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TEXTURAL AND CHEMICAL CHANGES DURING RIPENING OF PORT SALUT ARGENTINO LIGHT CHEESE WITH MILK PROTEIN CONCENTRATE AFTER LONG FROZEN STORAGE PERIOD

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ABSTRACT

Texture of a Port Salut Argentino light cheese with milk protein concentrate after 5 months of frozen storage was studied. Behavior of dynamic rheological measurements during ripening time (6, 20, 33, 47 and 68 days) with maturation index was analyzed. At 68 days of ripening, moisture decreased by 11% and total nitrogen content increased by 25% in frozen cheeses with respect to control cheeses. Between 6 and 68 days of ripening, maturation index was higher for frozen (17.8–29.1) than control cheeses (4.4–11.6). G′ was higher than G″ showing that elastic behavior was dominant. During ripening, these parameters decreased in control cheeses, but increased in frozen cheeses. According to power law model parameters, elastic properties were more sensitive to changes with frequency. Coefficient *a* was significantly affected for both freezing process and ripening time, coefficient *b* only by freezing process. Long frozen storage period affected significantly the physicochemical and textural characteristics.

PRACTICAL APPLICATIONS

Texture is important to the consumer to determine the quality and preference of a cheese variety. The effect of freezing on physicochemical and rheological properties of cheeses was widely studied due to its importance over the texture. Freezing can be a useful methodology to transport cheeses to long-distance commercial places without any modifications. This paper focuses on textural changes of Port Salut Argentino light cheese with milk protein concentrate after a long frozen storage period (5 months) and during the ripening time (expressed as maturation index). Relations between the power law parameters obtained from rheological measurements with maturation time were studied. Similar textural characteristics can be obtained during ripening after thawing for increasing commercial demand. The obtained results can be important to obtain equal cheese characteristics after being transported in frozen state.

INTRODUCTION

Texture is determinant of quality and preference by the consumer. Lawrence *et al.* (1987) identified two distinct phases in the development of cheese texture. During the first time (7–14 days), in soft cheeses, the relatively rapid hydrolysis of the α_{s1}-casein to the soluble α_{s1}-I-casein fraction by the residual coagulant enzymes reduces the rubbery texture of the cheese. The rest of the ripening involves a slower proteolysis by the coagulant enzymes, the native milk proteases and the released enzymes by the starter bacteria (Fox 1989). The chemical and physical changes that occur during ripening cause the body of the freshly cheese to lose its firmness, toughness and curdy texture (Tunick 2000). Cheese texture may also vary with a change in the physical state of the fats that are already present in the cheese (Watkinson *et al.* 1997; Dufour *et al.* 2000). In different cheese varieties, changes in moisture content, salt concentration, pH, fat and protein contents may affect cheese texture (Verdini and Rubiolo 2002a). Cheeses with high protein content, and therefore larger amount of casein, generate more water-soluble nitrogen during proteolysis, which contributes to flavor. From a commercial perspective, an understanding of tangible changes that occur during ripening could assist quality control and product development.

According to Lucey *et al.* (2003), all the textural characteristics of the cheese are a combination of measurable rheological properties. Increases in firmness, hardness, and elastic texture, and an increase in viscoelasticity, in cheeses with low fat/protein relation are commonly observed (Bryant *et al.* 1995; Ustunol *et al.* 1995). Specifically, as the fat content of cheese is lowered, moisture content increases and protein plays a greater role in texture development. To counter this, the moisture in nonfat substance of cheese is generally equal to that in full-fat cheese (Mistry and Anderson 1993). According to Mistry (2001), this behavior is related to the low-fat cheese manufacture employed, which leads to a lower retention of chymosin and lower plasmin activity.

Dynamic testing using small oscillatory deformations is one of the most important and fundamental methods for determining rheological properties of viscoelastic materials, and it has been used extensively to analyze the structure of cheese (Diefes *et al.* 1993; Ak and Gunasekaran 1996; Subramanian and Gunasekaran 1997a,b).

The continuous protein matrix has an open structure with dispersed fat globules, and the nature of this network determines fracture and rheological properties and the viscoelasticity of the cheese (Ustunol *et al.* 1995).

Freezing has been cited as an effective and traditional way of preserving cheese color, flavor, and nutritive value, and to extend the shelf life of dairy products (Lück 1977). However, it is only moderately effective for preserving cheese texture because it tends to affect the structural characteristics (Fennema 1972). The breakdown of the casein network could be generated by the ice crystal formation and freeze concentration produced during freezing process. Furthermore, freezing can damage the starter cells and the native structure of enzymes, liberating proteolytic enzymes to the media with almost different activity. These phenomena could increase the maturation index of cheeses as was reported by Graiver *et al.* (2004), Lück (1977) and Verdini *et al.* (2005).

The effect of freezing on physicochemical and rheological properties of several varieties of full-fat or traditional cheeses was investigated in previous publications (Califano and Bevilacqua 1999; Verdini *et al.* 2002, 2003, 2005; Graiver *et al.* 2004).

Meza *et al.* (2011), who worked with a low-fat soft cheese containing microparticulated whey proteins as fat replacer, reported that the freezing process contributes to the viscoelastic behavior during the ripening, improves the structural characteristics and extends the shelf life of the cheeses.

Cheeses with high protein content, characteristics like firm structure and low protein breakdown during ripening time, could help reduce the effect of freezing process and improve the viscoelastic characteristics (Fennema *et al.* 1973).

Milk protein concentrate (MPC) is a substance obtained by the removal of nonprotein constituents from pasteurized skim milk, so that the final product has a percentage of protein higher than 38%. Because protein and lactose levels are related, the more the protein content, the less the lactose content. The main features of this product are low lactose concentration, high content of calcium, excellent thermal and emulsion stability, besides the high nutritional value and its functionality. The MPC can be produced by physical methods such as precipitation, filtration (ultrafiltration) and dialysis. MPC is used in the manufacture of cheeses with skim milk to increase the protein and solid contents, and counteract the high water content of this kind of cheese.

The aim of this work was to analyze the texture of Port Salut Argentino light cheese with addition of MPC after a long freezing storage period (5 months) by rheological measurements during ripening time, and study the behavior of these parameters with maturation index.

MATERIALS AND METHODS

Cheeses: Freezing and Sampling Processes

Commercial reduced fat Port Salut Argentino cheeses were manufactured at a local factory (SanCor, Sunchales, Santa Fe, Argentina) by rennet coagulation, with addition of MPC. Square cheeses of 30 cm side and 7 cm high, in a heat-shrinkable plastic packing, were brought to the laboratory on ice bins for study and were randomly separated in two groups. One group of 12 cheeses was stored in a TABAI Comstar PR 4GM chamber (TABAI Espec Corp., Osaka, Japan) at –18C for 5 months, and then thawed at 8–10C and was stored at this temperature for ripening (frozen cheeses). The other group of 12 cheeses was stored under the usual conditions at 8–10C, and was used as control cheeses.

Physicochemical Analysis

Sampling procedure was performed in duplicate as described by Meza *et al.* (2011). From the original cheese it was obtained a central rectangular piece (7 cm × 7 cm × 30 cm) that was divided in three pieces. A central cubic piece of 7 cm wide was used in physicochemical analysis, and from the others two rectangular pieces were obtained the slices for rheological analysis (Fig. 1). Samples from both control and frozen cheeses were taken at different ripening times by duplicate, 6, 20, 33, 47, 68 and 100 days, using this procedure.

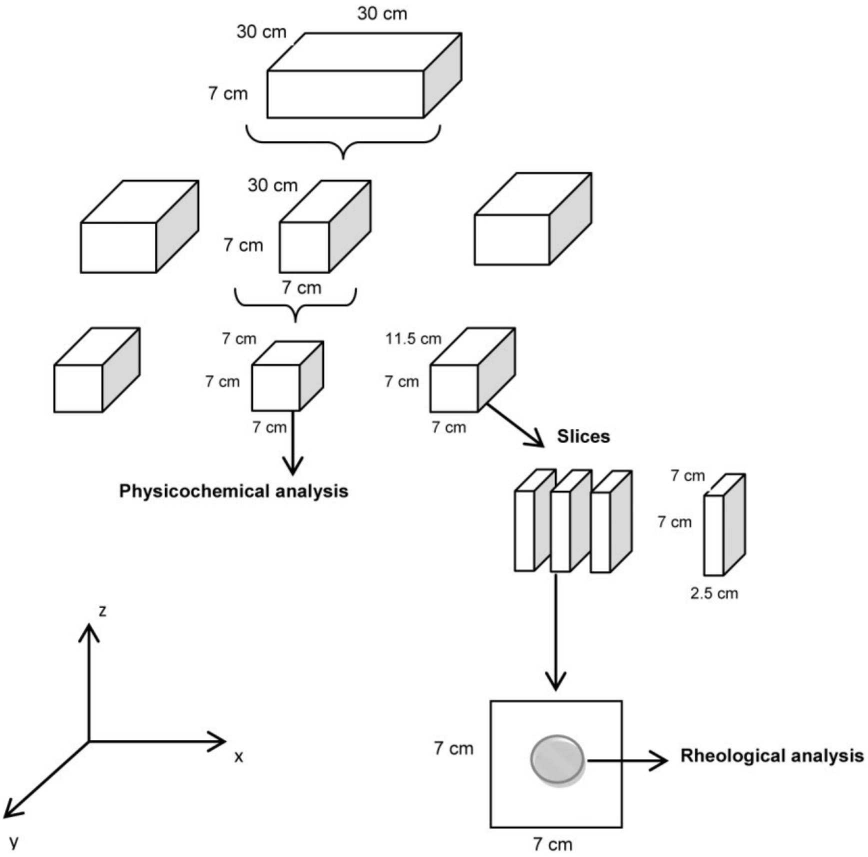

FIG. 1. SCHEMATIC REPRESENTATION OF THE SAMPLING OF PORT SALUT ARGENTINO LIGHT CHEESES WITH MILK PROTEIN CONCENTRATE (MPC)

Moisture content was measured with a microwave oven CEMAVC 80 and initial fat content was determined using standard procedures (AOAC 1990; IDF 1969). With a protocol developed by Kuchroo and Fox (1982) and modified by Verdini and Rubiolo (2002a), water-soluble fraction at pH 4.6 was extracted to determine the water-soluble nitrogen ($SN_{pH4.6}$). Total nitrogen (TN) and water-soluble nitrogen ($SN_{pH4.6}$) content were determined using a micro-Kjeldahl method with the same equipment previously used by Meza *et al.* (2010). Maturation index was expressed as a percentage of the $SN_{pH4.6}$ to the TN, according to Eq. (1). All analyses were made in triplicate:

$$MI = \frac{(SN_{\text{pH4.6}})}{TN} \times 100 \tag{1}$$

where MI is the maturation index, $SN_{\text{pH4.6}}$ is the pH 4.6 water-soluble nitrogen content, and TN is total nitrogen content.

Rheological Analysis

Dynamic Rheological Measurements. Dynamic rheological testing is normally performed by imposing a sinusoidally varying strain and measuring the resulting stress in the sample. The amplitude of strain is usually kept small to stay within a linear viscoelastic region (Ferry 1980).

Measurements were performed using a stress-controlled rheometer RheoStress 80 (Haake Inc. Instruments, Karlsruhe, Germany) with a plate-plate geometry test fixture (diameter: 20 mm; gap: 2.5 mm). Strain sweeps (0.001–0.1) at a frequency of 62.8 rad/s and at 20C were carried out to determine the linear viscoelastic region. Frequency sweeps (0.0628–62.8 rad/s) in the linear viscoelastic region at 20C and a constant strain of 0.005 were used to measure the rheological parameters: elastic modulus (G'), viscous modulus (G''), complex modulus ($|G^*|$) and tangent of phase angle (tan δ). All rheological tests were made in duplicate for each sample. For rheological testing, disks of 20-mm-diameter and 2.5 mm were cut with a borer from the cheese slices.

Rheological Parameters. Frequency (ω) dependence of G' and G'' at 20C of cheese samples was modeled with power law Eqs. (2) and (3) (Steffe 1992):

$$G' = a \times \omega^x \tag{2}$$

$$G'' = b \times \omega^y \tag{3}$$

where coefficients a and b represent the magnitude of G' and G'' at a frequency of 1 rad/s, and exponents x and y represent the slopes of the linear relationships between modulus and frequency.

Statistical Analysis

Analysis of variance (ANOVA) and Tukey's HSD (honest significant difference) of multiple ranks were applied when the effect of the factors was significant ($P < 0.05$). The corresponding rheological parameters derived from power law equations were determined using linear regression. The complete statistical analysis was carried out using Statgraphics (Statgraphics Inc., Rockville, MD).

RESULTS AND DISCUSSION

Physicochemical Properties

The initial pH, moisture, fat and TN contents of cheeses were 5.35, 56.08 ± 0.14%, 12.81 ± 0.09% and 25.22 ± 0.39%, respectively. Fat content and pH remain almost constant over the ripening time, even after frozen storage. Changes in moisture and TN content at different ripening time for control and frozen cheeses are shown in Table 1.

Moisture content changes significantly in frozen cheeses over the studied ripening period, being higher for control than frozen cheeses. The moisture content of frozen cheeses decreased at 2–7% with respect to control cheeses between 6 and 47 days, and 11% at 68 days of ripening. After 100 days of frozen storage period, water was not retained in cheese matrix upon thawing, causing the decrease in moisture and the consequent TN increment.

Because 100 days is a very long ripening time, usually not employed in the market for this kind of cheese, those values at this period were not included into statistical analysis.

Comparison between $SN_{pH4.6}$ with TN throughout the ripening time for control and frozen cheeses is shown in Fig. 2. The TN content changes significantly between 6 and 68 days in both control and frozen cheeses. Because frozen cheeses lost more water and had lower moisture content, they presented 12–25% more TN content than control cheeses. In control and frozen cheeses, $SN_{pH4.6}$ content

TABLE 1. CHANGES IN MOISTURE AND TOTAL NITROGEN (TN) CONTENTS DURING RIPENING FOR A COMMERCIAL PORT SALUT ARGENTINO LIGHT CHEESE CONTAINING MILK PROTEIN CONCENTRATE*

Ripening time (days)	Treatment	Moisture content (%w/w)	Total nitrogen, TN (%w/w)
6	Control	56.78 ± 0.82^{h}	24.48 ± 0.87^{a}
20		55.64 ± 0.43^{g,h}	25.75 ± 0.49^{a,b}
33		55.45 ± 0.94^{f,g,h}	25.03 ± 0.43^{a,b}
47		55.52 ± 0.44^{f,g,h}	26.76 ± 1.05^{a,b}
68		55.99 ± 0.06^{f,g,h}	26.85 ± 1.03^{b}
100		55.64 ± 0.01	28.72 ± 1.59
6	Frozen	53.69 ± 0.22^{f}	27.80 ± 2.47^{c}
20		51.62 ± 1.02^{e}	29.71 ± 2.14^{c,d}
33		54.08 ± 0.46^{f,g}	29.50 ± 1.01^{c,d}
47		51.45 ± 1.19^{e}	29.89 ± 1.42^{c,d}
68		49.96 ± 1.99^{e}	33.47 ± 4.78^{d}
100		47.97 ± 0.46	34.98 ± 1.01

* Mean values and standard deviations of three replicates. Means within a column with the same superscript letters are not significantly different ($P < 0.05$).

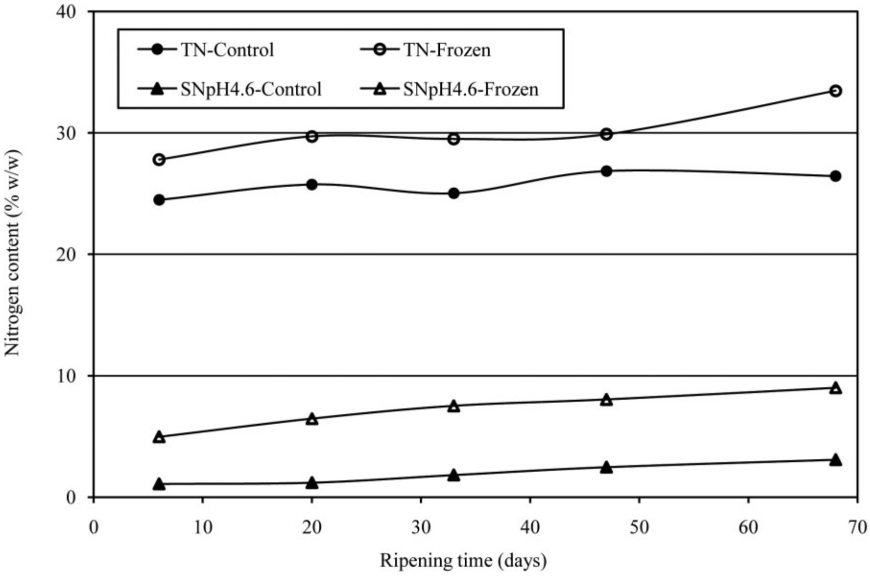

FIG. 2. CHANGES IN TOTAL NITROGEN (TN) AND 4.6 WATER-SOLUBLE NITROGEN (SNpH4.6) CONTENTS WITH RIPENING TIME FOR FROZEN AND CONTROL CHEESES

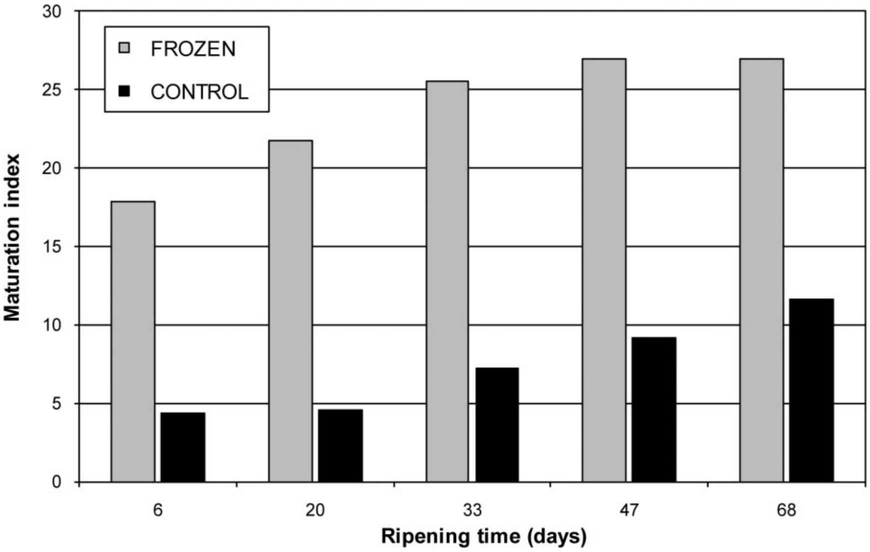

FIG. 3. CHANGES IN MATURATION INDEX WITH RIPENING TIME FOR FROZEN AND CONTROL CHEESES

increase, however, did not change statistically during ripening time. Even with that behavior, $SN_{pH4.6}$ content was significantly higher for frozen cheeses.

Bertola *et al.* (1996), who worked with a low-moisture mozzarella cheese, reported that frozen cheese matrix may be more susceptible to primary proteolysis, because freezing process can damage starter cell liberating high amounts of proteolytic enzymes, increasing the soluble nitrogen content. The $SN_{pH4.6}$ fraction contains peptides and free amino acids produced by the action of coagulating enzymes that hydrolyze preferentially α_{S1}-casein rather than β-casein (Bertola *et al.* 1991). Graiver *et al.* (2004) studied samples of low-moisture mozzarella cheese and reported higher values of $SN_{pH4.6}$ content in samples that were frozen before ripening. They found higher $SN_{pH4.6}$ concentration at the initial time produced by the freeze-thaw cycle, probably by the damage caused by ice crystals on the protein network, which favors the action of enzymes.

Figure 3 shows the maturation index for control and frozen cheeses. Between 6 and 68 days of ripening, the maturation index was much higher for frozen (from 17.8 to 29.1) than control cheeses (from 4.4 to 11.6). This behavior was expected by the higher $SN_{pH4.6}$ contents of frozen cheeses, increasing the relation between $SN_{pH4.6}$ and TN, and therefore higher maturation index. Meza *et al.* (2010, 2011) reported for low-fat soft cheeses held in frozen storage for 33 days, a maturation index from 4.1 to 13.0 at 1 and 76 days of ripening, respectively, and no significant moisture differences. On the other hand, Verdini *et al.* (2002) studied two sampling zones (central and external) of a commercial Port Salut Argentino cheese with 30 days on frozen storage: a maturation index from 7 to 17 for 1 and 56 days of ripening in the central zone, and from 6 to 13 for the same days in the external zone. The outcomes obtained in this study showed that the long frozen storage period has a significant difference with the proteolysis, increasing $SN_{pH4.6}$ and maturation index, and also decreasing moisture, which enlarge the difference between those values.

Region of Linear Viscoelasticity and Mechanical Spectra

The experimental conditions used for Port Salut Argentino light with MPC to determine the region of linear viscoelasticity were the same as indicated by Meza *et al.* (2010, 2011). Values of $|G^*|$ at any ripening time were not affected by the applied stress at a frequency of 62.8 rad/s. The linear relationship between stress and strain applied indicates that both control and frozen cheeses behaved as a linear viscoelastic material during dynamic tests. This result indicated that the extent of the linear viscoelastic region used was not affected by the freezing process.

The storage (G') and loss (G'') modulus during 6, 33 and 68 days of F and C Port Salut Argentino cheeses were determined as a function of frequency at 20C (Fig. 4). Frequency dependence of G' and G'' (on log-log scale) was nearly lineal in the range of 0.0628–62.8 rad/s for frozen and control cheeses as reported by Meza *et al.* (2011).

The value of G' was significantly greater than G'' at any given frequency over the entire ripening period, indicating a predominant elastic behavior for both control and frozen cheeses. On the other hand, those values showed that when the viscoelastic moduli are considered as a function of frequency, a viscoelastic material behaves like a liquid at low frequencies (Ferry 1980). G' and G'' values decreased with

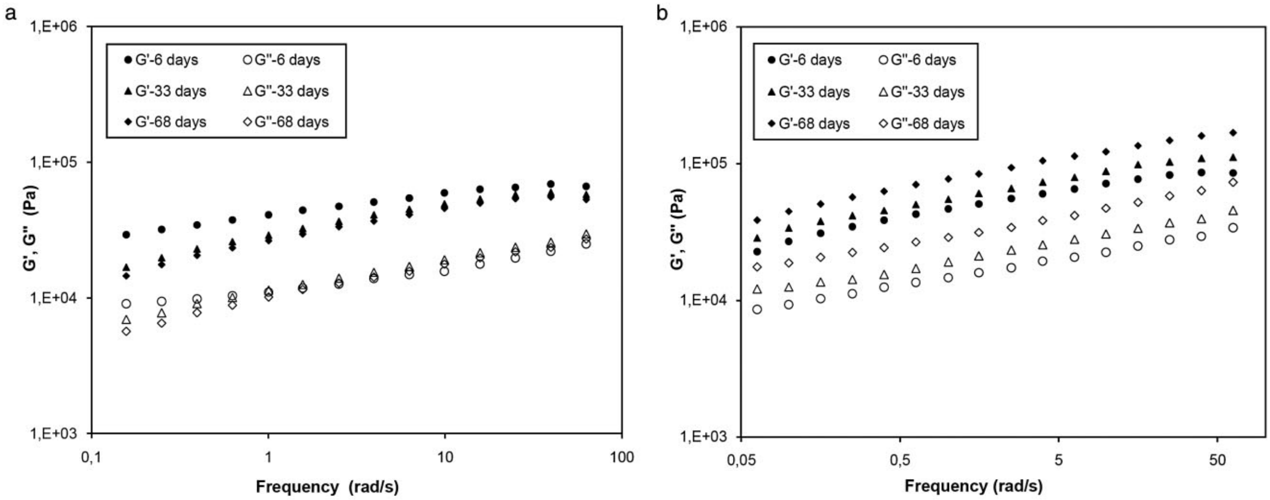

FIG. 4. FREQUENCY DEPENDENCE OF G′ FOR G″ FOR CONTROL CHEESES (A) AND FROZEN CHEESES (B) AT 6, 33 AND 68 DAYS OF RIPENING

ripening time in the case of control cheeses. However, in frozen cheeses, G′ and G″ increased with ripening time, showing that the elastic behavior was more dominant. The difference could be due to the disruption of fat globules and proteolysis, the protein matrix is rearranged, and a more compact texture is formed, containing aggregates of casein, as reported by Karami *et al.* (2009) in an Iranian UF feta cheese. Moreover, this process is enlarged in this case for the MPC aggregated and the largest moisture loss.

From a structural viewpoint, graphs of ln (G′, G″) as a function of ln ω do not exhibit changes in the slope when changes within the gel network are reduced. In weak gels, these slopes are positive, G′ being greater than G″ throughout the frequency range (Rao 1999). The positive slopes for ln (G′, G″) can be observed in Fig. 5 which are obtained for control and frozen Port Salut Argentino light cheeses. For control cheeses, the slopes, and also its changes with ripening time, were lower than in frozen cheeses, showing that protein changes were increased in this last case.

Values of tan δ as a function of frequency (log-log scale) of frozen and control cheeses at 6, 33 and 68 days of ripening are shown in Fig. 6. Tan δ is a value that compares the amount of energy lost during a test cycle to the amount of energy stored during this time (Ferry 1980). When tan $\delta < 1$, the elastic properties are predominant. In our study, this parameter increased with ripening time showing values between 0.31 and 0.51 for frozen cheeses and 0.26–0.52 for control cheeses; that confirms the elastic properties of the samples throughout the studied frequency range.

It was observed that with increasing ripening time, viscoelastic behavior is less dependent on frequency for control and frozen cheeses.

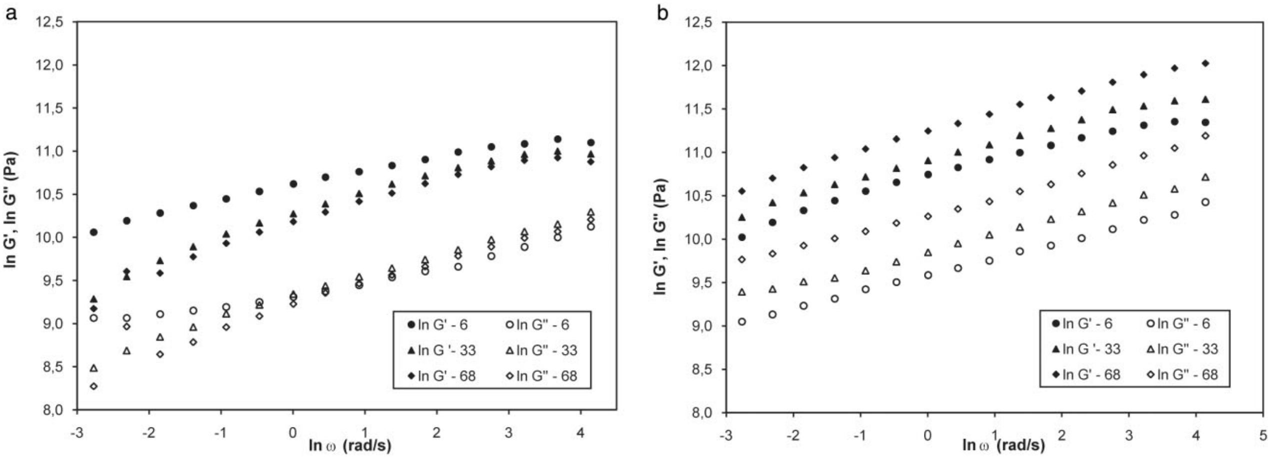

FIG. 5. LN (G′, G″) AGAINST LN Ω FOR CONTROL CHEESES (A) AND FROZEN CHEESES (B) AT 6, 33 AND 68 DAYS OF RIPENING

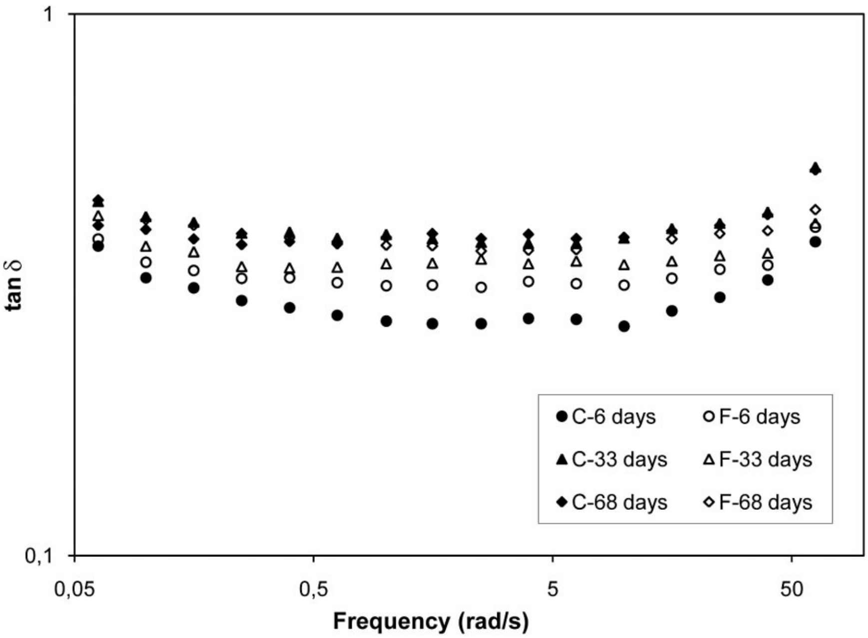

FIG. 6. FREQUENCY DEPENDENCE OF TAN Δ (LOG-LOG SCALE) FOR FROZEN (F) AND CONTROL (C) CHEESES AT 6, 33 AND 68 DAYS OF RIPENING

Rheological Parameters

Rheological parameters obtained from power law equations to analyze the frequency dependence of G′ and G″ of control and frozen cheeses are shown in Table 2.

Coefficient *a* was higher than *b* for frozen and control cheeses, showing that elastic properties were more sensitive to changes with frequency than viscous properties. Coefficient *a* was significantly affected both by freezing process and ripening time; however, coefficient *b* was only affected by freezing process.

A significant increase in coefficients *a* and *b* in frozen cheese at 68 days of ripening was observed (109.8 and 42.7 kPa, respectively). This could be related to the higher loss of water content (moisture content of 49.96% at 68 days) that acts as a plasticizer between protein chains, giving fluency to the protein network. According to Diefes *et al.* (1993) who have studied on low-fat part-skim mozzarella cheese that undergoes freezing, the local dehydration of proteins causes breaks in protein structure and produces small fat globules. The proteins become more compact or interact, forming disulfide bridges around new fat globules. Upon thawing, the proteins are unable to fully rebind water and this leads to a harder and more elastic-solid cheese structure with less free oil. Moreover, Bertola *et al.* (1996) reported that crystal diameters increase during freezing

TABLE 2. RHEOLOGICAL PARAMETERS FROM POWER LAW EQUATIONS FOR FROZEN AND CONTROL CHEESES OVER THE STUDIED RIPENING TIME*

Ripening time (days)	Treatment	*a* (kPa/s)	*x* (–)	R^2	*b* (kPa/s)	*y* (–)	R^2
6	Control	$56.4 \pm 13.1^{c,d}$	0.157 ± 0.003^{f}	0.96	16.5 ± 3.5^{k}	0.155 ± 0.003^{n}	0.96
20		71.9 ± 19.4^{d}	0.263 ± 0.028^{j}	0.95	27.7 ± 7.4^{k}	0.257 ± 0.019^{r}	0.98
33		41.0 ± 8.8^{c}	$0.240 \pm 0.031^{h,i,j}$	0.93	17.1 ± 3.6^{k}	0.247 ± 0.024^{r}	0.99
47		$48.0 \pm 9.2^{c,d}$	0.255 ± 0.018^{j}	0.95	19.2 ± 3.2^{k}	0.269 ± 0.015^{r}	0.99
68		37.9 ± 6.5^{c}	$0.251 \pm 0.013^{i,j}$	0.93	15.7 ± 2.7^{k}	0.271 ± 0.013^{r}	0.99
6	Frozen	$62.4 \pm 14.1^{c,d}$	$0.193 \pm 0.013^{f,g}$	0.97	20.8 ± 4.0^{m}	0.195 ± 0.011^{p}	1.00
20		$61.4 \pm 21.2^{c,d}$	$0.200 \pm 0.013^{g,h}$	0.97	23.5 ± 8.3^{m}	0.198 ± 0.056^{p}	0.99
33		$63.3 \pm 4.7^{c,d}$	$0.199 \pm 0.020^{f,g,h,i}$	0.98	22.8 ± 3.3^{m}	0.195 ± 0.017^{p}	0.99
47		$57.2 \pm 13.9^{c,d}$	$0.190 \pm 0.010^{f,g}$	0.97	20.5 ± 5.7^{m}	0.199 ± 0.015^{p}	1.00
68		109.8 ± 18.6^{e}	$0.211 \pm 0.009^{g,h,i}$	0.99	42.7 ± 7.0^{m}	0.204 ± 0.007^{p}	1.00

* Mean values and standard deviations of two replicates. Means within a column with the same superscript letters are not significantly different ($P < 0.05$).

process due to recrystallization, making more difficult the relocation of water at the lipid-casein interface after thawing, decreasing its lubricant effect and producing a harder cheese structure. This effect seems to be stronger after a long frozen storage.

Coefficients x and y of control cheeses increased significantly from 6 to 20 days of ripening, and did not change significantly between 20 and 68 days. According to Meza *et al.* (2011), these results mean those viscous and elastic moduli were more sensitive to changes with frequency during the first stage of ripening, showing a lower degree of structuring of the cheese matrix. However, for the frozen cheeses, both x and y were almost constant over the studied ripening period. This could be related to the degree of structuring of the protein matrix that is higher when the water content is low.

CONCLUSIONS

This work allowed the study of the influence of ripening time and long frozen storage period over the textural characteristics of Port Salut Argentino light cheese with addition of MPC.

The high TN and $SN_{pH4.6}$ content and the low moisture of the frozen cheeses affected the structure of the matrix cheese, not only increasing its maturation index but also changing its texture according to the rheological behavior observed. In the linear viscoelastic region, G' and G'' increased with ripening time because the capacity of the structure retention was reduced, a very different tendency from control cheeses with its values decreased.

The long frozen storage period (5 months) caused important changes in the moisture content, maturation index and rheological parameters, affecting significantly the textural characteristics and the common end use of this kind of cheese.

A simple sensory analysis showed a hardened cheese even at temperatures higher than ambient, that affect the meltability. The MPC added did not reduce the effect of long frozen storage period on the cheese matrix.

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REFERENCES

AK, M.M. and GUNASEKARAN, S. 1996. Dynamic rheological properties of mozzarella cheese during refrigerated storage. J. Food Sci. *61*, 566–576.

AOAC. 1990. *Official Methods of Analysis: Food Composition; Additives; Natural Contaminants*, Vol II, Moisture in cheese. AOAC Official Method 977.11. Association of Official Analytical Chemists Inc., Rockville, MD.

BERTOLA, N.C., BEVILACQUA, A.E. and ZARITZKY, N.E. 1991. Changes in rheological and viscoelastic properties and protein breakdown during the ripening of "Port Salut Argentino" cheese. Int. J. Food Sci. Technol. *26*, 467–478.

BERTOLA, N.C., CALIFANO, A.N., BEVILACQUA, A.E. and ZARITZKY, N.E. 1996. Textural changes and proteolysis of low-moisture mozzarella cheese frozen under various conditions. Lebensm. Wiss. Technol. *29*, 470–474.

BRYANT, A., USTUNOL, Z. and STEFFE, J. 1995. Texture of Cheddar cheese as influenced by fat reduction. J. Food Sci. *60*, 1216–1219, 1236.

CALIFANO, A.N. and BEVILACQUA, A.E. 1999. Freezing low moisture mozzarella cheese: Changes in organic acid content. Food Chem. *64*, 193–198.

DIEFES, H.A., RIZVI, S.H. and BARTSCH, J.A. 1993. Rheological behavior of frozen and thawed low-moisture part-skim mozzarella cheese. J. Food Sci. *58*, 764–769.

DUFOUR, E., MAZEROLLES, G., DEVAUX, M.F., DUBOZ, G., DUPLOYER, M.H. and MOUHOUS RIOU, N. 2000. Phase transition of triglycerides during semi-hard cheese ripening. Int. Dairy J. *10*, 81–93.

FENNEMA, O. 1972. Freezing of cheese – pros and cons. Western Milk Ice Cream News. 20 October. Greenbrae, Calif.: Leete. Cited in Diefes *et al.* (1993), Graiver *et al.* (2004).

FENNEMA, O.R., POWRIE, W.D. and MARTH, E.H. 1973. *Low-Temperature preservation of foods and living matter,* Chapter 5, Marcel Dekker, Inc., New York.

FERRY, J.D. 1980. *Viscoelastic Properties of Polymers*, pp. 33–35, John Wiley and Sons Inc., New York, NY.

FOX, P.F. 1989. Proteolysis during cheese manufacture and ripening. J. Dairy Sci. *72*, 1379–1400. Cited in Hort and Le Grys (2001), Verdini and Rubiolo (2002), Verdini *et al.* (2004).

GRAIVER, N.G., ZARITZKY, N.E. and CALIFANO, A.N. 2004. Viscoelastic behavior of refrigerated and frozen low-moisture mozzarella cheese. J. Food Sci. *9*, 123–128.

HORT, J. and LE GRYS, G. 2001. Developments in the textural and rheological properties of UK Cheddar cheese during ripening. Int. Dairy J. *11*, 475–481.

IDF. 1969. *Determination of the Fat Content of Cheese and of Processed Cheese Products. Standard 5A*, International Dairy Federation, Brussels, Belgium.

KARAMI, M., EHSANI, M.R., MOUSAVI, S.M., REZAEI, K. and SAFARI, M. 2009. Changes in rheological properties of Iranian UF-Feta cheese during ripening. Food Chem. *112*, 539–544.

KUCHROO, C.N. and FOX, P.F. 1982. Soluble nitrogen in Cheddar cheese: Comparison of extraction procedures. Milchwissenschaft *37*, 331–335.

LAWRENCE, R.C., CREAMER, L.K. and GILLES, J. 1987. Texture development during cheese ripening. J. Dairy Sci. *70*, 1748–1760. Cited in Hort *et al.* (2004.

LUCEY, J.A., JOHNSON, M.E. and HORNE, D.S. 2003. Invited review: Perspectives on the basis of the rheology and texture properties of cheese. J. Dairy Sci. *86*, 2725–2743. Cited in Meza *et al.* (2011).

LÜCK, H. 1977. Preservation of cheese and perishable products by freezing. S Afr. J. Dairy Technol. *9*, 127–132. Cited in Verdini and Rubiolo (2002), Verdini *et al.* (2002), Verdini *et al.* (2003), Verdini *et al.* (2005), Meza *et al.* (2011).

MEZA, B.E., VERDINI, R.A. and RUBIOLO, A.C. 2010. Viscoelastic behavior during the ripening of a commercial low-fat soft cheese. Dairy Sci. Technol. *90*, 589–599.

MEZA, B.E., VERDINI, R.A. and RUBIOLO, A.C. 2011. Effect of freezing on the viscoelastic behavior during the ripening of a commercial low-fat soft cheese. Int. Dairy J. *21*, 346–351.

MISTRY, V.V. 2001. Low-fat cheese technology. Int. Dairy J. *11*, 413–422.

MISTRY, V.V. and ANDERSON, D.L. 1993. Composition and microstructure of commercial full-fat and low-fat cheeses. Food Struct. *12*, 259–266. Cited in Mistry (2001).

RAO, M.A. 1999. *Rheology of Fluids and Semisolid Foods. Principles and Applications*, p. 443, Aspen, Gaithersburg.

STEFFE, J.F. 1992. *Rheological Methods in Food Process Engineering*, Freeman Press, Miami, FL.

SUBRAMANIAN, R. and GUNASEKARAN, S. 1997a. Small amplitude oscillatory shear studies on mozzarella cheese. Part I. Region of linear viscoelasticity. J. Texture Studies *28*, 633–642.

SUBRAMANIAN, R. and GUNASEKARAN, S. 1997b. Small amplitude oscillatory shear studies on mozzarella cheese. Part II. Relaxation spectrum. J. Texture Studies *28*, 643–656.

TUNICK, M.H. 2000. Rheology of dairy foods that gel, stretch, and fracture. J. Dairy Sci. *83*, 1892–1898. Cited in Karami *et al.* (2009).

USTUNOL, Z., KAWACHI, K. and STEFFE, J. 1995. Rheological properties of Cheddar cheese as influenced by fat reduction and ripening time. J. Food Sci. *60*, 1208–1210.

VERDINI, R.A. and RUBIOLO, A.C. 2002a. Texture changes during the ripening of Port Salut Argentino cheese in 2 sampling zones. J. Food Sci. *67*, 1808–1813.

VERDINI, R.A. and RUBIOLO, A.C. 2002b. Effect of frozen storage time on the proteolysis of soft cheeses studied b principal component analysis of proteolytic profiles. J. Food Sci. *67*, 963–967.

VERDINI, R.A., ZORRILLA, S.E. and RUBIOLO, A.C. 2002. Free amino acid profiles during ripening of Port Salut Argentino cheese after frozen storage. J. Food Sci. *67*, 3264–3270.

VERDINI, R.A., ZORRILLA, S.E. and RUBIOLO, A.C. 2003. Changes in equilibrium modulus and $\alpha - S_1$-casein breakdown during the ripening of Port Salut Argentino cheese as affected by frozen storage. J. Texture Studies *34*, 331–346.

VERDINI, R.A., ZORRILLA, S.E. and RUBIOLO, A.C. 2004. Characterization of soft cheese proteolysis by RP-HPLC analysis of its nitrogenous fractions. Effect of ripening time and sampling zone. Int. Dairy J. *14*, 445–454.

VERDINI, R.A., ZORRILLA, S.E. and RUBIOLO, A.C. 2005. Effects of the freezing process on proteolysis during the ripening of Port Salut Argentino cheeses. Int. Dairy J. *15*, 363–370.

WATKINSON, P., BOSTON, G., CAMPANELLA, O., COKER, C., JOHNSTON, K., LUCKMAN, M. and WHITE, N. 1997. Rheological properties and maturation of New Zealand Cheddar cheese. Lait *77*, 109–120.