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Effect of ripening temperature on low moisture Mozzarella cheese: 2. Texture and functionality

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Abstract — Low-moisture Mozzarella cheeses were made in duplicate and ripened for 70 d at 0, 4, 10 or 15 °C. For all temperatures, advance in ripening time resulted in significant ($P < 0.05$) decreases in the concentration of intact casein, firmness, melt time and apparent viscosity. In contrast, the flowability and stretchability of the molten cheeses increased significantly during storage. Increasing the ripening temperature from 0 to 15 °C resulted in a significant decrease in the mean concentration of intact casein and a decrease in the level of serum expressed on centrifugation. The latter changes were paralleled by a significant decrease in the mean melt time (time required for shred fusion) of the cheese over the 70 d ripening period, and a significant increase in the mean flowability and reduction in the mean apparent viscosity of the molten cheese. The effect of elevation of storage temperature on the functional attributes of the molten cheese was attributed mainly to the concomitant reduction in the content of intact casein which was negatively correlated with flowability and positively with apparent viscosity and melt time.

Ripening / temperature / Mozzarella / functionality / texture

Résumé — **Effets de la température d'affinage sur les fromages de Mozzarella à faible teneur en eau. 2. Texture et propriétés fonctionnelles.** Deux lots de fromages de Mozzarella à faible teneur en eau ont été fabriqués et maturés pendant 70 jours à 0, 4, 10 ou 15 °C. Pour toutes les températures, l'avancée dans le temps de maturation a entraîné une diminution significative ($P < 0,05$) de la concentration en caséine intacte, de la fermeté, du temps de fusion et de la viscosité apparente. Par opposition, l'aptitude à l'écoulement et au filant des fromages fondus a augmenté significativement pendant le stockage. L'augmentation de la température de maturation de 0 à 15 °C a résulté une diminution significative de la concentration moyenne en caséine intacte et une diminution de la quantité de sérum extraite par centrifugation. Parallèlement à ces derniers changements, on observait une diminution significative des temps moyens de fusion (temps exigé pour la fusion de brins de fromage râpé) au cours

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de la période de maturation de 70 jours, une augmentation significative de l'aptitude à l'écoulement et une diminution de la viscosité apparente moyenne du fromage fondu. L'effet de l'élévation de la température de stockage sur les caractéristiques fonctionnelles du fromage fondu a été attribué principalement à la réduction concomitante du contenu en caséine intacte qui a été négativement corrélé à l'aptitude à l'écoulement et positivement à la viscosité apparente et au point de fusion.

Maturation / température / Mozzarella / propriéte fonctionnelle / texture

1. INTRODUCTION

Cheese is used extensively in cooking applications, mainly because of its flavour and heat induced functionality, which is a composite of different attributes such as softening, flow and stretch. The functional attributes of cooked cheese generally have a major impact on the quality of foods in which cheese is included as an ingredient, e.g., pizza pie [12, 25]. Owing to its importance in cookery applications, numerous studies have been undertaken on the effects of different factors on the age related changes in the functionality of cooked cheese, especially Mozzarella, and to a lesser extent, Cheddar and processed cheese [18, 19, 26, 27, 29–31, 33, 34, 37, 38]. These studies have shown that the functionality of natural cheese is dynamic, with the different functional attributes undergoing marked changes during ripening. For a given cheese variety, there is a time window within which the different functional attributes fall within the ranges which afford the cheese with the overall desired functionality. The time at which the cheese becomes functional and the width of the window, and hence the functional shelf life, are affected by the extent of chemical changes including the increase in proteolysis and the ratio of bound to free moisture [19, 20]. The inverse relationships between the degree of proteolysis in the raw cheese and the flowability and apparent viscosity of the melted cheese for Cheddar, Mozzarella and model acid curd cheeses [1, 14, 18, 22, 24] are noteworthy.

Owing to the importance of proteolysis, various groups have investigated the effects of altering the degree of proteolysis in Cheddar and Mozzarella, by the use of either calf rennet or *Cryphonectria parasitica* proteinase as coagulant [3, 37, 38] or by the addition of proteinases (e.g., from *Bacillus subtilis*) to the ultrafiltration (UF) retentate used to prepare cheese from ultrafiltered milk [23]. *C. parasitica* proteinase resulted in greater degradation of β-CN and higher flowability, compared to calf rennet [23].

Numerous studies, especially on Cheddar cheese, have shown that raising the ripening temperature results in increased proteolysis of both α_s- and β-caseins [2, 10, 11]. Feeney et al. [8], found that increasing the storage temperature for Mozzarella cheese from 0 to 15 °C, resulted in a higher level of degradation of α_{s1}-casein but had little effect on the extent of breakdown of β-casein. The increased hydrolysis of α_{s1}-casein coincided with increases of approximately 2.5 and 4.0 fold in the level of pH 4.6 soluble N and 5% phosphotungstic acid soluble N, respectively, at 70 d [8]. Owing to the positive relationship between proteolysis and flowability in Cheddar cheese [1, 14], altering the ripening temperature would be expected to affect the extent of age related changes in functionality, and hence the functional shelf life of low moisture (LM) Mozzarella cheese. However, the authors are not aware of studies on the effects of ripening temperature on the functionality of cheese. The objective of the current study, which was undertaken simultaneously with that of Feeney et al. [8], was to investigate the effect of ripening temperature, in the range 0 to 15 °C, on the texture and heat induced functionality of LM Mozzarella cheese.

2. MATERIALS AND METHODS

2.1. Cheese manufacture

Mozzarella cheese was manufactured as described previously [15]. The milk was inoculated with a starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus helveticus* (Chr. Hansen's Laboratory Ireland Ltd, Little Island, Cork, Ireland), added at levels of 1.0 and 0.5 g·100 g^{-1}, respectively. When the milk pH reached 6.55, chymosin (Double Strength Chy-max, 50 000 MCU·mL^{-1}; Pfizer Inc, Milwaukee, WI, USA) was added at a level of 0.077 mL·kg^{-1} milk and the milk allowed to set at 36 °C. After cutting (~ 30 min later), the curds/whey mixture was cooked to 42 °C and pitched at pH 6.1. The curd from each vat was cheddared and milled when the pH reached ~ 5.15, dry salted at a level of 46 g·kg^{-1} and mellowed for 20 min. The salted curds (48 kg) were batch heated to 58 °C in hot (78 °C) water and batch plasticized, and moulded into rectangular 2.3 kg blocks which were cooled in water at ~2 °C to a surface temperature of 24 °C and a core temperature of 50 °C. The salted cheeses form each vat were vacuum wrapped and stored for 70 d at 0, 4, 10 or 15 °C. The ripening temperatures formed the treatments and are referred to as T0, T4, T10 and T15, respectively. Owing to the capacities of the cheese vats and plasticization equipment, it was not feasible to subdivide the plasticized curd from the milk of one vat into 4 sub-batches, each of which could be ripened at 0, 4, 10 or 15 °C. A curd weight of ~ 46 kg (~ equivalent to the yield of one vat) was required for batch plasticization and the delivery of 10 moulded 2.3 kg blocks of cheese all of which were required for analyses of age related changes in texture and functionality. Cheesemaking trials were performed in duplicate.

2.2. Sampling of cheese

Cheese was grated to particles of < 1 mm in a Krups Rotary 350 food processor, fitted with a universal blade (Robert Krups GmbH & Co, Postfach 190460, D-5650 Solingen 19, Germany). Shredded cheese was prepared by cutting cheese, stored at 4 °C, into 2.5 cm cubes (Cheese Blocker; Bos Kaasgereedschap, Bodengraven, Postbus 2410 AC Netherlands) and immediately shredding in a Hallde RG-350 machine (AB Hallde Maskiner, Kista, Sweeden) using the raw food grating disc (K) to yield shreds ≈ 25 mm long and ≈ 4 mm diameter. Cheeses (4 °C) were cut using a Berkel slicer (Model 300G; Avery Berkel Group; Foundry Lane, Smethwick, Warley, West Midlands B66 2LP, England), into slices of the required thickness (6.5 mm), from which discs (45.5 mm diam) were cut using a stainless steel borer.

2.3. Composition and proteolysis

Grated cheese were analyzed in duplicate for salt, fat, protein, moisture, pH, calcium, phosphorous and pH 4.6 soluble N, as described previously [14–16]. The level of intact casein (g·100 g^{-1} cheese) in the cheese was calculated using the following formula:

$$\text{Intact casein} = [\text{Total N (g·100 g}^{-1}) - \text{pH 4.6 soluble N (g·100 g}^{-1})] \times 6.38$$

where the factor 6.38 was used to convert N to casein. In the above equation it was assumed that all of the protein in the cheese is casein; undenatured whey protein accounts for only ~ 1 g·100 g^{-1} of total protein in the full fat cheese [9].

2.4. Cheese firmness

Four to six cylindrical samples (29-mm diameter) of each cheese were prepared using a cork borer and cut to the desired length (29 mm). The samples were placed in an airtight plastic bag and held at 8 °C overnight. Each sample was taken from storage and compressed immediately to 30% of the original height at a rate of 50 mm·min^{-1} between a 140 mm cross-head and base-plate on a Model 4301 Universal Instron Testing Machine (Instron Ltd, High Wycombe HP12 35Y, UK). The firmness was the force required to compress the cheese to 30% of the original height [7].

2.5. Expressible serum

Cheese serum was expressed by centrifugation at 12 500 *g* at 25 °C, as described by Guo and Kindstedt [17]. The expressed serum and fat were collected in a pre-weighed graduated cylinder and held at 4 °C until the surface layer solidified. The fat layer was punctured to release the subnatant serum, which was then weighed; the serum is expressed in g per 100 g cheese moisture.

2.6. Evaluation of cheese functionality on cooking

Melt time was defined as the time required for a fixed weight of shredded cheese (1.73 kg·m^{-2}) to melt and fuse into a molten mass free of shred identity, on heating at 280 °C [14, 16]. Flowability was defined as the percentage increase in the diameter of a disc of cheese on melting at 280 °C for 4 min [14, 16]. The stretchability of the molten cheese on baked pizza pie was measured by uniaxial extension at a velocity of 0.066 m·s^{-1} [14,16]; prior to heating, the shredded cheese was distributed uniformly at a fixed loading (2.5 kg·m^{-2}) onto a pizza base which was pre-cut in half. The maximum apparent viscosity of the molten cheese (at 70 °C) was measured using a helipath viscometer, as described by Guinee et al. [14, 16].

2.7. Statistical analysis

Two replicate cheesemaking trials were undertaken; in each trial four different batches of cheese were produced and ripened at 0, 4, 10 or 15 °C, and designated as treatments T0, T4, T10 and T15.

A randomized complete block design which incorporated the four treatments, and 2 blocks (replicate trials) was used for analysis of compositional data at 1 d. Analysis of variance (ANOVA) was carried out using a SAS procedure [32] where the effects of treatment and replicates were estimated for all response variables. Duncan's multiple-comparison test was used as a guide for pair comparisons of the treatment means. The level of significance was determined at $P < 0.05$.

A split plot design was used to monitor the effects of treatment, ripening time and their interaction on the response variables measured at different times throughout ripening, i.e., intact casein content, expressible serum, firmness, melt time, flowability, apparent viscosity and stretch. Analysis of variance for the split plot design was carried out using a general linear model (GLM) procedure of SAS [32]. Fisher's difference test was used to determine whether statistically significant differences occurred among means. All differences considered as significant are at least $P < 0.05$.

Where firmness, melt time, flowability and apparent viscosity of the melted cheese were plotted as a function of intact casein, linear regression of data (from all cheeses) with intercept was performed. The significance of the regressions was determined by applying Students t test to r^2 with $n - 2$ df where n is the actual number of data points.

3. RESULTS AND DISCUSSION

3.1. Cheese composition

The compositions of the various cheeses at 1 d have been reported by Feeney et al. [8]. There were no significant differences between any of the compositional parameters, apart from the salt-in-moisture content, which was significantly higher in T0.

Owing to the increase in the degree of proteolysis, which has been described previously for these cheeses [8], there was a decrease in the content of intact casein in all cheeses during maturation (Fig. 1). There was a significant effect of the interaction between ripening time and temperature on the level of intact casein (Tab. I), as indicated by the divergence in the intact casein/time curves. At 70 d, the mean levels in T10 and T15 were similar and significantly lower ($P < 0.05$) than those in T0 and T4, which also had similar mean levels. While this trend was also observed at ripening times < 70 d, differences were not significant. The decrease in intact casein content with elevation of the ripening temperature from 0 to 15 °C is consistent with the increase in pH 4.6 soluble N as ripening temperature is increased [10, 11, 36].

3.2. Cheese firmness

The firmness of all cheeses decreased significantly during ripening (Fig. 2; Tab. I). The decrease, which concurs with the results of previous studies on Mozzarella [14,38], is consistent with the decrease in the content of intact casein [4–6, 35]. The mean firmness over the 70 d ripening period was not significantly affected by temperature. A similar trend was noted by Yun et al. [37, 38] for the hardness and springiness of LM Mozzarella cheeses in which the level of pH 4.6 soluble N at 50 d was varied from ~ 7 to 14 g·100 g^{-1} through the use of

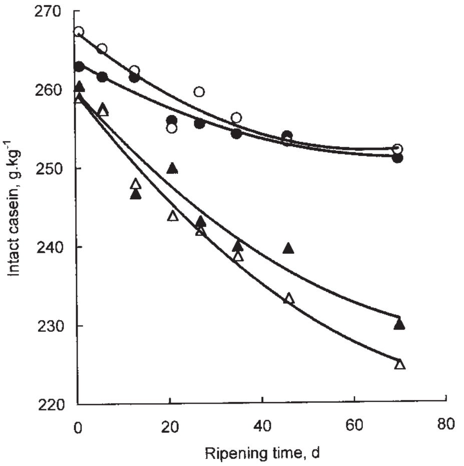

Figure 1. Age related changes in the concentration of intact casein in low-moisture Mozzarella cheese stored at 0 °C (●), 4 °C (○), 10 °C (▲) or 15 °C (△). Values presented are the means from duplicate trials.

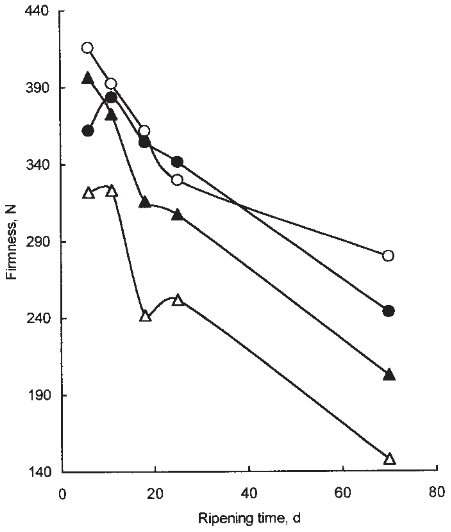

Figure 2. Age related changes in firmness of low-moisture Mozzarella cheese stored at 0 °C (●), 4 °C (○), 10 °C (▲) or 15 °C (△). Values presented are the means from duplicate trials.

Table I. Effect of storage temperature on the mean squares (MS) and probabilities (*P*) for aggregated changes in intact casein, expressible serum and firmness in unheated low-moisture Mozzarella cheese.

Factors	Intact casein ($g \cdot kg^{-1}$)			Expressible serum ($g \cdot 100\ g^{-1}$) total moisture			Firmness (N)		
	df	MS	*P*	df	MS	*P*	df	MS	*P*
Main plot									
Temperature	3	954	0.28	3	99.1	0.07	3	18 261	0.20
Error	3	452		3	13.3		3	6 180	
Sub-plot									
Time	7	480*	< 0.001	3	737.4*	< 0.001	4	31 379*	< 0.001
Interaction (temp × time)	21	29.8*	0.002	9	12.8	0.59	12	664	0.98
Error	28	8.8		12	15.3		16	2 355	

* Statistically significant ($P < 0.05$).
Intact casein and firmness were measured at various intervals over 70 d; expressible serum was monitored over 18 d.

different coagulants. However, the firmness of the T10 and T15 cheeses was numerically lower than those of the T0 and T4 cheeses at most ripening times, possibly because of the higher level of proteolysis of the former. Regression analysis of the firmness/intact casein data for all cheeses (Fig. 8) indicated a significant ($P < 0.05$) correlation between the level of intact casein and firmness (r = 0.84).

3.3. Functionality of heated cheese

Changes in the functional characteristics of the different cheeses are shown in Figures 3 to 6. There was a marked improvement in the functionality of the baked cheese during the first 30 d of ripening, as reflected by the significant increases in flowability and stretch and decreases in melt time and apparent viscosity. The improved functionality may be attributed in part to the age related reduction in the concentration of intact *para*-casein and the increased water binding capacity of the casein [20, 21]. The quantity of serum, as a percentage of cheese moisture, expressed on centrifugation decreased significantly

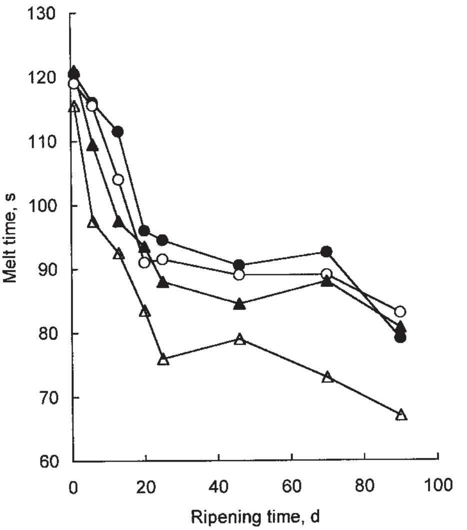

Figure 3. Age related changes in the melt time of low-moisture Mozzarella cheese stored at 0 ºC (●), 4 ºC (○), 10 ºC (▲) or 15 ºC (△). Values presented are the means from duplicate trials.

during the first 18 d of ripening (Fig. 7; Tab. I), indicating an increase in the water binding capacity of the protein [20]. An increase in the water binding capacity of the

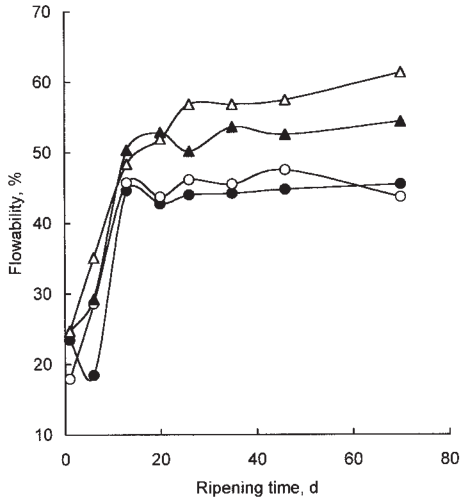

Figure 4. Age related changes in flowability of low-moisture Mozzarella cheese stored at 0 ºC (●), 4 ºC (○), 10 ºC (▲) or 15 ºC (△). Values presented are the means from duplicate trials.

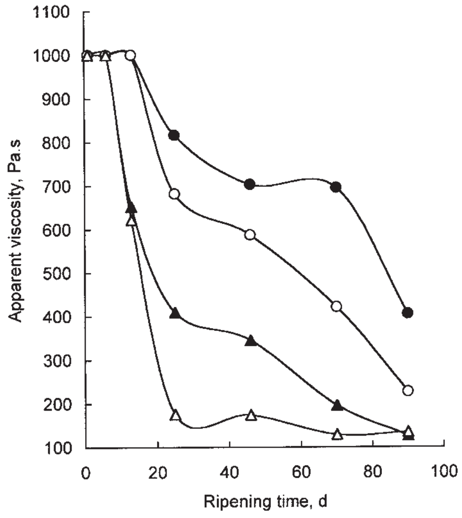

Figure 5. Age related changes in apparent viscosity of low-moisture Mozzarella cheese stored at 0 ºC (●), 4 ºC (○), 10 ºC (▲) or 15 ºC (△). Values presented are the means from duplicate trials.

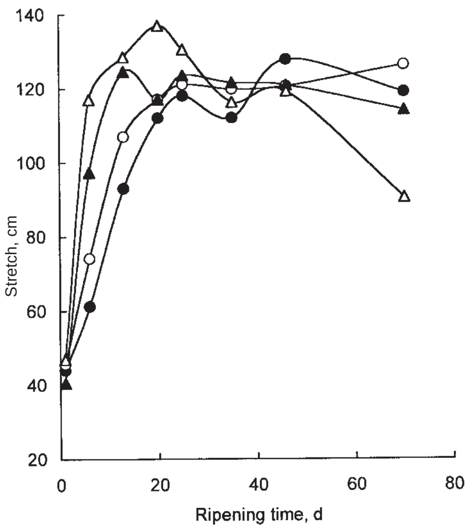

Figure 6. Age related changes in stretchability of low moisture Mozzarella cheese stored at 0 ºC (●), 4 ºC (○), 10 ºC (▲) or 15 ºC (△). Values presented are the means from duplicate trials.

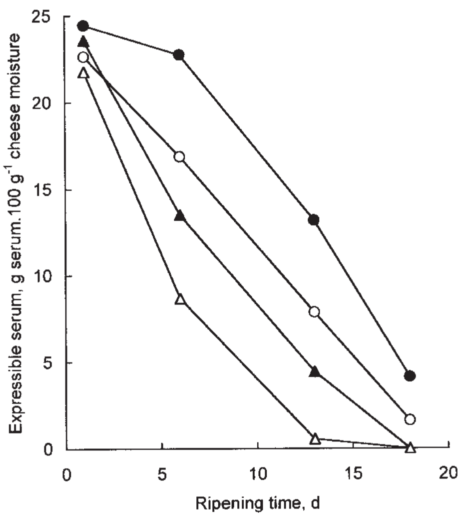

Figure 7. Age related changes in the level of serum expressed, on centrifugation at 12 500 g, from low-moisture Mozzarella cheese stored at 0 ºC (●), 4 ºC (○), 10 ºC (▲) or 15 ºC (△). Values presented are the means from duplicate trials.

Table II. Effect of storage temperature on the mean squares (MS) and probabilities (*P*) for aggregated changes in functionality of heated low-moisture Mozzarella cheese.

Factors	Melt time (s)			Flow (%)			Stretch (cm)			Apparent viscosity (Pa·s)		
	df	MS	*P*	df	MS	*P*	df	MS	*P*	df	MS	*P*
Main plot	3	657*	0.044	3	2721*	< 0.003	3	449	0.47	3	338 596*	0.003
Temperature	3	64.8		3	5.2		3	403		3	5 254	
Error												
Sub-plot												
Time	7	1 581*	< 0.001	7	1 024*	< 0.001	7	5 885*	< 0.001	6	787 490*	< 0.001
Interaction	21	19.2	0.82	21	17.1	0.48	21	311	0.248	18	31 645	0.63
(temp × time)												
Error	28	28.3		28	16.9		28	237		24	37 071	

* Statistically significant ($P < 0.05$).

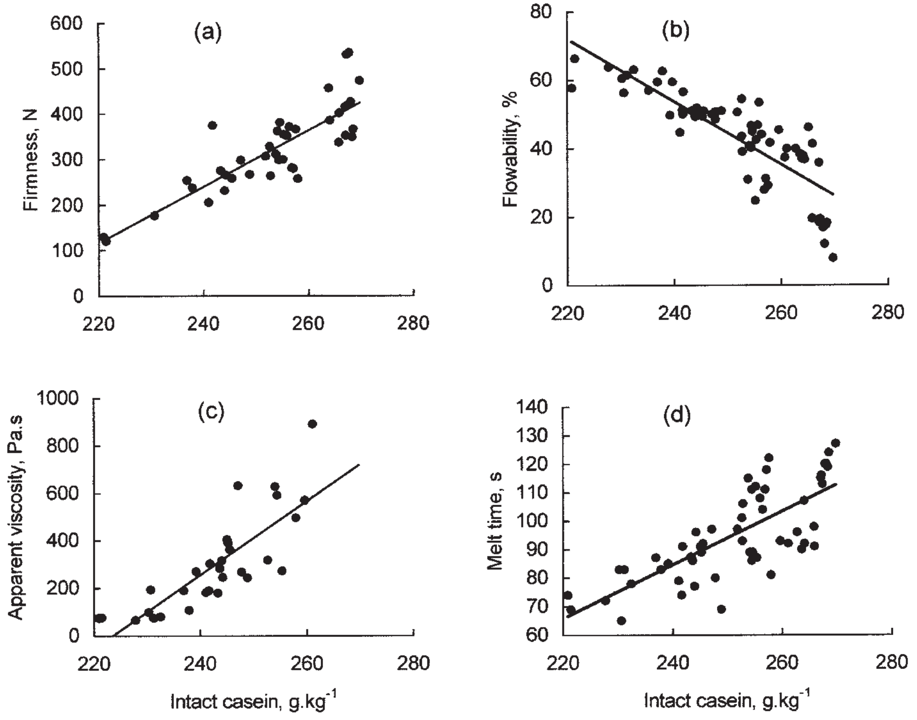

Figure 8. Relationship between the level of intact casein and firmness (a), flowability after baking at 280 °C for 4 min (b), apparent viscosity at 70 °C (c) and melt time on baking at 280 °C (d). The data were obtained from low-moisture Mozzarella cheeses produced in two replicate trials, ripened at 0, 4, 10 or 15 °C and analyzed over a 70 d ripening period; regression lines with intercept shown. Regression was significant ($P < 0.05$) in all cases.

para-casein is expected to enhance functionality as it is conducive to greater retention of moisture during baking of the pizza, which in turn limits the occurrence of defects associated with excessive dehydration, e.g., burning, crusting and poor flowability.

Increasing the storage temperature resulted in a significant ($P < 0.05$) increase in the mean flowability and significant decreases in the mean melt time and apparent viscosity during ripening (Tab. II). The effect of elevated storage temperature on these functional characteristics may be attributed to the reductions in the content of intact casein and the level of expressible serum [22, 37, 38]. Indeed, there were significant relationships ($P < 0.05$) between the content of intact casein and flowability (r = – 0.83), melt time (r = 0.75) and apparent viscosity (r = 0.81) (Fig. 8). Similarly, regression analysis revealed that the level of expressible serum was positively correlated with melt time, $P < 0.05$, r = 0.78) and negatively with flowability ($P < 0.05$; r = – 0.65). Similar correlations between the content of intact casein and the functional attributes of the melted cheese have been noted for Cheddar cheeses differing in fat content [14]. The above trends also concur with those of Yun et al. [37, 38] who reported that LM Mozzarella cheeses made using *C. parasitica* proteinase as coagulant had a higher degree of β-casein degradation, higher mean levels of pH 4.6 soluble N and 12% TCA soluble N, a higher mean flowability and a lower mean apparent viscosity than the corresponding cheeses made with chymosin or *Rhizomucor miehei* proteinase, during ripening. Similarly, Madsen and Qvist [23] reported that increased breakdown of α_{s1}- and β-caseins in Mozzarella cheeses prepared from high concentration ultrafiltered milk retentates containing 70 g·kg^{-1} whey protein and treated with a proteinase from *Bacillus licheniformis* or *Bacillus subtilis* (Neutrase®) resulted in significantly higher flowability at all stages of a 5 week ripening period. In the latter study, capillary electrophoresis indicated that the whey proteins in the Mozzarella prepared from the ultrafiltered milk retentates were not degraded during ripening.

Flowability and apparent viscosity may be defined as the different forms of displacement of contiguous planes of the structural *para*-casein matrix in the heated cheese, as affected by heat induced changes in the degrees of fat coalescence and

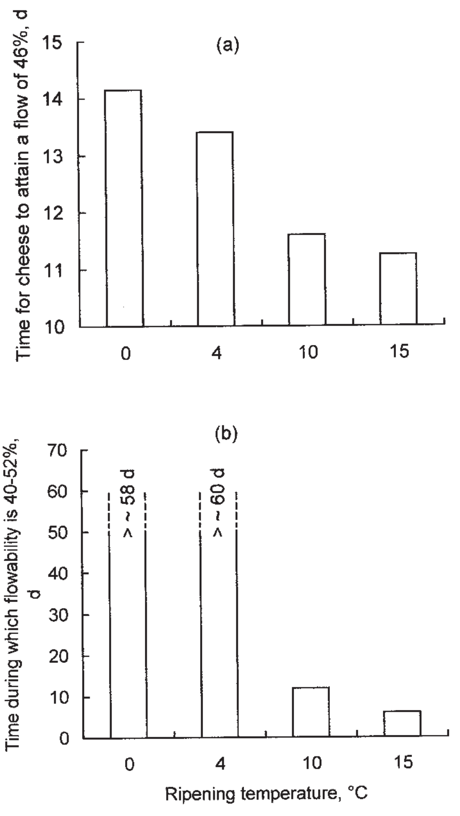

Figure 9. Effect of ripening temperature on the time (days) for low-moisture Mozzarella to attain a flowability of 46% (a) and duration of period for which flowability remained in the range 40–52% (b).

protein hydration, and the application of shear stress to the molten cheese mass [13, 28]. Hence, it is envisaged that an increase in the level of proteolysis and hydration of the *para*-casein matrix would weaken the degree of casein-casein interaction in the unheated cheese and thereby contribute to an increase in the level of displacement of the molten cheese obtained for a given shear stress [4].

Owing to its effects on proteolysis and protein hydration and hence, functionality of the heated cheeses, changing the ripening temperature has implications for the functional shelf life of Mozzarella. A recent survey of commercial cheeses, including Mozzarella [16], indicated that the functional attributes of heated Mozzarella fall within bands (e.g., flowability, ~ 40 to 70%; apparent viscosity, ~ 200–1 000 Pa·s). The current results show that temperature had a notable effect on the time that different functional attributes (e.g., flowability and apparent viscosity) attained values representative of commercial LM Mozzarella and the duration of the period for which functional attributes remained within a given band (e.g., 40–52% flowability, Figs. 9a and 9b). Hence, alteration of ripening temperature provides a convenient means by which the functional shelf life of LM Mozzarella cheese can be varied.

4. CONCLUSIONS

Increasing the ripening temperature from 0 to 15 °C resulted in a decrease in the level of intact casein, and an increase in the water binding capacity of casein, as reflected by the decrease in the level of expressible serum. These changes were paralleled by a decrease in the mean firmness of the uncooked cheese over the 70 d ripening period, and an increase in the mean flowability and reductions in the mean apparent viscosity and melt time of the cooked cheese.

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