International Dairy Journal 21 (2011) 346–351

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Effect of freezing on the viscoelastic behaviour during the ripening of a commercial low-fat soft cheese

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ARTICLE INFO

Article history:
Received 19 October 2010
Received in revised form
13 December 2010
Accepted 15 December 2010

ABSTRACT

The effect of the freezing process (freezing at −25 °C, frozen storage at −25 °C during 33 days, and thawing at 6 °C) on the viscoelastic behaviour of a commercial low-fat soft cheese containing microparticulated whey proteins as fat replacers at different ripening times (1, 21, 48, and 76 days) was studied. Frequency sweeps (0.01–10 Hz) at 20 °C in the linear viscoelastic region were performed. Frequency dependency of viscoelastic data was determined using power-law equations ($G' = a\omega^x$, $G'' = b\omega^y$). Freezing process produced an increase in the maturation index, the viscous behaviour and a decrease in values of coefficient *a*. However, the decay rate of coefficient *a* during the studied ripening period was not influenced by this process. A negative correlation between *a* and maturation index was obtained. The results of this work indicated that the freezing process is a factor that contributes to the viscoelastic behaviour of the studied cheeses.

1. Introduction

Cheese is one of the most important dairy products that is consumed around the world. It is a viscoelastic material, the textural, physicochemical, and sensorial characteristics whose are modified during ripening. According to Lucey, Johnson, and Horne (2003), all of its textural characteristics are a combination of measurable rheological properties. Recently, extensive rheological characterization of several varieties of cheeses was reported by Muliawan and Hatzikiriakos (2007, 2008).

The development of high quality products with low-fat content is a challenging experience and a priority for the food industry due to increasing consumer demand. However, in low-fat cheeses an increase in firmness and hardness, an over-firm and elastic texture, and an increase in viscoelasticity are commonly observed (Bryant, Ustunol, & Steffe, 1995; Olson & Johnson, 1990; Ustunol, Kawachi, & Steffe, 1995). As a result of the reduction of fat, protein and moisture content increase and proteins play a greater role in the development of low-fat cheese texture (Mistry, 2001). The rheological behaviour of this type of cheeses is also influenced by the low extent of protein breakdown along ripening. According to Mistry (2001), this behaviour is related to the low-fat cheese manufacture process, which leads to a lower retention of chymosin and lower plasmin activity.

To improve the texture of low-fat cheeses, the use of fat replacers (such as microparticulated whey proteins) in the low-fat cheesemaking process was proposed (Rodríguez, 1998). Microparticulated whey proteins can give a sense of lubricity and creaminess similar to fat by inserting themselves into the pores of the casein network like fat globules (Singer, 1996).

Freezing has been traditionally used as a preservation technique of raw and processed food. It was suggested as an alternative method to extend the shelf-life of dairy products like cheeses (Lück, 1977). The effect of freezing on physicochemical and rheological properties of several varieties of full-fat or traditional cheeses was investigated in previous publications (Califano & Bevilacqua, 1999; Gravier, Zaritzky, & Califano, 2004; Kuo & Gunasekaran, 2003; Van Hekken, Tunick, & Park, 2005; Verdini, Zorrilla, & Rubiolo, 2002, 2003, 2005). However, the effect of freezing on the rheological behaviour of low-fat cheeses has not been extensively studied. Changes produced during freezing (ice crystal formation and freeze-concentration) may lead to protein destabilization that generates the breakdown of the casein network. Furthermore, it is expected that freezing can produce the damage of starter cells and the liberation of proteolytic enzymes to the media. This phenomenon may increase the maturation rate of cheeses as was reported

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doi:10.1016/j.idairyj.2010.12.003

by Gravier et al. (2004), Lück (1977) and Verdini et al. (2005). It is interesting to notice that, although this preservation technique produces a deleterious effect on cheese quality, in the case of low-fat cheeses freezing could help to improve some characteristics like the firm structure and the low extent of protein breakdown during the ripening time.

The aim of this work was to study the effect of the freezing process (freezing, frozen storage, and thawing) on the viscoelastic behaviour during the ripening of a commercial low-fat soft cheese containing microparticulated whey proteins as fat replacers to improve textural quality.

2. Materials and methods

2.1. Cheeses

Commercial low-fat soft cheeses (LFSC) manufactured as described in Meza, Verdini, and Rubiolo (2010) containing microparticulated whey proteins as fat replacer (Simplesse® D100, NutraSweet Co., Deerfield, IL, USA) and packed in heat-shrinkable plastic bags until sampling were used for this study. Cheeses were 3.00 ± 0.10 kg weight, and the dimensions were 28.7 ± 0.31 cm × 11.6 ± 0.31 cm × 7.40 ± 0.20 cm. The initial composition of the LFSC was: 52.8 ± 0.5% moisture, 33.6 ± 0.0% proteins, 5.75 ± 0.04% fat, and 0.41 ± 0.01% salt; the pH was 5.27 ± 0.03.

2.2. Freezing process

Cheeses were transported in ice containers from the local factory to the testing laboratory and randomly separated in two groups. Cheeses were frozen in a Tabai Comstar PR 4 GM chamber (Tabai Espec Corp., Osaka, Japan) at −25 °C until the centre reached −25 °C, held in frozen storage at −25 °C for 33 days, and then thawed at 6 °C. After thawing, cheeses were held at 6 °C for ripening (frozen cheeses). The temperature of the cheeses during freezing and thawing was measured in the centre of one cheese using a thermocouple and a data acquisition system Tabai Comstar THP-18 (Tabai Espec Corp., Osaka, Japan). Cheeses of the same batch held at 6 °C, previously reported by Meza et al. (2010), were used as control samples (control cheeses).

2.3. Cheese sampling and physicochemical analysis

Samples were taken from frozen and control cheeses at different ripening times (1, 21, 48, and 76 days) in triplicate. The sampling procedure was performed as described in Fig. 1. Cubic pieces #1 were used for physicochemical analysis and slices were used for rheological analysis.

Moisture content and initial fat content were determined using standard procedures (AOAC, 1990; IDF, 1969). Salt concentration was determined applying the method proposed by Fox (1963). Water-soluble fraction at pH 4.6 was extracted with a protocol developed by Kuchroo and Fox (1982) and modified by Verdini and Rubiolo 2002. The pH, total nitrogen content (TN) and water-soluble nitrogen (WSN) content in the water-soluble fraction at pH 4.6 were determined using the same methods published previously (Meza et al., 2010). Maturation index (MI) was expressed as a percentage of the WSN to the TN. All the analysis was made in duplicate.

2.4. Rheological analysis

2.4.1. Dynamic rheological measurements

Dynamic rheological 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). Frequency sweeps (0.01–10 Hz) in the linear viscoelastic region at 20 °C and stress sweeps (64–640 Pa) at the maximum frequency (10 Hz) at 20 °C were carried out. For rheological testing, disks of 20 mm diameter were cut with a borer from the cheese slices (Fig. 1) and a thin film of silicone oil (100 cP) was applied to the exposed edges of the sample to prevent water vaporization during measurements.

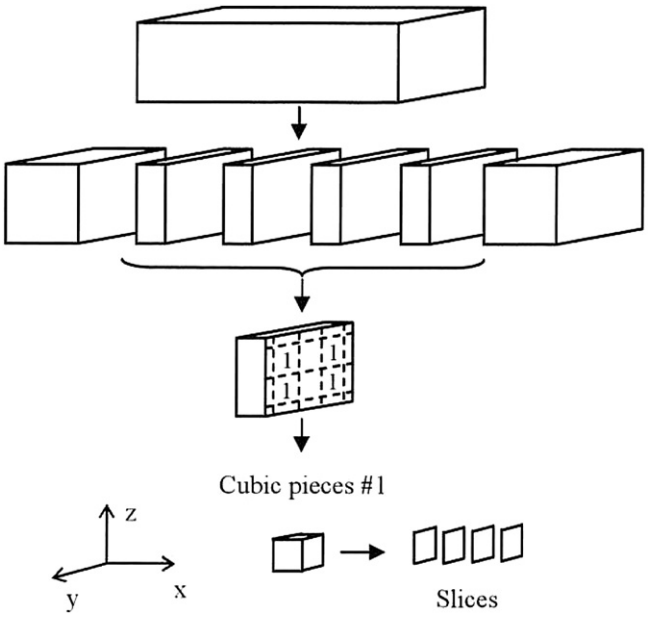

Fig. 1. Schematic view of the sampling of a commercial low-fat soft cheese containing microparticulated whey proteins as fat replacer.

Elastic modulus (G'), viscous modulus (G''), complex modulus ($|G^*|$), complex viscosity ($|\eta^*|$), and tangent of phase angle ($\tan\delta$) were measured in the linear viscoelastic region at a fixed stress amplitude of 318 Pa. All rheological tests were made in duplicate for each sample.

2.4.2. Rheological parameters

The frequency (ω) dependence of G' and G'' at 20 °C of cheese samples was determined using power-law equations (Steffe, 1992):

$$G'(\omega) = a\omega^x \quad (1)$$

$$G''(\omega) = b\omega^y \quad (2)$$

where a and b represent the magnitude of G' and G'' at a given frequency and x and y represent the slopes of the relationships between modulus and frequency.

A first order kinetic model was assumed to represent the rate of decay of the rheological coefficient a during the ripening (Verdini & Rubiolo, 2002):

$$P(\theta) = P_0 e^{-K\theta}$$

where $P(\theta)$ is the rheological coefficient during the ripening (Pa s^x), P_0 is the initial value of the rheological coefficient (Pa s^x), K is the kinetic rate constant ($days^{-1}$) and θ is the ripening time (days).

2.5. Statistical analysis

In this study, analysis of variance (ANOVA) was used and when the effect of the factors was significant ($P < 0.05$), the multiple ranks honestly significant difference (HSD) Tukey test was applied

with a 95% confidence level. The rheological parameters derived from power-law equations were determined using linear regression. In addition, simple and multiple regression analysis were applied to establish the relationships between physicochemical properties and rheological parameters. The complete statistical analysis was carried out using Minitab 13.20 (Minitab Inc., State College, PA, USA).

3. Results and discussion

3.1. Physicochemical properties

Changes in physicochemical properties of frozen cheeses are shown in Table 1. Moisture content of frozen cheeses did not change significantly over the studied ripening period and no significant differences were observed between the moisture contents values of frozen and control cheeses at any ripening time.

During brine salting of cheese, a salt gradient is developed from the surface to the centre of the cheese and salt equilibrium is reached during ripening by diffusion (Luna & Chavez, 1992). Salt concentration of frozen cheeses increased significantly over the ripening period studied, from 1 to 21 days, showing that salt was not evenly distributed in the sample at that point in time. However, salt concentration of frozen cheeses did not change significantly between 21 and 76 days (Table 1). They showed the same behaviour as the control cheeses. The moisture content did not change over the studied ripening period even salt concentration was distributed in the sample at the beginning of the maturation time (1–21 days). Water moves in counter-diffusion of salt and, because salt concentration values were low, the water movement in the cheese was not detected in the high moisture contents that are present in the studied samples. Also, no significant differences were observed between salt concentrations of frozen and control cheeses at any studied ripening time.

The pH of frozen cheeses increased significantly, from 1 to 21 days, and did not change between 21 and 76 days (Table 1). The pH of cheese during ripening is determined by the amount of lactic acid produced by the lactic acid starter, which causes a decrease in the pH, and the buffering capacity of the cheese, which resists this change (Lucey & Fox, 1993). Values of final pH affect cheese texture, enzyme activity and nonstarter microflora (Fox, Guinee, Cogan, & McSweeney, 2000). According to Guinee, Feeney, Auty, and Fox (2002), the increase in pH may be associated with the gradual increase in casein hydration. Furthermore, the casein hydration may be affected by migration of salt in moisture in contact with the protein phase (Cervantes, Lund, & Olson, 1983). During freezing, the solutes present in the aqueous phase (salts, sugars, acids) can be displaced from the ice moving front, creating regions of higher solute concentration. These phenomena could lead to the migration of salt from the solution to the protein in the non-frozen aqueous phase. In addition, the freezing process could produce the damage of lactic acid bacteria cells, lowering the production of organic acids and preventing the decrease of pH during the ripening of frozen cheeses.

Significant differences were observed between pH values of frozen and control cheeses from 21 to 76 days of ripening, where the pH of frozen cheeses was higher than the pH of control cheeses (Table 1). This result indicates that the freezing process produced an increase in the pH of the LFSC. However, values of pH obtained in this study (5.11–5.34) are expected according to results obtained by other authors for different varieties of soft cheeses (Metzger, Barbano, Kindstedt, & Guo, 2001; Upreti & Metzger, 2007).

Maturation index increased significantly during the ripening of frozen cheeses from 1 to 76 days, showing the same behaviour than control cheeses. This result is expected because MI is an index of primary proteolysis, which is often produced by residual chymosin in soft cheeses (Fox & McSweeney, 1996). Significant differences were found between MI of frozen and control cheeses at every studied ripening time, where values of frozen cheeses were higher than values of control cheeses. This result indicates that the freezing process produced an increase in the MI of the LFSC. Maturation indices for low-fat soft cheeses were not found in literature. However, Gravier et al. (2004), who have studied the effect of freezing in low-moisture Mozzarella cheese before ripening, reported higher values of water-soluble nitrogen content at pH 4.6 in samples that were frozen. In addition, Verdini et al. (2002) published that refrigerated and frozen storage conditions significantly affected the MI of Port Salut Argentino cheese, but a clear behaviour was not observed during a short ripening period (56 days). Bertola, Califano, Bevilacqua, and Zaritzky (1996) suggested that cheese matrix may be more susceptible to primary proteolysis in frozen cheeses. In addition, freezing can damage starter cells liberating high amounts of proteolytic enzymes to the media, increasing the MI.

Table 1
Physicochemical properties during the ripening of a commercial low-fat soft cheese containing microparticulated whey proteins as fat replacer.[a]

Ripening time (days)	Moisture content (%, w/w)	Salt concentration (%, w/w)	pH	MI (%)
Control cheeses				
1	52.80 ± 0.46^{a}	0.41 ± 0.00^{a}	5.27 ± 0.03^{c}	3.60 ± 0.67^{a}
21	52.26 ± 0.68^{a}	0.71 ± 0.05^{b}	5.18 ± 0.05^{b}	6.62 ± 0.21^{c}
48	51.76 ± 0.59^{a}	0.76 ± 0.14^{b}	5.11 ± 0.03^{a}	8.66 ± 1.05^{e}
76	52.43 ± 0.29^{a}	0.69 ± 0.13^{b}	5.16 ± 0.04^{b}	10.24 ± 0.54^{g}
Frozen cheeses				
1	53.49 ± 2.06^{a}	0.44 ± 0.01^{a}	5.28 ± 0.02^{c}	4.09 ± 0.66^{b}
21	53.33 ± 1.70^{a}	0.92 ± 0.22^{b}	5.34 ± 0.00^{d}	7.39 ± 0.95^{d}
48	52.56 ± 0.31^{a}	0.85 ± 0.28^{b}	5.34 ± 0.01^{d}	9.51 ± 0.50^{f}
76	53.16 ± 1.04^{a}	0.76 ± 0.07^{b}	5.32 ± 0.02^{d}	12.97 ± 1.02^{h}

[a] Mean values and standard deviations of three replications. Means within a column with the same superscript letter are not significantly different ($P < 0.05$).

3.2. Region of linear viscoelasticity

All tested stresses only produced deformation in the linear viscoelastic region and the magnitude of the applied stress did not affect the values of $|G^*|$ in frozen cheeses at any studied ripening

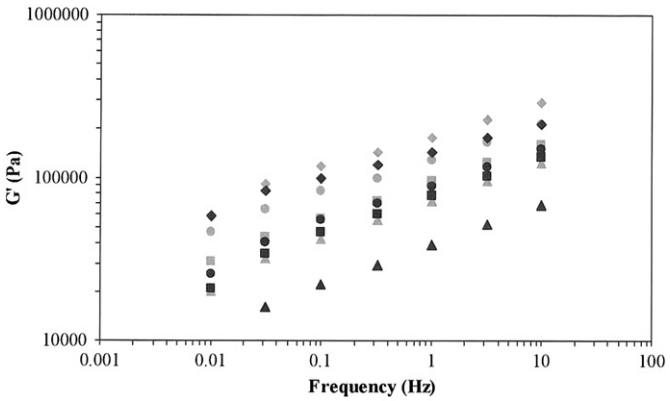

Fig. 2. Example of the data obtained from dynamic oscillatory measurement: frequency dependency of elastic modulus (G') at 20 °C of a commercial low-fat soft cheese containing microparticulated whey proteins as fat replacer. Data were obtained on days (◆, ◆) 1, (●, ●) 21, (■, ■) 48 and (▲, ▲) 76; grey symbols are control cheeses and black symbols are frozen cheeses. Fixed stress 318 Pa. Points represent individual values of one of the three samples.

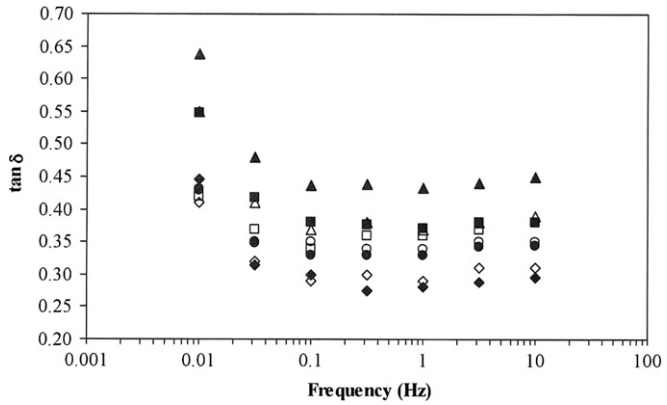

Fig. 3. Example of the data obtained from dynamic oscillatory measurement: tangent of phase angle (tan δ) at 20 °C of a commercial low-fat soft cheese containing microparticulated whey proteins as fat replacer. Data were obtained on days (◇, ◆) 1, (○, ●) 21, (□, ■) 48 and (△, ▲) 76; open symbols are control cheeses and closed symbols are frozen cheeses. Points represent individual values of one of the three samples.

time, showing the same behaviour that was previously observed for control cheeses (Meza et al., 2010). This result indicates that the freezing process did not produce modifications in the extent of the linear viscoelastic region of the LFSC. Similar behaviour was observed by Ribero, Zorrilla, and Rubiolo (2007), who have studied the influence of immersion freezing in NaCl solutions and of frozen storage on the viscoelastic behaviour of mozzarella cheese. The authors published that a critical strain of 0.005 at 1 Hz was obtained from strain sweeps for both refrigerated and frozen samples, indicating that freezing process did not produce modifications in the extent of the linear viscoelastic region of Mozzarella cheese.

3.3. Mechanical spectra

Changes in values of G' and tan δ as a function of frequency of frozen and control cheeses are shown in Figs. 2 and 3. Values of tan δ for frozen cheeses were between 0.28 and 0.65, indicating that elastic properties predominate in the samples throughout the studied frequency range. Similar results were observed by Ma, Drake, Barbosa-Canovas, and Swanson (1997) in a low-fat Cheddar cheese with whey proteins as fat replacer and by other authors in several types of low-fat cheeses (Subramanian & Gunasekaran, 1997).

In general, frozen cheeses presented higher values of tan δ than control cheeses (Fig. 3), suggesting that the freezing process produced an increase in the viscous properties of the LFSC. Also, values of G' (Fig. 2) and G'' decreased with ripening of frozen cheeses showing the same behaviour as control cheeses. A decrease in the linear viscoelastic data has been correlated with protein breakdown (Ak & Gunasekaran, 1996; Subramanian & Gunasekaran, 1997) that makes cheese more elastic–viscous with storage time (Diefes, Rizvi, & Bartsch, 1993). These observations are in agreement with the increase in the tan δ with ripening time (Fig. 3).

3.4. Rheological parameters

Rheological parameters derived from power-law equations that were used to analyze the frequency dependence of G' and G'' of studied cheeses are shown in Table 2. It can be observed that elastic properties were more sensitive to changes in frequency than viscous properties, because in all cases exponent x was higher than y. Coefficient a decreased significantly during the studied ripening period, from 1 to 76 days in frozen cheeses, showing the same response as control cheeses (Table 2). The decrease of coefficient a of elastic modulus could be produced by the loss of the protein network by proteolysis. According to Ak and Gunasekaran (1996), this phenomena produced a decrease in values of G' in Mozzarella cheese during refrigerated storage. However, coefficient b did not change significantly over the studied ripening period, from 1 to 48 days, but decreased significantly from 48 to 76 days in frozen cheeses (Table 2). The same behaviour was observed in control cheeses. The decrease of coefficient b of viscous modulus can be related to the generation of ionic groups due to hydrolysis of protein that binds water and would reduce the viscous dissipation (Ak & Gunasekaran, 1996; Creamer & Olson, 1982; Dave, Sharma, & Muthukumarappan, 2003).

Exponents x and y of frozen and control cheeses increased significantly during the studied ripening period, from 1 to 21 days, and did not change significantly between 21 and 76 days (Table 2). This result suggests that both viscous and elastic modulus were sensitive to changes in frequency during the first stage of ripening, showing a lower degree of structuring of the cheese matrix.

The only rheological parameter affected by the freezing process was coefficient a, where in general frozen cheeses presented lower values than control cheeses. This result indicates that only elastic modulus of the LFSC was affected by the freezing process. For this reason, coefficient a was selected to analyze their kinetic behaviour during the ripening and to perform correlations with the physicochemical properties.

3.5. Kinetic analysis of coefficient a

The kinetic analyses during ripening of coefficient a are shown in Fig. 4. The kinetic rate constant of frozen cheeses was 0.011 ± 0.009 days^{-1} and there was no significant difference

Table 2
Rheological parameters derived from power-law equations during the ripening for elastic ($G' = a\omega^x$) and viscous ($G'' = b\omega^y$) moduli of a commercial low-fat soft cheese containing microparticulated whey proteins as fat replacer.[a]

Ripening time (days)	a (kPa s^x)	x (−)	R^2	b (kPa s^y)	y (−)	R^2
Control cheeses						
1	121.1 ± 5.2^g	0.217 ± 0.001^a	0.99	39.5 ± 1.5^b	0.185 ± 0.002^a	0.99
21	79.2 ± 16.1^d	0.231 ± 0.011^b	0.99	28.8 ± 5.5^b	0.210 ± 0.011^b	0.99
48	72.1 ± 10.6^c	0.236 ± 0.008^b	0.99	25.6 ± 3.5^b	0.233 ± 0.008^b	0.99
76	43.9 ± 4.5^b	0.248 ± 0.017^b	0.99	17.7 ± 1.5^a	0.215 ± 0.011^b	0.99
Frozen cheeses						
1	85.5 ± 21.7^f	0.196 ± 0.019^a	0,99	29.5 ± 4.7^b	0.161 ± 0.019^a	0.98
21	77.1 ± 21.2^e	0.245 ± 0.015^b	0.99	29.5 ± 10.4^b	0.221 ± 0.015^b	0.98
48	53.8 ± 11.9^c	0.252 ± 0.020^b	0.99	26.5 ± 7.5^b	0.223 ± 0.009^b	0.99
76	38.4 ± 13.5^a	0.275 ± 0.013^b	0.99	18.3 ± 5.9^a	0.243 ± 0.005^b	0.99

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

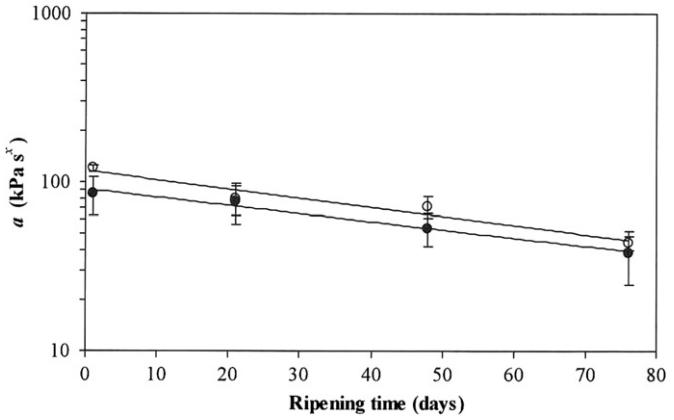

Fig. 4. Kinetic analysis of the coefficient *a* of power-law equation of a commercial low-fat soft cheese (○, control cheeses; ●, frozen cheeses) containing microparticulated whey proteins as fat replacer. Error bars are based on standard deviations and lines represent the fitted first order kinetic model.

between this value and the kinetic rate constant of control cheeses (0.012 ± 0.001 days^{-1}). These results indicate that, although frozen cheeses presented lower values of coefficient *a* than control cheeses (Section 3.4), the decay rate of coefficient *a* during the studied ripening period was not influenced by the freezing process. Similar results were obtained by Verdini et al. (2003), who have reported that freezing did not significantly affect the decay rates of asymptotic equilibrium modulus obtained by compression and stress relaxation tests in Port Salut Argentino cheese.

3.6. Correlations between physicochemical properties and coefficient a

Correlations between physicochemical properties and the coefficient *a* of power-law equation of frozen and control cheeses are shown in Table 3. The best simple correlation was obtained between MI and coefficient *a*; while the combination of salt concentration and pH with MI using multiple correlations did not improve the results ($P > 0.05$). In addition, salt concentration and pH whether in simple regression or in multiple regression analysis (when MI was excluded) with the coefficient *a* showed a poor correlation (Adjusted $R^2 < 0.40$). In agreement, coefficient errors are accurate only when a simple correlation between MI and coefficient *a* was applied. It is interesting to notice that control cheeses presented better correlations than frozen cheeses (Table 3). Significant effects were produced in the coefficient *a* by the freezing process (Section 3.4), indicating that this preservation technique is a process that contributes, along with physicochemical properties like MI, to the viscoelastic behaviour during the ripening of the LFSC.

Table 3
Correlation between coefficient *a* of power-law equation and physicochemical properties of a commercial low-fat soft cheese containing microparticulated whey proteins as fat replacer.

Regression equation	R^2 adjusted
Control cheeses	
$a = 153.5 \pm 12.0 - 10.2 \pm 1.5$ MI	0.79
$a = -156.7 \pm 450.9 + 58.3 \pm 84.7$ pH $- 9.1 \pm 2.3$ MI	0.78
$a = -311.2 \pm 536.1 + 26.6 \pm 44.9$ ClNa $+ 85.8 \pm 99.5$ pH $- 9.8 \pm 2.6$ MI	0.76
Frozen cheeses	
$a = 113.4 \pm 11.8 - 5.86 \pm 1.3$ MI	0.64
$a = -486.4 \pm 926.0 + 113.4 \pm 175.0$ pH $- 6.3 \pm 1.5$ MI	0.62
$a = -379.7 \pm 119.2 + 4.2 \pm 26.6$ ClNa $+ 92.8 \pm 226.8$ pH $- 6.3 \pm 1.6$ MI	0.57

4. Conclusions

In this work, the effect of the freezing process (freezing, frozen storage, and thawing) on viscoelastic behaviour of a commercial low-fat soft cheese containing microparticulated whey proteins as fat replacer was studied during ripening. The freezing process produced an increase in the viscous properties and the MI. The only rheological parameter derived from power-law equations affected by the freezing process was coefficient *a* from elastic modulus. However, the decay rate of coefficient *a* during the studied ripening period was not statistically influenced by freezing. In addition, a negative correlation between coefficient *a* and MI was obtained.

Taking into account that freezing process produced significant structural modification in the cheeses, it could be concluded that this preservation technique is a factor that contributes, along with physicochemical properties like MI, to the viscoelastic behaviour during the ripening of the cheeses. Therefore, the freezing process could be used to extend the shelf-life and to improve the structural characteristics of the commercial low-fat soft cheeses containing microparticulated whey proteins as fat replacer.

Acknowledgments

This work was done with the financial support of Universidad Nacional del Litoral, Consejo Nacional de Investigaciones Científicas y Técnicas and the Agencia Nacional de Promoción Científica y Tecnológica of Argentina. The authors acknowledge Daniel De Piante Vicin for technical assistance in the physicochemical and rheological tests.

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