INTRODUCTION

Yogurt has gained widespread consumer acceptance in the U.S. (Otto, 1988) and other developed countries, primarily by women, children and teenagers, who consume yogurt as a luncheon or snack food. These populations have high calcium requirements and are also frequently deficient in iron (Dallman et al., 1984). Even though yogurt is an excellent source of calcium and protein (United States Department of Agriculture, 1982), it contains very little iron (Blanc, 1981).

Fortification of iron in yogurt would help meet this nutritional need. Using dairy foods as a vehicle for supplementing iron seems to be an advantage because people who consume diets with low iron density usually consume more dairy products (Hekmat and McMahon, 1997). Furthermore, iron-fortified dairy foods have a relatively high iron bioavailability (Woestyne et al., 1991). However, before any such fortification is undertaken in yogurt, the effects of iron fortification on microbial physiology during manufacture and shelf-life of yogurt, oxidation of milk fat, and the effect of iron on sensory characteristics must be ascertained.

Iron in food is absorbed by the intestinal mucosa, and especially nonheme iron, the major dietary pool, is greatly influenced by meal composition. It is well known that vitamin C is a powerful enhancer of nonheme iron absorption (Lynch and Cook, 1980). Its influence may be pronounced in meals of iron availability (Dallman et al., 1984). Even though yogurt is an excellent source of calcium and protein (United States Department of Agriculture, 1982), it contains very little iron (Blanc, 1981).

Microencapsulation, which shows potential as a carrier of enzymes in the food industry, could be a good vehicle for the addition of iron to milk (Jackson and Lee, 1991; Bersen‘eva et al., 1990). Currently there is a considerable interest in developing encapsulated 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.

For microencapsulation several researchers have used coating materials such as milk fat, agar, and gelatin, etc. responsible for enzyme, flavor and iron microencapsulation in foods (Braun and Olson, 1986; Magee and Olson, 1981a, b), but no study has measured the efficiency of iron microencapsulation using fatty acid esters, and the stability of microcapsule itself and inside the body. Therefore, the objective of this study was to examine the effect of microencapsulated iron and/or vit C added yogurt on chemical and sensory aspects during storage.

MATERIALS AND METHODS

Materials

For the microencapsulation of iron complex, polyglycerol monostearate (PGMS) was used as a coating material. It was purchased from Il-Shin Emulsifier Co., LTD. (Seoul, Korea). As core materials, water-soluble iron complex ferric ammonium sulfate (FeNH4(SO4)2 4H2O) and L-ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Shinyo Pure Chemical Co. LTD (Osaka, Japan) and were in food grade.
Preparation of microcapsule
Microcapsules of iron were made by polyglycerol monostearate (PGMS), which was selected as a major coating material from our previous study (Kwak et al., 2001). Also, ferric ammonium sulfate and L-ascorbic acid were selected (Kwak and Yang, 2002). Other experimental factors were as follows: the ratio of coating material to core material was 5:1, and 50 mL distilled water was additionally added because PGMS solution was highly viscous. The spray solution was heated at 55°C for 20 min, and stirred at 1,200 rpm for 1 min during spraying. An airless paint sprayer (W-300, Wagner Spray Tech. Co., Markdorf, Germany) nebulized a coating material-iron emulsion at 45°C 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. The chilled fluid was centrifuged at 2,490 × g for 10 min to separate unwashed microcapsule suspension. Microcapsules were formed as lipid solidified in the chilled fluid. The microencapsulation of iron and ascorbic acid were done in triplicate.

Yogurt preparation
A commercial homogenized and pasteurized milk containing 3.4% fat and 13.4% total solids was fortified with 2.7% (w/v) skim milk powder to increase viscosity of yogurt and then homogenized (HC-5000 Homogenizer, Microfluidics Corp., Newton, MA, USA) at 150 kg/cm² (60°C) and cooled to 42°C. A 0.2% commercial YC-380 starter culture (Chr. Hansen Pty. Ltd. Bayswater, Australia) in freeze-dried direct-to-vat set form containing Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus thermophilus was inoculated and fermented at 42°C until pH was 4.3. After fermentation, some yogurt samples were initially removed and incubated at 4°C for 24 h. The remaining samples were stored at 4°C for 20 d to study the changes in chemical, microbial and sensory aspects during prolonged fermentation of yogurt at 5 d intervals.

Treatments
Five different groups in this experiment were as follows: 1) no addition as control (Trt 1); 2) 20 ppm uncapsulated iron added (Trt 2); 3) 20 ppm capsulated iron added (Trt 3); 4) 20 ppm capsulated iron and 100 ppm uncapsulated vit C added; (Trt 4 and 5) 20 ppm capsulated iron and 100 ppm capsulated vit C.

Efficiency of microencapsulation
For iron measurement, the dispersion fluid was assayed for untrapped iron during microencapsulation. One milliliter of the dispersion fluid was taken and diluted ten times and total iron content was measured at 259.94 nm wave length by inductively coupled plasma spectrometer (ICP). Lactam 8440 Model spectrometer (Plasmalab, Victoria, Australia) was used. A sample measurement was run in triplicate.

Total vit C was analyzed spectrophotometrically using DNP (2,4-dinitrophenyl hydrazine) test (Korea Food Code, 2002). Samples were prepared immediately before analyses and kept cool and protected against daylight during analysis. A vit C stock solution was prepared daily by dissolving 10 mg of vit C in 100 mL of deionized water (100 µg/mL). It was diluted with deionized water to obtain the final concentration of 10, 20, 30, 40 and 50 µg/mL. Total vit C was determined using the calibration graph based on concentration (µg/mL) vs absorbance, prepared daily running fresh standard solutions.

Chemical analyses

* pH and titratable acidity (TA) : pH and titratable acidity (determined by titration to pH 8.3) of the yogurt samples were measured at room temperature using a pH meter (Sartorius, Germany). The TA was determined after mixing the 9 mL yogurt sample with 18 mL distilled water and titrating with 0.1 N NaOH using a 0.5% phenolphthalein indicator to an end point of faint pink color.

* Thiobarbituric acid (TBA) test : Oxidation products were analyzed spectrophotometrically using the thiobarbituric acid (TBA) test (Hegenauer et al., 1979). The TBA reagent was prepared immediately before use by mixing equal volumes of freshly prepared 0.025 M TBA (neutralized with NaOH) and 2 M H₃PO₄/ 2 M citric acid. Reactions were terminated by pipetting 5.0 mL of yogurt sample containing iron microcapsules into a glass centrifuge tube and mixed thoroughly with 2.5 mL TBA reagent. The mixture was heated immediately in a boiling water bath for exactly 10 min, and then cooled on ice. Then 10 mL cyclohexanone and 1 mL of 4M ammonium sulfate were added and centrifuged at 2,490 × g for 5 min at room temperature. The orange-red cyclohexanone supernatant was decanted and its absorbance at 532 nm was measured spectrophotometrically in a 1 cm light path. All measurements were run in triplicate.

Viscosity
Viscosity measurement was made with Bostwick consistometer (CSC Scientific Company, Seoul, Korea). Sample (100 g) was placed and flow distance (cm) for 1 min was expressed as viscosity.

Microbiological analyses
Lactic acid bacteria were determined from the colony counts on specific lactic agar: MRS agar (pH 5.4) for Lactobacillus delbrueckii subsp. bulgaricus and M17 agar for Streptococcus thermophilus; 1 g yogurt samples stored for 0, 5, 10, 15, and 20 d were diluted with 9 mL of sterile peptone and water diluent. Subsequent serial dilutions of
each sample were plated in triplicate and incubated at 41°C for 48 h.

**Sensory evaluation**

For the storage test, 10 mL drink yogurt containing capsulated or uncapsulated iron and vit C was stored at 4°C for 0, 5, 10, 15 and 20 d. An eleven-person panel semi-experienced in judging dairy products recruited from faculty and graduate students in the Department of Food Science and Technology at Sejong University evaluated the yogurt samples throughout the study.

The intensity of taste aspects (bitterness, astringency, and sourness) were scored on a nine-point scale (1=none, 3=slight, 5=moderate, 7=strong and 9=very strong), and overall preference were scored on a nine-point scale (1=dislike extremely, 3=dislike moderate, 5=neither like or dislike, 7=like moderate, and 9=like extremely). A randomized, balanced, complete block design was used (Cochran and Cox, 1957) that resulted in two replications for all samples.

**Statistical analysis**

Data from each experiment were analyzed by analysis of variance (ANOVA) using an SAS program (1985) and differences among treatments were determined by Student-Newman-Keuls comparison test at p<0.05, unless otherwise stated.

**RESULTS AND DISCUSSION**

**Microencapsulation**

In the present study, the yield of iron and vit C microencapsulation were 73% and 95%, respectively. In our laboratory, PGMS was appeared to be hard to spray and we used the optimum ratio of PGMS to deionized water to reduce the viscosity of PGMS solution, found in our previous study (Kwak et al., in press). The ratio of PGMS to iron to distilled water was 5:1:50 (w/w/v); efficiency of the microencapsulation was 75% as the highest value.

The size of microencapsulated iron or vit C with PGMS was irregular from nano to micrometer, and the average size was in the range of 2 to 5 µm. Microscopic examination of microcapsules revealed spherical particles. Microcapsules containing iron or vit C had smooth surfaces and evenly distributed pockets. The shape of the microcapsules was likely affected by encapsulating conditions. Magee and Olson (1981a), and Braun and Olson (1986) found that lipid and cooling fluid temperatures affected the shape of microcapsule by controlling the cooling rate of lipid coatings. They observed that microcapsules were cylindrical when the lipid coating was rapidly cooled and spherical when the lipid was slowly cooled.

**The change of pH and titratable acidity (TA)**

Iron fortifications did not affect fermentation time required for the yogurt mixes to reach pH 4.10-4.20 (Figure 1). After 5 h fermentation, further trends of pH changes during storage were also similar: the pH values of control and fortified samples reached 4.00-4.07 after 1 d and 3.95-4.07 after 20 d.

The change of pH was as shown in Figure 1. pH was the highest in control group (Trt 1); it was 4.20 at 0 d and decreased to 4.07 at 5 d and plateaued thereafter up to 20 d storage in control.

When compared with uncapsulated iron added group (Trt 2) and capsulated iron groups (Trt 3), pH was significantly lower in Trt 2 at every time interval. Uncapsulated iron resulted in high level of acidity during fermentation (0 d storage) and further storage, showed that iron capsulation had a large effect on pH at every time interval.

Between capsulated iron with uncapsulated vit C (Trt 4) and capaulated iron with capsulated vit C (Trt 5), there was no difference at every time interval until 10 d storage. Uncapsulated vit C showed a certain protective effect on pH decrease. These results indicated that uncapsulated iron addition decreased the pH of yogurt during storage.
exchange between iron ions and micellar bound H⁺ (Gaucheron, 2000).

Titratable acidity (TA) increased with the storage time (Figure 2). The TA of control was the lowest and that of Trt 2 containing unencapsulated iron was the highest.

TBA test during storage

The effect of iron fortification in yogurt on chemical oxidation (as measured by the TBA test) during 20 d storage is shown in Figure 3. When compared with unencapsulated (Trt 2) and capsulated (Trt 3) iron added group, TBA value was slightly higher in unencapsulated iron added groups at 0, 5, and 20 d storage. The difference in TBA value between the 2 groups increased dramatically at 10 and 15 d storage.

When compared with capsulated iron with unencapsulated vit C (Trt 4) and capsulated iron with capsulated vit C (Trt 5), the TBA value of Trt 5 was not significantly lower than that of Trt 4 in the early stage of storage (0, 5 and 10 d). However, the TBA value of Trt 5 containing capsulated vit C was significantly lower at 15 d storage.

TBA absorbance was significantly lower in capsulated groups than the in unencapsulated group, regardless of iron and vit C, during storage. These data indicated that oxidation process may be faster in yogurt samples containing unencapsulated iron than in those containing capsulated iron. Another study (Kwak and Yang, 2002) showed the effect of iron fortification in milk on chemical oxidation during 15 d storage. They reported that TBA absorbance was significantly lower in a capsulated group than in an unencapsulated group at 15 d.

Jackson and Lee (1991) indicated that samples containing unencapsulated iron (ferrous sulfate and ferric chloride) showed 2-3 times greater fatty acid production, compared with those containing capsulated iron complex when milk fat was used as a coating material. The reason why iron fortification caused several modifications in milk and yogurt could be that added iron interacts with casein, resulting in iron-casein complexes, and the presence of O₂ acts as a prooxidant; therefore, lipid oxidation in yogurt is accelerated.

Change of microbial counts during storage

The change of Lactobacillus delbrueckii ssp. bulgaricus in microencapsulated iron fortified drink yogurt at 4°C for 20 d storage is shown in Table 1. At 0 d, the mean counts of L. delbrueckii ssp. bulgaricus for control and other groups were not significantly different. Also, the mean counts in all groups showed a decrease trend during 20 d storage. L.
delbrueckii ssp. bulgaricus counts were about 10^7 cfu/ml.

The change of viable cells of Streptococcus salivarius ssp. thermophilus in microencapsulated iron fortified drink yogurt at 4°C for 20 d storage is shown in Table 2. At 0 d, the mean counts of S. salivarius ssp. thermophilus for control and other groups were not significantly different. Also, the mean counts in all groups did not show any change during 20 d storage. Meanwhile, S. salivarius ssp. thermophilus counts were about 10^5 cfu/ml.

Hekmat and McMahon (1997) reported that counts of L. delbrueckii ssp. bulgaricus and S. salivarius ssp. thermophilus after 1 d of storage in iron-fortified skim yogurt were 7.0×10^8 cfu/ml, which were not significantly different from counts in unfortified yogurts. Counts decreased to 2.5×10^8 cfu/ml and 1.9×10^8 cfu/ml for L. delbrueckii ssp. bulgaricus and S. salivarius ssp. thermophilus, respectively, after 30 d storage. Fortifying yogurt with iron did not affect the growth of Pseudomonas fluorescens or Escherichia coli.

**Viscosity**

When viscosity was measured by consistometer, flowing a longer distance indicated lower viscosity, as shown in Figure 4. Four treatments except Trt 2 showed a slightly decreasing trend of viscosity during 20 d storage.

Table 1. Changes of viable cells of Lactobacillus delbrueckii subsp. bulgaricus in microencapsulated iron fortified drink yogurt stored at 4°C for 20 days storage

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
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<tr>
<td>(cfu/ml)</td>
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<tr>
<td>Trt 1</td>
<td>2.1×10^8±0.1^a 2.4×10^8±0.3^a 2.4×10^8±0.1^a 2.4×10^8±0.1^a 2.4×10^8±0.1^a</td>
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<tr>
<td>Trt 2</td>
<td>2.2×10^8±0.2^b 2.5×10^8±0.4^b 2.2×10^8±0.1^b 2.3×10^8±0.3^b 2.2×10^8±0.2^b</td>
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<tr>
<td>Trt 3</td>
<td>2.6×10^8±0.2^c 2.4×10^8±0.2^c 2.1×10^8±0.3^c 2.3×10^8±0.2^c</td>
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<tr>
<td>Trt 4</td>
<td>2.5×10^8±0.2^d 2.3×10^8±0.1^d 2.3×10^8±0.2^d 2.2×10^8±0.2^d 2.6×10^8±0.0^d</td>
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<tr>
<td>Trt 5</td>
<td>2.3×10^8±0.4^e 2.4×10^8±0.3^e 2.2×10^8±0.2^e 2.3×10^8±0.3^e 2.2×10^8±0.1^e</td>
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2. Within the same column means with different superscripts are significantly different (p<0.05).

Table 2. Changes of viable cells of Streptococcus thermophilus in microencapsulated iron fortified drink yogurt stored at 4°C for 20 days storage

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
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<tbody>
<tr>
<td>(cfu/ml)</td>
<td></td>
<td></td>
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<tr>
<td>Trt 1</td>
<td>2.2×10^8±0.1^a 2.4×10^8±0.3^a 2.4×10^8±0.1^a 2.4×10^8±0.1^a 2.4×10^8±0.1^a</td>
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<tr>
<td>Trt 2</td>
<td>2.2×10^8±0.2^b 2.5×10^8±0.4^b 2.2×10^8±0.1^b 2.3×10^8±0.3^b 2.2×10^8±0.2^b</td>
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<tr>
<td>Trt 3</td>
<td>2.6×10^8±0.2^c 2.4×10^8±0.2^c 2.1×10^8±0.3^c 2.3×10^8±0.2^c</td>
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<tr>
<td>Trt 4</td>
<td>2.5×10^8±0.2^d 2.3×10^8±0.1^d 2.3×10^8±0.2^d 2.2×10^8±0.2^d 2.6×10^8±0.0^d</td>
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<tr>
<td>Trt 5</td>
<td>2.3×10^8±0.4^e 2.4×10^8±0.3^e 2.2×10^8±0.2^e 2.3×10^8±0.3^e 2.2×10^8±0.1^e</td>
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2. Within the same column means with different superscripts are significantly different (p<0.05).

**Sensory analysis**

The sensory characteristics of drink yogurts in five treatments are shown in Table 3. Bitterness, was not significantly different among treatments during 20 d storage.

Trt 2, uncapsulated iron fortified yogurt, had a slightly higher score than others.

For astringency, uncapsulated or capsulated iron containing drink yogurt (Trts 2 and 3) showed a higher score, compared with those of control and other treatments at 0 d. At 5 d storage, groups containing uncapsulated iron (Trt 2) and capsulated iron with vit C regardless of capsulation (Trts 4 and 5) showed a significantly higher astringency, compared with other groups. No difference was found among groups thereafter.

A significantly stronger sourness was observed in Trt 4, (capsulated iron and uncapsulated vit C) at every period except 10 d storage. Sourness increased with storage time in Trt 2 containing uncapsulated iron only showed no difference to other treatments until 10 d storage. But then decreased greatly from 15 d up to 20 d. This decrease (Trt 2) may be because an interaction of casein and whey protein with iron complex was developed in yogurt during storage (Sadler et al., 1973).
all groups. Comparison of Trt 2 with Trt 3, indicates the effect of iron microencapsulation on sourness score, and no significant difference was found. The only difference found was between Trt 4 and Trt 5, and it was caused by vit C whether encapsulated or not. Therefore, a dramatically high sourness score in Trt 4 resulted not from capsulated iron, but from unencapsulated vit C in the drink yogurt.

For overall preference, control (Trt 1) and capsulated iron (Trt 3) and/or vit C (Trt 5) showed a high consumer preference in all storage periods. The scores of Trts 2 and 4 (uncapsulated iron added, and capsulated iron and unencapsulated vit C added groups) were much lower compared with those of other three treatments. This result indicated that microencapsulation process is very effective to mask off-taste and flavor of iron and vit C.

The sensory quality of iron-fortified dairy foods has been shown to be well maintained by the capsulation of both iron and vit C. Two major off-flavors have been associated with dairy products: oxidized flavor resulted from catalysis of lipid oxidation by iron, and sourness contributed by vit C. Iron is known to catalyze lipid oxidation resulting in rancidity with development of an unpleasant odor and flavor. The TBA test has been extensively applied to food in which the absorbance of TBA reaction products correlates positively with sensory evaluation. Fortification with iron complex causes oxidized off-flavor and high TBA number. To avoid oxidized and metallic flavors and color changes, microencapsulation techniques are needed. (Gaucheron, 2000).

**CONCLUSION**

The present study demonstrated that the ratio of 5:1:50 (w/w/v) as coating (PGMS) to core material (iron complex) to distilled water showed a high efficiency of microencapsulation of iron and vit C, by as 73% and 76%, respectively. Our results indicated that lipid oxidation process measured by TBA test was significantly slower in encapsulated iron than in unencapsulated iron fortified yogurt. In sensory quality, no significantly adverse effect was found in microencapsulated iron and vit C fortified drink yogurt during 20 d in this experiment. Therefore, this study provides important evidence that microcapsules of iron and vit C are an effective means of fortification, and can be applied to drink yogurt without any changes in sensory aspects.

**ACKNOWLEDGEMENT**

This research was supported by the Small & Medium Business Administration (SMBA) in Seoul, Korea.

**REFERENCES**


**Table 3. Sensory scores of drinking yogurt fortified with microencapsulated iron fortified drink yogurt stored at 4°C for 20 day storage**

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>Bitterness</th>
<th>Sourness</th>
<th>Overall preference</th>
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<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3.6±1.6a</td>
<td>3.4±1.2b</td>
<td>3.2±1.3a</td>
</tr>
<tr>
<td>5</td>
<td>4.1±1.1a</td>
<td>4.0±1.3b</td>
<td>3.8±1.2a</td>
</tr>
<tr>
<td>10</td>
<td>3.3±1.4a</td>
<td>3.0±1.0b</td>
<td>2.6±0.9b</td>
</tr>
<tr>
<td>15</td>
<td>3.1±1.2b</td>
<td>3.0±0.9b</td>
<td>3.0±1.0b</td>
</tr>
<tr>
<td>20</td>
<td>3.2±1.1a</td>
<td>2.5±1.0b</td>
<td>2.6±0.8b</td>
</tr>
</tbody>
</table>

1. DY; II. DY-I; III. DY-MI; IV. DY-MI-C; V. DY-MI-MC.

1. Within the same column means with different superscripts are significantly different (p<0.05).


