

Influence of Immersion Freezing in NaCl Solutions and of Frozen Storage on the Viscoelastic Behavior of Mozzarella Cheese

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ABSTRACT: The freezing of Mozzarella cheese by immersion in NaCl solutions may be an innovative procedure for the dairy industry because it combines conveniently salting and freezing processes. In this work, the influence of this type of freezing method and of the frozen storage of samples on the viscoelastic behavior of Mozzarella cheese was studied. Slabs ($2 \times 10 \times 10 \text{ cm}^3$) were immersed in 23% w/w NaCl solutions (control samples: 4°C , 90 min; frozen samples: -15°C , 180 min). Half of the frozen samples were immediately thawed at 4°C . The other half was stored at -20°C for 2 mo and then was thawed at 4°C (frozen-stored samples). Samples were stored at 4°C and assayed at 1, 7, 14, 20, 27, 34, and 41 d. Rheological tests were carried out in oscillatory mode (parallel-plate geometry, diameter: 20 mm, gap: 1 mm, frequency: 1 Hz). Strain sweeps were run ($0.001 \leq \gamma_0 \leq 0.1$) at 20, 40, and 60°C , and temperature sweeps were run from 20 to 65°C ($1.33^\circ\text{C}/\text{min}$, $\gamma_0 = 0.005$). Similar crossover temperatures were observed after 20 d of ripening. The influence of temperature on complex viscosity was studied by an Arrhenius-type equation. Activation energy values of 15.9 ± 0.4 , 14.1 ± 0.5 , and $13.8 \pm 0.6 \text{ kcal/mol}$ were obtained at 41 d for control, frozen, and frozen-stored samples, respectively. Although the immersion freezing of Mozzarella cheese affects some of the studied parameters, the differences observed between frozen and frozen-stored samples with control samples were small. Therefore, it was considered that the immersion freezing might be useful for the manufacture and commercialization of Mozzarella cheese.

Keywords: immersion freezing, Mozzarella cheese, rheology, salting

Introduction

Cheese freezing is effective in extending its shelf life, but it may affect the final quality of the product. There have been several studies on how Mozzarella cheese is affected by the freezing process (Cervantes and others 1983; Tunick and others 1991; Diefes and others 1993; Chaves and others 1999). These studies have shown that the modification of physical properties of Mozzarella cheese due to the freezing process varies greatly depending on the methodology and operating conditions of freezing, frozen storage, and thawing. An interesting alternative for fast freezing of cheeses may be freezing by immersion in NaCl solutions (Zorrilla and Rubiolo 2005a, 2005b). This methodology has recognized advantages; it is one of the fastest freezing techniques, and it is associated with lower costs and higher quality of the final product as compared to traditional freezing methods (Lucas and Raoult-Wack 1998). Moreover, in the case of Mozzarella cheese, immersion freezing would allow decreasing the production time.

Mozzarella is a *pasta filata* type cheese. The manufacture of this cheese is characterized by a unique texturization process where the curd is continuously kneaded and stretched in hot water until a smooth, fibrous structure is obtained. Mozzarella cheese must have certain characteristics for use on pizzas and other foods. The desirable characteristics of this cheese in the solid and melted states are known as “functional properties,” which, in a way, express con-

sumer expectations of how the cheese should behave when it is used as an ingredient. The functional properties of Mozzarella cheese include shreddability for the solid cheese, and meltability, stretchability, elasticity, free oil formation, and browning for the melted cheese. Clearly, a majority of these functional properties are associated with the rheology of the solid and melted cheese (Gunasekaran and Ak 2003). For example, Ustunol and others (1994), Zhou and Mulvaney (1998), Lucey and others (2003), and Montesinos-Herrero and others (2006) related some rheological parameters, particularly viscoelastic parameters, to some functional properties.

Composition and structural characteristics of cheese affect its rheological properties and they undergo notable changes during maturation (Subramanian and Gunasekaran 1997), proteolysis being the principal driving force behind age-dependent functional changes in Mozzarella cheese (Kindstedt 2004). Dynamic testing is one of the most important and fundamental methods for determining rheological properties of viscoelastic materials and it has been used extensively to probe the structure of cheese (Tunick and others 1993a, 1993b, 1995; Subramanian and Gunasekaran 1997; Yu and Gunasekaran 2005).

Our objective was to evaluate the influence of the freezing of Mozzarella cheese by immersion in NaCl solutions and of the frozen storage on its viscoelastic behavior using the small amplitude oscillatory shear (SAOS) technique.

Materials and Methods

Cheese sampling and immersion brine

Unsalted fresh Mozzarella cheeses (3500 g weight, $28 \times 10 \times 10 \text{ cm}^3$ size) were provided by a local factory. Samples were slabs of 2-cm thickness, which were cut perpendicularly to the principal axis

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of the cheese block. The immersion brine was a NaCl solution of 23% w/w (0.55% Ca²⁺, pH 5.2).

Treatments

Twenty-one slabs were immersed in the brine at 4 °C and removed at 90 min (control samples) and 42 slabs were immersed in the brine at -15 °C and removed at 180 min. Samples were carefully wiped with a paper towel. Twenty-one frozen samples were thawed at 4 °C immediately after the freezing process was finished (frozen samples), while 21 frozen samples were stored at -20 °C for 2 mo and then were thawed at 4 °C (frozen-stored samples). All samples were packed under vacuum and stored at 4 °C during 6 wk. Three slabs per treatment were randomly selected at 1, 7, 14, 20, 27, 34, and 41 d of ripening. Each slab of Mozzarella cheese was cut parallel to the principal axis in 2 equal parts. In one of the halves, pH and contents of chloride, moisture, total nitrogen, and soluble nitrogen in water at pH 4.6 were determined; in the other half, the rheological tests were carried out.

Chemical analysis

The pH was determined with an electrode for solid foods (PH Spear, OAKTON Instruments, Vernon Hills, Ill., U.S.A.) in duplicate. Chloride content was determined as suggested by Fox (1963) with an automatic titrator model DL40 RC (Mettler Instrumente AG, Greifensee, Switzerland) in 5 replicates. Moisture content was determined in a microwave CEM AVC 80 (CEM, Mattheus, N.C., U.S.A.) in duplicate. Water-soluble fraction extraction at pH 4.6 was performed with a modified procedure developed by Kuchroo and Fox (1982) (Sihufe and others 2003). Total nitrogen (TN) and water-soluble nitrogen (WSN) were determined in duplicate using the micro-Kjeldahl method with an automatic digester model 430, a distillation unit model 322, and a control unit model 342 (Büchi Labortechnik AG, 1998, Flawil, Switzerland), and a DL40 RC titrator (Mettler Instrumente AG, Greifensee, Switzerland). Maturation index was calculated as a percentage of WSF of the cheese TN (WSN × 100/TN), and it was used to follow the proteolysis degree during ripening. Fat content was determined for initial composition in duplicate (Intl. Dairy Federation 1969).

Rheological measurements

Cheese slices (approximately 1.5 mm thickness) were cut from the half slab chosen for rheological tests and perpendicularly to the principal axis of the cheese block, placed into plastic bags to prevent dehydration, and held refrigerated until testing. The 1st 3 slices were discarded and disks (20 mm dia) were cut using a borer. Disks were obtained discarding the external region of approximately 10 mm from cheese surface. A rheometer Haake RheoStress RS80 (Haake Instrument Inc., Paramus, N.J., U.S.A.) with parallel plates (20 mm dia, 1 mm gap) at a frequency of 1 Hz was used for rheological measurements. The temperature of the lower plate of the measuring system was maintained by circulating water from water bath. The disk-shaped cheese sample was placed on the lower plate and then the upper plate was brought in contact with the sample to reach

temperature equilibrium. A thin film of silicone oil (20 Cp) covered the edge of samples to avoid evaporation during measurements.

The dynamic rheological data obtained included the 2 components of complex shear modulus (G^*): the storage modulus (G'), which is a measure of the energy stored and recovered per cycle of deformation (elastic component) and the loss modulus (G''), which is a measure of the energy dissipated or lost as heat per cycle of deformation (viscous component) (Gunasekaran and Ak 2003). These parameters are related as follows:

$$|G^*|^2 = (G')^2 + (G'')^2 \quad (1)$$

Strain sweep. Sweep tests of strain amplitude (γ_0) were performed from 0.001 to 0.1 at 20, 40, and 60 °C, to determine the limits of linear viscoelasticity.

Temperature sweep. The temperature sweep tests were carried out from 20 to 65 °C (1.33 °C/min) at a strain amplitude of 0.005 ± 5 × 10⁻⁵. The loss tangent ($\tan \delta = G''/G'$) changing with temperature and the temperature at crossover modulus ($G' = G''$) were determined. Moreover, the effect of temperature on complex viscosity ($|\eta^*| = G^*/\omega$, ω : frequency of oscillation) was studied by an Arrhenius-type equation:

$$\eta^* = A_{VISC} \exp\left(\frac{E_a}{RT}\right) \quad (2)$$

where A_{VISC} is the pre-exponential factor, E_a is the activation energy (cal/mol), R is the gas constant (1.9872 cal/mol K), and T is the temperature (K) (Rao 1999; Tunick 2000).

Statistics analysis

Data were analyzed by ANOVA using Minitab (Minitab Inc., State College, Pa., U.S.A.). When differences between treatment effects were significant ($P < 0.05$), a multiple comparison of means was performed using the Tukey test.

Results and Discussion

Chemical characteristics

The initial composition and the mean composition for each treatment are shown in Table 1. It can be observed that the control samples underwent the higher dehydration. Expected values of total protein, fat, and chloride contents and of pH were observed (Diefes and others 1993; Yun and others 1993a, 1993b; Guo and Kindstedt 1995; Bertola and others 1996a, 1996b; Subramanian and Gunasekaran 1997; Chaves and others 1999; McMahan and others 1999; Muthukumarappan and others 1999; Guinee and others 2001).

The proteolysis of α_{S1} and β -casein in Mozzarella cheese is mainly caused by the residual coagulant and plasmin (Fox and Guinee 1987; Farkye and others 1991). In this case, maturation index increases during the ripening period studied, approximately from 2.8% to 7.3% (Figure 1). As reported by Yun and others (1993a), Bertola and others (1996b), Guinee and others (2000), and Feeney and others (2001),

Table 1 – Average chemical composition of cheese samples

Component	Initial sample ^a	Control samples ^b	Frozen samples ^b	Frozen-stored samples ^b
Moisture (%)	50.2 ± 0.3	47.7 ± 0.7	49.3 ± 0.4	49.3 ± 0.5
Fat (%)	19.7 ± 0.4	–	–	–
Total protein (%)	22 ± 2	23.1 ± 0.6	22.2 ± 0.9	22.1 ± 0.6
Chloride in moisture (%)	0.143 ± 0.004	2.43 ± 0.05	2.66 ± 0.09	2.8 ± 0.1
pH	5.43 ± 0.03	5.28 ± 0.04	5.29 ± 0.03	5.30 ± 0.07

^a Average values and standard deviations.

^b Average values and standard deviations considering all the samples for each treatment.

the low level of proteolysis may be explained by the partial thermal inactivation of the coagulant during kneading and stretching process.

Viscoelastic behavior

Cheese is a viscoelastic material because it exhibits both elastic and viscous behavior depending on the time scale of the deformation. During short time scales, the behavior of cheese is mainly elastic; that is, a test piece almost recovers its original form after the removal of the stress applied. However, at long time scales, the behavior is mainly viscous; that is, most of the deformation remains after the removal of the stress applied. The simplest type of viscoelastic behavior is linear viscoelasticity, which will be observed if the relative deformation is smaller than about 2% (Dave and others 2003; Gunasekaran and Ak 2003).

Dynamic rheological measurements are useful in enhancing the understanding of the viscoelastic behavior of Mozzarella cheese (Ak and Gunasekaran 1996; Subramanian and Gunasekaran 1997). In the small amplitude oscillatory shear analysis (SAOS), a small strain is applied to the sample to measure the strength and flexibility of internal bonds and provides quantitative values for the viscoelastic properties of the cheese matrices (Diefes and others 1993; Van Hekken and others 2004). The measurements yield true engineering values for elasticity and viscosity in contrast to the approximations obtained from other test methods (Dave and others 2003).

Strain sweep. Typical strain sweep results for the Mozzarella cheese samples studied are shown in Figure 2. It can be observed that the modulus decreases with the ripening time, which agrees with the results reported by Diefes and others (1993), Ak and Gunasekaran (1996), and Subramanian and Gunasekaran (1997). This behavior may be explained taking into account the changes that occur during ripening. Loss of protein network by proteolysis implies a decrease in G' during ripening (Ak and Gunasekaran 1996). Although it is expected that G'' increases with the ripening time, this modulus decreases similarly to the elastic modulus during ripening. This may be related to the fact that proteolysis generates ionic groups that bind water and that would reduce viscous dissipation (Ak and Gunasekaran 1996).

It was also observed that the G' and G'' values decreased with increasing temperature of the rheological test (Figure 2), as suggested by Mounsey and O'Riordan (1999). Hennesly and others (2006) suggested that this may be caused by weakening of protein-protein interactions within the casein network with increasing tempera-

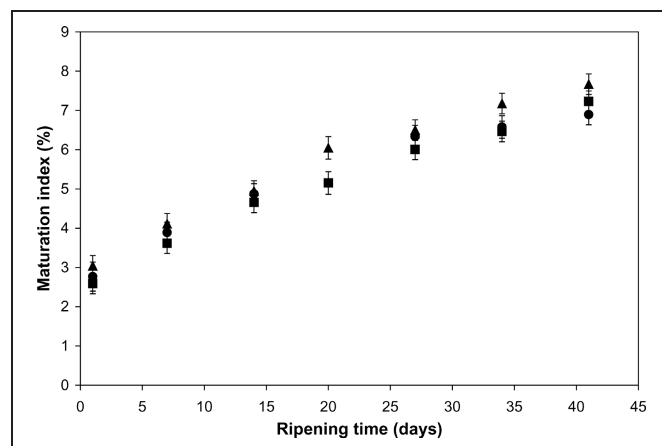


Figure 1 – Maturation index of control (■), frozen (●), and frozen-stored (▲) samples changing with ripening time. Bars indicate 95% confidence limits.

ture, which, accompanied by liquefaction and deformation of the fat globules, may plasticize the protein matrix and improve the flowability.

At 20 and 40 °C, G' is generally greater than G'' , indicating the dominant elastic behavior (Figure 2). This behavior was also observed at 60 °C and 1 d of ripening. However, at 14 and 41 d of ripening and at 60 °C, G' is generally greater than G'' , which is related to a viscous behavior. At 40 °C and 14 d of ripening, and for a strain amplitude greater than 0.02, G' and G'' gradually decreased. On one hand, this behavior may be related to the changes of fat with temperature, and on the other hand, to the structural change of protein matrix due to hydration and proteolysis during ripening. The fat, which acts as reinforcing filler at low temperatures (<40 °C), gradually becomes a diluting factor or plasticizer. This probably occurs above approximately 40 °C, when the casein network is substantially dissociated and the fat is completely liquid (Muthukumarappan and others 1999). At 41 d of ripening, the linear viscoelasticity region is expanded probably due to the water redistribution during ripening and the intermolecular interactions associated (McMahon and others 1999; Muthukumarappan and others 1999; Guinee and others 2001).

From the strain sweeps, it was observed that $\gamma_0 = 0.005$ ensures that the rheological determinations be carried out in the linear viscoelasticity region. Tunick and others (1993a), Ak and Gunasekaran (1996), Subramanian and Gunasekaran (1997), Gunasekaran and Ak (2000, 2003), and Yu and Gunasekaran (2005) reported similar strain amplitude limits of linear viscoelasticity region.

Loss and storage modulus values obtained at $\gamma_0 = 0.005$ were analyzed by ANOVA, using the temperature of the rheological assay (20, 40, and 60 °C), the ripening time (1, 7, 14, 20, 27, 34, and 41 d), and the preservation process (control, immersion freezing, and immersion freezing plus frozen storage) as main factors. The main factors and the interactions affected significantly both modulus values.

The decrease of modulus values with ripening time was more marked during the 1st week of ripening (Figure 3A and 4A). After 14 d of ripening, no differences between the treatments were observed. A similar behavior was observed at the different temperatures evaluated, the decrease at 20 °C being more marked (Figure 3B and 4B). The decrease of the average values of G' and G'' when temperature changes from 20 to 40 °C is more pronounced than when temperature changes from 40 to 60 °C (Figure 3C and 4C). Taking into account the preservation process, differences were not too marked, control samples showing modulus values slightly higher than modulus values of frozen samples and frozen-stored samples, but only at 20 °C.

Temperature sweep. Dynamic rheology may provide useful information related to the heat-induced changes in Mozzarella cheese. $\tan \delta$, which is a useful index to study the viscoelasticity of materials (Mounsey and O'Riordan 2001), was obtained from the temperature sweeps. In Figure 5, it can be observed that as ripening time increases, cheese shows a viscous behavior ($\tan \delta > 1$) at a lower temperature. Moreover, the most important changes in $\tan \delta$ were observed at temperatures greater than 40 °C.

The behavior of $\tan \delta$ with ripening time may be explained taking into account not only the proteolysis but also the changes in the states of water and cheese protein. On one hand, at the beginning of ripening, the relatively strong interactions between proteins are maintained when cheese is heated. Cheese resists the tendency to flow even though expressible water and fat are present within the fat-serum channels (McMahon and others 1999). On the other hand, during the 1st weeks of storage of Mozzarella cheese, expressible water is transferred from the fat-serum channels into the protein matrix. As the proteins that constitute the matrix become more hydrated, their hydrodynamic volume increases, and the matrix

begins to extend into the spaces between fat globules in the fat-serum channels. A more hydrated protein structure allows the proteins to slip past one another more easily and, when combined with the lubricating properties of the fat, it results in improved meltability (McMahon and others 1999).

The crossover temperature can be used to identify the solid-like to liquid-like phase transitions the cheese undergoes during melting (Gunasekaran and Ak 2003). In Figure 6, it can be observed that crossover temperature decreases as ripening time increases. Moreover, at ripening time higher than 20 d, crossover temperatures are similar for the 3 processes studied (control, immersion freezing, and immersion freezing plus frozen storage). In general,

frozen and frozen-stored samples showed higher crossover temperature than control samples. Besides, it can be observed that control samples show an initial decrease of the crossover temperature more marked than the frozen and frozen-stored samples. However, all the samples showed a similar crossover temperature after 20 d of ripening. The results obtained agree with those found in the bibliography for Mozzarella cheese (Bertola and others 1996a, 1996b; Bevilacqua 1997; Chaves and others 1999; Kuo and Gunasekaran 2003).

Values of E_a for the different studied conditions are shown in Figure 7. The values determined are in the order of those found in the bibliography. For example, Gunasekaran and Ak (2003) reported

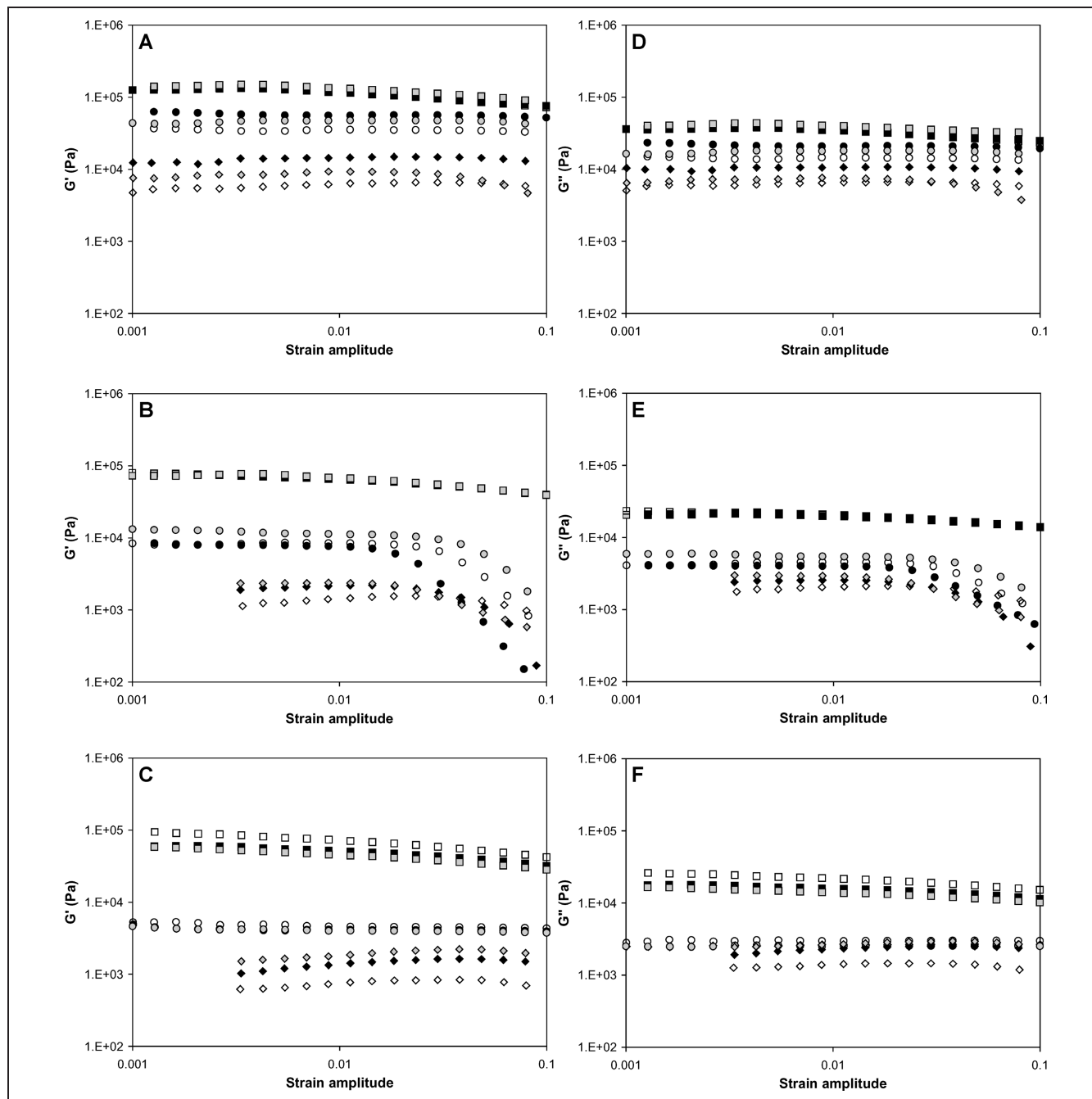


Figure 2—Sweeps of strain amplitude at 20 °C (□), 40 °C (○), and 60 °C (△); control (empty symbols), frozen (black symbols) and frozen-stored (gray symbols) samples. G' (A, B, C) and G'' (D, E, F) at 1 (A, D), 14 (B, E), and 41 (C, F) d of ripening.

values of 32 and 8 kcal/mol for cheddar and Mozzarella cheeses, respectively. In our case, the freezing process significantly affects the activation energy; higher E_a values at the end of the ripening period studied being observed for the control samples.

According to Diefes and others (1993), the frozen Mozzarella cheese shows a local dehydration of proteins that causes breaks in protein structures that allow small fat globules to contact each other and form granules. Moreover, the proteins become more compact or interact to form disulfide bridges around new fat granules. After thawing, the proteins are unable to fully rebind water. This leads to a harder and more elastic-solid cheese structure with less free oil. Numerous studies indicate that the effects of the processes of freezing-thawing-frozen storage change with cheese variety, process conditions, and ripening time (Tunick and others 1991; Oberg and

others 1992; Chaves and others 1999; Kuo and Gunasekaran 2003; Graiver and others 2004). In this case, although immersion freezing of Mozzarella cheese significantly affected some of the rheological parameters studied, the differences observed were small and therefore, the commercialization quality of Mozzarella cheese might not be noticeably modified.

Conclusions

The influence of freezing of Mozzarella cheese by immersion in NaCl solutions was evaluated analyzing its viscoelastic behavior. The freezing process used decreased the dehydration of cheeses and did not affect the physicochemical parameters studied. The rheological analysis of samples showed that the storage and loss

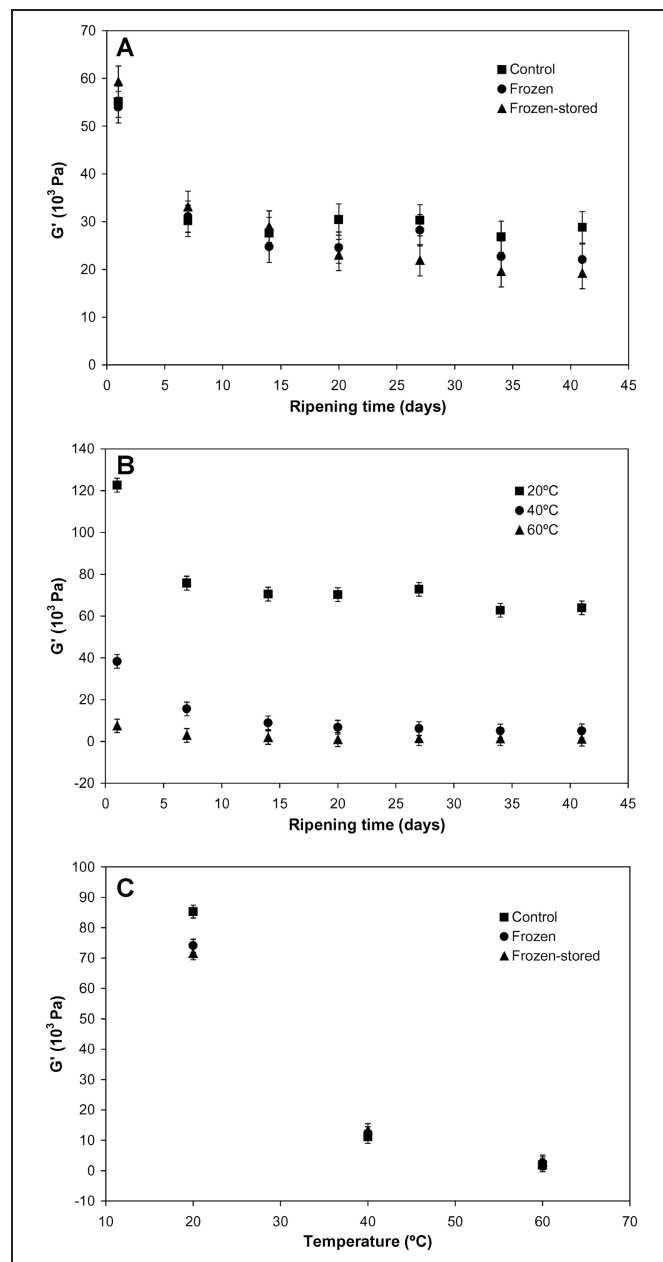


Figure 3—Interaction plots of the main factors obtained from ANOVA of the storage modulus. (A) Preservation process-time interaction; (B) temperature-time interaction; (C) preservation process-temperature interaction. Bars indicate 95% confidence limits.

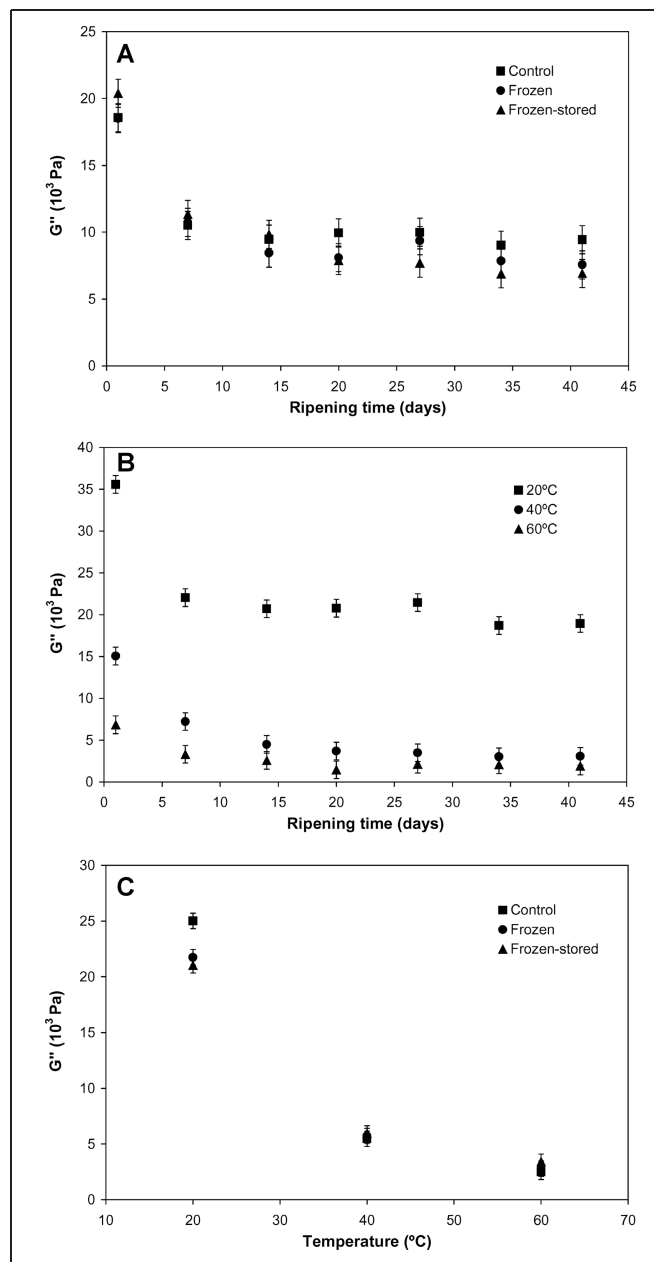


Figure 4—Interaction plots of the main factors obtained from ANOVA of the loss modulus. (A) Preservation process-time interaction; (B) temperature-time interaction; (C) preservation process-temperature interaction. Bars indicate 95% confidence limits.

modulus decreased with the temperature of the rheological assays and with the ripening time.

The crossover temperature decreased during cheese ripening. The frozen samples and the frozen-stored samples showed a higher crossover temperature than the control samples. However, the crossover temperatures were similar after 20 d of ripening. The relation of the complex viscosity with temperature by an Arrhenius-type equation was used to study the effect of the temperature on the viscoelastic properties. Control samples showed different activation energy than frozen samples and frozen-stored samples. Control samples showed significantly higher activation energy at the end of the ripening period studied, 15.9 ± 0.4 , 14.1 ± 0.5 , and 13.8 ± 0.6 kcal/mol at 41 d being observed for control, frozen, and frozen-stored samples, respectively.

The study of the viscoelastic behavior of Mozzarella cheese showed that although the immersion freezing significantly affected some of the parameters studied, the differences among the immersion freezing and the immersion freezing plus frozen storage treatments with the control treatment are relatively small. Therefore, the immersion freezing process may be useful for the manufacture and commercialization of Mozzarella cheese.

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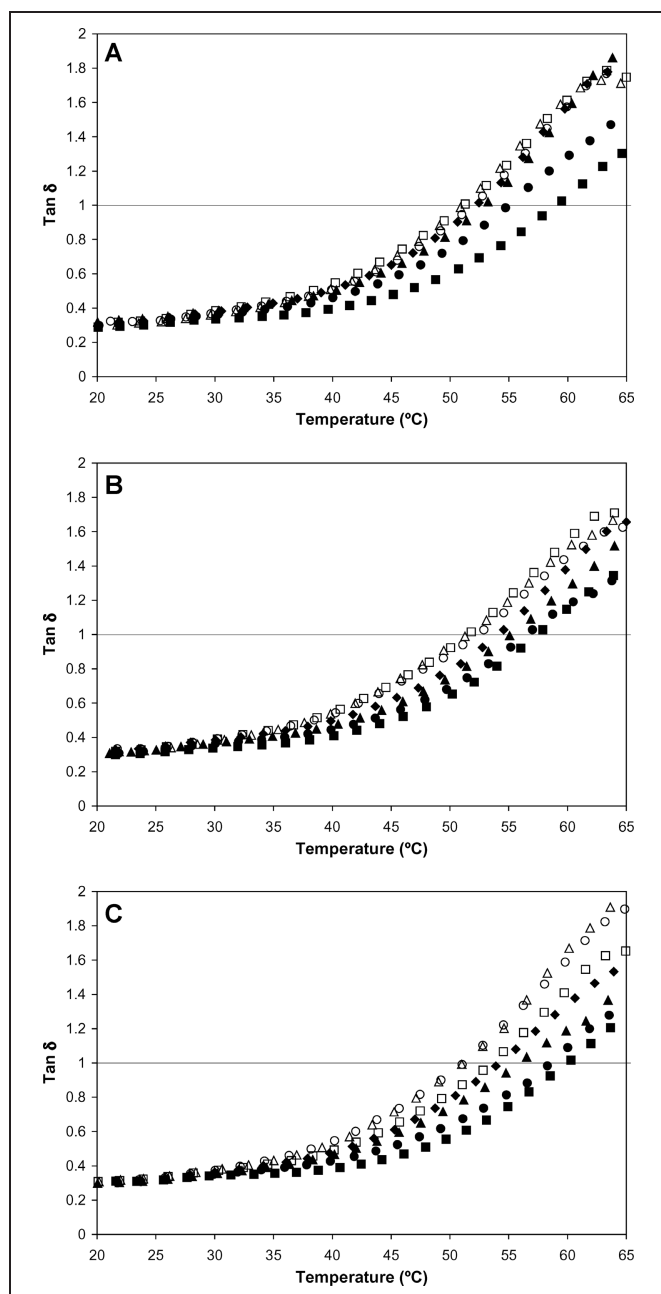


Figure 5—Temperature sweeps for control (A), frozen (B), and frozen-stored (C) samples at 1 (■), 7 (●), 14 (▲), 20 (◆), 27 (□), 34 (○), and 41 (△) d of ripening

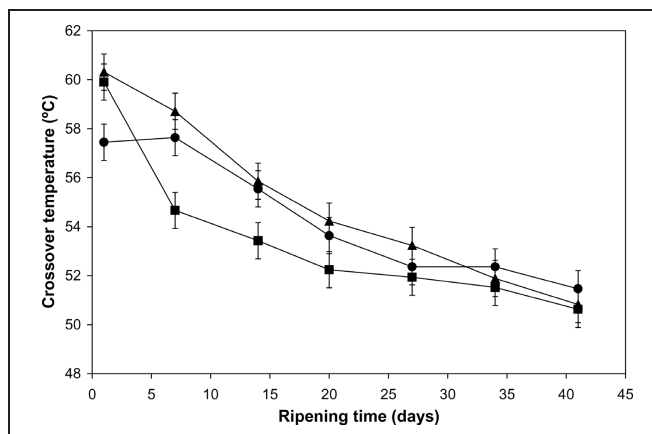


Figure 6—Crossover temperatures for control (■), frozen (●), and frozen-stored (▲). Bars indicate 95% confidence limits.

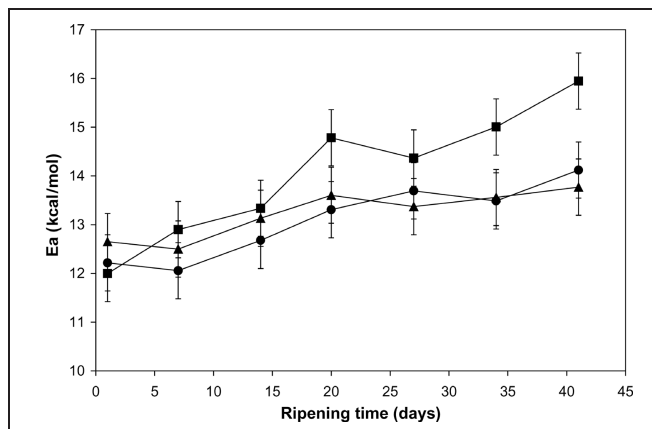


Figure 7—Activation energy for control (■), frozen (●), and frozen-stored (▲) samples. Bars indicate 95% confidence limits.

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