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# Biochemical and Functional Relationships in Cheese

T.P. Guinee and P.F. Fox

Reduced fat content and increased fat emulsification reduce the flowability, stretchability and fluidity of melted natural cheese (e.g. Cheddar) due to a reduction in heat-induced fat coalescence. Adjusting technological levers such as ripening temperature, cheese pH and calcium content offer convenient ways of altering the functional shelf-life of cheese, including low-moisture Mozzarella.



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## Compositional, biochemical and functional relationships of Mozzarella and other cheeses used in pizza pie

(Biochemical and Functional Relationships in Cheese)

Armis No. 4537

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## Summary and Conclusions

*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. 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. These studies have shown that the functionality of natural cheese is dynamic, with the different functional attributes undergoing marked changes during ripening, and, for a given cheese variety, the desired functional attributes are optimum within a specific time frame during maturation. 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.*

*The main aims of this project were to investigate the effects of the following on the age-related changes in heat-related functional attributes (e.g. stretchability, fluidity) of cheese:*

- \* fat reduction,*
- \* the degree of fat emulsification,*
- \* the pH and calcium content and their interaction,*
- \* the correlation between proteolysis and functional attributes, especially attributes other than flowability, e.g. rheological properties of raw cheese, stretchability of heated cheese, and*
- \* the age-related changes in the functionality of cheeses other than Mozzarella, e.g. analogue pizza cheese and Emmental.*

*At the outset of this project, comparatively little information was available on the effects of the above parameters on the age-related changes in heat-induced functional attributes (e.g. stretchability, fluidity) of cheese, especially for varieties other than Mozzarella.*

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### *Main Conclusions and Achievements*

Reduction in the fat level in Cheddar cheese, in the range 30 to 7%, w/w, resulted in:

- \* increases in stress required to fracture the cheese and the firmness of the raw cheese, and
- \* a marked deterioration of the functionality of the melted cheese, as reflected by decreases in the flowability, stretchability and fluidity, and an increase in apparent viscosity at different ripening times.

Increasing the degree of fat emulsification in natural cheese, by high pressure homogenisation of the cheese milk, resulted in:

- \* a marked decrease in the degree of fat globule clumping and coalescence on heating, and
- \* marked decreases in the stretchability, flowability and elasticity of the melted cheese.

Proteolysis in low-moisture Mozzarella cheese was:

- \* increased with elevation of storage temperature in the range 0 to 15°C,
- \* scarcely affected by increasing the level of rennet or by adding bovine trypsin to the cheese milk,
- \* negatively correlated with firmness of the raw cheese and the melt time and apparent viscosity (chewiness) of the melted cheese, and
- \* positively correlated with flowability of the melted cheese.

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The interaction between pH and calcium content in Mozzarella cheese:

- \* had a marked effect on the composition and texture of the unheated cheese and the functionality of the heated cheese during storage,
- \* affected the rate at which satisfactory heat-induced functionality is attained, and
- \* afforded the cheesemaker a means for altering the functional shelf-life of Mozzarella.

Evaluation of the age-related changes in a number of varieties (Cheddar, Emmental, Provolone, Mozzarella, Caciocavallo, Kashkaval) indicated that:

- \* heat functionality in these varieties is a dynamic property with the different functional attributes such as flowability and stretchability changing markedly with ripening time,
- \* the extent of age-related change is variety specific, and
- \* mature samples of these varieties had poorer stretchability, and comparable or higher flow than Mozzarella cheese.

Analysis of commercially-produced imitation pizza cheese revealed that:

- \* they exhibit batch-to-batch variation in the heat-induced functionality,
- \* compared to natural cheeses, they underwent only relatively small changes in proteolysis, texture, and functionality during storage.
- \* the inclusion of starch in their formulations generally impaired the flowability of melted cheese to an extent dependent on the starch type, and
- \* the products had an inferior stretchability compared to natural cheese.

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## Research and Results

### *Effects of fat content on the texture and heat-induced functional properties of Cheddar cheese*

Cheddar cheeses with the different fat contents (% w/w) were made at pilot-scale level and ripened at 7°C for 180 days. The cheeses were designated: full-fat (FFC), 30% (w/w); reduced-fat (RFC), 21.8% (w/w); half-fat (HFC), 17.1% (w/w); and low-fat (LFC, 6.0% [w/w]). A decrease in the fat content from 30.0 to  $\leq 17.1\%$  (w/w) resulted in significant decreases in content of moisture in non-fat substances and primary proteolysis (pH 4.6-soluble N [expressed as % total N]) and increases in the contents of moisture, protein, intact casein and free amino acids (*Table 1*). Reduction in fat content resulted in an increase in the volume fraction of the casein matrix and a decrease in the extent of fat globule clumping and coalescence.

*These changes in composition and proteolysis coincided with marked increases in the fracture stress (force required to break cheese). On baking the cheese, reduction in fat content resulted in significant increases in the mean melt time (time required for shred fusion) and apparent viscosity and a decrease in the mean flowability of the melted cheese (Fig. 1).*

The stretchability of the FFC cheese increased most rapidly and, at ~ 15 and 30 days, attained mean values which were similar to those of commercial Mozzarella and significantly higher than those of the other cheeses. Thereafter, the stretchability of the FFC decreased progressively to values that were significantly (i.e. at 150 days) lower than those of the RFC and HFC.

### *Effect of fat content and degree of fat emulsification on the structure-function relationship of Cheddar cheese*

A model study was undertaken to observe the effects of fat level and degree of fat emulsification, as affected by homogenisation of the cheesemilk, on the heat-induced changes in microstructure, viscoelasticity and functionality of Cheddar-type cheese. Cheddar-type cheeses of different fat contents (% w/w) were produced at pilot plant and denoted: full-fat (FFC), 30.6; half-fat (HFC), 17.4;

and skim cheese (SKC) 1.3. Full-fat Cheddar cheese was also prepared from milk which had been homogenised at first and second stage pressures of 25 and 5 MPa, respectively, and denoted as FFCH. Homogenisation of cheesemilk resulted in a slight increase in moisture content and an increase in primary proteolysis. Confocal laser scanning microscopy revealed that the extent of fat globule clumping and coalescence in both the unheated and heated (to 95°C) cheeses decreased with homogenisation of the cheesemilk and with fat reduction (Fig. 2). Homogenisation of the cheesemilk and reducing the fat content of the cheese resulted in a decrease in the flowability and stretchability of the melted cheese (Fig. 3). Dynamic measurement of the viscoelastic changes on heating the

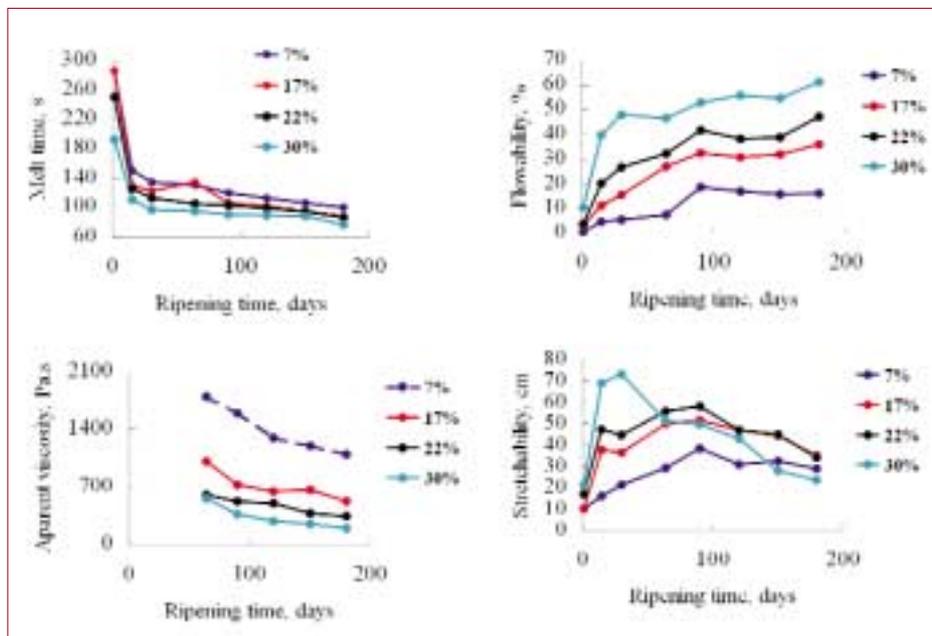
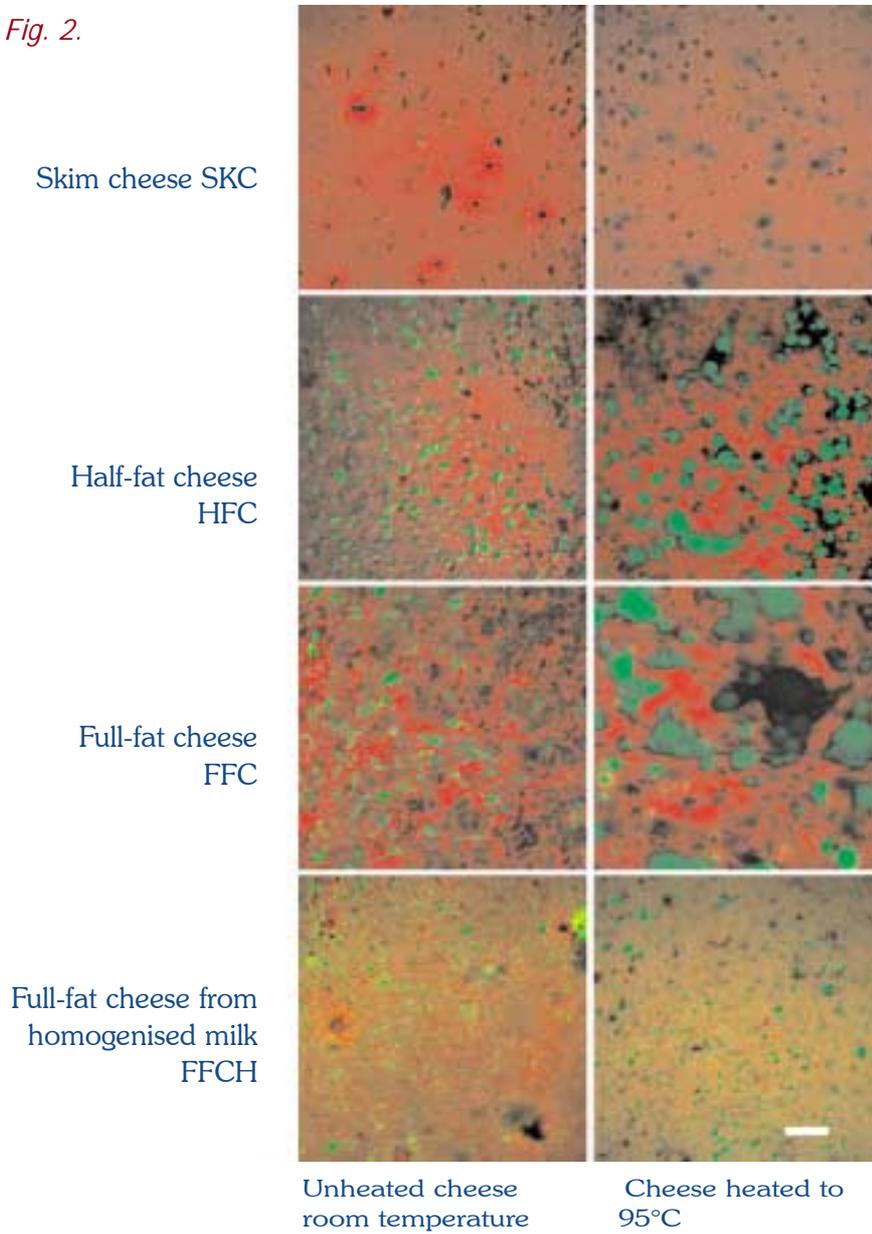


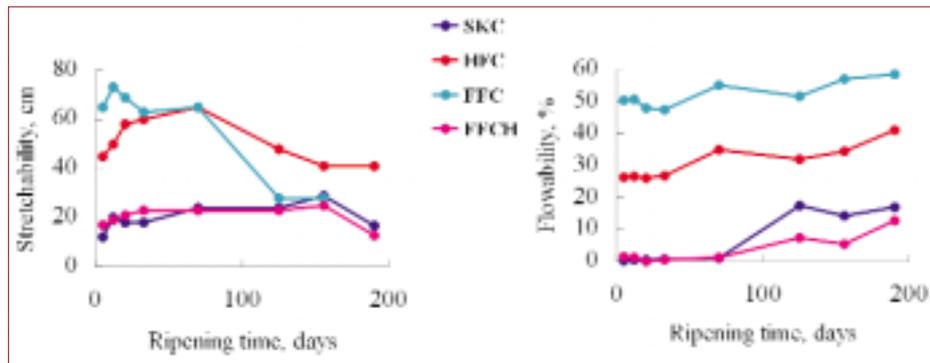
Fig. 1: Changes in the melting characteristics of Cheddar cheese of different fat content (7, 17, 22 and 30% w/w) during ripening at 7°C. The values presented are the means of three replicate trials.

cheese from 20 to 90°C showed that reduction of the fat content resulted in a decrease in the fluidity (as measured by the phase angle,  $\delta$ ) at temperatures > 50°C. Homogenisation resulted in a marked decrease in  $\delta$  at temperatures > 45 - 50°C (Fig. 4).

*Fig. 2.*



*Fig. 2:* Confocal laser scanning micrographs of 5 day-old Cheddar cheeses of different fat content, prepared from non-homogenised or homogenised milks, before and after heating. The micrographs show protein, as red areas, and fat, as green areas. Bar corresponds to 25 $\mu$ m.



*Fig. 3: Stretchability and flowability of Cheddar-type cheese of different fat contents, as a function of ripening time at 7°C, from non-homogenised milks, (Skim cheese, SKC; Half-fat cheese, HFC; and Full-fat cheese, FFC) or from full-fat homogenised milk, (Full-fat homogenised cheese, FFCH).*

The results indicated that heat-induced fat coalescence results in the formation of large oil pools which form a lubricating film between the protein layers and facilitate the mobility of the protein phase and leads to an overall reduction in the rigidity of the cheese on cooking.

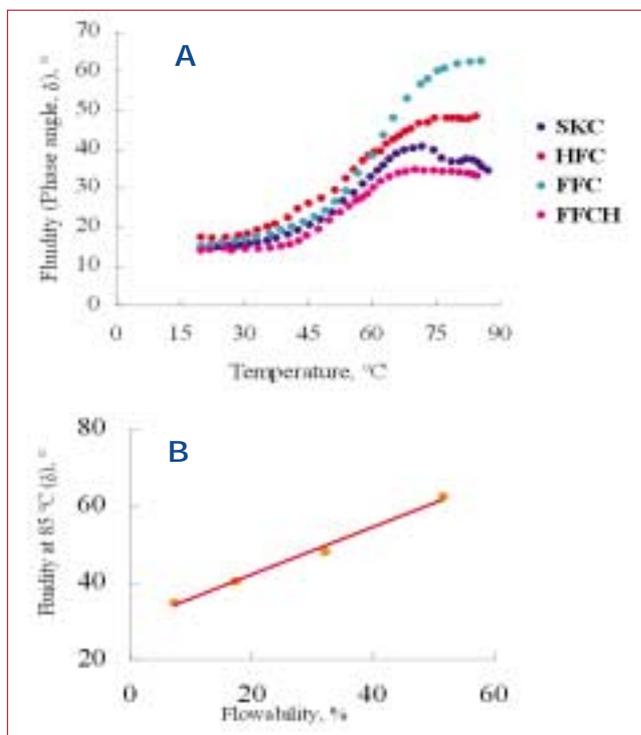
*Hence, the adverse effect of reducing the fat content and increasing the degree of fat emulsification on the fluidity and flowability of the melted cheese may be attributed largely to the concomitant reduction in the degree of heat-induced fat coalescence.*

These observations greatly enhance our understanding of the melting process and may be used to design processed/analogue cheese products with customised functionality, e.g. flow resistance.

*A high correlation existed between flowability and phase angle (Fig. 4), which indicates that viscoelastic analysis using low amplitude strain oscillation rheometry, is a useful technique for quantitatively assessing the meltability of natural cheeses.*

### Effects of pH and calcium content on Mozzarella cheese

Low-moisture Mozzarella (LMMC) cheese with different levels of calcium and pH were made using a starter culture (control, CL) or direct acidification (DA) with lactic acid or lactic acid and glucono- $\delta$ -lactone. Three Mozzarella cheeses with different calcium and pH values were produced and denoted CL, DA1 and DA2. The mean 1-day pH and calcium (mg/g protein) content of the various cheeses were CL - 5.42 and



**Fig. 4:** Viscoelastic analyses of 5 day-old Cheddar-type cheeses of different fat content, from non-homogenised milk (Skim Cheese, SKC; Half-fat cheese, HFC; and Full-fat cheese, FFC) or Full-fat homogenised milk (FFCH).

**A** = Fluidity (phase angle,  $\delta$ ) as a function of temperature of the cheeses.

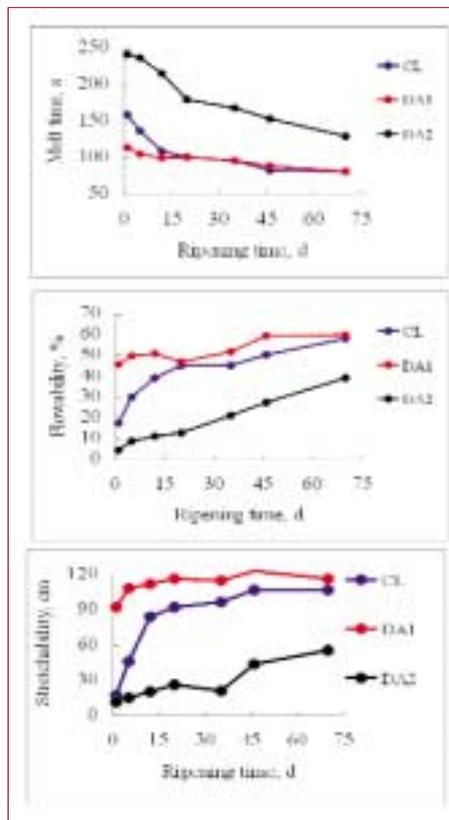
**B** = Relationship between fluidity and flowability in 5 day-old cheeses.

28; DA1 - 5.96 and 21.8; DA2 - 5.93 and 29.6. Reducing the calcium-to-casein ratio of the high-pH cheeses (i.e.  $\geq 5.9$ ) from 29.6 to 21.8 mg/g resulted in significant increases in moisture level and water binding capacity (WBC) in the raw cheese (Table 2) and in the stretchability, flowability and fluidity of the melted cheese over the 70-day ripening period (Figs. 5 and 6).

The melt time, flowability and stretchability of the low-calcium high-pH DA1 cheese at 1 day were similar to those attained by the CL cheese after storage times  $\geq 12$  days. In

*Fig. 5: Changes in the melting characteristics of Mozzarella cheese with different pH and calcium levels during storage at 4°C: low-pH, high-calcium control, CL; directly-acidified, high-pH, low-calcium, DA1; directly-acidified, high-pH, high-calcium, DA2. The values presented are the means of three replicate trials.*

contrast, raising the pH of Mozzarella with a high calcium-casein ratio resulted in a decrease in the WBC of the raw cheese and a slower development of flowability and stretchability in the melted cheese. Thus, the high-pH, high-calcium DA2 cheese required a period of 70 days to attain values of melt time, flowability and stretchability similar to those reached by the control CL cheese at 8 days.

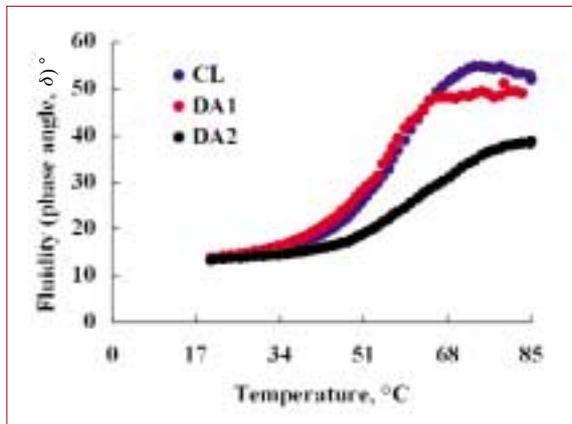


*Hence, exploitation of the interaction between cheese pH and calcium content affords the cheesemaker a convenient means by which to alter the storage time required for Mozzarella to attain satisfactory functionality.*

### ***Proteolysis in Mozzarella cheese and its impact on functionality***

Several means were exploited to increase the level of proteolysis in Mozzarella cheese with a view to altering the rate at which functional attributes, such as flowability, develop.

A three-fold increase in the level of chymosin (EC 3.4.23.1) addition to the cheesemilk resulted in an approximate 25% increase in the level of primary proteolysis after 45 days storage at 4°C, as measured by the level of pH 4.6-soluble N. However, this increase in proteolysis was not sufficient to



*Fig. 6: Fluidity, as a function of heating temperature, of 5 day-old Mozzarella cheeses with different pH and calcium level during storage at 4°C: low-pH, high-calcium control, CL; directly-acidified, high-pH, low-calcium, DA1; directly-acidified, high-pH, high-calcium, DA2. The values presented are the means of three replicate trials.*

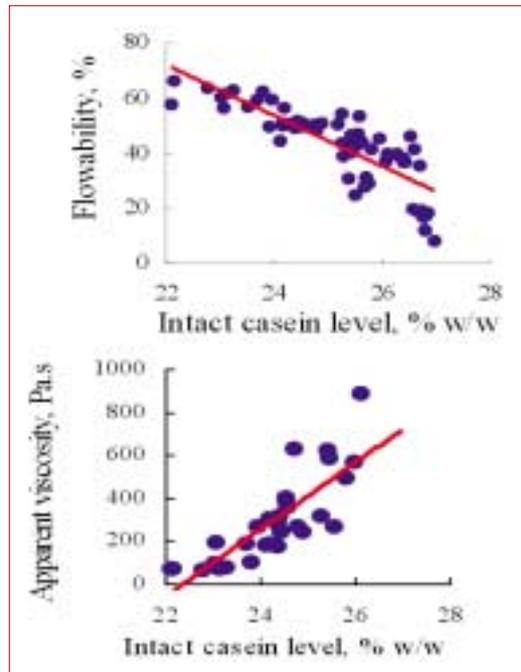
affect a significant change in the functionality of the melted cheese over a 70-day investigation period.

Addition of bovine trypsin (EC 3.4.21.4), at levels of 0.54 to 1.07% (w/w) of a commercial preparation (EC 3.4.21.4 activity of 0.5 Anson units/g) had little effect on the composition or on age-related changes in proteolysis of the unheated cheese or on functionality of the heated cheese. The above findings indicate substantial losses in the activities of chymosin and trypsin as a result of denaturation during curd manufacture and plasticisation.

Elevation of storage temperature proved to be the most effective means of increasing proteolysis in low-moisture Mozzarella. Raising the temperature, from 0 or 4°C, to 10 or 15°C, resulted in a significant increase in primary proteolysis, with the percentage increases in pH 4.6-soluble N at 35 days amounting to ~ 55% and 75%, respectively. The increased proteolysis, at this magnitude, resulted in a significant increase in flowability and decrease in the apparent viscosity. The effect of elevated storage temperature on the functional attributes of the molten cheese was attributed to the concomitant reduction in the content of intact casein which was negatively correlated with flowability and positively with apparent viscosity (*Fig 7*).

*Hence, alteration of ripening temperature provides a convenient means by which the functional shelf-life of LM Mozzarella cheese can be varied.*

*Fig. 7: Relationship between the level of intact casein and flowability after baking at 280°C for 4 min and apparent viscosity at 70°C. The data are for low-moisture Mozzarella cheeses produced in two replicate trials, ripened at 0, 4, 10 or 15°C and analysed over a 70-day ripening period. Regression was significant ( $P < 0.05$ ).*



### **Functionality of Different Varieties**

Evaluation of the age-related changes in a number of varieties (Cheddar, Emmental, Provolone, Mozzarella, Kashkaval) indicated that heat-induced functionality in natural cheeses is a dynamic property, where the different functional parameters change to varying degrees during storage. The extent of age-related change in functionality across the spectrum of cheeses depends on variety, composition, microstructure of the fat phase, extent of proteolysis and casein hydration, and ripening conditions.

In contrast to natural cheeses, imitation cheeses were relatively stable during storage, with only small changes in primary and secondary proteolysis, texture, and functionality being noted on storage for 180 days at 4°C. The inclusion of either maize or potato starch in imitation cheese at levels of ~ 3% w/w significantly impaired the flowability. However, starch had little effect on the stretchability of imitation cheese, which was inferior to that of low-moisture Mozzarella.

**Table 1:**  
**Characteristics of Cheddar cheeses of different fat content**

	Cheese Code <sup>1</sup>			
	LFC	HFC	RFC	FFC
<b>Composition</b>				
Moisture (% w/w)	46.1 <sup>a</sup>	43.0 <sup>b</sup>	40.9 <sup>c</sup>	37.8 <sup>d</sup>
Fat (% w/w)	7.2 <sup>d</sup>	17.2 <sup>c</sup>	21.9 <sup>b</sup>	30.0 <sup>a</sup>
Protein (% w/w)	38.5 <sup>a</sup>	33.3 <sup>b</sup>	31.0 <sup>a</sup>	26.4 <sup>d</sup>
<sup>2</sup> MNFS (% w/w)	49.6 <sup>b</sup>	51.9 <sup>b</sup>	52.4 <sup>a</sup>	54.0 <sup>a</sup>
Ca (mg/100 g)	1098 <sup>a</sup>	936 <sup>b</sup>	871 <sup>c</sup>	741 <sup>d</sup>
P (mg/100 g)	839 <sup>a</sup>	680 <sup>b</sup>	638 <sup>c</sup>	533 <sup>d</sup>
<b>Proteolysis</b>				
pH 4.6-soluble N (% total N) at:				
60 day	5.3 <sup>c</sup>	6.1 <sup>b</sup>	6.5 <sup>b</sup>	8.0 <sup>a</sup>
120 day	9.5 <sup>c</sup>	11.7 <sup>b</sup>	11.7 <sup>b</sup>	13.4 <sup>a</sup>
150 day	11.3 <sup>c</sup>	12.9 <sup>b</sup>	13.8 <sup>b</sup>	14.6 <sup>a</sup>
190 day	13.3 <sup>c</sup>	14.7 <sup>b</sup>	15.0 <sup>b</sup>	17.0 <sup>a</sup>

*a,b,c,d* Values within a row not sharing a common superscript differed significantly,  $P < 0.05$ . Results are means of three replicate trials.

<sup>1</sup>Codes. LFC = Low-fat Cheddar; HFC = Half-fat Cheddar;  
RFC = Reduced-fat Cheddar; FFC = Full-fat Cheddar.  
<sup>2</sup>MNFS = moisture in non-fat substances.

*Table 2:*  
**Compositional characteristics of Mozzarella cheeses**

	Cheese Code <sup>1</sup>		
	CL	DA1	DA2
<b>Composition</b>			
Moisture (% w/w)	48.0 <sup>b</sup>	56.7 <sup>a</sup>	49.4 <sup>b</sup>
Fat (% w/w)	20.3 <sup>a</sup>	15.9 <sup>b</sup>	16.9 <sup>b</sup>
Protein (% w/w)	27.0 <sup>a</sup>	23.5 <sup>b</sup>	28.5 <sup>a</sup>
<sup>2</sup> MNFS (% w/w)	60.3 <sup>b</sup>	67.4 <sup>a</sup>	59.4 <sup>b</sup>
Ca (mg/g/protein)	27.7 <sup>a</sup>	21.8 <sup>b</sup>	29.6 <sup>a</sup>
pH	5.45	5.96	5.93
<b>Water binding capacity</b> (g non-expressible serum/g protein) at:			
1 day	1.40 <sup>b</sup>	1.85 <sup>a</sup>	1.46 <sup>b</sup>
22 day	1.78 <sup>b</sup>	2.04 <sup>a</sup>	1.61 <sup>c</sup>
46 day	1.78 <sup>b</sup>	2.06 <sup>a</sup>	1.63 <sup>c</sup>

*a,b,c,d* Values within a row not sharing a common superscript differ significantly,  $P < 0.05$ . Results are means of 3 replicate trials.

<sup>1</sup>Codes. CL = Control; DA1 = Directly acidified 1;  
DA2 = Directly acidified 2.

<sup>2</sup>MNFS = moisture in non-fat substances.



Dr. T.P. Guinee



Prof. P.F. Fox

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