Optimization of Microencapsulation of *Inonotus obliquus* Extract Powder by Response Surface Methodology and Its Application into Milk

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Abstract

This study was carried out to optimize microencapsulation conditions for *Inonotus obliquus* extract powder with medium-chain triacylglycerol (MCT) using response surface methodology (RSM), and to determine the possibility of adding encapsulated *I. obliquus* extract to milk toward improving the functionality of milk. and to investigate the effects of added encapsulated *I. obliquus* extract on the physicochemical and sensory properties of the product. The microencapsulation efficiency of *I. obliquus* extract powder was investigated with respect to three variables (ratios of core material to coating material, amounts of water added, and spray pressure) in RSM. The optimal conditions for microencapsulation of *I. obliquus* extract powder were obtained from the ratio of core material to coating material (2.92:10) and the amount of water added (0.5 mL); the spray pressure had an insignificant effect on the microencapsulation ($p < 0.05$). Adding encapsulated *I. obliquus* extract into milk did not significantly affect either color (L, a and b values) or thiobarbituric acid (TBA) values. Sensory test revealed that there were significant improvements in brownish, herb flavor, and bitterness scores for encapsulated *I. obliquus* extract-added milk as compared with those for uncapsulated *I. obliquus* extract-added milk. Based on the data obtained from the present study, it is concluded that microcapsules of *I. obliquus* extract powder could be applicable to milk without remarkably adverse effects on physicochemical and sensory properties

Key words: *Inonotus obliquus*, microencapsulation, response surface methodology, milk

Introduction

Inonotus obliquus, also called Chaga mushroom, is a black parasitic fungus that belongs to family Hymenochaetaceae and grows on living trunks of the mature birch. It is widely distributed in North America, Europe, and Asia located at latitudes of 45-50°N. Traditionally, it has been used for the treatment of gastrointestinal cancer, cardiovascular disease and diabetes since the 16th century in Russia, Poland, and most of Baltic countries (Cui *et al.*, 2005; Hu *et al.*, 2009; Ju *et al.*, 2010; Lee *et al.*, 2007). Moreover, it has been reported that this fungus has the health-promoting functions, such as protection of DNA from the damage induced by oxidative stress (Park *et al.*, 2004), anti-inflammatory (Park *et al.*, 2005), antioxidant (Cui *et al.*, 2005), and antitumor activities (Kahlos *et al.*, 1986). Despite the health benefits of *I. obliquus* mentioned above, the mushroom has high viscosity, undesir-

able flavor and color. To overcome the problems, the microencapsulation technique can be a good application for *I. obliquus* into the food system.

Microencapsulation, a technique for encapsulating tiny particles or droplets by coating materials, has been widely applied in the food industry to mask the core off-taste, hide the core color, prevent oxidation and protect functional materials (Gharsallaoui *et al.*, 2007; Shahidi and Han, 1993). Even though milk is a nutritious food all over the world, some consumers require special functional properties in the milk. Therefore, over the years, many different food ingredients, such as L-ascorbic acid (Lee *et al.*, 2004), isoflavone (Jeon *et al.*, 2005), fish oil (Fonollá *et al.*, 2009), chitooligosaccharide (Choi *et al.*, 2006), and Korean mistletoe extract (Kim *et al.*, 2008) have been microencapsulated in milk formulation to improve the functional value.

To accomplish the microencapsulation of *I. obliquus*, the most important thing to be considered in the present study was the choice of a coating material to form the microcapsules. In the present study, medium-chain triacylglycerol (MCT) was used as a coating material for the microencapsulation of *I. obliquus* extract powder because

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this has been proven as an effective coating material in our previous studies (Jeon *et al.*, 2005; Kim *et al.*, 2008; Lee *et al.*, 2004).

So-called healthy foods, especially those with nutraceutical properties, are in great demand in our health conscious society. Nutraceutical milk could be a good vehicle in this respect if nutraceutical ingredients, such as *I. obliquus*, were to be added into milk. However, there is no report in the literature on the production of milk which incorporates the microencapsulated *I. obliquus*. Therefore, the objectives of the present study were to optimize the microencapsulation condition for *I. obliquus* extract powder with MCT using response surface methodology (RSM), and evaluate the effects of adding the microencapsulated *I. obliquus* extracted into milk on the physicochemical and sensory properties during storage.

Materials and Methods

Materials

As a core material, *I. obliquus* extract powder was provided from Hyun Communication (Seoul, Korea). The coating material, MCT and polyethylene sorbitan monostearate (Tween-60) were obtained from Il-Shin Emulsifier Co., Ltd. (Seoul, Korea). Market milk used was manufactured from Seoul Dairy Co-op (Ansan, Korea).

Experimental design for response surface methodology

RSM was employed to optimized the effective process of microencapsulation of *I. obliquus* powdered extract with respect to operating parameters [ratios of core material to coating material (X_1), amounts of water added (X_2) and spray pressure (X_3)], which were ranged from 0.5:10 to 5.5:10 (w/w), 0.5 to 11.5 mL, and 1,100 to 2,300 psi, respectively. The actual variable was coded to facilitate multiple regression analysis (Table 1). The least-square regression model was adequately fitted into

Table 1. Coded levels for independent variables used in experimental design for microencapsulation of *Inonotus obliquus* extract powder

Coded X_i	Variable	Coded level		
		-1	0	1
X_1	Ratio of coating material to core material	0.5:10	3.0:10	5.5:10
X_2	Water (mL)	0.5	6.0	11.5
X_3	Spray pressure (psi)	1,100	1,700	2,300

the responses taken from the experimental data and to define an optimization process of the microencapsulation efficiency.

Twenty experimental settings consisting of 6 star points (star distance is 0), 6 central points and 8 factorial points were generated with 3 factors and 3 levels by the principal of RSM using MINITAB release 15 (Korean version, Minitab Korea, Gunpo, Korea). The regression model equation for the microencapsulation efficiency (%) of *I. obliquus* extract powder could be predicted as follows:

Microencapsulation efficiency

$$= b_0 + \sum_{i=1}^3 b_i X_i + \sum_{i=1}^3 b_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 b_{ij} X_i X_j$$

where subscripts i and j took values from 1 to the number of variables (n); the b_0 was the intercept term; the b_i values were linear coefficient; the b_{ij} values were quadratic coefficient; X_i and X_j were uncoded independent variables.

Preparation of microcapsule

The microcapsule was prepared by the method from Kim *et al.* (2008). *I. obliquus* extract powder was mixed with water, and the suspension was mixed with MCT for 1 min at 1,200 rpm with a stirrer. An airless paint sprayer (W-300, Wagner Spray Tech. Co., Markdorf, Germany) nebulized the emulsion of the coating material-*I. obliquus* extract powder into a cylinder containing a 0.05% Tween-60 solution at 5°C. The diameter of the nozzle orifice used was 0.4 mm. Microcapsules were formed as lipid solidified in the chilled fluid.

To elucidate the microencapsulation efficiency, total polyphenol contents in *I. obliquus* extract powder and total polyphenol contents in residue after centrifuging the microcapsules were measured as follows. The chilled fluid was centrifuged at 4,500×g for 20 min to separate the supernatant (microcapsule suspension). After centrifuging, the residue was assayed for untrapped polyphenol contents in *I. obliquus* extract powder. The total polyphenol contents were evaluated by the Folin-Ciocalteu method as described by Wong and Chye (2009). The microencapsulation efficiency (%) was calculated as follows:

Microencapsulation efficiency (%) = $[1 - (\text{total polyphenol contents in residue} / \text{total polyphenol contents in } I. obliquus \text{ extract powder})] \times 100$

Microscopic observation

The microstructural image of the microcapsule was magnified by 1,000-folds with a light microscope (Eclipse 80i, Nikon, Tokyo, Japan). The measurement of capsule sizes was employed by Dixi imaging solution Ver. 2.89 (Dixioptics, Seoul).

Supplementation of microencapsulated or uncapsulated *I. obliquus* extract into milk

Encapsulated or uncapsulated *I. obliquus* extract was aseptically added into pasteurized milk (1%, v/v) on the basis of the preliminary study. Each milk sample was stored for 0, 3, and 6 d at 7°C to evaluate pH, color, TBA, and sensory properties as a short period of storage.

pH analysis

The pH values of each milk sample were measured at 7°C using a pH meter (Orion 900A, Boston, MA, USA).

Color measurement

Color values of each milk sample were investigated using a colorimeter (CR210; Minolta, Tokyo, Japan) after calibrating its original value with a standard plate (X=97.83, Y=81.58, Z=91.51). Measured CIE L*, a*, and b*-values were used as indicators of lightness, red-

ness and yellowness, respectively, in artificial light. This was triplicated.

Thiobarbituric acid (TBA) test

The absorbance changes by addition of encapsulated or uncapsulated *I. obliquus* extract into milk was measured using a thiobarbituric acid (TBA) test, as described by Stapelfeldt *et al.* (1997).

Sensory analysis

Eight-trained sensory panelists evaluated randomly coded milk samples. The color, rancidity, bitterness, astringency, and overall acceptability were investigated on a 7-point scale (1=very weak, 4=moderate, 7=very strong).

Statistical analysis

All statistical analyses were performed using SAS version 9.0 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was performed using the general linear models (GLM) procedure to determine significant differences among the samples. Means were compared by using Fisher's least significant difference (LSD) procedure. Significance was defined at the 5% level. This was triplicated.

Table 2. Central composite design for the optimization of microencapsulation of *Inonotus obliquus* extract powder using medium-chain triacylglycerol as a coating material

Run number	Coded variable ¹			Process variable			Microencapsulation efficiency (%)
	X_1	X_2	X_3	X_1	X_2	X_3	
1	1	1	-1	5.5:10	11.5	1100	92.87
2	-1	-1	-1	0.5:10	0.5	1100	93.05
3	-1	1	1	0.5:10	11.5	2300	86.63
4	1	-1	1	5.5:10	0.5	2300	92.17
5	0	0	0	3.0:10	6.0	1700	93.25
6	0	0	0	3.0:10	6.0	1700	93.34
7	1	-1	-1	5.5:10	0.5	1100	94.20
8	-1	-1	1	0.5:10	0.5	2300	92.98
9	0	0	0	3.0:10	6.0	1700	93.20
10	0	0	0	3.0:10	6.0	1700	93.05
11	1	1	1	5.5:10	11.5	2300	92.98
12	-1	1	-1	0.5:10	11.5	1100	85.88
13	1	0	0	5.5:10	6.0	1700	93.71
14	0	0	1	3.0:10	6.0	2300	94.26
15	0	-1	0	3.0:10	0.5	1700	95.05
16	0	0	0	3.0:10	6.0	1700	92.84
17	0	0	-1	3.0:10	6.0	1100	92.42
18	0	0	0	3.0:10	6.0	1700	92.88
19	1	1	0	5.5:10	11.5	1700	92.21
20	-1	0	0	0.5:10	6.0	1700	91.78

¹ X_1 , X_2 and X_3 are the ratio of coating material to core material, amount of water added and spray pressure, respectively.

Results and Discussion

Optimization of microencapsulation of *I. obliquus* extract powder by RSM

To measure the optimal condition of the microencapsulation of *I. obliquus* extract powder and the relationship between the response (microencapsulation efficiency) and the significant variables, statistical analyses of ANOVA was performed through a joint test of three parameters (Table 3). Among the linear, quadratic and cross-product forms of independent variables, X_1 , X_2 , X_1X_1 and X_1X_2 were significant at the level of $p < 0.05$. Thus, the regression model equation for the microencapsulation efficiency (%) could be predicted as follows:

$$\text{Microencapsulation efficiency (\%)} = 93.4109 + 1.5610X_1 - 1.6880X_2 - 1.1423X_1^2 + 1.6250X_1X_2$$

According to the regression coefficients and p -values, the linear term of ratios of core material to coating material (X_1) and amounts of water added (X_2) had significant effects on the microencapsulation efficiency ($p < 0.05$), whereas spray pressure (X_3) had a negligible effect on the microencapsulation efficiency ($p > 0.05$). Therefore, it is indicated in the present study that ratios of core material to coating material and amounts of water added could be the very critical parameters for the microencapsulation of *I. obliquus* extract powder with high microencapsulation efficiency.

Table 3. Values of regression coefficients calculated for microencapsulation of *Inonotus obliquus* extract powder using medium-chain triacylglycerol as a coating material

Independent variable ¹	Regression coefficient	Standard error coefficient	t -value	Significance level (p)
Constant	93.4109	0.2532	368.985	0.000
Linear				
X_1	1.5610	0.2329	6.070	0.000
X_2	-1.6880	0.2329	-7.249	0.000
X_3	0.0600	0.2329	0.258	0.802
Quadratic				
X_1X_1	-1.1423	0.4441	-2.572	0.028
X_2X_2	-0.2573	0.4441	-0.579	0.575
X_3X_3	-0.5473	0.4441	-1.232	0.246
Interaction				
X_1X_2	1.6250	0.2604	6.241	0.000
X_1X_3	-0.3250	0.2604	-1.248	0.240
X_2X_3	0.3700	0.2604	1.421	0.186
r^2				0.944

¹ X_1 , X_2 and X_3 are the ratio of coating material to core material, amount of water added and spray pressure, respectively.

The response surface graph for microencapsulation efficiency as a function of two selected parameters (X_1 and X_2) using significant effective factors for the microencapsulation efficiency is shown in Fig. 1 since the spray pressure had not significant effect on the microencapsulation. Increasing the ratio of core material to coating material from 0.5:10 to 3.0:10 increased the microencapsulation efficiency but the microencapsulation ratio reduced thereafter. An elevation of the amount of water added caused a reduction of microencapsulation.

Response optimization helps to identify the factor settings that optimize a single response or a set of responses. It is useful in determining the operating conditions that will result in a desired response. In the present study, the goal was to search the optimization condition for the encapsulation of *I. obliquus* extract powder. As shown in Fig. 2, the best combination of factor settings for achiev-

Fig. 1. Response surface for microencapsulation efficiency of *Inonotus obliquus* extract powder with respect to the ratio of core material to coating material and amount of water added at the spray pressure of 2,300 psi.

Fig. 2. Process optimization curve for microencapsulation of *Inonotus obliquus* extract powder. Current means optimum condition of the microencapsulation. y: the yield of the microcapsule, d: desirable score.

ing the desired response was found to be the ratio of core material to coating material (2.92:10) and amount of water added (0.5 mL) with a desirability score of 0.971 which means reaching maximum yield of the capsules.

Microscopic observation

A photograph of microencapsulated *I. obliquus* extract produced under the ratio of core material to coating material (3.0:10), amount of water added (0.5 mL) and spray pressure (1,700 psi) was illustrated in Fig. 3. The overall sizes of microcapsules produced ranged from 1 to 9 μm . The microscopic examination of microcapsules containing *I. obliquus* extract revealed spherical particles, and the microcapsules had rounded surfaces and evenly distributed pockets of *I. obliquus* extract solution in microcapsules made by MCT. According to Choi *et al.* (2006), the average sizes (50–200 μm) of the microcapsules were too sufficiently small to offer a gritty texture to the milk and float to the surface of the milk. In general, homogenized milk has about 2–5 μm in milk fat size, which make milk stable by fat dispersing in milk serum. Based on this phenomena, the capsule size (1–9 μm) also evenly disperse in milk, such a small microcapsule size is desirable. Therefore, the textural changes in the food system may be avoided. Finally, the present study showed the sufficiently small size of microcapsules for adding into milk.

Changes in pH, color, and TBA values

The microcapsules produced under the ratio of core material to coating material (3.0:10), amount of water added (0.5 mL) and spray pressure (1,700 psi) for microencapsulation of *I. obliquus* extract powder were added into milk. The changes of pH in encapsulated or uncapsulated

Fig. 3. Photomicrograph of microencapsulated *Inonotus obliquus* extract with medium-chain triacylglycerol as a coating material. The photograph was taken at 1,000 $\times$ magnification.

sulated *I. obliquus* extract-added milk stored at 7°C for 6 d are illustrated in Fig. 4. The pH values for all the milk samples were nearly constant during 6-d storage and ranged from 6.7 to 6.9, as a reflective of fresh state (Lee *et al.*, 2006).

Table 4 presents color values of milk samples with the addition of encapsulated or uncapsulated *I. obliquus* extract during the storage at 7°C for 6 d. The L-values significantly reduced with the addition of uncapsulated *I. obliquus* extract during all the storage periods. However, the incorporation of encapsulated *I. obliquus* extract into the milk samples did not significantly affect the L-values during all the storage periods. Elevating the storage periods from 0 to 6 d did not significantly influence the L-values for all the milk samples studied. According to Philips *et al.* (1995), the L-value of milk has been demon-

Fig. 4. Changes of pH in encapsulated (EC) or uncapsulated (UC) *Inonotus obliquus* extract-added milk stored at 7°C during 6 d. Control: market milk.

Table 4. Changes of color for encapsulated (EC) or uncapsulated (UC) *Inonotus obliquus* extract-added milk stored at 7°C during 6 d¹

Treatment	Storage period (d)	Color value		
		L*	a*	b*
Control ²		91.17 ^a	−3.36 ^e	5.61 ^d
EC	0	90.65 ^{ab}	−3.06 ^d	6.08 ^c
UC	0	75.17 ^c	0.96 ^a	18.12 ^a
Control	3	90.33 ^b	−3.53 ^f	6.20 ^c
EC	3	90.27 ^b	−3.04 ^d	6.32 ^c
UC	3	76.71 ^c	0.62 ^b	17.38 ^b
Control	6	90.66 ^{ab}	−3.34 ^e	5.80 ^d
EC	6	90.10 ^b	−2.84 ^c	6.22 ^c
UC	6	76.42 ^c	0.72 ^b	17.35 ^b

¹Values with different letters within the same column differ significantly ($p < 0.05$).

²Control: market milk.

strated to have the most positive impact on consumer appeal. Accordingly, in the present study, it is speculated that the incorporation of the encapsulated *I. obliquus* extract into the milk samples as well as the prolonged storage (6 d) of the milk samples could not remarkably influence the consumer appeal.

The CIE a^* - and b^* -values slightly increased when encapsulated *I. obliquus* extract was added into the milk samples. On the other hand, there were remarkable differences between the control and uncapsulated *I. obliquus* extract-added milk samples during 6-d storage because *I. obliquus* extract powder is originally dark brown. On the basis of all of the results obtained from the present study (CIE L^* -, a^* -, and b^* - values), it is suggested that the changes in color values were markedly masked by micro-

encapsulation of *I. obliquus* extract powder, even though slight differences were found in color values between the control and encapsulated *I. obliquus* extract-added milk groups.

The effects of encapsulated or uncapsulated *I. obliquus* extract addition into milk on chemical oxidation (as measured by the TBA test) during 6 d of storage at 7°C are shown in Fig. 5. TBA absorbance values for the uncapsulated *I. obliquus* extract-added milk sample was slightly lower than control milk. This result could be explained by that *I. obliquus* extract contains polyphenol which acts as an antioxidant (Ju *et al.*, 2010). In the case of encapsulated *I. obliquus* extract-added milk samples, the very slight increase in the values of TBA absorbance was found from 0.13 to 0.16. This result could be explained by that medium-chain triacylglyceride (MCT) used as a coating material may be oxidized during storage of the milk. Therefore, the results indicated that there was no considerable chemical oxidation in the encapsulated or uncapsulated *I. obliquus* extract-added milk samples during the storage.

Sensory analysis

The sensory properties of encapsulated or uncapsulated *I. obliquus* extract-added milk stored at 7°C for 6 d are listed in Table 5. When uncapsulated *I. obliquus* extract was added into milk at 0-d storage, brownish scores significantly increased due to the original dark brown color of *I. obliquus* extract powder. On the other hand, there were no differences in the brownish scores between encapsulated *I. obliquus* extract-added milk and control samples, confirming the color masking effect of microen-

Fig. 5. Changes of thiobarbituric acid (TBA) values in encapsulated (EC) or uncapsulated (UC) *Inonotus obliquus* extract-added milk stored at 7°C during 6 d. Control: market milk.

Table 5. Sensory characteristics of encapsulated (EC) or uncapsulated (UC) *Inonotus obliquus* extract-added milk stored at 7°C during 6 d¹

Treatment	Storage period (d)	Sensory description ³					
		Brownish	Rancid	Herb flavor	Bitterness	Oily	Overall acceptability
Control ²	0	1.0 ^b	1.0 ^c	1.0 ^c	1.0 ^c	1.0 ^a	5.2 ^{ab}
EC		1.5 ^b	1.3 ^{bc}	2.0 ^c	1.2 ^c	1.6 ^a	5.5 ^{ab}
UC		6.0 ^a	1.2 ^c	4.8 ^a	3.7 ^a	1.8 ^a	3.8 ^b
Control	3	1.0 ^b	1.0 ^c	1.0 ^c	1.0 ^c	1.0 ^a	6.0 ^a
EC		1.2 ^b	1.3 ^{bc}	1.3 ^c	1.2 ^c	1.7 ^a	5.3 ^{ab}
UC		5.2 ^a	2.1 ^{ab}	3.3 ^b	3.0 ^{ab}	2.0 ^a	4.0 ^{ab}
Control	6	1.0 ^b	1.0 ^c	1.0 ^c	1.0 ^c	1.0 ^a	5.7 ^{ab}
EC		1.8 ^b	2.6 ^a	1.8 ^c	1.8 ^{bc}	2.1 ^a	4.1 ^{ab}
UC		5.2 ^a	1.7 ^{bc}	3.5 ^b	2.7 ^{ab}	2.2 ^a	4.8 ^{ab}

¹Values with different letters within the same column differ significantly ($p < 0.05$).

²Control: market milk.

³The scale of brownish, rancid, herb flavor, bitterness and oily scores: 1=very weak, 4=moderate, 7=very strong. The scale of overall acceptability scores: 1=dislike very much, 4=either like, 7=like very much.

capsulation. Rancidity and oily scores for the milk samples at 0-d storage were not significantly affected by the addition of encapsulated or unencapsulated *I. obliquus* extract. Herb flavor and bitterness scores were significantly increased at the entire storage periods when unencapsulated *I. obliquus* extract powder was used for the production of unencapsulated *I. obliquus* extract-added milk samples, probably due to the original herb flavor and bitter taste of *I. obliquus* extract powder. On the other hand, herb flavor and bitterness scores for encapsulated *I. obliquus* extract-added milk were not significantly different from the control milk sample, indicating masking of the original herb flavor and bitter taste of *I. obliquus* extract by microencapsulation technique. Overall scores for all the samples studied were not significantly different. Based on all the sensory data obtained from the present study, it is suggested that inappropriate properties of *I. obliquus* extract powder in sensory aspects, such as dark brown color, herb flavor, and bitter taste, can be masked effectively by microencapsulation technique. Thus, the present study indicates the possibility of adding *I. obliquus* extract powder into milk.

Response surface methodology was successfully used to optimize the microencapsulation conditions for *I. obliquus* extract powder. Based on the results obtained from RSM, the ratio of core material to coating material and amount of water added were the critical parameters to be considered for the microencapsulation of *I. obliquus* extract powder. The data on the pH, TBA, color, and sensory analysis obtained from the present study indicated that the quality properties of encapsulated *I. obliquus* extract-added milk were not remarkably different from the control. The production of the milk which incorporates encapsulated *I. obliquus* extract can broaden the utilization of *I. obliquus* extract powder, and the products can be regarded as possible health-promoting nutraceutical foods.

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