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Original article

Salt-induced structural changes in Mozzarella cheese and the impact upon free oil formation in ripening cheese

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Abstract – Mozzarella cheese was manufactured to different salt levels by a combination of dry- and brine-salting. Free oil formation was lower, apparent viscosity of melted cheese was higher, and expressible serum was lower for cheese at the higher salt levels. Salt had no effect on the extent of primary proteolysis as measured by pH 4.6 sodium acetate buffer or 120 $g \cdot L^{-1}$ trichloroacetic acid soluble peptides. Fat globule size decreased in the cheese after 20 d of ripening. Additional Mozzarella curd was salted separately in a brine bath without additional dry-salting. At the centre of the brined block of curd where the salt content was lowest, free oil was highest and apparent viscosity was lowest. These results are discussed in relation to how salt affects free oil formation by altering the protein and water phases during ripening.

Salt / mozzarella / cheese / fat globule / free oil

Résumé – Modifications structurelles de la Mozzarella induites par le sel, et impact sur la formation d'huile au cours de la maturation du fromage. De la Mozzarella a été fabriquée avec différentes concentrations de sel, par une combinaison de salage à sec et par saumure. À niveau de sel élevé, la formation d'huile et la libération de sérum étaient faibles tandis que la viscosité apparente de fusion était élevée. Le sel n'avait aucun effet sur la protéolyse primaire, basée sur les peptides solubles mesurés soit dans un tampon acétate de sodium (pH 4,6) ou dans 120 $g \cdot L^{-1}$ d'acide trichloroacétique. La taille des globules gras dans le fromage diminuait après 20 j de maturation. Du caillé de Mozzarella a été également salé à différents degrés dans des bains de saumure. Au centre de ce caillé, là où la quantité du sel était la plus faible, la concentration en huile libre était la plus élevée et la viscosité apparente la plus faible. Les effets du sel sur la formation d'huile libre au cours de la maturation sont discutés en relation avec les effets du sel sur les protéines et la phase aqueuse.

Sel / fromage / mozzarella / globule gras / huile libre

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1. INTRODUCTION

Functional properties of Mozzarella cheese, such as meltability, stretchability, viscosity of melted cheese, hardness of unmelted cheese, and propensity for release of free oil upon heating change rapidly in the first few weeks after manufacture. During cheese ripening, meltability increases [11, 28], apparent viscosity decreases [7, 13], and free oil formation increases [14] and becomes constant two to three weeks after manufacture [27, 28].

Free oil formation in cheese increases considerably at a fat-in-dry-matter exceeding 410 $g \cdot kg^{-1}$ [12]. Insufficient free oil has been suggested as causing excessive browning and poor melt properties due, in part, to dehydration of the cheese during heating [24]. However, to adjust the fat content to control free oil formation in cheese is of limited use, as excessive or too little free oil can cause serious defects in the cheese.

Salting of cheese curds improves meltability of non-fat Mozzarella [20]. The effect of salt on proteolysis is not as clear. Studies have reported that salt does not affect the amount of soluble peptides [6] or the extent of hydrolysis of α_{s1}-casein or β-casein [4], however another study has reported that salt enhances proteolysis [11], probably confounded by a higher level of moisture. The level of soluble peptides is not affected by the method of salting, such as brine- or dry-salting, however dry-salted cheese is softer, perhaps due to a higher moisture level [6].

The level of soluble peptides, an indicator of ripening, increases as cheese ages [28]. Hydrolysis of α_{s1}-casein increases over the first 50 d of ripening, however β-casein remains relatively unchanged during this time [15]. A faster rate of hydrolysis of β-casein using a more proteolytic coagulant results in free oil continuing to increase over time, rather than reaching a plateau value as occurs when using chymosin [27]. A lower amount of coagulant results in less proteolysis of the finished cheese over time, concomitant with a greater amount of free oil [15]. This contrasts with the observation of higher amounts of free oil in ripened cheese that has undergone more extensive proteolysis [28], but supports the observation of higher free oil with less proteolysis at a higher cooking temperature [26]. Lower moisture may have caused the lower extent of proteolysis in the latter case.

Proteolysis impacts positively upon meltability [1, 25] but the effect on free oil formation is not as clear. The level of salt is reported to be more important than moisture in dictating free oil formation [14] but this relationship may not involve proteolysis. Certainly there is no clear correlation between primary proteolysis and the level of salt [4].

The poor water-holding capacity of Mozzarella cheese in the first two weeks after manufacture, quantified by the amount of expressible serum, results in a cheese with poor shredding characteristics [8]. Salting of curds causes protein to swell over time which reduces the amount of expressible serum in non-fat [20] and low moisture, part skim Mozzarella [9]. The number and size of channels containing the expressible serum is proportional to the fat content, indicating that the serum channels serve to trap fat globules, squeezing them as the protein component swells during ripening [19]. Brine-salted Mozzarella cheese has significantly lower levels of expressible serum than unsalted cheese, and during ageing, the levels of expressible serum decrease more rapidly in the brine-salted cheese compared to the unsalted cheese [9].

Brine-salted Mozzarella initially has a higher salt content at the surface compared to the centre. This salt gradient may persist for several weeks, depending upon the size of the block of cheese during the brining process. The effect is for the apparent viscosity to be higher, and the free oil to be lower at the surface [14]. Free oil increases at the centre of a brine-salted cheese despite the increasing salt content over time, indicating an effect due to proteolysis [14].

The degree of salt-induced protein swelling may impact upon free oil formation.

The effect may be confounded by the salt gradient creating a moisture gradient, thus decreasing the size and number of serum channels, squeezing the fat globules and producing free oil. This may explain the observation of increasing free oil at the centre of brine-salted cheese during ripening [14].

One day after manufacture of Mozzarella cheese, higher amounts of free oil results in a more meltable cheese with larger and numerically more serum phase channels [19]. This same study found that the salt-induced swelling of the protein matrix also results in a more meltable cheese, but with less expressible serum, indicating that the effect of salt on meltability differs for freshly made cheese compared to ripened cheese.

Lower melting point fractions of milk fat homogenised into skim milk were shown to increase free oil formation after subsequent cheese manufacture [21]. Brine-salt or a combination of brine- and dry-salt does not affect free oil formation, but higher levels of salt will decrease expressible serum [23]. This study also showed that a decrease in the cooling temperature of curd blocks immediately after salting decreases free oil and increases expressible serum. The average size of fat globules decreases during ripening [7], probably due to coalescence of the larger globules to form pools of free oil that are not classified as fat globules under the microscope.

There is some suggestion that salt-induced protein swelling, as quantified by the amount of expressible serum, impacts upon free oil formation in cheese by a mechanism of squeezing and rupturing of the fat globules during the first day after manufacture. To determine if this mechanism is one of the factors affecting free oil formation at later stages of cheese ripening, Mozzarella cheese was manufactured at different salt levels and the apparent viscosity, expressible serum, soluble peptide concentration, and milk fat globule size and shape were examined over time. The aim of this study was to examine the effect of salt on the functionality of ripening Mozzarella cheese and to explain the observations in terms of protein and fat globule structure. In addition, these factors were considered at both the surface and the centre of salted cheese blocks.

2. MATERIALS AND METHODS

2.1. Mozzarella cheese manufacture

Mozzarella cheese was manufactured by a combination of dry- and brine-salting of the curd as previously described [16, 23]. Curd (40 kg per vat) was manufactured from milk in two vats (435 L vats, model 5MX, Kusel Equipment Co., Watertown, WI, USA) and dry-salted (20 $g \cdot kg^{-1}$). The curd was divided into 8 × 10 kg portions and each stretched using a horizontal single auger pilot-scale Mozzarella cooker-stretcher (Johnson and Nelles, Windsor, WI, USA) at a screw speed of 12 rpm and a curd feed rate of 1.5 kg per minute at 65 °C. The volume of stretching water was 50 L and the curd residency time was 600 s. The temperature of the stretching water was 65 °C, leading to a curd temperature upon exit of 60 °C. The brine solution in the cooker stretcher was either 0 $g \cdot kg^{-1}$ for low salt cheese, 30 $g \cdot kg^{-1}$ for the medium salt cheese or 60 $g \cdot kg^{-1}$ for the high salt cheese. The high and low salt cheeses were manufactured in triplicate and the medium salt cheese in duplicate. Curd blocks (1 kg) were cooled for 60 min in iced water, vacuum-packed in barrier bags (Cryovac, Hamilton, New Zealand) and stored at 4 °C. On days 3, 8, 20, 33, and 45 after manufacture the cheese samples were taken from refrigerated storage and prepared for free oil, expressible serum, apparent viscosity and soluble peptides measurement, confocal laser scanning microscopy (CLSM) and compositional analyses.

2.2. Effect of salt at different locations within cheese blocks

To examine the effect of salt at different locations within a block of ripening Mozzarella cheese, four samples of cheese

(5 kg blocks, 15 × 15 × 35 cm) were obtained from a commercial manufacturer. The samples were taken directly from the brine bath and transported to refrigerated storage (4 °C) on the same day. To examine the effect of different salt concentrations in different parts of the cheese, a 5 cm deep surface layer of each 5 kg block was removed. The surface sections and the remaining centre pieces were separately vacuum packed in barrier bags and stored at 4 °C. Cheese blocks were taken from refrigerated storage and prepared for free oil and apparent viscosity measurement, and compositional analyses at two intervals after manufacture (3 and 28 d).

2.3. Compositional analysis, free oil, viscosity and fat globule microstructure

Cheese samples were cut into 2 cm cubes, then mixed and shredded for compositional analyses. Moisture, fat and protein were measured using near infra-red spectroscopy (Infralab TM 5000E, Infrared Engineering, Irwindale, CA, USA) as previously described [21]. Salt was measured using a potentiometric technique [10]. Free oil content, expressed as either a proportion of the cheese weight or the total fat in the cheese [12] and apparent viscosity of cheese [21] were measured as previously described. Fat globule microstructure was examined using CLSM after staining the aqueous phase with rhodamine B and the lipid phase with Nile Blue. The size and shape of globules were then quantified as cross-sectional area, maximum diameter, circularity and elongation factor using computerised image analysis [2, 22]. Maximum diameter was defined as the longest line passing through the centre of the two-dimensional cross-sectional area of the fat globule. Circularity was defined as a measure of the degree of distortion of the fat globules. A circularity of 0 indicates complete distortion, whereas 1 indicates a perfect circle. Elongation factor was defined as the ratio of the longest to the shortest apparent diameters of the cross-sectional area.

2.4. Soluble peptides

The extent of proteolysis was quantified by measuring the concentration of soluble peptides in a 120 $g \cdot L^{-1}$ trichloroacetic acid (TCA) solution, and in a pH 4.6 sodium acetate buffer. Low molecular weight peptides and amino acids produced by the action of chymosin, and proteases and peptidases from the starter culture are soluble in 120 $g \cdot L^{-1}$ TCA, whereas these, plus the higher molecular weight peptides, are soluble in the pH 4.6 buffer [18]. The concentration of lower molecular weight soluble peptides was measured by grinding cheese (1.5 g) in a mortar and pestle with 120 $g \cdot L^{-1}$ TCA solution (20 mL), followed by filtering through Whatman #42 paper. The concentration of soluble peptides at pH 4.6 was measured by grinding cheese (3 g) in Sharp's reagent [17]. Both soluble peptide measurements were made in duplicate by Kjeldahl digestion.

2.5. Expressible serum

The expressible serum was measured in duplicate [8]. Shredded cheese (160 g) was placed in a 250 mL flat bottom centrifuge tube. The cheese was centrifuged at 12 500 × g for 75 min at 25 °C. The serum was decanted and the volumes of the aqueous and fat phases were measured. Expressible serum was defined as the volume of the aqueous phase.

2.6. Statistical analysis

A randomised block design was used to evaluate the effect of the treatments on the dependent variables, using SPSS 8.0 for Windows (SPSS, Chicago, IL, USA). Statistical significance was determined at $P \leq 0.05$. Bonferroni comparisons were used to analyse the differences between means. The treatment was the level of salt, and the vat replicates were blocked. Statistical interactions between the treatment and the age of the cheese were analysed and significant differences indicate that the salt effect on the relevant variable during ripening is different for each salt level.

Table I. The effect of salt content and ripening time at 4 °C on the gross composition of Mozzarella cheese.

Salt level	Low	Medium	High	Ripening	Salt	Interaction
Salt ($g \cdot kg^{-1}$)	10.8[a]	13.6[b]	15.9[c]	NS	***	NS
Moisture ($g \cdot kg^{-1}$)	463	464	464	NS	NS	NS
Salt-in-moisture	0.0234[a]	0.0293[b]	0.0342[b]	NS	***	NS
Fat ($g \cdot kg^{-1}$)	186	185	185	NS	NS	NS
Fat-in-dry-matter	0.347	0.345	0.344	NS	NS	NS
Protein ($g \cdot kg^{-1}$)	273	270	268	NS	NS	NS

[abc] Means (n = 3) within a row for each variable with different superscripts differ at $P \leq 0.05$.
Significance: *** $P \leq 0.001$; NS: not significant.

3. RESULTS AND DISCUSSION

3.1. Effect of salt on gross composition during ripening

Dry-salting the Mozzarella cheese to different salt contents resulted in an expected difference ($P \leq 0.05$) in the salt and salt-in-moisture contents (Tab. I), but did not affect any of the other compositional parameters measured. Previous unpublished work from this laboratory using the same cooker/stretcher and with an exit temperature of 65 °C has shown that pH does not change over 50 d of storage at 4 °C, but drifts upwards by 0.1 to 0.2 pH units from 50 to 120 d after manufacture. Therefore pH would not be expected to be a factor to account for differences in cheese functionality for the first 50 d of ripening.

As expected, there was no effect ($P \leq$ 0.05) of the ripening time on gross composition. Any affect that the ripening time has on functionality would therefore not be attributable to differences in gross composition during ripening. Differences in salt and salt-in-moisture content are therefore a possible factor in affecting any observed changes in functionality.

3.2. Effect of salt on functionality during ripening

Free oil formation in Mozzarella cheese increased over time ($P \leq 0.001$) (Tab. II and Fig. 1). The cheese with the lowest salt level showed the greatest increase in free oil during the first eight days of ripening (Fig. 1). Apparent viscosity decreased ($P \leq 0.001$) during ripening, most rapidly during the first 20 d of ripening (Tab. II and Fig. 2).

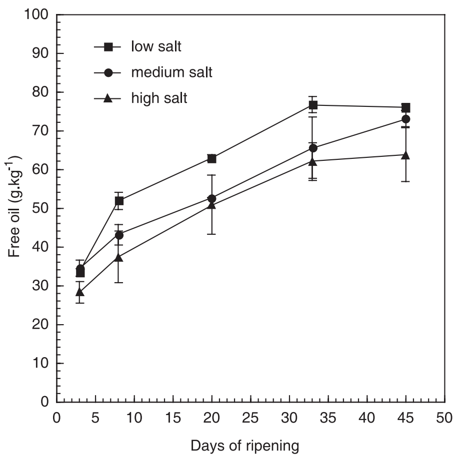

Figure 1. The effect of salt on free oil content (expressed as $g \cdot kg^{-1}$ of cheese) of Mozzarella cheese during ripening at 4 °C. See Table I for salt contents.

Salt content had a significant effect ($P \leq 0.05$) on free oil formation (Tab. II). Lower salt levels resulted in higher amounts of free oil (Fig. 1). One day after manufacture of Mozzarella cheese, salt content was found

Table II. Analysis of variance for functionality of Mozzarella cheese as a function of salt and age of cheese.

Source of variation		Free oil (by weight of cheese)		Free oil (by weight of fat)		Apparent viscosity (Pa·s)	
	df[†]	MS[§]	*P*	MS[§]	*P*	MS[§]	*P*
Treatment (salt level) (T)	2	546	0.01	1.29×10^4	0.013	6.93×10^{11}	0.017
Vat (blocked) (V)	1	91	0.16	1130	0.31	1.02×10^{11}	0.24
Error (T × V)	4	31	–	820	–	5.25×10^{10}	–
Age (A)	5	1960	< 0.001	4.51×10^4	< 0.001	1.24×10^{13}	< 0.001
A × T	10	27	0.92	840	0.95	1.18×10^{11}	0.96
Error	22	71	–	2530	–	3.95×10^{11}	–
Corrected total	47						

[†] Degrees of freedom. [§] Mean square.

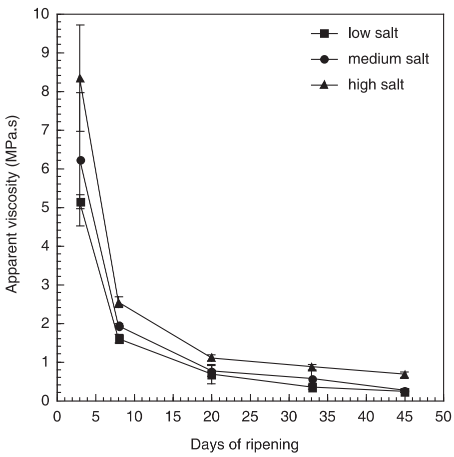

Figure 2. The effect of salt on apparent viscosity of Mozzarella cheese during ripening at 4 °C. See Table I for salt contents.

to have no significant effect on free oil formation [23] suggesting that if there is any effect of salt content on free oil formation, it will only become more pronounced during the subsequent ripening period. The interaction between age and salt treatment (A × T, Tab. II) is not significant for free oil or apparent viscosity, demonstrating that the trend over time is the same for all three salt treatments.

After eight days of ripening, no expressible serum could be extracted from the high or medium salt Mozzarella cheese and no serum could be expressed after 20 d from the low salt Mozzarella cheese (Fig. 3). These results indicate that protein swelling took place during the ripening period, as has been previously reported [9, 20].

At one day after manufacture free oil formation is not affected by the level of salt in Mozzarella cheese, although cold brining results in higher free oil compared to dry- and hot brine-salted cheese at the equivalent salt level [23]. In the present study, higher amounts of salt decreased the expressible serum and free oil. The extent of proteolysis was greater as cheese aged, and this was manifested by a lower apparent viscosity. This salt effect can be explained by a synergistic relationship between proteolysis and protein swelling. At higher salt levels the degree of protein swelling is enhanced, as measured by reduced expressible serum, and this will exert pressure on fat globules within the serum channels such that the globules are squeezed together within the protein matrix. This has been observed using scanning electron microscopy of cheese after 21 d of ripening [19]. However the free

Table III. Analysis of variance for fat globule structure of Mozzarella cheese as a function of salt content and age of cheese.

		Diameter		Cross-sectional area		Circularity		Elongation factor‡	
	df†	MS§	*P*	MS§	*P*	MS§	*P*	MS§	*P*
Treatment (salt level) (T)	2	1.37	0.37	77.5	0.093	0.0007	0.42	0.092	0.43
Vat (blocked) (V)	1	8.83	0.045	282	0.015	0.005	0.056	0.26	0.15
Error (T × V)	4	1.07	–	17.1	–	0.0007	–	0.084	–
Age (A)	5	10.2	0.001	791	0.002	0.01	0.025	0.082	0.24
A × T	10	1.80	0.33	108	0.55	0.001	0.91	0.13	0.04
Error	22	1.52	–	124	–	0.003	–	0.054	–
Corrected total	47								

† Degrees of freedom. § Mean square. ‡ Elongation factor = (maximum diameter)/(minimum diameter).

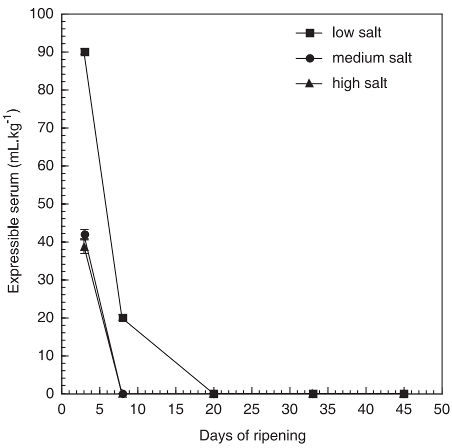

Figure 3. The effect of salt on expressible serum (expressed as mL·kg^{-1} of cheese) of Mozzarella cheese during ripening at 4 °C. See Table I for salt contents.

oil did not increase as a result of swelling of the protein matrix, but rather decreased at the higher salt levels. The swollen protein matrix may be sufficiently hydrolysed as the cheese ages such that the smallest fat globules are embedded into the softening protein matrix wall without rupture and subsequent free oil formation. This evidently does not take place in young cheese where the extent of proteolysis is much less, and where pressure applied to the smallest fat globules in the serum phase against a harder protein matrix wall may cause rupture with subsequent free oil upon heating [23]. This is a key difference between freshly made and aged Mozzarella.

The increase in apparent viscosity at higher salt concentration should not be ruled out as a possible contributor to melting behaviour and free oil formation. A less viscous melted cheese (with lower salt content) is likely to enhance the movement of adjacent protein layers under flow conditions, thus facilitating the movement of fat globules occluded within the protein matrix and increasing the propensity for coalescence and rupture to form larger pools of oil [5]. This mechanism is consistent with the data in this study and may be acting in conjunction with the previously described mechanism of protein swelling.

3.3. Effect of salt on milk fat globule structure during ripening

Salt content had no effect on the size or shape of fat globules (Tab. III). Although the fat globule elongation factor is positively correlated with free oil formation [22], and higher levels of salt decreased the

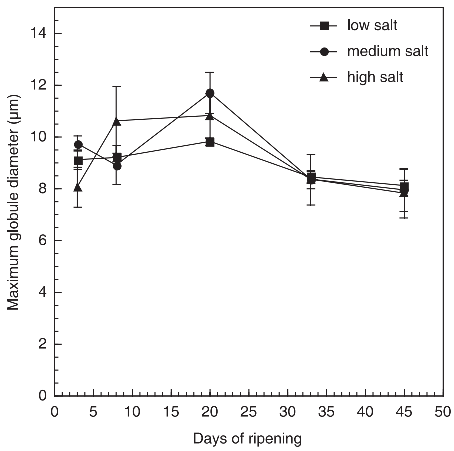

Figure 4. The effect of salt on the milk fat globule maximum diameter in Mozzarella cheese during ripening at 4 °C. See Table I for salt contents.

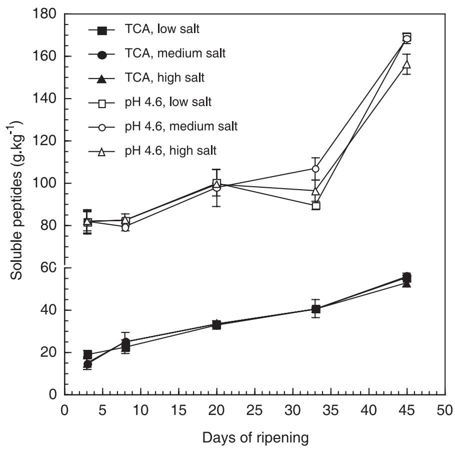

Figure 5. The effect of salt on release of 120 g·L^{-1} trichloroacetic acid (TCA) soluble and pH 4.6 soluble peptides in Mozzarella cheese during ripening at 4 °C. Results are expressed as soluble peptides (g·kg^{-1} of cheese). See Table I for salt contents.

amount of free oil (Fig. 1), there was no effect of salt on the elongation factor.

The diameter and the cross-sectional area of fat globules in Mozzarella were significantly affected ($P \leq 0.01$) by the age of the cheese (Tab. III). The diameter of fat globules decreased after 20 d of ripening (Fig. 4). Similar results were obtained for the cross-sectional area of the fat globule (data not shown), although in both cases the changes were minimal and may not be significant. Fat globule size has been reported to be smaller at 20 d compared to globules in freshly made Mozzarella cheese [7]. The circularity of the fat globule, which indicates a transition from emulsified fat globules to free pools of oil within the cheese protein matrix [3], was significantly affected ($P \leq 0.05$) by the number of days of ripening (Tab. III), but not in a consistent manner, as the levels fluctuated over the ripening period (data not shown). Elongation factor did not change during the ripening period.

The decrease in fat globule size after 20 d (Fig. 4) may be related to protein swelling that takes place during ripening of Mozzarella cheese, concomitant with reduced expressible serum. Free oil increased (Fig. 1) and expressible serum decreased (Fig. 3) during ripening. The increased swelling of the protein matrix may have contributed to rupture of the larger fat globules to form pools of free oil, and these would be underrepresented in the number-weighted average of fat globule size, thus giving an effective reduction in diameter and cross-sectional area. The increased volume of ruptured larger fat globules may be the primary contributor to free oil formation. Larger globules are of similar size to the chains of casein aggregates in the cheese, therefore are more likely to deform and rupture as protein rearrangements take place during ripening. Smaller globules are more likely to be occluded and remain intact within inhomogeneities of the larger protein structure.

3.4. Effect of salt on proteolysis during ripening

The concentration of soluble peptides in 120 g·L^{-1} TCA increased ($P \leq 0.001$) during ripening of cheese (Tab. IV and Fig. 5). The salt content did not affect the concentration of TCA soluble peptides during ripening. The concentration of soluble peptides in the

Table IV. Analysis of variance for proteolysis of Mozzarella cheese as a function of salt content and age of cheese.

	df†	Soluble N in TCA*		Soluble N in pH 4.6	
		MS§	*P*	MS§	*P*
Treatment (salt level) (T)	2	5.0×10^{-4}	0.072	2.3×10^{-3}	0.054
Vat (blocked) (V)	1	3.0×10^{-3}	0.005	2.0×10^{-4}	0.54
Error (T × V)	4	1.0×10^{-4}	–	3.0×10^{-4}	–
Age (A)	4	0.16	< 0.001	0.85	< 0.001
A × T	8	5.0×10^{-4}	0.99	8.5×10^{-3}	0.94
Error	20	4.1×10^{-3}	–	2.4×10^{-2}	–
Corrected total	39				

* Trichloroacetic acid (120 $g \cdot L^{-1}$). † Degrees of freedom. § Mean square.

pH 4.6 buffer also increased ($P \leq 0.001$) during ripening, but again there was no effect from the salt content. The interaction between treatment (salt) and age of the cheese was not significantly different (A × T in Tab. IV), indicating that the trends over time were the same for each salt level.

No effect of salt content on proteolysis was found, even though the amount of free oil was lower in the high-salt cheese during ripening. Other researchers have also found no differences in proteolysis in Mozzarella cheese at different salt levels during ripening [4]. The present results indicate that proteolysis may not be the only factor (if at all) which affects free oil formation during ripening, and that free oil formation is perhaps governed in part by the degree of protein swelling, as quantified by the amount of expressible serum.

3.5. Effect of salt at different locations within cheese blocks

The surface of the cheese (to a depth of 5 cm) had a lower moisture ($P \leq 0.001$) and a higher salt content ($P \leq 0.001$) at 3 and 28 d after manufacture (Tab. V). Fat content was not affected by the age of the cheese. The salt gradient was inversely related to the amount of free oil formed, with higher salt content resulting in a lower amount of free oil (Tab. V).

A salt gradient exists between the surface (higher salt) and the centre (lower salt) of a block of brine-salted Mozzarella cheese, and a more rapid increase in free oil occurs at the centre of the cheese during ripening [14]. This is the opposite of what would be expected if salt diffused into the centre of the cheese and reduced the amount of free oil by a putative mechanism involving only proteolysis. The diffusing salt creates an osmotic pressure difference [20], which decreases as the cheese ages, and this may provide the necessary pressure to deform fat globules as moisture migrates towards the surface, rupturing the globules and creating pools of free oil upon heating.

The apparent viscosity of the cheese was lower ($P \leq 0.001$) at the centre compared to the surface (Tab. V). A high apparent viscosity was observed at the surface where the salt content was higher. The free oil content expressed as a percentage of the total fat increased ($P \leq 0.001$) and the apparent viscosity decreased ($P \leq 0.001$) after 28 d of ripening at both the centre and the surface. These results have been previously reported for Mozzarella cheese during ripening [14], and are possibly a consequence of increased proteolysis over time at a point when there

Table V. Composition, free oil, and apparent viscosity of Mozzarella cheese at the surface and centre.

Location	Surface		Centre		Significance		
Days after manufacture	3	28	3	28	Age	Location	Interaction
Moisture (g·kg^{-1})	426^{a}	432^{b}	438^{c}	444^{d}	***	***	NS
Fat (g·kg^{-1})	231	242	228	236	NS	NS	NS
Fat-in-dry-matter	0.416	0.427	0.406	0.424	NS	NS	NS
Protein (g·kg^{-1})	244^{a}	244^{a}	241^{a}	247^{b}	***	NS	NS
Salt (g·kg^{-1})	20^{a}	20^{a}	11^{b}	11^{b}	NS	***	NS
Salt-in-moisture	0.081^{a}	0.083^{a}	0.045^{b}	0.043^{b}	NS	***	NS
Free oil (g·kg^{-1} of cheese)	62^{a}	83^{b}	70^{c}	101^{d}	***	***	*
Free oil (g·kg^{-1} of fat)	268^{a}	346^{b}	306^{a}	429^{d}	***	*	**
Apparent viscosity (MPa·s)	5.00^{a}	1.61^{b}	3.53^{c}	0.71^{d}	***	***	*

^{abcd} Means (n = 4) within a row for each variable with different superscripts differ at $P \leq 0.05$.
Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; NS: not significant.

is a much reduced osmotic pressure difference between the surface and the centre due to salt equilibration throughout the cheese.

4. CONCLUSION

Free oil formation increased during cheese ripening and occurred at a lower level where the salt content was higher. This inverse relationship between free oil and salt is not evident at one day after manufacture, indicating that there is an age effect on the formation of free oil. Higher amounts of salt resulted in a swelling of the protein matrix surrounding the serum channels, as measured by the reduction in expressible serum and the increase in apparent viscosity. The resultant increase in pressure upon fat globules located within the serum channels embedded the smaller globules into the softened protein matrix, limiting the propensity for rupture and the subsequent free oil formation. These results indicate that there is a synergistic relationship between proteolysis and protein swelling – both of which increase during ripening – to reduce the amount of free oil in melted cheese. This conclusion is valid for cheese that has undergone sufficient proteolysis during ripening. Although proteolysis alone is not sufficient to explain the mechanism of free oil formation, it is a necessary condition to cause a softening of the protein matrix to allow the embedding of fat globules, with concomitant reduction of free oil formation at higher salt levels.

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REFERENCES

[1] Dave R.I., McMahon D.J., Oberg C.J., Broadbent J.R., Influence of coagulant level on proteolysis and functionality of Mozzarella cheeses made using direct acidification, J. Dairy Sci. 86 (2003) 114–126.

[2] Everett D.W., Olson N.F., Free oil and rheology of Cheddar cheese containing fat globules stabilized with different proteins, J. Dairy Sci. 86 (2003) 755–763.

[3] Everett D.W., Ding K., Olson N.F., Gunasekaran S., Applications of confocal microscopy to fat globule structure in cheese, in: Malin E.L., Tunick M.H. (Eds.), Chemistry of Structure-Function Relationships in Cheese, Plenum Press, New York, NY, USA, 1995, pp. 321–330.

[4] Farkye N.Y., Kiely L.J., Allshouse R.D., Kindstedt P.S., Proteolysis in Mozzarella cheese during refrigerated storage, J. Dairy Sci. 74 (1991) 1433–1438.

[5] Guinee T., The functionality of cheese as an ingredient: a review, Aust. J. Dairy Technol. 57 (2002) 79–91.

[6] Guinee T.P., Mulholland E.O., Mullins C., Corcoran M.O., Effect of salting method on the composition, yield and functionality of low moisture Mozzarella cheese, Milchwissenschaft 55 (2000) 135–138.

[7] Guinee T.P., Feeney E.P., Auty M.A.E., Fox P.F., Effect of pH and calcium concentration on some textural and functional properties of Mozzarella cheese, J. Dairy Sci. 85 (2002) 1655–1669.

[8] Guo M.R., Kindstedt P.S., Age-related changes in the water phase of Mozzarella cheese, J. Dairy Sci. 78 (1995) 2099–2107.

[9] Guo M.R., Gilmore J.A., Kindstedt P.S., Effect of sodium chloride on the serum phase of Mozzarella cheese, J. Dairy Sci. 80 (1997) 3092–3098.

[10] International Dairy Federation, Determination of chloride content. Potentiometric titration method, Standard 88A, Int. Dairy Fed., Brussels, Belgium, 1988.

[11] Joshi N.S., Muthukumarappan K., Dave R.I., Role of soluble and colloidal calcium contents on functionality of salted and unsalted part-skim mozzarella cheese, Aust. J. Dairy Technol. 57 (2002) 203–210.

[12] Kindstedt P.S., Rippe J.K., Rapid quantitative test for free oil (oiling off) in melted Mozzarella cheese, J. Dairy Sci. 73 (1990) 867–873.

[13] Kindstedt P.S., Rippe J.K., Duthie C.M., Application of helical viscometry to study commercial Mozzarella cheese melting properties, J. Dairy Sci. 72 (1989) 3123–3128.

[14] Kindstedt P.S., Kiely L.J., Gilmore J.A., Variation in composition and functional properties within brine-salted Mozzarella cheese, J. Dairy Sci. 75 (1992) 2913–2921.

[15] Kindstedt P.S., Yun J.J., Barbano D.M., Larose K.L., Mozzarella cheese: Impact of coagulant concentration on chemical composition, proteolysis and functional properties, J. Dairy Sci. 78 (1995) 2591–2597.

[16] Kindstedt P.S., Rowney M., Roupas P., Technology, biochemistry and functionality of pasta filata/pizza cheese, in: Law B.A. (Ed.), Technology of Cheesemaking, Academic Press, Sheffield, UK, 1999, pp. 193–221.

[17] Kosikowski F.V., Cheese and Fermented Milk Foods, F.V. Kosikowski and Associates, Brooktondale, NY, 1982.

[18] Lau K.Y., Barbano D.M., Rasmussen R.R., Influence of pasteurization of milk protein breakdown in cheddar cheese during ageing, J. Dairy Sci. 74 (1991) 727–740.

[19] McMahon D.J., Fife R.L., Oberg C.J., Water partitioning in Mozzarella cheese and its relationship to cheese meltability, J. Dairy Sci. 82 (1999) 1361–1369.

[20] Paulson B.M., McMahon D.J., Oberg C.J., Influence of sodium chloride on appearance, functionality, and protein arrangements in nonfat Mozzarella cheese, J. Dairy Sci. 81 (1998) 2053–2064.

[21] Rowney M.K., Hickey M.W., Roupas P., Everett D.W., The effect of homogenization and milk fat fractions on the functionality of Mozzarella cheese, J. Dairy Sci. 86 (2003) 712–718.

[22] Rowney M.K., Roupas P., Hickey M.W., Everett D.W., The effect of compression, stretching, and cooking temperature on free oil formation in Mozzarella curd, J. Dairy Sci. 86 (2003) 449–456.

[23] Rowney M.K., Roupas P., Hickey M.W., Everett D.W., Salt-induced structural changes in one-day old Mozzarella cheese and the impact upon free oil formation, Int. Dairy J. 14 (2004) 809–816.

[24] Rudan M.A., Barbano D.M., A model of Mozzarella cheese melting and browning during pizza baking, J. Dairy Sci. 81 (1998) 2312–2319.

[25] Rudan M.A., Barbano D.M., Yun J.J., Kindstedt P.S., Effect of fat reduction on chemical composition, proteolysis, functionality, and yield of Mozzarella cheese, J. Dairy Sci. 82 (1999) 661–672.

[26] Yun J.J., Kiely L.J., Barbano D.M., Kindstedt P.S., Mozzarella cheese: impact of cooking temperature on chemical composition, proteolysis, and functional properties, J. Dairy Sci. 76 (1993) 3664–3673.

[27] Yun J.J., Kiely L.J., Kindstedt P.S., Barbano D.M., Mozzarella cheese: impact of coagulant type on functional properties, J. Dairy Sci. 76 (1993) 3657–3663.

[28] Yun J.J., Barbano D.M., Kindstedt P.S., Larose K.L., Mozzarella cheese: impact of whey pH at draining on chemical composition, proteolysis, and functional properties, J. Dairy Sci. 78 (1995) 1–7.