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The Effects of Microencapsulated Chitooligosaccharide on Physical and Sensory Properties of the Milk

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ABSTRACT : Effects of microencapsulated chitooligosaccharide addition in milk were evaluated by determination of the efficiency of microencapsulation, cholesterol removal, color, viscosity and sensory properties. Coating material was polyglycerol monostearate (PGMS) and the efficiency of microencapsulation was 88.08% at a 10:1 ratio of coating to core materials (w/w). When 0.5% of microencapsulated chitooligosaccharide was added into milk, the color values (L, a, and b) and viscosity were significantly different from those of noncapsulated chitooligosaccharide-added groups ($p<0.05$). The release of chitooligosaccharide from microcapsules was 7.6% in milk at 4°C for 15-day storage. In both 0.5 and 1.5% microencapsulation addition, the scores of all sensory characteristics except for off-flavor were significantly different between encapsulated chitooligosaccharide and noncapsulated chitooligosaccharide-added groups during all periods of storage. The present study indicated that chitooligosaccharide microcapsules could be applicable into commercial milk with little adverse effects on physical and sensory properties. (**Key Words :** Chitooligosaccharide, Microencapsulation, Milk)

INTRODUCTION

Chitosan, as one of the most abundant biochemical substances, is an excellent material utilizable in the biomedical, pharmacological, agricultural and biotechnological applications. Because of the unique properties of chitosan for their use, many scientists have isolated, purified and modified these biopolymers and evaluate their behavior. Futhermore, the hypolipidemic and hypocholesterolemic activity of chitosan in rats has been broadly reported (Sugano et al., 1978; Nagyvary et al., 1979). Since a strong positive correlation exists between increased serum cholesterol concentrations and risk of coronary heart disease, consumers are greatly concerned about high intake of cholesterol (Grundy et al., 1982; Gurr, 1992). The hypocholesterolemic action of orally administratered chitosan was first reported by Sugano et al. (1978), and has been confirmed later by several investigators with an increase of the excretion of cholic acid into feces in rats and in human (Kobayashi et al., 1979; Maezaki et al., 1993). One of the main reasons for the applicability of this substance may be its insolubility in practical solvents (Knorr, 1984). For this aim, the food processing industry uses polysaccharides to alter or control functional properties of foods (OTA, 1982). Even though chitooligosaccharide is a fine functional food ingredient for use in various foods, it is not suitable to apply into milk due to its bitter taste, off-flavor and color. To overcome this problem, microencapsulation technique could be used.

Microencapsulation shows a potential as carrier of enzymes in the food industry, which could be a good vehicle for the addition of chitooligosaccharide (Jackson and Lee, 1991). Recently, a considerable interest has been evolved in developing encapsulated nutrients, flavors and enzyme systems. 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. Ideally, oral delivery systems designed to transport a compound of interest through the stomach would be characterized as having a high encapsulation efficiency, provide maximal stability and therefore, limited release in acidic pH ranges and rapid release in neutral conditions (Vandenberg et al., 2001).

In our previous studies (Kwak et al., 2001; Kwak et al., 2003), there has been shown that emulsifiers can be used as

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an effective material to microencapsulate lactase, iron, water-soluble isoflavone and ascorbic acid. Results from those studies also have indicated that microcapsules would be convenient tools for fortified milk due to an increase of nutrient absorption by favoring the uptake and effective release in the intestine.

To manufacture cholesterol-reduced milk, β-cyclodextrin (β-CD) was used, which has positive attributes because β-CD is nontoxic, edible, non-hygroscopic, chemically stable and easy to separate (Nagamoto, 1985) and has proven in several other studies (Oakenfull and Sidhu 1991; Makoto et al., 1992; Ahn and Kwak, 1999; Lee et al., 1999; Shim et al., 2004; Hwang et al., 2005). Therefore, the objectives of this study were to evaluate effects of microencapsulated chitooligosaccharide on physical and sensory properties on milk and create a new healthy milk with hypocholesterolemic effect by providing microencapsulated chitooligosaccharide.

MATERIALS AND METHODS

Preparation of milk and chemicals

Raw milk (3.5% milk fat) was obtained from Binggre Dairy Plant (Kyonggi-do, Korea). Chitooligosaccharide was purchased from Kaidi Fine Chemical Industrial Co., Ltd. (Wuhan, China). Commercial β-cyclodextrin (purity 99.1%) was purchased from Nihon Shokuhin Kaku Co., Ltd. (Osaka, Japan). Cholesterol, 5-α cholestane and other materials were purchased from Sigma Chemical Co. (St Louis, MO, USA) and all solvents were gas chromatographic grade.

Preparation of cholesterol-reduced milk

Bulk raw milk was stirred with 1% β-CD at 800 rpm with a blender (Tops: Misung Co., Seoul, Korea) in a temperature-controlled water bath at 10°C (Kwak et al., 2003). To separate cholesterol-entrapped β-CD, each sample was centrifuged with 166×g for 15 min at 20°C (Kwak et al., 2003).

Determination of cholesterol

Cholesterol was extracted from the milk sample using the method of Adams et al. (1986) and stored at -20°C until analysis. Total cholesterol was determined on a fused silica capillary column (HP-5, 30 m×0.32 mm I.D.×0.25 μm thickness) using Hewlett-Packard 5890A gas chromatograph (Palo Alto, CA, USA) equipped with a flame ionization detector. The temperatures of injector and detector were 270 and 300°C, respectively. The oven temperatures were programmed from 200 to 300°C at 10°C/min and held for 20 min. Nitrogen was used as a carrier gas at a total flow rate of 30 ml with a split ratio of 1/50. Quantitation of cholesterol was performed by comparing the peak areas with a response of an internal standard (5-α cholestane).

The percentage of cholesterol reduction was calculated as followed: cholesterol reduction (%) = 100-(amount of cholesterol in β-CD-treated milk×100/amount of cholesterol in market milk). Cholesterol determination for control was averaged with 3 batches of treatment.

Preparation of microcapsule

Microcapsules of chitooligosaccharide were made by polyacylglycerol monostearate (PGMS), which was selected as a major coating material from our previous study (Kwak et al., 2001). Subsequently, the spray solution containing different ratios of coating to core materials as 5:1, 10:1, 15:1 and 20:1 (w/w) with ten times distilled water of core material was prepared.

The spray solution was heated at 55°C for 20 min, and mixed thoroughly with stirring at 1,200 rpm. An airless paint sprayer (W-300, Wagner Spray Tech. Co., Markdorf, Germany) nebulized a coating material-chitooligosaccharide 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 2,490×g for 10 min at 20°C (HMR-220IV, Hanil Industiral Co., Seoul, Korea) to separate chitooligosaccharide microcapsules. The microencapsulation of chitooligosaccharide was done in triplicate.

Efficiency of chitooligosaccharide microencapsulation

The dispersion fluid of microencapsulation was assayed for untrapped chitooligosaccharide by PGMS. One milliliter of the dispersion fluid was measured in triplicates at 530 nm by spectrophotometer (DU 650, Beckman Instruments Inc., Fullerton, CA, USA).

Color measurement on milk samples

Samples containing 0.5, 1.0, 1.5 and 2.0% encapsulated or uncapsulated chitooligosaccharide were measured for color measurement at 4°C using a colorimeter (Minolta CT-310, Konica Minolta Sensing, Inc., Osaka, Japan). Each product was analyzed in 5 replicate for Hunter L-values (100, perfect white; 0, black) for lightness, a-values (+, redness; -, greenness) and b-values (+, yellowness, -, blueness) for chromaticities.

Viscosity of milk samples

Samples containing 0.5, 1.0, 1.5 and 2.0% encapsulated or noncapsulated chitooligosaccharide were measured for viscosity using Brookfield viscometer (Model LVDV I+ Version 30, Stinghton, MA, USA) with spindle No. 2 at 100 rpm at 4°C.

Table 1. Efficiency of chitooligosacchride microencapsulation with different ratios of PGMS to chtooligosaccharide[1]

PGMS[2]	Ratio (w/v) Chitooligosaccharide	Efficiency (%)
5	1	83.67±1.7[b]
10	1	88.08±2.1[a]
15	1	84.84±1.9[b]
20	1	80.49±1.2[c]

[1] Means of triplicate. Means in a column without the same letter are significantly different ($p<0.05$).
[2] Polyacylglycerol monostearate.

Stability of microcapsules

To measure the stability of chitooligosaccharide, 10 ml of commercial milk was added into the same amount of microcapsule solution (8 g encapsulated chitooligosaccharide containing 1 g chitooligosaccharide/100 ml), stored at 4, 20 and 30°C for 15 days and measured the stability at 3 day interval. The samples were centrifuged, the supernatant was collected and the amount of chitooligosaccharide released from the microcapsules was analyzed in triplicates using a spectrophotometer (DU 650, Beckman Instruments Inc., Fullerton, CA, USA) at 530 nm.

Sensory analysis

Commercial whole milk (at every period) containing 0.5, 1, 1.5 and 2% encapsulated or noncapsulated chitooligosaccharide was stored at 4°C for 0, 3, 5, 8, 12 and 15 days. Seven semi-trained sensory panelists were 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 astringency, bitterness, yellowish, off-flavor, and viscosity were scored on a seven-point scale (1 = very slight, 3 = slight, 5 = strong and 7 = very strong), and overall quality were also scored on a seven-point scale (1 = dislike extremely, 3 = dislike moderately, 5 = like moderately and 7 = like extremely).

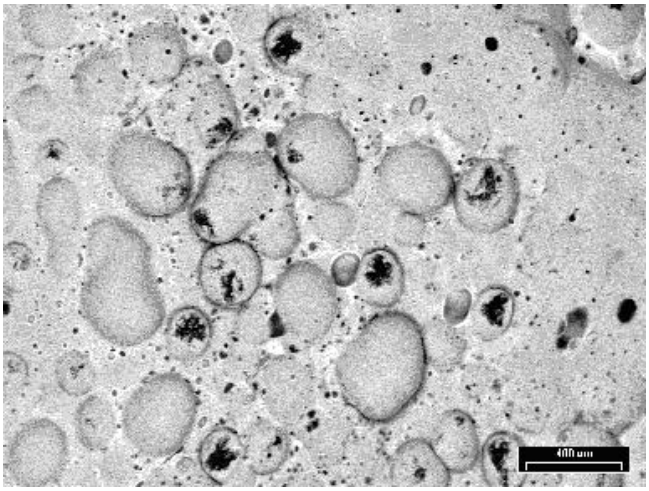

Figure 1. Photomicrograph of microencapsulated chitooligosaccharide with polyacylglycerol monostearate. The photograph was taken at 50×magnification.

Scanning electron microscopy (SEM)

For visualization of the morphological characterization of the extruded product, the microcapsules were freeze-dried, coated with silver using an ion sputting device (E-1030, Hitachi, Tokyo, Japan) and observed using a scanning electron microscope (JSM 5410LV, Jeol, Japan) at 25 kV and 15 tilt.

Statistical analysis

Experimental data from the milk samples, one-way ANOVA using a SAS program (1985) was used. The significance of the results was analyzed by the least significant difference (LSD) test. Difference of $p<0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Experimental groups were as followed: 1) Control; control as the market milk without β-CD treatment and

Table 2. Effect of different concentrations of encapsulated or noncapsulated chitooligosaccharide on color in cholesterol-reduced milk[1]

Treatment	Concentration (%)	L-value	a-value	b-value
Control[2]	0	5.7±0.2[a]	0.02±0.0[f]	3.49±0.2[a]
Noncapsulated	0.5	2.5±0.1[f]	4.04±0.1[b]	1.56±0.1[f]
	1.0	1.6±0.1[g]	5.89±0.1[a]	0.79±0.0[g]
	1.5	NA[4]	NA	NA
	2.0	NA	NA	NA
Encapsulated[3]	0.5	5.3±0.3[b]	1.14±0.1[e]	3.25±0.3[b]
	1.0	4.9±0.1[c]	2.14±0.1[d]	2.87±0.2[c]
	1.5	4.4±0.1[d]	2.44±0.1[d]	2.75±0.1[d]
	2.0	4.0±0.1[e]	3.19±0.2[c]	2.54±0.2[e]

[1] Means of 5 replicate. Means in a column without the same letter are significantly different ($p<0.05$).
[2] Market milk stored at 4°C for a day.
[3] Microencapsulated chitooligosaccharide by polyacylglycerol monostearate.
[4] NA: not available due to too dark samples.

Table 3. Effect of different concentrations of encapsulated or noncapsulated chitooligosaccharide on viscosity in cholesterol-reduced milk[1]

Treatment	Concentration (%)	Viscosity (cps)
Control	0	0.75±0.1[f]
Noncapsulated	0.5	3.24±0.4[d]
	1.0	30.27±1.7[c]
	1.5	43.00±1.1[b]
	2.0	49.30±1.0[a]
Encapsulated	0.5	0.79±0.1[f]
	1.0	1.34±0.1[e]
	1.5	1.59±0.0[e]
	2.0	3.88±0.1[d]

[1] Means of triplicate. Means in a column without the same letter are significantly different ($p<0.05$).
[2] Market milk stored at 4°C for a day.
[3] Microencapsulated chitooligosaccharide by polyacylglycerol monostearate.

chitooligosaccharide addition, 2) Noncapsulated; noncapsulated chitooligosaccharide-added after β-CD treatment and 3) Encapsulated; encapsulated chitooligosaccharide-added after β-CD treatment. Cholesterol-reduced milk was manufactured by 1% β-CD addition, mixing for 10 min with 800 rpm at 10°C, and centrifuged 166×g for 10 min at 10°C.

The rate of cholesterol removal and the efficiency of microencapsulation

The removal of cholesterol in milk sample reached 92.1% (Lee et al., 1999). When the ratio of polyacylglycerol monostearate (PGMS) to chitooligosaccharide was 10:1 (w/w) with 50 ml of distilled water, the efficiency of the microencapsulation increased up to 88.08%, the highest value and decreased thereafter (Table 1). Therefore, the optimum ratio of PGMS to chitooligosaccharide was found to be 10:1.

Several studies (Kwak et al., 2003; Lee et al., 2003; Lee et al., 2004) have reported the possibility of PGMS as a coating material for efficient formation of microcapsules. When iron was microencapsulated by PGMS, the efficiency was 75% and rate of release from microcapsules were dramatically increased in simulated intestinal fluid (Kwak et al., 2003). Other study using ascorbic acid as a core material (Lee et al., 2003) indicated the similar results, which indicated that 94.2% of microencapsulation efficiency was found and most sensory aspects were not significantly different from the market milk and ascorbic acid fortified milk during 5 day storage (Lee et al., 2004). Those studies indicated that PGMS can be effective means for fortifying milk by efficient microencapsulation and for enhancing bioavailability of core materials.

Microscopic observation

A microphotograph of microencapsulated chitooligosaccharide with PGMS is shown in Figure 1. The average size was in the range of 50-200 μm. Microscopic examination of microcapsules containing chitooligosaccharide revealed spherical and smooth surface particles and evenly distributed pockets of chitooligosaccharide solution.

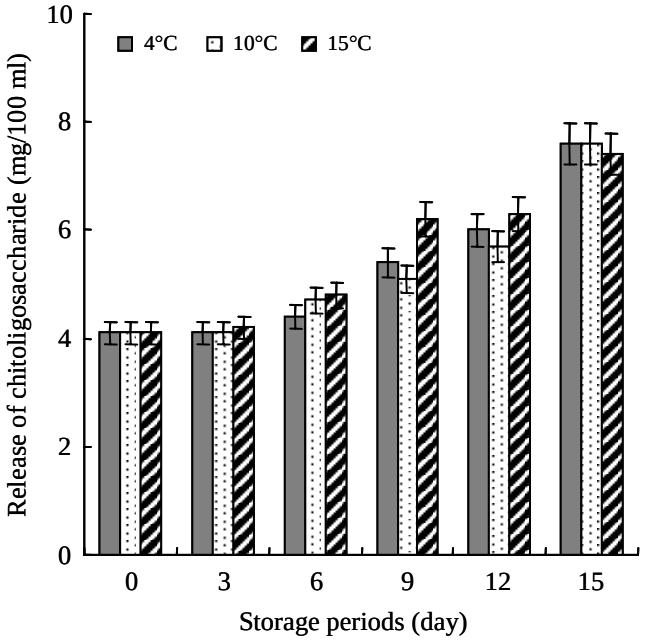

Figure 2. Effects of time and temperature on the release of chitooligosaccharide from microcapsules stored for 15 days in cholesterol-reduced milk.

For chitooligosaccharide addition into 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 the textural changes in the food system may be avoided. The present study showed the sufficiently small size of microcapsules and smooth surface of milk after fortifying chitooligosaccharide microcapsules. The shape of microcapsules was likely affected by encapsulating conditions (Magee and Olson, 1981; Braun and Olson, 1986), therefore, the optimum conditions may be applied for the present study.

Color of milk samples

The color values of milk samples with the addition of different amounts encapsulated or noncapsulated chitooligosaccharide were shown in Table 2. The color values were changed dramatically with noncapsulated chitooligosaccharide, while a slight change in color values was found with encapsulated chitooligosaccharide. The color values could not be measured in milk samples containing more than 1.5% noncapsulated chitooligosaccharide.

Regardless of microencapsulation, the L-value in

chitooligosaccharide-added groups decreased with an increased amount of chitooligosaccharide. Especially, noncapsulated chitooligosaccharide-addition showed a markedly lower L-value, compared with those of encapsulated chitooligosaccharide groups and control. More than 1.5% of noncapsulated chitooligosaccharide addition resulted in too low value to be measured. Even though L-value was significantly lower in encapsulated chitooligosaccharide-added group than that in control ($p< 0.05$), the difference was smaller, compared to that between noncapsulated chitooligosaccharide-added and control groups.

The a-value increased proportionally to both encapsulated and noncapsulated chitooligosaccharide additions, which indicated more brown color with higher amount of chitooligosaccharide. Also, the milk samples containing over 1.5% noncapsulated chitooligosaccharide was too dark to be measured. Since chitooligosaccharide normally is brown, chitooligosaccharide addition resulted in more intense change of a-value. The b-value change in samples was adverse to that of a-value. These results indicated that the changes of color value were remarkably masked by microencapsulation, although difference was found in color values between control and encapsulated chitooligosaccharide-added groups.

Viscosity of milk samples

When 0.5% of noncapsulated or encapsulated chitooliogosaccharide was added into milk samples, viscosity was 3.24 or 0.79 cps, respectively (Table 3). The viscosity of milk with noncapsulated chitooligosaccharide addition was significantly different from those of control and encapsulated chitooligosaccharide-added group ($p< 0.05$). With an increasing rate of chitooliogosaccharide addition, the viscosity increased dramatically. Since there was no difference between 0.5% of encapsulated chitooliogosaccharide-added group and control, this result may indicate that microencapsulation of

Table 4. Sensory scores of cholesterol-reduced milk containing different concentrations of encapsulated or noncapsulated chitooligosaccharide at refrigerated temperature for 15 day storage[1]

Sensory description	Treatment		Storage period (day)					
			0	3	6	9	12	15
Astringency	Control		4.0^{a}	4.1^{a}	3.9^{a}	4.1^{a}	4.0^{a}	4.2^{a}
	Noncapsulated	0.5%	4.9^{c}	4.8^{b}	5.0^{b}	5.1^{b}	4.7^{b}	5.2^{b}
		1.5%	6.1^{c}	6.3^{c}	6.1^{c}	6.2^{c}	6.2^{c}	6.0^{c}
	Encapsulated	0.5%	3.9^{a}	4.1^{a}	4.1^{a}	4.3^{a}	4.1^{a}	4.1^{a}
		1.5%	4.3^{b}	4.2^{a}	4.5^{a}	4.4^{a}	4.3^{a}	4.4^{a}
Bitterness	Control		4.1^{a}	4 .0^{a}	4.0^{a}	4.1^{a}	3.9^{a}	4.2^{a}
	Noncapsulated	0.5%	5.0^{b}	4.7^{b}	4.9^{b}	5.0^{b}	4.9^{b}	5.2^{b}
		1.5%	6.1^{c}	6.1^{c}	5.9^{c}	5.6^{c}	6.0^{c}	5.8^{c}
	Encapsulated	0.5%	4.0^{a}	3.7^{a}	4.0^{a}	4.1^{a}	4.0^{a}	4.0^{a}
		1.5%	4.6^{ab}	4.0^{a}	4.4^{a}	4.0^{a}	4.3^{a}	4.3^{a}
Off-flavor	Control		4.1^{a}	4.0^{a}	4.0^{a}	4.1^{a}	3.9^{a}	4.1^{a}
	Noncapsulated	0.5%	4.1^{a}	4.1^{b}	4.3^{b}	4.2^{a}	4.3^{a}	4.3^{a}
		1.5%	5.4^{b}	4.5^{c}	5.1^{c}	4.4^{b}	5.1^{b}	4.8^{b}
	Encapsulated	0.5%	3.8^{a}	3.6^{a}	3.7^{a}	4.0^{a}	4.1^{a}	4.0^{a}
		1.5%	4.1^{a}	4.0^{b}	4.0^{a}	4.0^{a}	4.1^{a}	4.1^{a}
Color	Control		4.0^{a}	4.0^{a}	3.9^{a}	4.0^{a}	3.9^{a}	4.2^{a}
	Noncapsulated	0.5%	5.2^{b}	5.2^{b}	5.5^{b}	5.3^{b}	5.4^{b}	5.4^{b}
		1.5%	6.5^{c}	6.6^{c}	6.6^{c}	6.8^{c}	6.6^{c}	6.4^{c}
	Encapsulated	0.5%	4.1^{a}	4.2^{a}	4.1^{a}	4.0^{a}	4.2^{a}	4.1^{a}
		1.5%	4.3^{a}	4.4^{a}	4.3^{a}	4.5^{a}	4.3^{a}	4.5^{a}
Viscosity	Control		4.1^{a}	4.1^{a}	4.0^{a}	4.1^{a}	3.9^{a}	4.0^{a}
	Noncapsulated	0.5%	4.7^{b}	4.9^{b}	4.9^{b}	5.4^{b}	4.9^{b}	4.8^{b}
		1.5%	6.4^{c}	6.4^{c}	6.2^{c}	6.4^{c}	6.2^{c}	5.8^{c}
	Encapsulated	0.5%	4.0^{a}	3.9^{a}	4.0^{a}	3.9^{a}	4.0^{a}	4.0^{a}
		1.5%	4.0^{a}	4.0^{a}	4.1^{a}	4.1^{a}	4.0^{a}	4.0^{a}
Overall[2]	Control		3.9^{a}	4.0^{a}	4.1^{a}	4.0^{a}	3.9^{a}	4.2 ^{a}
	Noncapsulated	0.5%	5.5^{b}	5.5^{b}	5.5^{b}	5.8^{c}	5.0^{b}	5.5^{b}
		1.5%	6.9^{c}	6.9^{c}	6.7^{c}	6.6^{d}	6.5^{c}	6.4^{c}
	Encapsulated	0.5%	4.1^{a}	4.0^{a}	4.1^{a}	3.8^{a}	4.1^{a}	4.0^{a}
		1.5%	4.5^{a}	4.5^{a}	4.1^{a}	4.5^{b}	4.2^{a}	4.5^{a}

[1] Sensory descriptions scoring except for overall: 1, very slight; 3, slight; 5, slight strong; 7, strong. Means of eight replicate. In each sensory description, means in a column without the same letter are significantly different ($p>0.05$).

[2] Overall scoring: 1, dislike extremely; 3, dislike moderately; 5, like moderately; 7, like extremely.

chitooligosaccharide could prevent the viscosity change significantly in milk.

Stability of microcapsules

The release of chitooligosaccharide from microcapsules maintained about 4 mg/100 ml up to 3 days and the rate was increased thereafter (Figure 2). In addition, the release of chitooligosaccharide was dramatically increased from 9th day of storage in all temperatures. At 9th day of storage, 5.4 mg/100 ml milk of chitooliogosaccharide was released, while 7.6 mg/100 ml milk at 15th day, which was significantly different when it was stored at 4°C. As expected, the release of chitooliogosaccharide was affected by storage period, but not by storage temperature, except for 9 day storage.

Sensory analysis

All of sensory scores in encapsulated chitooligosaccharide-added groups were not different from those in control group (Table 4). The astringency scores between encapsulated and noncapsulated chitooligosaccharide-added groups (both 0.5 and 1.5%) were significantly different even at 0 day ($p<0.05$). Even 0.5% noncapsulated chitooligosaccharide-added group showed high astringency score during 15 day storage. Also, 1.5% encapsulated chitooligosaccharide-added milk showed a significantly lower astringency compared with 0.5% noncapsulated group. Within groups, no difference was found with 15 day storage period. Similar trends were found in other aspects including bitterness, off-flavor, color, viscosity and overall quality. This study indicated that microencapsulation could protect off-flavor and other sensory defects.

The present above data indicated that inappropriate properties of chitooligosaccharide in physical and sensory aspects such as dark color, viscosity, off-flavor and bitter taste were masked effectively by microencapsulation technique. Therefore, this study showed the possibility of chitooligosaccharide application in milk.

CONCLUSION

Effects of microencapsulation of chitooligosaccharide and its addition to milk were evaluated to determine the efficiency of microencapsulation, cholesterol removal, color, viscosity and sensory properties of the market milk. The most physical properties of microencacapsulated chitooligosaccharide-added milk were significantly different from those of noncapsulated chitooligosaccharide addition. However, addition of microencapsulated chitooligosaccharide did not affect the sensory properties of milk, including astringency, bitterness and color, showing no significantly different from control group. Therefore, the present study provides a possible solution for addition of chitooligosaccharide in milk and other dairy products by microencapsulation.

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