

Microencapsulated Iron Fortification and Flavor Development in Cheddar Cheese

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ABSTRACT : This study was designed to examine the effect of microencapsulated iron-fortified Cheddar cheese and L-ascorbic acid as a bioavailable helper of iron on chemical and sensory aspects. Coating material was PGMS, and ferric ammonium sulfate and L-ascorbic acid were selected as core materials. The highest efficiency of microencapsulation of iron and L-ascorbic acid were 72 and 94%, respectively, with 5:1:50 ratio (w/w/v) as coating to core material to distilled water. TBA absorbance was significantly lower in microencapsulated treatments than those in uncapsulated treatments during ripening. The productions of short-chain free fatty acid and neutral volatile compound were not significantly different among treatments during ripening periods. In sensory aspects, bitterness, astringency and sourness were higher in Cheddar cheese fortified with microencapsulated iron and uncapsulated L-ascorbic acid than others. The present study indicated that fortification of iron as well as L-ascorbic acid did not show any defect problem to Cheddar cheese, and suggested the possibility of iron fortification of Cheddar cheese. (*Asian-Aust. J. Anim. Sci.* 2003. Vol 16, No. 8 : 1205-1211)

Key Words : Cheddar Cheese, Microencapsulated Iron, PGMS, L-ascorbic acid

INTRODUCTION

Today, cheese, a major product made of milk, has gained widespread popularity in the world, even its nutritional and culinary specialty has been known a long time ago (Siggelkow, 1981). Also, the consumption of cheese and cheese products has been gradually increasing over the past few years. A great percentage of that increase has been in the consumption of cheese for snacks or lunches (Wendorff, 1981).

Although cheese is an excellent source of calcium and protein, it contains very little iron (Blanc, 1981). Fortification of iron in cheese would help meeting 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 cheese, the effects of iron fortification on oxidation of milk fat, and 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 L-ascorbic acid is a powerful enhancer of nonheme iron absorption (Lynch and Cook, 1980). Its influence may be pronounced in meals of iron availability. L-ascorbic acid

facilitates iron absorption by forming a chelate with ferric iron at acid pH that remains soluble at the alkaline pH of the duodenum. However, the addition of L-ascorbic acid influences on the quality of yogurt due to its high acid. Therefore, iron and L-ascorbic acid need microencapsulation.

Microencapsulation, which shows potential as carriers of enzymes in the food industry, could be a good vehicle for the addition of iron to milk (Bersen'eva et al., 1990; Jackson and Lee, 1991). 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-although several researchers have used coating materials such as milk fat, agar, and gelatin, etc. responsible for enzyme, flavor and iron microencapsulation in foods (Magee and Olson, 1981a, b; Braun and Olson, 1986), 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 L-ascorbic acid added Cheddar cheese on chemical and sensory aspects during ripening.

MATERIALS AND METHODS

Materials

For the microencapsulation of iron complex, polyglycerol monostearate (PGMS) was used as a coating

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material. It was purchased from Il-Shin Emulsifier Co., LTD. (Seoul, Korea). As core materials, water-soluble iron complex, ferric ammonium sulfate ($\text{FeNH}_4(\text{SO}_4)_2 \cdot 4\text{H}_2\text{O}$) 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 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 (Kim et al., 2003). Other experimental factors were as follows: the ratio of coating material to core material was 5 g:1 g 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 with 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 cyclinder 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 L-ascorbic acid were done in triplicate.

Treatments

Five different groups in this experiment were as followed: 1) no addition as control (C), 2) 20 ppm uncapsulated iron added (I), 3) 20 ppm microencapsulated iron added (MI), 4) 20 ppm microencapsulated iron and 100 ppm uncapsulated L-ascorbic acid added (MIUC), and 5) 20 ppm microencapsulated iron and 100 ppm microencapsulated added L-ascorbic acid (MIMC).

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 L-ascorbic acid was analyzed spectrophotometrically using DNP (2,4-dinitrophenyl hydrazine) test described (Korea Food Code, 2002). Samples were prepared immediately before analyses and kept cold and protected against daylight during analysis. A L-ascorbic acid stock solution was prepared daily by dissolving 10 mg of L-ascorbic acid in 100 mL of deionized

water (100 g/mL). It was diluted with deionized water to obtain the final concentration of 10, 20, 30, 40 and 50 g/mL. Total L-ascorbic acid was determined using the calibration graph based on concentration (g/mL) vs absorbance, prepared daily running fresh standard solutions:

Manufacture of Cheddar cheese

Cheese making process was described by Metzger and Mistry (1994). Regardless of microencapsulation, ferric ammonium sulfate and L-ascorbic acid were added right before rennet addition. After cheese manufacturing, cheeses were weighed, vacuum packaged in a barrier bag and ripened at 5°C for 0, 1, 3, 5 and 7 mo. The cheese samples stored in refrigerator for 12 h were 0 mo. The cheese making experiment was triplicate on different days using different batches of treatments.

Chemical composition and cheese yield

Cheese was analyzed for moisture, fat, protein and ash using the methods of Association of Official Analytical Chemists (AOAC, 1990). Cheese yield was determined as wt. cheese×100/wt. milk.

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 (brought into by neutralized with NaOH) and 2 M H_3PO_4 /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 throughly 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 4 M 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 an 1 cm light path. All measurements were run in triplicate.

Analysis of short-chain free fatty acid

Cheese samples (1 g) were removed periodically and extracted with diethylether and hexane for 2 hr and eluted through a 10mm i.d. glass column containing neutral alumina as described by Kwak et al. (1990). A Hewlett-Packard Model 5880A GC equipped with a flame ionization detector was used. The preparation of FFA was achieved using a 15 m×0.53 mm i.d. Nukol fused-silica capillary column (Supleco Inc., Bellefonte, PA, USA). The GC was operated with helium carrier gas at 2 ml/min, hydrogen gas

Table 1. Mean chemical composition of iron fortified Cheddar cheese¹

Treatment	Component (%)				
	Moisture	Fat	Protein	Ash	Yield
Control	35.0	26.4	18.0	3.7	10.6 ^a
Iron-treatment	36.0	25.0	19.1	4.0	8.8 ^b

¹ Means within column by the same capital letter are not significantly different ($p < 0.05$).

37 ml/min, and air at 300 ml/min. The column oven was programmed as an initial holding for 1 min at 110°C and first level holding to 180°C at 5°C/min for 10 min and holding for 20 min. Both temperatures for injector and detector were 250°C. All quantitative analyses were done by relating each peak area of individual FFA to the peak area of tridecanoic acid as an internal standard. Each FFA was identified by the retention time of standard.

Analysis of neutral volatile compounds

Samples of cheese (40 g) were removed periodically and added with 10 ml distilled water. Two ml of each distillate was used to take headspace gas sample as described by Bassette & Ward (1975). A Hewlett-Packard Model 5880A GC equipped with a flame ionization detector was used. Headspace gas samples were analyzed on a capillary column (Supelcowax 10, 30m×0.32 mm I.D. Bellefonte, PA, USA). The column was operated with nitrogen carrier gas at a flow rate of 1.2 ml/min; hydrogen gas flow rate was 30.0 ml/min; air was 300.0 ml/min. Temperature for both injector port and detector was maintained at 230°C. The column oven was programmed at three temperature levels: initial holding for 5 min at 35°C/min and heating to 140°C at 15°C/min, holding for 30 min. The concentrations of volatile compounds were estimated by analyzing cheese samples that contained the known concentrations and those of containing no added standards. The difference between the two treatments was used for the estimation of concentrations of individual volatile compounds.

Sensory analysis

Seven trained sensory panelists evaluated randomly coded cheeses. Texture was evaluated on a 5 point scale (1=poor to 5=excellent). Typical Cheddar cheese flavor, acid, and bitterness were scored on a 5 point scale (1=low intensity to 5=high intensity).

Statistical analysis

Data from the determination of optimum conditions of the cheese, one-way ANOVA (SAS Institute Inc., Cary, NC, USA 1985) was used. The significance of the results was analyzed by the least significant difference (LSD) test. Differences of $p < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Microencapsulation

In the present study, the yields of iron and L-ascorbic acid microencapsulation were 72 and 94%, respectively. In our laboratory, PGMS was appeared to be hard to spray, therefore, we found the optimum ratio of PGMS to deionized water to reduce the viscosity of PGMS solution. In our previous study, 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 (Kwak et al., 2001). In addition, Kim et al. (2003) reported the efficiencies of microencapsulation for iron was 73 and 95% for L-ascorbic acid.

The size of microencapsulated iron or L-ascorbic acid with PGMS was irregular from nano to micrometer, and the average size was in the range of 2 to 5 μm (pictures not shown). Microscopic examination of microcapsules revealed spherical particles. Microcapsules containing iron or L-ascorbic acid had smooth surfaces and evenly distributed pockets. The shape of the microcapsules was likely affected by encapsulated 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

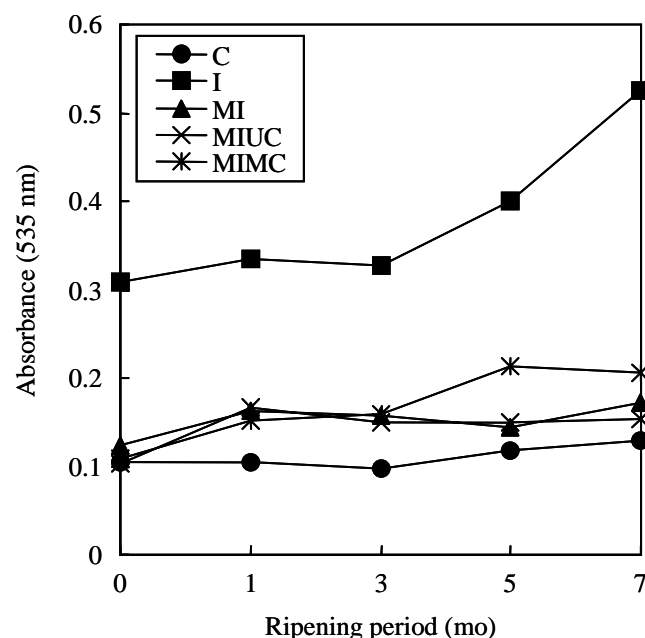


Figure 1. Changes of TBA absorbance in microencapsulated iron-fortified Cheddar cheese stored at 7°C for 7 mo. C; control (no addition), I; 20 ppm uncapsulated iron, MI; 20 ppm capsulated iron, MIUC; 20 ppm capsulated iron and 10 ppm uncapsulated L-ascorbic acid, MIMC; 20 ppm capsulated iron and 100 ppm uncapsulated L-ascorbic acid.

Table 2. Concentrations of short-chain fatty acids in iron and/or L-ascorbic acid fortified Cheddar cheese ripened at 7°C for 7 mo¹

Treat-ment	Ripening period (mo)	FFA concentration (ppm)				
		C4	C6	C8	C10	Total
C ²	0	24.4 ^b	45.2 ^b	47.3 ^b	51.3 ^c	168.2 ^b
	1	30.2 ^{ab}	54.4 ^b	54.9 ^b	58.4 ^c	197.9 ^b
	3	31.0 ^{ab}	68.0 ^{ab}	70.6 ^{ab}	96.6 ^{ab}	266.2 ^{ab}
	5	62.3 ^a	84.6 ^a	97.6 ^a	131.1 ^a	375.6 ^a
I ³	0	26.9 ^b	42.8 ^b	44.5 ^b	52.2 ^b	168.9 ^b
	1	28.2 ^b	43.8 ^b	43.3 ^b	49.6 ^b	180.7 ^b
	3	25.0 ^b	47.6 ^b	50.0 ^b	57.8 ^b	180.4 ^b
	5	56.7 ^a	73.5 ^{ab}	78.2 ^{ab}	83.6 ^{ab}	292.0 ^{ab}
MI ⁴	0	20.8 ^b	45.1 ^b	46.2 ^b	53.0 ^b	165.1 ^b
	1	26.1 ^b	50.7 ^b	59.3 ^b	72.4 ^b	208.5 ^b
	3	35.3 ^{ab}	70.5 ^{ab}	76.9 ^{ab}	109.6 ^{ab}	292.3 ^{ab}
	5	65.5 ^a	104.6 ^a	106.2 ^a	137.7 ^a	414.0 ^a
MIUC ⁵	0	19.3 ^b	40.6 ^b	41.2 ^b	44.2 ^b	145.3 ^b
	1	21.2 ^b	46.1 ^b	46.6 ^b	51.1 ^b	165.0 ^b
	3	27.6 ^b	67.1 ^{ab}	71.6 ^{ab}	95.2 ^{ab}	261.5 ^{ab}
	5	59.3 ^a	93.6 ^a	104.2 ^a	109.2 ^{ab}	366.3 ^a
MIMC ⁶	0	18.5 ^b	41.3 ^b	44.8 ^b	49.4 ^b	154.0 ^b
	1	19.5 ^b	47.8 ^b	51.1 ^b	59.2 ^b	178.0 ^b
	3	20.3 ^b	51.3 ^b	53.3 ^b	64.6 ^b	189.5 ^b
	5	62.1 ^a	85.9 ^a	86.7 ^a	85.2 ^{ab}	319.9 ^{ab}
	7	68.3 ^a	93.7 ^a	95.1 ^a	97.8 ^{ab}	354.9 ^a

¹ Means within column by the same capital letter are not significantly different ($p < 0.05$).

²C: control, ³I: iron added, ⁴MI: microencapsulated iron added, ⁵MIUC: microencapsulated iron and uncapsulated L-ascorbic acid added, ⁶MIMC: microencapsulated iron and microencapsulated L-ascorbic acid added.

cylindrical when the lipid coating was rapidly cooled and spherical when the lipid was slowly cooled.

Chemical composition and yield of Cheddar cheese

The composition of the cheese was presented in Table 1. Moisture content of cheese was ranged from 35.0 to 36.0%, fat from 25.0 to 26.4%, protein from 18.0 to 19.1% and ash from 3.7 to 4.0%. In the composition of the Cheddar cheeses, no difference was found between control and iron-fortified cheese. The yield of the treated cheese (8.8%) was lower than the control (10.6%). Ferric ammonium sulfate was 13.14 mg/100 g cheese and L-ascorbic acid was 77.3 mg/100 g cheese.

TBA test during ripening

The effect of iron fortification in Cheddar cheese on chemical oxidation (as measured by the TBA test) during 7 mo ripening was shown in Figure 1. In uncapsulated iron added group (I), TBA value increased dramatically from 0.31 (0 mo) to 0.53 (7 mo). In Comparison of microencapsulated iron added groups (MI, MIUC and

Table 3. The production of neutral volatile flavor compounds in iron and/or L-ascorbic acid fortified Cheddar cheese ripened at 7°C for 7 mo¹

Treat-ment	Ripening period (mo)	Acet-Aldehyde (ppm)	Acetone (ppm)	2-Buta-none (ppm)	Ethanol (ppm)	2-
						Hepta-none (ppm)
C ²	0	0.08 ^c	6.70 ^a	1.18 ^b	3.39 ^c	2.79 ^b
	1	0.18 ^b	6.68 ^a	1.19 ^b	6.40 ^c	2.86 ^b
	3	0.21 ^b	6.94 ^a	1.38 ^b	116.80 ^{ab}	2.78 ^b
	5	0.47 ^a	6.86 ^a	1.63 ^b	170.30 ^a	2.72 ^b
I ³	0	0.07 ^c	6.93 ^a	1.74 ^b	182.00 ^a	2.88 ^b
	1	0.21 ^b	6.71 ^a	1.20 ^b	5.54 ^c	2.82 ^b
	3	0.20 ^b	7.71 ^a	1.56 ^b	5.50 ^c	2.77 ^b
	5	0.50 ^a	6.74 ^a	1.69 ^b	73.77 ^b	2.68 ^b
MI ⁴	0	0.13 ^c	6.83 ^a	1.60 ^b	78.69 ^b	3.12 ^b
	1	0.23 ^b	6.84 ^a	1.69 ^b	96.25 ^b	2.94 ^b
	3	0.34 ^{ab}	6.68 ^a	1.18 ^b	4.77 ^c	2.79 ^b
	5	0.41 ^a	6.80 ^a	1.18 ^b	3.86 ^c	2.73 ^b
MIUC ⁵	0	0.16 ^b	6.85 ^a	2.96 ^a	50.11 ^c	2.94 ^b
	1	0.33 ^{ab}	6.77 ^a	1.32 ^b	127.70 ^{ab}	4.04 ^a
	3	0.23 ^b	6.87 ^a	1.42 ^b	134.13 ^{ab}	3.00 ^b
	5	0.52 ^a	6.78 ^a	1.19 ^b	12.00 ^c	2.94 ^b
MIMC ⁶	0	0.16 ^b	6.75 ^a	2.33 ^a	41.07 ^b	2.92 ^b
	1	0.23 ^b	6.73 ^a	2.50 ^a	79.55 ^b	2.81 ^b
	3	0.18 ^b	6.73 ^a	2.50 ^a	79.55 ^b	2.81 ^b
	5	0.18 ^b	6.91 ^a	1.96 ^b	126.33 ^{ab}	2.71 ^b
MIUC ⁵	0	0.16 ^b	6.79 ^a	2.20 ^a	132.65 ^{ab}	2.96 ^b
	1	0.20 ^b	6.68 ^a	1.19 ^b	4.63 ^c	2.78 ^b
	3	0.35 ^{ab}	6.78 ^a	2.05 ^a	5.63 ^c	2.86 ^b
	5	0.27 ^b	6.83 ^a	1.22 ^b	104.55 ^{ab}	2.65 ^b
MIMC ⁶	0	0.07 ^c	6.79 ^a	1.82 ^b	109.17 ^{ab}	2.70 ^b
	1	0.20 ^b	6.92 ^a	1.98 ^b	116.21 ^{ab}	2.52 ^b
	3	0.35 ^{ab}	6.92 ^a	1.98 ^b	116.21 ^{ab}	2.52 ^b
	5	0.27 ^b	6.92 ^a	1.98 ^b	116.21 ^{ab}	2.52 ^b

¹Means within column by the same capital letter are not significantly different ($p < 0.05$).

²C: control, ³I: iron added, ⁴MI: microencapsulated iron added, ⁵MIUC: microencapsulated iron and uncapsulated L-ascorbic acid added, ⁶MIMC: microencapsulated iron and microencapsulated L-ascorbic acid added.

MIMC), no difference was found as 0.12 (0 mo) and 0.16 (7 mo). When compared with microencapsulated iron with uncapsulated L-ascorbic acid (MIUC) and microencapsulated iron with microencapsulated L-ascorbic acid (MIMC), the TBA value was not significantly different during 7 mo ripening periods.

In this experiment, TBA absorbance was significantly lower in capsulated groups than those in uncapsulated group, regardless of iron and L-ascorbic acid, during storage. These data indicated that oxidation process may be faster in cheese samples containing uncapsulated iron than in those containing microencapsulated iron.

Our previous study (Kwak et al., 2001) showed the effect of iron fortification in milk on chemical oxidation during 15 d storage. We reported that TBA absorbance was significantly lower in capsulated group than that in uncapsulated group at 15 d. Similar result was observed in another study (Kim et al., 2003) indicating oxidation process may be faster in yogurt samples containing

uncapsulated iron than in those containing microencapsulated iron.

Jackson and Lee (1991) indicated that samples containing uncapsulated iron (ferrous sulfate and ferric chloride) showed 2-3 times high in fatty acid production, compared with those containing microencapsulated iron complex when milk fat was used as a coating material. The reason why iron fortification caused several modifications in dairy products could be explained that added iron may interact with casein, resulting in iron-casein complexes and the presence of O₂ acts as a preoxidant, therefore, lipid oxidation in Cheddar cheese can be accelerated.

Production of short-chain free fatty acids (FFA)

It is well known that short-chain free fatty acids (C₄ through C₁₀) constitute the backbone of Cheddar flavor (Lin and Jeon, 1987). Therefore, the production of short-chain FFA profiles was considered to be an important aspect in this study. The productions of short-chain FFA in control and experimental cheeses ripened at 7°C for 7 mo were shown in Table 2. Among control and treatments, no difference was found ($p>0.05$) at every period points.

During 7 mo ripening period, the total release of short-chain FFA production was not significantly different from 0 and 1 mo ripening period, however, the release increased from 3 mo ripening in groups C, MI, and MIUC. Total amount of short-chain FFA was in the range of 324.5 to 428.0 ppm. These results indicated that lipolysis process, which contributes the development of the short-chain FFA, in iron-fortified cheese was not different from in control.

Production of neutral volatile flavor compounds

The production of neutral volatile compounds was observed in iron-fortified cheese in Table 3. In groups containing no L-ascorbic acid (C, I, and MI), acetaldehyde production increased steadily up to 0.50-0.62 ppm at 7 mo. In comparison, L-ascorbic acid containing groups, regardless of microencapsulation (MIUC and MIMC), acetaldehyde production was 0.26-0.35 at 7 mo ripening.

Ethanol production was the highest among flavor compounds measured and showed a similar trend in all groups. Also, the ethanol production increased dramatically after 1 mo upto 7 mo in all groups.

Other neutral flavor compounds detected were acetone,

Table 4. Sensory characteristics in iron and/or L-ascorbic acid fortified Cheddar cheese ripened at 7°C for 7 mo¹

Treat-ment	Ripening period (mo)	Sensory description							
		Bitter	Acidic	Astrin-gency	Metallic	Color	Cheddar flavor	Texture	Overall
C ²	0	3.00 ^b	3.00 ^b	3.00 ^b	3.00 ^b	3.00 ^{ab}	3.00 ^c	3.00 ^{ab}	3.00 ^b
	1	3.17 ^b	3.00 ^b	3.17 ^b	2.83 ^c	3.33 ^{ab}	3.50 ^b	3.00 ^{ab}	3.33 ^{ab}
	3	3.33 ^b	3.17 ^b	3.17 ^b	3.00 ^b	3.50 ^a	3.50 ^b	3.15 ^{ab}	3.50 ^a
	5	3.33 ^b	3.33 ^b	3.33 ^b	3.17 ^b	3.50 ^a	3.67 ^{ab}	3.33 ^a	3.50 ^a
	7	3.50 ^b	3.50 ^{ab}	3.33 ^b	3.17 ^b	3.67 ^a	3.83 ^{ab}	3.50 ^a	3.50 ^a
I ³	0	3.33 ^b	3.00 ^b	3.33 ^b	3.33 ^b	2.17 ^{bc}	3.00 ^c	2.83 ^b	2.17 ^c
	1	4.67 ^a	3.17 ^b	3.67 ^{ab}	3.83 ^a	1.50 ^c	3.83 ^{ab}	3.17 ^{ab}	1.83 ^{cd}
	3	4.50 ^a	3.33 ^b	3.83 ^a	3.67 ^{ab}	1.83 ^c	3.83 ^{ab}	3.00 ^{ab}	1.33 ^d
	5	4.16 ^{ab}	3.33 ^b	3.83 ^a	3.83 ^a	2.67 ^b	2.67 ^c	2.83 ^b	2.33 ^c
	7	4.30 ^a	3.40 ^{ab}	4.00 ^a	4.15 ^a	1.25 ^c	1.50 ^d	2.25 ^c	1.00 ^e
MI ⁴	0	2.83 ^{bc}	3.00 ^b	2.83 ^b	2.83 ^c	3.17 ^{ab}	3.67 ^{ab}	3.67 ^a	2.67 ^{bc}
	1	4.17 ^{ab}	3.00 ^b	3.17 ^b	3.00 ^b	3.50 ^a	3.50 ^b	2.67 ^b	3.00 ^b
	3	4.33 ^a	3.50 ^{ab}	3.17 ^b	3.33 ^b	3.33 ^{ab}	3.50 ^b	2.33 ^c	2.83 ^{bc}
	5	3.67 ^{ab}	3.50 ^{ab}	3.50 ^{ab}	3.50 ^{ab}	3.00 ^{ab}	4.17 ^a	2.67 ^b	3.00 ^b
	7	3.75 ^{ab}	3.67 ^a	3.75 ^{ab}	3.75 ^a	3.50 ^a	4.33 ^a	2.25 ^c	3.25 ^{ab}
MIUC ⁵	0	2.83 ^{bc}	3.33 ^b	2.50 ^c	2.33 ^c	3.17 ^{ab}	2.83 ^c	3.67 ^a	2.67 ^{bc}
	1	3.83 ^{ab}	3.50 ^{ab}	3.17 ^b	2.33 ^c	3.17 ^{ab}	3.33 ^{bc}	3.00 ^{ab}	3.00 ^b
	3	2.83 ^{bc}	3.67 ^a	3.33 ^b	2.67 ^c	3.17 ^{ab}	3.33 ^{bc}	3.33 ^a	2.33 ^c
	5	2.67 ^{bc}	3.67 ^a	3.33 ^b	3.17 ^b	2.67 ^b	3.17 ^c	3.33 ^a	3.33 ^{ab}
	7	2.25 ^c	4.00 ^a	3.50 ^{ab}	3.33 ^b	1.75 ^c	3.00 ^c	3.00 ^{ab}	3.75 ^a
MIMC ⁶	0	2.83 ^{bc}	3.17 ^b	2.50 ^c	2.67 ^c	3.50 ^a	3.00 ^c	2.33 ^c	3.00 ^b
	1	3.83 ^{ab}	3.17 ^b	3.17 ^b	3.00 ^b	3.33 ^{ab}	3.50 ^b	2.33 ^c	3.33 ^{ab}
	3	3.17 ^b	3.33 ^b	3.83 ^a	3.00 ^b	3.67 ^a	3.83 ^{ab}	2.67 ^b	3.00 ^b
	5	3.50 ^b	3.33 ^b	3.83 ^a	3.33 ^b	3.17 ^{ab}	3.83 ^{ab}	2.00 ^c	3.20 ^{ab}
	7	3.75 ^{ab}	3.75 ^a	3.83 ^a	3.33 ^b	3.00 ^{ab}	3.83 ^{ab}	1.75 ^d	3.33 ^{ab}

¹Means within column by the same capital letter are not significantly different ($p<0.05$). Initial sensory score of control was 3. The scale of bitterness, acidic, astringency, metallic, and color, Cheddar flavor, texture, overall scores: 1=none, 3=moderate, 5=very strong. The scale of overall scores: 1=dislike very much, 3=neither like nor dislike, 5=like very much.

²C: control, ³I: iron added, ⁴MI: microencapsulated iron added, ⁵MIUC: microencapsulated iron and uncapsulated L-ascorbic acid added, ⁶MIMC: microencapsulated iron and microencapsulated L-ascorbic acid added.

2-butanone, and 2-heptanone. During 7 mo ripening, these compound productions were not significantly different in neither by ripening periods nor within 5 different groups. This study indicated that neutral volatile flavor compounds in iron-fortified Cheddar cheese were not different from that of the control Cheddar cheese.

Sensory analysis

The sensory characteristics in five treatments were shown in Table 4. For bitter taste, it was not significantly different among treatments during 7 mo ripening. However, Group I, which was uncapsulated iron-fortified cheese, showed a significant increase in bitter taste at 1 mo and thereafter. Also, microencapsulated iron-fortified Cheddar cheese (MI) showed a higher score at 3 mo ripening, compared with those of other groups.

For acidic taste, L-ascorbic acid added groups (MIUC and MIMC, regardless of iron microencapsulation) showed significantly higher scores at 3 mo, and at 7 mo, respectively, than those of others. For astringency, uncapsulated iron-fortified group (I), and microencapsulated iron and L-ascorbic acid-fortified group (MIMC) showed higher scores at 3 mo and thereafter than other values.

For metallic taste, iron-fortified groups without L-ascorbic acid (I and MI), regardless of microencapsulation, showed a higher score. Especially, uncapsulated iron-fortified group (I) increased dramatically the metallic taste even at 1 mo.

The major difference among control and experimental groups was observed in color. Uncapsulated iron and L-ascorbic acid added groups (I and MIUC) showed a profound color change to yellowish green. Group I was highly changed from 0 mo upto 7 mo ripening, while group MIUC showed color change at 5 mo ripening and thereafter. Interestingly, no difference was found between control and microencapsulated iron-added groups regardless of L-ascorbic acid (MI and MIMC) through all ripening periods.

Cheddar flavor was developed without a difference with ripening period in all groups, except for uncapsulated iron-fortified cheese (I) ripened at 5 and 7 mo ripenings. In uncapsulated iron-fortified Cheddar cheese, Cheddar flavor decreased with ripening time. Texture score increased with ripening period in control, however, those decreased in all experimental cheeses.

For overall preference test, control (C) and microencapsulated iron (MI) and/or L-ascorbic acid (MIUC and MIMC) containing treatments showed a high consumer preference in all storage periods. However, the scores of uncapsulated iron containing group (I) were dramatically lower compared with those of other treatments in all ripening periods. This result indicated that the process of microencapsulation was very effective to mask off-taste and off-flavor of iron and L-ascorbic acid.

The sensory quality of iron-fortified dairy foods has been shown to be effective by the microencapsulation of both iron and L-ascorbic acid. Two major off-flavors have been associated with dairy products: oxidized flavor resulted from catalysis of lipid oxidation by iron, and sourness contributed by L-ascorbic acid.

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 were 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 or L-ascorbic acid) to distilled water showed a high efficiency of microencapsulation as 72% and 94%, respectively. Our results indicated that lipid oxidation process measured by TBA test was significantly slower in capsulated iron than in uncapsulated iron-fortified Cheddar cheese. In sensory, we need to point out that no significantly adverse effects was found in microencapsulated iron and L-ascorbic acid-fortified Cheddar cheese during 7 mo ripening in this experiment. Therefore, the present study provides an important evidence that microcapsules of iron and L-ascorbic acid were an effective means of fortification, and can be applied to Cheddar cheese without any changes in sensory aspects.

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REFERENCES

- AOAC. Official methods of Analysis, 15th Ed. Association of Official Analytical Chemists: Arlington, VA, U.S.A. 1990; Method 948. 12, pp. 840-844.
- Basatte, R. and G. Ward. 1975. Measuring part per billion of volatile materials in milk. *J. Dairy Sci.* 58:428-430.
- Bersen'eva, E. A., A. A. Inanoy, T. P. Sansonova, E. M. Chernova and N. I. Oragvelidze. 1990. Microencapsulated aromatizers for tea. *Pishchevaya Paomyshlennost, USSR.* 1:57-59.
- Blanc, B. 1981. Biochemical aspects of human milk comparison with bovine milk. *World Rev. Nutr. Diet.* 36:1.
- Braun, S. D. and N. F. Olson. 1986. Encapsulation of proteins and peptides In milk fat: Encapsulation efficiency and temperature and freezing stability. *J. Microencapsulation.* 3(2): 115-126.

- Gaucheron, F. 2000. Iron fortification in dairy industry. Trends in Food Science and Technology. 11:403-409.
- Hegenauer, J., P. Saltman, D. Ludwig, L. Ripley and P. Bajo. 1979. Effects of supplemental iron and copper on lipid oxidation in milk. 1. Comparison of metal complexes in emulsified and homogenized milk. J. Agric. Food Chem. 27(4):860-867.
- Hekmat, S. and D. J. McMahon. 1997. Manufacture and quality of iron-fortified yogurt, J. Dairy Sci. 80:3114-3122.
- Jackson, L. S. and K. Lee. 1991. Microencapsulated iron for food fortification. J. Food Sci. 56:1047-1050.
- Kim, S. J., J. Ahn, J. S. Seok and H. S. Kwak. 2003. Microencapsulated iron for drink yogurt fortification. Asian-Aust. J. Anim. Sci. 16(4):581-587.
- Korea Food Code. 2002. pp. 321-323.
- Kwak, H. S., I. J. Jeon. and J. Park. 1990. Effects of food grade porcine pancreatic lipase on the production of short-chain fatty acids and its contribution. Kor. J. Food Sci. and Technol. 22:248-254.
- Kwak, H. S., M. R. Ihm and J. Ahn. 2001. Microencapsulation of β -galactosidase with fatty acid esters. J. Dairy Sci. 84:1576-1582.
- Lin, J. C. C. and I. J. Jeon. 1987. Effects of commercial food grade enzymes on fatty acid profiles in granular cheese. J. Food. Sci. 52:72-82.
- Lynch, S. R. and J. D. Cook. 1980. Interaction of vitamin C and iron. Annals of the New York Academy of Sciences. 365:32-44.
- Magee, E. L. Jr. and N. F. Olson. 1981a. Microencapsulation of cheese ripening systems: Formation of microcapsules. J. Dairy Sci. 64:600-610.
- Magee, E. L. Jr. and N. F. Olson. 1981b. Microencapsulation of cheese ripening systems: Stability of microcapsules. J. Dairy Sci. 64:611-615.
- Metzger, L. E. and V. V. Mistry. 1994. A new approach using homogenization of cream in the manufacture of reduced fat Cheddar cheese. I. Manufacture, composition, and yield. J. Dairy Sci. 77:3506-3515.
- SAS Institute Inc. 1989. SAS/STAT User's Guide: Version 6. 4th edn. SAS Institute Inc., Cary, North Carolina.
- Siggelkow, M. A. 1981. The continual process in soft cheese making. Proceedings from the second biennial Marschall international cheese conference. Madison, Wisconsin, U.S.A. 16:102-106.
- Wendorff, W. L. 1981. Current developments in the use of natural smoke flavors in cheese. Proceedings from the second biennial Marschall international cheese conference. Madison, Wisconsin, U.S.A. 22:149-154.
- Woestyne, M. V., B. Bruyneel, M. Mergeay and W. Verstraeta. 1991. The Fe chelator proferrerosamine A is essential for the siderophore-mediated uptake of iron by *Psuedomonas fluorescences*. Appl. Environ. Microbiol. 57:949.