستاتیک بین ماکرومولکول ها تضعیف کرد؛ که عمدتاً به دلیل غلظت زیاد نمک بود. ویژگی های حرارتی و طیف FTIR نمونه های پنیر با افزودن پلی ساکارید و دوره رسیدن تغییر کرد.