DAIRY FOODS

Whiteness Change During Heating and Cooling of Mozzarella Cheese

L. E. Metzger, D. M. Barbano, M. A. Rudan, P. S. Kindstedt, 2 and M. R. Guo 2
Northeast Dairy Foods Research Center, Department of Food Science, Cornell University, Ithaca, NY 14853

ABSTRACT

Whiteness (L-value) changes in low-fat and low-moisture, part-skim Mozzarella cheeses during heating (7 to 60°C) and cooling (60 to 7°C) were evaluated. In low-fat Mozzarella, a large increase in whiteness was observed during heating, and a decrease in whiteness was observed during cooling. In low-moisture, part-skim Mozzarella, the whiteness changes during heating and cooling were smaller. Serum phase was removed from low-fat and low-moisture, part-skim Mozzarella cheeses. White protein gels were formed when the isolated serum phase from either low-fat or low-moisture, part-skim Mozzarella was heated. The white gel that formed was composed predominantly of casein and casein proteolysis products. The gel might have been produced by heat-induced, hydrophobic protein-protein interactions, and it tended to dissociate when cooled. Formation of a gel during heating increased light scattering, which increased the L-value. The gel dissociated during cooling and no longer scattered light, which decreased the L-value. We hypothesized that a gel, which was reversible, formed in the serum phase of cheese during heating and might have been responsible for the observed changes in the L-value of low-fat Mozzarella cheese during heating and cooling. The additional fat in low-moisture, part-skim Mozzarella compared with low-fat Mozzarella masked some of the color changes in the serum phase of low-moisture, part-skim Mozzarella. A model was developed to describe the contributions of the casein matrix plus serum phase of Mozzarella cheese and the contribution of fat to the changes in whiteness of Mozzarella cheese during heating and cooling.

INTRODUCTION

Mozzarella cheese is predominately used as an ingredient in pizza (10). Mozzarella cheese undergoes significant change in temperature during baking on a pizza. A cheese temperature of 60 to 70°C is observed at the end of baking under typical conditions (e.g., 232°C for 5 min using a commercial forced-air pizza oven) (12). After baking, the cheese begins to cool and is normally consumed at 40 to 50°C. The wide temperature range during baking and subsequent cooling can have an impact on the appearance of Mozzarella cheese. Recently, it was determined that melting and browning characteristics of fat-free, low-fat, and reduced-fat Mozzarella cheeses could be controlled by preventing surface drying and case hardening of shreds during baking by using a hydrophobic surface coating (17). Significant changes in cheese melting and whiteness during baking have been reported with the use of a hydrophobic surface coating (17).

In a preliminary experiment, a pizza, topped with sauce and low-fat Mozzarella cheese, was baked in a continuous forced-air commercial food service pizza oven (Impinger model 1132; Lincoln Food Service Products, Inc., Fort Wayne, IN) at 232°C for 5 min. Immediately after baking, cheese on the pizza was very white (Figure 1a). However, upon cooling to 37°C the cheese became translucent (Figure 1b). The red to brown appearance (Figure 1b) of the cheese was due to the red color of the tomato sauce that is visible through the translucent layer of cool cheese on top of the tomato sauce. When the pizza was placed in the oven and reheated, the cheese became white again (Figure 1c), and the red color of the tomato sauce was no longer visible through the cheese. The whiteness of the cheese is influenced by several factors including light scattering of fat and protein particles (18). The reversibility of the observed whiteness change during heating and cooling indicated that a reversible, heat-induced interaction
occurred in the cheese. Changes in the composition of the serum phase of cheese are thought to be responsible for observed decreases in the whiteness (i.e., L-value) of unmelted, reduced-fat Mozzarella cheese during refrigerated storage (18). It is possible that changes in the serum phase of cheese also caused the observed color change in low-fat Mozzarella cheese during heating and cooling.

Because the whiteness of low-fat Mozzarella cheese changes during pizza baking, the objective of this research was to quantify changes in whiteness that occur when Mozzarella cheese undergoes a change in temper-

Figure 1. Pizza topped with low-fat Mozzarella cheese immediately after baking (cheese temperature of 65°C) (a), pizza topped with low-fat Mozzarella cheese after cooling (cheese temperature of 37°C) (b), and pizza topped with low-fat Mozzarella cheese after reheating (cheese temperature of 65°C) (c).
ature and to determine the components in the cheese responsible for that change.

MATERIALS AND METHODS

Experimental Design

Two experiments were conducted to evaluate whiteness changes during heating and cooling of Mozzarella cheese. In the first experiment, the L-values of four low-fat (ca. 6% fat) Mozzarella cheeses, manufactured using the no-brine stirred-curd method as described previously (2), and four commercial low-moisture, part-skim (LMPS) Mozzarella cheeses were measured during heating and cooling. In the second experiment, the serum phase (of two of the low-fat and two of the LMPS cheeses evaluated in the first experiment) was removed by centrifugation (12,500 × g for 75 min at 25°C) after 3 d of refrigerated storage (6). The effect of temperature on the L-value of the cheese serum was determined.

Cheese and Cheese Serum Composition

To prepare the cheese for compositional analyses, samples were ground in a blender (model 31BL92; Waring, New Hartford, CT) to a particle size of 2 to 3 mm. The ground cheese particles were packed into 50-ml plastic, snap-lid vials and were stored at 4°C until analyses. Fat content was determined by ether extraction ((1); method number 33.2.26, 989.05) except that 1 g of cheese and 9 ml of distilled water were used instead of a 10-ml milk sample and 3 ml of ammonium hydroxide. Moisture was determined gravimetrically by drying 2 g of cheese at 100°C in a forced-air oven (model OV-490A-2; Blue M, Blue Island, IL) for 24 h. Total nitrogen, salt, and pH of the cheese and cheese serum were determined by Kjeldahl (1); method number 33.2.11, 991.20), Volhard test (13); method number 15.5.B.), and Xerolyte electrode (model HA405, In gold electrode, Willmington, MA and Accumet pH meter, model 915, Fisher Scientific, Springfield, NJ), respectively.

Cheese calcium content was determined by an atomic absorption spectroscopy procedure adapted from Brooks et al. (3). A 15-g sample of cheese was mixed with 45 g of 12% (wt/vol) trichloroacetic acid (Mallinckrodt Baker Inc., Paris, KY). The cheese and trichloroacetic acid mixture were blended for 30 s with an Omni mixer-homogenizer (model 17105; Omni International, Waterbury, CT), and after 30 min the mixture was filtered (Whatman 541; Whatman International Ltd., Maidstone, England). A 1-g sample of the filtrate was added to 9 g of 12% trichloroacetic acid, 9.6 g of distilled water, and 0.4 g of a 5% (wt/vol) solution of lanthanum oxide (Sigma Chemical Co., St. Louis, MO). The final concentrations of trichloroacetic acid and lanthanum oxide in cheese samples were 6% (wt/vol) and 0.02% (wt/vol), respectively. The sample was aspirated into an atomic absorption spectrophotometer (model 2380; Perkin Elmer Corp., Norwalk, CT) fitted with a calcium lamp (0303-6017; Perkin Elmer Corp., Norwalk, CT) for calcium determination. The atomic absorption spectrophotometer was calibrated with reference standards prepared from calcium reference solution (SC191-500; Fisher Scientific, Fair Lawn, NJ). The appropriate amounts of calcium reference solution, trichloroacetic acid, 5% lanthanum oxide, and distilled water were mixed in a volumetric flask to obtain reference standards containing 0, 4, 6, 8, 10, 12, and 16 ppm of calcium. All reference standards contained 6% (wt/vol) trichloroacetic acid and 0.02% (wt/vol) lanthanum oxide.

Whiteness Measurement

Cheese. The Hunter L-value was determined using a Macbeth Color-Eye spectrophotometer (model 2020; Kollmorgen Instrument Corp., Newburgh, NY) by placing the vacuum-sealed sample in the viewing port. Before measurements, the spectrophotometer was calibrated with a white reference tile. The L-value was computed from the diffuse reflectance data in the range of 360 to 740 nm, based on illuminant A. The L-value corresponds to whiteness, and higher L-values indicate whiter products (5). In a preliminary experiment, the reference tile was vacuum-sealed in a clear barrier bag. The bag had minimal effect on the L-value of the reference tile (<0.1 change of an L-value of 93.5). A sample (5 × 5 × 1cm) of each cheese was vacuum-sealed (Multi Vac, model 160; Koch, Kansas City, MI) in a clear barrier bag (6 × 10 cm, model B150; Cryovac, Duncan, SC) and was immersed in water at 7°C for 20 min. The same cheese was then sequentially transferred to water baths (model 220A; National Appliance Co., Portland, OR) at 24, 38, 49, 60, 49, 38, 24, and 7°C. At each temperature the cheese was allowed to equilibrate for 20 min before the L-value was determined in duplicate. This analysis provided a profile of changes in the L-value of low-fat and LMPS Mozzarella cheeses during heating from 7 to 60°C followed by cooling to 7°C. This analysis was performed at 15, 30, 60, and 90 d of refrigerated storage at 4°C.

The effect of temperature on whiteness during heating and cooling was determined separately for each cheese type (i.e., low fat and LMPS). The L-values of each cheese at 7, 38, and 60°C during heating and at 38, 24, and 7°C during cooling were compared using two-way ANOVA. Sources of variation included replication and temperature and were analyzed as class variables. If the F-test for the statistical model was significant (P < 0.05) the means were compared using least
Table 1. Mean composition of low-fat and low-moisture, part-skim (LMPS) Mozzarella cheeses (n = 4) and cheese serum (n = 2).

<table>
<thead>
<tr>
<th>Component</th>
<th>Cheese</th>
<th>Cheese serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low fat</td>
<td>LMPS</td>
</tr>
<tr>
<td>Moisture, %</td>
<td>55.10</td>
<td>47.16</td>
</tr>
<tr>
<td>Fat, %</td>
<td>6.03</td>
<td>20.19</td>
</tr>
<tr>
<td>Protein1, %</td>
<td>32.32</td>
<td>26.15</td>
</tr>
<tr>
<td>Salt, %</td>
<td>1.31</td>
<td>1.18</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>0.976</td>
<td>0.869</td>
</tr>
<tr>
<td>pH</td>
<td>5.21</td>
<td>5.19</td>
</tr>
</tbody>
</table>

1Protein = TN × 6.38.

significant difference test (P = 0.05). The effect of refrigerated storage on whiteness at each temperature was determined separately for each cheese type. The L-value of each cheese at 15, 30, 60, and 90 d of refrigerated storage was compared using two-way ANOVA. Sources of variation included replicate and day of refrigerated storage and were analyzed as class variables. If the F-test for the model was significant (P < 0.05) the means were compared using least significant difference test (P = 0.05). The PROC GLM procedure of SAS was used for all data analysis (20).

Cheese serum. After serum was removed (6) from two of the low-fat and two of the LMPS cheeses, the cheese serum was tempered to 7°C. The L-value was determined as described previously for cheese, except samples were poured into 5 × 5 × 1 cm glass cuvettes (Fisher Scientific, Springfield, NJ) and were placed in the viewing port of the spectrophotometer. The cuvette containing cheese serum was sequentially transferred to water baths at 49 and 71°C. After being allowed to equilibrate for 20 min, the L-value was measured at each temperature. However, during heating from 49 to 71°C, a white gel formed, followed by formation of white precipitate for both low-fat and LMPS cheese sera. This phenomenon made it impossible to determine the L-value of the cheese serum at 71°C. The white precipitate, which formed during heating to 71°C, was removed by filtration (Whatman 1, Whatman International Ltd., Maidstone, England).

With SDS-PAGE (22), we analyzed protein fractions present in cheese before serum removal, cheese serum before heating, and precipitate and filtrate that formed when serum was heated to 71°C to determine which protein fractions in the cheese serum formed the white precipitate during heating. The approximate total protein loaded per slot was 18 to 25 µg.

RESULTS

Composition

The composition of the low-fat and LMPS cheeses are shown in Table 1. The low-fat Mozzarella cheese contained 55.10% moisture and 6.03% fat, and LMPS Mozzarella cheese contained 47.16% moisture and 20.19% fat. The compositions of the low-fat and LMPS Mozzarella cheeses evaluated were typical for these products. Compositions of the sera removed from the low-fat and LMPS cheeses after 3 d of refrigerated storage are shown in Table 1. The low-fat and LMPS sera contained substantial protein (ca. 3.5%), which was similar to the concentration of protein typically found in milk. Similar results have been reported for the protein content of reduced-fat and LMPS cheese sera removed after 3 d of refrigerated storage (6, 7, 8). The pH of the cheese serum was similar to the pH of the cheese before the serum was removed. Salt content was 2.06 and 1.84%, respectively, for the low-fat and LMPS Mozzarella cheese sera.

Effect of Temperature on Cheese Whiteness

A profile of L-values during heating from 7 to 60°C and the subsequent cooling to 7°C is shown in Figure 2 for low-fat Mozzarella cheese and in Figure 3 for LMPS Mozzarella cheese. Each line represents the mean values of four cheeses. The different lines within each figure represent four different times of refrigerated storage (i.e., 15, 30, 60, and 90 d) before analysis. The mean values at 7, 38, and 60°C during heating and at 38, 24, and 7°C during cooling at each time are shown in Table 2 and Table 3 for low-fat and LMPS Mozzarella cheeses, respectively. The L-value of low-fat Mozzarella at all times of refrigerated storage (Figure 2) increased dramatically during heating from 7 to 60°C and decreased during the subsequent cooling to 7°C. The L-value of the unmelted cheese at 7°C before heating and

Figure 2. Effect of heating and cooling on L-values of low-fat Mozzarella cheese at various durations of storage at 4°C (d 15 ●, d 30 ■, d 60 ▲, and d 90 ▲).
Figure 3. Effect of heating and cooling on L-values of low-moisture, part-skim Mozzarella cheese at various durations storage at 4°C (d 15 ●, d 30 ●, d 60 ■, and d 90 ▲).

the L-value at 7°C after heating decreased as the time of refrigerated storage of the unmelted cheese increased. However, the L-value at 60°C did not change during the first 60 d of refrigerated storage. A reduction in L-value of unmelted cheese during refrigerated storage has also been reported for reduced-fat Mozzarella cheese (18).

Change in the L-value of LMPS Mozzarella during heating and cooling (Figure 3) was different than that for low-fat Mozzarella. At 7°C before heating, the L-value of LMPS was 82 to 83, which was substantially higher than that of low-fat cheese. The L-value of LMPS Mozzarella at 7°C before heating was not significantly different (P > 0.05) from the L-value after heating to 60°C. However, during heating, the L-value decreased (P < 0.05) between 7 and 38°C and increased (P < 0.05) between 38 and 60°C. As was observed with low fat, the L-value decreased (P < 0.05) during cooling from 60 to 7°C. The L-value of the LMPS Mozzarella after cooling to 7°C was always less (P < 0.05) than the L-value at 7°C before heating.

Table 2. Mean (n = 4) L-value of low-fat Mozzarella cheese during heating and cooling at 15, 30, 60, and 90 d of refrigerated storage.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>SEM</th>
<th>F-test</th>
<th>LSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heating</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7°C</td>
<td>73.9a</td>
<td>71.9b</td>
<td>70.0c</td>
<td>68.0d</td>
<td>0.49</td>
<td>&lt;0.01</td>
<td>1.6</td>
</tr>
<tr>
<td>38°C</td>
<td>85.1b</td>
<td>86.3bc</td>
<td>80.1bc</td>
<td>78.2bc</td>
<td>3.05</td>
<td>0.08</td>
<td>NS</td>
</tr>
<tr>
<td>60°C</td>
<td>88.1c</td>
<td>87.8b</td>
<td>87.6a</td>
<td>86.7a</td>
<td>0.23</td>
<td>&lt;0.01</td>
<td>0.7</td>
</tr>
<tr>
<td>Cooling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38°C</td>
<td>87.8a</td>
<td>87.3ab</td>
<td>85.9ab</td>
<td>83.8ab</td>
<td>0.95</td>
<td>0.08</td>
<td>NS</td>
</tr>
<tr>
<td>24°C</td>
<td>86.6ab</td>
<td>84.9c</td>
<td>81.8bc</td>
<td>78.4bc</td>
<td>2.05</td>
<td>0.07</td>
<td>NS</td>
</tr>
<tr>
<td>7°C</td>
<td>85.1ab</td>
<td>81.9bc</td>
<td>78.3bc</td>
<td>76.4bc</td>
<td>1.45</td>
<td>0.05</td>
<td>6.2</td>
</tr>
<tr>
<td>SEM</td>
<td>0.51</td>
<td>0.46</td>
<td>2.25</td>
<td>2.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F test</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>1.53</td>
<td>1.40</td>
<td>6.78</td>
<td>6.85</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a,b,c,d*Means within same row not sharing common superscripts differ (P < 0.05).
*A,B,C,D,E*Means within same column not sharing common subscripts differ (P < 0.05).

1Least significant difference (P = 0.05).

Effect of Temperature on Cheese Serum

A dramatic change in the appearance of low-fat and LMPS Mozzarella cheese sera was observed when heated from 7 to 49°C (Figure 4, upper). The solutions became cloudy and increased in whiteness, but there was no separation of a precipitate. Similar changes in appearance of cheese sera, when heated, were observed for serum removed from cheese of any age (data not shown). The observable change in whiteness (shown in Figure 4, upper) corresponded to an increase in L-value from 35 (at 7°C) to 66 (at 49°C) for the low-fat cheese serum and from 27 (at 7°C) to 55 (at 49°C) for the LMPS cheese serum. When the low-fat and LMPS cheese sera were heated from 49 to 71°C, they separated into two phases, a white precipitate and a clear supernatant (Figure 4, lower). As can be seen in Figure 4 (lower), the top two petri dishes contain unheated serum. The lower two petri dishes contain the serum that was heated in a cylindrical, 35-ml plastic vial. The contents of the vials were emptied into the petri dishes. The low-fat serum, when heated to 71°C, formed a white gel that was firmer and retained the shape of the vial with some free liquid, whereas the gel from LMPS serum was not as firm and did not hold its shape as well. If the cheese serum was cooled to 7°C, the white precipitate did not immediately dissociate. However, after storage for several days at 7°C, the precipitate once
Table 3. Mean (n = 4) L-value of low-moisture, part-skim Mozzarella cheese during heating and cooling at 15, 30, 60, and 90 d of refrigerated storage.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>15°C</th>
<th>30°C</th>
<th>60°C</th>
<th>90°C</th>
<th>SEM</th>
<th>F-test</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heating</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7°C</td>
<td>82.8&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>81.7&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>82.1&lt;sup&gt;A&lt;/sup&gt;</td>
<td>82.2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.35</td>
<td>0.14</td>
<td>NS</td>
</tr>
<tr>
<td>38°C</td>
<td>79.6&lt;sup&gt;B&lt;/sup&gt;,&lt;sup&gt;CD&lt;/sup&gt;</td>
<td>75.4&lt;sup&gt;B&lt;/sup&gt;,&lt;sup&gt;DC&lt;/sup&gt;</td>
<td>73.0&lt;sup&gt;B&lt;/sup&gt;</td>
<td>72.5&lt;sup&gt;B&lt;/sup&gt;,&lt;sup&gt;C&lt;/sup&gt;</td>
<td>1.11</td>
<td>&lt;0.01</td>
<td>3.6</td>
</tr>
<tr>
<td>60°C</td>
<td>85.2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>84.5&lt;sup&gt;A&lt;/sup&gt;</td>
<td>82.8&lt;sup&gt;A&lt;/sup&gt;</td>
<td>83.3&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.12</td>
<td>0.74</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Cooling</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38°C</td>
<td>81.6&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>79.2&lt;sup&gt;b,BC&lt;/sup&gt;</td>
<td>75.1&lt;sup&gt;B&lt;/sup&gt;</td>
<td>78.2&lt;sup&gt;b,B&lt;/sup&gt;</td>
<td>1.90</td>
<td>0.05</td>
<td>6.1</td>
</tr>
<tr>
<td>24°C</td>
<td>78.3&lt;sup&gt;C,D&lt;/sup&gt;</td>
<td>74.0&lt;sup&gt;b,D&lt;/sup&gt;</td>
<td>73.2&lt;sup&gt;B&lt;/sup&gt;</td>
<td>74.6&lt;sup&gt;B&lt;/sup&gt;,&lt;sup&gt;KC&lt;/sup&gt;</td>
<td>1.14</td>
<td>&lt;0.01</td>
<td>3.7</td>
</tr>
<tr>
<td>7°C</td>
<td>78.2&lt;sup&gt;CD&lt;/sup&gt;</td>
<td>77.2&lt;sup&gt;b,DC&lt;/sup&gt;</td>
<td>75.4&lt;sup&gt;B&lt;/sup&gt;</td>
<td>75.6&lt;sup&gt;b,BC&lt;/sup&gt;</td>
<td>0.82</td>
<td>0.05</td>
<td>2.6</td>
</tr>
</tbody>
</table>

SEM 1.03 1.34 1.34 1.32  
F-test <0.01 <0.01 <0.01 <0.01  
LSD 3.10 4.03 4.03 4.00  

<sup>a,b</sup>Means within same row not sharing common superscripts differ (P < 0.05).  
<sup>A,B,C,D</sup>Means within same column not sharing common subscripts differ (P < 0.05).  
<sup>1</sup>Least significant difference (P = 0.05).

again became soluble, and the L-value of the serum returned to its original level.

### Electrophoresis

Results for SDS-PAGE analysis are shown in Figure 5 (low-fat Mozzarella cheese; low-fat cheese serum; and the white precipitate and filtrate, which formed when low-fat cheese serum was heated) and in Figure 6 (LMPS Mozzarella cheese; LMPS cheese serum; and the white precipitate and filtrate, which formed when LMPS cheese serum was heated). A milk standard was placed at the right, outside lane in both electrophoresis gels. The major proteins present in milk are identified for the milk standard. The low-fat cheese and LMPS cheese were typical of 3-d-old Mozzarella cheese (19), and a majority of the protein present in the cheese was intact α<sub>s</sub>- and β-caseins plus a band for para-κ-casein that migrated between β-LG and α-LA. The proteins in both the low-fat and LMPS Mozzarella cheese sera were similar to previously reported results for LMPS cheese serum (6) and reduced-fat cheese serum (8). Low-fat and LMPS Mozzarella cheese sera contained intact casein and peptides derived from casein as well as whey proteins (6). Substantial sections of casein contain hydrophobic amino acids that can interact intra- and intermolecularly via hydrophobic interactions (16, 21). When the temperature of a solution is increased, an increase occurs in inter polymer hydrophobic bonding as a result of a thermal disruption of the water hydration shell around individual polymer molecules (4). The increase in hydrophobic interactions as temperature increases can result in thermogelation, which occurs when a solution is heated and forms a gel, which reverts back to a solution upon cooling (4). It is possible that the casein and peptides derived from casein in the cheese serum interact hydrophobically as the cheese serum is heated, resulting in the formation of white particles in colloidal suspension and eventually a gel. These particles may cause the cheese to increase in whiteness during heating because they increase the total amount of light reflected from the cheese (9). Therefore, increase in the L-value of low-fat Mozzarella cheese again became soluble, and the L-value of the serum returned to its original level.

### DISCUSSION

#### Role of Cheese Serum in Cheese Whiteness During Heating

Changes in the L-value and whiteness of low-fat Mozzarella cheese during heating might have been related to the changes in the L-value and whiteness of the cheese serum during heating. The cheese sera from low-fat and LMPS Mozzarella cheeses contained a substantial amount of protein. Protein in the cheese sera consisted of intact casein and peptides derived from casein as well as whey proteins (6). Substantial sections of casein contain hydrophobic amino acids that can interact intra- and intermolecularly via hydrophobic interactions (16, 21). When the temperature of a solution is increased, an increase occurs in inter polymer hydrophobic bonding as a result of a thermal disruption of the water hydration shell around individual polymer molecules (4). The increase in hydrophobic interactions as temperature increases can result in thermogelation, which occurs when a solution is heated and forms a gel, which reverts back to a solution upon cooling (4). It is possible that the casein and peptides derived from casein in the cheese serum interact hydrophobically as the cheese serum is heated, resulting in the formation of white particles in colloidal suspension and eventually a gel. These particles may cause the cheese to increase in whiteness during heating because they increase the total amount of light reflected from the cheese (9). Therefore, increase in the L-value of low-fat Mozzarella cheese again became soluble, and the L-value of the serum returned to its original level.

### Electrophoresis

Results for SDS-PAGE analysis are shown in Figure 5 (low-fat Mozzarella cheese; low-fat cheese serum; and the white precipitate and filtrate, which formed when low-fat cheese serum was heated) and in Figure 6 (LMPS Mozzarella cheese; LMPS cheese serum; and the white precipitate and filtrate, which formed when LMPS cheese serum was heated). A milk standard was placed at the right, outside lane in both electrophoresis gels. The major proteins present in milk are identified for the milk standard. The low-fat cheese and LMPS cheese were typical of 3-d-old Mozzarella cheese (19), and a majority of the protein present in the cheese was intact α<sub>s</sub>- and β-caseins plus a band for para-κ-casein that migrated between β-LG and α-LA. The proteins in both the low-fat and LMPS Mozzarella cheese sera were similar to previously reported results for LMPS cheese serum (6) and reduced-fat cheese serum (8). Low-fat and LMPS Mozzarella cheese sera contained intact casein and peptides derived from casein as well as whey proteins (6). Substantial sections of casein contain hydrophobic amino acids that can interact intra- and intermolecularly via hydrophobic interactions (16, 21). When the temperature of a solution is increased, an increase occurs in inter polymer hydrophobic bonding as a result of a thermal disruption of the water hydration shell around individual polymer molecules (4). The increase in hydrophobic interactions as temperature increases can result in thermogelation, which occurs when a solution is heated and forms a gel, which reverts back to a solution upon cooling (4). It is possible that the casein and peptides derived from casein in the cheese serum interact hydrophobically as the cheese serum is heated, resulting in the formation of white particles in colloidal suspension and eventually a gel. These particles may cause the cheese to increase in whiteness during heating because they increase the total amount of light reflected from the cheese (9). Therefore, increase in the L-value of low-fat Mozzarella cheese again became soluble, and the L-value of the serum returned to its original level.
cheese during heating (Figure 2) may be a result of the formation of light scattering protein gel particles in the serum phase of the cheese.

The observed reduction in the L-value of low-fat Mozzarella cheese during cooling might have been related to changes in the serum phase of cheese. If the proteins that interact to form gel particles during heating dissociate during cooling, then visible light would no longer be scattered, and the cheese would no longer appear white. As mentioned previously, the gel, which formed at 71°C when the isolated cheese serum from 3-d-old Mozzarella was heated, did not immediately dissociate upon cooling. This finding was not surprising because minimal proteolysis of casein has occurred after 3 d of refrigerated storage, and casein and peptides derived from casein in the cheese serum are largely intact (6). These intact caseins may have extensive hydrophobic interactions during heating, and as a result they would not immediately dissociate during cooling. During refrigerated storage, the caseins in cheese are cleaved into smaller peptides (19, 23, 24). Similarly, an increase in pH 4.6-soluble nitrogen is normal in the cheese serum phase with increasing time of refrigerated storage of cheese (6, 7). The casein peptides formed during increasing times of refrigerated storage may not form such strong hydrophobic interactions during heating.

Figure 4. Change in appearance of low-fat and low-moisture, part-skim (LMPS) Mozzarella cheese sera during heating from 7 to 49°C (upper). White gel formed when low-fat and low-moisture, part-skim (LMPS) Mozzarella cheese sera are heated from 7 to 71°C (lower).

Figure 5. An SDS-PAGE gel of low-fat Mozzarella: (from right to left) milk reference standard, cheese before serum removal, cheese serum before heating, precipitate formed when serum phase was heated to 71°C, and filtrate from the serum phase at 71°C.

Figure 6. An SDS-PAGE gel of low-moisture, part-skim Mozzarella for (from right to left) milk reference standard, cheese before serum removal cheese, serum before heating, precipitate formed when serum phase was heated to 71°C, and filtrate from the serum phase at 71°C.
and would dissociate more quickly than intact caseins during cooling. This finding may explain why the decrease in the L-value during cooling of low-fat Mozzarella became greater as storage time increased (Figure 2).

**Role of Fat in Cheese Whiteness During Heating**

The change in L-value for LMPS Mozzarella cheese (Figure 3) during heating and cooling was different than that for low-fat Mozzarella cheese (Figure 2). These differences were observed even though similar hydrophobically induced protein-protein interactions were observed in the cheese serum of both cheese types (Figure 4). Substantial differences in whiteness changes during heating and cooling between the low-fat and LMPS Mozzarella may be related to the additional fat in LMPS Mozzarella (20.2% for LMPS and 6.0% for low fat). In Mozzarella cheese, fat exists in particles that have lost part or all of their original membrane and are embedded in the casein matrix (14, 18). Fat can contribute to the L-value of dairy products by scattering light (15, 18). As a result, the L-value of unmelted Mozzarella cheese is influenced by the amount and particle size distribution of fat in cheese (18). Therefore, additional fat in LMPS Mozzarella cheese caused the L-value of unmelted LMPS Mozzarella to be much higher than that of low-fat Mozzarella before heating. However, during heating from 7 to 38°C, the L-value of LMPS Mozzarella decreased. This decrease became larger with increasing time of refrigerated storage of the cheese before heating. In this temperature range, the fat in Mozzarella cheese changes from a solid to a clear liquid, which will reduce its contribution to light scattering. This phenomenon is particularly true for larger fat droplets in the structure formed by coalescence during stretching. In addition, the particle size distribution of fat within the structure of cheese changes during heating with a portion of the fat coalescing and migrating to the surface as a clear free oil during heating (11). The amount of free oil lost during heating increases in LMPS Mozzarella with increasing time of refrigerated storage (11, 19), which may explain why the decrease in L-value of LMPS cheese between 7 and 38°C increased as the time of refrigerated storage increased. During cooling, the fat would revert from a liquid to a solid and, once again, increase the L-value, but not to the same degree as when it was originally in the cheese matrix before heating. As a result, the contribution of fat to the L-value of LMPS Mozzarella cheese before heating is larger than the contribution to the L-value after heating and cooling.

When Figures 2 and 3 are compared, the following points are observed: 1) the whiteness of unmelted low-fat Mozzarella was lower than LMPS; 2) the maximum level of whiteness achieved by low-fat Mozzarella was higher than for LMPS, and 3) the decrease in whiteness with time of refrigerated storage was larger for low-fat cheese than for LMPS Mozzarella both before and after heating.

The whiteness values for LMPS Mozzarella before heating are primarily due to the light scattering caused by the large amount of fat particles distributed throughout the matrix of LMPS Mozzarella cheese. The whiteness of low-fat Mozzarella before heating is due to light scattering by the small number of fat globules and the casein matrix. Fat-free Mozzarella was previously reported (18) to have an L-value of 65, which reflects the combined contribution of the casein matrix and serum phase to whiteness. The decrease in L-value of the unmelted low-fat Mozzarella during refrigerated storage was due to degradation of the casein matrix of the cheese and the increased absorbance of light by the increasing concentration of soluble peptides in the serum phase of the cheese (Figure 2). It has been reported previously (18) that the L-value of unmelted fat-free Mozzarella decreases at an even faster rate than that of low-fat or LMPS during refrigerated storage, indicating a decreased contribution of the casein matrix caused by proteolysis during refrigerated storage.

As both low-fat and LMPS Mozzarella cheeses are heated, the contribution of fat to whiteness decreased, and the contribution of the heat-induced protein gel formation in the serum phase increased. The low-fat Mozzarella has more cheese serum phase per unit volume, which may explain the higher L-values for low-fat Mozzarella at 60°C than for LMPS Mozzarella. During cooling after heating the decrease in whiteness of the low-fat Mozzarella was driven mostly by the dissociation of the protein-protein interactions in the serum phase. The decrease in whiteness of low-fat Mozzarella post heating was greater as time of storage increased and degradation of the casein matrix increased. In LMPS Mozzarella, this phenomenon is partially countered by the solidification of the milk fat during cooling and its positive contribution to whiteness at 7°C.

**Proposed Model for Temperature-Induced Whiteness Changes in Mozzarella Cheese**

Based on the results of this study, a model is proposed to describe the contributions of fat, serum, and serum-casein matrix interactions to the L-value of cheese during heating and cooling (Figure 7). This model assumes that the L-value of Mozzarella cheese during heating and cooling will be influenced by both the state (i.e., particle size) and the amount of fat in Mozzarella cheese and state (i.e., protein concentration) and amount of
serum phase. When cheese is heated, some of the protein in the serum phase interacts to form gel particles, which scatter light, and cause the cheese to become whiter. The cheese serum contains a large amount of intact β-casein, which has a large hydrophobic domain. At the temperature of the cheese serum during baking, the caseins dissolved in the cheese serum can interact hydrophobically to form a gel. When the cheese cools, the proteins in the cheese serum dissociate, no longer scatter light, and the cheese is less white. In addition, there may also be reversible interactions between the proteins in the cheese serum and casein matrix during heating, which also contribute to whiteness during heating and cooling. Thus, the line for the contribution of matrix plus cheese serum to whiteness (Figure 7) reflects the formation of a gel in the serum phase that increases cheese whiteness at peak heating temperature. The contribution of the serum to L-value will be greater after heating than before heating if the gel in the serum does not completely dissociate.

Prior to heating, fat in Mozzarella cheese is contained in small particles, which scatter light and cause the cheese to appear white. However, during heating the fat melts, particularly fat that no longer has a milk-fat globule membrane, and its contribution to L-value is reduced (Figure 7). As the cheese cools, fat solidifies, and it scatters light and contributes to an increase in the L-value. The contribution of fat to the L-value after heating is less than before because a portion of the fat is lost as free oil.

CONCLUSIONS

Whiteness of low-fat Mozzarella cheese increases dramatically during heating from 7 to 60°C. Whiteness decreases during cooling from 60 to 7°C. White protein gels were formed when the serum phase was removed from low-fat and LMPS Mozzarella and then heated. The white gel that formed was composed primarily of casein and casein proteolysis products. The gel formation was probably due to reversible, heat-induced, hydrophobic protein-protein interactions of intact caseins and large peptides derived from casein. The formation of a gel during heating increases light scattering, which increases L-value. During cooling the gel dissociates and no longer scatters light, which decreases L-value. This temperature change in whiteness of isolated cheese sera corresponds to changes in whiteness observed in the cheese during heating and cooling. A model was developed to describe the contributions of the casein matrix plus serum phase of Mozzarella cheese and the contribution of fat to the changes in whiteness of Mozzarella cheese during heating and cooling.

ACKNOWLEDGMENTS

The authors thank Joanna Lynch, Maureen Chapman, Laura Landolf, and Pat Wood for technical support and the Northeast Dairy Foods Research Center and Dairy Management Inc. (Rosemont, IL) for financial support.

REFERENCES


