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Microencapsulation and properties of the silkworm pupal oil with soybean protein isolate/β-cyclodextrin

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ABSTRACT

Microencapsulation process make the small droplets of oil are surrounded by a shell coating of proteins and carbohydrate resulting in small dry granules, an extended shelf life, and eliminating the unpleasant taste and odor. Response surface methodology (RSM) was applied to optimize the parameters of microencapsulation of the silkworm pupal oil. The linear term of the ratio of wall material and core material, and the quadratics of the mass fraction of solids, the ratio of wall material and core material, and homogeneous time, as well as the interactions between the mass fraction of solids and homogeneous time showed significant effects on microencapsulate efficiency. The optimal condition for microencapsulation of oil within the experimental range of the variables researched was at 6.13min, 2.37 wall material:1 core material, 20.37% of the mass fraction of solids. At this condition, the predicted value of microencapsulates efficiency reached 53.95%. The freeze-dried microcapsules have two types of structures, small spherical units of less than 10μm and agglomerates less than 5μm.

Key words: Microencapsulation; silkworm pupal oil; Response surface methodology; optimization

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INTRODUCTION

Silkworms are well-known as an efficient large-scale producer of silk thread. The mulberry silkworm (*Bombyx mori* L.) and two non- mulberry silkworm, the oak silkworm (*Antheraea pernyi*) and the eri silkworm (*Samia cynthia ricini*), are the main species around the world [1]. The pupae are rich in protein, containing 18 known amino acids, including all of the essential amino acids and sulphur-containing amino acids, and are of high quality according to the amino acid profile recommended by FAO/WHO [2-4]. In recent years, silkworm pupae have been put on the list of ''novel food resources managed as common food'' by Ministry of Health PR China [5]. The silkworm pupal oil is abundant in unsaturated fatty acid, reaching 75% of total fat, while polyunsaturated acid especially the alpha-linolenic acid (ALA) accounts for a high percentage as well. The long chain n-3 fatty acids, e.g. ALA, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and n-9 fatty acids (e.g. oleic acid) are wildly added into the diet. The ALA and oleic acid are rich in three silkworm pupae oil, with 27.99% ALA and 33.26% oleic acid in mulberry silkworm pupal oil [6], 34.27% ALA and 30.97% oleic acid in oak silkworm pupal oil, and 50.52% ALA and 9.13% oleic acid in eri silkworm pupal oil [7], respectively. There is a huge volume of literature supporting the positive benefits of including the long chain n-3 fatty acids in the diet. Thus, the silkworm pupal oils are new sources of long chain n-3 and n-9 fatty acids. However, the slight flavors and lipid oxidation in the silkworm pupal oils may limit the incorporation these oils into food formulations.

The microencapsulation of the fish oil can provide many benefits such as providing an oxygen barrier resulting in an extended shelf life, a taste profile barrier eliminating fish oil taste and odor, high nutritional density and nutritional availability, and a protective barrier from shear and temperature changes when incorporated into foods [8]. Microencapsulation is also a strategy that has been used to stabilize and deliver bioactive and to protect n-3 fatty acids after they have been isolated from their source [9-11]. Recently, the microencapsulation had been used in oils process, e.g. citronella oil [12], tea tree oil [13], sweet orange oil [14], clove bud oil and red thyme oil [15], and extra-virgin olive oil [16].

The coating materials, which were used by oil microencapsulated, include zein, sugar beet pectin [17], the gelatin/gum Arabic [18], soybean protein isolate/gum Arabic [14], silica gel [19], soybean soluble polysaccharide and octenyl succinic anhydride [20]. The capsule wall components and the addition of antioxidant additives affected the shelf life and chemical alteration [21]. In present research, the soybean protein isolate/β-cyclodextrin was selected due to the low cost and the biocompatible nature of the ingredients involved, which is also most desirable in oil intended for human use. A larger literature indicated that even the best combination of biopolymers for microencapsulating oil used with different drying techniques can produce both stable and unstable produces [20]. A commonly method such as spay drying or freeze drying has been used for microencapsulation. Spray-drying, which is a particularly simple and economically effective means of microencapsulating chemically reactive oils and flavor compounds [22], may produce individual particles, but the low physical strength of the wall and the conditions used in this process may limit the use of this method [23].

Microencapsulation process makes it possible to transform the oil into a powder, where the small droplets of oil are surrounded by a shell coating of proteins and carbohydrate resulting in small dry granules that have powder like flow characteristics [8]. The objectivity of this research was to explore the feasibility of using isolated soy protein and β-cyclodextrin as wall material, to develop and optimize the microencapsulation process for silkworm pupae oil. The response surface methodology (RSM) was used to optimize the operating parameters on the microencapsulate efficiency (ME) of oil, including homogeneous time, the ratio of wall material and core material, the mass fraction of solids. The apparent diameter of microencapsulated silkworm pupal oil was observed by scanning electron microscope.

EXPERIMENTAL SECTION

MATERIALS

The oak silkworm pupal used in present research was extracted followed the description by Wei et al (2009) [6]. The oak silkworm pupal was obtained from Shenyang University of agricultural (ShenYang, China). The extracted pupal oil contains 34.27% α-linolenic acid, 30.97% oleic acid, 19.92% palmitic acid, 6.89% linoleic acid, 4.77% palmitoleic acid, 1.99% Stearic acid, 0.60% heptadecanoic acid and 0.39% 0(Z),13(Z),16(Z)-nonadecatrienoic acid [7]. The isolated soy protein and β-cyclodextrin was purchased in the market.

REAGENTS

Carbon dioxide (99.99% purity), contained in a cylinder, was purchased from Jinwang Gas Co. (Anhui, China). Petroleum ether, with boiling range from 30°C to 60°C, was purchased from Hefei Haze Biotechnology Co., LTD (Anhui, China). Other solvents and chemicals were obtained commercially and were of analytical grade.

PREPARATION OF ENCAPSULATION MATERIALS AND EMULSIONS FOR DRYING

The β-cyclodextrin suspensions was dissolved in 50°C water for 10 min until the emulsion was transparent, and then, was mixed with the isolated soy protein. The mixture was homogenized with the oak silkworm pupal oil for some minutes by a high shear homogenizer IKAT18 (Shanghai, China). The microcapsule emulsion including oil was freeze-dried using a freeze-drier LGJ (Beijing, China). Then, the microencapsulated oak silkworm pupal oil become powder and stored at 4 °C for further use. The microencapsulate efficiency (ME) of the oak silkworm pupal oil was determined by a modified method described by Tan et al (2005) [24]. Hexane (100 mL) was added to 10g microencapsulated oak silkworm pupal oil powder and stirred for 10 min. The suspension was filtered and the residue rinsed three times by hexane through each time. The residual powder was air dried at 80°C for 30 min and weighed. The amount of surface oil (O_S) was calculated by as follows [8, 25]:

$$O_S = \text{Original weight} - \text{Final Weight of microspheres} \quad (1)$$

The total oil (O_T) includes the encapsulated oil (O_E) and O_S. It was determined using the Soxhlet extraction unit. 10 g microencapsulated oak silkworm pupae oil powder was extracted using 60 mL hexane for 5 h. After extraction, the oil exhausted powder was air-dried to constant weight. The O_T and the ME can be calculated as follows:

$$O_T = \text{Original weight} - \text{Weight of Soxhlet extracted microspheres} \quad (2)$$

$$O_E = O_T - O_S \quad (3)$$

$$ME = O_E / O_T \times 100\% \quad (4)$$

COLOR OF MICROCAPSULES CONTAINING OAK SILKWORM PUPAL OIL

Color of the microcapsules containing oak silkworm pupal oil was determined using the chroma meter Hunter Lab (USA). Color data were reported in CIELAB color scales (L*value is degree of lightness to darkness, a*value is degree of redness to greenness, and b* value is degree of yellowness to blueness). The values of chroma and hue angle were calculated as following [8]:

$$\text{Chroma} = [a^{*2} + b^{*2}]^{1/2} \qquad (5)$$

$$\text{Hue angle} = \tan^{-1}(b^*/a^*) \qquad (6)$$

EXPERIMENTAL DESIGN FOR RESPONSE SURFACE METHODOLOGY

To obtain a high microencapsulate efficiency, the response surface methodology (RSM) and Box-Behnken design was used for optimizing the microencapsulation process, including homogeneous time, the ratio of wall material and core material and the mass fraction of solids. Three independent variables studied were the mass fraction of solids (%, X1), the ratio of wall material and core material (X2), and homogeneous time (min, X3), for uncoded variable levels (Table 1). These independent variables and their levels were selected based on the preliminary experiments (data not shown). The experimental design needed 15 experimental settings with 12 factorial points and 3 central points, which was showed in Table 2. Response (Y), i.e. microencapsulate efficiency at each design point was recorded. Triplicate extractions were carried out at all the design points. Experiments at the center point were conducted for evaluation of the experimental error. A second-order polynomial equation was used to express the microencapsulate efficiency (Y) as a function of the independent variables. The experiments were run in random order to minimize the effects of unexpected variability in the observed responses due to extraneous factors. The experimental design included 15 experiments of three variables at three levels (−1, 0, +1). Table 1 gives the range of variables employed. The actual set of experiments performed (experimental runs 1–15) and the microencapsulate efficiency of the oak silkworm pupal oil obtained are listed in Table 2.

A second-order polynomial equation was developed to study the effects of variables on the microencapsulate efficiency. The equation indicates the effect of variables in terms of linear, quadratic, and cross-product terms: The second-order polynomial fitted was [6]:

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii} X_i^2 + \sum\sum_{i<j=1}^{3}\beta_{ij} X_i X_j \qquad (5)$$

where Y is the microencapsulate efficiency (ME) of the oak silkworm pupal oil (%), Xi and Xj are the levels of variables (the mass fraction of solids, the ratio of wall material and core material, and homogeneous time), β_0 the constant term, β_i the coefficient of the linear terms, β_{ii} the coefficient of the quadratic terms, and β_{ij} the coefficient of the cross-product terms. The experimental results were analyzed using SAS9.2 software to build and evaluate models and to plot the three-dimensional response surface curves.

Table 1 Uncoded and coded levels of independent variables used in the RSM design.

Independent variables	Symbols	variables Levels		
		-1	0	1
The mass fraction of solids (%)	X1	15	20	25
The ratio of wall material and core material	X2	1	2	3
Homogeneous time (min)	X3	4	6	8

Table 2 Experimental scheme and results obtained from RSM for the oil microencapsulate efficiency.

Design point	Coded variable			Process variable			ME
	X1	X2	X3	X1	X2	X3	Y (%)
1	-1	-1	0	15	1	6	43.50
2	-1	1	0	15	3	6	46.70
3	1	-1	0	25	1	6	41.17
4	1	1	0	25	3	6	49.15
5	0	-1	-1	20	1	4	45.12
6	0	-1	1	20	1	8	44.36
7	0	1	-1	20	3	4	48.90
8	0	1	1	20	3	8	49.09
9	-1	0	-1	15	2	4	41.05
10	1	0	-1	25	2	4	47.38
11	-1	0	1	15	2	8	48.02
12	1	0	1	25	2	8	45.00
13	0	0	0	20	2	6	52.25
14	0	0	0	20	2	6	53.58
15	0	0	0	20	2	6	54.55

RESULTS AND DISCUSSION

FITTING THE MODEL

The experimental design was adopted on the basis of coded level from three independent variables (Table 1) to minimize the experimental runs and the time for optimizing microencapsulation conditions for oak silkworm pupal oil, resulting in a 15 simplified experimental set (Table 2). To obtain a regression equation that could predict the response within the given range, the independent and dependent variables were analyzed. The regression coefficients of intercept, linear, quadratic, and interaction terms of the model are presented in Table 3, which were calculated by the least square technique. In order to check the significance of each coefficient and the interaction strength of each parameter, the means of the ANOVA (F-test) and p-value are used (Table 3). The p-value of the model was 0.0038, which confirmed that the model was suitable for this experiment. Meanwhile, the ‘‘lack of fit” of this model with the p-value was insignificant (0.4477). The above results indicate that the accuracy and general availability of the polynomial model are adequate, where the coefficient of determination (R^2) and adjusted coefficient of determination (Adj.R^2) are 0.9655 and 0.9033, respectively.

To calculate the estimation of the coefficients of the second-order polynomial equation and the obtained regression coefficients, multivariable linear regression, whose significance was determined by the F-test and p-value, was used (Table 4). The corresponding variable will be more significant if the absolute value of F becomes larger and the p-value becomes smaller. Neglecting the non-significant parameters, the final predictive equation obtained is given as below:

$$Y1 = -92.7 + 10.61875X2 - 0.1967X1X1 - 0.23375X1X3 - 3.4125X2X2 - 0.795X3X3 \quad (6)$$

From Table 4 and Eq. (6), it was evident that the factor with the largest effect on the microencapsulate efficiency of oil was the one linear term of the ratio of wall material and core material ($p < 0.01$), followed by the three quadratic term of the mass fraction of solids, the ratio of wall material and core material, and homogeneous time ($p < 0.01$). The interactions between the mass fraction of solids and homogeneous time also had high significant effects on the microencapsulate efficiency. Based on the above model, the optimal condition for microencapsulate of oak silkworm oil was at homogeneous time 6.13min, the ratio of wall material and core material 2.37:1, the mass fraction of solids 20.37%, and the microencapsulate efficiency of oak silkworm pupal oil was 53.95% under this condition.

Table 3 Analysis of variance of regression parameters for the response surface model

Source	df	SS	MS	F	P
Model	9	228.1754	25.35283	15.53877	0.0038
Lack of fit	3	5.491325	1.830442	1.372866	0.4477
Pure error	2	2.6666	1.3333		
Cor total	14	236.334			
R^2=96.55%，Adj.R^2=90.33%					

Table 4 Regression coefficients of predicated second-order polynomial model for the response variables

Source	DF	SS	MS	F	P
X_1	1	1.470612	1.470612	0.90134	0.386
X_2	1	48.46201	48.46201	29.70241	0.0028
X_3	1	2.02005	2.02005	1.238091	0.3165
X_1*X_1	1	89.28667	89.28667	54.72388	0.0007
X_1*X_2	1	5.7121	5.7121	3.500952	0.1202
X_1*X_3	1	21.85563	21.85563	13.39533	0.0146
X2*X2	1	42.9975	42.9975	26.35321	0.0037
X2*X3	1	0.225625	0.225625	0.138286	0.7252
X3*X3	1	37.33809	37.33809	22.88455	0.0050

ANALYSIS OF RESPONSE SURFACE

According to Eq. (6), three dimensional response surface curves and contour plots were plotted to determine their optimum values and the interactions among the various selected factors for attaining the maximum microencapsulate efficiency. The best way to visualize the influence of the independent variables on the dependent one is to draw surface response plots of the model. The plots were generated by plotting the response using the z-axis against two independent variables with keeping the other two independent variables at their zero level (Fig. 1-3).

Fig. 1 shows the interaction between the mass fraction of solids and the ratio of wall material and core material on microencapsulate efficiency for oak silkworm pupae oil, while homogeneous time is fixed at 6 min. The microencapsulate efficiency significantly increased with increasing the ratio of wall material and core material, then decreased slowly with increasing the ratio of wall material and core material after the fixed mass fraction of solids reach the center point, and this trend became more obvious at lower mass fraction of solids tested.

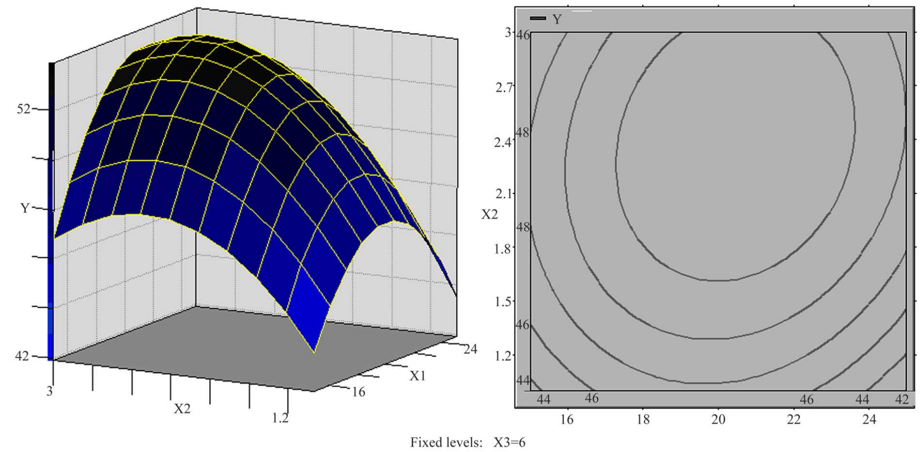

Fig. 1. Response surface plots showing the effects of the ratio of wall material and core material and the mass fraction of solids on microencapsulate efficiency. X1: The mass fraction of solids (%); X2: The ratio of wall material and core material; Y: The Microencapsulate efficiency.

The effect of the mass fraction of solids and homogeneous time on microencapsulate efficiency was showed in Fig. 2. The results indicated that the microencapsulate efficiency increased dramatically with the increase of homogeneous time at a lower fixed mass fraction of solids, while the microencapsulate efficiency increased gradually with the increase of mass fraction of solids at the lower homogeneous time. When the value of the mass fraction of solids lies in the center point up and down, the microencapsulate efficiency increased slowly with the increase of homogeneous time, while the microencapsulate efficiency decreased flatly with the increase of homogeneous time.

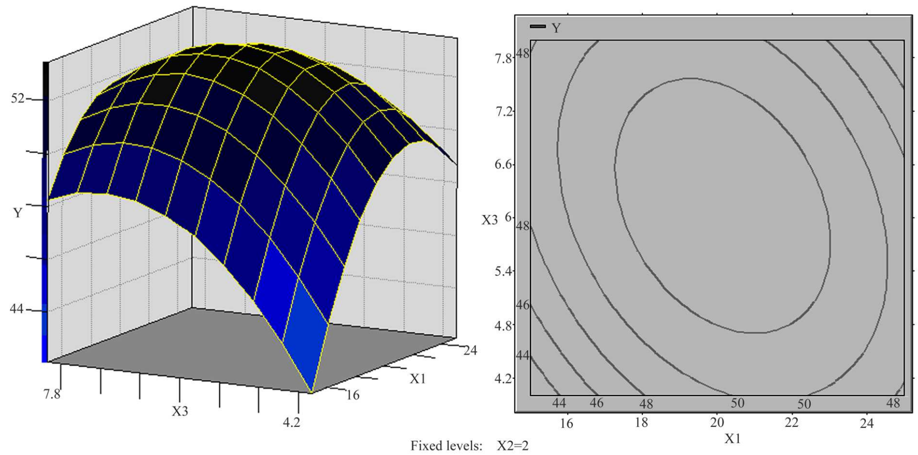

Fig. 2. Response surface plots showing the effects of Homogeneous time and the mass fraction of solids on microencapsulate efficiency. X1: The mass fraction of solids (%); X3: Homogeneous time (min); Y: The Microencapsulate efficiency.

The interaction between the ratio of wall material and core material and homogeneous time showed (Fig. 3) that the oak silkworm oil microencapsulate efficiency increased gradually with the increase of homogeneous time at a fixed the ratio of wall material and core material, then decrease gradually with homogeneous time.

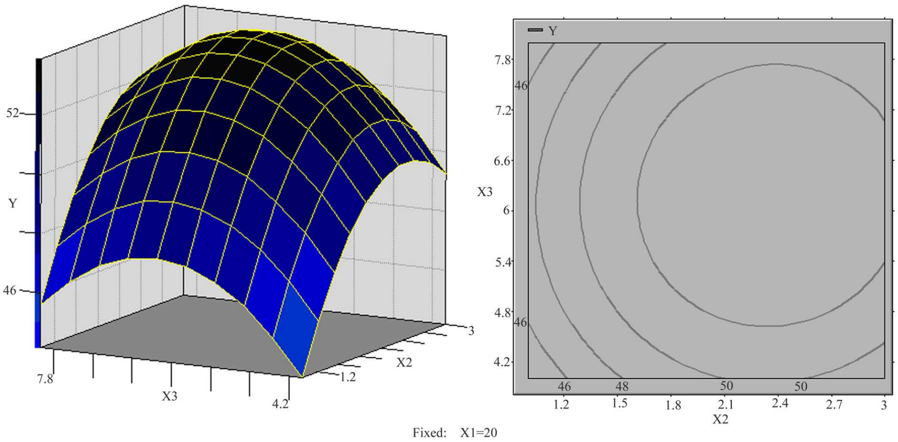

Fig. 3. Response surface plots showing the effects of Homogeneous time and the ratio of wall material and core material on microencapsulate efficiency. X2: The ratio of wall material and core material; X3: Homogeneous time (min); Y: The Microencapsulate efficiency.

COLOR OF OAK SILKWORM PUPAL OIL MICROCAPSULES

We used two different drying techniques, including spay drying and freeze drying, to produce oak silkworm pupal oil microcapsules. The color of two microcapsule samples was determined using the chroma meter Hunter Lab. The values of L*, a*, b*, chroma and hue angle were shown in Table 5. The result indicated that the microcapsule sample using spay drying was more darkness and more yellow than the microcapsule sample using freeze drying.

Table 5 The color of microcapsules containing oak silkworm pupae oil

Color	wall	freeze drying	spay drying
L*	90.09	86.26	73.93
a*	0.76	4.70	10.30
b*	16.08	5.94	0.87
chroma	16.12	7.57	10.34
hue angle	2.58	72.46	118.39

MICROCAPSULES CHARACTERIZATION

Microcapsules containing oak silkworm pupal oil were characterized by electronic microscopy after freeze-dried. The blackberry-like morphology of the microcapsules in afresh slurry and their apparent diameter was all varied less than 10μm. The microphotographs of freeze-dried microcapsules of oak silkworm pupal oil clearly show two types of structures: small spherical units of less than 10μm and agglomerates less than 5μm (Fig. 4a), and all of the microcapsules of oak silkworm pupal oil was smaller the coating materials, such as the soybean protein isolate (Fig. 4b) and the β-cyclodextrin (Fig. 4c).

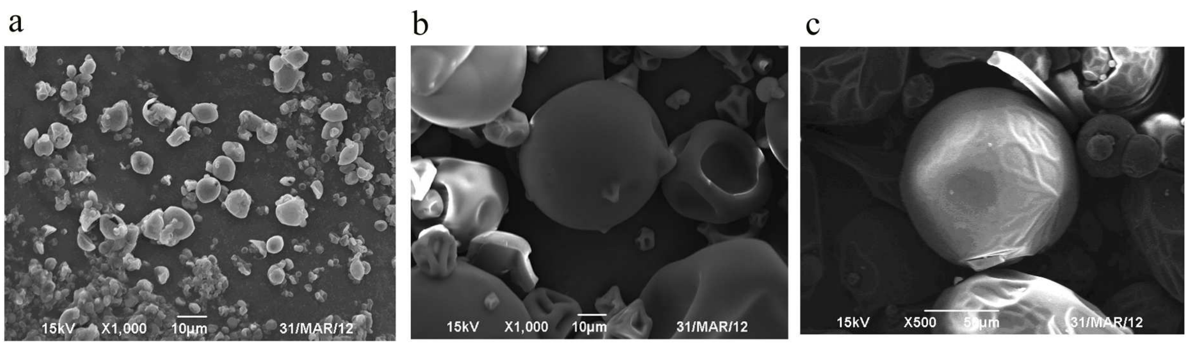

Fig. 4. Scanning electron microphotographs of the freeze-dried microcapsules.

a, microcapsules containing oak silkworm pupae oil (Magnified × 1000); b, microphotographs of the soybean protein isolate (Magnified × 1000); c, microphotographs of the β-cyclodextrin (Magnified × 500).

CONCLUSION

For the conditions used in this work, we conclude that the microencapsulation for oak silkworm pupal oil was dependent on the linear term of the ratio of wall material and core material, and the quadratics of the mass fraction of solids, the ratio of wall material and core material, and homogeneous time, as well as the interactions between the mass fraction of solids and homogeneous time. A polynomial regression model was established to describe the experimental results, the optimal condition for microencapsulate efficiency was at 6.13min, 2.37:1, 20.37%. At this condition, the predicted value of microencapsulates efficiency reached 53.95%. The freeze-dried microcapsules have two types of structures, small spherical units of less than 10μm and agglomerates less than 5μm.

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