Chemical, Physical, and Sensory Characteristics of Mozzarella Cheese Fortified Using Protein-Chelated Iron or Ferric Chloride

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ABSTRACT

Mozzarella cheese containing 25 and 50 mg of iron/kg of cheese was manufactured from milk that had been fortified with casein-chelated iron, whey protein-chelated iron, or FeCl₃. Chemical, physical, and sensory characteristics were compared with those of a control cheese. Physical properties were assessed by testing melting, apparent viscosity, and browning of heated cheese. Cheeses were evaluated by trained panelists for the presence of metallic flavors, oxidized flavors, and other undesirable flavors.

Addition of 25 mg iron/kg of cheese had no effects on the physical properties of Mozzarella cheese. Apparent viscosity of cheese fortified with 50 mg of iron/kg of cheese tended to be slightly higher than the control cheese, although this difference was not statistically significant at all storage times. Cook color was not affected by iron fortification. No increase in chemical oxidation (measured using thiobarbituric acid assay) was observed between the control and iron-fortified cheeses. Slight but statistically significant increases in metallic flavors, oxidized flavors, and off-flavors in the iron-fortified cheese were observed by the trained sensory panel, but the flavor defects were of very low intensity. For metallic flavors, oxidized flavors, and off-flavors, the control cheese scored 1.5, 1.5, and 1.3, respectively; the iron-fortified cheese scored 2.1, 2.0, and 1.6 based on a nine-point scale (where 1 = not perceptible to 3 = slightly perceptible). Sensory scores for iron-fortified cheese made using casein-chelated iron or whey protein-chelated iron was not significantly different from those of cheese made using ferric chloride. When used on pizza, consumer panels rated the iron-fortified cheeses as comparable with the control cheese.

Key words: Mozzarella cheese, iron, fortified, pizza

Abbreviation key: Fe₅CN = casein-chelated iron, FeWP = whey protein-chelated iron, TBA = thiobarbituric acid.

INTRODUCTION

Calcium and iron are two nutrients that are essential for growth. Both are also needed in higher amounts by women, especially because of increased requirements during pregnancy and because of their higher risk for osteoporosis and iron-deficiency anemia (4, 5). Dairy products contribute many nutrients to the diet (calcium, riboflavin, protein, magnesium, and vitamins A, B₆, and B₁₂) but provide very little iron (0.6 mg of iron/kg of milk) (6). Fortification of dairy products with iron has thus been frequently considered as a potential means for preventing iron deficiency (12, 19, 25). MacPhail and Bothwell (15) recommended that, before such a decision is made the need for supplemental iron in a well-defined population should be established, an appropriate and acceptable food vehicle fortified with a stable iron source should be used, and the iron source should have an established bioavailability.

These criteria were used by Zhang and Mahoney (25, 26, 27) in their design of a fortification scheme involving Cheddar cheese. All three criteria would also be satisfied if Mozzarella cheese were used as the food vehicle for iron fortification. Iron deficiency anemia is the most prevalent nutritional deficiency in the world and has been recognized as endemic in women of childbearing years, in adolescents, and in children (10). Consumption of Mozzarella cheese has increased annually for the last 10 yr, reflecting increased consumer demands for pizza (18), and the National Health and Nutrition Examination Survey data indicate that dairy products are well accepted by populations at risk for iron-deficiency anemia (8).
Finally, studies of rats (25) and humans (A. W. Mahoney, 1992, unpublished data) have shown that the iron in iron-fortified cheese is readily bioavailable. There have been a variety of cheeses that have been iron fortified. Sadler et al. (23) fortified cottage cheese with 60 mg of iron/kg of cheese and observed no detectable off-flavors after 2 mo of storage. Jackson and Lee (11, 12) fortified Havarti cheese using FeCl₃ at 140 mg/kg and observed better organoleptic quality when the iron was microencapsulated. Zhang and Mahoney (26) produced iron-fortified Cheddar cheese but did not observe any differences in fat oxidation when compared with that of unfortified cheese. The bioavailability of iron-fortified Cheddar cheese was subsequently studied at Utah State University (A. W. Mahoney, 1992, unpublished data) in which a group of menstruating women was fed test meals of ⁵⁹Fe-labeled cheese. About 6 to 8% of the iron was absorbed, which is similar to the degree of iron bioavailability of other iron-fortified nonheme foods.

Loss of iron in the whey during the manufacture of iron-fortified cheese is another factor of importance. Jackson and Lee (11, 12) observed that less iron was retained in Havarti cheese curd when it was microencapsulated than when free FeCl₃ was used (70% vs. 90% retention). The objective of this study was to make Mozzarella cheese fortified with iron at 25 and 50 mg/kg by adding FeCl₃ or protein-chelated iron to milk. Two iron-protein complexes were prepared with iron chelated to either whey proteins (FeWP) or casein (FeCN). Cheese quality was then determined by chemical, physical, and sensory characteristics and compared with a control cheese that had not been fortified.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

Ferric chloride and protein-chelated iron (FeCN and FeWP) were used to produce Mozzarella cheese that had been fortified with 25 and 50 mg of iron/kg of cheese. No iron was added to the control cheeses. Cheese was made from 6 kg of milk (in triplicate) to determine the retention of iron in the cheeses, and the amount of each iron source that was needed to reach target concentration of iron in cheese of 25 and 50 mg/kg, to test for chemical oxidation during storage, and to conduct sensory analysis by a trained panel. Each trial consisted of a control cheese and iron-fortified cheeses representing each iron source at both levels of fortification. Cheeses were then made from 68 kg of milk and tested by untrained taste panelists for physical and organoleptic properties when used on pizza. These trials were repeated each week (using an iron source at 25 and 50 mg/kg and a control per week) for a total of 6 wk, until two replications of cheese using each iron source were manufactured.

Cheese composition and iron analysis were determined on d 0. Chemical oxidation, cheese flavor, melt, and apparent viscosity were tested on d 0, 7, 14, 21, and 28. Browning was measured on d 0, 14, and 28. Cheese from d 15 (replicate 2) was cooked on pizza and was tested by consumer sensory panels. A split-plot design was used to determine the statistical significance of mean comparisons between dependent variables for all tests and to compare control cheese against iron-fortified cheeses using Minitab 7.2 (Minitab Inc., State College, PA). Statistical significance was declared at P ≤ 0.05.

Materials

Ferric chloride (FeCl₃·6H₂O) was obtained from Sigma Chemical Company (St. Louis, MO); FeCN and FeWP were prepared according to the method described by Zhang and Mahoney (26). Prior to the addition of FeCN and FeWP to milk, the iron sources were assayed in triplicate for iron content; FeWP contained 135 mg of iron/g with an iron recovery rate of 88%, and the FeCN contained 60.4 mg of iron/g with an iron recovery rate of 51%.

Raw whole and skim milks were obtained from the Gary H. Richardson Dairy Products Laboratory (Utah State University, Logan), pasteurized at 63°C for 30 min, mixed to produce a standardized milk with casein to fat ratio of 1.2, and cooled to 34°C. Single-strength calf rennet and lyophilized cultures of Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus were obtained from Rhône-Poulenc Inc. (Madison, WI). All chemicals were reagent grade, and deionized water was used in the chemical analysis.

Manufacture of Mozzarella Cheese

Small vats. Six kilograms of milk were poured into each of seven stainless steel vats (21 × 21 × 21 cm) that were maintained at 34°C in a water bath; 0.75 g of each culture was added to each vat, and the milk was allowed to ripen for 1.5 h. Iron sources (FeCl₃, FeCN, or FeWP) were stirred into individual vats at levels to yield 25 and 50 mg of iron/kg of cheese; no iron was added to the control vat. The location of the vats in the water bath was randomized...
at each trial. Each vat was stirred vigorously for 5
min with plastic ladles to disperse the iron sources.

Five milliliters of rennet (diluted 1:40 with cold
potable water) were added, and the milk was agi-
tated for 5 min and allowed to set. Approximately 30
min after rennet addition, the curd was cut with
1.9-cm wire knives and then allowed to heel in the
whey for 30 min with periodic gentle agitation to
prevent curd matting. The curd was then heated from
34 to 40.5°C over 30 min. The whey was drained, and
the curd was matted and turned every 20 min until
the titratable acidity increased to 0.60% (pH 5.2 to
5.3). The curd was milled and stretched in 82°C
water for 5 min until a uniform and elastic cheese
consistency was achieved. The cheeses were placed in
stainless steel molds (9 × 9 × 9 cm), cooled in an ice
bath to ambient temperature, removed from the
molds, and then brined (saturated NaCl at 4°C) for 4
h.

After brining, the surface of each cheese was dried
with paper towels, and the block was cut into eight
equal pieces. One piece was used for the initial analy-
sis (d 0), and the seven remaining pieces were
vacuum-packaged into individual packages (semi-
permeable packaging film; Boise Cascade Company,
Burley, ID). Four samples were used for weekly thio-
barbituric acid (TBA) analysis, one for proximate
analysis, and one for ferrozine analysis; an extra
package was stored for any follow-up analysis. A
separate trial was conducted to make cheese for or-
ganoleptic testing by trained judges.

**Large vats.** Sixty-eight kilograms of milk were
used to make cheese following the procedure just
described except that milled curd was dry-salted, dis-
pensed into an Alfa-Laval Cooker Stretcher (Tetra-
Pak, Greenwood, IN), and stretched using 82°C
water. Samples of cheese from the middle portion of
curd from each vat were placed in molds and im-
mersed in iced water. When the cheese blocks had
cooled to about 34°C, they were removed from the
molds and brined (25% NaCl, 4°C) for 4 h.

**Chemical Analysis**

Moisture was determined using a microwave oven
(model AVC 80; CEM Corp., Matthews, NC) (2). Fat
was analyzed by the Babcock method (2). Protein
was analyzed by the Kjeldahl method (2). Iron con-
tent was determined by the ferrozine method (7). Fat
oxidation was estimated using the TBA assay (3).

**Physical Analysis**

Apparent viscosity was measured as described by
Merrill et al. (16) except that cheese was heated to
80°C. Fifteen grams of shredded cheese were weighed
into 25-mm × 150-mm glass test tubes. The samples
were tapped down and melted for 10 min in an 80°C
water bath. The spindle of a heliopath viscometer
(model DV-II; Brookfield Engineering Lab, Inc.,
Stoughton, MA) was inserted in the cheese, and ap-
parent viscosity was recorded every 5 s for 10 min as
the spindle moved vertically up the heliopath.

Melt was determined using a modified tube test
(18, 20, 21). Cheese was shredded and put into
30-mm × 250-mm glass tubes that were plugged with
number 7 rubber stoppers. The cheeses were taped
down in the glass tube to 4 cm from the end of the
rubber stopper. The opposite end of the tubes were
capped with number 7 stoppers that had holes in the
center to allow air to escape. The tubes were set
horizontally on a stainless steel tray and held at 4°C
for 30 min. They were then transferred to a forced-air
oven at 110°C and held for 60 min. The tubes were
cooled to ambient temperature (ca. 25°C), and melt
was measured as the distance (in centimeters) of
cheese flow.

Cook color of cheese after heating for 40 min at
95°C was determined as extent of browning (b*)
using a chromameter (model CR-221B; Minolta Cor-
poration, Ramsey, NJ) as described by Oberg et al.
(18).

**Sensory Analysis**

**Trained sensory panels.** Panelists were recruited
from the faculty and graduate students in the Depart-
ment of Nutrition and Food Sciences at Utah State
University. Panelists were trained to detect metallic
and oxidized flavors using cheese that had been forti-
fied with high concentrations of iron (40 and 80 mg/
kg) as FeSO₄, which is known to cause oxidative
flavors in foods (24). Panelists who were unable to
detect oxidized and metallic flavors were eliminated
from the panel. Only the scores of the 10 judges (6
men and 4 women) who attended every taste panel
were used for statistical analysis.

Cheeses were cut into slices (approximately 2.5 ×
2.5 cm) at 1 h prior to serving and placed in plastic
cups (Solo Cup Co., Urbana, IL); cheeses were ran-
domized to avoid positional bias. Duplicate trays of
samples (6 iron-fortified cheeses and a control
cheese) were presented at 10-min intervals to the
panel. The intensity of metallic flavors, oxidized
flavors, and off-flavors were rated using a nine-point
scale (9 = extremely strong to 1 = not perceptible).

**Consumer sensory panels.** Three consumer
panels (23 ± 8 panelists) were recruited to judge the
flavors of cheese cooked on pizza at 260°C for 5 min in
TABLE 1. Mean concentration of iron, protein, moisture, and fat of iron-fortified Mozzarella cheese made using 6 kg of milk per vat and three different iron sources and control cheese made without fortification.

<table>
<thead>
<tr>
<th>Iron source</th>
<th>Target Iron (mg/kg)</th>
<th>Target Protein (%)</th>
<th>Target Moisture (%)</th>
<th>Target Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.5 1.6</td>
<td>25.1 2.8</td>
<td>47.2 0.9</td>
<td>19.3 2.4</td>
</tr>
<tr>
<td>FeCl$_3$</td>
<td>25 27.9 1.2</td>
<td>23.9 0.8</td>
<td>47.5 1.1</td>
<td>18.7 1.9</td>
</tr>
<tr>
<td>FeCl$_3$</td>
<td>50 50.8 4.0</td>
<td>23.3 1.2</td>
<td>45.4 1.4</td>
<td>19.3 1.8</td>
</tr>
<tr>
<td>FeCN</td>
<td>25 26.9 1.0</td>
<td>21.8 0.5</td>
<td>47.4 1.6</td>
<td>18.7 1.3</td>
</tr>
<tr>
<td>FeCN</td>
<td>50 50.7 1.9</td>
<td>22.9 0.6</td>
<td>47.3 1.7</td>
<td>16.0 1.2</td>
</tr>
<tr>
<td>FeWP</td>
<td>25 20.1 0.7</td>
<td>22.9 0.8</td>
<td>47.3 1.2</td>
<td>17.3 1.3</td>
</tr>
<tr>
<td>FeWP</td>
<td>50 50.4 3.2</td>
<td>25.2 0.9</td>
<td>47.6 0.8</td>
<td>16.0 0.0</td>
</tr>
</tbody>
</table>

$^1$Fe$_{CN}$ = Casein-chelated iron; Fe$_{WP}$ = whey protein-chelated iron.

RESULTS

Cheese Composition

Composition of cheese is shown in Tables 1 and 2. The amount of FeCl$_3$, Fe$_{CN}$, or Fe$_{WP}$ required for addition to milk in the large (68-kg) vats, so as to obtain cheese that contained 25 or 50 mg of iron/kg of cheese, was determined by calculation of iron retention obtained in trials using the small (6-kg) vats. Most cheeses made in both the large (68-kg) and small (6-kg) vats fitted the criteria for low moisture, part-skim Mozzarella cheese. There were some variations in moisture, protein, and fat content although the only observed trends were for the cheeses fortified with Fe$_{CN}$ or Fe$_{WP}$ to have lower fat contents and the cheese fortified with FeCl$_3$ to 50 mg of iron/kg of cheese to have the lowest moisture content. Cheese made in the larger vats had lower moisture and fat contents probably because of increased mechanical action imparted to the cheese curd during mechanized cooking and stretching. Mean iron retention in the cheese made from milk fortified using FeCl$_3$, Fe$_{CN}$ or Fe$_{WP}$ was 91, 66, and 61%, respectively. The control cheese contained 7.5 mg of iron/kg of cheese, and fortified cheeses were close to their targets of 25 and 50 mg of iron/kg of cheese.

TABLE 2. Mean concentration of iron, protein, moisture, and fat of iron-fortified Mozzarella cheese made using 68 kg of milk per vat and three different iron sources and control cheese made without fortification.

<table>
<thead>
<tr>
<th>Iron source</th>
<th>Iron fortification (mg/kg)</th>
<th>Protein (%)</th>
<th>Moisture (%)</th>
<th>Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeCl$_3$</td>
<td>02</td>
<td>21.9 1.8</td>
<td>44.3 1.6</td>
<td>18.5 0.5</td>
</tr>
<tr>
<td>FeCl$_3$</td>
<td>25</td>
<td>23.0 1.3</td>
<td>46.3 3.7</td>
<td>20.0 2.0</td>
</tr>
<tr>
<td>FeCl$_3$</td>
<td>50</td>
<td>24.6 1.0</td>
<td>43.5 0.4</td>
<td>19.0 1.0</td>
</tr>
<tr>
<td>Fe$_{CN}$</td>
<td>0</td>
<td>25.3 2.1</td>
<td>46.8 0.8</td>
<td>18.0 2.0</td>
</tr>
<tr>
<td>Fe$_{CN}$</td>
<td>25</td>
<td>21.6 0.6</td>
<td>44.7 0.3</td>
<td>15.0 1.0</td>
</tr>
<tr>
<td>Fe$_{CN}$</td>
<td>50</td>
<td>20.1 2.1</td>
<td>44.7 0.3</td>
<td>14.0 0.1</td>
</tr>
<tr>
<td>Fe$_{WP}$</td>
<td>0</td>
<td>23.8 2.6</td>
<td>47.4 1.4</td>
<td>17.0 1.0</td>
</tr>
<tr>
<td>Fe$_{WP}$</td>
<td>25</td>
<td>24.0 3.0</td>
<td>44.7 0.5</td>
<td>14.5 1.5</td>
</tr>
<tr>
<td>Fe$_{WP}$</td>
<td>50</td>
<td>23.0 2.6</td>
<td>46.6 1.5</td>
<td>16.0 0.1</td>
</tr>
</tbody>
</table>

$^1$Fe$_{CN}$ = Casein-chelated iron; Fe$_{WP}$ = whey protein-chelated iron.

$^2$Control.
Chemical Oxidation

No significant differences were observed in fat oxidation (as measured by TBA assay) based on iron source or level (Table 3). The only factor that was significant was storage time. All cheeses (fortified and unfortified) showed a slight increase in TBA value during 28 d of refrigerated storage (Table 4).

Physical Properties

The physical properties of the iron-fortified cheeses followed the same trends that had been observed previously for Mozzarella cheese (16, 17, 18). As the cheese aged, it generally had lower apparent viscosity and higher melt values (Figures 1, 2, and 3). Apparent viscosity decreased about 50% during the first 14 d of storage and decreased only slightly during further storage to 28 d. Melt increased proportionally during each 7-d period. Fortification of cheese at 25 mg of iron/kg of cheese did not affect either melt or apparent viscosity. There was a tendency for cheese fortified to 50 mg of iron/kg of cheese using FeCl₃ or FeCN to have slightly higher apparent viscosity and lower melt. Significant differences in apparent viscosity were observed for cheese made using FeCl₃ at d 0, 7, 20, and 28 (Figure 1A) and for cheese made using FeCN at d 0 and 14 (Figure 2A). There were no significant differences in apparent viscosity or melt for cheese made using FeWP.

There were no significant differences in browning or cook color between any of the cheeses. Cook color

significantly increased for all cheeses during storage as shown in Figure 4. The change in cook color was greatest between d 14 and 28.

Sensory Evaluation

Compared with the control Mozzarella cheese with no added iron, iron-fortified Mozzarella cheese was rated as having slightly more oxidized flavor, metallic flavor, and other off-flavors (Table 5), although intensity of these flavors in iron-fortified cheeses were only rated between slightly perceptible and not per-

![Figure 1. Apparent viscosity (A) and melt behavior (B) during 28 d of refrigerated storage of control (unfortified) Mozzarella cheese (◊) and Mozzarella cheese that had been fortified with FeCl₃ to attain 25 mg/kg (●) or 50 mg/kg (○). Error bars represent standard errors of the mean.]

### TABLE 3. The ANOVA for chemical oxidation measured by thiobarbituric acid assay of iron-fortified Mozzarella cheese made using three different iron sources (FeCl₃, casein-chelated iron, and whey protein-chelated iron) or made without iron fortification (control).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>0.133***</td>
</tr>
<tr>
<td>Treatment (T)</td>
<td>6</td>
<td>0.011NS</td>
</tr>
<tr>
<td>Control vs. fortified</td>
<td>1</td>
<td>0.025NS</td>
</tr>
<tr>
<td>Among fortified</td>
<td>5</td>
<td>0.008NS</td>
</tr>
<tr>
<td>Source (S)</td>
<td>2</td>
<td>0.010NS</td>
</tr>
<tr>
<td>Level (L)</td>
<td>1</td>
<td>0.002NS</td>
</tr>
<tr>
<td>S × L</td>
<td>2</td>
<td>0.009NS</td>
</tr>
<tr>
<td>Error a</td>
<td>12</td>
<td>0.009</td>
</tr>
<tr>
<td>Day (D)</td>
<td>4</td>
<td>0.069*</td>
</tr>
<tr>
<td>T × D</td>
<td>24</td>
<td>0.006NS</td>
</tr>
<tr>
<td>S × D</td>
<td>8</td>
<td>0.008NS</td>
</tr>
<tr>
<td>L × D</td>
<td>4</td>
<td>0.002NS</td>
</tr>
<tr>
<td>S × L × D</td>
<td>8</td>
<td>0.006NS</td>
</tr>
<tr>
<td>Error b</td>
<td>56</td>
<td>0.020</td>
</tr>
<tr>
<td>Total</td>
<td>104</td>
<td></td>
</tr>
</tbody>
</table>

*P ≤ 0.05.

**P ≤ 0.001.
ceptible. There were no significant flavor differences between cheeses fortified at 25 and 50 mg of iron/kg of cheese or between cheeses made using chelated or unchelated iron. Also, no significant changes in oxidized flavor during 28 d of storage were detected by the panels.

When the Mozzarella cheese was cooked on pizza, no significant differences in flavor between the control and iron-fortified cheeses were detected by the consumer panel. The mean scores for appearance, mouthfeel, flavor, and overall rating were 6.4 ± 1.7, 6.3 ± 1.7, 6.5 ± 1.7, and 6.5 ± 1.6, respectively (6 = like slightly to 7 = like moderately).

DISCUSSION

Of the three iron sources tested (FeCl$_3$, Fe$_{CN}$, and Fe$_{WP}$), only FeCl$_3$ did not require prior preparation of

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Figure 2. Apparent viscosity (A) and melt behavior (B) during 28 d of refrigerated storage of control (unfortified) Mozzarella cheese (◊) and Mozzarella cheese that had been fortified with casein-chelated iron to attain 25 mg/kg (○) or 50 mg/kg (□). Error bars represent standard errors of the mean.

Figure 3. Apparent viscosity (A) and melt behavior (B) during 28 d of refrigerated storage of control (unfortified) Mozzarella cheese (◊) and Mozzarella cheese that had been fortified with whey protein-chelated iron to attain 25 mg/kg (○) or 50 mg/kg (□). Error bars represent standard errors of the mean.
TABLE 4. Mean fat oxidation measured by thiobarbituric acid assay of iron-fortified cheese made using
three different iron sources or made without iron fortification.

<table>
<thead>
<tr>
<th>Iron source</th>
<th>Iron fortification</th>
<th>Absorbance at 535 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg/kg)</td>
<td>d 0</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0.104</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>25</td>
<td>0.112</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>50</td>
<td>0.101</td>
</tr>
<tr>
<td>FeCN</td>
<td>25</td>
<td>0.102</td>
</tr>
<tr>
<td>FeCN</td>
<td>50</td>
<td>0.097</td>
</tr>
<tr>
<td>FeWP</td>
<td>25</td>
<td>0.113</td>
</tr>
<tr>
<td>FeWP</td>
<td>50</td>
<td>0.166</td>
</tr>
</tbody>
</table>

₁FeCN = Casein-chelated iron; FeWP = whey protein-chelated iron.

Figure 4. Cook color (browning as measured by b* value) during 28 d of refrigerated storage of Mozzarella cheese that had been fortified with FeCl₃ to attain 0, 25, 50 mg of iron/kg of cheese.

an iron-protein complex. Chelation of the iron with protein prior to its addition to milk appeared to be unnecessary to minimize iron-induced oxidation of fat when making iron-fortified cheese. When FeCl₃ is added to milk, 85% is complexed to casein milk proteins (22) and, in particular, is bound to αs₁-CN, which has a higher iron-binding capacity than the other milk proteins. Thus, most of the iron added to milk as FeCl₃ binds strongly to the proteins, minimizing the amount of iron that exists in the ionic form (e.g., Fe²⁺). It has also been shown that the binding of iron to casein is independent of pH (9, 22) at the pH values encountered during cheese making.

Less iron was lost in the whey when FeCl₃ was used (9% for FeCl₃ vs. 39% for FeWP and 34% for FeCN). This higher retention of iron from FeCl₃ supports previous findings (9) that, when FeCl₃ is added to milk, the iron is predominantly bound to the casein micelles in preference to whey proteins. During the manufacture of Mozzarella cheese, the lower iron retention with FeWP and FeCN may have resulted from the lack of association of the iron-protein complexes with the casein micelles when the milk was coagulated by rennet; thus, more iron would be lost in the whey.

Strong binding of iron to milk proteins may also explain why the manufacture of iron-fortified cheese using FeCl₃ had no effect on the production of fat oxidative products, such as malondialdehyde, that are measured as part of the TBA assay. Binding of iron by the milk proteins would reduce the ability of iron to participate in iron-catalyzed hydroxyl radical formation and lipid peroxidation by restricting change in the oxidation state between Fe²⁺ and Fe³⁺. Judges who were trained to detect oxidized and metallic flavors were more sensitive than the TBA assay in detecting differences in oxidation flavors in cheese, suggesting that the TBA assay is not a sufficiently sensitive indicator of oxidation in a food such as cheese.

The higher oxidative and metallic flavors of the iron-fortified cheeses that were observed by the trained panelists were much less than expected, given the role of iron in promoting lipid peroxidation. Although the slightly increased oxidation that was observed in iron-fortified Mozzarella cheese was statistically significant, this increase may have little practical importance given the consumption patterns for Mozzarella cheese. Unlike Cheddar cheese, the primary use of Mozzarella cheese is for cooking rather than for use in sandwiches or for snacking. Mozzarella cheese is used primarily on pizza (1), which makes melting and functional performance more imp-

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portant than flavor. Typically, Mozzarella is stored for 7 to 14 d to allow development of desired melt properties before use. During this time, apparent viscosity of the cheese decreases greatly with corresponding increase in melting properties.

There are two possible explanations for the reduced melting properties observed for cheese that had been fortified using FeCl₃ to 50 mg of iron/kg of cheese. How well a cheese melts depends on the state of the proteins in the cheese, which is affected by pH, calcium content, and moisture content of the cheese (13). Mozzarella cheese is usually stretched at a target pH of 5.2 to 5.3 when enough calcium has been solubilized to convert the proteins in the cheese curd from a dicalcium paracaseinate to a monocalcium caseinate (14). If insufficient calcium is removed (such as when curd pH is too high), the curd will not melt and fuse when stretched in hot water. It has been observed that the addition of iron to milk retards demineralization of casein micelles as milk is acidified (9). When milk was acidified and then ultracentrifuged (52,000×g for 1 h at 20°C), more calcium (expressed as milligrams of Ca per gram of protein) was retained in the micelle pellet of iron-fortified milk at pH 6.2, 5.8, and 5.3 than in unfortified milk. There was no differences at pH 6.7, 4.5, or 4.0. Retention of phosphorus in the micelle pellet followed a similar trend. The same effects of iron fortification could have occurred in the present study resulting in slightly more calcium being retained in an iron-fortified cheese than in a corresponding unfortified cheese. Or differences in meltability may have been caused by slight variations in fat and moisture content of the cheeses. Of the cheeses that showed the most reduction in meltability, the cheese that had been fortified using FeCl₃ to 50 mg of iron/kg of cheese had the lowest moisture content, and the cheese that had been fortified using FeCN to 50 mg of iron/kg of cheese had the lowest fat content. However, there were similar differences in fat and moisture contents in the cheeses made using FeWP without differences occurring in meltability.

**TABLE 5. Mean intensities of metallic flavor, oxidized flavor, and off-flavors in Mozzarella cheese during 28 d of storage.**

| Flavor attribute | Iron-fortified cheese (n = 600) | Control cheese (n = 100) | P <  
|------------------|-------------------------------|-------------------------|--------
| Metallic         | 2.1                           | 1.5                     | 0.05   
| Oxidized         | 2.0                           | 1.5                     | 0.05   
| Off-flavor       | 1.6                           | 1.3                     | 0.05   

1Trained taste panelists used a nine-point scoring scale (1 = not perceptible and 3 = slightly perceptible).

**CONCLUSIONS**

Cheeses that are minimally aged, so that there is only a minor risk of off-flavors produced by oxidation, can be successfully fortified to contain 25 or 50 mg of iron/kg of cheese. Fortification of milk with FeCl₃ prior to renneting is simple, and use of a protein-chelated iron source did not provide any advantages with regard to fat oxidation that would justify the additional expense and time required for their preparation. Furthermore, more iron was retained in the cheese when FeCl₃ was used. During the manufacture of iron-fortified Mozzarella cheese, the procedure may need to be altered to achieve the fat, moisture, and calcium contents necessary for optimum cheese melting properties. The popularity of pizza and the lower fat content of Mozzarella cheese relative to Cheddar cheese make Mozzarella cheese an ideal cheese for iron fortification.

**ACKNOWLEDGMENTS**

The authors thank Don V. Sisson for help in statistical analysis, Richard K. Merrill for technical assistance on cheese making, and Sharareh Hekmat for technical assistance with iron fortification. The authors also acknowledge the work of the late Arthur W. Mahoney, who initiated work at Utah State University on iron-fortified cheese.

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