DAIRY FOODS

Functionality of Low Fat Mozzarella Cheese

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ABSTRACT

Low fat Mozzarella cheese was made from milks containing casein to fat ratios of 3.0, 5.0, 7.0, and 8.0. Prior to addition of rennet, milk was pasteurized at 79°C for 28 s and then acidified to pH 6.0 with lactic acid. Three replicates of each cheese were made in 7-L vats and stored at 4°C. Functional properties as pizza cheese were evaluated. Cheese moisture and fat contents were evaluated at 1 d. Apparent viscosity and extent of flow of melted cheese, cook color, and proteolysis were evaluated at 1, 7, 14, and 28 d. Moisture content measured by a rapid microwave oven method underestimated the moisture content of low fat cheeses; probable moisture was calculated by component analysis. The part-skim Mozzarella control with 19% fat had a moisture content of 51%; the moisture contents of the low fat cheeses containing 2 to 5% fat were 63%. Low fat cheeses did not melt as well as did the part-skim Mozzarella cheese, although the differences between the cheeses with 2 and 5% fat were insignificant. Storage for 28 d only marginally increased the meltability of low fat cheese. Lower fat content also increased cook color. The amount of intact ε-CN decreased by at least 48% in all cheeses as a result of proteolysis during 28 d of storage.

(Key words: Mozzarella cheese, moisture, low fat, functionality)

Abbreviation key: AV = apparent viscosity, C:F = casein to fat ratio, MFFC = moisture in fat-free cheese.

INTRODUCTION

Dietary awareness of consumers and their desire to follow nutritional guidelines (16) by reducing total fat intake have prompted the cheese industry to investigate fat reduction in Mozzarella cheese (13). Reduced-fat, low fat, and nonfat cheese variations are now being developed as potential alternatives to part-skim Mozzarella cheese.

The pizza restaurant industry uses cheese with fat content typical of, or higher than, low moisture, part-skim Mozzarella and represents the largest category of Mozzarella users in the US (1, 26). In a recent survey, over 50% of pizza restaurants reported occasional to frequent problems in quality, including melting (67%), poor shredding (55%), and blistering or browning (50%)(26).

Two important characteristics of Mozzarella cheese used as pizza topping are its ability to melt and stretch (22). As fat or moisture is removed, functional defects appear. Masi and Addeo (12) found that increases in the fat and moisture contents of Mozzarella cheese are accompanied by a decrease in the modulus of elasticity (an indication of rigidity), resulting in softening of the cheese body and difficulty in shredding. Konstance and Holsinger (9) found that reducing moisture or fat results in increased hardness, elevated springiness values, and decreased meltability. Defects associated with low fat Mozzarella cheese include a rubbery, tough texture, lack of flavor, paleness or green tint, inability to melt, and poor stretchability (15, 29).

Merrill et al. (14) described a method for manufacturing reduced-fat Mozzarella cheese containing 50% less fat than part-skim Mozzarella. A higher pasteurization temperature, milk preacidification, larger curd size, reduced cook temperature, minimal stirring during cooking, and less frequent turning during cheddaring were used to retain more moisture in the curd. No significant differences occurred between the two types of cheese in moisture, apparent viscosity, melting, or browning over 28 d of refrigerated storage.

The objective of this study was to determine the effect of lowering fat content of part-skim mozzarella cheese by 75 to 90% on cheese functionality over 28 d of refrigerated storage. A melt test was used to measure the extent of cheese flow when cheese was melted, and an apparent viscosity (AV) test was used to
provide information on stretchability. Browning of the cheese when heated was used to evaluate cook color, and degree of proteolysis during storage was also measured.

MATERIALS AND METHODS

Milk, Cultures, and Rennet

Pasteurized (79°C for 29 s) skim milk and 2% milk were obtained from the Gary H. Richardson Dairy Products Laboratory at Utah State University. Milk was cooled to 4°C; the protein and fat levels were determined and then were standardized to a casein to fat ratio (C:F) of 1.2, 3.0, 5.0, 7.0, or 8.0. Lyophilized direct-set cultures of Lactobacillus helveticus LH 100 and Streptococcus thermophilus TA 061 and single-strength calf rennet extract were obtained from Rhône-Poulenc Marschall Products (Madison, WI).

Manufacturing Procedure

Low fat cheeses were made from milk with C:F of 3.0, 5.0, 7.0, and 8.0 using the method of Merrill et al. (14) and compared with a control cheese made from milk having C:F of 1.2. Five stainless steel vats (22 x 22 x 22 cm), each containing 7 L of milk, were used to manufacture cheese. The milks with C:F 3.0, 5.0, 7.0, and 8.0 were adjusted to pH 6.0 (at <10°C) using 85% lactic acid (EM Industries, Inc., Cherry Hill, NJ) that had been diluted 1:2 (vol/vol) with distilled water. The pH of the control milk was not adjusted. The 7-L vats were warmed simultaneously in a water bath to 34°C, inoculated with 0.75 g of each culture, and allowed to ripen for 45 min. After ripening, 3 ml of rennet, diluted in 30 ml of distilled water, were added. The curd was cut using 1.9-cm knives at 10 min (for milk with C:F 1.2 because it was not pre-acidified) after rennet addition, heated for 15 min, and then gently agitated for 30 s. The temperature of all vats (both control and low fat) was raised to 38°C over 10 min with periodic gentle agitation. The whey was drained at pH 6.0 (whey pH), and the curds were hand-cheddared (turning every 20 min) at 38°C until the curd pH reached 5.2. Curds were cut and stretched in hot water (83°C) until they were elastic and smooth. The stretched cheese (approximately 700 g from each vat) was placed into stainless steel molds (9 x 9 x 9 cm) and immersed in ice water for 30 min to cool. The six resulting cheese loaves from each vat were removed from the molds, immersed in individual refrigerated (4°C) brines (saturated NaCl, pH 5.0) for 4 h, and then individually vacuum-packed. Cheeses were stored at 4°C.

Chemical and Physical Analysis of Cheese

Moisture content was measured by microwave oven (model AVC 80; CEM Corp., Matthews, NC), using 50% power for 5 min, and fat content was determined using a modified Babcock method (27). The extent of melting was measured by the tube test of Olson and Price (21), modified by increasing the oven temperature to 150°C. Cook color was measured by reflectance colorimetry (Minolta Chroma Meter CR-100; Minolta Corp., Ramsey, NJ) (19). Total protein (N x 6.25) was measured by the semi-micro-Kjeldahl method [AOAC method 920.123; (21)] and ash using a dry ash method (AOAC method 935.42; (21)]. Melt characteristics and AV of melted cheese, cook color, and proteolysis were measured at 1, 7, 14, and 28 d. Curd pH, moisture, total protein, and fat were measured at 1 d.

Apparent viscosity. A Brookfield DV II+ helical viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA) fitted with a T-bar spindle (T-F with a 9.0 mm crossbar) was used to measure AV of melted cheese using the method of Kindstedt et al. (8) with modifications. A 25-cm section of rod was added to the helipath rod so that the total distance of travel was 25 cm (total helipath rod height, 50 cm). A jacketed test tube holder was attached to the rod to allow use of a remote water bath. Hot water (80°C) was circulated through the jacket during evaluations to maintain a uniform sample temperature. Fifteen grams of shredded cheese were tamped into a 25-mm x 150-mm test tube, tempered for 10 min at 80°C, and then placed in the water-jacketed test tube holder. The T-bar was lowered until it reached a position just above the bottom of the test tube and the viscometer was activated (1.5 rpm). An IBM-compatible computer, equipped with Brookfield DV Gather+ 1.0 software, was used to record AV every 5 s for 10 min. The mean of the readings on the AV profile curve from 0.5 to 1.5 min were reported as AV.

Gel electrophoresis. One-dimensional SDS-PAGE was carried out on a PhastSystem™ using PhastGel™ homogeneous 12.5 gels (Pharmacia LKB Biotechnology, Piscataway, NJ). Cheese samples (25 mg) were solubilized by adding a mixture of 1 ml of Tris (10 mM)-EDTA (1 mM) buffer, pH 8.0, 350 µl of SDS (10%), and 50 µl of β-mercaptoethanol; samples were then placed in boiling water for 5 min, mixed by vortexing for 5 s, and boiled an additional 5 min (24, 25). Bromophenol blue (3 µl of 4.5%, wt/vol, solution)
TABLE 1. Mean (±SEM) percentages of fat, protein, ash, moisture contents estimated by a microwave oven method, probable moisture, and moisture in the fat-free cheese (MFFC) in Mozzarella cheeses made from milk of various casein to fat ratios (C:F).

<table>
<thead>
<tr>
<th>C:F</th>
<th>Fat X SEM</th>
<th>Protein X SEM</th>
<th>Ash X SEM</th>
<th>Moisture microwave oven1</th>
<th>Probable moisture2</th>
<th>MFFC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>19.3 0.4</td>
<td>24.6 0.3</td>
<td>3.0 0.03</td>
<td>51.2 2.1</td>
<td>52.1</td>
<td>64.5</td>
</tr>
<tr>
<td>3.0</td>
<td>5.0 0.6</td>
<td>28.2 0.1</td>
<td>2.9 0.01</td>
<td>52.5 1.0</td>
<td>62.9</td>
<td>66.2</td>
</tr>
<tr>
<td>5.0</td>
<td>3.1 0.5</td>
<td>30.4 0.4</td>
<td>3.0 0.01</td>
<td>51.7 0.8</td>
<td>62.5</td>
<td>64.5</td>
</tr>
<tr>
<td>7.0</td>
<td>2.5 0.4</td>
<td>30.4 0.6</td>
<td>3.0 0.02</td>
<td>54.6 1.1</td>
<td>63.1</td>
<td>64.7</td>
</tr>
<tr>
<td>8.0</td>
<td>2.2 0.5</td>
<td>30.1 0.3</td>
<td>3.1 0.01</td>
<td>55.3 0.7</td>
<td>63.6</td>
<td>65.0</td>
</tr>
</tbody>
</table>

1Microwave oven method for part-skim and reduced fat Mozzarella cheese.
2Calculated as 100% - (percentage of fat + percentage of protein + percentage of ash + 1.0%). The 1.0% accounts for lactic acid in the cheese and other solids lost during ashing.
3Calculated as the percentage of probable moisture/(100% - percentage of fat).

was added as a tracking dye, and 0.5 µl of sample was loaded on the gel (4). Skim milk, αs-CN, β-CN, κ-CN, and whole casein served as controls. Gels were stained with Coomassie blue-R (23). Relative peak areas were determined using PhastImage™ software (Pharmacia LKB Biotechnology).

Statistical Analysis

Analysis of variance was run for the variables: melt, AV, and cook color. Three independent replicates for each C:F were run using a split plot design; C:F was the whole-plot effect, and storage time was the split-plot effect. Statistical analysis was done using Minitab 7.2 (Minitab Inc., State College, PA).

RESULTS

Composition

Low fat cheeses were made from milks with C:F of 3.0, 5.0, 7.0, and 8.0 that had fat contents between 2.2 and 5.0% (Table 1). The low fat cheeses contained from 4 to 6% more protein than the control, but ash contents were similar. The microwave oven method [which had been successfully used in previous studies for part-skim Mozzarella cheese (17, 18, 19)] estimated the moisture contents of all cheeses to be between 51.2 to 54.6%. However, when a mass balance was used to validate the moisture measurements, it was observed that the microwave oven method had underestimated the moisture contents of the low fat cheeses. Probable moisture content (5) was calculated based on measurements of protein, fat, and ash and allowing 1% for lactic acid and other solids in the cheese that were volatilized during ashing (Table 1). On this basis, the moisture contents of the low fat cheeses ranged from 62.5 to 63.6%. Moisture contents were similar even though fat contents ranged from 2 to 5%. When moisture in the fat-free cheese (MFFC) was calculated based on probable moisture content, the MFFC of the low fat cheeses were comparable with the MFFC of the control cheese (all between 64.5 and 66.2%).

Melt

When an overall analysis of variance was conducted for the 28-d storage period, melting was not statistically different (P > 0.05) among the cheeses with different fat contents (Table 2). Some differences in the melt were observed at individual storage times (Figure 1). At d 1, the cheeses melted to the same extent (i.e., P > 0.05). After 7 d of storage, the melting improved, but the low fat cheeses melted less well (P < 0.05) than the control. No further increases in melting were observed. At d 14, the melt of the control cheese was still higher (P < 0.05) than that of the low fat cheeses, although no difference (P > 0.05) was observed in the melt among the cheeses after 28 d, perhaps because of the large variance in melting of the control cheese. Variation in melting was less for the low fat cheeses. For all cheeses, melting was significantly affected (P < 0.05) by storage time.

AV

The AV remained relatively constant (Figure 2) as the T-bar raised through the melted cheese (area 1) and, in some instances, increased slightly as the T-bar approached and exited the cheese column surface (area 2). Mean AV decreased sharply as the T-bar was raised above the cheese surface (area 3), draw-
Characteristics differed, AV profiles of the low fat cheeses and part-skim control were similar.

**Cook Color**

Cook color was affected ($P < 0.05$) by fat content of the cheese (Table 2). All low fat cheeses had lower $b^*$ values (a greenish tint) than the part-skim control cheese. The overall effect of storage time on cook color was not significant ($P > 0.05$), although all of the cheeses had lower $b^*$ values after 28 d of refrigerated storage (Figure 4).

**Proteolysis**

Peak densitometry indicated that approximately 50% of the $\alpha_s$-CN in all samples was hydrolyzed after 28 d of storage (Figure 5). The part-skim Mozzarella cheese (which contained 19.3% fat) had a 52% loss of $\alpha_s$-CN. The low fat Mozzarella cheeses (containing 5.0, 3.1, 2.5, and 2.2% fat) lost 47, 55, 77, and 61% of the $\alpha_s$-CN, respectively, in agreement with the observations of Kieley et al. (6). Hydrolysis of $\alpha_s$-CN in all cheeses, regardless of fat level, was greatest between d 1 and d 14; little change occurred after d 14.

**DISCUSSION**

When a microwave oven is used to dry cheese, the amount of microwave energy absorbed per unit of weight is dependent on the fat, salt, and water contents of the cheese. Low fat cheeses, with less fat and lower salt concentration in the water phase, absorb less energy than higher fat cheeses containing less moisture. Thus, low fat cheeses would reach a lower maximal temperature than the higher fat cheeses, given the same microwave power settings, and possibly contribute to underestimating the moisture con-
tent. Increasing the power setting, however, does not correct this underestimation. (In a follow-up study, the microwave oven method was modified by increasing power setting from 50% to 100%. The moisture content values increased by 1.5% but still left unaccounted for 10% of the proximate analysis.) The inability of the microwave oven method we used to volatilize all of the water in the low fat cheeses indicates that some of the water remained trapped within the protein matrix. Assuming that the difference between the measured moisture content and the probable moisture (based on protein, fat, and ash content) represents water that is trapped in the protein matrix (as vicinal, multilayer, and entrapped water), then the portion of such water in low fat cheese is much greater than that in part-skim Mozzarella cheese.

This result demonstrates the importance of measuring other components in cheese (protein, fat, minerals, and carbohydrates) as well as moisture. Emmons (5) showed that cheese moisture content may also be underestimated even when using a forced-draft oven method. Whatever method is used, procedural changes may be necessary when cheeses of vastly different compositions are being compared, particularly when low fat cheeses are being compared with full fat cheeses by a microwave oven method.

The melt test measures the ability of cheese particles to flow past one another when heated. Fat or unbound water acts as a lubricant and increases the ability of cheese particles to flow (28, 29). Assuming that a relationship exists between how tightly water is bound and how well the cheese melts, the moisture content of low fat cheese becomes a predominant issue in developing low fat cheeses that melt well. Furthermore, even though moisture content in the low fat cheeses (63 to 64%) was higher than in the part-skim cheese (52%), when considered on a fat-free basis, the moisture contents were all similar (65 to 66%). Thus, the protein to moisture ratio appears to remain constant even after fat content is reduced from 19 to 2%, reflecting the water-holding capacity of the proteins in cheese. Therefore, the state of the water in the cheese, as well as water on a fat-free basis, needs to be considered when functional properties of low fat Mozzarella cheese are investigated.

The trend of large increases in melting during the first 7 d of refrigerated storage of part-skim Moz-
zarella cheese, followed by a little increase from d 7 to 28 (Figure 1), is consistent with prior observations (17, 18, 19). There is, however, a difference in the rate of aging of low fat (2 to 5% fat) versus part-skim (19% fat) cheeses (14, 28). Because the rates of hydrolysis of $\alpha_s$-CN were similar (Figure 5) and because the fat content did not affect initial melting (Figure 1), the explanation for this difference in aging probably lies in some fundamental differences in how the protein and fat are structurally arranged in the cheese. A better understanding of cheese structure, especially on the molecular level, would help elucidate the parameters that control cheese melting.

The AV profiles that are derived from helical viscometry (Figure 2) are the combined result of cheese viscosity, the ability of milk proteins to form strands around the T-bar, and the ability of those strands to deform when stretched (7, 8). For both the control and low fat cheeses, the AV was constant while the T-bar remained in the melted cheese. An increase in AV as the T-bar exited the cheese could result from partial drying of the cheese surface. Then, as the cheese strand attached to the T-bar is stretched and reduced in thickness, the AV rapidly decreases. Differences in this “tail” section (area 3 of the AV profile) were considerable. The thickness of the strand and the time it remained attached to the T-bar were recorded as differences in height and duration of the AV profile. Cheese that was capable of forming strong, yet elastic strands frequently remained attached to the T-bar for the duration of the test (10 min) with fibers extending up to 25 cm. No correlation was apparent between the amount of cheese pulled up by the T-bar and the extent to which it remained attached to the T-bar. Only the portion of the AV profiles from 0.5 to 1.5 min were thus used to calculate mean AV values. This procedure eliminated variation from differences in surface hardening and tail sections. However, further study of cheese strand characteristics after the T-bar leaves the melted cheese column may better describe the term “stretch” as it is used by the pizza industry and may help to elucidate rheological characteristics of the cheese.

The primary structural protein in Cheddar cheese is $\alpha_s$-CN, which undergoes hydrolysis during refrigerated storage and is associated with textural changes during aging (10, 21). $\alpha_s$-Casein probably performs a similar structural role in Mozzarella cheese. Residual milk-clotting enzymes, endogenous milk protease (plasmin), and proteolytic activity of starter cultures have been shown (6, 10) to improve meltability through proteolytic degradation of the milk protein matrix. Hydrolysis of $\beta$-CN by chymosin proceeds more slowly. Basch et al. (3) calculated half-lives for the loss of $\alpha_s$-CN and $\beta$-CN as 2 and 37 wk. Kiely et al. (6) also found that, after 29 d, 50% of the $\alpha_s$-CN in low moisture, part-skim (20% fat) Mozzarella cheese was hydrolyzed, but $\beta$-CN remained virtually unchanged.

Interestingly, all five cheeses melted the same on d 1, which suggests that fat content is not the prime factor controlling cheese meltability. The part-skim Mozzarella cheeses had 5 to 10 times more fat, but protein to moisture ratios were the same. The marked increase in melt and the decrease in AV of the part-

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**Figure 4.** Mean (±SEM) cook color measurements of Mozzarella cheese (n = 3) made from milk with a casein to fat ratio of 1.2 (solid bar), 3.0 (open bar), 5.0 (diagonally striped bar), 7.0 (horizontally striped bar), and 8.0 (gray bar) during 28 d of storage.

**Figure 5.** Amount of intact $\alpha_s$-CN, expressed as a percentage of d 1, during 28 d of storage at 4°C of Mozzarella cheeses (n = 1) made from milk with a casein to fat ratio of 1.2 (solid bar), 3.0 (open bar), 5.0 (diagonally striped bar), 7.0 (horizontally striped bar), and 8.0 (gray bar).
skim Mozzarella cheese during the 1st wk of storage had been assumed to be a function of proteolysis. However, the cases in the low fat cheese underwent the same degree of proteolysis (or more), yet melting was not improved. Therefore, hydrolysis of αs-CN, although probably contributing to improved melting, was not the prime factor in the increase in melting that occurred during storage of Mozzarella cheese.

After 7 d, the control cheese showed no increase in melting, but, between d 7 and d 14, the disappearance of αs-CN was greatest.

Cook color is influenced both by proteolytic activity (producing amino acids and small peptides) and carbohydrate utilization by the starter cultures used for Mozzarella cheese production. Galactose-fermenting strains of *S. thermophilus* and *L. helveticus* reduce the amount of browning during cook compared with nongalactose-fermenting strains (20). Cook color decreases with time when *L. delbrueckii* ssp. *bulgaricus* is replaced by *L. helveticus* in Mozzarella cheese starters (19). The slightly higher b* value for the low fat cheeses, although not statistically significant, may have been due to the higher moisture content of the low fat cheeses, which resulted in slightly more retention of lactose in the cheese. Cook color would also be expected to decrease during storage as residual sugars in the cheese are utilized by bacteria.

When cool, the low fat cheeses had a slightly greenish tint that became less noticeable upon heating and returned when cooled. This greenish tint is associated with fewer light-scattering centers (less fat globules) and is a common defect of low fat cheeses. Some manufacturers overcome this by adding a whitening agent, such as titanium dioxide, and others have looked to microparticulated fat mimetics as a potential way to impart more opaqueness to low fat and nonfat cheeses.

**CONCLUSIONS**

Low fat Mozzarella cheeses containing between 2 and 5% fat were produced. Their moisture contents were estimated, based on component analysis, to be 61 to 63%. An underestimation of moisture content in the low fat cheeses occurred when a rapid microwave oven test method was used to determine moisture content. This result reinforces the need to confirm moisture measurements by component analysis and mass balance, especially when low fat cheeses are being analyzed. Melting characteristics of the low fat Mozzarella were affected by the reduction in fat. By increasing water retention in low fat Mozzarella cheese, some improvement can be made in melting characteristics. However, further work is needed to produce low fat Mozzarella cheese that meets the FDA requirement of less than 6% fat and has acceptable functionality during cooking. These low fat cheeses may require the addition of fat mimetics that entrap water and also provide lubricant properties typically provided by fat globules.

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