Effect of Milk Preacidification on Low Fat Mozzarella Cheese: II. Chemical and Functional Properties During Storage

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ABSTRACT

The effect of milk preacidification on cheese manufacturing, chemical properties, and functional properties of low fat Mozzarella cheese was determined. Four vats of cheese were made in 1 d using no preacidification (control), preacidification to pH 6.0 and pH 5.8 with acetic acid, and preacidification to pH 5.8 with citric acid. This process was replicated four times. Modifications in the typical Mozzarella manufacturing procedures were necessary to accommodate milk preacidification. The chemical composition of the cheeses was similar among the treatments, except the calcium content and calcium as a percentage of protein were lower in the preacidified treatments. During refrigerated storage, the chemical and functional properties of low fat Mozzarella were affected the most by milk preacidification. The chemical composition of the cheeses was similar among the treatments, except the calcium content and calcium as a percentage of protein were lower in the preacidified treatments. During refrigerated storage, the chemical and functional properties of low fat Mozzarella were affected the most by milk preacidification to pH 5.8 with citric acid. The amount of expressible serum, unmelted cheese whiteness, initial unmelted hardness, and initial apparent viscosity were lower with preacidification. The reduction in initial unmelted cheese hardness and initial apparent viscosity in the pH 5.8 citric treatments represents an improvement in the quality of low fat Mozzarella cheese that allows the cheese to have better pizza bake characteristics with shorter time of refrigerated storage.

(Key words: low fat, Mozzarella cheese, preacidification, functional and chemical properties)

Abbreviation key: AV = apparent viscosity, CA:PROT = calcium as a percentage of protein, TPA = texture profile analysis.

INTRODUCTION

The overall functionality of low fat and fat-free Mozzarella cheese is not equivalent to Mozzarella with higher fat content. Generally, the cheese is too hard before and after baking. Some quality attributes of fat-free, low, and reduced fat Mozzarella cheese have been improved (i.e., melting and browning characteristics during baking on a pizza; Rudan and Barbano, 1998). Other researchers (Fife et al., 1996; Merrill et al., 1994) have used milk preacidification in the manufacture of low and reduced fat Mozzarella cheese. In these studies, modifications in manufacturing procedures included one level of milk preacidification (pH 6.0) with lactic acid. An improvement in the functionality (melting and stretching characteristics) of reduced fat Mozzarella cheese was observed with this manufacturing procedure (Merrill et al., 1994). The functionality of low fat Mozzarella produced by preacidification to pH 6.0 with lactic acid remains inferior (poor melting characteristics) compared with low-moisture, part-skim Mozzarella cheese (Fife et al., 1996). However, no cheese calcium data were presented in these studies, and the effect of different acid types and the level of preacidification were not determined. Furthermore, the improvement in cheese functionality of reduced fat Mozzarella was attributed to higher cheese moisture, not calcium reduction (Merrill et al., 1994). Keller et al. (1974) determined that the type of acid used to manufacture direct acid low-moisture, part-skim Mozzarella has an effect on cheese calcium content and rheological characteristics. Their research indicates that the type of acid used for preacidification is important and may influence the effect of preacidification on low fat Mozzarella cheese functionality.

Rudan et al. (1999) found that the calcium content of Mozzarella cheese increased progressively as fat content was reduced, with low fat containing 50% more calcium per gram than low-moisture, part-skim Mozzarella. Calcium plays an important role in micelle structure as well as cheese texture by crosslinking protein (Lucey et al., 1993; Solorza and Bell, 1995; Yun et al., 1995b). Reductions in calcium might reduce the hard-
ness of the cheese and improve the functional properties of low fat Mozzarella cheese. The objective of this study was to determine whether various levels of calcium reduction, caused by milk preacidification with different acids, would influence the manufacturing characteristics, chemical properties, and unmelted and melted functional properties of low fat Mozzarella cheese.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

Four treatments were employed: control (no preacidification), pH 6.0 acetic (preacidification to pH 6.0 with acetic acid), pH 5.8 acetic (preacidification to pH 5.8 with acetic acid), and pH 5.8 citric (preacidification to pH 5.8 with citric acid). Cheese manufacture was repeated on four different days using a 4 × 4 randomized-complete block design. The data for proximate analyses were analyzed using two-way ANOVA with treatment and day of manufacture analyzed as class variables. Changes in chemical composition and functionality parameters during refrigerated storage were analyzed using a split plot design with treatment (preacidification level) as the whole plot factor. For the whole plot factor, treatment was analyzed as a class variable and the day of cheese manufacture was blocked. For the subplot factor, age, and age × age were analyzed as quantitative variables. Additionally, at each time period, chemical and functionality data were analyzed using two-way ANOVA with treatment and day of manufacture analyzed as class variables. The level of significance was P < 0.05 throughout the paper. The PROC GLM procedure of SAS was used for all data analysis (SAS, 1990).

Cheese Manufacture

The cheese was manufactured from 0.5% fat milk (230 kg/vat) using a no-brine, stirred curd Mozzarella manufacturing procedure (Barbano et al., 1994). Prior to manufacture acetic or citric acid was added to the preacidified treatments (milk temperature of 4°C) to adjust the milk pH to the appropriate level. The details of milk standardization, preacidification, and cheese making were described previously (Metzger et al., 2000). Direct-to-vat Thermococcus C120 (Streptococcus sp. thermophilus) and Thermodor R160 (Lactobacillus delbrueckii ssp. bulgaricus) starter cultures (Rhones-Poulenc, Madison, WI) were added in a 1:1 ratio. Each starter culture was added at a rate of 0.40 ml/kg of milk and a 60-min ripening time was used for all treatments. The salting pH in the preacidified treatments was 5.35, while the control salting pH was 5.5. Based on preliminary work, it was established that milk preacidification decreased the rate of acid production by starter and caused an increase in make time relative to a non-preacidified control. Thus, to achieve the same curd pH (i.e., 5.3) at the beginning of stretching for all treatments, it was necessary to add salt to the control at pH 5.5. A target stretching temperature of 65°C for the temperature of the cheese exiting the mixer was used for all treatments.

A 30-min period for rennet coagulation was used for the control treatment, and 15 min was used for all preacidified treatments. The shorter coagulation time was used (i.e., time from rennet addition to cut) because of the rapid firming of the coagulum in the preacidified treatments. The whey was drained for the control at pH 6.2, while the preacidified treatments were stirred for 30 min after cutting and before whey drainage. Cheese-making characteristics including the time from rennet addition to salting and from the end of whey removal to salting, pH at the beginning of whey removal and salting, and the time from beginning to end of stretching and cheese temperature during stretching (temperature of the cheese exiting the stretcher at the midpoint of stretching) were determined. After manufacture, the cheese was cooled in an ice bath and stored at 4°C, as previously described (Metzger et al., 2000).

Cheese Analysis

Chemical properties. The fat, protein, salt, moisture, and calcium contents of the cheese were determined as described previously (Metzger et al., 2000). Cheese pH (Xerolyt electrode, model HA405, Ingold electrode, Willmington, MA and Accumet pH meter, model 915, Fisher Scientific, Springfield, NJ) and pH 4.6 acetate buffer and 12% TCA soluble N (Bynum and Barbano, 1985) were determined at 2, 15, 30, 60, and 90 d of refrigerated storage. Soluble nitrogen was expressed as a percentage of the total nitrogen content of the cheese. The amount (g/100 g of cheese) of expressible serum was determined at 2, 4, 6, 8, and 10 d of refrigerated storage. The cheese serum was removed by centrifugation at 12,500 × g for 75 min at 25°C (Guo and Kindstedt, 1995).

Functional properties. The color of the cheese was determined with a Macbeth Color-Eye Spectrophotometer (model 220; Kollmorgen Instrument Corp., Newburgh, NY). The L, a, and b values of the cheese, which correspond to whiteness, red-green, and blue-yellow were measured in triplicate at 4°C using illuminate A (incandescent lamp) and were calculated from the diffuse reflectance data in the range of 360 to 740 nm. Immediately before analysis the cheese was cut (5 mm thick × 3.6 cm diameter discs) with a wire and placed in the spectrophotometer viewing port to determine whiteness. The texture profile analysis (TPA) hard-
ness, as described by Bourne (1978), was measured by determining the force necessary to compress a cylinder of unmelted cheese (diameter 2 cm, height 2 cm) to 75% of its original height with an Instron Universal Testing Machine (model TM; Instron Corp., Canton, MA) at 10°C using a cross head speed of 12.7 cm/min. Each cheese core was tempered to 10°C in a refrigerated water bath for 30 min, removed from the bath, placed on the Instron at ambient temperature, and compressed immediately. The compression took about 20 s.

The apparent viscosity (AV) of the melted cheese was determined by helical viscometry (Kindstedt and Kiley, 1992; Kindstedt et al., 1989) and cheese meltability was determined by a modified Schreiber test (Yun et al., 1993). Apparent viscosity was determined at 6, 15, 30, 60, and 90 d of refrigerated storage. Color, TPA hardness, and meltability were determined at 2, 15, 30, 60, and 90 d of refrigerated storage. Shred melting and browning during baking were evaluated using a pizza bake test with and without a hydrophobic surface coating (Rudan and Barbano, 1998). The pizza bake test was performed at 30, 60, and 90 d of refrigerated storage.

**RESULTS AND DISCUSSION**

**Cheese Manufacture**

Table 1 shows selected cheese manufacturing characteristics including: cheese manufacturing time from rennet addition to beginning of salting and end of whey removal to the beginning of salting, pH at the beginning of whey removal and at the beginning of salting, and total time of stretching and cheese temperature during stretching. Mozzarella cheese making depends on a symbiotic relationship between the thermophilic starter cultures (Radke-Mitchell and Sandine, 1984; Sanders, 1991). Initially during cheese making, acid production is due largely to *Streptococcus thermophilus*, whereas *Lactobacillus delbrueckii* ssp. *bulgaricus* becomes dominant toward the end of manufacture (Radke-Mitchell and Sandine, 1984). Preacidification with acetic or citric acid caused the cultures to produce acid more slowly than the control, as seen from the longer total cheese-making time required to reach pH 5.3. The lower pH in the preacidified treatments when the starter was added may have affected the symbiotic relationship between the thermophilic starter culture strains (Yun et al., 1995a) and resulted in the slower acid production and longer cheese-making time (Table 1) from whey draining to salting. The pH at the beginning of whey removal was significantly lower ($P < 0.05$) in the preacidified treatments than the control, as we intended (Table 1).

The operational conditions (i.e., curd feed rate, mixer temperature, screw speed, etc.) were the same for all treatments. However, the preacidified treatments had a lower ($P < 0.05$) cheese temperature (Table 1) during stretching than the control. The stretching time was not affected ($P > 0.05$) by preacidification. Although the target salting pH was lower in the preacidified treatments than for the control, the pH during stretching was the same (i.e., pH 5.3) for all treatments. Calcium reduction in the preacidified treatments affect the melt and flow properties of cheese (i.e., the cheese was visibly softer), which could influence heat transfer during stretching and result in the observed differences in cheese temperature during stretching. The lower

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>pH 6.0 acetic</th>
<th>pH 5.8 acetic</th>
<th>pH 5.8 citric</th>
<th>SEM</th>
<th>F-test</th>
<th>LSD 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total time², min</td>
<td>99.0b</td>
<td>123.5a</td>
<td>124.3a</td>
<td>117.0a</td>
<td>3.08</td>
<td>&lt;0.01</td>
<td>9.85</td>
</tr>
<tr>
<td>Whey draining to salt³, min</td>
<td>37.25b</td>
<td>74.3a</td>
<td>75.5a</td>
<td>68.3a</td>
<td>3.16</td>
<td>&lt;0.01</td>
<td>10.10</td>
</tr>
<tr>
<td>Whey draining pH⁴</td>
<td>6.22a</td>
<td>5.90b</td>
<td>5.72c</td>
<td>5.71c</td>
<td>0.01</td>
<td>&lt;0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Salting pH⁵</td>
<td>5.48b</td>
<td>5.36b</td>
<td>5.36b</td>
<td>5.33b</td>
<td>0.01</td>
<td>&lt;0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Stretching time⁶, min</td>
<td>11.3</td>
<td>13.5</td>
<td>12.8</td>
<td>9.3</td>
<td>1.16</td>
<td>0.24</td>
<td>NS ²</td>
</tr>
<tr>
<td>Cheese temp⁸, °C</td>
<td>62.3a</td>
<td>59.8b</td>
<td>60.1b</td>
<td>59.0b</td>
<td>0.59</td>
<td>0.03</td>
<td>1.89</td>
</tr>
</tbody>
</table>

²Means within same row not sharing common superscripts are different ($P < 0.05$).
³Least significant difference ($P = 0.05$).
⁴Total cheese-making time from rennet addition to the beginning of stretching at pH 5.3.
⁵Cheese-making time from end of whey removal to beginning of salting.
⁶Whey pH at beginning of whey removal.
⁷Cheese pH at beginning of salting.
⁸Total stretching time from the beginning to end of stretching.
²NS = Not significant.
CHEESE COMPOSITION AND MOZZARELLA FUNCTIONALITY

Preacidification and Mozzarella Functionality

Cheese temperature during stretching in the preacidified treatments may affect changes in chemical and functional properties of the cheese during refrigerated storage (Kindstedt et al. 1995).

Chemical Properties

Cheese composition. The compositions of the cheeses are shown in Table 2. The calcium content and the calcium as a percentage of protein (Ca:protein) decreased \((P < 0.05)\) with all levels of preacidification. Preacidification to pH 5.8 with acetic acid decreased \((P < 0.05)\) calcium and Ca:protein more than preacidification to pH 6.0 with acetic acid. Preacidification with citric acid to pH 5.8 caused a larger decrease \((P < 0.05)\) in calcium and Ca:protein than preacidification to pH 5.8 with acetic acid, even though the whey pH at draining (Table 1) was not different. The observed differences in calcium and Ca:protein between citric and acetic acid may be a result of differences in the binding affinity of calcium for acetic and citric acid (Inczeáz, 1976). Differences in calcium content as a result of acid type have also been reported in directly acidified low-moisture, part-skim Mozzarella (Keller et al., 1974). The largest level of calcium reduction in the pH 5.8 citric treatment still had calcium content within the nutritional label tolerances for full fat Mozzarella cheese (Posati, et al. 1976). There were small but significant \((P < 0.05)\) differences in fat, protein, and fat on a dry basis (Table 2). However, moisture, salt, moisture to protein ratio, moisture in nonfat substance and salt to moisture ratio were not affected \((P > 0.05)\).

Table 2. Mean \((N = 4)\) initial composition of Mozzarella cheeses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>pH 6.0 acetic</th>
<th>pH 5.8 acetic</th>
<th>pH 5.8 citric</th>
<th>SEM</th>
<th>F-test</th>
<th>LSD1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture, %</td>
<td>55.10</td>
<td>53.58</td>
<td>54.34</td>
<td>55.30</td>
<td>0.384</td>
<td>0.09</td>
<td>NS2</td>
</tr>
<tr>
<td>Fat, %</td>
<td>6.03c</td>
<td>6.51a</td>
<td>6.55a</td>
<td>6.19b</td>
<td>0.035</td>
<td>&lt;0.01</td>
<td>0.11</td>
</tr>
<tr>
<td>Protein, %</td>
<td>32.32b</td>
<td>33.80a</td>
<td>33.52a</td>
<td>33.03ab</td>
<td>0.349</td>
<td>0.04</td>
<td>1.12</td>
</tr>
<tr>
<td>Salt, %</td>
<td>1.31</td>
<td>1.29</td>
<td>1.23</td>
<td>1.48</td>
<td>0.072</td>
<td>0.39</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>0.976a</td>
<td>0.872b</td>
<td>0.754a</td>
<td>0.578d</td>
<td>0.013</td>
<td>&lt;0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>FDB3, %</td>
<td>13.43c</td>
<td>14.02ab</td>
<td>14.34c</td>
<td>13.84b</td>
<td>0.103</td>
<td>&lt;0.01</td>
<td>0.33</td>
</tr>
<tr>
<td>M:F4</td>
<td>1.71</td>
<td>1.59</td>
<td>1.62</td>
<td>1.68</td>
<td>0.029</td>
<td>0.07</td>
<td>NS</td>
</tr>
<tr>
<td>MNFS5, %</td>
<td>58.64</td>
<td>57.31</td>
<td>58.14</td>
<td>58.95</td>
<td>0.399</td>
<td>0.09</td>
<td>NS</td>
</tr>
<tr>
<td>SM6, %</td>
<td>2.37</td>
<td>2.42</td>
<td>2.26</td>
<td>2.67</td>
<td>0.141</td>
<td>0.55</td>
<td>NS</td>
</tr>
<tr>
<td>Ca:protein7, %</td>
<td>3.02a</td>
<td>2.58b</td>
<td>2.25c</td>
<td>1.75d</td>
<td>0.020</td>
<td>&lt;0.01</td>
<td>0.06</td>
</tr>
</tbody>
</table>

\(a,b,c,d\)Means within same row not sharing common superscripts are different \((P < 0.05)\).

1Least significant difference \((P = 0.05)\).

2NS = Not significant.

3Fat content on a dry weight basis.

4Ratio of moisture to protein.

5Moisture in the nonfat substance of the cheese.

6Percentage of salt in moisture in the cheese.

7Calcium as a percentage of protein.

Cheese pH. Cheese pH was affected \((P < 0.05)\) by treatment and the interaction of treatment \(\times\) age (Table 3). The pH of the cheese for all the treatments at 2 d of refrigerated storage were similar (Figure 1), as intended. However, during storage, the cheese pH for the preacidified treatments remained stable or decreased, while the control increased (Figure 1). The observed differences in cheese pH between the control and preacidified treatments may be related to a difference in cheese buffering capacities as a result of different calcium levels. In addition, the higher cheese temperature during stretching in the control (Table 1) may also have contributed to the higher cheese pH during refrigerated storage in the control due to more thermal inactivation of culture organisms (Kindstedt et al., 1995).

Proteolysis. Treatment had an effect \((P < 0.05)\) on both pH 4.6 and 12% TCA soluble N (Table 3). Age (i.e., storage time) had a large impact on both pH 4.6 and 12% TCA soluble N (Figures 2 and 3). In addition, the interaction of treatment \(\times\) age was also significant (Table 3) for both pH 4.6 and 12% TCA soluble N. The preacidified treatments had higher pH 4.6 and 12% TCA soluble N than the control. As was the case with cheese pH, the lower soluble N in the control may have been caused by the higher cheese stretching temperature (Table 1) in the control (Kindstedt et al., 1995). The higher stretching temperature in the control would inactivate more chymosin (Kindstedt et al., 1995) and reduce the pH 4.6 soluble N. The lower pH 4.6 soluble N would provide less medium molecular weight peptides for starter culture enzymes; therefore, the 12%
Table 3. Mean squares and probabilities (in parentheses) of chemical, unmelted, and melted functional properties for low fat Mozzarella cheese during storage at 4°C.

<table>
<thead>
<tr>
<th>Factors</th>
<th>df</th>
<th>Expressible serum</th>
<th>pH</th>
<th>pH 4.6 soluble N</th>
<th>12% TCA soluble N</th>
<th>L-value</th>
<th>TPA (^1) hardness</th>
<th>Apparent viscosity (x 10(^6))</th>
<th>Meltability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-plot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (T)</td>
<td>3</td>
<td>412.75*</td>
<td>0.066*</td>
<td>16.10*</td>
<td>3.69*</td>
<td>68.69*</td>
<td>208.02</td>
<td>5.489</td>
<td>14.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.001)</td>
<td>(0.002)</td>
<td>(0.001)</td>
<td>(0.01)</td>
<td>(0.47)</td>
<td>(0.18)</td>
<td>(0.19)</td>
<td></td>
</tr>
<tr>
<td>Day of cheese</td>
<td>3</td>
<td>1.61</td>
<td>0.152*</td>
<td>37.82*</td>
<td>3.40*</td>
<td>12.29*</td>
<td>265.15</td>
<td>0.882</td>
<td>13.16</td>
</tr>
<tr>
<td>Manufacture</td>
<td></td>
<td>(0.72)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.02)</td>
<td>(0.03)</td>
<td>(0.37)</td>
<td>(0.81)</td>
<td>(0.22)</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>3.49</td>
<td>0.011</td>
<td>1.73</td>
<td>0.56</td>
<td>2.56</td>
<td>225.38</td>
<td>2.703</td>
<td>7.35</td>
</tr>
<tr>
<td>Subplot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (A)</td>
<td>1</td>
<td>2.83*</td>
<td>0.003</td>
<td>1125.60*</td>
<td>380.38*</td>
<td>330.53*</td>
<td>602.49*</td>
<td>16.801*</td>
<td>193.37*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.03)</td>
<td>(0.39)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
</tr>
<tr>
<td>A × A</td>
<td>1</td>
<td>2.55*</td>
<td>0.002</td>
<td>31.84*</td>
<td>5.87*</td>
<td>81.95*</td>
<td>386.18*</td>
<td>25.995*</td>
<td>1.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.04)</td>
<td>(0.47)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.52)</td>
</tr>
<tr>
<td>T × A</td>
<td>3</td>
<td>0.381</td>
<td>0.058*</td>
<td>9.36*</td>
<td>2.96*</td>
<td>41.88*</td>
<td>187.00*</td>
<td>11.440*</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.55)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.85)</td>
</tr>
<tr>
<td>T × (A × A)</td>
<td>3</td>
<td>0.383</td>
<td>0.005</td>
<td>0.17</td>
<td>0.03</td>
<td>6.11</td>
<td>9.24</td>
<td>2.744*</td>
<td>3.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.58)</td>
<td>(0.24)</td>
<td>(0.90)</td>
<td>(0.93)</td>
<td>(0.10)</td>
<td>(0.80)</td>
<td>(0.01)</td>
<td>(0.52)</td>
</tr>
<tr>
<td>Error</td>
<td>56</td>
<td>0.574</td>
<td>0.004</td>
<td>0.86</td>
<td>0.18</td>
<td>2.83</td>
<td>27.2</td>
<td>0.552</td>
<td>4.17</td>
</tr>
</tbody>
</table>

\(^1\)Texture profile analysis.

\(^*\)Statistically significant (\(P < 0.05\)).

TCA soluble N for the control would be expected to be lower (Barbano et al., 1993).

**Expressible serum.** The method to remove a portion of the water phase of Mozzarella cheese uses centrifugation at 12,500 \(×\) g for 75 min at 25°C (Guo and Kindstedt, 1995). After centrifugation, an insoluble pellet, an aqueous fraction, and a layer of oil are obtained. The aqueous fraction is referred to as the expressible cheese serum. Using this method, the amount and composition of expressible serum in Mozzarella cheese changes during refrigerated storage (Guo and Kindstedt, 1995, 1997; Guo et al., 1998). Two days after manufacture, a large amount of expressible serum is obtained (up to 25 g of serum per 100 g of Mozzarella cheese), and this expressible serum contains approximately 3 to 5% protein (Guo and Kindstedt, 1995). During the first 2 to 14 d of refrigerated storage, the amount of expressible serum in a

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**Figure 1.** Effect of milk preacidification (control: ■, pH 6.0 acetic □, pH 5.8 acetic; ●, pH 3.8 citric; ○) on cheese pH (SEM = 0.008) during storage at 4°C (LSD = 0.08, 0.08, 0.08, 0.11, and 0.13, at d 2, 15, 30, 60, and 90, respectively).

**Figure 2.** Effect of milk preacidification (control: ■, pH 6.0 acetic □, pH 5.8 acetic; ●, pH 3.8 citric; ○) on pH 4.6 soluble N content of cheese (SEM = 0.125%) during storage at 4°C (LSD = 0.38, 0.77, 1.04, 0.94, and 2.65, at d 2, 15, 30, 60, and 90, respectively).
PREACIDIFICATION AND MOZZARELLA FUNCTIONALITY

Figure 3. Effect of milk preacidification (control: ◆, pH 6.0 acetic □, pH 5.8 acetic ■, pH 3.8 citric ●) on 12% TCA soluble N (SEM = 0.057%) during storage at 4°C (LSD = 0.23, 0.23, 0.71, 0.91, and 1.19, at d 2, 15, 30, 60, and 90, respectively).

Figure 4. Effect of milk preacidification (control: ◆, pH 6.0 acetic □, pH 5.8 acetic ■, pH 3.8 citric ●) on expressible serum of cheese (SEM = 0.102 g/100 g) during storage at 4°C (LSD = 1.92, 1.69, 1.30, 1.53, and 1.74 at d 2, 4, 6, 8, and 10, respectively).

typical Mozzarella cheese decreases, and the amount of protein in the expressible serum increases (Guo and Kindstedt, 1995; Guo et al., 1998). The inability of the cheese to express serum after extended storage indicates that the water phase contains enough protein and other constituents to prevent its release from the cheese under the conditions of the test.

During refrigerated storage, the insoluble casein matrix of Mozzarella cheese appears to start to solubilize and interact with the water phase of the cheese (Guo and Kindstedt, 1995, 1997). The authors hypothesize that the interaction of solubilized casein with the water phase of Mozzarella cheese results in the observed decrease in the amount of expressible serum and the increase in the concentration of protein in the expressible serum (Guo and Kindstedt, 1995). Casein proteolysis products and intact caseins increase in concentration in the water phase with time of storage, while the concentration of whey proteins does not (Guo and Kindstedt, 1995). The constituents of the water phase appear to progressively reach concentrations that allow them to form a gel that resists removal of water (i.e., expressible serum) under the conditions of the test (i.e., 25°C and 12,500 × g).

The expressible serum (g/100 g of cheese) was affected (P < 0.05) by treatment and age (Table 3). In addition, there was an interaction (P < 0.05) of age × treatment (Table 3). The amount of expressible serum was lower in all the preacidified treatments compared with the control (Figure 4). Preacidification to pH 5.8 with citric acid decreased the amount of expressible serum more than preacidification to either pH 6.0 or 5.8 with acetic acid. In fact, no expressible serum could be removed from the pH 5.8 citric treatments at any time at 12,500 × g at 25°C.

The addition of acid to milk has an effect on micelle structure (Dalgleish and Law, 1988; Sing et al. 1996; Van Hooydonk et al. 1986). As the pH of milk is lowered, a substantial portion of the micellar casein is solubilized (Dalgleish and Law, 1988; Van Hooydonk et al., 1986). As a result, milk preacidification should increase the level of solubilized casein in the water phase of the cheese, and the lower the pH of preacidification, the higher the concentration of solubilized casein in the water phase. More casein was lost in the whey (Metzger et al., 2000) as preacidification increased, which is consistent with a higher protein concentration in the water phase of the cheese with increasing preacidification. Higher concentrations of solubilized casein have been attributed to a decrease in the amount of expressible serum (Guo and Kindstedt, 1995), and, as a result, milk preacidification may decrease the amount of expressible serum due to casein solubilization and formation of casein gel in the water phase of the cheese. This would explain why the amount of expressible serum decreased as the level of preacidification increased. The differences in amount of expressible serum between the pH 5.8 citric and pH 5.8 acetic treatments may be related to the differences in the binding affinity of calcium for the two acid types which may affect the casein matrix and the amount of casein soluble in the water phase of the cheese.

Additionally, a slower decline in the amount of expressible serum and a lower rate of increase in concentration of protein in the expressible serum of low-moisture, part-skim Mozzarella cheese during refrigerated...
storage was observed when the cheese temperature during stretching was increased from 62 to 66°C (Kindstedt et al. 1995). Therefore, it appears that any factor that increases the concentration of intact casein or casein proteolysis products in the water phase of Mozzarella cheese will decrease the quantity of expressible serum. We hypothesized that the intact caseins and casein proteolysis products interact with soluble calcium to form a calcium-induced gel that increases in strength as protein concentration in the water phase increases or as temperature decreases. Thus, time of refrigerated storage, lower cheese temperature (Guo and Kindstedt, 1995), and milk preacidification reduce the amount of expressible serum that can be removed from Mozzarella cheese by centrifugation.

**Functional Properties**

**Unmelted cheese.** The unmelted cheese whiteness (L-value) was affected \( (P < 0.05) \) by treatment, age, and age \( \times \) age (Table 3). No effect of treatment on \( a \) or \( b \) values was detected (data not reported). In addition, the unmelted cheese whiteness was affected \( (P < 0.05) \) by the interaction of treatment \( \times \) age (Table 3). The L-value of the control cheese was higher than for the preacidified cheeses at all times and the initial (i.e., d 2) L-value decreased as preacidification level increased (Figure 5). The general trend was for the L-values to decrease during refrigerated storage. However, the reduction in L-value was larger for the control than the other treatments. A decrease in L-value during storage has also been observed in reduced fat Mozzarella cheese, and this reduction is thought to be caused by time-dependent changes in the serum phase of the cheese (Rudan et al., 1998). Because differences in the amount of expressible serum as a result of preacidification were observed in this study (Figure 4), it is not surprising that the L-value of the unmelted cheese was also affected by preacidification. In general, when there is less expressible serum at 12,500 \( \times g \) at 25°C and a higher concentration of soluble caseins in the serum phase, the L-value of the unmelted cheese is lower. The lower unmelted cheese whiteness in the preacidified treatments is not a desirable characteristic of low fat Mozzarella cheese. However, modifications in fat particle size distribution by using homogenization of cream before cheese making (Rudan et al., 1998) could be used to counter the observed reduction in unmelted cheese whiteness caused by milk preacidification and maintain a higher unmelted cheese L-value throughout refrigerated storage. The net effect of these two treatments (i.e., preacidification and homogenization) needs further investigation.

TPA hardness was affected \( (P < 0.05) \) by age and age \( \times \) age and the interaction of treatment \( \times \) age (Table 3). The TPA hardness of the pH 5.8 citric treatment was initially lower (2 d) than the other treatments (Figure 6). The pH 5.8 citric treatments had the lowest calcium content and ca:protein (Table 1); this is consistent with their significantly lower TPA hardness at 2 d of storage (Figure 6). The much lower initial TPA hardness in the pH 5.8 citric treatment versus the other treatments may be a result of modifications in protein-protein interactions due to calcium reduction. Calcium plays a critical role in cheese texture by crosslinking protein and...
a reduction in cheese calcium decreases crosslinking among protein fibers, which causes the cheese to become softer (Geurts et al. 1972; Sing et al., 1996; Van Hooydonk et al. 1986). The relatively small reduction in calcium in the pH 6.0 acetic and pH 5.8 acetic treatments had a minimal effect on TPA hardness. The effect of calcium reduction on d 2 TPA hardness is not linear (Figure 6), and a critical amount of calcium needs to be removed before initial TPA hardness decreases.

**Melted cheese.** The AV was affected ($P < 0.05$) by age and age $\times$ age and the interaction treatment $\times$ age and treatment $\times$ (age $\times$ age) (Table 3). The results for AV were similar to TPA hardness in that the AV for the pH 5.8 citric treatment was initially lower than the other treatments, but during refrigerated storage the AV of all treatments converged (Figure 7). The lower initial AV in the pH 5.8 citric treatment may be caused by decreased crosslinking among casein fibers as a result of calcium reduction. Additionally, the effect of calcium reduction on the initial AV does not appear to be linear, and the relatively small reduction in calcium in the pH 6.0 acetic treatments (Table 1) had a minimal effect on the initial AV. Other research also found that preacidification to pH 6.0 with acetic acid had little effect on the AV of low fat Mozzarella cheese, although cheese calcium content was not reported (Fife et al., 1996). However, even though large differences existed in initial AV, there was little difference among treatments after 60 to 90 d of refrigerated storage. The reduction in AV during refrigerated storage is a result of proteolysis (Kindstedt and Kiley, 1992) and may also be influenced by changing protein-water interactions that cause the amount of expressible serum to decrease.

Meltability (as measured with the modified Schreiber melt test) was not affected ($P > 0.05$) by preacidification treatment but was affected ($P < 0.05$) by age (Table 3). The meltability increased during refrigerated storage in all treatments (Figure 8).

**Baking characteristics.** Use of a hydrophobic surface coating has been shown to improve the melting and browning characteristics of low fat and fat-free Mozzarella cheese (Rudan and Barbano, 1998). After 60 d of refrigerated storage, the results of a pizza bake test without and with a hydrophobic surface coating indicated (based on visual observation) that all treatments had poor shred melt and excessive burning without a hydrophobic surface coating, and excellent melt and normal browning with the hydrophobic surface coating. Preacidification to reduce the calcium content of the cheese had little influence on the melting and browning characteristics of low fat Mozzarella during pizza baking. The use of the hydrophobic surface coating during pizza baking demonstrated that the inherent meltability of the cheeses at 50 to 60 d was excellent and similar because all four treatments demonstrated complete melting and shred fusion.

**CONCLUSIONS**

Milk preacidification reduced the total calcium content of low fat Mozzarella cheese and given equal milk pH at rennet addition and equal pH at whey draining, citric acid caused larger reductions in cheese calcium content than acetic acid. The chemical and functional properties during refrigerated storage of low fat Mozzarella cheese were affected by milk preacidification. The

**Figure 7.** Effect of milk preacidification (control: ◆, pH 6.0 acetic □, pH 5.8 acetic: ■, pH 3.8 citric: ○) on apparent viscosity of melted cheese (SEM = 100 Pa.s) during storage at 4°C (LSD = 941, 1834, 1974, 1298, and 1259, at d 6, 15, 30, 60, and 90, respectively).

**Figure 8.** Effect of milk preacidification (control: ◆, pH 6.0 acetic □, pH 5.8 acetic: ■, pH 3.8 citric: ○) on modified Schreiber test meltability (SEM = 0.275 mm) during storage at 4°C (LSD = 1.46, 1.54, 2.27, 2.47, and 5.60, at d 2, 15, 30, 60, and 90, respectively).
largest level of calcium reduction and modification of chemical and functional properties occurred in the pH 5.8 citric treatment. Preacidification to pH 5.8 with citric acid reduced the amount of expressible serum, unmelted cheese whiteness, initial TPA hardness, and initial AV compared with control and other preacidified treatments. The reduction in the initial TPA hardness and AV of low fat Mozzarella cheese are beneficial and represent an improvement in low fat Mozzarella cheese quality. The d 2 TPA hardness and initial AV in the pH 5.8 citric treatment represent values typical of low-moisture, part-skim Mozzarella after several weeks of refrigerated storage. This is significant and may allow for the manufacture of the low fat Mozzarella cheese, which could perform satisfactorily on a pizza within a few days of manufacture. Preacidification reduced the unmelted cheese whiteness, which is undesirable. However, if preacidification is combined with a reduction in the fat globule size (Rudan et al., 1998), the reduction in unmelted cheese whiteness caused by preacidification may be reduced. Further work is needed on the influence of the combination of preacidification and homogenization of cream on low fat Mozzarella cheese functionality.

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