

MICROSTRUCTURE AND TEXTURE OF PANELA TYPE CHEESE-LIKE PRODUCTS: USE OF LOW METHOXYL PECTIN AND CANOLA OIL AS MILK-FAT SUBSTITUTES

MICROESTRUCTURA Y TEXTURA DE PRODUCTOS IMITACION DE QUESO PANELA: USO DE PECTINA DE BAJO METOXILO Y ACEITE DE CANOLA COMO SUSTITUTOS DE LA GRASA LACTEA

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Abstract

Three Panela type white fresh cheese-like products (PCLP) were prepared using skim milk incorporated with 6.8 g L⁻¹ of emulsified canola oil (CO) and different concentrations of low methoxyl pectin (P): 0, 1.25 and 2.5 g L⁻¹. In addition, full-milk fat (FFC) and low-milk fat (LFC) Panela type cheeses were manufactured from milk containing 27 and 6.8 g L⁻¹ of milk-fat (MF), respectively. After 72 h of storage (4 °C), the cheese systems were subjected to proximal chemical analysis, instrumental texture determination and microstructure observation using Scanning Electron Microscopy. The cheese systems showed differences in their chemical composition, structural arrangement and textural characteristics, due to chemical composition variation of the milk from which they were elaborated. Complete substitution of MF by emulsified CO, resulted in cheese-like products having a highly porous protein network, interrupted by numerous small drops of oil. P contributed to the openness of the protein network of the cheese-like products conferring it the ability to retain greater initial amounts of whey in comparison with those retained into the protein network of the FFC. Polynomial models that estimated the textural characteristics of the cheese systems as a function of P, CO and MF concentrations in the milk used to prepare them. Increases in the concentration of P, CO and MF were related with lower values of hardness and chewiness, whilst lower springiness values in the cheese systems were associated with increasing of the P and MF concentrations. The cohesiveness of the cheese systems was negatively affected by CO.

Keywords: white fresh cheese, cheese-like products, fat substitutes, cheese microstructure, oil-in-water emulsions.

Resumen

Se prepararon tres productos imitación de queso tipo Panela (PCLP) a partir de leche descremada incorporada con 6.8 g L⁻¹ de aceite de canola (CO) emulsionado y diferentes concentraciones de pectina de bajo metoxilo (P): 0, 1.25 y 2.5 g L⁻¹. Adicionalmente se elaboraron como referencias, un queso tipo Panela completo en grasa láctea (FFC) y uno bajo en grasa láctea (LFC), a partir de leche conteniendo 27 y 6.8 g L⁻¹ de grasa láctea (MF), respectivamente. Los sistemas de queso, después de 72 h de almacenamiento (4 °C), fueron sometidos a análisis químico proximal, determinación instrumental de textura y observación microestructural mediante Microscopía Electrónica de Barrido. Los sistemas de queso mostraron diferencias en su composición química, arreglo estructural y características texturales, debido a variaciones en la composición de la leche usada en su preparación. La sustitución completa de MF por CO, resultó en productos tipo queso con una matriz proteínica altamente porosa, interrumpida por numerosas gotas pequeñas de aceite. P, promovió la abertura de la red proteínica de los productos tipo queso, confiriéndole la habilidad de retener mayores cantidades iniciales de lactosuero, en comparación con aquellas retenidas en la red proteínica del FFC. Se obtuvieron modelos polinomiales que estiman las características texturales de los sistemas de queso, en función de las concentraciones de MF, CO y P presentes en la leche usada para su elaboración. Aumentos en las concentraciones de P, CO y MF, estuvieron relacionados con disminuciones de los valores de dureza y masticabilidad; mientras que valores de elasticidad más bajos en los sistemas de queso, estuvieron asociados con incrementos en las concentraciones de P y MF. La cohesividad de los sistemas de queso fue afectada negativamente por CO.

Palabras clave: queso tipo panela, productos imitación queso, sustitutos de grasa, microestructura de queso, emulsiones aceite-en-agua.

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1. Introduction

One of the most popular cheeses in Mexico is a white fresh cheese known as "Panela" due to its mild fresh-milk slightly salty flavor (NOM-F-1987), and because it is considered as "healthy" by the consumers due to its low fat content. The production of Panela type cheeses was 14 227 Ton and represented 12 % of the total cheese production in the year 2004 in Mexico (SIAP, 2004). Because these type of cheeses are metastable systems, pronounced changes in moisture content occur over time (Vega, 1997), so that manufacturers frequently use water binders, such as gums and starches during their production, in order to prevent or reduce the syneresis phenomenon and contribute to achieve an acceptable product texture and body (Lobato-Calleros et al., 2000). Likewise, changes in the chemical composition of these cheeses has been proposed, specially those regarding the fat content, due to the hypercholesterolemic effect of milk-fat, associated cardiovascular diseases (Ney, 1991; Sloan, 2003). Nevertheless, the reduction of milk-fat in cheeses results in products that exhibit considerable higher mechanical-sensory characteristics, such as hardness, springiness, chewiness and cohesiveness, than those found in their full milk-fat counterpart (Bryant et al., 1995). The modifications in the mechanical behavior of reduced fat cheeses has been attributed to the formation of a protein network that is relatively closed and compact due to higher protein density, in contrast to the more open, relaxed, highly interrupted by milk-fat globules protein network occurring in a full milk-fat cheese (Bryant et al., 1995; McMahon and Oberg, 1998; Lobato-Calleros et al., 2001). When developing new products, it is of the upmost importance that the mechanical properties of these products resemble as closely as possible those of the products they seek to displace from the market, so that they will be acceptable to the consumer (Drake et al., 1996; Lobato-Calleros et al., 2001). The use of fat substitutes has been proposed in order to improve the mechanical characteristics of reduced fat cheeses (Clark, 1994; McMahon et al., 1996; Bhaskaracharya and Shan, 2001). These ingredients, such as low methoxyl pectin, are polar, water soluble compounds exhibit that functional characteristics, such as high water binding capacity, that favor the formation of more open and relaxed protein networks in reduced fat cheeses (Lobato-Calleros et al., 2001). However, the functionality of fat substitutes is limited and best results are achieved when substitution of milk-fat is only partially done in cheeses (Bruhn et al., 1992). Recent researches have explored the possible total substitution of milk-fat by polyunsaturated vegetable oils so as to achieve functional products with healthier saturated/unsaturated fatty acid balance that may contribute to improve consumers' well being (Yu and Hammond, 2000). The substitution of milkfat by polyunsaturated vegetable oils in a 1:1 ratio results in cheeses with lower mechanical characteristics than those exhibited by the full-milk fat cheese, probably due in part to the liquid state and lower melting point of the vegetable oils ((Lobato-Calleros et al., 2002). This behavior is in opposition to that occurring when milk-fat is reduced in cheeses, without compensating this reduction with other type of ingredient.

The aim of this work was to manufacture Panela cheese-like products in which the milk-fat was substituted by either emulsified canola oil or by a blend of emulsified canola oil and low methoxyl pectin in different ratios, and to evaluate the effect of these substitutions on the chemical composition, microstructure and instrumental textural characteristics when compared with full-milk fat and reduced milk-fat cheeses.

2. Materials and methods

2.1. Cheese formulations

Five Panela type white fresh cheeses were manufactured: (1) A full-milk fat cheese (FFC) made from milk containing 27 g L⁻¹ milk-fat (MF); (2) A low-fat cheese (LFC) made from milk containing 6.8 g L⁻¹ MF, and (3) three Panela cheese-like products (PCLP)_{canola oil, pectin}, from skim milk (0.1 g MF L⁻¹), incorporated with 6.8 g L⁻¹ of emulsified canola oil (C) (Capullo®, Unilever de Mexico, S.A de C.V) and different quantities (0.00, 1.25 and 2.50 g L⁻¹) of low methoxyl pectin (P) (Slendid 200®, Copenhagen Pectin A/S, Division de Quimica Hercules, Mexico, S.A. de C.V.) (Table 1).

Table 1. Canola oil and low methoxyl pectin content of the different Panela cheese-like products.

Cheese code (PCLP) _{canola}	(g CO L ⁻¹ skim milk)	(g P L ⁻¹ skim milk)
PLCP _{6.8, 0.0}	6.8	0.00
PLCP _{6.8, 1.25}	6.8	1.25
PLCP _{6.8, 2.50}	6.8	2.50

CO: canola oil; P: low methoxyl pectin.

2.2. Preparation of cheese milk formulations

All of the cheese milk formulations were prepared from raw milk (37 g L⁻¹ MF) provided by the Unidad de Tecnologia (UTL). the Universidad of Autonoma Chapingo. The MF content of raw milk was standardized in accordance to the cheese being manufactured: 27 g L⁻¹ for FFC, 6.8 g L⁻¹ LFC and 0.1 g L⁻¹ for PCLP_{canola oil}, pectin. Likewise, a canola oil-in-skim milk (0.1 g L⁻¹ MF) emulsion, and a low methoxyl pectin in distilled water dispersion (3.75% weight) were prepared, and afterwards added to the respective standardized milks in the

requisite amounts, in order to achieve the desired concentrations of MF, CO and/or P in the milk cheese required for manufacturing the different PCLP_{canola oil, pectin} (Table 1). Each 100 g of emulsion contained 10.2 g of CO, 88.68 g skim milk and 1.12 g of low molecular weight emulsifiers mixture made up by sorbitan monostearate (SM) and glycerol monostearate (GM) both lipophilic in nature, and polyoxyethylene sorbitan monostearate (PSM) hydrophilic in nature (Canamex, S.A de C.V., Mexico), in weight proportions of 0.5PSM-0.2SM-0.3GM. The CO-in-skim milk emulsion (60 \pm 1 °C) was added drop-wise to the standardized milk (60 ± 1 °C with the help of an Ultra-Turrax ® T50 basic homogenizer (IKA ® Works, Inc., Wilmington, USA) operated at 4000 rpm during 5 min.

Each 100 g of P were prepared by adding 3.75 g of pectin to 96.25 g distilled water (20 \pm 2 °C), with vigorous stirring using a CAFRAMO mechanical stirrer (model RZR1, Cole-Parmer, Vernon Hills, IL, USA) until a homogeneous dispersion was obtained. Then the dispersion was heated up to 75 \pm 1 °C, during 10 min and let to cool for 24 h at room temperature, in order to achieve complete hydration of the P.

2.3. Cheese manufacture

All cheeses were manufactured from 15 L batches of their respective cheese milk formulations using a complete randomized design and were done in triplicate. The procedure followed to manufacture the cheeses was in all cases the same: i) cheese milk was pasteurized (63 \pm 1 °C, 30 min); ii) cooled down to 36 \pm 1 °C; iii) 0.15 g L⁻¹ of calcium chloride were added (previously dissolved in distilled water); iv) cheese milk coagulation (36 \pm 1 °C, 30 min) was done by adding 0.15 mL L⁻¹ rennet (chimosin, strength 1:10 000, Cuamex, S.A. de C.V., Mexico) diluted in 50 mL distilled water; v)

curd cutting into cubes ~ 1 cm³; vi) mild stirring of curd during 5 min; vii) curd grain left to stand (10 min); viii) elimination of around 50 % whey; ix) curd salting (13 g salt L⁻¹ milk); x) curd left to stand (10 min); xi) dewheying; xii) moulding of curd in 1 kg containers; xiii) cheeses let to stand (20 \pm 2 °C, 30 min); xiv) turn around and dewheying of cheeses (20 \pm 2 °C, 2 h); xv) withdrawal of cheeses from moulds and maintenance 20 ± 2 °C, 2 h; and xvi) packaging of cheeses in polyethylene bags thermally sealed. Cheeses kept under refrigeration at 4 ± 0.5 °C during 3 days until required for characterization for chemical analyses: fat, crude protein and pH and moisture content after 3, 6 and 9 days of manufactured (AOAC, 1995).

2.4. Cheese microstructure

Cheese microstructure was studied by scanning electron microscopy (SEM) using cylindrical samples (0.5 cm diameter) taken from the central part of the cheeses with a borer and adjusting their height to ~ 1 cm with a razor. Samples were prepared as indicated by Lobato-Calleros et al. (1998), i.e., by fixing them in a 2 % glutaraldehyde solution in 2 % phosphate buffer (0.1 M, pH 7.2) for 6 h, dehydration by immersion in aqueous ethanol solutions of increasing concentration (50, 60, 70, 80, 90 and 100 %, 60 min in each), and immersion in acetone for 1 h. Samples were critical point dried (Sample Drying at Critical Point Samdri 780 A; Tousimis, Rockville Research Corporation, MD, USA) and covered with gold (Fine Coat Ion Sputter JFC 1100, Jeol Ltd., Akishima, Japan). Samples were then observed with a high vacuum JMS-35 Jeol scanning electrón microscope (Jeol Ltd., Akishima, Japón), at 15 KV and magnification of 2 000 X.

2.5. Textural characteristics

The textural characteristics of the cheeses (Szczesniak, 1963) were determined

with Stable Micro Systems Mod. TA-XT2i Texturometer (Texture Technologies Corp., White Plains, N.Y.), using Texture Profile Analysis (TPA) (Bourne, 1982). Cylindrical samples were obtained from the center of cheeses with help of a 0.5 inch borer, and their height was adjusted to ~ 1.1 cm with a razor, at day 3 after manufacture. Samples were put into Petri dishes and kept at room temperature (20 \pm 2 °C) for 2 h. Afterwards compressed 50%, in two cycles, using the P50 probe and a crosshead speed of 120 mm min⁻¹. Each repetition of the cheeses was analyzed in triplicate. The values of the textural characteristics of hardness, springiness, cohesiveness and chewiness of the cheeses were obtained with the equipment software.

2.6. Data analyses

Chemical composition and textural characteristics data of the cheeses were subjected to one way analysis of variance, and when appropriate to Tukey's means comparison test. Ascending multiple regression analysis was used to determine polynomial models that estimated the cheeses textural characteristics values in terms of MF. CO and P contained in cheese milk. Data carried analysis was out using StatgraphicsPlus 5.1 (Statistical Graphics Corp. Manugistics, Inc., Cambridge, M.A., USA) software package.

3. Results and discussion

3.1. Chemical composition

All the cheeses showed significant differences in their fat, protein and moisture contents (p≤0.05) (Table 2), due to variations in the cheese milk composition from which they were made. FFC had significant higher fat content than the rest of the cheeses as it was made from cheese milk with 27 g MF L⁻¹ compared to 6.8 g MF L⁻¹ in the LFC and 6.8 g CO L⁻¹ in the PCLP_{canola oil, pectin}. FFC had a

lower protein and moisture content ($p \le 0.05$) than the PLCP_{canola oil, pectin} (Table 2). Reduction in the fat content of different cheese types such as "pasta filata" (Pagliarini y Beatrice, 1994); Cheddar (Drake et al., 1996) and Manchego (Lobato-Calleros et al., 2001), has been related to percent increases in their moisture and protein content. PLCP_{6.8, 1.25} and PLCP_{6.8, 2.50}, that included P in their formulation, showed a significant higher moisture content ($p \le 0.05$) than the rest of the cheeses after 3 days of manufacture (Table 2). However, moisture content in both cheeses containing pectin experienced a moisture content decay after 6 and 9 days so that no time. significant differences in moisture content (p>0.05) were observed with the other two low fat cheeses that did not include pectin in their formulation: PLCP_{6.8, 0.0} and LFC (Table 2). Low methoxyl pectin in the presence of calcium ions has the ability to form gelled structures, which bind large amounts of water equivalent to 50 fold-times their weight (Singhal et al., 1991). Nevertheless, during storage time fresh cheeses experience syneresis due to a reordering of their protein network, which results in expelling of considerable large amounts of whey (Lobato-Calleros et al., 2000). This phenomenon probably occurred as well in the pectin gel structure. The lower protein content (p ≤ 0.05) in the PLCP_{6.8, 1.25} and PLCP_{6.8, 2.50} compared to that of PLCP_{6.8, 0.0}, can be explained in terms of the higher moisture contents of the cheeses at day 3 when analyses were performed.

Table 2. Chemical composition (means \pm SD, n = 9) of cheeses.

Cheese code*	Fat (%)	Protein (%)	Moisture (3 days)	Moisture (6 days)	Moisture (9 days)
FFC	15.7 ± 0.4^{b}	15.7 ± 0.1^{a}	60.5 ± 0.1^{a}	56.6 ± 1.8^{a}	54.3 ± 1.8^{a}
LFC	3.8 ± 0.2^a	20.9 ± 0.2^{d}	65.6 ± 0.5^{b}	61.4 ± 0.7^{ab}	59.5 ± 1.2^{b}
PLCP _{6.8, 0.0}	3.9 ± 0.2^a	19.4 ± 0.3^{c}	66.2 ± 1.0^{b}	60.7 ± 0.9^{ab}	59.0 ± 0.3^{b}
PLCP _{6.8, 1.25}	4.0 ± 0.0^{a}	18.3 ± 0.6^{b}	68.8 ± 0.8^{c}	64.5 ± 1.3^{b}	61.0 ± 1.7^{b}
PLCP _{6.8, 2.50}	3.9 ± 0.1^a	18.4 ± 0.3^{b}	68.9 ± 0.4^{c}	63.4 ± 3.0^{b}	61.2 ± 1.9^{b}

^{*} In (PCLP)_{canola oil, pectin}: CO: canola oil; P: low methoxyl pectin; a,b,c,d,e: different superindexes in the same column indicate that means are significantly different ($p \le 0.05$).

Table 3. Textural characteristics (means \pm SD, n = 9) of cheeses.

Cheese code*	Hardness (N)	Springiness (mm)	Chewiness (N.mm)	Cohesiveness (Dimensionless)
FFC	7.7 ± 0.6^{b}	2.8 ± 0.0^{a}	17.2 ± 1.3^{b}	0.8 ± 0.0^{c}
LFC	13.5 ± 0.2^{e}	3.8 ± 0.1^{c}	39.0 ± 1.9^{e}	$0.8 \pm 0.0^{\rm c}$
PLCP _{6.8, 0.0}	11.8 ± 0.2^d	5.2 ± 0.1^d	29.2 ± 2.1^d	0.5 ± 0.1^{a}
PLCP _{6.8, 1.25}	9.7 ± 0.7^{c}	3.8 ± 0.1^{c}	24.9 ± 0.6^{c}	0.7 ± 0.0^{b}
PLCP _{6.8, 2.50}	4.2 ± 0.2^a	3.2 ± 0.2^{b}	6.5 ± 0.3^{a}	0.5 ± 0.1^{b}

In (PCLP)_{canola oil, pectin}: CO: canola oil; P: low methoxyl pectin; a,b,c,d,e: different superindexes in the same column indicate that means are significantly different ($p \le 0.05$).

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Textural characteristic	Model*	R^2
Hardness	15.51 – 0.46 CO – 3.04 P – 0.29 MF	94.5
Springiness	4.17 + 0.13 CO - 0.79 P - 0.05 MF	96.1
Chewiness	46.41 – 2.18 CO – 9.1 P – 1.08 MF	92.1
Cohesiveness	0.77 – 0.03 CO	64.2

^{*}CO: canola oil (g L^{-1} cheese milk); P: low methoxyl pectin (g L^{-1} cheese milk); MF: milk fat (g L^{-1} cheese milk). In all cases p < 0.001.

3.2. Microstructure and texture

Variations in the chemical composition of the cheese milks, and thus in the cheeses obtained from them (Table 2), resulted in arrangements different structural that produced different textural characteristics of springiness, chewiness cohesiveness (p \leq 0.05) (Table 3). Table 4 shows the polynomial models that explain the effect of the cheese milk variables composition upon the mechanical behavior of the cheeses. The models describing the behavior of cheeses hardness and chewiness indicate that increases in the concentration of P, CO and MF caused a significant decrease $(p \le 0.05)$ in the values of these textural characteristics (Table 4). The magnitude of the effect of such composition variables had the following order: P > CO > MF (Table 4). P, CO and MF caused different structural characteristics in the cheeses (Figs. 1 to 5), which were responsible for the variations in the mechanical behavior. Reduction of 75 % of MF content in cheese milk resulted in a LFC with increased hardness and chewiness $(p \le 0.05)$ than those exhibited by FFC. The quantities of relatively high MF contents in the FFC contributed to the formation of a highly interrupted by MF globules protein network (Fig. 1). As can be appreciated from the void spaces that they originally occupied 1) MF globules morphology semispherical and highly heterogeneous in size. It has been informed that the size of the globules of MF contained in unhomogenized

milk exhibits great variability (Buchheim andDejmek, 1997). Reduction of MF in LFC promoted the formation of a more compact protein structure (Fig. 2), where relatively large areas of protein dominance are observable, than that exhibited by FFC (Fig. 1). High protein densities in cheese have been associated with increased hardness (Bryant et al., 1995). Substitution of MF by CO decreased the values of hardness and chewiness in cheeses. For example, PCLP_{6.8}. $_{0.00}$ was significantly less (p \leq 0.05) hard chewy than the LFC, both containing the same amount of fat, but in the former it was CO and in the latter MF (Table 3). Substitution of MF by CO induced the formation of a noticeable different structure in PCLP_{6.8, 0.00} (Fig. 3) from that of LFC (Fig. 2). In PCLP_{6.8, 0.00} a highly porous protein network was formed, due to the presence of numerous, very small CO droplets, which had the effect of lowering the degree of association occurring between the casein chains. These structural characteristics are in accordance to those found Manchego cheeselikeproducts containing CO in substitution of MF (Lobato-Calleros et al., 2002). The presence of numerous oil droplets interrupted the protein network conferring a greater softness and lower chewiness to the cheese. The incorporation of P into PLCP_{6.8, 1.25} and PLCP_{6.8, 2.50} also lowered their hardness and chewiness (Table 3).

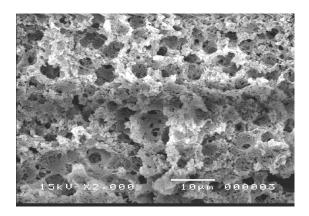


Fig. 1. SEM micrograph of panela cheese-like product (FFC) made from 27 g MF L⁻¹ cheese milk.

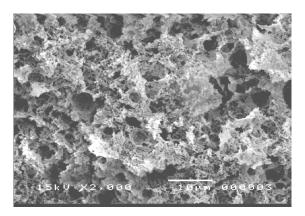


Fig. 2. SEM micrograph of panela cheese-like product (LFC) made with canola oil (6.8 g MF L⁻¹ cheese milk)

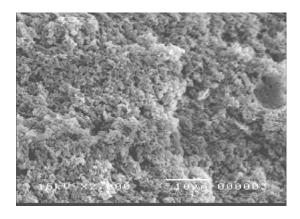


Fig. 3. SEM micrograph of Panela cheese-like product (PLCP_{6.8, 0.0}) made with canola oil (6.8 g L⁻¹ cheese milk).

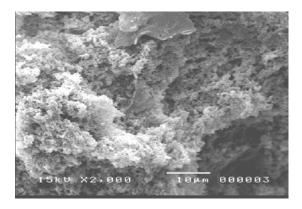


Fig. 4. SEM micrograph of panela cheese-like product (PLCP_{6.8, 1.25}) made with canola oil (6.8 g L⁻¹ cheese milk) and low methoxyl pectin (1.25 g L⁻¹ cheese milk).

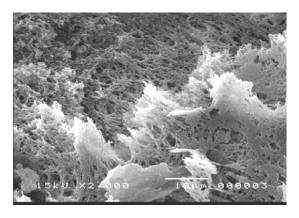


Fig. 5. SEM micrograph of panela cheese-like product (PLCP_{6.8, 2.50}) made with canola oil (6.8 g L⁻¹ cheese milk) and low methoxyl pectin (2.50 g L⁻¹ cheese milk).

The higher the concentration of P, lower was the hardness and exhibited by the cheese (Table 3). P promoted the formation of a more open protein network in both PLCP_{6.8, 1.25} (Fig. 4) and PLCP_{6.8, 2.50} (Fig. 5), causing it to exhibit a "sponge" like appearance in which the CO droplets were distributed. These highly porous like of the protein networks can be explained on terms of the great water binding capacity of P, that in conjunction with the Ca⁺² ions, tend to form calcium pectate gels, that may retain whey within themselves (Lobato-Calleros *et al.*, 2001).

The higher amount of pectin used in PLCP_{6.8, 2.50} (Fig. 5) resulted in a more open protein matrix than in PLCP_{6.8, 1.25} (Fig. 4). Increases in P and MF concentration decreased springiness in cheeses (Table 4). although the effect of MF was much lower than that of P, whereas CO showed a small positive contribution. These results indicate that P decreased the flexibility and protein network recovery capacity after a deforming stress is withdrawn, probable due to the interruption of casein-casein chains interactions caused by the highly hydrated P molecules. The positive contribution of CO to springiness compared with the negative effect of MF to this textural characteristic can probably be explained on terms of the much smaller droplet sizes exhibited by CO than MF. Marshall (1990) reported that cheese analogues with smaller fat droplet sizes exhibited greater springiness. The cohesiveness mathematical model fitted badly the experimental cheese milk composition variables (Table 4). Cohesiveness was significantly decreased (p \leq 0.05) in cheeses containing CO, and this result is in accordance with those reported by Yu and Hammond who found that cohesiveness was lower in cheese-like products where MF had been substituted by vegetable oils.

Conclusions

Substitution of milk fat by canola oil and low methoxyl pectin modified the microstructure of Panela type cheese-like products, thus affecting their mechanical properties. Both, canola oil and low methoxyl pectin promote the formation of finer protein networks, with smaller pore sizes, that result in textural characteristics that resemble more closely that of a low-milk fat cheese, than those of a low milk-fat cheese.

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