Asian-Aust. J. Anim. Sci.
Vol. 21, No. 2 : 299 - 306
February 2008

www.ajas.info

Microencapsulation of Korean Mistletoe (*Viscum album* var. *coloratum*) Extract and Its Application into Milk

N. C. Kim, J. B. Kim[1] and H. S. Kwak*

Department of Food Science and Technology, Sejong University, 98 Kunja-dong, Kwangjin-ku, Seoul 143-747, Korea

ABSTRACT : This study was designed to develop microencapsulated Korean mistletoe extract, to determine the stability *in vitro* and to examine its application in milk. Coating materials used were polyglycerol monostearate (PGMS) and medium-chain triacylglyderol (MCT). The highest efficiency of microencapsulation was 78.3% with 15:1:40 (w/w/v) as PGMS : mistletoe extract : distilled water and 66.1% with 15:1 (w/w) as MCT : mistletoe extract. The size of microcapsule was about 30.0 and 19.5 μm with PGMS and MCT, respectively. When microcapsules of mistletoe extract were incubated in simulated gastric fluid at pH 2 for 60 min, 14.8 and 17.2% of lectin was released from capsules which were coated with PGMS and MCT, respectively. Comparatively, 83.2 and 87.3% of lectin was released in simulated intestinal fluid (pH 8) after 60 min incubation of capsules coated with PGMS and MCT, respectively. The subsequent study determined the changes of physicochemical and sensory characteristics of milk with fortification of the mistletoe extract microcapsules during 12 day storage. TBA value was significantly lower in microcapsule-added groups than in the uncapsulated mistletoe extract-added group during the storage. When 100 ppm microencapsulated mistletoe extract was added, the L-, a- and b-values and viscosity were not significantly different from those of the control. In addition, the release of lectin from mistletoe extract over 12 days was 8.3 and 9.5 mg/100 ml in milk containing microcapsules made by PGMS and MCT, respectively. All sensory attributes showed a significant difference in uncapsulated mistletoe extract-added milk compared with other groups. The present study indicated that microcapsules of Korean mistletoe extract could be applied to milk and microcapsules coated with PGMS were effectively released in a simulated intestinal environment. (**Key Words** : Microencapsulation, Korean Mistletoe Extract, Polyglycerol Monostearate, Medium-chain Triacylglycerol, Milk)

INTRODUCTION

Mistletoe is a semi-obligate parasite plant that lives on many different kinds of trees including oak trees. The mistletoe has been utilized for a long period of time as a folk medicine for hypertension and cancer in Far East Asian countries, as well as in Europe (Park et al., 2001). Extracts from the European mistletoe (*Viscum album* L.) are widely used in cancer prevention and in an adjuvant chemotherapy of human cancer (Stoeva et al., 2001). The favorable effects of extracts from the European mistletoe have been known for over 70 years for the treatment of hypertonia, inflammatory diseases and also cancer (Kwaja et al., 1986; Hajto et al., 1989; Franz, 1991; Kuttan, 1993; Gabius et al., 1994).

Although mistletoe extracts are known for chemotherapy of various diseases, there is very little knowledge about its stability inside the body and biological and physiological functions. Also, regardless of its roles, lectin, which is the main constituent, could be instable and destroyed in the processing by temperature and pH, etc. The Korean mistletoe extract has high viscosity, undesirable flavor and color. In order to overcome these problems, the microencapsulation technique may be a good application for mistletoe fortification to food system.

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

* Corresponding Author: H. S. Kwak. Tel: +82-2-3408-3226, Fax: +82-2-3408-3319, E-mail: kwakhs@sejong.ac.kr

[1] School of Bioscienec and Food Technology, Handong Global University, Namsong-ri, Heunghae-up, Puk-ku, Pohang, Kyungpuk 791-780, Korea.

Received June 28, 2007; Accepted September 15, 2007

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). In the present study, fatty acid esters such as polyglycerol monostearate and medium-chain triglyceride were used since those have been proven as effective coating materials in out previous studies (Kwak et al., 2001; Kwak et al., 2003). However, no study has examined the efficiency of mistletoe extract microencapsulation using fatty acid esters, and the stability *in vitro*. Therefore, the objectives of this study were to develop the optimum conditions for microencapsulated Korean mistletoe extract, to examine the stability of microcapsules in simulated gastrointestinal fluids *in vitro*, and to apply the capsules into milk for development of functional milk.

MATERIALS AND METHODS

Materials

For the microencapsulation of Korean mistletoe (*Viscum album coloratum*) extract, polyglycerol monostearate (PGMS) and medium-chain triacylglycerol (MCT) were used as coating materials. Those were purchased from Il-Shin Emulsifier Co., LTD. (Seoul, Korea).

Preparation of plant materials of the Korean mistletoe

Mistletoes, living on oak trees, were collected on January in the Andong area (Korea). Upon collecting, the mistletoe leaves were immediately stored at -80°C. Distilled water through the ion-exchange column was added into the freezed Korean mistletoe leaves, cut by mixer for 2 min and then stirred for 16 h at 4°C. The supernatant was obtained by centrifugation (10,000 rpm, 30 min, 4°C), filtered through membrane filter and then freeze-dried for brown powdered form.

Preparation of microcapsule

Microcapsules of Korean mistletoe extract were made by MCT or PGMS, which were selected as major coating materials from our previous study (Kwak et al., 2001). The ratios of coating material to core material were 5:1, 10:1, 15:1 and 20:1 to maximize Korean mistletoe extract content and stability of microcapsules, and mixed at 1,200 rpm for 2 min with a stirrer. An airless paint sprayer (W-300, Wagner Spray tech. Co., Markdorf, Germany) nebulized a coating material-Korean mistletoe emulsion at 40°C into a cylinder containing a 0.05% polyethylene sorbitan monostearate (Tween-60) solution at 5°C. The diameter of the nozzle orifice was 0.4 mm. The chilled fluid was centrifuged at 4,520×g for 12 min to separate microcapsule suspension. Microcapsules were formed as lipid solidified in the chilled fluid.

For PGMS microencapsulation, the distilled water was additionally added because PGMS is highly viscous as described in the previous study (Kwak et al., 2003). The PGMS and distilled water were mixed with the ratio of 15:40 (w/v), heated to 55°C for 20 min, cooled to 40°C. Mistletoe extract was then added into the emulsion and stirred with 1,200 rpm for 30 sec for spraying. Both MCT and PGMS microencapsulation were done in triplicate.

Microencapsulation efficiency of mistletoe extract

The dispersion fluid of microencapsulation was assayed for untrapped lectin in Korean mistletoe extract (0.05% of Korean mistletoe extract is assumed as lectin). One milliliter of the dispersion fluid was taken and filtered through Whatman No. 540, followed by membrane filtration (di. 0.4 μm, Whatman International Limited, Madistone, England). The solute was stored in the refrigerator until analysis.

Lectin assay in Korean mistletoe extract were measured by enzyme-linked immunosorbent assay (ELISA) by the method described by Yoon et al. (2001). Flat-bottomed microtitration plates were coated with 100 μl per well of 1st antibody (rabbit anti-KML-C polyclonal antibody) in 50 mM carbonate-bicarbonate buffer (pH 9.6) by overnight incubation at 4°C or 2 h at 37°C. Excess antibody was washed off with distilled water containing 0.05% Tween-20 and the coated wells were blocked with 200 μl per well of 2% bovine serum albumin (BSA) in saline for 2 h at 37°C. Thereafter, sample or corresponding standard required for the calibration curve (KML-C, top 2 μg/ml serial dilution) were added to each well in duplicate. The plate was incubated at 37°C for 2 h. After washing, 2nd antibody that mouse anti-KML-C monoclonal antibody peroxidase conjugate at working strength (1:3,000) in PBS was added into each well and incubated at 37°C for 1 h. After three washings with 0.05% Tween-20 in distilled water, the antibody-antigen reaction was revealed by adding 100 ml of substrate to each well (0.25 M 3,3',5,5' tetramethylbenzidine, hydrogen peroxide in 50 mmol sodium acetate buffer pH 5.2). The reaction was stopped after 15 min by the addition of 50 ml of 2 M sulfuric acid per well. The absorbance was measured at 450 nm.

Microscopic observation

The micro-structural image of the capsule was magnified by 1000-folds with a light microscope (Olympus Optical Co., LTD., Japan) and photographed.

In vitro study

To determine the stability of lectin in Korean mistletoe extract in the stomach and small intestine, the simulated gastrointestinal solutions were prepared as follows: 1)

Table 1. Microencapsulation efficiency of Korean mistletoe extract with different ratios of coating to core materials[1]

Ratio (w/w)		Microencapsulation efficiency (%)	
Coating material	Core material	PGMS[2]	MCT[3]
5	1	60.5[d]	43.1[b]
10	1	69.4[b]	54.4[a]
15	1	74.5[a]	52.9[a]
20	1	66.4[c]	39.6[b]

[1] Means of triplicate. Means in a column without the same letter are significantly different ($p<0.05$). Other experimental factors: mixing temperature: 40°C, mixing speed: 1,200 rpm, centrifugal force: 2,490×g, and centrifugal time: 15 min.
[2] Polyacylglycerol monostearate, 40 ml of distilled water was added.
[3] Medium-chain triacylglycerol.

gastric fluid prepared in sample solution containing pepsin (pH 1.2) and simulated into pH 2 using by 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 pancreatin, 5 mg lipase, 10 mM cholic acid and 10mM deoxycholic acid, and simulated as pH 8.

In gastric fluid, the microcapsules of mistletoe extract in distilled water were incubated at 37°C with 100 rpm shaking, and in intestinal fluid, they were incubated at 37°C with the sample collecting at 10 min interval. The treated samples were centrifuged at 2,490×g and the supernatant was measured for mistletoe extract content released from the microcapsules. All treatments were triplicate.

Stability of microcapsules

Thiobarbituric acid (TBA) test : The absorbance change by addition of mistletoe into milk was measured using a TBA test at 5°C for 12 days. 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 mistletoe extract encapsulated or uncapsulated into a glass centrifuge tube and mixed thoroughly with 2.5 ml of TBA reagent. The mixture was heated immediately in a boiling water bath for exactly 10 min and cooled in ice. Ten millilters of 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 measured spectrometrically in a 1-cm light path. All measurements were run in triplicate.

Lectin release during storage : To measure the stability of lectin in Korean mistletoe extract microcapsules, 10 mg of mistletoe extract regardless of encapsulated or uncapsulated, was added to make 100 ppm, the mixture was stored at 5°C for 12 days, and its stability was measured at 2 day intervals. The samples were centrifuged, and the collected supernatant was analyzed for the determination of lectin content released from microcapsules. All measurements were made in triplicate.

Color analysis

Color value was measured using a colorimeter (CR210, Tokyo, Japan).

Viscosity analysis

The viscosity of 50 ml milk samples was measured at 5°C using a viscometer (VISCO STAR-L:J.P. Selecta S.A., Spain) with a single spindle at 200 rpm. All samples were measured in triplicate.

Sensory analysis

Commercial whole milk (at every period) containing 100 ppm uncapsulated and encapsulated Korean mistletoe extract was stored at 5°C for 0, 2, 4, 6, 8, 10 and 12 days. Seven semi-trained sensory panelists were recruited from faculty and graduated students in the Department of Food Science and Technology at Sejong University and evaluated in the milk samples throughout the study.

The astringency, bitterness, yellowish, off-flavor and herb flavor were scored on a seven-point scale (1 = very slight, 3 = slight, 5 = strong and 7 = very strong), and overall acceptability were also scored on a seven-point scale (1 = dislike extremely, 3 = dislike moderately, 5 = like moderately and 7 = like extremely).

Statistical analysis

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

RESULTS

Microencapsulation

PGMS : When the ratio of PGMS to distilled water was 15g:40 ml, the optimum ratio of PGMS to mistletoe extract, (5:1, 10:1, 15:1 and 20:1) was examined as shown in Table 1. Efficiency of the microencapsulation increased up to 15:1 (w/w) (coating to core ratio) with 40 ml distilled water in spray solution and decreased with 20:1 ratio. When PGMS was 15 g and mistletoe extract was 1 g, the efficiency of microencapsulation was 74.5% as the highest value. This result was in accordance to our previous study (Kwak et al., 2001), indicating that too much addition of distilled water for micorencapsulation reaulted in a weak-microcapsule coat, therefore, efficiency was decreased.

Several studies (Kwak et al., 2003; Lee et al., 2003; Choi et al., 2006) have reported the possibility of PGMS as a coating material for efficient formation of microcapsules.

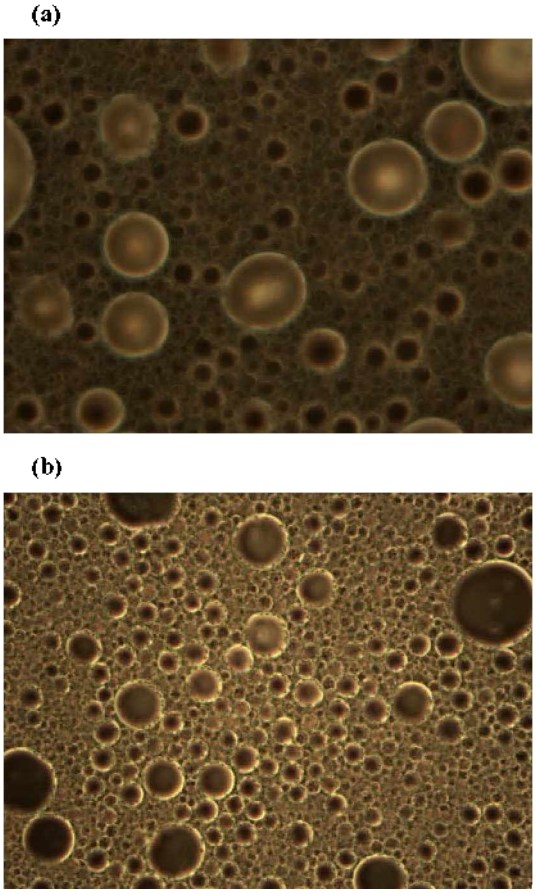

Figure 1. Photomicrograph of microencapsulated Korean mistletoe extract with polyglycerol monostearate (a) and medium-chain triacylglyerol (b). The photograph was taken at 400× magnification.

When iron was microencapsulated by PGMS, the efficiency was 75% (Kwak et al., 2003). Other studies using ascorbic acid (Lee et al., 2003) and chitooligosaccharide (Choi et al., 2006) as a core material indicated that 94.2% and 92.1% of microencapsulation efficiency was found, respectively.

MCT : The efficiency of microencapsulation by MCT is also shown in Table 1. The efficiency was the highest (54.4%) when the coat to core ratio was 10:1. Significant differences were not found between those of 10:1 (54.4%) and 15:1 (52.9%) ($p>0.05$). In the case of 20:1, MCT was left over in the upper layer of dispersion fluid after centrifugation. Therefore, the optimum ratio of MCT to mistletoe was found to be 15:1, even though left over MCT was still found in the upper layer.

Our previous study indicated that the efficiency of isoflavone microencapsulation increased steadily up to 15:1 of coat-to-core ratio and was the greatest (70.2%) when isoflavone was microencapsulated with MCT (Kim et al., 2006). Since the microencapsulation efficiency with MCT has been lower than that with PGMS in our studies, it is indicated that MCT may not be the adequate coating material for microencapsulation.

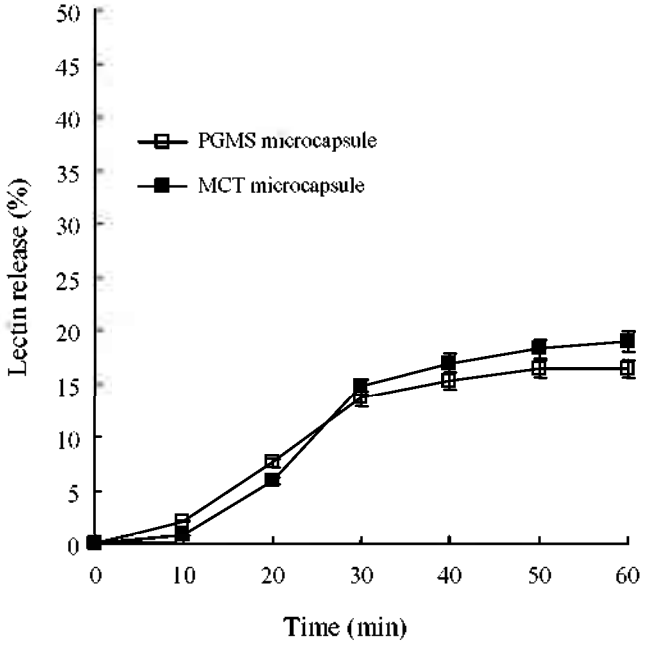

Figure 2. Korean mistletoe extract release from microcapsules with PGMS or MCT incubated in simulated gastric fluid *in vitro*. Simulated gastric fluid containing pepsin and adjusted to different pHs with HCl and NaOH at incubated at 37°C for 60 min. Each bar represents an average of three trials. Each bar indicates a standard deviation and bar with different letters are significantly different ($p<0.05$).

Microscopic observation

Photomicrograph of microencapsulated mistletoe with PGMS or MCT is shown in Figure 1. In the case of both PGMS and MCT microcapsules, the sizes were various and the average sizes were about 30 and 19.5 μm, respectively (Figure 1). Microscopic examination of microcapsules revealed spherical particles and microcapsules containing mistletoe extract had smooth surfaces and evenly distributed pockets of mistletoe solution in microcapsules made by both PGMS and MCT.

***In vitro* study**

This study was conducted to determine whether the microcapsules released Korean mistletoe extract during simulated gastric intestinal conditions (Figure 2). When incubated at pH 2, less than 3% lectin was released from the microcapsules at 10 min incubation and it was increased up to 13-15% at 30 min and plateaued thereafter. From 1-30 min incubation, there was a dramatic increase from 0 to 15%.

To determine how effectively mistletoe extract was released in the intestine, a simulated intestinal fluid (pH 8) was prepared with the presence of pancreatin and bile salts, and incubated during at 37°C for 60 min (Figure 3). When both pH and the duration of incubation increased, lectin release from mistletoe microcapsules increased dramatically.

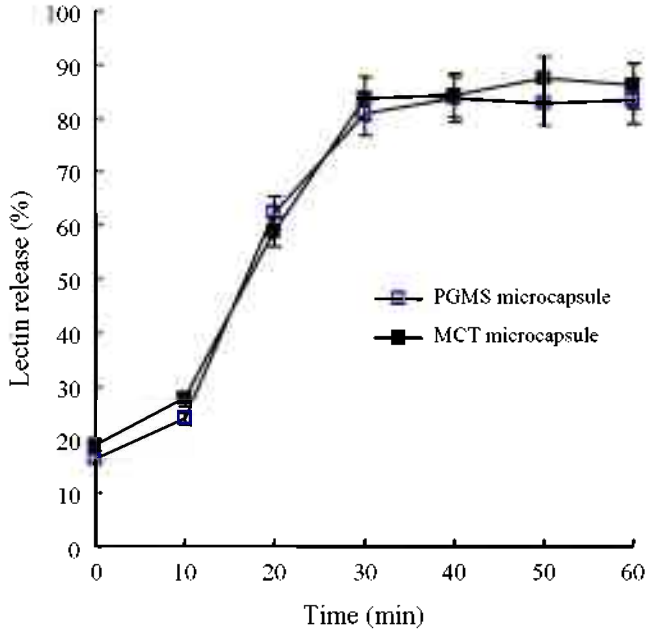

Figure 3. Korean mistletoe extract release from microcapsules with PGMS or MCT incubated in simulated intestinal fluid *in vitro*. Simulated intestinal condition included enzymes such as lipase (5 mg) and pancreatin (20 mg) and incubated at 37°C for 60 min. Each bar represents an average of three trials. Each bar indicates a standard deviation and bar with different letters are significantly different ($p<0.05$).

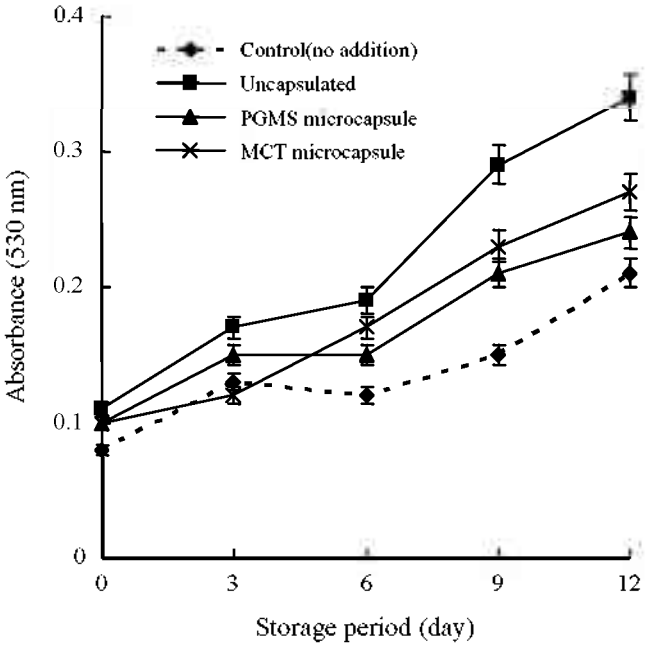

Figure 4. Effect of microencapsulated Korean mistletoe extract on absorbance change (TBA value) in milk stored at 5°C for 12 days.

Less than 30% of the entrapped mistletoe extract was released at pH 8 from 0 to 10 min and a dramatic increase (about 3 times) was observed at 30 min incubation and maintained thereafter. When incubated at pH 8, 80-82% lectin was released from microcapsules made by MCT or PGMS at 30 min incubation and thereafter, respectively.

Stability of microcapsules during storage

TBA test : The effect of mistletoe extract fortification in milk on chemical oxidation (as measured by the TBA test) during 12 days of storage is shown in Figure 4. The treatment was divided into 4 different groups as follows: (1) control, no addition of mistletoe extract microcapsules; (2) Uncapsulated, 100 ppm of uncapsulated Korean mistletoe extract, 10 mg/100 ml mistletoe extract-added milk; (3) PGMS microcapsule, 100 ppm of PGMS encapsulated mistletoe extract; (4) MCT microcapsule, 100 ppm of MCT encapsulated mistletoe extract.

In all groups, TBA absorbance increased proportionally to storage period. In the 100 ppm of uncapsulated mistletoe extract-added group, TBA absorbance increased dramatically from 0.11 to 0.34 from 0 to 12 days. TBA absorbance was significantly lower in the encapsulated group than in uncapsulated group at 12 days of storage. When 100 ppm of mistletoe extract was added, the TBA absorbance difference between uncapsulated (0.34) and encapsulated groups (0.24 and 0.27 for PGMS and MCT,

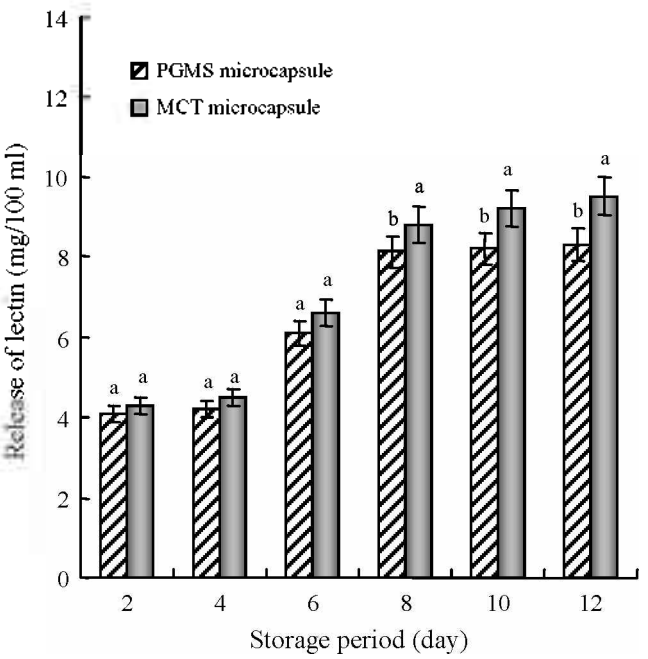

Figure 5. Release of lectin from Korean mistletoe extract microcapsules in milk stored at 5°C for 12 days.

respectively) was significant at 6 days of storage and thereafter. This result indicated that chemical lipid oxidation proceeded more rapidly in milk with uncapsulated mistletoe extract added than in milk with encapsulated mistletoe extract added regardless of coating materials.

Lectin release : To examine the stability of microencapsules during the storage, 10 ml of a microencapsulated solution (1 mg/ml) was mixed with 10 ml of commercial milk, and the mixture stored at 5°C for 12

Table 2. Color value of microencapsulated Korean mistletoe extract in milk stored at 5°C for 12 days[1]

Treatment	L-value	a-value	b-value
Control[2]	87.61[a]	-3.34[b]	5.32[a]
Uncapsulated	84.12[b]	-1.94[a]	6.94[b]
PGMS microcapsule	87.08[a]	-3.10[b]	5.50[a]
MCT microcapsule	87.19[a]	-3.13[b]	5.47[a]

[1] Means of triplicates. Means in a column with same letter are not significantly different ($p<0.05$).
[2] Market milk stored at 5°C for 1 day (no microcapsule added).

days. The release of lectin from the mistletoe extract microcapsules was then determined after 0, 2, 4, 6, 8, 10 and 12 day as shown in Figure 5.

The release of lectin (%) from mistletoe extract microcapsules maintained about 4 mg/100 ml up to 4 days and increased thereafter. When PGMS was used as a coating material, 6.1% of lectin was released at 6 days and increased up to 8.3% at 12 days. Comparatively, when MCT was used, 6.6 and 9.5% lectin were released at 6 and 12 days of storage, respectively. In addition, a significant difference in lectin release between the mistletoe extract microcapsules was found.

Color

The color values of milk samples with the addition of encapsulated and uncapsulated mistletoe extract were shown in Table 2. The color change was significant with uncapsulated mistletoe extract, while slight but not significant change was found with both encapsulated mistletoe extract samples. The L-value in uncapsulated mistletoe extract-added group was significantly lower than all other encapsulated-added and control groups ($p<0.05$). No difference was found between encapsulated microcapsule-added groups with both PGMS and MCT microcapsule groups and control group.

The a-value was significantly lower in uncapsulated mistletoe extract-added group than those in other groups ($p<0.05$), which indicated less brown color with mistletoe extract. Korean mistletoe extract microcapsule-added groups with PGMS and MCT were not significantly different from the control group, which was the commercial milk without any addition. The b-value change was similar to those of L- and a-values. These results indicated that the changes of color value were remarkably masked by the microencapsulation.

Viscosity

The viscosity of uncapsulated mistletoe extract-added group was significantly higher than those in encapsulated-added and the control groups at every storage periods ($p<0.05$) (Figure 6). When 100 ppm of uncapsulated mistletoe extract was added into milk samples, viscosity was 1.23 cps at the initial period and kept increasing

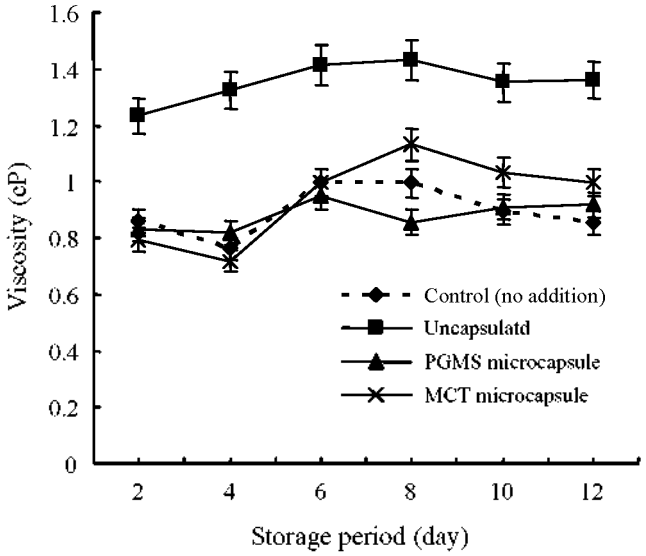

Figure 6. Effect of microencapsulated Korean mistletoe extract on viscosity in milk stored at 5°C for 12 days.

throughout the storage period. However, viscosity values in milk samples added by encapsulated mistletoe extract with PGMS and MCT were about 0.83 and 0.79 cps at the initial period, respectively, which was similar to that of the control (0.86 cps). Viscosity value increased steadily throughout the storage period and reached up to 0.92 cps (PGMS) and 0.99 cps (MCT) at 11 day storage and those were not significantly different from that of the control (0.84 cps). These results may indicate that microencapsulation of mistletoe extract could prevent significant change of viscosity in milk.

Sensory analysis

The sensory properties of mistletoe extract-fortified milk stored at 5°C for 12 days were evaluated (Table 3). When 100 ppm of microencapsulated mistletoe extract had been added into milk, astringency score was significantly different from that of the control and Korean mistletoe extract-added groups regardless of microencpasulation throughout the storage ($p<0.05$). When 100 ppm of uncapsulated mistletoe extract had been added, astringency score was dramatically higher than those of microencapsulated groups with PGMS and MCT ($p<0.05$). The scores of bitterness and yellowish, no difference was found between the control and microencapsulated groups, however, those were significantly higher in milk-added uncapsulated Korean mistletoe extract compared with other groups ($p<0.05$). Not much difference was found in off-flavor and herb flavor among groups. In respect of the overall acceptability, the control and 100 ppm microencapsulated mistletoe extract-added groups showed high consumer preference throughout the storage, while uncapsulated mistletoe extract-added milk showed less than

Table 3. Sensory scores of Korean mistletoe extract microcapsule-added milk stored at 4°C for 12 days[1]

Storage period (day)	Treatment[2]	Sensory description[3]					
		Astringency	Bitterness	Off-flavor	Herb flavor	Yellowish[4]	Overall acceptability
0	Control	1.00^{c}	1.00^{b}	1.50^{a}	1.00^{a}	1.00^{b}	5.14^{a}
	Uncapsulated	3.83^{a}	3.80^{a}	1.56^{a}	1.20^{a}	2.60^{a}	1.80^{c}
	PGMS microcapsule	1.36^{b}	1.14^{b}	1.64^{a}	1.00^{a}	1.40^{b}	4.16^{b}
	MCT microcapsule	1.47^{b}	1.34^{b}	1.66^{a}	1.00^{a}	1.50^{b}	4.00^{b}
2	Control	1.00^{c}	1.20^{b}	1.76^{a}	1.00^{a}	1.20^{c}	4.89^{a}
	Uncapsulated	4.38^{a}	3.80^{a}	2.35^{a}	1.00^{a}	2.82^{a}	1.56^{c}
	PGMS microcapsule	1.50^{b}	1.60^{b}	1.79^{a}	1.00^{a}	1.78^{b}	4.03^{b}
	MCT microcapsule	1.51^{b}	1.50^{b}	1.95^{a}	1.00^{a}	1.80^{b}	3.78^{b}
4	Control	1.25^{c}	1.20^{c}	1.67^{b}	1.00^{a}	1.44^{b}	4.89^{a}
	Uncapsulated	5.03^{a}	4.35^{a}	2.47^{a}	1.68^{a}	2.96^{a}	1.51^{c}
	PGMS microcapsule	1.58^{b}	1.74^{b}	1.83^{b}	1.24^{a}	1.75^{b}	3.89^{b}
	MCT microcapsule	1.66^{b}	2.18^{b}	1.79^{b}	1.50^{a}	1.82^{b}	3.96^{b}
6	Control	1.41^{c}	1.22^{c}	1.63^{c}	1.00^{b}	1.35^{c}	4.38^{a}
	Uncapsulated	5.37^{a}	4.23^{a}	2.89^{a}	1.74^{a}	2.85^{a}	1.42^{c}
	PGMS microcapsule	1.65^{b}	1.76^{b}	1.93^{bc}	1.24^{b}	1.85^{b}	3.66^{b}
	MCT microcapsule	1.69^{b}	2.15^{b}	2.28^{b}	1.20^{b}	1.96^{b}	3.29^{b}
8	Control	1.39^{c}	1.54^{b}	1.76^{c}	1.00^{b}	1.47^{c}	4.36^{a}
	Uncapsulated	5.48^{a}	4.65^{a}	3.18^{a}	2.15^{a}	2.67^{a}	1.58^{c}
	PGMS microcapsule	1.56^{c}	1.81^{b}	2.15^{bc}	1.74^{a}	1.87^{bc}	3.69^{b}
	MCT microcapsule	1.96^{b}	1.91^{b}	2.46^{b}	1.76^{a}	1.99^{b}	3.34^{b}
10	Control	1.42^{c}	1.51^{b}	2.05^{b}	1.00^{c}	1.41^{c}	4.07^{a}
	Uncapsulated	5.56^{a}	5.33^{a}	4.44^{a}	2.03^{a}	2.73^{a}	1.66^{c}
	PGMS microcapsule	1.83^{b}	2.14^{b}	2.63^{b}	1.75^{b}	1.87^{b}	3.72^{ab}
	MCT microcapsule	1.92^{b}	2.18^{b}	2.79^{b}	1.95^{a}	1.92^{b}	3.58^{b}
12	Control	1.67^{c}	1.70^{c}	2.01^{c}	1.00^{c}	1.68^{b}	3.95^{a}
	Uncapsulated	5.25^{a}	5.69^{a}	5.19^{a}	2.80^{a}	3.35^{a}	1.40^{c}
	PGMS microcapsule	1.8^{bc}	2.23^{bc}	3.13^{b}	2.03^{b}	1.87^{b}	3.40^{b}
	MCT microcapsule	2.1^{b}	2.49^{b}	3.36^{b}	2.39^{ab}	2.18^{b}	3.36^{b}

[1] Means within column by the same letter at each storage period are not significantly different ($p<0.05$).

[2] Control: market milk, Uncapsulated: uncapsulated mistletoe extract-added milk, PGMS microcapsule: microencapsulated mistletoe extract by polyglycerol monostearate-added milk, MCT microcapsule: microencapsulated mistletoe extract by medium-chain triglyceride-added milk.

[3] The scale of astrigency, bitterness, off-flavor, herbs flavor, color scores: 1 = very slight, 2 = slight, 3 = slight-moderate, 4 = moderate, 5 = moderate-strong, 6 = strong, 7 = very strong. The scale of overall acceptability score: 1 = dislike extremely, 4 = neither like nor dislike, 7 = like extremely.

[4] Yellow color.

score 2 throughout the storage. These resulted indicated that Korean mistletoe extract-added milk was shown to be well maintained by the encapsulation of mistletoe extract during 12 day storage.

DISCUSSION

It is well known that the crude extract of the Korean mistletoe (*Viscum album coloratum*) contains immunomodulatory effects. The extract induces mouse macrophages to produce cytokines, which leads to anti-cancer activity in a mouse metastasis model system (Yoon et al., 1995). The lectin that was purified from the extract proved to be responsible for the production of the sytokines and the anti-tumor activity. Additionally, the lectin also induced apoptotic cell death of several cancer cell lines (Yoon et al., 1999). On the basis of the above information, we decided to apply the Korean mistletoe extract for milk fortification using microencapsulation process to overcome the undesirable flavor, color and stability.

When microencapsulation efficiency of PGMS or MCT was determined, the efficiency was the highest with 15:1 or 10:1 (w/w) of coat-to-core ratio, respectively. In the case of 20:1 (w/w), both PGMS and MCT were leftover in the upper layer of the dispersion fluid after centrifugation. Our previous study (Kwak et al., 2003) showed the highest efficiency (75%) with 5:1:30 ratio (w/w/v) as coating to core materials to distilled water. That study showed that the conditions for microencapsulation depended on the ratio of coating to core materials, the viscosity of the spray solution, kinds of coating and core materials, and food system to which it was to be applied.

Lectin in Korean mistletoe extract has been recognized that may confer significant long-term benefits if incorporated into the diet either naturally as an integral part of the food or as a food supplement. It is the concept that has resulted in the development of the relatively new field of functional foods. Therefore, an experiment should be

performed to determine how stable the microcapsules were in the stomach and how effectively they were released in the intestine.

It is generally accepted that for an effective uptake if nutritional effect from microcapsules, several problems need to be solved such as the capsules have to contain as much activity as possible, have to resist the gastric and intestinal fluids, and have to be captured by the enterocytes before being released into the blood circulation.

As expected, the present study indicated that a little amount of lectin in Korean mistletoe extract was released at low pH. Comparatively, those releases increased dramatically in neutral pH, which was a similar condition to that of the intestine. These results also indicated that microcapsules would be a convenient tool for lectin fortification because of an increase of absorption by flavoring the uptake and effective release in the intestine. The present study showed a possible application in encapsulated lectin in Korean mistletoe extract fortification using MCT or PGMS, which may be used effectively in the food system.

ACKNOWLEDGMENT

This research was supported by a grand of local industry new technology fusion development project, the Small & Medium Business Administration (SMBA) in Daegu, Korea.

REFERENCES

Bersen'eva, E. A., A. A. Inanov, T. P. Sansonova, E. M. Chernova, and N. I. Oragvelidze. 1990. Microencapsulated aromatizers for tea. Pishchevaya Paomyshlennost, USSR 1:57-59.

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.

Choi, H. J., J. Ahn, N. C. Kim and H. S. Kwak. 2006. The effects of microencapsulated chitooligosaccharide on physical and sensory properties of the milk. Asian-Aust. J. Anim. Sci. 19:1347-1353.

Franz, H. 1991. In: Advances in lectin research (Ed. H. Franz, Kichi Kasai, J. Kochaurek, S. Olsnes, L. M. Shannon). pp. 4:33-50. Verlag Gesundheit and Springer Verlag, Berlin.

Gabius, H. J., S. Gabius and S. S. Joshi. 1994. Form ill-defined to the immunomodulatory lectin: will there be a reason for oncological of mistletoe: Planta Med. 60:2-7.

Hajto, T., K. Hostanska, and H. J. Gabius. 1989. Modulatory potency of the β-galactosidase-specific lectin from mistletoe (Iscador) on the host defense system *in vivo* in rabbits and patients. Cancer Res. 49:4803-4808.

Jackson, L. S. and K. Lee. 1991. Microencpasulated iron food fortification. J. Food Sci. 56:1047-1050.

Kim, N. C., B. J. Jeon, J. Ahn and H. S. Kwak. 2006. *In vitro* study of microencapsulated isoflavone and β-galactosidase. J. Agric. Food Chem. 54:2582-2586.

Kuttan, G. 1993. Tumoricidal activity of mouse peripheral macrophages treated with *Viscum album* extract. Immunol. Investig. 22:431-440.

Kwaja, T. A., C. B. Dias and S. Pentecost. 1986. Recent studies on the anticancer activities of mistletoe (*Viscum album*) and its alkaloids. Oncology 43:42-50.

Kwak, H. S., M. R. Ihm and J. Ahn. 2001. Microencapsulation of β-galactosidase with fatty acid esters. J. Dairy Sci. 84:1576-1582.

Kwak, H. S., K. M. Yang and J. Ahn. 2003. Microencpasulated iron for milk fortification. J. Agric. Food Chem. 51:7770-7774.

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.

Lee, J. B., J. Ahn, J. H. Lee and H. S. Kwak. 2003. The microencapsulated ascorbic acid released *in vitro* and its effect on iron bioavailability. Arch. Pharm. Res. 26:874-879.

Park, C. H., D. W. Lee, T. B. Kang, K. H. Lee, T. J. Yoon, J. B. Kim, M. S. Do and S. K. Song. 2001. cDNA cloning and sequence analysis of the lectin genes of the Korean mistletoe (*Viscum album coloratum*). Mol. Cells 12:215-220.

SAS. 1985. User's Guide: Statistics, Version 5 Edition. SAS Institute, Inc., Cary, NC.

Stoeva, S., M. Franz, R. Wacker, R. Krauspenhaar, E. Gutholrlein, A. Mikhailov, C. Betzel and W. Voelter. 2001. Promary structure, isoforms, and molecular modeling of a chitin-binding mistletoe lectin. Arch. Biochem. Biophys. 392:23-31.

Yoon, T. J., Y. C. Yoo, O. B. Choi, M. S. Do, T. B. Kang, S. W. Lee, I. Azuma and J. B. Kim. 1995. Inhibitory effect of Korean mistletoe (*Viscum album coloratum*) extract on tumor angiogenesis and metastasis of haematogenous and non-haematogenous tumor cells in mice. Cancer Lett. 97:83-91.

Yoon, T. J., Y. C. Yoo, T. B. Kang, E. Her, S. H. Kim, K. Kim, I. Azuma and J. B. Kim. 2001. Cellular and humoral adjuvant activity of lectins isolated from Korean mistletoe (*Viscum album coloratum*). Int. Immunopharmacol. 1:881-889.

Yoon, T. J., Y. C. Yoo, T. B. Kang, K. Shimazaki, S. K. Song, K. H. Lee, S. H. Kim, C. H. Park, I. Azuma and J. B. Kim. 1999. Lectins isolated from Korean mistletoe (*Viscum album coloratum*) induce apoptosis in tumor cells. Cancer Lett. 136:33-40.