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R. I. Dave

Donald J. McMahon
Utah State University

J. R. Broadbent

C. J. Oberg

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Reversibility of the Temperature-Dependent Opacity of Nonfat Mozzarella Cheese

Rajiv I. Dave, Donald J. McMahon, Jeffery R. Broadbent, and Craig J. Oberg

Abstract

Salted and unsalted nonfat mozzarella cheese was made by direct acidification and stored at 4°C over 60 d. Changes in cheese opacity were measured by using reflectance L* values while the cheese was heated from 10 to 90°C, then cooled to 10°C, and reheated to 90°C. A characteristic opacity transition temperature (TOP) was obtained for each cheese. Both salt content and storage time influenced TOP. Opacity during heating, cooling, and reheating formed a hysteresis. At d 1, the unsalted cheese became opaque when heated to 20°C, but the salted cheese required heating to 40°C. As the salted cheese was aged, its TOP increased so that by 60 d the cheese did not become opaque until it was heated to 70°C.

Key words: cheese, opacity, salt, storage, direct acidification

Abbreviation key: L* = whiteness value in the L*a*b* color system, TOP = opacity transition temperature.

Introduction

The structure of mozzarella cheese consists of protein fibers (formed and oriented during the cooking/stretching/molding process) separated by channels containing serum, fat globules, and bacteria (Oberg et al., 1993). The extent of this fiber formation depends on whether the cheese is stretched and molded, and on the fat content of the cheese. If fat content is decreased, fewer fat/serum channels will be formed, and the protein matrix occupies a larger proportion of the observed cheese structure (McMahon et al., 1999; Merrill et al., 1996). We have also observed that the amount of expressible serum in cheese can be correlated with fat content (McMahon et al., 1999), suggesting that the volume of serum contained in the fat/serum channels decreases when mozzarella cheese is made from milk with a reduced fat content. Thus, the microstructure of nonfat mozzarella cheese typically shows no fiber orientation and no fat/serum channels (McMahon et al., 1993; Paulson et al., 1998).

When lower-fat cheeses are manufactured, it has been observed that these cheeses suffer from a loss of opacity and appear more translucent than full-fat cheeses (Fife et al., 1996; Merrill et al., 1996). Mozzarella cheese made with either 10% fat or 6% fat has been reported to have a greenish tint that was attributed to decreased fat content (Merrill et al., 1994; Rudan et al., 1998a; Tunick et al., 1993). Also, Rudan et al. (1998b) showed that when a fat mimetic was incorporated into reduced-fat mozzarella cheese, the cheese was whiter than the control reduced-fat cheese. They also noted that the whiteness of the cheeses decreased during storage. Such loss of opacity is even more apparent in nonfat cheeses, and if no colloidal or particulate colorants are added to compensate for the absence of fat globules, the cheese becomes translucent (Paulson et al., 1998). Paulson et al. (1998) also found that the translucent appearance of nonfat cheese only developed when the cheese was cooled, or when a low-calcium cheese was salted. When the nonfat cheese curd was heated during cooking and stretching, it appeared white and was completely opaque. After overnight refrigeration at 4°C, the cheese became translucent. Further observations on the temperature dependency of cheese opacity were also reported by McMahon and Oberg (1998) and Metzger et al. (2000). The lower the fat content of the cheese, the greater the difference in opacity between cold and heated cheese. At cold temperatures (e.g., 4°C), cheeses containing a higher level of fat (>10%) retain their opacity, presumably because the fat globules scatter light. In nonfat cheese, no such light scattering occurs unless...
a colorant, such as titanium oxide, has been added to the cheese. At high temperatures (e.g., 80°C), cheeses of varying fat contents reach the same level of opacity as shown by their L* values (whiteness values in the L*a*b* color system) (McMahon and Oberg, 1998; Metzger et al., 2000). When nonfat cheese is cooked on a pizza, it has the opaqueness of regular (i.e., low moisture, part skim) mozzarella cheese but loses this opacity during cooling and takes on a glassy appearance when cold. When the pizza is reheated, however, the nonfat cheese again becomes opaque.

In summary, the heating-induced opacity of mozzarella cheese is reversible and appears influenced by fat and salt content of the cheese. To learn more about the role of milk proteins in cheese opacity, we studied the opacity of nonfat cheese so as to avoid changes in opacity that have been attributed to melting of fat during heating of the cheese (Metzger et al., 2000). The objectives of our research were to determine the extent to which the opacity changes induced during heating are reversible, and to determine how age of the cheese effects the temperature at which opacity can be induced. We also tested unsalted and salted cheese to see if salt concentration influenced the opacity transition temperature ($T_{OP}$).

**MATERIALS AND METHODS**

**Cheese Manufacture**

Cheese was manufactured in the Gary H. Richardson Dairy Products Laboratory at Utah State University. Four replicates of cheese were made by using 15 kg of skim milk (fortified with 2% nonfat dry milk) in small (34 × 22 × 22 cm) stainless steel vats. While the milk was cold (<10°C), it was acidified by adding 15 g of citric acid, followed by sufficient acetic acid (diluted 1:10, vol/vol, with distilled water) to bring the milk to pH 5.7. The milk was then warmed to 37°C and sufficient double-strength calf rennet (Chr. Hansen Inc., Milwaukee, WI) diluted (1:20) in water was added to coagulate the milk and produce a firm set within 20 to 25 min. The curd was cut, allowed to heal for 10 to 15 min, then gently stirred to facilitate curd syneresis. After an additional 10 to 15 min, approximately 50% of the whey was removed and 40 g of glucono-δ-lactone was added to the remaining whey/curd in the vat to further lower pH. The vat temperature was gradually increased to 44°C over 20 min with continued gentle stirring of the curd. Another 25% of whey (based on original milk volume) was removed, and an additional 25 g of glucono-δ-lactone was added. After approximately another 30 min, when the whey pH had dropped to 4.4 (and curd pH was about 5.2), the remaining whey was drained. The curd was then divided into two lots. One lot was salted by adding 1% (wt/wt) of dry salt then hand stretched in a 5% salt brine at 75°C. The unsalted curd was stretched in hot water at the same temperature. Both cheeses were placed in small stainless steel molds and immersed in ice water for 1 h. After cooling, the cheese blocks were cut into three pieces, individually vacuum-packaged, and stored at 4°C.

**Cheese Composition**

Fat was measured by using a modified Babcock method (Richardson, 1985). Cheese moisture was measured by vacuum-oven AOAC method 926.08. Protein was determined in AOAC method 920.123, with a factor of 6.38 used to convert nitrogen to protein content. Salt concentration was determined by blending grated cheese with distilled water (dilution factor of 1:20), filtering through a Whatman #1 filter paper (Whatman International Ltd., Maidstone, England) and measuring chloride content according to AOAC method 971.19 (model 926 salt analyzer; Corning, Medfield, MA). Calcium was determined by inductively coupled plasma atomic emission spectroscopy (US Environmental Protection Agency, 1992). Cheese pH was measured with a glass electrode. All measurements were performed in duplicate.

**Cheese Proteolysis**

The extent of proteolysis during cheese storage was monitored by measuring nonprotein nitrogen after 1, 30, and 60 d. Samples of grated cheese (1.5 g) were blended uniformly with 25 ml of 12% TCA for 45 s, then transferred to a beaker. The blender jar was rinsed with an additional 20 ml of 12% TCA, and the entire contents were allowed to stand for 10 min before filtering through Whatman #42 filter paper. The filtrate was then measured for nitrogen content by the Kjeldahl method, and converted to percent protein by using a factor of 6.38.

**Cheese Melt**

Melt was measured in duplicate by using a modified procedure based on the method of Bogenrief and Olson (1995). Cheese plugs weighing approximately 15 g were placed into glass tubes, which were sealed with rubber stoppers. Sample tubes were immersed horizontally in hot mineral oil (90°C), and the distance in millimeters that the cheese melted was measured at 4, 8, 12, and 16 min.

**Cheese Opacity**

Cheese opacity was determined by measuring reflectance L* values of the cheese by using a flat-bed
color scanner with LabSmart software (Westcor, Logan, UT). The $L^*a^*b^*$ color system uses $L^*$ to measure lightness on a scale of 0 to 100, with 0 being black and 100 being white.

Cylindrical samples of cheese were obtained from the cheese blocks using a #10 cork borer (18 mm i.d.) and cut to 4-cm length. The cheese samples were then placed into 4-dram glass sample vials (Cat. #60975D-4, Kimble Co., Vineland, NJ) so that the cheese was in contact with the bottom of the vial. The vials were then sealed with rubber stoppers to avoid loss of moisture. The cheeses were tempered to $10^\circ C$ for 30 min, placed on the scanner and a reflectance color scan conducted. Color values were obtained as the average of three 0.5-cm circular areas on the cheese surface.

The cheese was then consecutively tempered at 10-min intervals to 20, 30, 40, 50, 60, 70, 80, and 90$^\circ$ C, and color measurements were obtained at each temperature. The cheeses were then cooled to $10^\circ$ C, with color measurements recorded at the same temperature intervals. After cooling, the cheese was stored overnight at 4$^\circ$ C, tempered to $10^\circ$ C for 30 min, and then reheated to 90$^\circ$ C; $L^*$ values were measured as described above. The $L^*$ value was considered as indicative of the whiteness (or opacity) of the cheese. All measurements were performed in triplicate.

**Statistical Analyses**

ANOVA was performed by using SAS (SAS, 1991) to determine the effects of salt and storage time on melt, proteolysis, and opacity. A split plot design (replicates $= 4$) was used with salting as the whole plot treatment ($n = 2$) and aging as the split plot ($n = 3$). Separate ANOVA were done for color measures at each temperature for both heating and cooling. Two-sample t-tests were used to determine the influence of salting on the composition of d-1 cheese. Significance was declared at $P \leq 0.05$.

**RESULTS AND DISCUSSION**

**Cheese Composition**

Cheese composition is shown in Table 1. There were no significant differences in fat or calcium content. The salted cheese was higher in moisture content, lower in protein content, and had a significantly lower pH than the unsalted cheese. These differences were attributed to the hydrating effect salt has on cheese made by direct acidification as previously described by Paulson et al. (1998). Such increased water binding (or moisture retention) by milk protein gels and cheese curd, as a result of salt addition, has also been shown by others (Creamer, 1985; Ovanova et al., 1971; Robertson, et al., 1975). The cheeses made by Paulson et al. (1998) had a calcium content of only 0.3%, which was considerably lower than the 0.6% calcium reported in this study. However, when considered on a calcium:protein basis, both the unsalted and salted cheese still had lower calcium than is normally present in mozzarella cheese made by fermentation.

The lower pH of the salted cheese was probably a consequence of the change in water-binding properties of the curd when salt is added to a cheese that has a low calcium:protein ratio. For the salted cheese, there was virtually no additional curd syneresis after the whey was drained and the curd salted. Thus, more of the acidified whey would have been retained in the cheese. The difference in pH between the salted and unsalted cheese was not thought to be influencing opacity as we had previously observed that structure and functionality of cheese were not affected by pH providing the calcium content of the cheese was not altered (McMahon and Oberg, 1999). In this experiment, there was no significant difference in calcium content of the cheeses.

**Cheese Proteolysis**

The extent of proteolysis, as measured by TCA-soluble nitrogen, was higher in salted cheese than in unsalted cheese (Figure 1). Both salting and storage, as well as their interaction had a significant effect on proteolysis. At d 1, the difference was small, but by day 30, the amount of TCA-soluble nitrogen in the salted cheese had doubled, whereas there was only a very small increase in TCA-soluble nitrogen in the unsalted cheese. After 30 d there was evidence of proteolysis taking place in the unsalted cheese but not at the same rate as in the salted cheese. At 60 d, the unsalted cheese reached a level of proteolysis comparable with the salted cheese after 30 d of storage. The salted cheese continued to undergo proteolysis and by 60 d contained twice as much TCA-soluble protein as the unsalted cheese.

The retardation of proteolysis that occurred in the unsalted cheese suggests that the susceptible peptide bonds in the proteins were less accessible to enzymes present in the cheese. There was a difference in moisture between the two cheeses that could also cause this difference in proteolysis, but at 58% moisture it was expected that more proteolysis would have occurred by 30 d than was observed. We have shown in the past (Paulson et al., 1998) that adding salt to nonfat cheese causes the protein microaggregates, which are the basis of the cheese matrix, to become smaller and less dense. This relaxation of the cheese matrix structure would allow peptide bonds of proteins, which are normally
buried within the microaggregates, to be more accessible to cleavage by residual coagulant, plasmin, and any other proteolytic enzymes that may be present in the cheese.

### Cheese Melt

The pattern of cheese melting, as a function of storage time and salting, paralleled the extent of proteolysis (Figure 2). The salted cheese melted significantly more than the unsalted cheese, and melting for both cheeses increased significantly during aging. The heated cheeses flowed about fifty percent further after aging to 60 d than they did at d 1. While both salting and aging were significant factors throughout the 16-min melt test (i.e., they were significant for 4-, 8-, 12-, and 16-min measurements), the interaction of salting × aging was only significant for the 4- and 8-min measurements. Thus, initial flow of the cheese (4 to 8 min) was affected by this interaction, whereas the final flow distance was not (i.e., $P > 0.05$ for salting × aging interaction at 12 and 16 min of melting).

### Cheese Opacity

When both cheeses were cold ($\leq 10^\circ$C), they were translucent in appearance and had L* values of $\leq 80$. As they were heated (to $90^\circ$C), they all became opaque and had L* values of $\geq 90$. Based on visual observations

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**Table 1.** Mean composition and pH of unsalted and salted nonfat mozzarella cheese.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unsalted cheese</th>
<th>SD</th>
<th>Salted cheese</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>58.7*</td>
<td>0.21</td>
<td>60.1*</td>
<td>0.25</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>40.0*</td>
<td>0.35</td>
<td>37.4*</td>
<td>0.74</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>0.2</td>
<td>0</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>Salt (%)</td>
<td>0.15*</td>
<td>0.008</td>
<td>1.54*</td>
<td>0.094</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.66</td>
<td>0.017</td>
<td>0.63</td>
<td>0.041</td>
</tr>
<tr>
<td>pH</td>
<td>5.46*</td>
<td>0.023</td>
<td>5.27*</td>
<td>0.068</td>
</tr>
</tbody>
</table>

*Means were significantly different ($P \leq 0.05$).
of the cheese during heating, a noticeable decrease in translucency (with a corresponding increase in opacity) occurred when \( L^* \) values reached approximately 82. At an \( L^* \) value of about 85, the cheeses became opaque. The maximum \( L^* \) value reached by all cheeses during heating was very similar (\( L^* \) values of approximately 92). However, the temperature at which the cheeses underwent the transition from translucent to opaque was dependent on the age of the cheese and whether the cheese was unsalted (Figure 3) or salted (Figure 4).

**Influence of salt.** The salt content of the cheese had a significant effect on opacity at 20 to 60°C and at 90°C. Aging was significant at 10 to 70°C, and the interaction of salting \( \times \) aging was significant at temperatures in the range of 10 to 80°C. Similar results were obtained for cooling of cheese, with all three effects mentioned above being significant at most of the temperatures. These differences were a result of the transition from translucency to opacity occurring at different temperatures.

At d 1, both unsalted and salted cheeses were translucent at 10°C. After heating to 30°C, both cheeses became opaque. As the cheeses aged, the temperature required to induce opacity increased. For example, it was necessary to heat 60-d-old salted cheese to 70°C to induce opacity. In contrast, there was only a slight change in the opacity transition of the unsalted cheese, and after 60 d of storage the unsalted cheese still became opaque when heated to 30°C.

**\( \beta \)-Casein.** Metzger et al. (2000) suggested the increase in cheese opacity during heating results from precipitation of \( \beta \)-casein. At cold temperatures, \( \beta \)-casein becomes more soluble (Bingham, 1971), and its concentration in the serum phase of mozzarella cheese increases during the first few days of refrigerated storage (Guo and Kindstedt, 1995). This follows well-established observations that \( \beta \)-casein dissociates from casein micelles in milk at low temperatures (Bloomfield and Morr, 1973; Pierre and Brule, 1981). Such dissociation is indicative of about 50% of \( \beta \)-casein interacting only loosely, via hydrophobic interactions, with the other proteins in the casein micelles (McMahon and Brown, 1984a).

When expressible serum from mozzarella cheese is heated, the \( \beta \)-casein present in the serum becomes insoluble and an opaque gel is formed (Metzger et al., 2000). Based on this observation, they proposed that upon heating of cheese, a similar gel forms in the serum pockets distributed throughout the cheese matrix. These gel particles then increase light scattering in the cheese and the cheese becomes opaque. Such gelation, however, may not explain the change in opacity of non-fat cheese because such cheese does not contain serum pockets (McMahon et al., 1993; Paulson et al., 1998).

![Figure 3](image-url) Effect of heating (■), cooling (●), and reheating (□) on \( L^* \) values of unsalted nonfat mozzarella cheese that had been stored at 4°C for (A) 1 d, (B) 30 d, and (C) 60 d. An \( L^* \) value of 82 (dashed line) was indicative of when the cheese changes from being translucent to opaque and was used to determine the opacity transition temperature. Error bars are SEM (most are hidden by symbols).
An alternate mechanism by which the temperature-induced insolubility of $\beta$-casein could cause opacity is via interactions of $\beta$-casein with other proteins. In general, the caseins strongly interact with each other and do not exist as monomers under native conditions (McMahon and Brown, 1984a). In addition to self-polymerization, $\beta$-casein forms aggregates with other caseins via both hydrophobic interactions and calcium-phosphate linkages (Green, 1971; McMahon and Brown, 1984a, 1984b). Thus, it would be expected that cold-solubilized $\beta$-casein molecules would interact with other proteins in the curd matrix (perhaps, especially the very hydrophobic para-$\kappa$-casein) when cheese is heated. It can be concluded that the change in opacity of cheese during heating is most likely a function of the temperature sensitivity of $\beta$-casein, but whether it results in a separate protein gel being formed or changes the structure of the protein matrix requires further research.

**Opacity transition temperature.** By using an $L^*$ value of 82 as a measurement of when the cheese changes from being translucent to opaque, a $T_{\text{OP}}$ was determined for each cheese. At d 1, the $T_{\text{OP}}$ for unsalted cheese was 13°C. This transition increased as the cheese was aged and occurred at 23°C by d 30 and 26°C by d 60 (see Figure 3). The salted cheese had $T_{\text{OP}}$ values of 30, 53, and 63°C for cheese stored for 1, 30, and 60 d, respectively (see Figure 4). The temperatures at which the cheeses became completely opaque were slightly higher (corresponding to an $L^*$ value of 85) and were calculated to be 17, 25, and 27°C for unsalted cheese stored for 1, 30, and 60 d. For the salted cheeses, complete opacity was attained at 33, 57, and 67°C for the same storage times. Maximum $L^*$ values were attained at about 30, 30, and 40°C for unsalted cheese, and 40, 60, and 80°C for salted cheese stored for 1, 30, and 60 d, respectively.

Salted cheese aged to 30 or 60 d exhibited an initial decrease in $L^*$ value as the cheese was heated (Figure 4). For example, the 60-d-old cheese had an initial $L^*$ value at 10°C of 78. This decreased to 76 at 20°C and reached a minimum of 74 at 40°C just before the beginning of the opacity transition. A similar decrease in whiteness had been observed by Metzger et al. (2000), although they attributed this decrease to changes occurring in the fat portion of cheese. In our study, however, the cheese contained only 0.2% fat, suggesting that this decrease may also be related to changes in the protein portion of cheese. Interestingly, no decrease in $L^*$ value was observed in the unsalted cheese. However, it is not possible to determine if this is a distinct difference in protein functionality in the absence of salt, or a consequence of the opacity transition beginning at a low temperature, i.e., approximately 10°C.

![Figure 4](image-url)
**Opacity hysteresis.** The transition from translucency to opacity was observed to be completely reversible. After heating and cooling the cheese, the cheeses exhibited virtually the same TOP values when they were reheated (see Figures 3 and 4). However, the reverse transition (from opacity to translucency) occurred at a temperature 10 to 15°C lower than the respective TOP. Thus, after cooling to 10°C at the same rate as the cheese was heated, the L* value at 10°C was slightly higher than the initial L* value before heating. A similar observation was made by Metzger et al. (2000). When the cheese was held overnight (approximately 18 h), it regained its initial translucency and L* value.

When the cheese was reheated, its L* value versus temperature curve was virtually identical to that obtained the first time the cheese was heated. The retardation in transitioning from opacity to translucency as the cheese was cooled suggests that the changes taking place within the cheese when it is heated require a longer time (or colder temperature) to revert to their initial state. Assuming the change in opacity is related to change in solubility of β-casein, the delay in transition during cooling reflects the longer time required to resolubilize β-casein compared with the rate at which aggregates are formed during heating. Without additional data it is not possible to determine whether the change involves formation of β-casein homopolymers, or heteropolymers of β-casein in some combination with the other proteins present in the cheese (i.e., αs1-casein, αs2-casein, and para-κ-casein).

The temperature at which the transition occurs represents the ease at which aggregates or a precipitate are formed. A low TOP (as observed in unsalted cheese) indicates sufficient protein-protein interactions are already present in the cheese at low temperatures so that a small increase in hydrophobic interactions will cause polymerization. The difference in TOP between unsalted and salted cheese indicates that electrostatic interactions are also important in this solubility-induced transition. Any such interactions that are temperature dependent, such as those between calcium and phosphate, could also influence protein solubility. The caseins (αs1-casein, αs2-casein, and β-casein) all contain protein-bound phosphate, and so the balance of interactions between calcium and sodium and phosphate would also be expected to influence cheese opacity.

**Proteolysis.** The increase in TOP as the salted cheese was aged suggests that proteolysis occurring during aging acts as a deterrent to polymerization. More protein breakdown occurred in the d-30 and d-60 cheeses compared with the d-1 cheese. Consequently, the d-30 and d-60 cheeses had to be heated to higher temperatures to bring about the transition that leads to cheese opacity. Perhaps portions of the proteins being cleaved and released contain hydrophobic amino acid side chains that would have initially been involved in the aggregation process of d-1 cheese.

## CONCLUSIONS

Temperature-induced opacity observed during heating of nonfat mozzarella cheese was shown to be completely reversible and is presumably a function of the temperature dependence of hydrophobic interactions between proteins. The temperature (TOP) at which the cheese becomes opaque is dependent on the chemical composition of the cheese and the status of the proteins. Salted cheese must be heated to a higher temperature to induce opacity compared with unsalted cheese. Storage of cheese also increases TOP values and can be related to the protein hydrolysis that occurs during storage.

## ACKNOWLEDGMENTS

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## REFERENCES


