L-Ascorbic Acid Microencapsulated with Polyacylglycerol Monostearate for Milk Fortification

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Efficiency was examined of microencapsulating l-ascorbic acid by polyglycerol monostearate (PGMS), and changes in the chemical and sensorial aspects of l-ascorbic acid and/or iron-fortified milk during storage were evaluated. The selected core materials were ferric ammonium sulfate and l-ascorbic acid. The highest efficiency (94.2%) of microencapsulation was found with the ratio of 5:1 as the coating to core material. The release of ascorbic acid from the microcapsules increased sharply from 1.6 to 6.7% up to 5 d of storage. The TBA value was the lowest in the milk sample with added encapsulated iron and unencapsulated l-ascorbic acid up to 5 d of storage in comparison with the other treated samples. A sensory analysis showed that most aspects were not significantly different between the control and fortified samples encapsulated with ascorbic acid after 5 d of storage. The results indicate that l-ascorbic acid microencapsulated with PGMS can be applied to fortify milk and acceptable milk products can be prepared with microencapsulated l-ascorbic acid and iron.

Key words: microencapsulation; l-ascorbic acid; polyacylglycerol monostearate; milk; fortification

Milk is a universal and nutritious food, although, it has an extremely low iron content.¹ ² According to recent nutrition surveys, iron-deficiency anemia is a highly prevalent and seemingly considerable problem, resulting from an inadequate intake of iron, particularly among young children, adolescents, and women of menstrual age throughout the world.³ ⁴ Pediatricians and nutritionists now universally recommend the addition of iron to milk-based formulas and foods to improve the hematological status.⁵ ⁶ However, iron fortification is difficult in food processing due to the potential for oxidized off-flavor,⁵ ⁷ color change,⁶ ⁸ sedimentation and metallic flavor,⁶ ⁹ probably as a result of the lipid peroxidation of milk fat.⁶ ¹⁰ ¹¹

Ascorbic acid is known to be involved in iron metabolism in animals.¹² Ascorbic acid enhances the absorption of iron from the intestines by reducing ferric iron to the ferrous state, a more soluble form that is easily absorbed.¹³ Ascorbic acid is also involved with adenosine triphosphate (ATP) in the release and reduction of ferric iron from ferritin, and its subsequent incorporation with the iron-binding proteins, apoferritin and transferrin, into tissue ferritin.¹⁴ Regardless of its roles, ascorbic acid is known to be very unstable and easily destroyed during processing by temperature, pH, oxygen, UV light, etc. In order to overcome some of these shortcomings of ascorbic acid, the microencapsulation technique may be suitable for ascorbic acid.

Microencapsulation, which has potential as a carrier in the food system, could be a good method for adding ascorbic acid and iron to dairy products.⁸ ¹⁵ There is considerable correct interest in developing encapsulated flavors and enzymes. Among several factors, the choice of coating material, the chemical and physical properties of the core material, the process used to form microcapsules and the ultimate properties desired of the microcapsules have to be considered.

Although several researchers have used various coating materials, such as milk fat, agar and gelatin to encapsulate enzymes, flavor components and iron in foods,¹⁶–¹⁸ no study has presented the possibility of using fatty acid emulsifiers as the coating material. The objectives of this present were to examine the efficiency of microencapsulating ascorbic acid with polyacylglycerol monostearate (PGMS) and to investigate whether ascorbic acid would prevent the adverse chemical and sensory effects of iron addition during storage.

Materials and Methods

Materials. Polyacylglycerol monostearate (PGMS) was used as a coating material for microencapsulating the ascorbic acid and iron complex. PGMS was purchased from Il-Shin Emulsifier Co. (Seoul, Korea). L-Ascorbic acid and water-soluble iron complex,
ferric ammonium sulfate (FeNH$_4$(SO$_4$)$_3$·4H$_2$O), were respectively purchased as core materials from Sigma Chemical Co. (St. Louis, MO, USA) and Shinyo Pure Chemical Co. (Osaka, Japan) and were of food grade.

**Preparation of microcapsules.** Microcapsules of ascorbic acid and iron were made by coating with PGMS, 50 ml of distilled water being added to 5 g of PGMS to reduce its highly viscous nature as described. The solution of PGMS and distilled water was heated to 55°C for 20 min and then stirred at 1,200 rpm for 30 sec before spraying. The ratios of PGMS as the coating material to distilled water to core material of 5:50:1 (w/v/w), 10:50:1, 15:50:1 and 20:50:1 were tested to maximize the core content and stability of the microcapsules, and were mixed at 1,200 rpm for 1 min with a stirrer.

An airless paint sprayer (W-300, Wagner Spray Tech. Co., Markdorf, Germany) were used to nebulize the coating material-core emulsion at 45°C in a cyclinder containing a 0.05% polyethylene sorbitan monostearate (Tween 60) solution at 5°C. The diameter of the nozzle orifice was 0.4 mm. The chilled fluid was centrifuged at 450 × g for 10 min to separate the microcapsule which had formed as solidified lipid in the chilled fluid.

**Efficiency of microencapsulation.** The dispersion fluid was assayed for untrapped L-ascorbic acid. To measure the total L-ascorbic acid content, 5 ml of the dispersion fluid was mixed with 100 ml of a metaphosphoric acid:glacial acetic acid:distilled water mixture (15:40:200, w/v/v), centrifuged, and supernatant collected. Total L-ascorbic acid was spectrophotometrically analyzed by the 2,4-dinitrophenyl hydrazine (DNP) test. Each sample was prepared immediately before the analysis, kept cold and protected by dissolving the analysis, Stock L-ascorbic acid stock solution was prepared daily by dissolving 10 mg of L-ascorbic acid in 10 ml of deionized water (100 μg/ml). This stock solution was diluted with deionized water to obtain final concentrations of 10, 20, 30, 40 and 50 μg/ml. Total L-ascorbic acid was determined by using a calibration curve based on the concentration (μg/ml) vs. absorbance of freshly prepared daily standard solutions.

**Stability of the microcapsules.**

**L-Ascorbic acid released during storage.** To measure the stability of L-ascorbic acid in the microcapsules, 10 ml of milk was added the same amount of the microcapsule solution (1 mg of ascorbic acid /1 ml), the mixture being, stored at 4°C for 12 d and the stability measured at 3-d intervals. Each sample was centrifuged, and the collected supernatant was analyzed to determine the amount of L-ascorbic acid released from the microcapsules. Each measurement was made in triplicate.

**Thiobarbituric acid (TBA) test.** The effect of microencapsulated L-ascorbic acid in iron-fortified milk was measured by using the TBA test during storage at 4°C for 12 d. The oxidized products were analyzed spectrophotometrically. The reagent for the TBA test was prepared immediately before use by mixing equal volumes of freshly prepared 0.025 M TBA, which had been neutralized with NaOH, and 2 M H$_3$PO$_4$ /2 M citric acid. The reaction for the TBA test was started by pipetting 5.0 ml of milk containing encapsulated or unencapsulated iron into a glass centrifuge tube and mixing thoroughly with 2.5 ml of the TBA reagent. The mixture was immediately heated in a boiling-water bath for exactly 10 min and then cooled on ice. Ten ml of cyclohexanone and 1 ml of 4 M ammonium sulfate were added, and the mixture centrifuged at 2,490 × g for 5 min at room temperature. The supernatant of orange-red cyclohexanone was decanted, and its absorbance at 532 nm was measured spectrophotometrically with a 1-cm light path. All measurements were made in triplicate.

**Sensory evaluation.** The sensory test was conducted on commercial whole milk containing encapsulated L-ascorbic acid (0, 100 or 250 ppm) that had been stored at 4°C for 1, 3, 5, 8 or 12 d. Ten panelists with some experienced in judging dairy products were recruited from the faculty members and graduate students in the Department of Food Science and Technology at Sejong University to evaluate the milk samples throughout the study.

The intensity of off-flavor and sourness were scored on a 5-point scale (1=none, 2=slight, 3=moderate, 4=strong and 5=very strong), and overall preference was scored on a 5-point scale (1=like extremely, 2=like moderately, 3=neither like nor dislike, 4=dislike moderately, and 5=dislike extremely). A random and balanced complete block design was used that resulted in replications for all samples.

**Statistical analysis.** Data from each experiment were subjected to an analysis of variance (ANOVA) with the SAS program, and differences among treatments were determined by LSD at p < 0.05, unless otherwise stated.

**Results and Discussion**

**Microencapsulation** PGMS is a solid at room temperature, so it was first heated for ease of spraying, before distilled water was added to reduce the viscosity of the solution that was sprayed to encapsulate L-ascorbic acid and/or iron. The results of our previous study showed that 50 ml of distilled water was sufficient for spraying, so we examined the effect of different ratios of the coating to core material in 50 ml of distilled water addition on the efficiency of L-ascorbic acid microencapsulation. A ratio of PGMS to L-ascorbic acid of 5:1 gave the greatest microencapsulation efficiency of 94.2%, this being significantly different than that from the other ratios (p < 0.05). The encapsulation efficiency decreased with increasing ratio of the coating
Our previous study has reported that the microencapsulation efficiency of L-ascorbic acid was 95.0% with a 15:1 ratio of coating to core material when medium-chain fatty acid (MCT) was used as the coating material. Based on these two results, PGMS may be more appropriate as the coating material due to quality concerns.

Similar studies have reported the optimum ratios of the coating (agar, gelatin, soluble starch, and milk fat) to core material (ω-3 fatty acid, iron, flavor, etc.) for efficient microcapsule formation. When ω-3 fatty acid was microencapsulated by milk fat, the ratio of the coating to core material of 8:2 resulted in an efficiency of 95.6%. In addition, Sankarikutty et al. have indicated that a 7:3 ratio of cardamon oil to a mixture of gum acacia and maltodextrin resulted in the highest efficiency among other ratios. Those studies indicated that the optimum conditions of the ratio of coating and core materials, viscosity of the spray solution, and method of microencapsulation varied with kind of coating, core material and food to be applied.

**Storage**

**L-Ascorbic acid release**

To examine the stability of microencapsulation during storage, ten ml of a microencapsulated solution (1 mg/ml) was mixed with 10 ml of commercial milk, and the mixture stored at 4°C for 12 d. The release of L-ascorbic acid from the microcapsules was then determined after 1, 3, 5, 8 and 12 d as shown in Fig. 1.

The stability of microencapsulation was adversely affected during storage. The release of L-ascorbic acid (%) increased sharply up to 5 d of storage, and then increased slowly up to 12 d. After 5 d of storage, 6.6% of L-ascorbic acid was released, while that was 9.2% at 12 d.

**TBA test**

The effect of L-ascorbic acid fortification of milk on preventing lipid oxidation by iron (as measured by the TBA test) during 12 d of storage is shown in Fig. 2. The test was run on 5 different milk samples as follows: 1) Control, raw milk; Trt 1, 2) 100 ppm of unencapsulated iron; Trt 2, 3) 100 ppm of microencapsulated iron added; Trt 3, 4) 100 ppm of microencapsulated iron and 100 ppm unencapsulated L-ascorbic acid added; and Trt 4, 5) 100 ppm of microencapsulated iron and 100 ppm microencapsulated L-ascorbic acid added.

As expected for the control group, to which nothing had been added, the TBA value did not change up to 8 d of storage and increased slowly thereafter (0.08 after
12 d). There was no difference between the control and Trts 3 and 4 up to 8 days of storage, although a significant difference was found when compared with the values for Trts 1 and 2 after 3 d of storage.

The iron-added groups without L-ascorbic acid addition (Trts 1 and 2) showed a TBA absorbance that increased proportionally with the storage period. The TBA absorbance was significantly lower in the L-ascorbic acid-added groups, regardless of microencapsulation, than in the groups without added L-ascorbic acid. This result indicates that the milk fat in raw milk may be oxidized even at 4°C during 12 d of storage. The 100 ppm unencapsulated iron-added group showed a TBA absorbance that increased dramatically from 0.06 to 0.15 from 0 to 12 d. Even microencapsulated iron addition had an adverse effect on lipid oxidation throughout the 12 d of storage.

When 100 ppm of unencapsulated iron with no L-ascorbic acid was added (Trt 1), the TBA value was the highest among the treatments throughout the storage period; it was 0.06 at the beginning, and dramatically increased thereafter. When 100 ppm of encapsulated iron with no L-ascorbic acid was added (Trt 2), the TBA value also increased up to 12 d of storage (0.13), although the rate of increase was lower than that of the 100 ppm unencapsulated iron-added group (Trt 1).

Compared with those results, in the case of the group with 100 ppm encapsulated iron and unencapsulated L-ascorbic acid added (Trt 3), the TBA value did not change between 0 and 8 d of storage, being the lowest at every storage interval, but then increased sharply to 0.115 after 12 d. The fact that the TBA value increased after 5 d may have been due to partial destruction of the unencapsulated L-ascorbic acid by environmental factors. When 100 ppm of encapsulated iron and encapsulated L-ascorbic acid was added (Trt 4), the TBA value was stable up to 8 d of storage and increased thereafter.

The change of TBA value with 250 ppm of microencapsulated L-ascorbic acid and 100 ppm of iron in milk is shown in Fig. 3. When only 100 ppm of unencapsulated iron had been added (Trt 1), the trend is similar to that in Fig. 2. In the control, Trt 1 and Trt 2 groups, the changes in TBA value during storage were not different. A significant result is that the TBA value for the Trt 3 group (100 ppm of microencapsulated iron with 250 ppm of unencapsulated L-ascorbic acid) decreased dramatically up to 5 d, and then increased sharply thereafter. When the TBA value was compared between the 100 ppm and 250 ppm of unencapsulated ascorbic acid addition (Trt 3 in Figs. 2 and 3), that for the 250 ppm-added group was lower at 0.075 than the 0.10 value for the 100 ppm-added group. This result indicates that an amount of L-ascorbic acid may help to effectively prevent the oxidation of milk fat.

Unencapsulated L-ascorbic acid acted as an antioxidant during the early stage of storage (up to 5 d), although it may have been inactivated during longer-term storage by other factors. In comparison, when microencapsulated L-ascorbic acid had been added, the TBA value was stable up to 8 d of storage, and increased thereafter. This can be explained by the fact that ascorbic acid is known to be very unstable and easily destroyed during processing involving temperature, pH, oxygen, UV light, etc.

A similar study19) has examined the effect of iron fortification in milk on its chemical oxidation during 12 d of storage. The TBA absorbance increased proportionally to the storage period. When 100 ppm of unencapsulated iron had been added to milk, the TBA absorbance increased dramatically at 0.17 to 0.58 from 0 to 12 d. The TBA absorbance was significantly lower for the encapsulated iron-added group than that in the unencapsulated group after 12 d of storage (0.35). Their data show that chemical lipid oxidation processed faster in milk with unencapsulated iron added than in milk with encapsulated iron added, regardless of the iron content.

**Sensory analysis**

The sensory properties of L-ascorbic acid-fortified milk stored at 4°C for 12 d were evaluated (Table 2). When 100 ppm of microencapsulated L-ascorbic acid had been added to milk, the sourness score was not significantly different from that of the control up to 5 d (p > 0.05), but increased thereafter up to 12 d. When 250 ppm of microencapsulated L-ascorbic acid had been added, sourness increased and showed a significant difference after 5 d and longer.

In respect of the off-taste, both the L-ascorbic acid-
treated groups showed a significant difference after 8 d of storage when compared with that of control score. In respect of the overall preference, the control and 100 ppm microencapsulated L-ascorbic acid added groups showed high consumer preference up to 5 d of storage, while 250 ppm addition impaired the consumer’s milk preference, even after 3 d of storage and thereafter. The sensory quality of L-ascorbic acid-fortified milk was shown to be well maintained by the encapsulation of both L-ascorbic acid and iron.

Acknowledgment

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References


Table 2. Sensory Scores for Milk Containing Microencapsulated L-Ascorbic Acid Stored for up to 12 d at 4°C1

<table>
<thead>
<tr>
<th>Sensory description</th>
<th>Treatment</th>
<th>Storage period (d)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Soreness</td>
<td>Control</td>
<td>1.0a</td>
</tr>
<tr>
<td></td>
<td>100 ppm</td>
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<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.3</td>
</tr>
<tr>
<td>Off-taste</td>
<td>Control</td>
<td>1.0a</td>
</tr>
<tr>
<td></td>
<td>100 ppm</td>
<td>1.2a</td>
</tr>
<tr>
<td></td>
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<td>1.4a</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
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</tr>
<tr>
<td>Overall preference</td>
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<tr>
<td></td>
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<td></td>
<td>250 ppm</td>
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<tr>
<td></td>
<td>SEM</td>
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1Sourness and off-taste scoring: 1, none; 2, slight; 3, moderate; 4, slightly strong; 5, strong. Overall preference scoring: 1, dislike extremely; 2, dislike moderately; 3, neither like or dislike; 4, like moderately; 5, like extremely. Means of 8 replicates are shown. Means in a column without the same letter are significantly different (p < 0.05).