



Temperature dependency of linear viscoelastic properties of a commercial low-fat soft cheese after frozen storage

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ABSTRACT

The temperature dependency of linear viscoelastic properties of a commercial low-fat soft cheese containing microparticulated whey protein as fat replacer was studied considering the effect of freezing. After thawing, cheeses were held at 6 °C during 48 days for ripening. Refrigerated cheeses (stored at 6 °C for 48 days) were used as control samples. Frequency sweeps (0.01–10 Hz) in the linear viscoelastic region at 10, 20, 30, 40, and 50 °C were performed. Activation energies for complex viscosity at 1 Hz were obtained from an Arrhenius-type equation. Also, the time–temperature superposition method, the modified Cole–Cole analysis and the weak gel model for foods were used to compare the behavior of frozen and refrigerated cheeses. The results obtained in this work indicated that the viscoelastic properties of the studied cheeses obtained at different temperatures were influenced by freezing.

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1. Introduction

Nowadays there is a growing tendency to consume low-fat cheeses around the world. Despite the known benefits for human health, the reduction of the lipid content has a negative effect on the sensory characteristics of food products. Consequently, some strategies can be used in the cheese-making process in order to improve the texture of low-fat cheeses, being one example the addition of microparticulated whey proteins as fat replacers (Hinrichs, 2001).

Depending on cheese variety, it may be necessary to use a preservation technique to extend their self life. The traditional methodology to preserve fresh and soft cheeses is the refrigerated storage at low temperatures (4–6 °C). However, freezing was used as an alternative preservation technology for extending the self life of fresh and soft cheeses (Califano and Bevilacqua, 1999; Verdini et al., 2002, 2003, 2005; Meza et al., 2011). It was published that freezing may influence the texture and the rheological properties of cheeses (Dieses et al., 1993; Gravier et al., 2004; Verdini et al., 2003).

Recently, a study concerning on the effect of freezing on the viscoelastic behavior at 20 °C during the ripening of a commercial low-fat soft cheese has been reported (Meza et al., 2011). In that study, the freezing process was proposed as a preservation technique to improve some of the low-fat cheese quality defects, like the firm structure and the low extent of protein breakdown

during cheese ripening. As consequence of the freeze-concentration and the ice crystal formation produced during freezing, increased breakdown of casein network occurs and an increase in the maturation rate of cheeses is observed (Gravier et al., 2004; Verdini et al., 2005; Meza et al., 2011).

According to Muliawan and Hatzikiriakos (2007), cheese can be described as multiphase system that exhibits a solid-like behavior, changing gradually to a liquid-like behavior as the temperature increases. Because rheological properties of cheese are strongly dependent on temperature, Gunasekaran and Ak (2003) recommended that measurements at different temperatures must be made for a good rheological characterization of the cheese behavior. Several methodologies, like the time–temperature superposition method (TTS) and the modified Cole–Cole analysis (MCC), have been used to analyze the effect of temperature on the linear viscoelastic properties of cheeses (Singh et al., 2006; Udyarajan et al., 2007; Muliawan and Hatzikiriakos, 2007). However, no information about the application of this methodology to low-fat cheeses that were underwent frozen storage was found in literature.

The solid-like behavior of cheeses at temperatures below 40 °C has been documented. Udyarajan et al. (2007) observed that cheeses may show a low frequency dependence of rheological properties at temperatures lower than 40 °C, indicating that this material behaves like a strong gel. In addition, those authors published that cheeses behave as a critical gel according to the Winter and Chambon (1986) criteria in the temperature region from 10 to 20 °C. Thus, the rheological behavior of cheeses could be evaluated

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Nomenclature

<i>Roman</i>			
A	pre-exponential factor (Eq. (1))	G'_p	corrected elastic modulus (Pa) (Eq. (2))
A_F	gel strength (Eq. (6))	G''_p	corrected viscous modulus (Pa) (Eq. (3))
a_T	shift factor of time–temperature superposition method (Eq. (4))	MCC	modified Cole–Cole analysis
E_a	activation energy (kJ/mol) (Eq. (1))	R	universal gas constant (J/mol K)
E_A	activation energy (kJ/mol) (Eq. (5))	T	absolute temperature (K)
G	elastic or viscous moduli at the absolute temperature T (Pa) (Eq. (4))	T_0	absolute reference temperature (K)
G'	elastic modulus (Pa)	TTS	time–temperature superposition method
G''	viscous modulus (Pa)	z	interaction factor (Eq. (6))
$ G^* $	complex modulus (Pa)	<i>Greek</i>	
G_0	elastic or viscous moduli at the absolute temperature T_0 (Pa) (Eq. (4))	$ \eta^* $	complex viscosity (Pa s)
G_p	corrected elastic or viscous moduli (Pa) (Eq. (4))	ρ	density at the temperature T (kg/m ³)
		ρ_0	density at the temperature T_0 (kg/m ³)
		ω	frequency (rad/s)

using mathematical models that allow to understand the material microstructure. In this sense, a weak gel model for foods has been developed in order to characterize the rheological behavior of food systems (Gabriele et al., 2001). Only few publications were found in literature related to the analysis of cheese rheological properties using the weak gel model for foods (Mackù et al., 2008).

The objective of this work was to analyze the temperature dependency of linear viscoelastic properties of a commercial low-fat soft cheese containing microparticulated whey protein as fat replacer. The particular interest in this study was to identify possible differences between frozen and refrigerated cheeses (used as control samples).

2. Materials and methods

2.1. Cheese samples

Seven commercial low-fat soft cheeses elaborated with microparticulated whey proteins as fat replacer (Simplesse® D100, NutraSweet Co., Deerfield, IL, USA) were used. Cheeses were manufactured for this research work at a local factory according to regional legislation, packed in heat-shrinkable plastic bags, and transported on ice bins to the testing laboratory for further ripening and analysis as described in Meza et al. (2010a). The dimensions of the cheeses were: 28.7 ± 0.3 by 11.6 ± 0.3 cm side, and 7.4 ± 0.2 cm height and the weight of the cheeses was 3.0 ± 0.1 kg.

Four cheeses were frozen in a Tabai Comstar PR 4GM chamber (Tabai Espec Corp., Osaka, Japan) at -25 °C at a freezing rate of 1.46 °C/h until the center reached -25 °C. After that, cheeses were held in frozen storage at -25 °C for 33 days, and then thawed at 6 °C. The temperature of the cheeses during freezing and thawing was measured in the center of one cheese using a thermocouple and a data acquisition system Tabai Comstar THP-18 (Tabai Espec Corp., Osaka, Japan). After thawing, cheeses were held at 6 °C and 60% of relative humidity during 48 days for ripening (fully developed cheeses). Those samples were called frozen cheeses. Also, other three cheeses were held at 6 °C and 60% of relative humidity during 48 days for ripening and used as control samples (refrigerated cheeses). Representative samples were taken from both frozen and refrigerated cheeses using the sampling procedure described by Meza et al. (2011).

2.2. Initial composition of cheeses

The total protein content was estimated from total nitrogen content determined by the Kjeldahl method, using a Büchi 430 auto-

matic digester (Büchi, Flawil, Switzerland), a Büchi 322 distillation unit, and a Mettler DL40RC automatic titrator (Mettler Instrumente AG, Greifensee, Switzerland). Fat and moisture content were determined using standard procedures (IDF, 1969; AOAC, 1990). The potentiometric method proposed by Fox (1963) was used to calculate the salt concentration. The pH was measured as described by Meza et al. (2010a). All the analysis were made in duplicate. Initial compositions of both frozen and refrigerated cheeses are shown in Table 1.

2.3. Rheological measurements

Disks of 20 mm diameter and 3 mm thickness, obtained from representative cheese slices according to a previously published procedure (Meza et al., 2011), were used as samples. Rheological measurements were carried out using a stress controlled rheometer RheoStress 80 (Haake Instruments Inc., Karlsruhe, Germany) with a plate–plate geometry test fixture with a diameter and gap of 20 and 2.5 mm, respectively. The temperature of the lower plate was maintained by circulating water from a HaakeT thermostatic water bath (Haake Instrument Inc., Paramus, NJ, USA). The cheese sample was placed on the lower plate and the upper plate was put in contact with the sample during 3 min in order to allow cheese relaxation and to reach temperature equilibrium. A silicone oil (100 cP) thin film was applied to the exposed cheese sample edges to prevent water vaporization.

Stress sweeps at 10 Hz in the range of 64–640 Pa were performed in order to determine the region of linear viscoelasticity. Frequency sweeps (0.01–10 Hz) in the linear viscoelastic region were done at 10.0, 20.0, 30.0, 40.0, and 50.0 ± 0.5 °C at fixed stress amplitude (318 Pa). Elastic modulus (G'), viscous modulus (G''), complex modulus ($|G^*|$), and complex viscosity ($|\eta^*|$) were determined. Rheological measurements were made in triplicate in all cases.

2.4. Rheological analysis

2.4.1. Complex viscosity as function of temperature

The temperature dependency of complex viscosity can be expressed by an Arrhenius-type equation:

$$|\eta^*| = A \exp\left(\frac{E_a}{RT}\right) \quad (1)$$

where A is the pre-exponential factor, E_a is the activation energy, R is the universal gas constant, and T is the absolute temperature. The

Table 1Initial composition of frozen and refrigerated commercial low-fat soft cheeses containing microparticulated whey proteins as fat replacer.^a

Samples	Total protein content (%)	Fat content (%)	Moisture content (%)	Salt concentration (%)	pH
Frozen cheeses	32.9 ± 0.7	5.75 ± 0.04	53.5 ± 2.1	0.44 ± 0.01	5.28 ± 0.02
Refrigerated cheeses	33.6 ± 0.0	5.75 ± 0.04	52.8 ± 0.5	0.41 ± 0.00	5.27 ± 0.03

^a Mean values and standard deviations of two samples.

activation energy controls the rate of molecular motion that is related with the material flow behavior (Rao, 1999).

2.4.2. Time–temperature superposition method

The TTS is a procedure that allows overlap isothermal data of viscoelastic variables into a single master curve, using a shift factor a_T (Ferry, 1980). In this work, the superposition of G' and G'' at five temperatures (10.0, 20.0, 30.0, 40.0, and 50.0 ± 0.5 °C) was obtained by calculating:

$$G'_p = G' \left(\frac{T_0 \rho_0}{T \rho} \right) \quad (2)$$

$$G''_p = G'' \left(\frac{T_0 \rho_0}{T \rho} \right) \quad (3)$$

where G'_p and G''_p are the corrected elastic and viscous moduli; while G' , G'' , and ρ are the elastic modulus, the viscous modulus and the density of the material, respectively, at the absolute temperature T . In addition, ρ_0 is the density of the same material at the absolute reference temperature T_0 that was chosen conveniently (20 °C). The thermal density ratio (ρ_0/ρ) was considered negligible.

Taking into account the Eqs. (2) and (3), shift factor a_T can be expressed by:

$$a_T = \frac{G_{p|T}}{G_{p|T_0}} = \frac{G}{G_0} \left(\frac{T_0}{T} \right) \quad (4)$$

where G_p is the corrected elastic or viscous moduli, G is the elastic or viscous moduli at the absolute temperature T , and G_0 is the elastic or viscous moduli at the absolute reference temperature T_0 . Values of shift factor a_T determined from Eq. (4) were different depending on frequency. Thus, the shift factor which superimposes the majority G'_p and G''_p points in the range of studied frequencies was chosen using a numerical shift procedure proposed by Rosati et al. (2000) and used previously (Meza et al., 2010b).

Temperature dependence of a_T can be established using a modified Arrhenius-type equation (Ferry, 1980):

$$\log a_T = \left(\frac{E_A}{2.303R} \right) \left(\frac{1}{T} - \frac{1}{T_0} \right) \quad (5)$$

where E_A is the activation energy that describes the energy associated with relaxation mechanism at cross linking points (Kasapis and Sworn, 2000; Nickerson et al., 2004).

2.4.3. Modified Cole–Cole analysis

The MCC consists of plotting G' and G'' values measured at several temperatures against each other on logarithmic coordinates (Baek and Han, 1995). In this study, the superposition of moduli at five temperatures (10.0, 20.0, 30.0, 40.0, and 50.0 ± 0.5 °C) was obtained using this procedure. Departure from superposition using the MCC suggests that relaxation times are influenced by temperature dependent factors. This analysis complements the TTS and it might be used to select the T_0 , giving an approximation where the TTS is valid.

2.4.4. Weak gel model for foods

The weak gel model for foods was developed considering that the structure of the food corresponds to a cooperative arrangement

of flow units forming interacting strands (Gabriele et al., 2001). According to this model, values of $|G^*|$ as function of frequency (ω) can be expressed by:

$$|G^*| = A_F \omega^{1/z} \quad (6)$$

where z is the interaction factor (number of flow units interacting with one another in the food structure) and A_F is the gel strength (related to the strength of the interactions between flow units).

2.5. Statistical analysis

Analysis of variance was used in this study. When the effect of the factors was significant ($P < 0.05$), the Tukey's honestly significant difference test was applied (95% of confidence level). Linear regression was used to determine the weak gel model for foods parameters (A_F and z) and the activation energies from Arrhenius-type equations (Eqs. (1) and (5)). All the statistical analysis was performed using Minitab 13.20 (Minitab Inc., State College, PA, USA).

3. Results and discussion

3.1. Complex viscosity as function of temperature

Values of $|\eta^*|$ at different temperatures as function of frequency for frozen and refrigerated cheeses are shown in Fig. 1. Complex viscosity decreased as frequency increased at all temperatures, showing a shear-thinning behavior. The shear-thinning nature of cheese has been previously reported (Gunasekaran and Ak, 2003). It is known that cheese is an example of an oil-in-water emulsion, consisting of milk fat globules suspended in an aqueous semi-solid phase of aggregated casein proteins (Fox et al., 2000; McClements, 2005). According to McClements (2005), the shear-thinning behavior in food emulsions may occur for several reasons. For example, particles or flocs may be aligned, deformed and/or disrupted by the shear flow field.

A plot of $\ln |\eta^*|$ at 1 Hz as function of the reciprocal of absolute temperature (Fig. 2) generated a straight line, following the Arrhenius-type equation (Eq. (1)). The E_a values of $|\eta^*|$ at 1 Hz for frozen and refrigerated cheeses were 100.4 ± 9.7 and 142.5 ± 6.4 kJ/mol, respectively, and a statistical significant difference was observed between both values. According to Tunick et al. (1990), higher values of E_a of cheeses can be related to more resistance to break down of its body. In this study, the lower value of E_a for frozen cheeses could indicate that its structure breaks down more easily than refrigerated cheeses. These findings may have potential relationship with sensorial data like meltability, because activation energy provides an objective means of measure the flow of cheese (Tunick, 2010).

Activation energy analysis has been applied to liquid foods (Rao, 1999) and was used to examine cheese flow when temperature increases (Tunick, 2010). From the molecular point of view, according to fluids flow theory, the activation energy can be interpreted as the energy required by a molecule to jump from one equilibrium position to another position in the liquid medium (Fox et al., 1956; Monkos, 1997). The comparison of E_a numerical values obtained in this work, with data available in literature for several protein solutions,

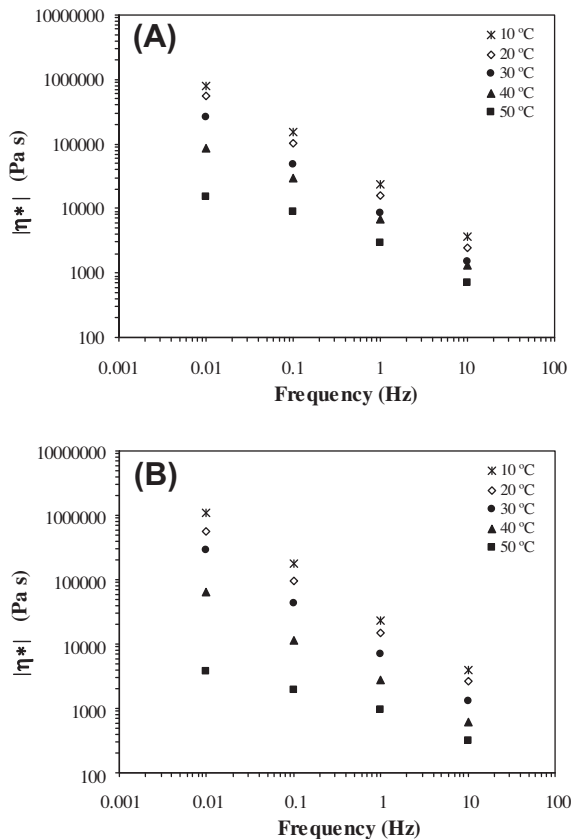


Fig. 1. Example of complex viscosity ($|\eta^*|$) as function of frequency at different temperatures for frozen (A) and refrigerated (B) commercial low-fat soft cheeses containing microparticulated whey proteins as fat replacer. Points represent individual values of one of the three replicates. Stress fixed amplitude of 318 Pa.

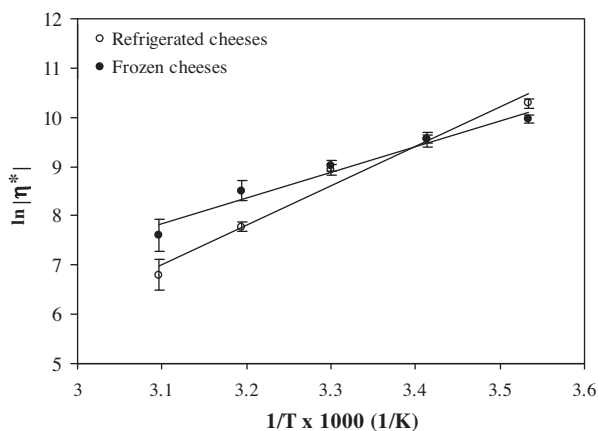


Fig. 2. Arrhenius plot of complex viscosity ($|\eta^*|$) at 1 Hz for a frozen and refrigerated commercial low-fat soft cheeses containing microparticulated whey proteins as fat replacer. Lines represent fitted curves and bars are based on standard deviations.

suggests that activation energy values depend on the weight and dimension of the molecules. For example, for lysozyme solutions ($24.9\text{--}342.6\text{ kg m}^{-3}$) the activation energy was $3.97 \times 10^{-4}\text{ kJ mol}^{-1}$ (Monkos, 1997) and for bovine serum albumin solutions ($17.6\text{--}363.4\text{ kg m}^{-3}$) the E_a was $5.37 \times 10^{-5}\text{ kJ mol}^{-1}$ (Monkos, 1996). However, the activation energies for sodium caseinate solutions varied from 7.5 to 12.1 kJ mol^{-1} as the concentration of the solution changed from 10.5% to 13.0% (w/w), respectively (Barreto et al., 2003).

3.2. Time-temperature superposition method

Values of a_T as a function of temperature for frozen and refrigerated cheeses are shown in Table 2. According to Ferry (1980), in order for the TTS to be valid, the same shift factor a_T must superpose successfully all viscoelastic moduli. In addition, satisfactory application of the TTS suggests that the material is thermorheologically simple, where relaxation times for all mechanisms change in the same way with temperature. In this study, superposition of isothermal frequency data gave similar a_T values at low temperatures ($10\text{--}30\text{ }^\circ\text{C}$) for both G' and G'' (Table 2). This result indicates that, in that low-temperature region, both frozen and refrigerated cheeses behave like a thermorheologically simple material. However, superposition of isothermal frequency data did not give similar a_T values at high temperatures ($40\text{--}50\text{ }^\circ\text{C}$) for both G' and G'' (Table 2). The high-temperature data exhibited slight deviation from the TTS in master curves of G' and G'' when mean values of a_T were used to superimpose moduli (Figs. 3 and 4). Subramanian et al. (2006) successfully applied the TTS in the range of $10\text{--}50\text{ }^\circ\text{C}$ for both regular and reduced-fat pasteurized processed cheese. Nevertheless, failure in the TTS application at high temperatures was reported by other authors. Singh et al. (2006) published that the amount of overlap for G' and G'' data of Mozzarella cheese at different temperatures decreased when temperature increased, where master curves at 40 and $50\text{ }^\circ\text{C}$ showed less overlap in comparison to curves obtained at 20 and $30\text{ }^\circ\text{C}$. Muliawan and Hatzikiriakos (2007) indicated that, at temperatures higher than $40\text{ }^\circ\text{C}$, Mozzarella cheese started to melt undergoing structural changes. For those authors, viscoelastic data showed that cheese behaved as a physically different material depending on temperature.

Cheese is a complex food with a casein network entrapping fat and serum. At high temperatures, protein matrix could shrink accompanied by solid fat transition into liquid oil, generating the expulsion of oil and serum phases. Udyarajan et al. (2007) hypothesized that during heating, calcium and phosphate present in the cheese serum phase may form new insoluble calcium phosphate, which could interact with caseins. According to those authors, changes in casein-casein interactions at high temperatures may exist, generating changes in the rheological properties of cheeses and producing the TTS failure.

Values of a_T monotonically decreased with temperature (Table 2). Similar results were observed in other studies (Subramanian et al., 2006). In the region where the TTS was successfully applied ($10\text{--}30\text{ }^\circ\text{C}$), a_T factors were analyzed to determine whether the temperature dependence of relaxation times can be described by an Arrhenius-type equation. Activation energies of a_T from Eq. (5) for frozen and refrigerated cheeses were 165.7 ± 15.6 and $158.1 \pm 8.8\text{ kJ/mol}$, respectively, and no statistical significant differences were obtained between both values.

According to Lucey et al. (2003), cheese melting process will take place if bond-breaking events (or relaxation) occur at the cross-linking points in the protein cheese matrix. On the other hand, melting process will not occur if the activation energy required for the disentanglements and the relaxation mechanisms is high. This way, the estimation of E_A from Eq. (5) can be used as an indicator of the melting process of cheeses. In this study, at temperatures between 10 and $30\text{ }^\circ\text{C}$, where melting did not occur, both frozen and refrigerated cheeses behaved like a thermorheologically simple material with similar activation energies.

3.3. Modified Cole-Cole analysis

Modified Cole-Cole plots for frozen and refrigerated cheeses are shown in Fig. 5. Elastic and viscous moduli could be superimposed at temperatures between 10 and $30\text{ }^\circ\text{C}$. However, moduli failed to superpose at temperatures higher than $30\text{ }^\circ\text{C}$, indicating

Table 2

Values of shift factors (a_T) as a function of temperature for frozen and refrigerated commercial low-fat soft cheeses containing microparticulated whey proteins as fat replacer.^a

Samples	Temperature (°C)	$\log a_T$ (G') ^a	$\log a_T$ (G'') ^a	Mean $\log a_T$
Frozen cheeses	10	0.30	0.30	0.30
	20	0.00	0.00	0.00
	30	-1.70	-1.70	-1.70
	40	-2.10	-2.70	-2.40
	50	-2.70	-3.70	-3.20
Refrigerated cheeses	10	0.78	0.78	0.78
	20	0.00	0.00	0.00
	30	-1.22	-1.22	-1.22
	40	-2.40	-3.40	-2.90
	50	-3.22	-4.70	-3.96

^a Values correspond to one of three replicates.

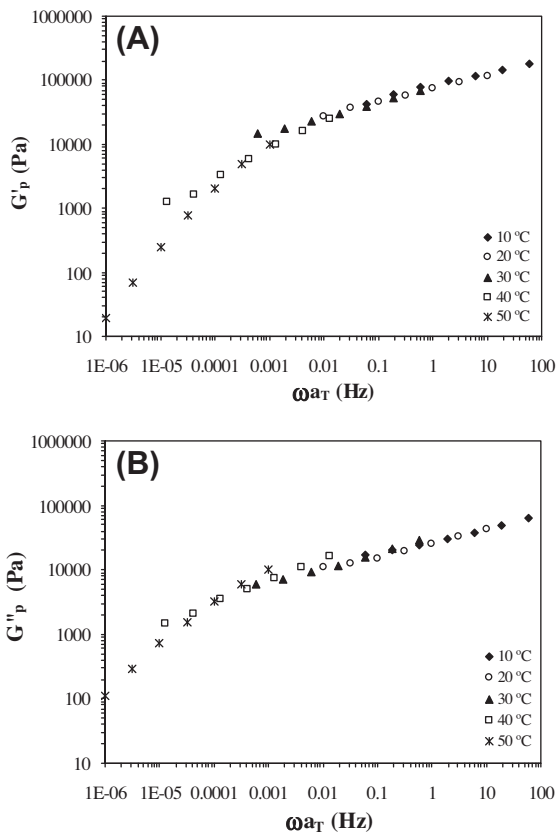


Fig. 3. Example of master curves for frozen commercial low-fat soft cheeses containing microparticulated whey proteins as fat replacer: (A) elastic modulus (G') and (B) viscous modulus (G''). Points represent individual values of one of the three replicates. Stress fixed amplitude of 318 Pa.

temperature-dependent morphological changes in the cheese matrix. The MCC further supported the results obtained by the application of the TTS. Therefore, in the temperature range between 10 and 30 °C, both moduli displayed similar temperature dependence, allowing successful superposition with both the TTS and the MCC.

In addition, a linear trend was observed in the temperature range of successful superposition (Fig. 5). The shape of the modified Cole–Cole plot can give information of the polydispersity within the material. In this case, a linear trend without an inflection point suggests that the material is amorphous (Han and Kim, 1993; Nickerson et al., 2004).

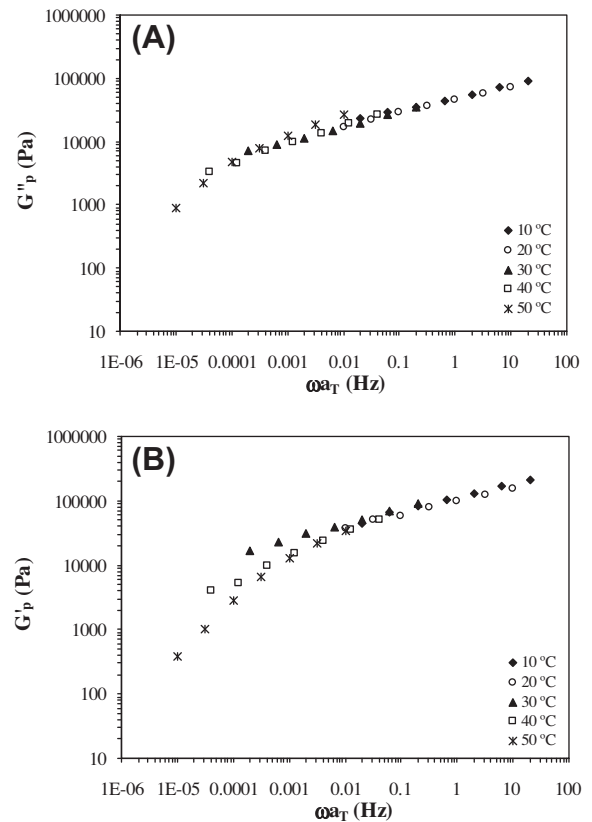


Fig. 4. Example of master curves for refrigerated commercial low-fat soft cheeses containing microparticulated whey proteins as fat replacer: (A) elastic modulus (G') and (B) viscous modulus (G''). Points represent individual values of one of the three replicates. Stress fixed amplitude of 318 Pa.

3.4. Weak gel model for foods

Values of A_F and z factors of weak gel model for foods at different temperatures for frozen and refrigerated cheeses are shown in Table 3.

Values of z and A_F decreased as temperature increased from 10 to 50 °C for both frozen and refrigerated cheeses. In this case, lower z values indicate a minor number of interactions and also, the interactions turned out weak because values of A_F were smaller at high temperatures. According to Lucey et al. (2003), when temperature increases, a decrease in the total number and/or strength of bonds in the cheese matrix is expected. Those authors have applied the “Horne model” to describe the behavior of the cheese during heating. This approach suggested that the strength of protein bonds in the cheese matrix is the result of a balance between attractive interactions (hydrophobic) and electrostatic repulsion. Although hydrophobic interactions increase with increasing temperature, the net result may be a weakening of the protein matrix due to a reduction of contact area between casein molecules. Also, attractive interactions (like hydrogen bonds) decrease with increasing temperature (Bryant and McClements, 1998).

In general, values of z corresponding to frozen cheeses were lower than for refrigerated cheeses, suggesting that frozen cheeses presented lower number of interactions, but with the same strength according to A_F values (Table 3). This result was probably due to a mechanical damage, produced by crystal formation, and a higher proteolysis degree. In our previous work (Meza et al., 2011) it was observed that maturation indexes (related to the primary proteolysis degree during ripening) for frozen cheeses were higher than for refrigerated cheeses at any ripening time. All these events could gen-

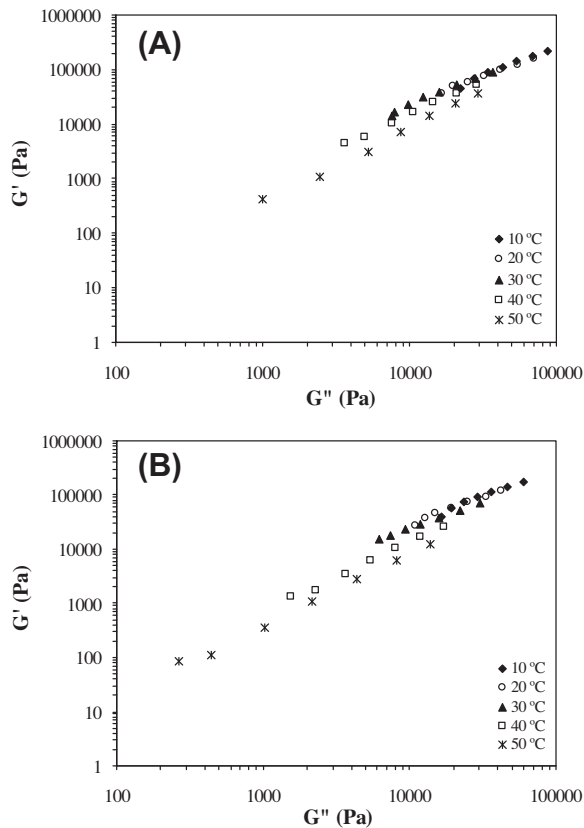


Fig. 5. Modified Cole–Cole plot for frozen (A) and refrigerated (B) commercial low-fat soft cheeses containing microparticulated whey proteins as fat replacer. Points represent individual values of one of the three replicates.

Table 3

Values of gel strength (A_f) and interaction factor (z) of weak gel model for frozen and refrigerated commercial low-fat soft cheeses containing microparticulated whey proteins as fat replacer.

Samples	Temperature (°C)	A_f^A (kPa s ^{1/z})	z^A (-)	R^2
Frozen cheeses	10	134.0 ± 10.0 ^e	4.53 ± 0.20 ⁱ	0.99
	20	87.5 ± 12.5 ^d	4.44 ± 0.29 ^g	0.99
	30	51.1 ± 5.4 ^c	3.64 ± 0.32 ^e	0.99
	40	30.0 ± 5.9 ^b	2.52 ± 0.17 ^c	0.99
	50	11.6 ± 4.0 ^a	1.79 ± 0.06 ^b	0.99
Refrigerated cheeses	10	141.0 ± 16.0 ^e	5.09 ± 0.41 ^j	0.99
	20	90.1 ± 6.6 ^d	4.81 ± 0.13 ^h	0.99
	30	48.4 ± 5.2 ^c	4.41 ± 0.08 ^f	0.99
	40	14.7 ± 1.5 ^b	2.84 ± 0.23 ^d	0.99
	50	5.3 ± 2.0 ^a	1.61 ± 0.20 ^a	0.99

^{a–g}Means within a column with the same letter are not significantly different ($P < 0.05$).

^A Mean values and standard deviations of three samples.

erate the breakdown of casein network, diminishing the number of interactions between protein molecules in frozen cheeses.

4. Conclusions

The results obtained in this work indicated that the viscoelastic properties of the studied cheeses obtained at different temperatures were influenced by freezing. Activation energy of $|\eta^*|$ at 1 Hz for frozen cheeses was lower than for refrigerated cheeses, suggesting less resistance to break down of cheese body.

Superposition of elastic and viscous moduli using the TTS was successful at low temperatures (10–30 °C), indicating that both frozen and refrigerated cheeses behave like a thermorheologically simple material. In this temperature region, a_T factors could be analyzed with an Arrhenius-type equation. In addition, the application of the MCC confirmed the results obtained by the TTS. Parameters obtained from the weak gel model for foods indicate that z and A_f values decreased as temperature increased from 10 to 50 °C. Also, z values for frozen cheeses were lower than for refrigerated cheeses, suggesting that frozen cheeses presented lower number of interactions (but with the same strength according to A_f values) than refrigerated cheeses.

Taking into account that freezing process has produced significant modification in the viscoelastic properties of the studied cheeses, this preservation technology could be used to extend their self-life and to improve their structural characteristics.

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References

- AOAC, 1990. Moisture in Cheese, Official Method 977.11. Official Methods of Analysis: Food Composition; Additives; Natural Contaminants. Association of Official Analytical Chemists Inc., Virginia, USA.
- Baek, D.M., Han, C.D., 1995. Rheological behavior of binary mixtures of polystyrene-block-polyisoprene copolymers in the disordered state. *Polymer* 36, 4833–4839.
- Barreto, P.L.M., Roeder, J., Crespo, J.S., Maciel, G.R., Terenzi, H., Pires, A.T.N., Soldi, V., 2003. Effect of concentration, temperature and plasticizer content on rheological properties of and glass transition of their films. *Food Chemistry* 82, 425–431.
- Bryant, C.M., McClements, D.J., 1998. Molecular basis of protein functionality with special consideration of cold-set gels derived from heat-denatured whey. *Trends in Food Science and Technology* 9, 143–151.
- Califano, A.N., Bevilacqua, A.E., 1999. Freezing low moisture Mozzarella cheese: changes in organic acid content. *Food Chemistry* 64, 193–198.
- Diefes, H.A., Rizvi, S.S.H., Bartsch, J.A., 1993. Rheological behavior of frozen and thawed low-moisture, part-skim Mozzarella cheese. *Journal of Food Science* 58, 764–769.
- Ferry, J.D., 1980. *Viscoelastic Properties of Polymers*. John Wiley and Sons, New York, USA.
- Fox, P.F., 1963. Potentiometric determination of salt in cheese. *Journal of Dairy Science* 46, 744–745.
- Fox, T.G., Gratch, S., Loshaek, S., 1956. Viscosity relationships for polymers in bulk and in concentrated solutions. In: Eirich, F.R. (Ed.), *Rheology: Theory and Applications*, vol. 1. Academic Press, New York, USA.
- Fox, P.F., Guinee, T.P., Cogan, T.M., McSweeney, P.L.H., 2000. *Fundamentals of cheese science*. Aspen Publishers, Maryland, USA.
- Gabriele, D., de Cindio, B., D'Antona, P., 2001. A weak gel model for foods. *Rheological Acta* 40, 120–127.
- Gravier, N.G., Zaritzky, N.E., Califano, A.N., 2004. Viscoelastic behavior of refrigerated and frozen low-moisture Mozzarella cheese. *Journal of Food Science* 9, 123–128.
- Gunasekaran, S., Ak, M.M., 2003. *Cheese Rheology and Texture*. CRC Press LLC, Florida, USA.
- Han, C.D., Kim, J.K., 1993. On the use of time–temperature superposition in multicomponent/multiphase polymer systems. *Polymer* 34, 2533–2539.
- Hinrichs, J., 2001. Incorporation of whey proteins in cheese. *International Dairy Journal* 11, 495–503.
- IDF, 1969. Determination of the fat content of cheese and of processed cheese products. Standard 5A. International Dairy Federation, Brussels, Belgium.
- Kasapis, S., Sworn, G., 2000. Separation of the variables of time and temperature in the mechanical properties of high sugar/polysaccharide mixtures. *Biopolymers* 53, 40–45.
- Lucey, J.A., Johnson, M.E., Horne, D.S., 2003. Invited review: perspectives on the basis of the rheology and texture properties of cheese. *Journal of Dairy Science* 86, 2725–2743.
- Macků, I., Buňka, F., Pavlínek, V., Leciánová, P., Hrabě, J., 2008. The effect of pectin concentration on viscoelastic and sensory properties of processed cheese. *International Journal of Food Science and Technology* 43, 1663–1670.

- McClements, D.J., 2005. Food emulsions: principles, practices, and Techniques. CRC Press LLC, Florida, USA.
- Meza, B.E., Verdini, R.A., Rubiolo, A.C., 2010a. Viscoelastic behavior during the ripening of a commercial low-fat soft cheese. *Dairy Science and Technology* 90, 589–599.
- Meza, B.E., Verdini, R.A., Rubiolo, A.C., 2010b. Effect of freezing on the viscoelastic behaviour of whey protein concentrate suspensions. *Food Hydrocolloids* 24, 414–423.
- Meza, B.E., Verdini, R.A., Rubiolo, A.C., 2011. Effect of freezing on the viscoelastic behavior during the ripening of a commercial low-fat soft cheese. *International Dairy Journal* 21, 346–351.
- Monkos, K., 1996. Viscosity of bovine serum albumin aqueous solutions as a function of temperature and concentration. *International Journal of Biological Macromolecules* 18, 61–68.
- Monkos, K., 1997. Concentration and temperature dependence of viscosity in lysozyme aqueous solutions. *Biochimica et Biophysica Acta* 1339, 304–310.
- Muliawan, E.B., Hatzikiriakos, S.G., 2007. Rheology of Mozzarella cheese. *International Dairy Journal* 17, 1063–1072.
- Nickerson, M.T., Paulson, A.T., Speers, R.A., 2004. A time–temperature rheological approach for examining food polymer gelation. *Trends in Food Science and Technology* 15, 569–574.
- Rao, A.M., 1999. *Rheology of Fluid and Semisolid Foods: Principles and Applications*. Aspen Publishers, Maryland, USA.
- Rosati, D., Van Loon, B., Navard, P., 2000. Rheology of strongly segregated poly(styrene-dimethylsiloxane) block copolymers. *Polymer* 41, 367–375.
- Singh, A.P., Lakes, R.S., Gunasekaran, S., 2006. Viscoelastic characterization of selected foods over an extended frequency range. *Rheological Acta* 46, 131–142.
- Subramanian, R., Muthukumarappan, K., Gunasekaran, S., 2006. Linear viscoelastic properties of regular and reduced-fat pasteurized process cheese during heating and cooling. *International Journal of Food Properties* 9, 377–393.
- Tunick, M.H., 2010. Activation energy measurements in rheological analysis of cheese. *International Dairy Journal* 20, 680–685.
- Tunick, M.H., Nolan, E.J., Shieh, J.J., Basch, J.J., Thompson, M.P., Maleeff, V.E., Holsinger, V.H., 1990. Cheddar and Cheshire cheese rheology. *Journal of Dairy Science* 73, 1671–1675.
- Udyarajan, C.T., Horne, D.S., Lucey, J.A., 2007. Use of time–temperature superposition to study the rheological properties of cheese during heating and cooling. *International Journal of Food Science and Technology* 42, 686–698.
- Verdini, R.A., Zorrilla, S.E., Rubiolo, A.C., 2002. Free amino acid profiles during ripening of Port Salut Argentino cheese after frozen storage. *Journal of Food Science* 67, 3264–3270.
- Verdini, R.A., Zorrilla, S.E., Rubiolo, A.C., 2003. Changes in equilibrium modulus and α_{S1} -casein breakdown during the ripening of Port Salut Argentino cheese as affected by frozen storage. *Journal of Texture Studies* 34, 331–346.
- Verdini, R.A., Zorrilla, A.E., Rubiolo, A.C., 2005. Effects of the freezing process on proteolysis during the ripening of Port Salut Argentino cheeses. *International Dairy Journal* 15, 363–370.
- Winter, H.H., Chambon, F., 1986. Analysis of linear viscoelasticity of a crosslinking polymer at gel point. *Journal of Rheology* 30, 367–382.