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The Microencapsulated Ascorbic Acid Release *in vitro* and Its Effect on Iron Bioavailability

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The present study was carried out to examine the stability of microencapsulated ascorbic acid in simulated-gastric and intestinal situation *in vitro* and the effect of microencapsulated ascorbic acid on iron bioavailability. Coating materials used were polyglycerol monostearate (PGMS) and medium-chain triacylglycerol (MCT), and core materials were L-ascorbic acid and ferric ammonium sulfate. When ascorbic acid was microencapsulated by MCT, the release of ascorbic acid was 6.3% at pH 5 and 1.32% at pH 2 in simulated-gastric fluids during 60 min. When ascorbic acid was microencapsulated by PGMS, the more ascorbic acid was released in the range of 9.5 to 16.0%. Comparatively, ascorbic acid release increased significantly as 94.7% and 83.8% coated by MCT and PGMS, respectively, for 60 min incubation in simulated-intestinal fluid. In the subsequent study, we tested whether ascorbic acid enhanced the iron bioavailability or not. In results, serum iron content and transferring saturation increased dramatically when subjects consumed milks containing both encapsulated iron and encapsulated ascorbic acid, compared with those when consumed uncapsulated iron or encapsulated iron without ascorbic acid. Therefore, the present data indicated that microencapsulated ascorbic acid with both PGMS and MCT were effective means for fortifying ascorbic acid into milk and for enhancing the iron bioavailability

Key words: Microencapsulation, Ascorbic acid, Iron availability, Polyacylglycerol monostearate, Medium-chain triacylglycerol

INTRODUCTION

Milk is the universal and nutritious food, however, it contains an extremely low content of iron (Hegenauer *et al.*, 1979). According to the recent nutrition surveys, iron deficiency anemia is a highly prevalent and seemingly considerable problem, resulting from inadequate intake of iron, particularly among young children, adolescents, and women of menstrual age all over the world (HANES, 1974; Nutrition Canada 1973). Recently, pediatricians and nutritionists universally recommend the addition of iron to milk-based formulas and foods to improve the hematological status (Hegenauer *et al.*, 1979). However, iron fortification is difficult in food processing due to potential oxidized off-flavors, color changes, sedimentation and metallic flavors (Jackson and Lee, 1991) probably as a result of lipid peroxidation of milk fat (Edmonson *et al.*, 1971).

Ascorbic acid is known to be involved in the metabolism of iron in animals (NRC, 1993). Ascorbic acid enhances the absorption of iron from the intestine by reducing ferric iron to the ferrous state, a more soluble form that is easily absorbed (Monsen, 1982). Ascorbic acid is also involved with adenosine triphosphate (ATP) in the release and reduction of the ferric iron from ferritin, and its subsequent incorporation with iron-binding protein, apoferritin and transferrin, into tissue ferritin (Lim *et al.* 2000). Regardless of its roles, ascorbic acid is known to be very unstable and easily destroyed in the processing by temperature, pH, oxygen, UV light etc. In order to overcome some of these shortcomings of ascorbic acid, the microencapsulation technique may be a good application for ascorbic acid.

Microencapsulation, which shows potential as a carrier in food system, could be a good method for the addition of ascorbic acid and iron to dairy products (Jackson and Lee, 1991; Berseneva *et al.* 1990). Currently, there is a

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considerable interest in developing encapsulated flavors and enzymes. Uddin *et al.* (2001) indicated that microencapsulated ascorbic acid could prevent color change by ascorbic acid, retard its release rate, and mask its acid taste. Also, iron is known to catalyze lipid oxidation resulting in rancidity with development of an unpleasant odor and flavor. The most important reason using iron microencapsulation to fortify to milk products derived from potential oxidized off-flavors (Jackson and Lee, 1991), probably due to lipid prooxidation of milk (Edmonson, 1971).

It is generally accepted that for an effective uptake of nutritional effect from microcapsules, several problems need to be resolved: The capsules have to contain as much as nutrition as possible and have to resist the gastric and intestinal fluids can be captured by the enterocytes being released into the blood circulation. Therefore, the objective of this study was to examine the stability of microencapsulated ascorbic acid in simulated-gastric acid intestinal situation *in vitro*, and the effect of microencapsulated ascorbic acid on iron availability.

MATARIALS AND METHODS

Materials

For the microencapsulation of ascorbic acid and iron, medium-chain triacylglycerol (MCT) and polyacylglycerol monostearate (PGMS) were used as coating materials. Those were purchased from Il-Shin Emulsifier Co., LTD. (Seoul, Korea). As core materials, L-ascorbic acid and water-soluble iron complex, ferric ammonium sulfate ($FeNH_4(SO_4)_2 \cdot 4H_2O$), were purchased from Sigma Chemical Co., (St. Louis, MO, USA) and Shinyo Pure Chemical Co. LTD. (Osaka, Japan), respectively, and were in food grade.

Preparation of microcapsule

Microcapsules of ascorbic acid were made by MCT or PGMS. For MCT, the different ratios of coating to core materials were 5:1, 10:1, 15:1, and 20:1 (w/w) to maximize core contents and stability of microcapsules, and mixed at 1,200 rpm for 1 min with stirrer. For PGMS, 50 mL distilled water was added to 5 g PGMS because PGMS was highly viscous, as described (Kwak *et al.*, 2003). The mixture of PGMS and distilled water was heated to 55°C for 20 min, and stirred with 1,200 rpm for 30 sec for spraying. The different ratios of coating to core materials were 5:50:1 (w/v/w), 10:50:1, 15:50:1, and 20:50:1, and further procedure was followed as same as MCT. Iron microencapsulation was achieved using PGMS.

For spraying, an airless paint sprayer (W-300, Wagner Spray Tech. Co., Markdorf, Germany) nebulized a coating material-core emulsion at 45°C into 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 microcapsules, which were formed as lipid solidified in the chilled fluid.

Stability of microencapsule *in vitro*

To determine the stability in the stomach and intestine, as indirect method, the simulated-gastrointestinal solutions were prepared as follows: 1) gastric fluid prepared in sample solution containing pepsin (pH 1.2) and simulated into 4 different fluids with pHs 2, 3, 4 and 5 using by 2 N HCl and NaOH, and 2) intestinal fluid was prepared in 0.1M PBS buffer (100 mL, pH 3.4) containing 20 mg pancreatin. 5 mg lipase, 10 mM cholic acid and 10 mM deoxycholic acid, and simulated into 3 different intestinal solutions as pHs 6, 7 and 8.

In both gastric and intestinal fluids, the microcapsules of ascorbic acid in distilled water (total ascorbic acid content: 100 ppm) were incubated at 37°C with the sample collecting 0, 20, 40 and 60 min. The treated samples were centrifuged and the collected supernatant was analyzed for determination of ascorbic acid content released from microcapsules. All treatments were triplicate.

Determination of iron bioavailability

Volunteered fifteen healthy college women, aged 20 to 25 years, were selected from Sejong University, Seoul, Korea. The some drinks such as coffee and green tea, which act as an inhibitor of iron absorption, were prohibited.

Three different milk samples were consumed consequently with 2 h-interval: firstly, commercial milk with no addition (100 mL) was consumed to determine their own iron status, secondly, 100 ppm uncapsulated iron added milk was consumed, thirdly, 100 ppm encapsulated iron added milk, and finally, 100 ppm encapsulated iron and 250 ppm encapsulated ascorbic acid with MCT.

Fasting morning blood samples (5 mL) were drawn for the baseline and after 1 h when they were receiving 5 different milk samples. Blood was drawn with plastic syringes with stainless steel needles, placed in trace metal free plastic tubes, and promptly centrifuged to separate red cells from plasma. Serum iron, serum total iron-binding capacity, and transferring saturation were determined by Sigma kit (Sigma Co., St. Louis, MO, USA) with spectrophotometer.

Statistical analysis

Data from each experiment were analyzed by analysis of variance (ANOVA) using a SAS program (1985) and differences among treatments were determined by LSD at $p<0.05$, unless otherwise stated.

RESULTS AND DISCUSSION

In vitro study

Since iron deficiency anemia is a highly prevalent and seemingly considerable experiment should be performed to determine how stable the microcapsules were in the stomach and how effectively released in the intestine, which in the primary site of iron absorption and regulation. It is generally accepted that for an effectively uptake of nutritional effect from microcapsules, several problems need to be resolved: the capsules have to contain as much nutrition as possible, have to resist the gastric and intestinal fluids and be captured by the enterocytes before being released into the blood circulation.

Ascorbic acid release in simulated-gastric fluid

This study was conducted to determine whether the microcapsules released iron during simulated-gastric and -intestinal conditions. In simulated-gastric fluid, the iron release showed a similar trend in every pHs (2, 3, 4 and 5). With a change of pH from 2 to 5, the release of ascorbic acid became smaller at every incubation time points, indicating the higher stability of microcapsules in higher pH. In addition, there was a dramatic increased in ascorbic acid released in first 20 min interval.

With microencapsulated by MCT, 12.5% iron was released from the microcapsules at 20 min, and slightly increased to 13.2% at 60 min at pH 2 (Fig. 1). Incubation at pHs 3 and 4 at 37°C showed 9.9 and 6.8% release at 20 min and 9.3 and 11.1% at 60 min, respectively. At pH 5, the ascorbic acid release was not dramatic during incubation period, and also it was the lowest among treatments as 4.7% at 20 min, and 6.3% at 60 min.

Since PGMS is a solid type in room temperature, an additional procedure was applied based on the method described as followed: firstly, the heating process was applied for ease of spraying, and secondly, distilled water was added to reduce the viscosity of spray solution for encapsulating L-ascorbic acid and/or iron (Kwak *et al.*, 2001). From our previous study (Kwak *et al.*, 2001), 50 mL of distilled water was sufficient to spray, therefore, we examined the effect of different ratios of coating to core material with 50 mL distilled water addition on the efficiency of ascorbic acid microencapsulation.

When ascorbic acid was microencapsulated by PGMS, the higher release of ascorbic acid was found in all pHs and incubation periods (Fig. 2). However, the trend of release was not different from that with MCT. At pH 2, 13.5% iron was released from the microcapsules at 20 min, and slightly increased to 16.0% at 60 min. Incubation at pHs 3 and 4 at 37°C showed 12.2 and 10.1% release at 20 min, and 14.5 and 13.2% at 60 min, respectively. At pH 5, the ascorbic acid release was similar to other treatments as 8,5% at 20 min, and 10.0% at 60 min.

The present results indicated that the release of ascorbic acid from microcapsules made by MCT showed a much higher stability in simulated-gastric fluid, compared with that by PGMS. In addition, the higher percentage of ascorbic acid released in lower pHs may probably due to a highly acidic condition, which resulted in capsule breakage. Similar study was conducted with iron microcapsules made by with PGMS (Kwak *et al.*, 2003). In their study, most of iron was released within 20 min at every pHs (3, 4, 5 and 6), which reached in the range of 13.0 to 14.7%.

Similar studies (Kwak *et al.*, 2001; Kim *et al.*, 1996; Jackson and Lee, 1991; Magee and Olson, 1981) have reported the optimum ratios of coating (agar, gelatin, soluble starch, milk fat) to core material (-3 fatty acid, iron, flavor etc.) for efficient microcapsule formation. When -3 fatty acid was microencapsulated by milk fat, the ratio of

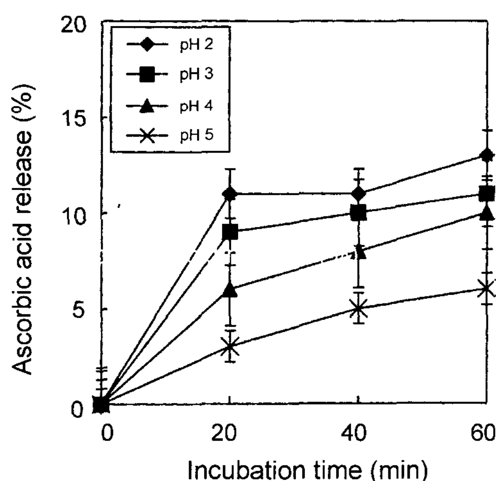

Fig. 1. The ascorbic acid release from microcapsules made with MCT in a simulated-gastric fluid during 60 min incubation.

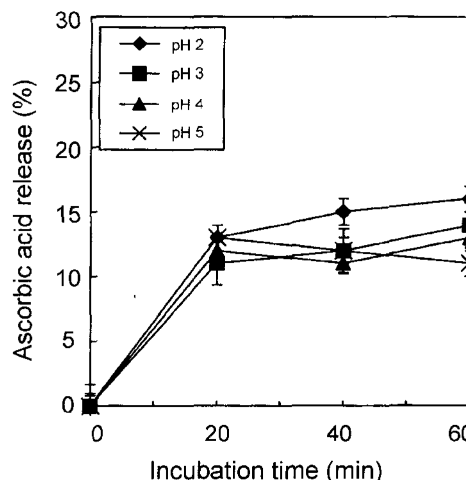

Fig. 2. The ascorbic acid release from microcapsules made with PGMS in a simulated-gastric fluid during 60 min incubation.

coating to core material was 8:2 and the efficiency was 95.6% (Kim *et al.*, 1996). In addition, Sankarikutty *et al.* (1988) indicated that the 7:3 ratio of cardamon oil to the mixture of gum acacia and maltodextrin showed the highest efficiency among other ratios. Those studies indicated that the optimum conditions including the ratio of coating and core materials, the viscosity of spray solution, the method of microencapsulation varied with kinds of coating, core materials and food to be applied.

Ascorbic acid release in simulated-intestinal fluid

With microencapsulated by MCT, 50.0% iron was released from the microcapsules at 20 min. Iron release continuously increased to 91.0% at 40 min and slightly increased thereafter (94.7%) at pH 8 (Fig. 3). Incubation at pH 7 at 37°C showed about 41.8% release at 20 min, and 84.5% at 60 min, respectively. At pH 6, the ascorbic acid release was not dramatic after 20 min incubation, and also it was the lowest among treatments as 44.1% at 40 min, and 53.9% at 60 min.

When ascorbic acid was microencapsulated by PGMS, about 3-17% lower release of ascorbic acid was found, compared with those made by MCT (Fig. 4), however, the trend of release was not different. At pH 6, 34.8% iron was released from the microcapsules at 20 min, and increased to 49.9% at 60 min. Incubation at pHs 7 and 8 at 37°C showed 28.9 and 46.2% release at 20 min and 77.8 and 83.8% at 60 min, respectively.

The present results indicated that the release of ascorbic acid from microcapsules made by MCT was higher in simulated-intestinal fluid, compared with that by PGMS. In addition the higher percentage of ascorbic acid released in pH 8 may probably due to an optimum pH condition, which resulted in effective capsule breakage. Similar study

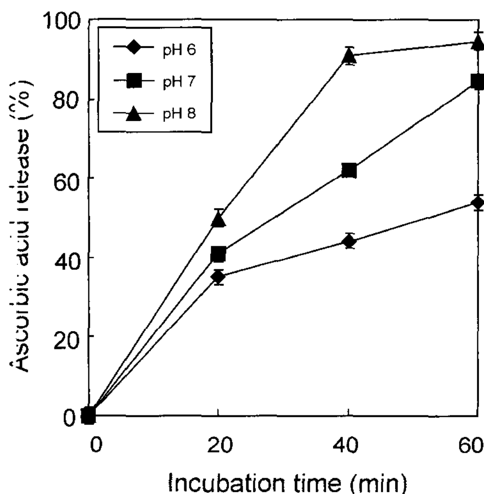

Fig. 3. The ascorbic acid release from microcapsules made with MCT in a simulated-intestinal fluid during 60 min incubation.

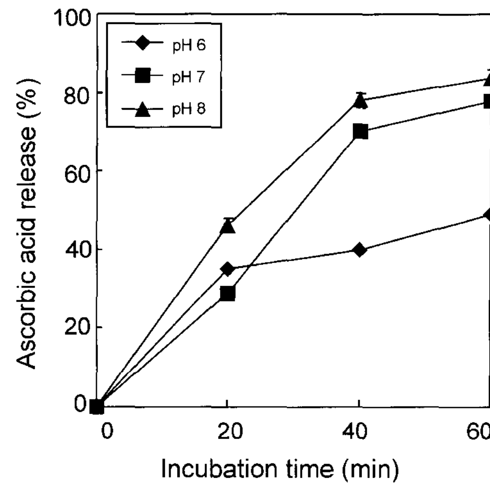

Fig. 4. The ascorbic acid release from microcapsules made with PGMS in a simulated-intestinal fluid during 60 min incubation.

was conducted with iron microcapsules made with PGMS (Kwak *et al.*, 2003). In their study, most of iron was released within 40 min at pHs 7 and 8, which reached in the range of 82.0 to 92.0%.

As we expected, the present study indicated that a little amount of iron was released in low pH. Comparatively, iron release increased dramatically in neutral pH, which in a similar condition to that of the intestine. These results suggested that microcapsules would be convenient tools for iron-fortified milk due to an increase of iron absorption by favoring the uptake and effective release in the intestine.

Bioavailability of iron

Iron absorption in fortified foods depends on one hand on the iron nutritional status of subjects and on the other hand on the composition of the food. In this case, we evaluated the iron absorption using milk as vehicle, which has high concentrations of calcium, phosphorus and casein, known as inhibitors of iron absorption (Uicich *et al.* 1999; Deehr *et al.*, 1990).

Table I presents mean values of serum iron (SI), total iron binding capacity (TIBC), and transferrin saturation (TS) as determined in blood samples obtained from the 15 subjects. The mean serum iron value was 78±22 μg/100 mL milk after consuming a commercial milk as control. The serum iron concentration increased with addition of uncapsulated iron by 11 μg/100 mL milk. It was slightly higher than that of control.

Comparatively, when subjects consumed the milk containing 100 ppm of encapsulated iron, serum iron concentration increased dramatically upto 106±27 μg/100 mL. As expected, when ascorbic acid microcapsules were additionally added, serum iron concentration was 119±30 μg/100 mL, which was a profound increase than that of control and may represent an effective iron absorption with ascorbic

Table I. Mean values of serum iron, total binding capacity, and transferring saturation with microencapsulated iron and/or ascorbic acid added milk intake[1]

Treatments	Serum iron (μg/100 mL)	Total iron binding capacity (μg/100 mL)	Transferrin saturation (%)
Control[2]	78±22	395±72	19±5
Trt 1[3]	89±24	358±75	24±4
Trt 2[4]	106±27	337±71	31±3
Trt 3[5]	119±30	279±51	42±6

[1]Means of 5 replicates. Means in a column without the same letter are significantly different ($p < 0.05$).
[2]Control: no addition of iron or ascorbic acid
[3]100 ppm uncapsulated iron added
[4]100 ppm microencapsulated iron added
[5]100 ppm microencapsulated iron and 250 ppm microencapsulated by ascorbic acid made by MCT

acid.

The other parameters determined were total iron binding capacity (TIBC), and transferrin saturation (TS). The value of TIBC was inverse of that of SI concentration. The control group showed 395±72 μg/100 mL, which was significantly higher than that of group consumed encapsulated iron and ascorbic acid (279±51 μg/100 mL). In addition, the highest TS value was found in group consumed encapsulated iron and ascorbic acid. Above results indicated that microencapsulated iron and/or ascorbic acid showed a good bioavailability, and it is an effective method for the fortification of milk.

CONCLUSION

The present study indicated that microcapsules of ascorbic acid made by PGMS or MCT were very effective for enhancing the iron availability. The release of ascorbic acid from microcapsules made by MCT was higher in simulated-gastric fluid, compared with that by PGMS. In addition, the higher percentage of ascorbic acid released in lower pHs may probably due to a highly acidic condition, which resulted in capsule breakage. In simulated-intestinal fluid, the higher amount of ascorbic acid was released from microcapsules made by MCT. In bioavailability study, serum iron content and transferrin saturation increased dramatically when consumed both encapsulated iron and ascorbic acid together. Above results suggested that microcapsules would be a convenient tool for iron-fortified milk due to an increase of iron absorption by favoring the uptake and effective release in the intestine.

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