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Microencapsulated Iron for Milk Fortification

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This study was designed to develop a microencapsulated iron that could be used to fortify milk and to determine the sensory properties of milk fortified with microencapsulated iron. Coating material was polyglycerol monostearate (PGMS), and selected core material was ferric ammonium sulfate. The highest efficiency of microencapsulation was 75% with 5:1:30 ratio (w/w/v) as coating to core materials to distilled water. Iron release was 12% when stored at 4 °C for 3 days. The TBA value was the lowest when 100 ppm of capsulated iron was added into milk and was significantly lower in capsulated groups compared with that in uncapsulated groups. In an in vitro study, only 3−5% of iron was released in simulated gastric fluid (pH 3, 4, 5, and 6). Comparatively, iron release increased dramatically from 12.3% (pH 5) to 95.7% (pH 8) for 60 min of incubation in simulated intestinal fluid. In a sensory analysis, most aspects except for metallic taste and color were not significantly different between control and capsulated iron fortified milk at 3 days of storage. However, between capsulated and uncapsulated groups, astringency, metallic, color, and overall scores were significantly different. The present study indicated that the use of microencapsulated iron with PGMS is effective for fortifying milk.

KEYWORDS: Microencapsulation; iron; polyglycerol monostearate; milk

INTRODUCTION

Milk is a universal and nutritious food; however, it has an extremely low content of iron (*1*). According to 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 (*2*, *3*).

Recently, pediatricians and nutritionists have now universally recommended the addition of iron to milk-based formulas and foods to improve the hematological status (*1*). The RDA of iron for men is 10 mg/day, so the reasonable intake from iron-fortified products might be suggested. However, iron fortification is difficult in food processing due to potential oxidized off-flavors, color changes, and metallic flavors (*4*), probably as a result of lipid prooxidation of milk fat (*5*).

Microencapsulation, which shows potential as a carrier of enzymes in the food industry, could be a good vehicle for the addition of iron to dairy products (*4*, *6*). Currently, there is considerable interest in developing capsulated flavors and enzymes. Among several factors to be considered, choice of coating material is the most important and depends on the chemical and physical properties of the core material, the process used to form microcapsules, and the ultimate properties desired in microcapsules.

Another potential carrier of iron could be liposome formation (*7*, *8*), which has great potential in pharmaceutical applications for drug delivery (*9*). However, encapsulation efficiency of liposomes is only ∼25%, and liposome dispersion lacks long-term stability (*9*).

For microencapsulation, although several researchers have used coating materials such as milk fat, agar, and gelatin, responsible for enzyme, flavor and iron microencapsulation in foods (*10*−*12*), no study has measured the efficiency of iron microencapsulation using fatty acid esters and the stability during storage. Therefore, the objectives of this study were to develop a microencapsulated iron source for fortification of milk and to measure the sensory properties of milk fortified with microencapsulated iron.

MATERIALS AND METHODS

Materials. For the microencapsulation of iron compounds, polyglycerol monostearate (PGMS) was used as a coating material. It was purchased from Il-Shin Emulsifier Co., Ltd. (Seoul, Korea). As a core material, six different iron complexes were tested in this experiment as follows: ferrous chloride, ferrous lactate, ferrous ammonium sulfate, ferric ammonium citrate, ferric sulfate, and ferric ammonium sulfate. These were purchased from Sigma Chemical Co. (St. Louis, MO) and were of food grade.

Preparation of Microcapsule. Microcapsules of iron were made by PGMS, which was selected as a major coating material from our previous study (*13*). Subsequently, six different iron compounds were tested for microcapsule preparation; spray solutions containing different ratios of coating to core material of 3:1, 5:1, 10:1, 15:1, and 20:1 (coating to core) with 50 mL of distilled water were tested.

The spray solution was heated at 55 °C for 20 min and mixed thoroughly with stirring at 1200 rpm. An airless paint sprayer (W-300,

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Wagner Spray Tech. Co., Markdorf, Germany) nebulized a coating material−iron emulsion into a cylinder containing a 0.05% polyethylene sorbitan monostearate (Tween 60) solution at 5 °C. The diameter of the nozzle orifice was 0.33 mm. Microcapsules were formed as lipid solidified in the chilled fluid. The chilled fluid was centrifuged at 2490*g* for 10 min to separate iron microcapsules. The microencapsulation of iron was done in triplicate.

Efficiency of Iron Microencapsulation. The dispersion fluid of microencapsulation was assayed for untrapped iron by PGMS. One milliliter of the dispersion fluid was taken and diluted 10 times, and total iron content was measured at 248.3 nm by atomic absorption spectrophotometry (AAS; Aln IL 751 model spectrophotometer, Instrumental Laboratory Spectrophotometer, Wilmington, MA). A sample measurement was run in triplicate.

Microscopic Observation. The microstructural image of the capsule was magnified by 1000-fold with a light microscope (Olympus Optical Co., Ltd.) and photographed.

Stability of Microcapsules. *Thiobarbituric Acid (TBA) Test.* The absorbance change by addition of iron into milk was measured using a TBA test at 4 °C for 12 days (*1*). Oxidation 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 was neutralized with NaOH and 2 M H_3PO_4/2 M citric acid. Reactions of the TBA test were started by pipetting 5.0 mL of milk containing iron capsulated or uncapsulated into a glass centrifuge tube and mixed thoughly with 2.5 mL of TBA reagent. The mixture was heated immediately in a boiling water bath for exactly 10 min and cooled on ice. Ten milliliters of cyclohexanone and 1 mL of 4 M ammonium sulfate were added and centrifuged at 2490*g* for 5 min at room temperature. The orange-red cyclohexanone supernatant was decanted and its absorbance at 532 nm measured spectrophotometically in a 1-cm light path. All measurements were run in triplicate.

Iron Release during Storage. To measure the stability of iron microcapsules, 10 mL of commercial milk was added into the same amount of microcapsule solution (1 mg of Fe/mL), the mixture was stored at 4 and 20 °C for 12 days, and its stability was measured at 3 day intervals. The samples were centrifuged, and the collected supernatant was analyzed for the determination of iron content released from microcapsules. All measurements were made in triplicate.

In Vitro Study. To determine the stability in the stomach and intestine, simulated gastrointestinal solutions were prepared as follows: (1) gastric fluid prepared in sample solution containing pepsin (pH 1.2) and simulated into four different fluids with pH 3, 4, 5, and 6 using 2 N HCl and NaOH; and (2) intestinal fluid was prepared in 0.1 M PBS buffer (100 mL, pH 7.4) containing 20 mg of pancreatin, 5 mg of lipase, 10 mM cholic acid, and 10 mM deoxycholic acid, and simulated into four different intestinal solutions at pH 5, 6, 7, and 8.

In gastric fluid, the microcapsules of iron in distilled water (total iron content = 100 ppm) were incubated at 37 °C with 100 rpm shaking for 10 min, and in intestinal fluid, they were incubated at 37 °C for 30 min with sample collection at 20 min intervals. The treated samples were centrifuged at 2490*g*, and the supernatant was measured for iron content released from the microcapsules. All treatments were made in triplicate.

Sensory Evaluation. For the storage test, 100 mL of commercial whole milk (at every period) containing capsulated or uncapsulated iron (10 mg) was stored at 4 °C for 1, 3, 6, 9, and 12 days of storage. A 10-person panel, semiexperienced in judging dairy products, was recruited from faculty and graduate students in the Department of Food Science and Technology at Sejong University and evaluated the milk samples throughout the study.

The intensity of off-flavors (metallic and acidic flavors), taste aspects (astringency and bitterness), and color were scored on a nine-point scale (1 = none, 3 = slight, 5 = moderate, 7 = strong, and 9 = very strong), and overall quality was also scored on a nine-point scale (1 = like extremely, 3 = like moderate, 5 = neither like or dislike, 7 = dislike moderate, and 9 = dislike extremely).

Statistical Analysis. Data from each experiment were analyzed by analysis of variance (ANOVA) using an SAS program (*14*), and differences among treatments were determined by Duncan's multiple test at $p < 0.05$, unless otherwise stated.

Table 1. Microencapsulation Efficiency of Iron with Different Ratios of PGMS to Iron Complex[a]

ratio (w/w)		microencapsulation efficiency (%)
PGMS[b]	iron complex[c]	
3	1	61c
5	1	75a
10	1	67b
15	1	57cd
20	1	53c
SEM		3.8

[a] Means of triplicate. Means in a column without the same letter are significantly different ($p < 0.05$). Other experimental factors: distilled water added into PGMS, 50 mL; mixing temperature, 55 °C; mixing speed, 1200 rpm; centrifugal force, 2490*g*; centrifugal time, 10 min. [b] Polyacylglycerol monostearate. [c] Ferric ammonium sulfate.

RESULTS

Core Material. On the basis of our previous study for microencapsulation of β-galactosidase (*13*), PGMS was chosen as a coating material in the present study. To find an appropriate iron source as a core material for microencapsulation, six different iron complexes of food grade were examined. Although there was no difference among iron complexes in microencapsulation efficiency (69−74%), we selected ferric ammonium sulfate as a core material on the basis of its sensory aspects including color and metallic taste (data not shown). Ferric ammonium sulfate showed the highest efficiency at 74%.

Microencapsulation. Even though PGMS was heated to 55 °C, it appeared to be hard to spray as described in our previous study (*13*). From a preliminary experiment, when the ratio of coating (PGMS) to core material (ferric ammonium sulfate) to distilled water was 5 g:1 g:50 mL, the highest efficiency was 74%. The addition of too much distilled water for microencapsulation resulted in a weak microcapsule coating; therefore, efficiency was decreased in our preliminary study (data not shown).

When the ratio of PGMS to distilled water was 5 g:50 mL (w/v), the optimum ratio of PGMS to iron complex, ferric ammonium sulfate (3:1, 5:1, 10:1, 15:1, and 20:1), was examined as shown in **Table 1**. The efficiency of the microencapsulation increased up to 5:1 (w/w) (coating-to-core ratio) with 50 mL of distilled water in spray solution and decreased thereafter when the amount of coating material increased. When PGMS was 5 g and iron complex was 1 g, the yield of microencapsulation was 75%, the highest value.

Microscopic Observation. A photomicrograph of microencapsulated iron with PGMS is shown in **Figure 1**. The size of microcapsules was irregular, and the average size was in the range of 2−5 μm. Microscopic examination of microcapsules revealed spherical particles. Microcapsules containing iron solutions had smooth surfaces and evenly distributed pockets of iron solution.

Stability of Microcapsules. *TBA Test during Storage.* The effect of iron fortification in milk on chemical oxidation (as measured by the TBA test) during 12 days of storage is shown in **Figure 2**. The treatment was divided into four different groups as follows: (1) 100 ppm of uncapsulated iron, 100 mg/100 mL iron-added milk; (2) 100 ppm of capsulated iron; (3) 200 ppm of uncapsulated iron; and (4) 200 ppm of capsulated iron.

In all groups, TBA absorbance increased proportionally to storage period. In the 100 ppm of uncapsulated iron-added group, TBA absorbance increased dramatically from 0.17 to 0.58 from 0 to 12 days. TBA absorbance was significantly lower in

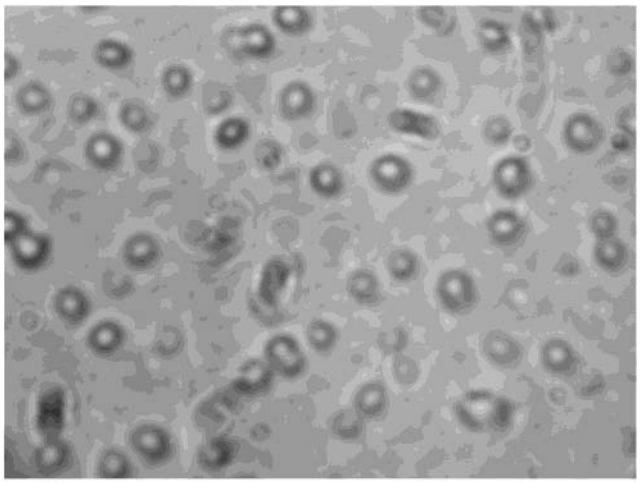

Figure 1. Photomicrograph of microencapsulated iron with polyglycerol monostearate. The photograph was taken at 1000× magnification and is shown here at 80% of the original.

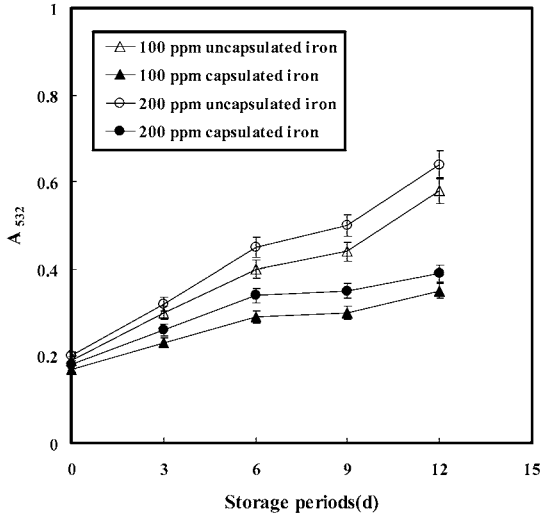

Figure 2. Changes of TBA absorbance of milk added by microencapsulated iron stored at 4 °C for 15 days. Ferric ammonium sulfate was used as core material.

the capsulated group than in uncapsulated group at 12 days of storage (0.35). When 200 ppm of iron was added, the TBA absorbance difference between uncapsulated (0.64) and capsulated groups (0.38) was significant at 12 days of storage and thereafter. This result indicated that chemical lipid oxidation proceeded more rapidly in milk with uncapsulated iron added than in milk with capsulated iron added regardless of iron content.

Iron Release during Storage. Microcapsules were examined for the ability to retain iron at different temperatures during storage (**Figure 3**). Ten milliliters of ferric ammonium sulfate microcapsule solution (1 mg/mL) was mixed with 9 mL of commercial milk and stored at 4 and 20 °C for 12 days. In all samples, the iron lost (percent) increased with the storage period. Significantly more iron was released from microcapsules at the higher temperature (20 °C) at every 3 day interval than at the lower temperature (4 °C). At 3 days of storage, iron lost was 12 and 16% at 4 and 20 °C, respectively, which was not significantly different. However, at 12 days of storage, 18 and 21% of iron was released at 4 and 20 °C, respectively.

In Vitro Study. This study was conducted to determine whether the microcapsules released iron during simulated gastric

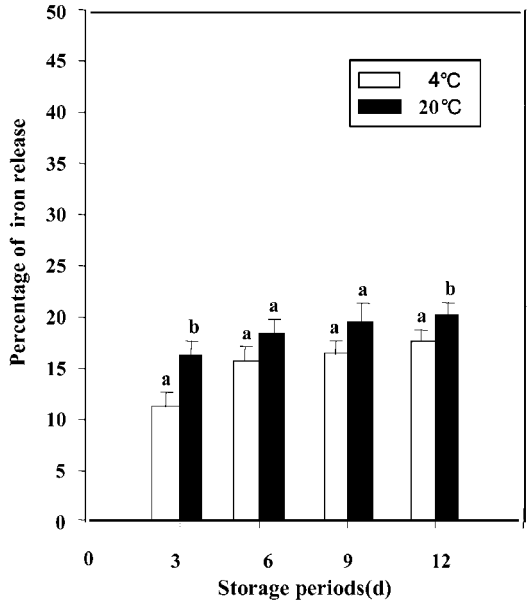

Figure 3. Effect of temperatures on iron release from microcapsules stored for 30 days in milk.

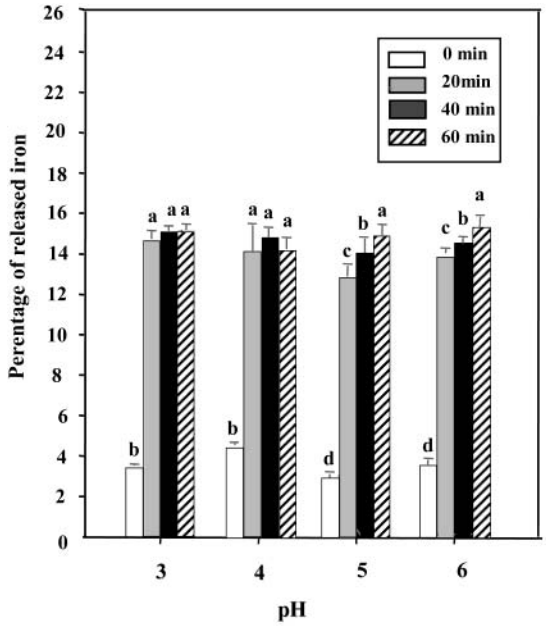

Figure 4. Effect of different pH values on iron release from microcapsules incubated under simulated gastric condition in vitro. Coating material, polyglycerol monostearate; core material, ferric ammonium sulfate. Simulated gastric fluid contained pepsin and was adjusted to different pH values with HCl and NaOH and incubated at 37 °C for 60 min. Each bar represents an average of three trials. Each bar indicates a standard deviation, and bars with different letters are significantly different ($p < 0.05$).

intestinal conditions. The iron release showed a similar trend at all pH values (3, 4, 5, and 6) (**Figure 4**) during incubation in simulated gastric fluid. Under incubation at pH 3, <4% of iron was released from the microcapsules at the initial time (0 min), and this increased to 14.7% at 20 min and plateaued thereafter. Incubation at pH 4, 5, and 6 at 37 °C at the initial time showed a 3.0−4.5% iron release. There was a dramatic increase to 14.2, 13.0, and 14.0% at pH 4, 5, and 6, respectively, during 20 min of incubation. The higher percentage of iron release at lower

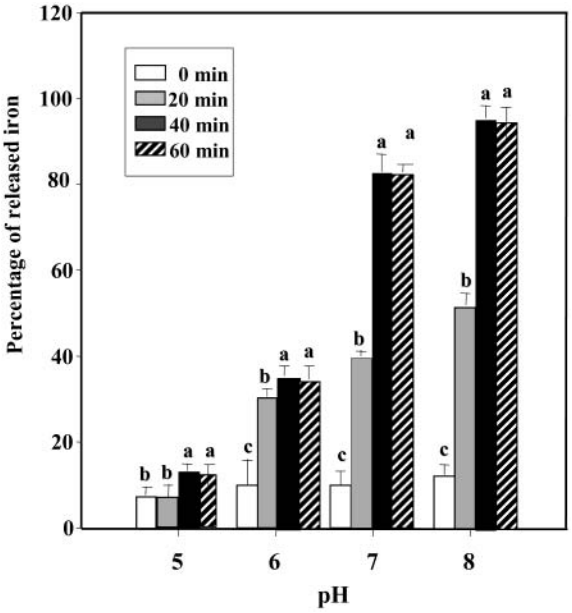

Figure 5. Effects of different pH values on iron release from microcapsules incubated in simulated intestinal fluid in vitro. Coating material, polyglycerol monostearate; core material, ferric ammonium sulfate. Simulated intestinal condition included enzymes such as lipase (5 mg) and pancreatin (20 mg) and was incubated at 37 °C for 60 min. Each bar represents an average of three trials. Each bar indicates a standard deviation, and bars with different letters are significantly different ($p < 0.05$).

pH (3 and 4) than at higher pH (5 and 6) may probably due to a highly acidic condition, which resulted in capsule breakage.

To determine how effectively iron was released in the intestine, a simulated intestinal fluid was prepared with the presence of pancreatin and bile salts and incubated during at 37 °C for 60 min (**Figure 5**). When both pH and the duration of incubation increased, iron release increased dramatically, especially at pH 7 and 8. Less than 15% of the entrapped iron was released at pH 5 at every time period (0, 20, 40, and 60 min), and not much increase was found until 60 min of incubation. Under incubation at pH 6, a dramatic increase (~2 times) was observed between 0 and 20 min and maintained thereafter. With incubation at pH 7 and 8, iron release from microcapsules at 40 min of incubation and thereafter, respectively, was 82 and 92%.

Sensory Analysis. The sensory properties of milks with three different treatments were evaluated for 12 days of storage at refrigerated temperature (**Table 2**). Three treatments were performed as follows: (1) control, commercial market milk; (2) capsulated, 100 ppm of capsulated iron-added milk; and (3) uncapsulated, 100 ppm of uncapsulated iron-added milk.

The astringency score of capsulated iron-added milk was not different from that of control until 3 days but increased thereafter until 12 days. Uncapsulated iron-added milk also showed a significantly higher astringency at 3 days compared with control and capsulated iron-added groups. For bitterness, the capsulated iron group did not show a significant difference at 3 days of storage with other groups, whereas bitterness in the uncapsulated iron-containing group was higher at 6 days and thereafter. A strong metallic taste was detected even at 1 day of storage in uncapsulated iron. The acidic taste was a little higher in iron microcapsule-added groups but not significantly different among groups during 12 days of storage. Uncapsulated iron changed milk color even at 1 day of storage. However, when capsulated iron was added, significant color change compared with the control at 6 days of storage was observed. Between the control and capsulated iron groups, the overall scores were not different until 3 days of storage. The uncapsulated iron group had significantly higher overall scores even at 1 day of storage, probably due to strong metallic taste and color.

Table 2. Sensory Scores of Different Microencapsulated Iron-Fortified Milks at Refrigerated Temperature for 12 Days of Storage[a]

sensory description	treatment	storage period 1 day	3 days	6 days	9 days	12 days
astringency	control	1.0a	1.0a	1.0a	1.0a	1.0a
	capsulated	1.9ab	1.6a	2.2b	3.8c	4.8c
	uncapsulated	2.0ab	3.0bc	4.2c	4.8c	6.0d
bitterness	control	1.0a	1.0a	1.0a	1.0a	1.0a
	capsulated	1.4a	1.0a	1.6a	2.4b	3.0bc
	uncapsulated	1.5a	1.4a	2.2b	2.6b	3.0bc
metallic	control	1.0a	1.0a	1.0a	1.0a	1.0a
	capsulated	2.3b	2.7ab	3.6c	5.2d	7.2e
	uncapsulated	3.4c	4.9d	5.6d	7.0e	8.2e
acidic	control	1.0a	1.0a	1.0a	1.6a	2.2ab
	capsulated	1.3a	1.2a	1.4a	2.2ab	3.4b
	uncapsulated	1.3a	1.4a	1.8a	2.6ab	3.6b
color	control	1.0a	1.0a	1.2a	1.8ab	3.6b
	capsulated	1.3a	1.2a	2.0b	2.6b	6.2c
	uncapsulated	2.0b	2.1b	3.4c	4.6b	6.0c
overall[b]	control	1.0a	1.0a	1.6a	2.0ab	3.1b
	capsulated	1.3ab	1.4a	3.2b	4.8c	8.0e
	uncapsulated	3.8bc	3.0b	4.0c	6.2d	8.2e

[a] Sensory descriptions scoring except for overall: 1, none; 3, slight; 5, moderate; 7, slightly strong; 9, strong. Means of eight replicates. Means in a column without the same letter are significantly different ($p > 0.05$). [b] Overall scoring: 1, like extremely; 3, like moderately; 5, neither like nor dislike; 7, dislike moderately; 9, dislike extremely.

DISCUSSION

For microencapsulation of iron to fortify milk, microcapsules should not impart a gritty texture to the milk or float to the surface of the milk. Generally, small microcapsules are desired, so that textural changes in the food system may be avoided. Thus, the size and shape of the microcapsule were a considerable aspect in the present study. When milk was fortified with the iron microcapsules made, adverse effects were not profound, probably due to the sufficiently small and smooth surface of the microcapsules.

Jackson and Lee (*4*) compared the shape and size of microcapsules between commercial microcapsules and theirs made by aqueous $FeSO_4$ and high milk fat. They indicated that their capsulated iron solutions had a diameter of 6.00 ± 3.40 μm and that of the commercial microcapsules was 200 ± 30 μm, which were irregularly shaped and had rough surfaces.

To develop a microencapsulated iron source that could be used to fortified milk, first, core materials should be tested. Ferrous lactate showed a strong off-flavor, and ferrous chloride, ferrous ammonium sulfate, and ferrous sulfate·$7H_2O$ showed a black color. In addition, the microcapsules made by ferric ammonium citrate were too light to be separated by centrifugation, resulting in a low yield of microcapsule from a dispersion fluid. Therefore, ferric ammonium sulfate was selected as an appropriate core material with acceptable sensory aspects.

Second, the microencapsulation efficiency of PGMS as a coating material was determined. The efficiency of microencapsulation was greatest when the coat-to-core ratio to distilled water was 5:1:50 (w/w/v). In the case of 20:1:50 (w/w/v), PGMS was left over in the upper layer of the dispersion fluid after centrifugation.

Similar studies (*4*, *12*, *13*, *15*) have reported the ratios of coating (fatty acid esters, agar, gelatin, soluble starch, milk fat, etc.) and core material (lactase, $\omega-3$ fatty acid, iron, flavor, etc.) for an efficient microcapsule formation. When $\omega-3$ fatty acid was microencapsulated by milk fat, the ratio of coating to core material was 8:2 and the efficiency was 95.6% (*15*). In addition, Sankarikutty et al. (*16*) indicated that the 7:3 ratio of cardamom oil to the mixture of gum acacia and maltodextrin showed the highest efficiency among other ratios. Our previous study (*13*) showed that the highest efficiency (72.8%) was found with a ratio of 15:1 (w/w, PGMS to lactase). Those studies showed that the conditions for microencapsulation depended on the ratio of coating and core materials, the viscosity of the spray solution, kinds of coating and core materials, and food system to which it was to be applied.

Iron is known to catalyze lipid oxidation, resulting in rancidity with the development of an unpleasant odor and flavor. The most important reason for using iron microencapsulation to fortify to milk products derived from the potential of oxidized off-flavors (*4*), probably due to lipid prooxidation of milk (*5*). Therefore, we determined the effect of fat oxidation of iron microencapsulation by TBA test during 9 days of storage. This study showed a satisfactory protective effect from lipid oxidation in capsulated iron-fortified milk compared with uncapsulated iron-fortified milk.

A similar study conducted by Jackson and Lee (*4*) reported that samples containing uncapsulated iron (ferrous sulfate and ferric chloride) showed 2−3 times higher fatty acid production compared with those containing capsulated iron complex when milk fat was used as a coating material. Modifications in iron-fortified milk and yogurt could be explained by iron's interaction with casein, resulting in iron−casein complexes, and the presence of O_2, which acts as a prooxidant, accelerating lipid oxidation in milk (*17*).

Because iron-deficiency anemia is a highly prevalent and seemingly considerable problem, a considerable experiment should be performed to determine how stable the microcapsules are in the stomach and how effectively they are released in the intestine, which is the primary site of iron absorption and regulation.

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 nutrition as possible, they have to resist gastric and intestinal fluids, and they must be captured by the enterocytes before being released into the blood circulation. As we expected, the present study indicated that a small amount of iron was released at low pH. Comparatively, iron release increased dramatically at neutral pH, which is 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.

A final aspect we needed to consider was the unpleasant off-taste usually derived from fat oxidation with iron or iron itself. Generally, the sensory quality of iron-fortified dairy foods has been shown to be affected by the type of iron source used, the amount of iron added, and the properties of the dairy products being fortified. The most affected aspects on sensory analysis were metallic taste and color, which were significantly different at 1 day in capsulated iron-fortified milk. However, there was not much significant adverse effect of microencapsulated iron on milk taste and flavor during 5 days of storage. The present results showed a possible application in capsulated iron fortification using PGMS, which may be used effectively in food systems.

ABBREVIATIONS USED

PGMS, polyglycerol monostearate; TBA, thiobarbituric acid.

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