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In Vitro Study of Microencapsulated Isoflavone and β-Galactosidase

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This study was designed to find the optimum conditions for isoflavone or β-galactosidase microencapsulation and to examine the release efficiency of microcapsules in simulated gastrointestinal conditions. Coating materials were either medium-chain triacylglycerol (MCT) or polyglycerol monostearate (PGMS). The highest rate of microencapsulation was found at 15:1 (w/w) ratio of MCT to isoflavone or β-galactosidase as 70.2 or 75.4%, respectively. When PGMS was used as the coating material, 91.5% β-galactosidase was microencapsulated with 15:1 mixture (w/w). *In vitro* study, less than 6.3−9.3% of isoflavone was released in simulated gastric fluid (pH 2−5) during 1 h incubation. Comparatively, isoflavone release increased dramatically to 87.8% at pH 8 for 1 h incubation in simulated intestinal fluid and was maintained thereafter. The release of β-galactosidase showed a similar trend to that of isoflavone. It appeared in the range of 12.3−15.2% at pH 2−5; however, it increased significantly to 80.6% as the highest value at pH 8. Among the released isoflavones, 53.5% was converted into the aglycone form of isoflavone at pH 8 for 3 h incubation. The present study indicated that isoflavone or β-galactosidase could be microencapsulated with fatty acid esters and released effectively in simulated intestinal condition.

KEYWORDS: Microencapsulation; isoflavone; β-galactosidase; fatty acid esters

INTRODUCTION

In recent years, the health benefits of soybean-based products have been widely recognized all over the world. Epidemiological studies and clinical trials have revealed that isoflavones and oligosaccharides in soybean are effective for the prevention of various chronic diseases and hormone-associated health disorders (*1*). Genistein, daidzein, and their derivatives such as aglyclones are the major forms found in the soybean grains and in nonfermented foods (*2*, *3*). To establish a relation between isoflavone intake and its proposed biological activity, the absorption, distribution, metabolism, and excretion isoflavones from the glycone and aglycone forms have been investigated in animals and humans (*4*, *5*). After ingestion, the glucoside forms of isoflavones are hydrolyzed to the aglycone forms by β-glucosidase (*4*, *6*) including β-galactosidase in the jejunum (*7*). The released aglycone forms of isoflavones are either absorbed intact by the intestine or are further metabolized by intestinal microflora into several other products (*8*, *9*). Moreover, since the benefits of soybean are more easily achieved with the isoflavones in their aglycone forms, fermented soybean products could provide a better source of food factors.

Soy milk is one of the popular traditional products of soy beans and has been consumed widely as a nutritious and economical protein food. However, the soy milk manufactured by the traditional process has not been accepted because of its off-flavor which is characterized by beany flavor and an objectionable aftertaste (*7*). Among several investigations on the objectionable aftertaste of soybeans, Arai et al. (*10*) identified many phenolic acids from defatted soy flour and showed that these phenolic acids had sour, bitter, and astringent taste. Sessa et al. (*11*) reported that oxidized phosphatidylcholine contributed to the bitter taste in soy flakes. Huang et al. (*12*) identified isoflavone compounds from defatted soy flour, as daidzein, genistein, and glycitein, and stated that these isoflavones might be responsible for the undesirable bitterness and astringency of soybean products.

To mask those sensory defects, microencapsulation could be a good vehicle for the addition of isoflavone to food products (*13*, *14*). Among several factors to be considered for this technique, the 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.

In addition, β-galactosidase microencapsulation could be a means of employing higher rates of bioavailability. It may prevent the hydrolysis of lactose in foods, which is mostly contained in dairy products, and may provide the increased absorption rate in intestinal condition.

Although several researchers have used coating materials such as milk fat, agar, gelatin, and so forth responsible for enzyme, flavor, and iron microencapsulation in foods (*15*−*18*), no study

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has measured the efficiency of isoflavone or β-galactosidase microencapsulation using fatty acid esters and their stability *in vitro*. Therefore, the objectives of this study were to find the optimum conditions for isoflavone or β-galactosidase microencapsulation and to examine the release efficiency of microcapsules in simulated gastrointestinal conditions *in vitro*.

MATERIALS AND METHODS

Materials. For microencapsulation, polyglycerol monostearate (PGMS) or medium-chain triacylglycerol (MCT) was used as the coating material. It was purchased from Il-Shin Emulsifier Co., Ltd. (Seoul, Korea). One core material was isoflavone, which was the glycone form extracted from soybean and purchased from Amore Pacific Co. Ltd (Seoul, Korea). The other one was β-galactosidase, fungal lactase (activity, 80,000 unit/g) purchased from Amano Enzyme, Inc. (Nagoya, Japan). The six individual standards for isoflavone, genistin, daidzin, genistein, and daidzein were obtained from Sigma Chemical Co. (St. Louis, MO), and glycitein was purchased from Fujico Co. (Tokyo, Japan). Other materials for *in vitro* study were purchased from Sigma Chemical Co. (St. Louis, MO).

Preparation of Microcapsule. Microcapsules of isoflavone were made by MCT, and β-galactosidase was made by either MCT or PGMS. Subsequently, the glycone form of isoflavone or β-galactosidase was mixed with MCT in the ratios of 5:1, 10:1, 15:1, and 20:1 (coating to core), and the conditions were the same with PGMS except for distilled water addition with four-fifths of the coating material. The spray solution was heated at 55 °C for 20 min and was mixed thoroughly with stirring at 1200 rpm.

An airless paint sprayer (W-300, Wagner Spray Tech. Co., Markdorf, Germany) nebulized a coating and core material 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 to separate isoflavone or β-galactosidase microcapsules. The microencapsulation of the core materials was done in triplicate.

Efficiency of Microencapsulation. The dispersion fluid of microencapsulation was assayed for untrapped isoflavone by MCT. One milliliter of the dispersion fluid was taken and diluted 10 times and the total isoflavone content was measured by HPLC (Waters 600, ETL Testing Lab. Inc., Cortland, NY). A sample measurement was run in triplicate.

The dispersion fluid was assayed for untrapped β-galactosidase according to a modified procedure of Shin et al. (*19*). Two milliliters of the dispersion fluid was filtered by Whatman no. 540, followed by membrane filteration (id. 1.0 μm, Whatman International Ltd., Madistone, England). The 2 mL of 5 mM *o*-nitrophenol β-galactosidase (Sigma Chemicals Co., St. Louis, MO) heated to 37 °C for 15 min was added to 0.5 mL in a water bath for 20 min. The reaction was stopped by adding 0.5 mL of 500 mM Na_2CO_3. The color intensity was read at 420 nm with Beckman DU 650 Spectrophotometer (Beckman Instruments Inc., Fullerton, CA). Microencapsulation efficiency was calculated as follows: 1 − (specific activity of residual in the dispersion fluid/initial specific activity of enzyme in spray solution) × 100. The dispersion fluid was centrifuged at 200 × *g* to remove the intact capsules from the fluid. Sample measurements were run in triplicate.

***In Vitro* Study.** To determine the stability in the stomach and intestine, the simulated gastrointestinal solutions were prepared as follows: (1) gastric fluid prepared in sample solution containing pepsin (pH 1.2) and simulated into five different fluids with pHs 2−6 using 1 N HCl and 1 N NaOH. 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 10 mM deoxycholic acid and was simulated into four different intestinal solutions as pHs 6−9.

In gastric and intestinal fluids, the microcapsules of isoflavone or β-galactosidase in distilled water were incubated at 37 °C with 100 rpm shaking for 10 min; in intestinal fluid, they were incubated at 37 °C for 30 min with the sample collecting at 1 h interval. The treated samples were centrifuged at 2,490 × *g*, and the supernatant was measured for isoflavone or β-galactosidase content released from the microcapsules. All treatments were triplicate.

Statistical Analysis. Data from each experiment were analyzed by analysis of variance (ANOVA) using a SAS program (*20*), and differences among treatments were determined by Duncan′ multiple test at $p < 0.05$, unless otherwise stated.

Table 1. Microencapsulation Efficiency of Isoflavone with Different Ratios of MCT to Isoflavone[a]

ratio (w/w)		
MCT[b]	isoflavone	efficiency (%)
5	1	57.3d
10	1	63.1c
15	1	70.2a
20	1	68.6b

[a] Means of triplicate. Means in a column without the same letter are significantly different ($p < 0.05$). [b] Medium-chain triacylglycerol.

Table 2. Microencapsulation Efficiency of β-Galactosidase with Different Ratios of Coating Materials to β-Galactosidase[a]

ratio (w/w)		efficiency (%)	
coating material	β-galactosidase	MCT[b]	PGMS[c]
5	1	50.2d	61.4d
10	1	63.7c	73.9c
15	1	75.4a	91.5a
20	1	70.2b	79.5b

[a] Means of triplicate. Means in a column without the same letter are not significantly different ($p<0.05$). [b] Medium-chain triacylglycerol. [c] PGMS (polyacylglycerol monostearate):distilled water = 5:4.

RESULTS

Microencapsulation of Isoflavone. The efficiency of microencapsulation made by MCT is shown in **Table 1**. Efficiency of isoflavone microencapsulation increased steadily up to 15:1 of coat-to-core ratio and was the greatest (70.2%) at that ratio. A significant difference was not found between 15:1 (70.2%) and 20:1 (68.6%). In the ratio of 20:1, MCT was leftover in the upper layer of dispersion fluid after centrifugation. Therefore, the optimum ratio of MCT to isoflavone was 15:1, even though leftover was still found in the upper layer.

Microencapsulation of β-Galactosidase. The efficiency of microencapsulation for β-galactosidase is shown in **Table 2**. Microencapsulation efficiency made by MCT also increased up to 15:1 of coat-to-core ratio. The highest efficiency was 75.4% in that ratio. Significant difference was not found between ratios of 15:1 (75.4%) and 20:1 (70.2%). MCT with β-galactosidase was also dispersed in the upper layer after centrifugation similar to that of isoflavone. Therefore, the optimum ratio of MCT to β-galactosidase was found to be 15:1, even though leftover was still found in the upper layer.

Even though PGMS was heated to 55 °C, it appeared to be hard to spray as described in a previous study (*18*). From preliminary experiment, when the ratio of coating (PGMS) to core material to distilled water was 5 g:1 g:30 mL, the highest efficiency was 75%. Too much addition of distilled water for microrencapsulation resulted in a weak microcapsule coat; therefore, the efficiency was decreased in our preliminary studies (*18*, *21*).

When the ratio of PGMS to distilled water was 5:4 (w/v), the optimum ratio of PGMS to β-galactosidase, 5:1, 10:1, 15:1, and 20:1, was examined as shown in **Table 2**. Efficiency of the microencapsulation increased up to 15:1 (w/w) (coating-

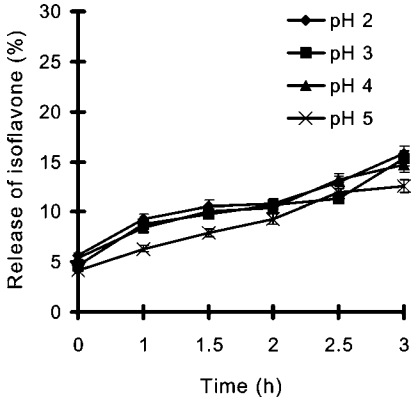

Figure 1. Effect of different pH values on isoflavone release from microcapsules incubated under simulated gastric condition *in vitro*.

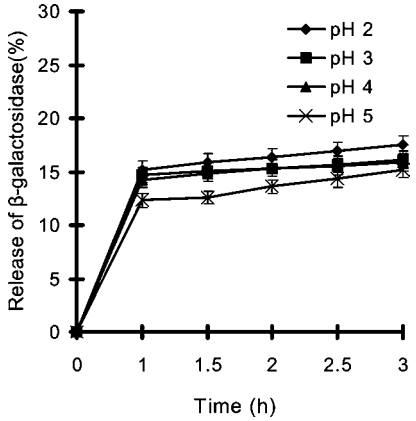

Figure 2. Effect of different pH values on β-galactosidase release from microcapsules incubated under simulated gastric condition *in vitro*.

to-core ratio) and decreased thereafter when the amount of coating material increased. When the ratio was 15:1, the microencapsulation efficiency was 91.5% as the highest value.

Since PGMS is a solid type in room temperature, an additional procedure was applied on the basis of the method for MCT microencapsulation; first, the heating process was applied for ease of spraying, and, second, distilled water was added to reduce the viscosity of spray solution for the encapsulating lactase.

***In Vitro* Study.** *(1) Simulated Gastric Condition.* This study was conducted to determine whether the microcapsules released isoflavone or β-galactosidase during simulated gastric conditions. The isoflavone release showed a similar trend in every pH (2−5) (**Figure 1**) when incubated in simulated gastric fluid. When incubated at pH 3, only about 3−5% isoflavone was released from the microcapsules at the initial time (0 h), and it was increased up to 8.8% at 1 h and plateaued thereafter. Incubation at pHs 4 and 5 at 37 °C at the initial time also showed the release of 3−5% isoflavone. There was a dramatic increase to the range of 12.5−15.8% at pHs 2−5 during 1 h incubation. The relatively higher percentage of isoflavone release in lower pHs (2 and 3) than higher pH 5 may probably be due to highly acidic condition, which resulted in capsule breakage.

A similar trend was also found in β-galactosidase case. The release of β-galactosidase showed a similar trend in every pH (2−5) (**Figure 2**) when incubated in simulated gastric fluid. When incubated at pH 2, none was released from the microcapsules at the initial time (0 h), and it was increased up to 15.2% at 1 h and plateaued thereafter. Incubation at pHs 3 and 5 at 37 °C at the initial time showed also no enzyme release. There was a dramatic increase as 12.3−15.2% at pHs 2−5 during 3 h incubation. The higher percentage of β-galactosidase release in lower pHs (3 and 4) than higher pH (5) may probably be due to a highly acidic condition, which resulted in capsule breakage.

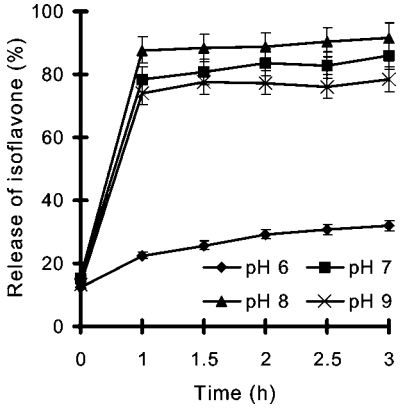

Figure 3. Effect of different pH values on isoflavone release from microcapsules incubated under simulated intestinal condition *in vitro*.

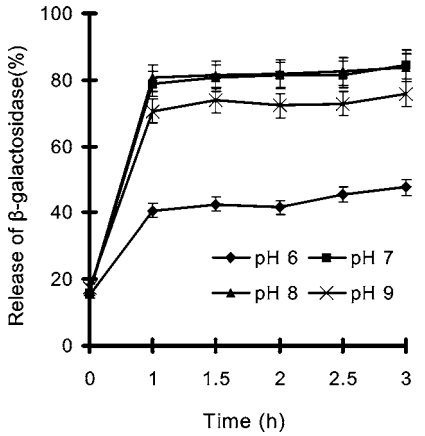

Figure 4. Effect of different pH values on β-galactosidase release from microcapsules incubated under simulated intestinal condition *in vitro*.

(2) Simulated Intestinal Condition. To determine how effectively isoflavone or β-galactosidase was released in the intestine, a simulated intestinal fluid was prepared with the presence of pancreatin and bile salts and was incubated at 37 °C for 3 h (**Figures 3** and **4**). When both pH and the duration of incubation increased, the release of isoflavone increased dramatically, especially in pHs 7 and 8. Less than 30% of the entrapped isoflavone was released at pH 6 at every time period (0−3 h), and not much increase was found until 1 h incubation. When incubated at pHs 7−9, a dramatic increase (over 4 times) was observed between 0 and 1 h incubation and was maintained thereafter. When incubated at pHs 7 and 8, 88 and 79% isoflavone were released from microcapsules at 1 h incubation and thereafter, respectively.

In the case of β-galactosidase, a similar trend was found as expected. When both the pH and the duration of incubation increased, the release of enzyme increased dramatically, especially at pHs 7 and 8. Less than 45% of the entrapped β-galactosidase was released at pH 6 at every time period (1−3 h); however, twice as much increase was found until 1 h incubation. When incubated at pHs 7−9, a dramatic increase (over 2 times) was observed between 0 and 1 h incubation and was maintained thereafter. When incubated at pHs 7 and 8, 78.8 and 80.6% β-galactosidase were released from microcapsules at 1 h incubation and thereafter, respectively.

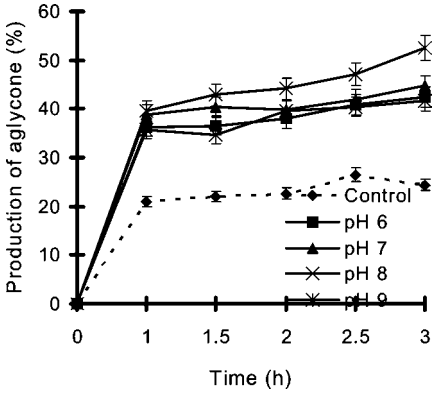

Figure 5. The production of aglycones by released β-galactosidase from microcapsules when incubated in simulated intestinal conditions.

In addition, the present study examined the conversion of released isoflavone into aglycone when incubated in simulated intestinal conditions with different pHs and times (**Figure 5**). At 1 h incubation, 35.8−39.7% of isoflavone released from microcapsules was converted into the aglycone form at pHs 6−9. The conversion rate increased to 41.7−52.5%, and the highest conversion was found at pH 8, which may be the optimum pH for released β-galactosidase. The present study indicated that the microencapsulated β-galactosidase effectively hydrolyzed isoflavone from glycoside to aglycone forms in simulated intestinal condition.

DISCUSSION

Large quantities of potentially beneficial or toxic plant phenolics are present in human diets. Although the range of plant phenolics is diverse, the majority is glycosylated, and this affects uptake, metabolism, and subsequent biological activity. Glycosylation of the (iso)flavonoids had been thought to delay intestinal absorption until the large intestine where metabolism by colonic microflora releases aglycones (*22*).

In our recent study (*23*), high hydrolysis for isoflavone conversion from glycones to aglycones occurred by exogenous β-galactosidase. Certain flavonoid glycosides such as genistein and daidzein are absorbed from the small intestine in man within a short period (*22*).

On the basis of the above information, we decided to find the effective form of isoflavone for fortification. The first thing we needed to do was to mask the objectional flavor of isoflavone, and the second was to find how to increase the isoflavone absorption or hydrolysis of isoflavone existing intact or by adding in the food system. To meet these conditions, the microencapsulation technique could be the most effective process in the present study.

When microencapsulation efficiency of PGMS or MCT was determined, the efficiency was the highest with 15:1:4 (w/w/v) of coat-to-core ratio to distilled water. In the case of 20:1 (w/w), both PGMS and MCT were leftover in the upper layer of the dispersion fluid after centrifugation. We presumed that low efficiency for isoflavone attributed to the low solubility of isoflavone in water-soluble solvent rather than the characteristics of coating material.

Isoflavone has been recognized that the human diet contains a complex array of naturally occurring bioactive non-nutrients that may confer significant long-term health 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, which is the primary site of isoflavone absorption and regulation.

It is generally accepted that for an effective uptake of nutritional effect from microcapsules, several problems need to be solved such as the capsules have to contain as much nutrition 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 isoflavone and β-galactosidase 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 isoflavone and β-galactosidase fortification because of an increase of absorption by favoring the uptake and effective release in the intestine. The present study showed a possible application in encapsulated isoflavone and β-galactosidase fortification using MCT or PGMS, which may be used effectively in the food system.

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