

# CHANGES IN EQUILIBRIUM MODULUS AND $\alpha_{s1}$ -CASEIN BREAKDOWN DURING THE RIPENING OF PORT SALUT ARGENTINO CHEESE AS AFFECTED BY FROZEN STORAGE

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## ABSTRACT

*The effect of the freezing, frozen storage and thawing on textural parameters and  $\alpha_{s1}$ -casein breakdown during the ripening of Port Salut Argentino cheese was studied. Moisture content, salt concentration, casein profiles and asymptotic equilibrium modulus were monitored in control cheeses ripened at 5C and in cheeses, stored at -22C for 30 days, thawed and ripened at 5C, for different ripening times (1, 6, 13, 27 and 56 days) and two sampling zones (central and external). The freezing process significantly increased the rate of  $\alpha_{s1}$ -casein and  $\alpha_{s1}$ -I-casein hydrolysis. This process may affect the susceptibility of  $\alpha_{s1}$ -casein to chymosin attack and also the availability of hydrolytic enzymes released by damaged microorganisms, which may contribute to the faster hydrolysis of  $\alpha_{s1}$ -I-casein. The freezing process did not significantly affect the decay rates of asymptotic equilibrium modulus. First order kinetics constants for decay of the asymptotic equilibrium modulus were  $3.71 \cdot 10^2 \text{ day}^{-1}$  (control cheeses, central zone),  $8.48 \cdot 10^2 \text{ day}^{-1}$  (control cheeses, external zone),  $4.52 \cdot 10^2 \text{ day}^{-1}$  (frozen cheeses, central zone), and  $11.43 \cdot 10^2 \text{ day}^{-1}$  (frozen cheeses, external zone). Significant differences in the decay rates of asymptotic equilibrium modulus were found between central and external zones in control*

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and frozen cheeses primarily due to differences in moisture contents of the sampling zones.

## INTRODUCTION

Port Salut Argentino cheese is one of the most popular soft cheese varieties in Argentina (Zalazar *et al.* 1999). The Food Code (CAA 1981) describes Port Salut Argentino as a semicooked cheese, produced from pasteurized milk, acidified by lactic bacteria, coagulated by rennet and/or other specific enzymes and ripened for a short period.

Throughout manufacture and ripening a series of biochemical events lead to characteristic flavour and textural attributes of a cheese (Fox 1987). All cheeses contain three major constituents (casein, fat and water) that contribute to cheese structure and therefore to rheological properties (Prentice 1987). Relationships between protein breakdown and texture during the ripening of Port Salut Argentino cheese were determined by Bertola *et al.* (1991) who found that the rigidity of the protein matrix decreased as  $\alpha_{s1}$ -casein was degraded during ripening.

Compression and relaxation tests are useful for characterizing cheese texture (Bertola *et al.* 1991, 1996). Verdini and Rubiolo (2002a) measured texture changes during the ripening of Port Salut Argentino cheese modeling stress relaxation curves. Asymptotic equilibrium modulus was useful to characterize texture changes during cheese ripening. A first order kinetic model was proposed for the decay of the asymptotic equilibrium modulus.

Expanding commercialization of Port Salut Argentino cheese increased the interest in extending its shelf-life. Freezing of dairy products is an appropriate preservation technique. However, cheese freezing is normally avoided because of the tendency towards physical breakdown in body and structural characteristics caused by ice crystal formation (Webb and Arbuckle 1977). Lück (1977) reviewed cheese preservation by freezing and reported that Camembert cheeses frozen immediately after making, defrosted and then ripened under normal conditions for up to 3 weeks were acceptable based on sensory analysis. The quality of full cream Gouda cheeses was satisfactory to good after being stored at -20C. Cervantes *et al.* (1983) found that cheeses that underwent rapid freeze-thaw cycles and stored at -15C for a week showed no significant changes in textural characteristics based on compression tests and sensory analysis. Diefes *et al.* (1993) found that frozen and thawed cheeses were less elastic and viscous than refrigerated samples of equal age. Chaves *et al.* (1999) studied the effects of slow freezing on Mozzarella cheese and showed that there was no difference in the extent of proteolysis after thawing and tempering. Verdini and Rubiolo (2002b) studied Port Salut Argentino cheeses that were previously matured

before freezing and stored frozen for up to 60 days and reported no effect of frozen storage time on peptide profiles of the water-soluble fraction. However, no studies of the effect of the freezing process (freezing, frozen storage and thawing) on Port Salut Argentino cheese texture were found.

Salt content also influences the rate of casein hydrolysis and therefore, cheese texture. Cheeses salted by immersion in brine have a large salt gradient from the surface to the center (Zorrilla and Rubiolo 1994). Salt concentration changing with position in the cheese and ripening time affects the primary casein degradation (Zorrilla and Rubiolo 1997). Moisture content affects textural properties of cheese. Verdini and Rubiolo (2002a) reported that sampling location in the cheese affected textural parameters of Port Salut Argentino cheese mainly due to moisture content differences. Therefore, it is also of great interest to analyze casein hydrolysis and textural properties at different sampling zones.

The objective of this work was to study casein hydrolysis and textural parameters at different ripening times and sampling zones in Port Salut Argentino cheeses that were frozen at the beginning of the ripening.

## MATERIAL AND METHODS

### Cheese Samples

Commercial Port Salut Argentino cheeses ( $3.6 \pm 0.1$  kg weight,  $23.2 \pm 0.3$  cm diameter,  $7.7 \pm 0.3$  cm height) were manufactured at a local factory. Cheeses were salted in a brine solution (20-22% w/w) for 3 h at 3C, stored for 20 h and packed in heat-shrinkable plastic bags. The initial composition of cheeses was:  $28.7 \pm 0.7\%$  w/w fat,  $20.4 \pm 0.9\%$  w/w total protein, and  $48.8 \pm 2.6\%$  w/w moisture.

Thirty cheeses were transported in insulated boxes with ice from the factory to our laboratory and randomly separated in two groups. Fifteen cheeses were held at 5C for ripening (cheeses R). Fifteen cheeses were frozen in a Tabai Comstar PR 4GM chamber (Tabai Espec Corp., Osaka, Japan) at -30C until the center reached -22C. Cheeses were held in frozen storage at -22C for 30 days, then thawed at 5C. After thawing, cheeses were held at 5C for ripening (cheeses F).

Cheeses R and F were sampled at different ripening times (1, 6, 13, 27, and 56 days) in triplicate. Cubic pieces of 25 mm were cut as described by Creamer and Olson (1982) from two different cheese zones, central zone (C) and external zone (E), as shown in Fig. 1.

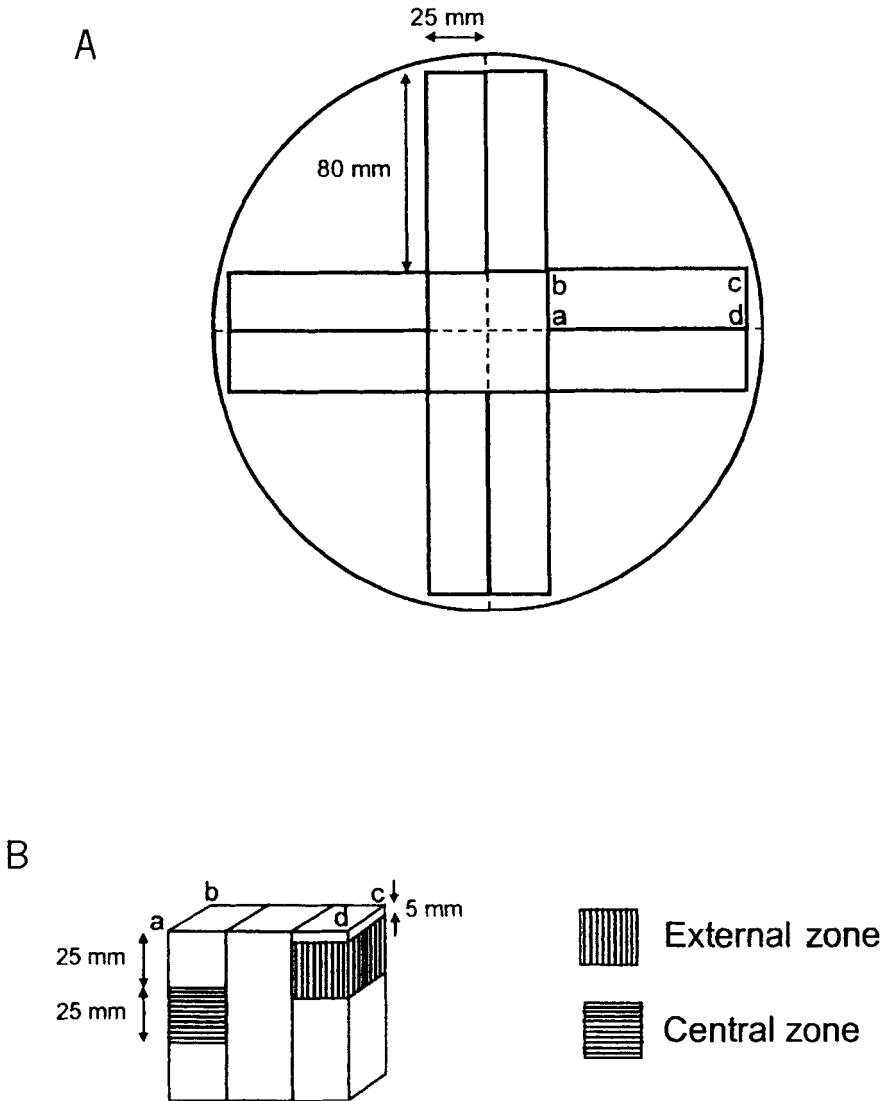


FIG. 1. SCHEMATIC VIEW OF SAMPLING  
(A) Top-down view.(B) Side-on view.

### Moisture and Chloride Analysis

Moisture content was measured with a microwave oven CEM AVC 80 (CEM, Mattheus, NC). Chloride concentration was determined with an Automatic Titrator model DL40RC (Mettler Instrumente AG, Greifensee, Switzerland) as proposed by Fox (1963).

### Casein Extraction and Analysis

Grated cheese (10 g) mixed with three times sample weight of water was homogenized using an Ultra-Turrax® T25 (IKA® Werke, Janke & Kunkel GmbH & Co KG, Staufen, Germany) homogenizer for 2 min (Kuchroo and Fox 1982). The homogenate was held at 40C for 1 h, pH was adjusted to 4.4-4.6, and the suspension was centrifuged for 30 min at 5C and 4800 rpm (Biofuge 28RS, Heraeus Sepatech, Osterode, Germany). After centrifugation, the supernatant was removed. The precipitate was dissolved in 7 M urea. The resulting solution was dialyzed against water for 48 h using Cellu Sep 5000 MWCO membranes (Membrane Filtration, Inc., San Antonio, TX), lyophilized (Heto Lab Equipment, Allerød, Denmark) and stored in a freezer at -22C for chromatographic analysis.

Approximately 20 mg of the lyophilized powder was dissolved in 0.01 M imidazole (pH 7), 0.01 M dithioerythritol and 6.6 M urea (Christensen *et al.* 1989). The solution was mixed and filtered through a disposable 0.2- $\mu$ m filter (Alltech Associates, Deerfield, IL) before 100  $\mu$ L was injected. A chromatograph with a gradient programmer model 2360 (Isco, Inc., Lincoln, NE), a V<sup>4</sup>® variable wavelength absorbance detector and a SynChropak RPP (250  $\times$  4.6 mm) C<sub>18</sub>, 300 Å column (SynChrom, Inc., Lafayette, IN) at 30C were used for chromatographic separations. Detection was at 220 nm. Gradient elution was used with solvent A: 0.1% trifluoroacetic acid (TFA) in water and solvent B: 0.1% TFA in acetonitrile. The gradient program was: initial composition 0% B, isocratic step at 0% B for 5 min, linear step to 25% B in 5 min, linear step to 35% B in 30 min, linear step to 50% B in 5 min, isocratic step at 50% B for 10 min. The flow rate was 1.0 mL/min. All the reactants used were of HPLC grade. Data were processed with the Peak Simple II version 3.91 1994 (SRI Instruments, Torrance, CA).

### Compression and Stress Relaxation Tests

The samples were stored in plastic containers to prevent dehydration. Samples were left in the test room for 3 h to reach test temperature. Experiments were carried out at 21  $\pm$  1C. They were compressed using a Universal Testing Machine (Schimadzu DSS 10 T-S, Tokyo, Japan) with a 5-kg load cell. Relaxation curves were recorded for 8 min as suggested by Peleg (1979).

Compression ratio of 40% and crosshead speed of 10 mm/min were used. Data were collected with a personal computer throughout an analogical output of the Universal Testing Machine. Tests were carried out in duplicate. A program written in Visual Basic® language was used for the analysis of the recorded data.

### Stress Relaxation Analysis

The total force  $F(t)$  was measured and the true stress  $\sigma(t)$  was obtained from:

$$\sigma(t) = \frac{F(t)h}{A h^*} \quad (1)$$

where  $A$  is the original cross-sectional area,  $h$  is the original height and  $h^*$  is the height at the end of compression.

Stress relaxation data was normalized using Peleg's model with an empirical linear equation (Peleg 1979, 1980).

$$\frac{t\sigma_0}{(\sigma_0 - \sigma(t))} = k_1 + k_2 t \quad (2)$$

where  $\sigma_0$  is the stress at the beginning of the relaxation, and  $k_1$  and  $k_2$  are constants.

The asymptotic equilibrium modulus ( $E_A$ ) was obtained from the value of  $k_2$  as:

$$E_A = \frac{\sigma_0}{\varepsilon} \left( 1 - \frac{1}{k_2} \right) \quad (3)$$

where  $\varepsilon$  is

$$\varepsilon = \ln \left( \frac{h^*}{h} \right) \quad (4)$$

### Statistical Analysis

Data were analyzed using ANOVA with Minitab 13.20 (Minitab Inc., State College, PA). When differences between treatment effects were significant ( $P < 0.05$ ), a multiple comparison of means was performed using the Tukey's test. Slopes of the fitted curves were compared using the method proposed by Green and Margerison (1978).

## RESULTS AND DISCUSSION

### Moisture and Salt Contents

Moisture content during cheese ripening is shown in Fig. 2. The moisture content at the beginning of the ripening was higher in zone E than in zone C for cheeses R and F (51% and 46%, respectively). Despite the moisture gradient, both cheeses R and F did not reach a uniform moisture content during the 56 ripening days.

Sodium chloride content during cheese ripening is shown in Fig. 2. Salt concentration during ripening was significantly lower in zone C than in zone E until 13 days for both cheeses R and F. Salt concentration in zone C increased significantly from 1 to 13 days due to NaCl diffusion. A uniform salt concentration was reached in both zones and there were no significant differences in salt concentrations for 27 and 56 days for both cheeses R and F.

### Casein Hydrolysis

A chromatogram of a cheese casein extract is shown in Fig. 3. The  $\alpha_{s1}$ -casein peak was identified using a standard of  $\alpha_s$ -casein. The target of initial primary proteolysis by chymosin is  $\alpha_{s1}$ -casein yielding  $\alpha_{s1}$ -casein (f1-23) and  $\alpha_{s1}$ -casein (f24-199), which is known as  $\alpha_{s1}$ -I-casein (Exterkate *et al.* 1991, 1995; McSweeney *et al.* 1993). The  $\alpha_{s1}$ -I-casein peak was assigned comparing the retention time with those reported in literature (Hynes 1998) and according to its behavior during cheese ripening. Peak areas of  $\alpha_{s1}$  and  $\alpha_{s1}$ -I-casein per 100 g cheese are shown in Fig. 4.

A first-order kinetics model was assumed to represent the primary hydrolysis of  $\alpha_{s1}$ -casein (Zorrilla and Rubiolo 1997):

$$\alpha_{s1}(\theta) = \alpha_{s1}(0)e^{-K_a\theta} \quad (5)$$

where  $\alpha_{s1}(\theta)$  is the RP-HPLC peak area of  $\alpha_{s1}$ -casein/100 g cheese changing with ripening time ( $\theta$ ),  $\alpha_{s1}(0)$  is the initial value of  $\alpha_{s1}$ , and  $K_a$  is the kinetic

constant of  $\alpha_{s1}$ -casein hydrolysis. Values of  $K_a$  for Port Salut Argentino cheeses are shown in Table 1. The  $K_a$  values were in the same order of those reported by Zorrilla and Rubiolo (1997) for Fynbo cheese ripened at 12C.

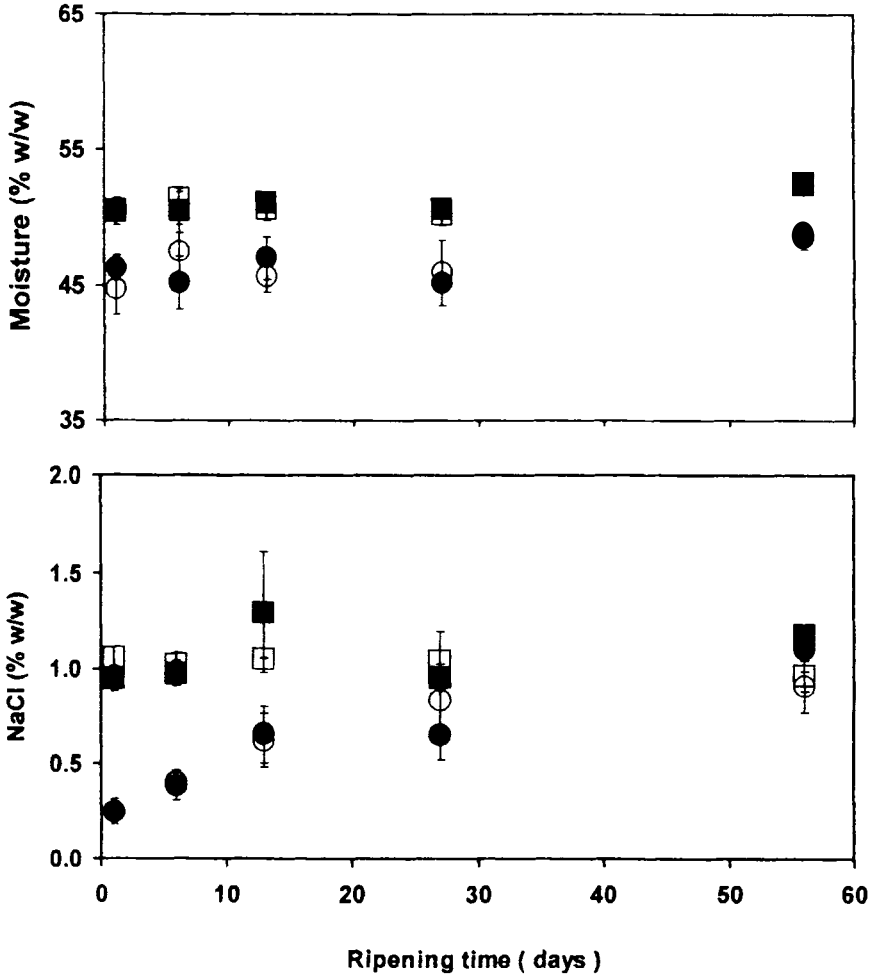


FIG. 2. MOISTURE AND NaCl CONTENTS DURING PORT SALUT ARGENTINO CHEESE RIPENING

(●) Cheeses R - Zone C; (■) Cheeses R - Zone E; (○) Cheeses F - Zone C;  
(□) Cheeses F - Zone E.



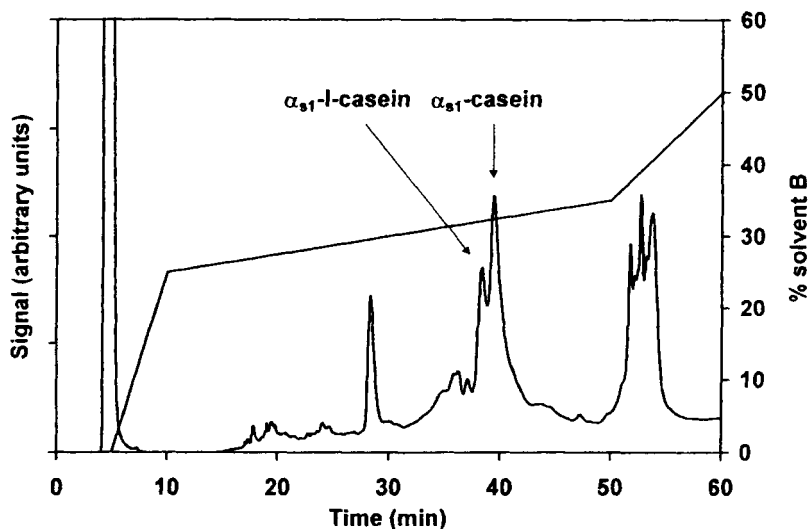


FIG. 3. TYPICAL CHROMATOGRAM OF A CHEESE SAMPLE

Considering the inhibitor effect of a higher salt concentration, differences in  $K_{\alpha}$  values according to sampling zones may be expected in cheeses salted by immersion. Zorrilla and Rubiolo (1997) reported lower values of the kinetics constant for the external zone than the central zone for Fynbo cheese ripened at 12C. For Port Salut Argentino cheese, no significant differences between the  $K_{\alpha}$  values for zones C and E were observed in cheeses R, although a significant salt concentration difference between sampling zones was found during the first stage of ripening, (Table 1). At 5C the rate of enzymatic reactions is slower than at 12C and therefore, salt inhibitor effect may be masked.

The  $K_{\alpha}$  values were significantly higher for cheeses F than for cheeses R (Table 1). Exterkate *et al.* (1997) suggested that the conformation of  $\alpha_{s1}$ -casein in the cheese matrix restrained rennet action. Proteins are destabilized by freezing and altered gradually during frozen storage (Fennema *et al.* 1973). Therefore, the increase in the kinetics constant after frozen storage may be related to conformational changes that may occur during the freezing process and may affect  $\alpha_{s1}$ -casein susceptibility to chymosin hydrolysis. Furthermore, the  $K_{\alpha}$  value for cheeses F was higher in zone E than in zone C (Table 1).

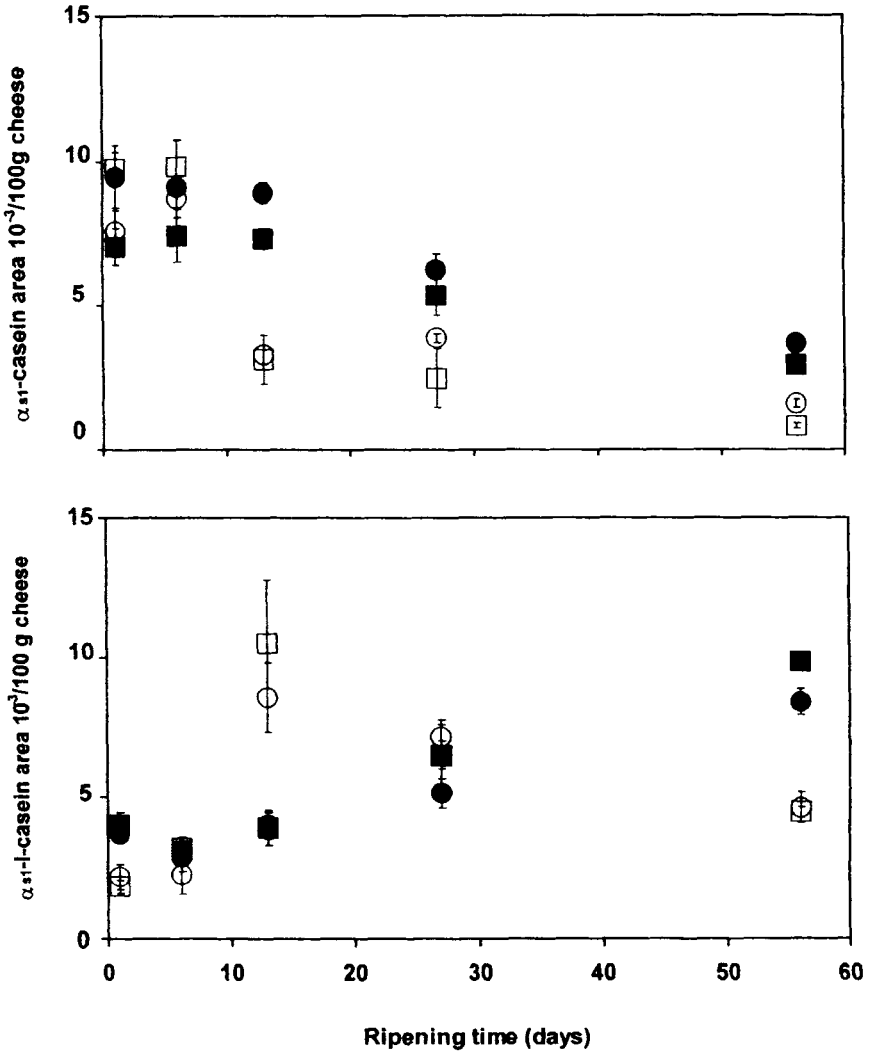


FIG. 4. AREAS OF  $\alpha_{s1}$ -CASEIN AND  $\alpha_{s1}$ -l-CASEIN DURING PORT SALUT ARGENTINO CHEESE RIPENING

(●) Cheeses R - Zone C; (■) Cheeses R - Zone E; (○) Cheeses F - Zone C;  
(□) Cheeses F - Zone E.

TABLE 1.  
KINETICS CONSTANTS OF  $\alpha_{s1}$ -CASEIN HYDROLYSIS

Cheese	Zone	$K_a \cdot 10^2 (\text{day}^{-1})^{(1)}$	$R^{2(2)}$
R	C	1.79 <sup>a</sup>	0.980
	E	1.74 <sup>a</sup>	0.943
F	C	2.82 <sup>b</sup>	0.828
	E	4.46 <sup>c</sup>	0.909

(1) Same letters within column are not significantly different ( $P < 0.05$ ).

(2) Determination coefficient.

The fragment of  $\alpha_{s1}$ -I-casein undergoes further degradation either by the coagulant or by other proteinases and peptidases of the starter bacteria (McSweeney *et al.* 1993). The content of  $\alpha_{s1}$ -I-casein increased for cheeses R during the studied ripening period (Fig. 4). In the case of cheeses F, the content of  $\alpha_{s1}$ -I-casein increased quickly for 13 days and then decreased (Fig. 4). Frozen storage increased the hydrolysis of  $\alpha_{s1}$ -casein, and also increased the degradation of  $\alpha_{s1}$ -I-casein. This may be related to a higher availability of hydrolytic enzymes released by microorganisms affected by the freezing process (Fennema *et al.* 1973).

### Stress Relaxation Analysis

The rheological properties of a cheese are largely dependent on  $\alpha_{s1}$ -casein hydrolysis and moisture content (de Jong 1978). Asymptotic equilibrium modulus is a measure of the ability of the sample to maintain stress for a fixed strain over time and therefore, it is an index of "solidity" (Barrett *et al.* 1998). Figure 5 shows that asymptotic equilibrium modulus diminished during the ripening period showing that cheese became less solid as long as  $\alpha_{s1}$ -casein was hydrolyzed (Bertola *et al.* 1991; Verdini and Rubiolo 2002a).

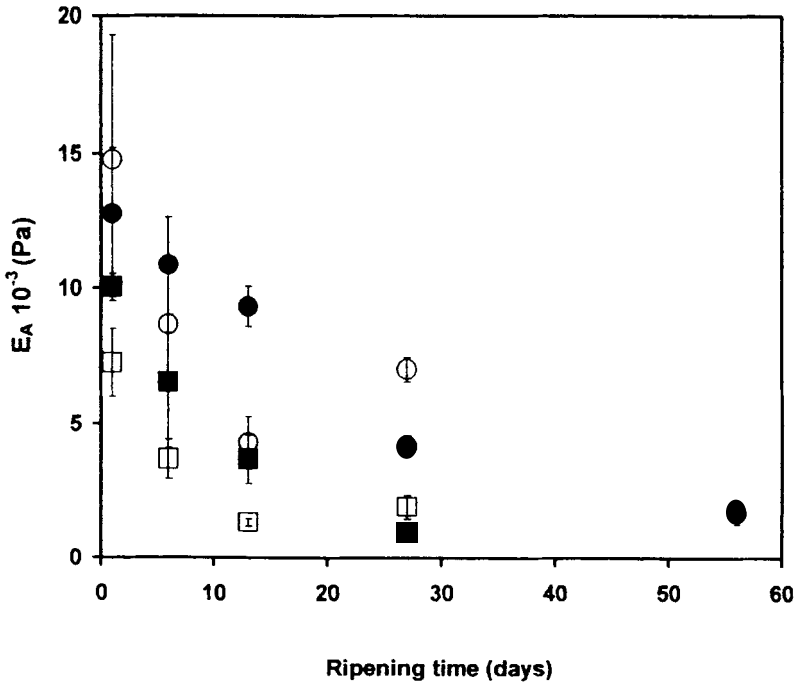


FIG. 5. ASYMPTOTIC EQUILIBRIUM MODULI DURING PORT SALUT ARGENTINO CHEESE RIPENING  
 (●) Cheeses R - Zone C; (■) Cheeses R - Zone E; (○) Cheeses F - Zone C;  
 (□) Cheeses F - Zone E.

A first-order kinetics model was assumed to represent the decay of  $E_A$  during ripening (Verdini and Rubiolo 2002a):

$$E_A(\theta) = E_A(0)e^{-K_E\theta} \quad (6)$$

where  $E_A(\theta)$  is the asymptotic equilibrium modulus during ripening time ( $\theta$ ),  $E_A(0)$  is the initial value of the asymptotic equilibrium modulus, and  $K_E$  is the kinetics constant of  $E_A$  decay.

Values of  $K_E$  for Port Salut Argentino cheeses are shown in Table 2. Values of  $K_E$  were higher in zone E than in zone C for both cheeses R and F. Differences in moisture contents between sampling zones during the studied ripening period can be considered responsible for the differences observed in the  $K_E$  values (Verdini and Rubiolo 2002a).

TABLE 2.  
KINETICS CONSTANTS OF THE DECAY OF THE ASYMPTOTIC  
EQUILIBRIUM MODULUS

Cheese	Zone	$K_{EA} 10^2 (\text{day}^{-1})^{(1)}$	$R^{2(2)}$
R	C	3.71 <sup>a</sup>	0.980
	E	8.48 <sup>b</sup>	0.999
F	C	4.52 <sup>a</sup>	0.774
	E	11.43 <sup>b</sup>	0.901

(1) Same letters within column are not significantly different ( $P < 0.05$ ).

(2) Determination coefficient.

No significant differences were observed in the  $K_E$  values between cheeses F and R (Table 2). Characterization of the stress relaxation curves is one of the most useful rheological tests for solid foods (Peleg 1979) and the asymptotic equilibrium modulus is a valuable parameter to study texture changes during cheese ripening (Bertola *et al.* 1991; Verdini and Rubiolo 2002a). Consequently, Port Salut Argentino cheeses undergoing the freezing process may have textural characteristics similar to control cheeses.

## CONCLUSIONS

The freezing, frozen storage and thawing process increased the rate of  $\alpha_{s1}$ -casein and  $\alpha_{s1}$ -I-casein hydrolysis of Port Salut Argentino cheese. Some differences in the susceptibility of  $\alpha_{s1}$ -casein to chymosin attack and in the availability of hydrolytic enzymes released by microorganisms might explain these results. However, the decay rates of asymptotic equilibrium modulus were not significantly different due to the freezing process. Taking into account that this parameter is a good indicator of textural changes during cheese ripening, it can be considered that Port Salut Argentino cheeses undergoing the freezing process may have textural characteristics similar to control cheeses.

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## REFERENCES

- BARRETT, A.H., BRIGGS, J., RICHARDSON, M. and REED, T. 1998. Texture and storage stability of processed beef as affected by glycerol and moisture levels. *J. Food Sci.* **63**, 84–87.
- 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. *Intern. J. Food Sci. Tech.* **26**, 467–478.
- BERTOLA, N.C., CALIFANO, A.N., BEVILACQUA, A.E. and ZARITZKY, N.E. 1996. Effect of freezing conditions on functional properties of low moisture Mozzarella cheese. *J. Dairy Sci.* **79**, 185–190.
- CAA. 1981. *Código Alimentario Argentino Actualizado*. De La Canal y Asociados SRL, Buenos Aires, Argentina.
- CERVANTES, M.A., LUND, D.B. and OLSON, N.F. 1983. Effect of salt concentration and freezing on Mozzarella cheese texture. *J. Dairy Sci.* **66**, 204–213.
- CHAVES, VIOTTO, W.H. and GROSSO, C.R.F. 1999. Proteolysis and functional properties of Mozzarella cheese as affected by refrigerated storage. *J. Food Sci.* **64**, 202–205.
- CHRISTENSEN, T.M.I.E., KRISTIANSEN, K.R. and MADSEN, J.S. 1989. Proteolysis in cheese investigated by high performance liquid chromatography. *J. Dairy Res.* **56**, 823–828.
- CREAMER, L.K. and OLSON, N.F. 1982. Rheological evaluation of maturing Cheddar cheese. *J. Food Sci.* **47**, 631–636, 646.
- DE JONG, L. 1978. The influence of the moisture content on the consistency and protein breakdown of cheese. *Neth. Milk Dairy J.* **32**, 1–14.
- DIEFES, H.A., RIZVI, S.S.H. and BARTSCH, J.A. 1993. Rheological behavior of frozen and thawed low moisture Mozzarella cheese. *J. Food Sci.* **58**, 764–769.

- EXTERKATE, F.A., ALTING, A.C. and SLANGEN, C.J. 1991. Specificity of two genetically related cell-envelope proteinases of *Lactococcus lactis* subsp. *cremoris* towards  $\alpha_{s1}$ -casein-(1-23)-fragment. *Biochem. J.* 273, 135-139.
- EXTERKATE, F.A., ALTING, A.C. and SLANGEN, C.J. 1995. Conversion of  $\alpha_{s1}$ -casein-(24-199)-fragment and  $\beta$ -casein under cheese conditions by chymosin and starter peptidases. *System Appl. Microbiol.* 18, 7-12.
- EXTERKATE, F.A., LAGERWERF, F.M., HAVERKAMP, J. and VAN SCHALKWIJK, S. 1997. The selectivity of chymosin action on  $\alpha_{s1}$ - and  $\beta$ -caseins in solution is modulated in cheese. *Intern. Dairy J.* 7, 47-54.
- FENNEMA, O.R., POWRIE, W.D. and MARTH, E.H. 1973. *Low Temperature Preservation of Foods and Living Matter*. Marcel Dekker, New York.
- FOX, P.F. 1963. Potentiometric determination of salt in cheese. *J. Dairy Sci.* 46, 744-745.
- FOX, P.F. 1987. *Cheese: Chemistry, Physics and Microbiology*. Vol 1. General Aspects. Elsevier Applied Science, London.
- GREEN, J.R. and MARGERISON, D. 1978. *Statistical Treatment of Experimental Data*. Elsevier Scientific Publishing Co., Amsterdam, Holland.
- HYNES, E.R. 1998. Estudio de la proteólisis durante la maduración de quesos blandos. Cremoso Argentino. Dphil thesis. Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santa Fe, Argentina.
- KUCHROO, C.N. and FOX, P.F. 1982. Soluble nitrogen in Cheddar cheese: comparison of extraction procedures. *Milchwissenschaft* 37, 331-335.
- LÜCK, H. 1977. Preservation of cheese and perishable products by freezing. *South Afr. J. Dairy Tech.* 9, 112-127.
- MCSWEENEY, P.L.H., OLSON, N.F., FOX, P.F., HEALY, A. and HØJRUP, P. 1993. Proteolytic specificity of chymosin on bovine  $\alpha_{s1}$ -casein. *J. Dairy Res.* 60, 401-412.
- PELEG, M. 1979. Characterization of the stress relaxation curves of solid foods. *J. Food Sci.* 44, 277-281.
- PELEG, M. 1980. Linearization of relaxation and creep curves of solid biological materials. *J. Rheol.* 24, 451-463.
- PRENTICE, J.H. 1987. Cheese Rheology. In *Cheese: Chemistry, Physics and Microbiology*, Vol. 1, General Aspects. Elsevier Applied Science, London.
- 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 by principal component analysis of proteolytic profiles. *J. Food Sci.* 67, 963-967.
- WEBB, B.H. and ARBUCKLE, W.S. 1977. Freezing of dairy products. In *Fundamentals of Food Freezing*. AVI Pub. Co., Westport, Conn.

- ZALAZAR, C., MEINARDI, C. and HYNES, E. 1999. Quesos típicos argentinos. Centro de Publicaciones Universidad Nacional del Litoral, Santa Fe, Argentina.
- ZORRILLA, S.E. and RUBIOLO, A.C. 1994. Fynbo cheese NaCl and KCl changes during ripening. *J. Food Sci.* 59, 972-975.
- ZORRILLA, S.E. and RUBIOLO, AC. 1997. Kinetics of casein degradation during ripening of Fynbo cheese salted with NaCl/KCl brine. *J. Food Sci.* 62, 386-389.