Distribution of Added Iron in Milk of Cows and Buffaloes and its Effect on Oxidized Flavor Development

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ABSTRACT

Association of added ferrous and ferric forms of iron in raw and pasteurized cow and buffalo milk is discussed in connection with its effect on oxidative deterioration of milk. Association of iron with sulphydryls is shown to accelerate oxidized flavor development. Heat treatment or a suitable reducing agent capable of rendering the metal to the reduced state appears to promote the catalytic activity of added iron in milk. Buffalo milk does not differ from cow milk in its resistance to iron-induced oxidative deterioration.

Recent reports on iron deficiency anemia (1, 12, 16, 17) have attracted the attention of nutritionists in finding effective means of enriching the human diet with sufficient iron. Since iron deficiency is highest in children, iron fortification of milk has attained special significance in recent years. Controversy has arisen over the effectiveness of different iron compounds that have been used for food fortification. The reduced forms of iron compounds such as ferrous sulfate are generally favored, presumably because of their greater solubility and probable availability. However, the dairy industry has accepted the idea of iron fortification with caution as to give a final concentration of 10 ppm when 1 ml was added to 100 ml milk.

To follow migration of iron between cream and skim milk, samples were centrifugally separated at room temperature (25 C) in an International Centrifuge, model SBV size 1., at 1500 x g. Centrifuged samples were cooled in an ice water bath and the hardened cream layer was punctured with a pointed glass rod and the skim milk drawn off. The percentage of fat in the cream was adjusted to 55% in each instance by adding the required quantity of skim milk obtained during the separation. The fat content of cream was determined by the Gerber method. Cream was washed by diluting with distilled water (45 C) to the volume of the original milk and reseparating. Cream was washed three times in this manner. The first wash water, which contained most of the skim milk associated with cream was added to the skim milk portion which was used for further fractionation. Casein was removed from the skim milk by isoelectric precipitation at pH 4.5, with 1 N HCl. The casein obtained was washed repeatedly with glass-distilled water and the washings added to whey. Alternatively, casein was obtained from skim milk by super centrifugation for 2 h at 25,000 x g in the International centrifuge. Part of whey proteins was precipitated by heating isoelectric whey at 85 C for 10 min. This precipitate was removed from whey by centrifugation and decantation.

Estimation of iron in milk fractions was done by a colorimetric method using 1, 10 phenanthroline (10). Protein estimation was done by micro-Kjeldahl method. Active sulphydryls were estimated by the method of Narang et al. (8) with the following modification: The absorbance was measured at 310nm instead of 300 nm as at this wave length there was less interference from substances that absorbed at 300 nm. Milk fat oxidation was followed by thiobarbituric acid (TBA) values suggested by King (4). A difference in the absorbance of 30 x 10^-4 indicated oxidized flavor.

RESULTS AND DISCUSSION

Distribution of added iron in raw milk fractions is given in Table 1. The difference in iron content between the fractions of iron-enriched samples and the corresponding fractions of non-enriched samples is taken to represent 'added iron' concentration. From data in the table it will be seen that about 9% of the added iron was found in the total cow cream, while about 12.5% was
associated with total cream in buffalo milk. Though the cream samples were standardized to 55% fat, buffalo milk contained larger amounts of skim milk because of its higher initial fat content. Therefore, the higher percentage of added iron found in buffalo cream could be due to larger amount of skim milk associated with it. This is supported by the fact that there was no significant difference between the added iron concentrations of cow and buffalo washed creams. However, the concentration of added iron associated per gram of washed cream was found to be significantly lower in buffalo samples, than in cow cream samples. This may be of importance as it has been suggested that the concentration of the metal associated with fat globules is an important factor in the oxidative deterioration of milk (5).

More than 90% of the added iron was associated with skim milk, mostly bound to casein; about 20% of the added iron remained with the whey. King et al. (6) found that added iron was partly dialysable at low pH. A part of the added iron dissociated from casein when the pH of milk was lowered to that of isoelectric point of casein. This would explain why the concentration of iron per unit weight was slightly more in centrifuged casein than in isoelectric casein. No attempt was made to determine the iron content of whey protein fractions, as it has been shown that low pH treatments and denaturation change the association of the whey proteins with metals (5, 6).

Table 2 gives the distribution of iron added to milk before pasteurization. A comparison of data in Tables 1 and 2 shows that the distribution pattern of added iron, ferrous or ferric, in both cow milk and buffalo milk was unaffected by subsequent pasteurization. The pasteurization process employed in this study would denature the whey proteins which might result in their coprecipitation with isoelectric casein. Protein determinations in whey showed a loss of about 5% of the protein due to pasteurization; since this constitutes only about 1% of the weight of casein, analytical results would not be influenced noticeably.

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Data on distribution of iron added to pasteurized milk are in Table 3. Though the amount of iron associated with cream was unaffected by the stage of addition, less iron was bound to casein in milk, if added after pasteurization than in raw milk. The iron added to milk before pasteurization was determined by determination of iron associated with casein, whey and skim milk fractions. Data in Table 3 shows that the distribution of iron added to pasteurized milk was influenced by the stage of addition.
pasteurization. This might be due to the strong affinity of iron towards sulfhydryl groups released from whey proteins during pasteurization. This difference in distribution of iron might also mean that iron once bound to casein is not released to combine with whey fractions at the temperature employed in this experiment. Estimation of iron in whey proteins precipitated by heat coagulation of isoelectric whey at 85°C for 10 min showed this fraction to be low in iron concentration. Further, no significant difference was noticed between iron concentrations of whey proteins isolated by this method from raw milk samples or samples to which iron was added either before or after pasteurization. However, this may not present a true picture of the association of iron with whey proteins in milk as the heat treatment employed to denature the proteins could have affected changes in their association.

The data presented do not give evidence of any effect of the oxidation state of iron on the distribution pattern of its salts, either in cow milk or in buffalo milk.

The oxidative stability of iron-enriched milk samples is given in Table 4. The TBA values were measured 48 h after addition of ferrous or ferric forms of iron. Analysis showed that significantly less iron had associated with the whey fraction of milk in the presence of NEM; in such samples, the TBA absorbance was less than $3 \times 10^{-3}$ even after 48 h of storage (Table 4). Demott (2) had noted less cooked flavor in ferric iron enriched milk samples which were subsequently pasteurized. The present study on pasteurized samples enriched with ferric iron showed a decrease of about 80% within 24 h in active sulfhydryl groups estimated by the NEM method. This would imply rapid oxidation of sulfhydryls by iron, getting itself reduced in the process to a reportedly stronger pro-oxidant ferrous form. Scanlan and Shipe (11) in a study of homogenized milk also showed that steam-vacuum treatment reduced ferric iron. Addition of hydrogen peroxide retarded the reduction of iron and prevented development of oxidized flavor. However, presence of iron in the reduced stage did not appear to be the controlling factor in determining its oxidative potency. This is evident from the fact that ferrous iron in raw milk was less potent than ferric iron added to pasteurized milk. This might mean that iron-sulfhydryl association has a more specific role than the mere conversion of ferric iron to the ferrous form. This appears analogous to the role of ascorbic acid in copper catalyzed oxidation of milk (13, 14). In this case ascorbic acid is not involved in milk lipid oxidation catalyzed by iron (15).

Pasteurization of milk after addition of iron increased the catalytic activity of both forms of iron. Statistical analysis of the data indicated that ferrous iron was significantly more potent than ferric iron in producing oxidative deterioration ($P<0.05$) (Table 4). Comparison of data in Tables 1 and 2 shows no marked difference in the distribution pattern of added iron. Demott (2) who employed a lower temperature for pasteurization (63°C for 30 min) which would not activate measurable quantities of sulfhydryls, also noticed oxidized flavor development in samples enriched with iron before pasteurization. This enhanced oxidative deterioration of milk appears to be due to activation of added iron during heat treatment. TBA values were higher in iron-enriched samples pasteurized at 63°C for 30 min compared to samples pasteurized at 81°C for 1 min. Prolonged heating at lower temperatures in comparison to short time heating at higher temperature appears to activate iron to induce oxidized flavors.

From the foregoing observations it may be concluded that heat treatment or the presence of a suitable reducing agent capable of rendering iron to its reduced form enhances the catalytic activity of iron. The TBA values show that unlike with copper, buffalo milk does not differ from cow milk in its resistance to oxidative deterioration induced by iron.

TABLE 4. Thiobarbituric acid values of milk samples stored 48 h at 5 ± 1°C after addition of ferrous or ferric forms of iron

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment</th>
<th>State of iron added</th>
<th>$A \times 10^3$</th>
<th>$\text{Fe}^{2+}$</th>
<th>$\text{Fe}^{3+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cow milk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>$\text{Fe}^{2+}$</td>
<td>24 ± 7</td>
<td>25 ± 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>$\text{Fe}^{2+}$</td>
<td>39 ± 7</td>
<td>37 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>$\text{Fe}^{3+}$</td>
<td>63 ± 7</td>
<td>44 ± 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>$\text{Fe}^{3+}$</td>
<td>76 ± 9</td>
<td>61 ± 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>$\text{Fe}^{3+}$</td>
<td>28 ± 6</td>
<td>22 ± 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Buffalo milk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>$\text{Fe}^{2+}$</td>
<td>24 ± 5</td>
<td>24 ± 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>$\text{Fe}^{2+}$</td>
<td>42 ± 8</td>
<td>42 ± 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>$\text{Fe}^{3+}$</td>
<td>61 ± 8</td>
<td>45 ± 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>$\text{Fe}^{3+}$</td>
<td>81 ± 8</td>
<td>66 ± 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>$\text{Fe}^{3+}$</td>
<td>27 ± 11</td>
<td>28 ± 5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Several percentages refer to the distribution pattern of added iron. Analysis showed that significantly less iron had associated with the whey fraction of milk in the presence of NEM; in such samples, the TBA absorbance was less than $3 \times 10^{-3}$ even after 48 h of storage (Table 4). Demott (2) had noted less cooked flavor in ferric iron enriched milk samples which were subsequently pasteurized. The present study on pasteurized samples enriched with ferric iron showed a decrease of about 80% within 24 h in active sulfhydryl groups estimated by the NEM method. This would imply rapid oxidation of sulfhydryls by iron, getting itself reduced in the process to a reportedly stronger pro-oxidant ferrous form. Scanlan and Shipe (11) in a study of homogenized milk also showed that steam-vacuum treatment reduced ferric iron. Addition of hydrogen peroxide retarded the reduction of iron and prevented development of oxidized flavor. However, presence of iron in the reduced stage did not appear to be the controlling factor in determining its oxidative potency. This is evident from the fact that ferrous iron in raw milk was less potent than ferric iron added to pasteurized milk. This might mean that iron-sulfhydryl association has a more specific role than the mere conversion of ferric iron to the ferrous form. This appears analogous to the role of ascorbic acid in copper catalyzed oxidation of milk (13, 14). In this case ascorbic acid is not involved in milk lipid oxidation catalyzed by iron (15).

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REFERENCES