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Microencapsulation of Water-Soluble Isoflavone and Physico-Chemical Property in Milk

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This study was carried out to investigate the addition of water-soluble isoflavone into milk by means of microencapsulation technique. The yield of microencapsulation, sensory attributes, and capsule stability of water-soluble isoflavone microcapsules in milk were measured. Coating materials used was polyglycerol monostearate (PGMS), and core material was water-soluble isoflavone. The encapsulation yield of water-soluble isoflavone with PGMS was 67.2% when the ratio of coating material to core material was 15 : 1. The rate of water-soluble isoflavone release from capsules was 18, 19, and 25% when stored at 4, 20, and 30°C for 12 days in milk, respectively. In sensory evaluation, beany flavor and color of microencapsuled water-soluble isoflavone added milk were significantly different from uncapsuled water-soluble isoflavone added milk, however, bitterness was not significantly different. *In vitro* study, micro-capsules of water-soluble isoflavone in simulated gastric fluid with the range of 3 to 6 pHs were released 3.0~15.0%, however, the capsules in simulated intestinal fluid with pH 7 were released 95.7% for 40 min incubation time. In conclusion, this study provided that PGMS as coating materials was suitable for the microencapsulation of water-soluble isoflavone, and the capsule containing milk was almost not affected with sensory attribute.

Key words: Microencapsulation, Water-soluble isoflavone, Polyglycerol monostearate, Milk

INTRODUCTION

Isoflavones are phytoestrogens which have potent biological activity that may reduce the risk of hormone-dependent disease (Adlercreutz, 1995). Isoflavones from soya significantly lengthened the follicular phase of the menstrual cycle (Cassidy *et al.*, 1994), an action which has been associated with a decreased risk of breast cancer.

Isoflavones are strikingly similar in chemical structure to mammalian estrogens and act as weak estrogens (Setchell and Cassidy, 1999). In women, menopause may accentuate the decline of bone mineral density and thereby increase the risk of osteoporosis (Cassidy *et al.*, 1994; Cassidy *et al.*, 1995). Bone mineral density is therefore strongly related to the estrogen levels in postmenopausal women (Setchell and Cassidy, 1999). Recent study (Fanti *et al.*, 1998) indicated that the beneficial effects of soy protein result from stimulation of bone formation rather than suppression of bone resorption.

A recent study proposed that isoflavones may also be the factor responsible for the cholesterol-lowering property of some soy-foods (Anthony *et al.*, 1996; Fukui *et al.*, 2002). Soy protein lowers total cholesterol, low-density lipoprotein cholesterol, and triglycerides in humans, and inhibits atherosclerosis in animals (Arliss and Biermann, 2002). Since a strong positive correlation exists between increased serum cholesterol concentrations and risk of coronary heart disease, most consumers are concerned about the excessive intake of cholesterol (Grundy *et al.*, 1982; Gurr, 1992). Therefore, physical, chemical, and biological methods to reduce cholesterol have been studied in foods including dairy products (Kwak, 2001; Ahn and Kwak, 1999; Lee *et al.*, 1999; Szejtli, 1988).

Milk is the universal and nutritious food including calcium. A good supply of calcium is needed during the childhood and teen years in order to build strong bones and help protecting against osteoporosis in later life.

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Since milk is a great source of calcium, isoflavone is necessary to be added into milk for postmenopausal women. However, it can not be added into milk directly because isoflavone contains bitterness, beany off-flavor and brown color. Especially aglycone which is water-soluble isoflavone can not get rid of these faults during processing, therefore, to solve these problems, microencapsulation technique is needed.

Microencapsulation, which shows potential as carriers of enzymes in the food industry, could be a good vehicle for the addition of iron into milk (Jackson and Lee, 1991; Bersenieva *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 although several researchers have used coating materials such as milk fat, agar, and gelatin, etc. responsible for enzyme, flavor and iron microencapsulation in foods (Kwak *et al.*, 2001), no study has measured the efficiency of water-soluble isoflavone microencapsulation, and the stability of microcapsule itself.

Therefore, the objectives of this study were to examine the optimum conditions of water-soluble isoflavone microencapsulation and to measure the stability of microcapsule retaining the isoflavone in milk during storage and *in vitro* study.

MATERIALS AND METHODS

Materials

Polyglyerol monostearate (PGMS) as a coating material was purchased from Il-Shin Emulsifier Co., LTD. (Seoul). Water-soluble isoflavone 30% purity as a core material was obtained from Imorepacific Co., LTD. (Seoul). Genistin (4', 5,7-trihydroxyisoflavone-7-glucoside) was purchased from Sigma Chem. Co. (St. Louis, MO, USA) and daidzin (4'-hydroxyisoflavone-7-glucoside) was purchased from Fujicon, Co., LTD. (Tokyo, Japan). These were in food grade.

Microencapsulation

Microcapsules of water-soluble isoflavone were made by PGMS, which was selected as coating material from the preliminary experiment (Kwak *et al.*, 2001). The ratios of coating material to core material were 5:1, 10:1, 15:1, and 20:1 to maximize the content of water-soluble isoflavone and the stability of microcapsules, and mixed at 1,200 rpm for 1 min with a stirrer. An airless paint sprayer (W-300, Wagner Spray Tech. Co., Markdorf, Germany) nebulized a coating material-water soluble isoflavone emulsion at 45°C into a cylinder containing a solution at 5°C (Kwak *et al.*, 2001). The diameter of the nozzle orifice was 0.4 mm. The chilled fluid was centri-fuged at 2,490×g for 10 min to separate unwashed microcapsule suspension. Microcapsules were formed as lipid solidified in the chilled fluid. To remove residual enzyme adhering to the outside walls of microcapsules, unwashed microcapsules were mixed with 0.05% Tween-60 solution again, centrifuged, and obtained one-time washed microcapsules. The procedure was repeated.

For PGMS microencapsulation, the distilled water was additionally added because PGMS is highly viscous. PGMS and distilled water were mixed with the different ratios (5:2, 5:3, 5:4, and 5:5, w/v), heated to 55°C for 20 min, and stirred with 1,200 rpm for 30 s for spraying. PGMS microencapsulation were done in triplicate.

HPLC analysis

Quantification and identification of isoflavone was performed using a μ-Bondapak C18 column (5 μm, 25× 4.6 mm, i.d.: Waters, USA) and two solvent system at a flow rate of 1 mL/min. The column was equilibrated with 20% methanol and after the injection of 20 μL of sample, the ratio of solvent B to A was increased by a linear gradient to 100% of solvent B in 50 min. Analyses were performed using a Shimazu solvent delivery system equipped with a UV detector (Shimazu, Japan) monitored absorbance at 254 nm.

The stock solution of isoflavone was prepared by dissolving pure standards in 70% methanol. Small portions of the solution were injected into the HPLC column and the purity of each isoflavone was confirmed based on the percentage area of the peak area in the HPLC chromatogram.

Stability of microcapsule during storage

To measure the stability of water-soluble isoflavone microcapsules, 10 mL of distilled water was added into the same amount of microcapsule solution, stored at 4, 20, and 30°C for 12 days. The samples were centrifuged and the collected supernatant was analyzed for determination of water-soluble isoflavone content released from microcapsules. All measurements were triplicate.

Sensory analysis

In the preliminary experiment, microcapsule added milk was prepared. An eight-person panel, semi-experienced in judging dairy products evaluated the milk samples throughout the study. Sensory characteristics of microcapsules with PGMS was added into the milk and stored for 1, 3, 6, 9, and 12 days at 4°C. Sensory characteristics were tested as follow.

The intensity of off-flavor (beany flavor) taste aspect

(bitterness) and color were scored on a nine-point scale (1=none, 3=slight, 5=moderate, 7=strong, and 9=very strong).

In vitro study

To determine the stability in the stomach and intestine of human beings, the simulated gastrointestinal solutions were prepared as follows: 1) gastric fluid prepared in sample solution containing pepsin (pH 1.2) and simulated into 5 different fluids with pH 2, 3, 4, 5 and 6 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, 10mM cholic acid and 10mM deoxycholic acid, and simulated into 4 different intestinal solutions as pH 5, 6, 7 and 8 (Freund *et al.*, 2000; Kwak *et al.*, 2002).

In gastric fluid, the microcapsules of water-soluble isoflavone in distilled water were incubated at 37°C with 100 rpm shaking for 10 min, while incubated for 60 min with the sample collecting at 20 min interval in intestinal fluid. The treated samples were centrifuged at 2,490×g and the supernatant was measured for water-soluble isoflavone content released from the microcapsules. All treatments were triplicate.

Statistical Analysis

Data from each experiment were analyzed by ANOVA using a SAS program (1985) and differences among treatments were determined by LSD at P<0.05, unless otherwise stated.

RESULTS AND DISCUSSION

Microencapsulation

Even though polyglycerol monostearate (PGMS) was heated to 55°C, it appeared to be hard to spray as described in our previous study (Kwak *et al.*, 2001). Therefore, we investigated the optimum ratio of PGMS to distilled water to reduce the viscosity of PGMS solution (Table I). When the ratio of coating to core material was 5:1, the highest efficiency of PGMS to distilled water was found at 5 g : 30 mL as 75% and the lowest at 5 g : 70 mL as 52%. The lower efficiency of microencapsulation was probably due to a weak coat of microcapsule by the addition of distilled water.

When the ratio of PGMS to distilled water was 5 g : 30 mL, the optimum ratio of PGMS to water-soluble isoflavone was examined as shown in Table II. Efficiency of the microencapsulation increased up to 15 : 1 (coating to core ratio) and decreased thereafter when the amount of coating material increased. When the ratio of PGMS to water-soluble isoflavone was 15 and 1 g, the yield of microencapsulation was 67.2% as the highest value.

Table I. Microencapsulation efficiency of water-soluble isoflavone with different addition of distilled water[1]

Ratio (v/w)			Microencapsulation efficiency (%)
PGMS[2]	Water-soluble isoflavone	Distilled water	
5	1	30	75[a]
5	1	40	69[ab]
5	1	50	70[a]
5	1	60	67[b]
5	1	70	52[c]
SEM			2.3

[1]Means of triplicate. Means in a column without the same letter are significantly different ($p<0.05$).
[2]Polyglycerol monostearate. Other experimental factors: PGMS : water-soluble isoflavone=5 : 1, mixing temperature : 55°C, mixing speed : 1,200 rpm, centrifugal force : 2,490×g, and centrifugal time : 10 min.

Table II. Microencapsulation efficiency with different ratios of PGMS to water-soluble isoflavone [1]

Ratio (w/w)		Microencapsulation efficiency (%)
PGMS[2]	water-soluble isoflavone	
5	1	43.4[c]
10	1	61.8[ab]
15	1	67.2[a]
20	1	50.0[b]
SEM		3.8

[1]Means of triplicate. Means in a column without the same letter are significantly different ($p<0.05$).
[2]Polyacylglycerol monostearate
Other experimental factors : distilled water added into PGMS : 50 mL, mixing temperature : 55°C, mixing speed : 1,200 rpm, centrifugal force : 2,490×g, and centrifugal time: 10 min.

Stability of microcapsule during storage

Microcapsules were examined for the ability to retain water-soluble isoflavone in different temperatures during storage as shown in Fig. 1. In all samples, the water-soluble isoflavone lost (%) was increased with the length of storage period. More water-soluble isoflavone was significantly released from microcapsules at the highest temperature (30°C) after 8 days storage than those at lower temperature (4 and 20°C). At 5 day storage, water-soluble isoflavone lost (%) was 17, 18, and 20% at 4, 20, and 30°C, respectively, which was not significantly different. However, at 12 day storage, 18, 19 and 25% of water-soluble isoflavone was released at 4, 20 and 30°C, respectively.

It was suggested that the storage at 30°C may have changed the lipid crystal structure reducing stability (Jackson and Lee, 1991). Percentage of water-soluble isoflavone loss from microcapsules stored at 20°C for 12 days did not differ from freshly prepared microcapsules

(4°C storage). These data indicated that microcapsules could be used immediately or stored for about two weeks below or at 20°C.

Sensory analysis

Milk containing microcapsules of water-soluble isoflavone was observed by sensory evaluation during 12 days of storage at 4°C as shown in Table III.

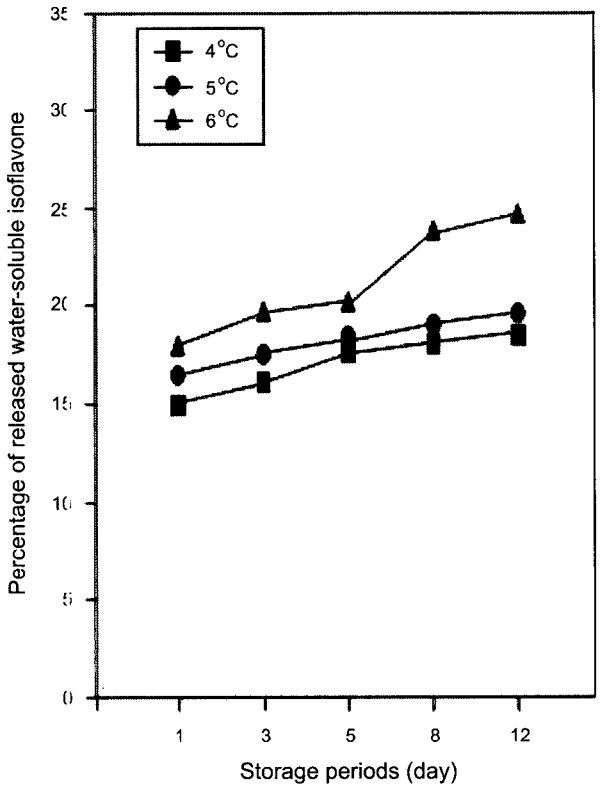

Fig. 1 Effect of different temperatures on water-soluble isoflavone release from microcapsules stored for 12 days. Coating material: Polyglycerol monostearate. Core material: Water-soluble isoflavone. Each bar represents an average of three trials.

For beany flavor, capsulated water-soluble isoflavone added milk showed a little difference compared with that of control until 6 days, and an increase thereafter. Uncapsulated water-soluble isoflavone added milk showed difference at 1 day remarkably, and a dramatic increase up to 12 day. For bitterness, it was not significant up to 3 day storage with both capsulated and uncapsulated water-soluble isoflavone addition, bitterness increased slowly to 6 day. Among sensory characteristics, color was observed with microcapsule added groups. Especial-ly, uncapsulated water-soluble isoflavone addition showed the highest yellow at every storage period.

This study provided that the capsule containing milk was almost not affected with sensory attribute.

In vitro study

Glycosylation of the isoflavonoids has been thought to delay intestinal absorption until the large intestine where metabolism by colonic microflora releases aglycones. However, metabolites of some flavonols and isoflavones appear in plasma within 30 min of ingestion, indicating rapid absorption in the small intestine. Therefore, a considerable experiment should be performed to determine how stable the microcapsules are in the stomach and how effectively release in the intestine, which is the primary site of water-soluble isoflavone absorption and regulation.

The release of water-soluble isoflavone from microcapsules showed a similar trend in every pH 3, 4, 5 and 6 (Fig. 2), when incubated in simulated gastric fluid. When incubated at pH 3, less than 4% water-soluble isoflavone was released from the microcapsules at the initial time and it was increased up to 14.7% at 20 min and plateaued thereafter. Incubation at pH 4, 5 and 6 at 37°C at the initial time showed 3.0-4.5% release of water-

Table III. Sensory scores of different microencapsulated water-soluble isoflavone added milk for 12 day storage in refrigerated temperature[1]

Sensory description	Treatments	Storage period (d)				
		1	3	6	9	12
Beany flavor	Control	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}
	Capsulated	1.7^{c}	2.0^{b}	2.1^{b}	2.4^{ab}	2.7^{a}
	Uncapsulated	4.3^{b}	3.9^{c}	4.7^{ab}	5.4^{a}	5.5^{a}
Bitterness	Control	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}
	Capsulated	1.17^{a}	1.3^{b}	1.5^{ab}	1.7^{a}	2.1^{a}
	Uncapsulated	1.5^{b}	1.4^{b}	2.2^{ab}	2.6^{a}	2.7^{a}
Color	Control	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}
	Capsulated	1.4^{c}	1.6^{c}	2.1^{b}	2.5^{ab}	2.7^{a}
	Uncapsulated	4.9^{c}	5.3^{b}	5.6^{a}	5.45^{ab}	5.9^{a}

[1]The scale of score : 1, none; 3, slight; 5, moderate; 7, slightly strong; 9, strong. Means of duplicate. Means in a column without the same letter are significantly different ($p<0.05$).

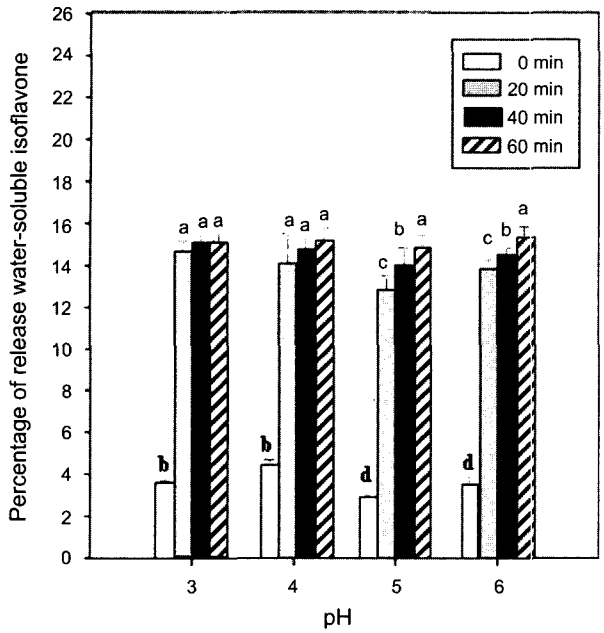

Fig. 2. Effect of different pHs on water-soluble isoflavone release from microcapsules incubated in simulated gastric fluid at 37°C for 60 min *in vitro*. Each bar represents an average of three trials. Each bar indicates a standard deviation and bar with different letters are s gnificantly different ($p<0.05$).

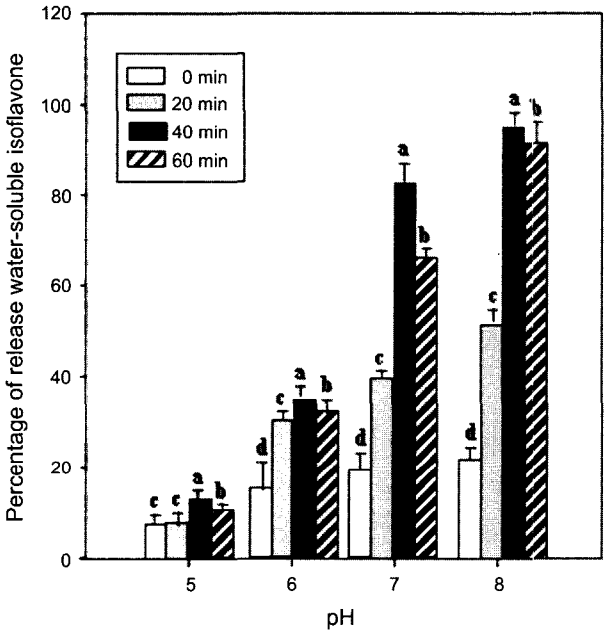

Fig. 3. Effects of different pHs on water-soluble isoflavone release from microcapsules incubated in simulated intestinal fluid at 37°C for 60 min *in vitro*. Each bar represents an average of three trials. Each bar indicates a standard deviation and bar with different letters are significantly different ($p<0.05$).

soluble isoflavone. There was a dramatic increase as 14.2, 13.0 and 14.0% at pH 4, 5, and 6 during 20 min incubation, respectively.

To determine how effectively water-soluble isoflavone was released in the intestine, a simulated intestinal fluid was prepared with the presence of pancreatin and bile salts and incubated at 37°C for 60 min (Fig. 3). With both an increase of pH and the duration of incubation, the release of water-soluble isoflavone increased dramatically, especially in pH 7 and 8. Less than 15% of the entrapped water-soluble isoflavone was released at pH 5 at every time period (0, 20, 40, and 60 min), and not much water-soluble isoflavone was released up to 60 min incubation. When incubated at pH 6, a dramatic increase (about 2 times) was observed between 0 and 20 min incubation and maintained thereafter. When incubated at pH 7 and 8, 82 and 92% water-soluble isoflavone were released from microcapsules at 40 min incubation, respectively.

A similar study (Freund *et al.*, 2000) was designed to appreciate the stability of spherulites (liposomes) in gastric or intestinal fluid in different incubation pHs. Less than 3% of spherulites was broken in acidic fluid (pH 3, 4, 5 and 6) at 37°C for 2 h incubation. Their results were in agreement with our *in vitro* results which demonstrated less than 15% release of microcapsules in an acidic condition.

It is generally accepted that for an effective uptake of nutritional effect from microcapsules, several problems need to be resolved: the capsules have to contain as much nutrition as possible, they have to resist the gastric and intestinal fluids and be captured by the enterocytes before being released into the general circulation.

Our results suggested that microcapsules would be convenient tools for water-soluble isoflavone addition due to an increase of water-soluble isoflavone absorption by favoring the uptake and effective release in the intestine.

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 satisfied efficiency of microencapsulatation such as 75%. In sensory aspects, there was no adverse effect on milk taste during 5 days in microcapsulation from PGMS. *In vitro* study, the rates of water-soluble isoflavone release from the capsules were significantly affected through the difference of pH in the environment. Therefore, we suggest that the result of this study provide evidence that microcapsulation of water-soluble isoflavone can be applied into milk.

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