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Carrageenan type effect on soybean oil/soy protein isolate emulsion employed as fat replacer in panela-type cheese

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SUMMARY: In order to modify the fatty acid profile of panela-type cheese (a Mexican fresh cheese), emulsified soybean oil with soy protein isolate and different carrageenan (iota, kappa or lambda) was employed as fat replacer. The replacement of milk fat in panela-type cheese resulted in higher cheese yield values and moisture content, besides a concomitant lower fat phase and higher protein content, due to a soy protein isolate in emulsified soybean oil. Fat replacement resulted in a harder but less cohesive, spring and resilient texture, where differences in texture could be attributed to the specific carrageenan-casein interactions within the rennet coagulated cheese matrix. The FTIR analysis showed that the milk fat replacement changed the fatty acid profile, also in function of the type of carrageenan employed. Lambda carrageenan containing emulsions improved moisture retention and maintained the textural properties of panela-type cheese.

KEYWORDS: *Carrageenan; Emulsified soybean oil; Fat replacement; Fourier Transform Infra-Red Spectroscopy; Panela-type cheese; Textural profile analysis*

RESUMEN: ***Efecto del tipo de carragenina en emulsiones de aceite de soja/aislado de proteína de soja utilizadas como sustituto de grasa en quesos tipo panela***. Para modificar el perfil de ácidos grasos de los quesos tipo panela (queso fresco popular en México), se utilizó aceite de soja emulsionado con aislado de proteína de soja y diferentes carrageninas (iota, kappa o lambda) como sustituto de la grasa. Reemplazar la grasa de la leche en el queso tipo panela resultó en mayor rendimiento quesero y mayor contenido de humedad, además de una concomitante menor fase grasa y mayor contenido de proteína, debido al aislado de proteína de soja en el aceite de soja emulsionado. La sustitución de la grasa dio como resultado una textura más dura, pero menos cohesiva, elástica y resiliente, donde estas diferencias podrían ser atribuidas a la interacción especifica entre carrageninas-caseinas en la matriz coagulada del queso. El análisis de FTIR muestra que reemplazar la grasa de la leche cambia el perfil de ácidos grasos, también en función del tipo de carragenina empleado. Las emulsiones con lambda carrageninas mejoraron la retención de humedad y mantuvieron las propiedades de textura del queso tipo panela.

PALABRAS CLAVE: *Aceite de soja emulsionado; Análisis del perfil de textura; Carrageninas; Infrarrojo con Transformada de Fourier; Queso tipo panela; Sustituto de grasa*

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1. INTRODUCTION

Dairy products like cheese are an important source of essential nutrients (mainly proteins, besides calcium, magnesium and potassium, riboflavin and vitamin B12). Nonetheless, dairy products like cheese are one of the major contributors of saturated fatty acid (SFA) intake, compounds associated with the development of chronic diseases such as cardiovascular disease, congestive heart failure, and obesity (Livingstone *et al.*, 2012). Consumer awareness of dietary fat has increased and the demand for low-fat foods, including cheese, has grown substantially, with a strong influence on the market. The removal of the fat from the casein network in low-fat cheese results in the formation of a more compact casein network that released more water and became tougher. To improve the textural characteristics of low-fat cheese, the moisture levels in curd must be increased (Banks, 2004). To compensate for these changes, fat substitutes or fat mimics can be employed. Fat substitutes are generally lipid-based macromolecules that physically and chemically resemble fats and oils such as sucrose fatty acid esters and polyesters, carbohydrate fatty acid esters, various emulsifiers (such as mono- and di-glycerides, lecithin), and structured lipids. Fat mimics are generally carbohydrate-based (modified starches and hydrocolloids) or protein-based macromolecules that are designed to mimic the organoleptic and physical properties of fats generally via the binding of water (Johnson *et al.*, 2009).

Fresh cheese was elaborated from natural cows' milk, pasteurized, non-acidified, and with an elevated water content (up to 58%). In 2014, cheeses production in Mexico was around 312,082 tons, where fresh and panela cheese represented 17.8 and 15.7%, respectively (SIAP, 2014). The higher consumption of fresh cheese calls for an improvement in quality characteristics by offering low fat fresh-type products. Processed cheese products manufactured by blending various edible oils, proteins and other ingredients can offer an attractive food-based delivery vehicle for lipids such as polyunsaturated fatty acids or omega-3 fatty acids (Ye *et al.*, 2009). The substitution of milk fat by vegetable oils can contribute to a healthier saturated/unsaturated fat balance in cheese (Yu and Hammond, 2000, Fathi Achachlouei *et al.*, 2013, Lobato-Calleros *et al.*, 2002, 2003, 2007), by lowering cholesterol levels and impacting human nutrition (Kesenkas *et al.*, 2009). Soybean is the dominant oilseed produced in the world, due to its favorable agronomic characteristics, high-quality protein, and valuable edible oil, contributing to the half of all oilseeds produced worldwide. Soybean oil's particular fatty acid composition is higher in linoleic acid and lower in linolenic acid, in comparison with the other major vegetable oils, both essential fatty acids for humans and therefore of dietary importance (Wang, 2002). In the same manner, soy protein ingredients possess appropriate functional properties for food applications due their high nutritional value and their ability to form and stabilize emulsions (Kinsella, 1979). In this view, since vegetable oils had been employed to replace fat in cheese elaborations, a viable alternative is to use an emulsion elaborated with soybean oil and soy protein isolate. The incorporation of polysaccharides to a protein solution improves the stability of oil droplets against creaming (Uruakpa and Arnfield, 2005). Polysaccharides such as carrageenans are widely employed in the dairy industry due to their specific reactivity with casein micelles (Drohan *et al.* 1997, Langendorff *et al.*, 1999, 2000). The incorporation of carrageenans in soybean oil/soy protein isolate emulsion, could, on one hand, results in a more stable emulsion during and after cheese processing, given that a synergistic effect between soy protein isolate and carrageenans had been reported (Molina Ortiz *et al.*, 2004). On the other hand, the presence of different types of carrageenans in emulsified soybean oil will affect the physicochemical and textural properties of panela-type cheese in a different way, due to reactivity between caseins and carrageenans. The aim of this work was to determine the effect of milk fat replacement with emulsified soybean oil, employing soy protein isolate and different carrageenans (iota, kappa or lambda) on the physicochemical, textural and FTIR spectroscopy of panela-type cheese.

2. MATERIALS AND METHODS

2.1. Emulsified soy oil and panela-type cheese elaboration

Soy protein isolate Appensol ISL (DVA Mexicana, Naucalpan, Mexico) was dissolved in distilled water (5% w/v) before being mixed with different types of carrageenans (1%, w/v): kappa-carrageenan Gelcarin GP8612, iota-carrageenan Viscarin SD389 or lambda-carrageenan Viscarin GP209 (FMC BioPolymers, Philadelphia, USA). To each protein-carrageenan suspension, Nutrioli® pure soy oil (Ragasa Industrial S.A de C.V., Monterrey, México) was incorporated in a proportion of 80:20 (v/v) and mixed employing an Oster homogenizer (Sumbean Mexicana, Mexico, 12,000 rpm) until obtaining a homogenous 'mayonnaise'. The emulsified oil was stored in plastic bags at −20 °C until use as a milk fat replacer.

Raw milk was obtained from dairy facilities of the Universidad Autonoma Estado Hidalgo at Tulancingo, Mexico. Milk (4.37% fat content) was filtered and pasteurized (63 °C for 30 min) then cooled at 42 °C. Part of the milk was skimmed in an Elecrem 3 cream separator (Fresnes, France) to a final milk fat content of 0.2%. Whole (full fat) and skimmed milk were mixed in order to obtain

TABLE 1. Milk fat replacement with emulsified soybean oil/soy protein isolate and carrageenans

Replacement (%)	Whole milk liters (4.37% fat)	Total fat in milk after skimming (g)	Skim milk liters (0.2% fat)	Emulsified soybean/soy protein isolate and carrageenans added to replace milk fat (g)	Total fat (%)
0	4	174.8	0	0	4.37
25	3	131.1	1	44.6	3.28
50	2	87.4	2	89.2	2.18
75	1	43.7	3	133.8	1.09

different milk fat proportions, incorporating the different emulsified soy oil formulations to replace and compensate for milk fat, according to Table 1. The different milk batches with different percentages of milk fat replaced with emulsified soybean oil/soy protein isolate and iota, kappa or lambda carrageenan were kept at a constant temperature of 40 °C and mixed before the addition of 0.01% v/v of Cuamex® rennet (Chr. Hansen de México S. A. de C. V., México). The curd was left to settle for 30 min and cut into 1 cm^3 cubes. The cut curd was then subjected to mild stirring during 30 min. The whey was drained and the upper layer of the whey and salt (0.8% w/v) were mixed with the curd. The curd was molded in 2 kg containers. The individual cheeses were pressed and the remaining whey was expelled. After 90 min, the cheeses were weighed to determine yield, and vacuum packed until further analysis. Each cheese formulation was elaborated in duplicate.

2.2. Cheese yield, moisture, fat phase and total protein content

Cheese yield (%) was calculated as the percent weight of the finished cheese divided by the weight of the milk employed for each batch (Drake *et al.*, 1996). Cheese moisture was determined according to the AOAC Official Method No. 926.08 (AOAC, 1998) employing aluminum pans (ca. 3 g of sample) dehydrated in an oven at 100 °C for 4 h. Fat content was determined by the acid butyric method of Van Gulik according to the ISO 3433 (ISO, 2011). Total protein content was determined in agreement with the AOAC Official Method 926 (AOAC, 1998) employing a conversion factor of 6.38. All analyses were performed in triplicate.

2.3. Textural profile analysis

The panela-type cheese texture analysis was performed in a texture analyzer LFRA 4500 (Brookfield Engineering, Middleboro, MA, USA). Samples were obtained from the middle of the whole cheese blocks (4x4 cm and 2 cm height) which were kept in plastic bags to avoid moisture loss at room temperature. The samples were consecutively compressed two times (30%) with a 7 cm diameter acrylic disk with a 5-second waiting period at a crosshead speed of 1 mm/s. From the force-deformation curves texture profile parameters were calculated as follows: hardness (force necessary to attain a given deformation, maximum force), adhesiveness (work necessary to overcome the attractive forces between the surface of the food and the surface of other materials with which the food comes in contact), cohesiveness (strength of the internal bonds making up the body of the product), springiness (degree to which a product returns to its original shape once it has been compressed), and resilience (capacity to recover its original shape after compression) (Szczesniak, 1963; Bourne, 1978). The results are the mean of at least three reproducible runs per cheese batch.

2.4. Fourier transformation infra-red spectroscopy analysis

A Fourier Transform Infrared spectrophotometer PerkinElmer model Spectrum GX (Perkin Elemer Inc, Walthman, MA, USA) equipped with a horizontal attenuated total reflection accessory and diamond point was employed to determinate changes in cheeses composition. Sixty-four scans were coded at a nominal resolution of 4 cm^{-1} in the spectral region 4000–550 cm^{-1}. Single beam spectra of the samples were collected against a background of air and presented in absorbance units. The cheese samples were placed in the HATR accessory to give total crystal coverage, cleaning the crystal between samples.

2.5. Experimental design and data analysis

In order to determinate the effect of milk fat replacement in panela-type cheese employing emulsified soy oil, a complete factorial design was employed. The proposed model for the data was:

$$y_{ij}=\mu+\alpha_i+\beta_j+\epsilon_{ij} \qquad \text{Eq. (1)}$$

where y_{ij} represents the variable response for the i-th level of fat replacement (0, 25, 50 and 75%), at the j-th type of carrageenan (kappa, iota or lambda); μ is the overall mean; α_i and β_j are the main effects of the level of substitution and carrageenan type; and $\in_{ij}$ is the residual or error terms assumed to be normally distributed with zero mean and variance σ^2 (Der and Everitt, 2002). The results were analyzed according to the PROC ANOVA procedure in SAS Software v 8.0 (SAS System, Cary). Significant differences means were determined by the Duncan means test.

3. RESULTS

3.1. Yield, moisture, fat phase and protein

The use of emulsified oil to replace milk fat resulted in a significantly ($p<0.05$) higher panela-type cheese yield, where a major proportion of replacement (up to 75%) resulted in higher yield values. In same manner, emulsified oil with lambda carrageenan increased the cheese yield significantly ($p<0.05$) (Table 2). Higher yield values were related to an increase in cheese moisture content and retention of this water into the coagulated system. Panela-type cheese moisture was significantly ($p<0.05$) higher when milk fat was replaced with emulsified oil above 50%. Lambda carrageenan containing emulsions resulted in significantly ($p<0.05$) higher cheese moisture contents (Table 2). As fat was replaced with emulsified oil, the fat phase decreased in the panela-type cheese. Replacing 75% of milk fat with emulsified oil resulted in significantly ($p<0.05$) lower fat content, with higher fat content in control (full-fat) samples. For the carrageenan type, emulsified oil with lambda carrageenans resulted in significantly ($p<0.05$) lower percentages of fat in the cheese (Table 2). The replacement of milk fat with emulsions made with soy protein isolate increased the protein content in the cheese. Total protein was significantly ($p<0.05$) higher when 75% of milk fat was replaced with emulsified oil. Lower protein content was obtained in control samples. For the carrageenan type, kappa and iota carrageenan treatments resulted in significantly ($p<0.05$) higher protein contents (Table 2).

3.2. Texture profile analysis

For cheese hardness, the replacement of 25 or 50% milk fat with emulsified soybean oil resulted in a significantly ($p<0.05$) harder texture. In the same way, the panela-type cheese texture was significantly ($p<0.05$) harder when iota or kappa carrageenan was employed in the emulsified oil (Table 3). Panela-type cheese adhesiveness was not significantly ($p>0.05$) different for the percent of milk fat replacement with emulsified oil. For the carrageenan type, the emulsified oil containing iota or kappa carrageenan was significantly ($p<0.05$) more adhesive (Table 3).

In contrast, elastic related textural parameters (cohesiveness, springiness and resilience) were reduced when emulsified oil was employed to replace milk fat. The cohesiveness of cheeses was significantly ($p<0.05$) lower when milk fat was replaced. In same manner, incorporating iota or kappa carrageenan to cheese elaboration significantly ($p<0.05$) decreased cheese cohesiveness (Table 3). The springiness of the panela-type cheese was significantly ($p<0.05$) lower when emulsified oil replaced milk fat. Iota or kappa carrageenan in emulsified soybean oil employed to replace milk fat decreased cheese springiness significantly ($p<0.05$) as well (Table 3). Significantly ($p<0.05$) lower resilience values were also observed when emulsified soybean oil replaced milk fat. Iota or kappa carrageenan in emulsified oil decreased sample resilience significantly ($p<0.05$) (Table 3).

TABLE 2. Physicochemical properties of fat-reduced panela-type cheese employing emulsified oil with carrageenans

Carrageenan type in emulsified soybean oil/soy protein isolate	Milk fat substitution (%)	Yield (%)	Moisture (%)	Fat (%)	Total protein (%)
Control	0	16.41±0.00^{d,C}	56.84±2.03^{c,C}	30.40±0.21^{a,A}	12.41±0.30^{c,C}
Iota	25	17.18±0.00^{c,B}	57.88±2.51^{b,B}	27.00±1.20^{b,B}	12.39±0.20^{c,A}
	50	17.47±0.17^{b,B}	58.71±2.25^{a,B}	26.50±0.60^{c,B}	11.83±0.14^{b,A}
	75	17.60±0.00^{a,B}	58.92±1.91^{a,B}	25.50±0.25^{d,B}	14.11±0.03^{a,A}
Kappa	25	15.50±0.00^{c,B}	58.74±1.95^{b,B}	27.20±0.10^{b,B}	12.48±0.36^{c,A}
	50	15.40±0.00^{b,B}	59.14±2.03^{a,B}	26.80±0.30^{c,B}	13.54±0.31^{b,A}
	75	16.22±0.00^{a,B}	59.81±2.38^{a,B}	25.64±0.30^{d,B}	13.41±0.05^{a,A}
Lambda	25	16.41±0.00^{c,A}	59.62±2.52^{b,A}	26.90±1.20^{b,C}	12.60±0.18^{c,B}
	50	17.70±0.00^{b,A}	60.40±2.12^{a,A}	25.85±1.73^{c,C}	13.65±0.17^{b,B}
	75	19.08±0.00^{a,A}	60.72±1.71^{a,A}	25.18±0.30^{d,C}	12.21±0.17^{a,B}

^{a,b,c,d}Means with the same letter in the same column are not significantly ($p>0.05$) different for the percent of milk fat substitution.
^{A,B,C}Means with the same letter in same column are not significantly ($p>0.05$) different for the carrageenan type.

TABLE 3. Textural profile analysis of fat-reduced panela-type cheese employing emulsified oil with carrageenans

Carrageenan type in emulsified soybean oil/soy protein isolate	Milk fat substitution (%)	Hardness (N)	Adhesiveness (N)	Cohesiveness (dimensionless)	Springiness (dimensionless)	Resilience
Control	0	$31.40\pm0.82^{b,C}$	$0.75\pm0.40^{a,B}$	$0.39\pm0.03^{a,A}$	$0.80\pm0.01^{a,A}$	$0.76\pm0.01^{a,A}$
Iota	25	$45.19\pm3.83^{a,A}$	$0.70\pm0.10^{a,A}$	$0.38\pm0.01^{b,C}$	$0.77\pm0.01^{b,C}$	$0.74\pm0.00^{b,C}$
	50	$45.81\pm8.43^{a,A}$	$0.75\pm0.13^{a,A}$	$0.34\pm0.04^{c,C}$	$0.75\pm0.00^{c,C}$	$0.72\pm0.02^{c,C}$
	75	$27.87\pm4.50^{b,A}$	$0.74\pm0.77^{a,A}$	$0.37\pm0.02^{c,C}$	$0.75\pm0.00^{c,C}$	$0.67\pm0.02^{d,C}$
Kappa	25	$41.13\pm2.17^{a,A}$	$0.80\pm0.17^{a,A}$	$0.34\pm0.01^{b,C}$	$0.76\pm0.01^{b,C}$	$0.71\pm0.02^{b,C}$
	50	$29.30\pm1.52^{a,A}$	$0.79\pm0.16^{a,A}$	$0.31\pm0.01^{c,C}$	$0.75\pm0.00^{c,C}$	$0.64\pm0.03^{c,C}$
	75	$39.25\pm2.93^{b,A}$	$0.76\pm0.17^{a,A}$	$0.26\pm0.02^{c,C}$	$0.75\pm0.03^{c,C}$	$0.59\pm0.04^{d,C}$
Lambda	25	$27.80\pm1.21^{a,B}$	$0.66\pm0.08^{a,C}$	$0.37\pm0.03^{b,B}$	$0.80\pm0.02^{b,B}$	$0.75\pm0.00^{b,B}$
	50	$39.63\pm0.89^{a,B}$	$0.67\pm0.05^{a,C}$	$0.39\pm0.01^{c,B}$	$0.73\pm0.02^{c,B}$	$0.75\pm0.01^{c,B}$
	75	$32.16\pm2.27^{b,B}$	$0.70\pm0.05^{a,C}$	$0.39\pm0.03^{c,B}$	$0.74\pm0.04^{c,B}$	$0.74\pm0.02^{d,B}$

^{a,b,c,d} means with same letter in same column are not significantly ($p>0.05$) different for the percent of milk fat substitution.
^{A,B,C} means with same letter in same column are not significantly ($p>0.05$) different for the carrageenan type.

3.3. Fourier transformation infra-red spectroscopy analysis

Figure 1 shows the MIR spectra for the different panela-type cheeses formulated with the different milk fat substitutions (0, 25, 50 and 75%) for each emulsified oil containing the different carrageenan types (iota, kappa and lambda). Arrows indicate intensity of absorption bands as a result of the increase in milk fat substitution by emulsified oil. MIR spectra present differences in the absorbance magnitude for each one of the panela-type cheese samples, since each band or spectral peak is directly related to the substitution and carrageenan type (iota, kappa or lambda). This reflects the changes in cheese chemical composition for the different milk fat replacement and the different carrageenans employed in emulsified oil formulation, contrasting the differences at the 2100–950 cm^{-1} region for iota (Fig. 1-a), kappa (Fig. 1-b) or lambda (Fig. 1-c) carrageenans.

4. DISCUSSION

4.1. Yield, moisture, fat phase and protein

The replacement of milk fat with emulsified oil affected the physicochemical properties of panela-type cheese. The main effect was regarding water retention improvement in the coagulated cheese curd. Since soybean oil emulsions were emulsified and stabilized with soy protein isolate and carrageenans, the hydration properties and interactions of both theese macromolecules with caseins (before, during and after being rennet coagulated) seems to be the explanation for this behavior, resulting in higher yield due to higher moisture retention.

On one hand, the use of emulsified soybean oil implies the presence of additional water in the system, along with proteins and polysaccharides that can retain water in the system and/or interact with milk proteins during cheese processing. Giroux *et al.* (2013) reported that higher moisture content was found in model cheese made from double emulsions, resulting in the incorporation of larger oil droplets, increasing the openness of the cheese matrix and creating more interstitial space, providing a reservoir for retaining additional moisture in cheese. Fat globules are occluded in the paracasein network pores of the cheese, physically limiting the aggregation of the surrounding paracasein network, reducing protein matrix contraction and moisture expulsion (Fox *et al.*, 2000; Lobato-Calleros *et al.*, 2007). Milk fat replacement is related to the generation of larger interstitial spaces, due to larger oil droplets, resulting in higher moisture retention. The moisture content of cheese was inversely proportional to milk fat content, and milk fat content reduction in the cheese increased moisture content (Romeih *et al.*, 2002). In addition, the more retained water resulted in higher cheese yield in samples with emulsified oil replacing milk fat. In the same manner, the control full-fat samples had higher fat contents than cheese made with emulsion since emulsions contained a lower true fat fraction, resulting in lower fat content and higher protein content (Giroux *et al.*, 2013). When more emulsified oil was incorporated to replace milk fat, a higher protein content was observed due to the soy protein isolate employed to formulate the emulsions. Higher protein content in low fat cheese increased the water binding capacity of the cheese matrix (Fathi Achachlouei *et al.*, 2013). The addition of a fat replacer increased the cheese yield probably due to its higher moisture retention ability (Sahan *et al.*, 2008). The presence of both soy

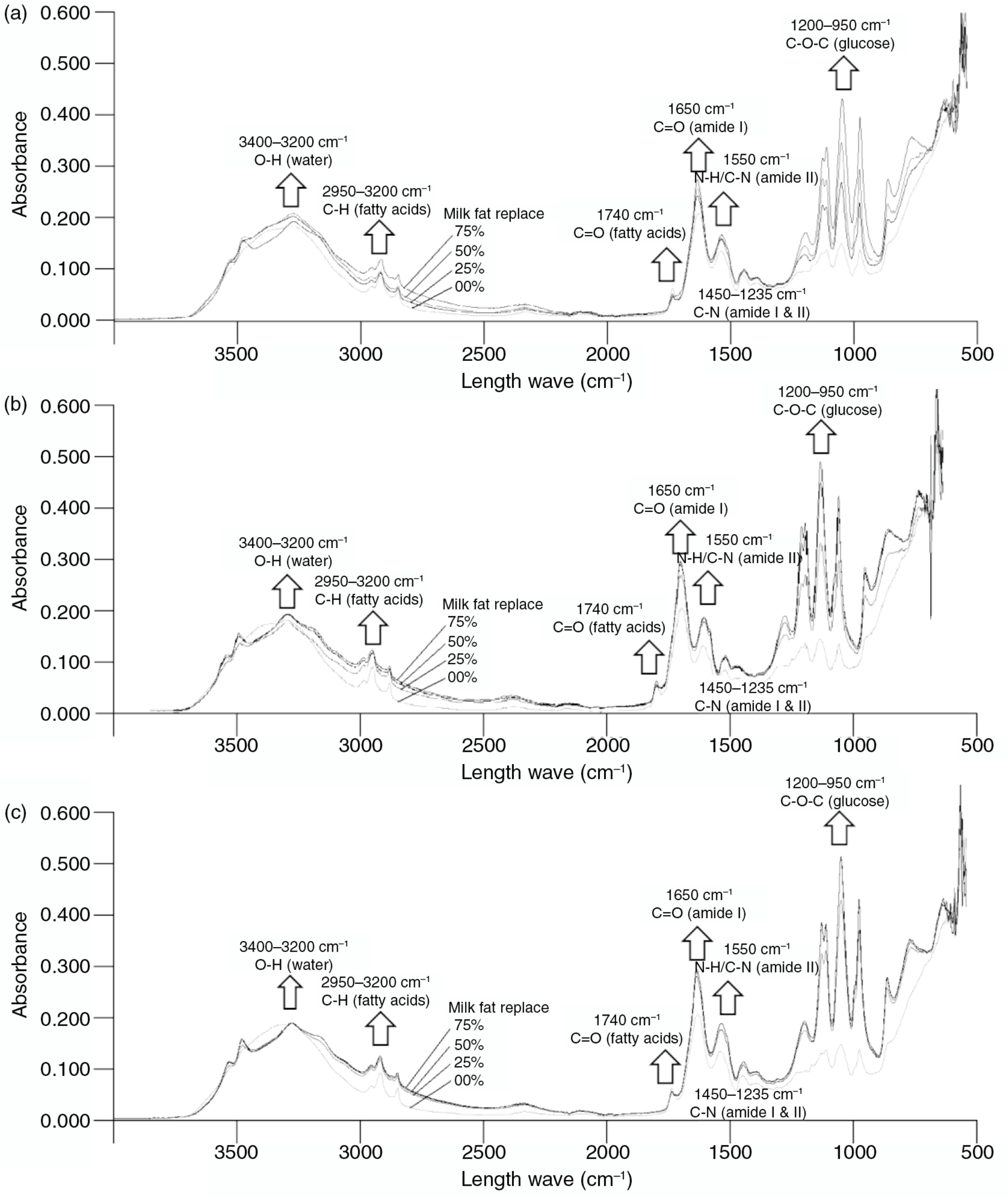

FIGURE 1. FTIR spectra for the different panela-type cheeses formulated with emulsified soybean oil/soy protein isolate and (a) iota-carrageenan, (b) lambda-carrageenan, and (c) kappa-carrageenan.

protein isolate and carrageenans helped to increase water retention and yield as well as protein content.

On the other hand, the associative interaction between carrageenans and casein micelles depends on the carrageenan's conformation. At neutral pH, kappa and iota carrageenans in helical conformation can stabilize milk proteins at a low concentration since the addition of carrageenan affected the formation of rennet-induced gels, mainly attributed to electrostatic interactions between kappa casein the positive patch and negative sulfate groups of carrageenans (Snoeren *et al.*, 1976; Thaiudom and Goff, 2003; Gu *et al.*, 2005; Corredig *et al.*, 2011; Wang *et al.*, 2014). Iota, kappa and lambda carrageenans adsorb onto casein micelles forming a cross-linking network below the coil-helix transition temperature (60 °C), probably due to the bridging by the helical parts of carrageenan chains (Dalgleish and Morris, 1998; Langendorff *et al.*, 2000; Gu *et al.*, 2005). Carrageenan conformation results in differences between adsorption behaviors due to different charge densities. Both charge density (lambda>iota>kappa) and polysaccharide conformation contribute to the structure forming event (Gu *et al*., 2005; Wang *et al*., 2014). Kappa and iota are in the helix form at ambient temperature, whereas lambda is in the random coil conformation (Nilsson and Piculell, 1991; Gu *et al*., 2005). Kappa carrageenan adsorption onto casein micelle is thermally reversible, whereas iota-carrageenan adsorption onto casein micelle is irreversible (Čĕrníková *et al*., 2008). The most charged lambda carrageenan does not show transition from coil to helix, being that it is a non-gelling carrageenan (Nilson and Piculell, 1991; Corredig *et al*., 2011).

4.2. Texture profile analysis

In general, fat replacement resulted in a harder and less ductile texture, where higher moisture retained in the cheese structure (as yield related to weight gain) and lower fat content affected texture. Substitution of part of the milk fat with non-milk fat modified textural properties of the processed cheese since fat globule size increased with the decrease in the distribution uniformity within the protein matrix (Cunha *et al*., 2010). Cheese structure is altered with a decrease in fat content, and lower-fat cheese has a more compact protein matrix with less open spaces than full-fat cheese since interstitial spaces are occupied by fat globules. When fat content is reduced, longer areas of uninterrupted protein matrix with less uniformly dispersed fat globules are formed (Guinee and McSweeney, 2006). This is associated with hard texture even when the moisture content is high (Gunasekaran and Ak, 2000). Fat reduction in fresh white cheese resulted in more complete protein zones comprising the protein structure, increasing the degree of protein molecules cross-linking in the three dimensional network, and increasing resistance to deformation (Lobato-Calleros *et al*., 2007). In same manner, cheese elaborated with milk fat was more cohesive than the vegetable fat blend cheese (Dinkçi *et al*., 2011), and cheese cohesiveness decreases with fat content (Gunasekaran and Ak, 2000). It has been reported that the addition of palm oil decreased cheese hardness, probably due to the presence of fatty acids with lower melting point (Cunha *et al*., 2010). Some fat mimics have been found to enhance the uniformity of fat distribution in reduced-fat cheese (Drake *et al*., 1996). Differences in texture could be attributed to the interactions of hydrocolloids with the rest of the matrix besides the ability of the given hydrocolloids to form gels (as in the case of kappa and iota-carrageenan, forming harder cheeses, followed by lambda) (Hanáková *et al*., 2013). An increasing concentration of kappa and iota carrageenans (more emulsified oil replacing milk fat) enhances interactions between carrageenan chains and leads to the formation of a denser network structure which increases the rigidity of processed cheese (Čĕrníková et al., 2008). In panela-type cheese at the experimental conditions employed, milk fat substitution resulted in a harder and less cohesive texture.

4.3. Fourier transformation infra-red spectroscopy analysis

In the MIR spectra of panela-type cheese formulated with different percentages of milk fat substitution with emulsified soybean oil/soy protein isolate with iota, kappa or lambda carrageenans, the same typical absorption bands can be observed but with different magnitude, reflecting changes in the composition of panela-type cheese. Changes in cheese IR spectra had been employed to determine changes in its composition, presenting several typical peaks and the assignments different wavelength ranges for the contributions of the hydroxyl groups, acids, esters, amide I and amide II, aliphatic chains of fatty acids and acidic amino acids, at specific regions: 3873–3000 cm^{-1} for O–H stretching modes of water absorbing, $-$C$-$H stretching in fatty acids (3000–2800 cm^{-1}), $-$C=O of acids and esters (1750–1650 cm^{-1}), amide I and amide II of proteins (1650–1450 cm^{-1}), esters and aliphatic chains of fatty acids (1460–1150 cm^{-1}) and C=O and C$-$C stretching of acids (1200–800 cm^{-1}), respectively (Cuibus *et al*., 2014). As can be observed, in the 1200–950 cm^{-1} region, bands seem to be closer with tension vibration of C–O–C bonds, a characteristic vibration attributed to carbohydrate content (Al-Jowder *et al*., 1999). The higher the percent of milk fat substitution (25–75%), the higher the bands' magnitude. This is a higher amount of C–O–C functional groups (from iota, kappa or lambda carrageenans) incorporated into cheese (by the emulsified oil replacing milk fat). In the same manner, the absorbance of C–O (~1175 cm^{-1}) and C=O (~1750 cm^{-1}) of the ester bonds of triacylglycerols and the acyl chain C–H (3000–2800 cm^{-1}) are commonly used to determine fat. The infra-red bands appearing in the 3000–2800 cm^{-1} region are particularly useful because they are sensitive to the content, the conformation and the packing of the triglycerides (Casal and Mantsch, 1984; Dufour and Riaublanc, 1997). Since the milk fat replacement with 25% of emulsified oil spectra is different to that with 75% of milk fat replacement, the differences in the spectra can be employed to identify carrageenan addition to cheese.

5. CONCLUSIONS

Fat content influences the volume and continuity of the casein matrix, which is interrupted by fat globules. When milk fat was replaced by emulsified soybean oil, structural changes resulted in an increase in moisture enhanced by the soy protein isolate and carrageenan-casein interactions retaining more water (decreasing curd volume due to water expelling). The panela-type cheese texture resulted as expected in a harder but less plastic structure, i.e., although a harder cheese was obtained, the lower cohesiveness, springiness and resilient values compensate the texture profile. Milk fat replacement changed the fatty acid profile, where the type of carrageenan also affected the MIR spectra. Lambda carrageenan containing emulsions improved moisture retention and maintained the textural properties of panela-type cheese.

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