Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 107 (2008) 98–105
Food Chemistry
www.elsevier.com/locate/foodchem

Optimization of microencapsulation of seed oil by response surface methodology

Jang-Hyuk Ahn [a,b], Young-Pil Kim [a], Yu-Mi Lee [b], Eun-Mi Seo [b], Ki-Woong Lee [b], Hak-Sung Kim [a,*]

[a] Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1, Gusung-dong, Yusung-gu, Daejeon 305-701, Republic of Korea
[b] Research and Development Center, Namyang Dairy Co., Ltd., Gongju 314-914, Republic of Korea

Received 3 May 2007; received in revised form 11 July 2007; accepted 19 July 2007

Abstract

The response surface methodology (RSM) was employed to optimize the microencapsulation condition of sunflower oil (SO) as a typical seed oil. The microencapsulation efficiency (MEE) of microencapsulated sunflower oil (MESO) was investigated with respect to four variables including SO concentration (X_1), proportion of milk protein isolates (MPI) to coating wall (X_2), soy lecithin concentration (X_3), and homogenizing pressure (X_4). As a result, a polynomial regression model equation was fitted as follows: $\text{MEE}\ (\%) = 4.137772 + 3.524183X_1 + 3.475205X_2 + 2.914167X_3 - 0.074532X_1^2 - 0.067482X_2^2$. Effect of homogenizing pressure was negligible. The optimal conditions for microencapsulation of SO were 23.6% SO, 19.0% MPI, 2.5% soy lecithin, and 54.8% dextrin, respectively. MESO under the optimized conditions gave rise to the highest MEE, approaching 96.6% of MEE. Compared to MESO showing a low MEE (70.2%), the peroxide value (POV) of the total oil from the MESO under the optimized conditions was significantly lowed even under the accelerated storage conditions at 60 ± 1 °C after 30 days, which indicates a promising feature of RSM-mediated microencapsulation process of seed oil.

Keywords: Response surface methodology; Microencapsulation; Seed oil; Lipid oxidation; Peroxide value

1. Introduction

Microencapsulation has been widely used for manufacturing of powdered edible oil products (Rosenberg & Lee, 1993; Rosenberg & Young, 1993) since it enables a prolonged shelf-life by protecting oils from oxidation. Using the appropriate encapsulating substances, core component oils in microcapsules can be protected from deterioration caused by adverse environmental conditions such as light, moisture and oxygen. Consequently, the shelf life of the products could be prolonged (Shahidi & Han, 1993). Despite protective effect of microencapsulation, severe lipid oxidation on the surface of the microcapsules could occur due to exposure to high temperature during the spray-drying process. It has been known that minimizing the fat content on the surface of microcapsules is crucial to the production of stable fat powders against oxidation. In the case of microencapsulation of milk fat using whey proteins, low level of fat content resulted in stable fat powders (Keogh & O'Kennedy, 1999). Residual oils on the surface of the microcapsules would influence a harmful effect on the oxidation of microencapsulated oils. In this regard, microencapsulation efficiency (MEE) has been used as an important parameter to assess the quality of microencapsulated oils.

Lipid oxidation during storage or food processing usually causes a deleterious effect on human health (Frankel, 1998) since it can lead to the rancidity (Gordon, 1991)

* Corresponding author. Tel.: +82 42 869 2616; fax: +82 42 869 2610.
E-mail address: hskim76@kaist.ac.kr (H.-S. Kim).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.07.067

and defective nutrition due to degradation products such as reactive oxygen species (Esterbauer, Schaur, & Zollner, 1991; Guardiola, Dutta, Codony, & Savage, 2002; Sanders, 1983). Protection of lipid oxidation is a critical factor to food quality and shelf-life of edible oils, and microencapsulation of oils has been widely adopted as an approach to address this issue. Nonetheless, to our knowledge, the factors affecting the MEE have not been investigated in detail with respect to compositions of the core oil, coating material, and emulsifier as well as fabrication conditions. Furthermore, few studies have been conducted regarding oxidation of microencapsulated sunflower oil (MESO) even though much attention has been paid to the oxidation of sunflower oil (SO) in the liquid form (Guilleän, Cabo, Ibargoitia, & Ruiz, 2005; Iqbal & Bhanger, 2007; Makhoul, Ghaddar, & Toufeili, 2006).

In the present study, we report the optimization of microencapsulation conditions of seed oils using response surface methodology (RSM). RSM was used in various fields of food chemistry studies such as optimization of the extraction of phenolic compounds from wheat (Liyana-Pathirana & Shahidi, 2005), and optimization of pectin hydrolysis enzymes (Rodríguez-Nogales, Ortega, Perez-Mateos, & Busto, 2007), among others. As a typical seed oil, SO was employed for microencapsulation in combination with coating materials including dextrin, milk protein isolates (MPI), and emulsifier (soy lecithin). To determine the optimal conditions for microencapsulation of SO, the effects of four variables (i.e., SO concentration, proportion of MPI to coating wall, soy lecithin concentration, and homogenizing pressure) on the microencapsulation efficiency were investigated and analyzed systematically. A quadratic polynomial model was introduced to correlate the MEE with the four variables. For validation of the correlation, the MEE was experimentally obtained by measuring the peroxide value (POV) for MESO, and compared with that predicted from the regression equation. Details are reported herein.

2. Materials and methods

2.1. Materials

Sunflower oil (SO) of *Helianthus annuus* was supplied by Tradin Organic Agriculture B.V. (Amsterdam, Netherlands) as a certified organic product. Fatty acid composition of SO was as follows; 0.08% (w/w) myristic acid (C14:0), 5.86% palmitic acid (C16:0), 3.28% stearic acid (C18:0), 37.84% oleic acid (C18:1n-9), 51.40% linoleic acid (C18:2n-6), 0.26% linolenic acid (C18:3n-3), 0.20% arachidic acid (C20:0), 0.14% eicosenoic acid (C20:1), 0.53% behenic acid (C22:0), and 0.17% lignoseric acid (C24:0). Spray-dried dextrin with dextrose equivalent (DE) 8–12 was supplied by Sunrich (Hope, MN, USA). Spray-dried milk protein isolate (MPI) containing 80.6% (w/w) protein and 4.9% (w/w) lactose, which was concentrated from skimmed milk pasteurized at 72 °C for 15 s, was supplied by Emmi Milch AG (Dagmersellen, Switzerland). The paste type soybean lecithin, prepared by drying after degumming of pressed soybean oil, was purchased from Clarkson Soy Products (Cerro Gordo, IL, USA). Dextrin contained 93.7% (w/w) carbohydrates according to product specifications, and was produced as part of a full-scale standard production. MPI contained 4.0% (w/w) lactose, 83.0% (w/w) milk protein, and 2.0% (w/w) milk fat. Soybean lecithin contained 36.0% (w/w) phospholipids and 10.0% (w/w) phosphatidylcholine.

Butanol, chloroform, petroleum ether and hexane were acquired from Fisher Scientific (Pittsburgh, PA, USA). Sodium thiosulphate was from Merck (Darmstadt, Germany). Acetic acid, potassium iodide and ρ-anisidine were purchased from Sigma–Aldrich (St. Louis, MO, USA). All solvents and reagents were appropriate grade for chromatographic analysis and purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Experimental design for response surface methodology (RSM)

Response surface methodology (RSM) was employed to investigate the variation of MEE with respect to operating parameters including SO concentration, proportion of MPI to coating wall, soy lecithin concentration, and homogenizing pressure. The composition of four variables was designed by central composite design (CCD) approach. CCD is a 2^k factorial design with star points and central points. The variables and their concentration ranges are: SO as a core material (X_1) from 20% to 40% (w/w), proportion of MPI in coating wall (X_2) from 10 to 30 (coating wall includes MPI, dextrin, and soy lecithin), concentration of soy lecithin as a supplemented emulsifier (X_3) from 0.5 to 2.5% (w/w), and homogenizing pressure (X_4) from 50 to 250 kg/cm^2. The actual variable was coded to facilitate multiple regression analysis (Table 1).

Thirty one experimental settings consisting of 8 star points (star distance is 0) and 3 central points were generated with 4 factors and 3 levels by the principal of RSM using MINITAB Release 14 (Korean version, Minitab Korea, Gunpo, Republic of Korea). The quadratic polynominal regression model was assumed for predicting Y variable (MEE = microencapsulation efficiency). The model proposed for the response of Y fitted Eq. (1) as follows:

$$Y = \beta_{k0} + \sum_{i=1}^{4} \beta_{ki} x_i + \sum_{i=1}^{4} \beta_{kji} x_i^2 + \sum_{i=1}^{3} \sum_{j=1+1}^{4} \beta_{kij} x_i x_j, \qquad (1)$$

where Y is response (MEE of microcapsules, %). β_{k0}, β_{ki}, β_{kiii}, and β_{kijj} are constant coefficients of intercept, linear, quadratic and interaction terms, respectively. X_i and X_j are uncoded independent variables (concentration of SO, ratio of MPI and dextrin, concentration of soy lecithin, and homogenizing pressure).

Table 1
Coded levels for independent variables used in experimental design for microencapsulation of SO with milk protein isolates and dextrin

Variables	Coded X_i	Coded level			ΔX[a]
		−1	0	1	
Concentration of sunflower oil (%, w/w)	X_1	25	30	35	5
Ratio of MPI:dextrin (w/w)	X_2	15	20	25	5
Concentraion of soy lecithin (%, w/w)	X_3	1.0	1.5	2.0	0.5
Homogenizing pressure (kg/cm^2)	X_4	100	150	200	50

[a] ΔX is the increment of each experiment factor values corresponding to one unit of the coded variables.

2.3. Microencapsulation of SO

Microencapsulation was carried out by a similar method as described elsewhere (Keogh & O'Kennedy, 1999). Briefly, a suspension of MPI and dextrin in deionized water was prepared by mixing with a homomixer (Ultra Turrax T-50, Janke & Kunkel Ika-Labortechnik, Staufen, Germany) for 20 min at 6000 g at 65 °C following addition of SO and soybean lecithin at the designed ratio as shown in Table 2. The emulsion mixture was then homogenized with a homogenizer (APV RANIE, Albertslund, Denmark) at a feeding rate of 1 L/min at 50–250 kg/cm^2 by 3 cycles, followed by immediate feeding into a spray drier (Niro Atomizer with disk type, Niro, Søborg, Denmark). Temperature at inlet and outlet of spray dryer were 160 ± 5 °C and 95 ± 5 °C, respectively, at a feeding rate of 1.6 L/h. At intervals, samples were taken and analyzed for peroxide value (POV). The remaining samples were stored in a freezer (−20 °C) under a nitrogen blanket to determine fatty acid composition and tocopherols. The

Table 2
Central composite design for the optimization of SO microencapsulation

Run number	Coded variable				Process variable				Measured MEE (%)
	X_1[a]	X_2[b]	X_3[c]	X_4[d]	X_1[a]	X_2[b]	X_3[c]	X_4[d]	
1	−1	−1	−1	−1	25	15	1	100	84.4
2	1	−1	−1	−1	35	15	1	100	77.8
3	−1	1	−1	−1	25	25	1	100	92.6
4	1	1	−1	−1	35	25	1	100	90.0
5	−1	−1	1	−1	25	15	2	100	90.0
6	1	−1	1	−1	35	15	2	100	79.8
7	−1	1	1	−1	25	25	2	100	94.4
8	1	1	1	−1	35	25	2	100	86.4
9	−1	−1	−1	1	25	15	1	200	83.9
10	1	−1	−1	1	35	15	1	200	79.6
11	−1	1	−1	1	25	25	1	200	93.6
12	1	1	−1	1	35	25	1	200	86.4
13	−1	−1	1	1	25	15	2	200	89.3
14	1	−1	1	1	35	15	2	200	79.3
15	−1	1	1	1	25	25	2	200	94.6
16	1	1	1	1	35	25	2	200	82.0
17	−2	0	0	0	20	20	1.5	150	93.9
18	2	0	0	0	40	20	1.5	150	70.2
19	0	−2	0	0	30	10	1.5	150	72.2
20	0	2	0	0	30	30	1.5	150	93.3
21	0	0	−2	0	30	20	0.5	150	81.5
22	0	0	2	0	30	20	2.5	150	92.7
23	0	0	0	−2	30	20	1.5	50	90.0
24	0	0	0	2	30	20	1.5	250	91.7
25	0	0	0	0	30	20	1.5	150	90.5
26	0	0	0	0	30	20	1.5	150	90.7
27	0	0	0	0	30	20	1.5	150	89.9
28	0	0	0	0	30	20	1.5	150	90.1
29	0	0	0	0	30	20	1.5	150	90.2
30	0	0	0	0	30	20	1.5	150	89.7
31	0	0	0	0	30	20	1.5	150	89.8

[a] Sunflower oil concentration.
[b] Proportion of MPI to coating wall (MPI, dextrin, soy lecithin).
[c] Soy lecithin concentration.
[d] Homogenizing pressure.

homogenized emulsions were immediately fed into a pilot-scale spray drier using disk type nozzle (Niro Atomizer, Niro, Søborg, Denmark) equipped with spray drying chamber with dimensions of 160 cm in height and 90 cm in diameter. The emulsion was fed into the chamber at a feeding rate of 1.6 L/h, atomized by the hot air (air velocity of 2 m/s).

2.4. Microencapsulation efficiency (MEE)

The microencapsulation efficiency (MEE) was calculated according to the method described elsewhere (Pauletti & Amestoy, 1999)

$$\mathrm{MEE} = \frac{(\text{total oil} - \text{extractable oil}) \times 100}{\text{total oil}}$$

The total oil content of the powder was determined by the Röse-Gottlib method (Int. Dairy Fed., 1993). The extractable oil was measured after gentle shaking according to the methods described elsewhere (Sankarikutty, Sreekumar, Narayanan, & Mathew, 1998; Velasco, Marmesat, Dobarganes, & Márquez-Ruiz, 2006). Briefly, 200 mL of light petroleum ether (60–80 °C) were added to 4 g of MESO in Erlenmeyer flask with stopper. Stirring was applied at room temperature for 15 min at 25 °C in the dark. The organic solution was passed through a Büchner funnel with a Whatman No. 4 filter, and collected in a round-bottom flask to evaporate using rotatory evaporator in a water bath at less than 30 °C to minimize influence of heating on lipid oxidation.

2.5. Antioxidant stability of the microcapsule by accelerated storage condition

The prepared microcapsules were stored at 60 °C for 30 days that was a same condition as the study of comparison of antioxidant and synergistic effects of rosemary extract with tocopherol, ascobyl palmitate, and citric acid in SO was performed (Hraś, Hadolin, Knez, & Bauman, 2000). Shelf life of edible oils is normally predicted from accelerated storage tests conducted at high temperatures ranging between 60 °C, in the Schaal oven test (Wanasundara & Shahidi, 1998), and 100 °C in tests utilizing the Rancimat (Frankel, 1998; Makhoul et al., 2006). Aliquots (250.0 g) of each sample were poured into each PYREX glass vessels (500 mL, 80 mm i.d.) with a cap in the incubator at 60 ± 1 °C. Samples of glass vessels were taken at intervals for peroxide value (POV) determination. The remaining samples were stored in a freezer (−20 °C) under a nitrogen blanket to determine fatty acid composition.

2.6. Analytical method for lipid oxidation

Extraction of free fat on the MESO was conducted according to the method described elsewhere (Sankarikutty et al., 1998). Extraction of total oil and inner encapsulated oil for POV test was performed by Pont method (Newstead & Headifen, 1981; Pont, 1955). In the case of encapsulated oil, extraction using Pont method was started from the MESO devoid of free oil dried to a constant weight after free oil was extracted. To release the fat from reconstituted MESO, de-emulsification reagent was used, and followed by heating and centrifugation. For preparation of de-emulsification reagent, 10 g sodium salicylate and 10 g sodium citrate were dissolved separately in double-distilled water, followed by mixing together with 18 mL *n*-butanol, and made up to 90 mL with double distilled water. Ten grams of MESO were mixed with 20 mL water at 50 °C in a 125 mL Erlenmeyer flask with stopper. After 15 mL de-emulsification reagent were added, the mixture was shaken vigorously and left to stand in a 70 °C water bath for 6 min. The resulting mixture was centrifuged at 3000*g* for 10 min, and the extracted fat was subjected to the POV test.

Hydroperoxides, primary oxidation products, were measured and represented as the peroxide value (POV) as described in the AOCS (AOCS Official Method, 1993). The POV was expressed as milliequivalents (mequiv) of active oxygen per kilogram of oil. Automatic titration was performed using potentiometric titration system (Model 799 GPT Titrino, 685 Dosimat, Pt Titrode) equipped with a sample changer (Metrohm, Herisau, Switzerland).

2.7. Active oxygen method (AOM) by Rancimat

Induction time to primary oxidation of surface free fat on the MESO was measured by the Rancimat method (Läubi & Bruttel, 1986) with Rancimat apparatus (Metrohm, Herisau, Switzerland). A flow of air (20 L/h) was bubbled through 5.0 g of oil heated to 98 °C. The volatile oxidation products were stripped from the oil and dissolved in cold water, increasing its conductivity. The time taken to reach an inflection point at the induction curve was measured for lipids in seed oils and on the surface of the MESO, respectively.

2.8. Analytical method for fatty acids

The fatty acid composition of SO was determined by capillary gas chromatograph (Agilent, 6890A Plus, Santa Clara, CA, USA) with a flame ionization detector and a DB-225 column (30 m × 0.25 mm i.d, 0.25 μm film thickness, J&W Scientific Agilent, Wilmington, DE, USA), using a standard methodology (AOCS Official Method, 1983). Temperature was programmed to increase from 140 to 220 °C with a 4 °C/min gradient. Flow rate of nitrogen as carrier gas was 0.8 mL/min. The injector temperature was 250 °C with air flow of 300 mL/min, and detector temperature was 260 °C with nitrogen flow of 30 mL/min, respectively. Content of each fatty acid was verified by comparison of retention time of test samples with those of reference standards.

2.9. Scanning electron microscope (SEM)

Field emission scanning electron microscope (FE-SEM, FEI, Sirion, Hillsboro, OR, USA) was used to examine the morphology and surface appearance of microcapsules. Microcapsule samples were attached with a two-sided adhesive tape to specimen stubs and then Pt-coated in a sputter coater (BAL-TEC, SCD 005, Witten, Germany) at 30 mA for 150 s. The coated microcapsules were examined in a Sirion SEM at 10 kV with 1.5 nm resolution (Rosenberg, Kopelman, & Talmon, 1985).

2.10. Particle size analysis

Particle size analyzer (Mastersizer 2000, MALVERN, UK) was used to determine the sizes of emulsions and microcapsules. Measurement time and snap for emulsions were 12 s and 12,000, respectively. For microcapsules, measurement time and snap were 10 s and 10,000, respectively. The background snap was 5000.

3. Results and discussion

3.1. Optimization of MESO by response surface methodology (RSM)

It has been demonstrated that response surface methodology (RSM) gave rise to the evaluation of effects of multiple parameters on response variables in lipid or enzyme process (Twu, Shih, Yen, Ling, & Shieh, 2005). To minimize the experimental runs and time for optimization of microencapsulation conditions of SO, a four-factor central composite design (CCD) was adopted on the basis of coded level from four independent variables (Table 1), resulting in thirty-one simplified experimental set (Table 2). The SO concentration, the proportion of MPI to the coating wall, soy lecithin concentration, and homogenizing pressure were investigated in the ranges of 20–40% (w/w), 10–30%, and 0.5–2.5%, and 50–250 kg/cm^2, respectively. Since the homogenizing pressure had a negligible effect on the MEE as shown in Table 3, the response surface graphs for MEE as a function of three selected parameters (X_1, X_2, and X_3) using significant effective factors for MEE are shown in Fig. 1. Microencapsulation of SO with the SO concentration of 25% and the MPI proportion of 25 gave rise to the highest MEE (~94.6%) (Fig. 1a). At any given concentration of soy lecithin (0.5–2.5%), an increase of SO concentration resulted in a decrease of the MEE (Fig. 1b), whereas an increase of MPI proportion to the coating wall increased the MEE (Fig. 1c).

To determine the optimal condition of MESO and the relationship between the response (MEE) and the significant variables, statistical analyses of ANOVA was performed through a joint test of four parameters (Table 3). Among the linear, quadratic, and cross-product forms of independent variables, X_1, X_2, X_3, X_1^2, and X_2^2 were significant at the level of $p < 0.01$. Thus, when the response

Table 3
Values of regression coefficients calculated for the sunflower oil microencapsulation

Independent variable	Regression coefficient	Standard error	t-value	Significance level (p)
Constant	−43.826845	28.3854	−1.544	0.142
Linear				
X_1	4.470643	1.1705	3.819	0.002
X_2	4.413929	1.0372	4.256	0.001
X_3	31.652381	9.8626	3.209	0.005
X_4	0.040590	0.0986	0.412	0.686
Quadratic				
X_1^2	−0.076457	0.0163	−4.687	0.000
X_2^2	−0.069407	0.0163	−4.255	0.001
X_3^2	−2.610655	1.6313	−1.600	0.129
X_4^2	0.000061	0.0002	0.374	0.714
Interaction				
X_1 X_2	−0.010425	0.0218	−0.478	0.639
X_1 X_3	−0.373750	0.2181	−1.714	0.106
X_1 X_4	−0.000413	0.0022	−0.189	0.852
X_2 X_3	−0.317250	0.2181	−1.455	0.165
X_2 X_4	−0.000488	0.0022	−0.244	0.826
X_3 X_4	−0.022325	0.0218	−1.024	0.321
r^2				0.939
F				17.500
Probability of F				0.000

MEE was experimentally determined under the thirty-one conditions, the regression coefficients were calculated for the MEE by RSREG analysis, and a polynomial regression model equation was fitted as follows: MEE (%) $= 4.137772 + 3.524183X_1 + 3.475205X_2 + 2.914167X_3 - 0.074532X_1^2 - 0.067482X_2^2$, where X_1 is the SO concentration, X_2 is the proportion of MPI to the coating wall, and X_3 is soy lecithin concentration. From the regression coefficients and p-value, the linear and quadratic term of SO concentration (X_1), proportion of MPI to the coating wall (X_2), and soy lecithin concentration (X_3) had significant effects on the microencapsulation efficiency ($p < 0.01$), whereas those of homogenizing pressure (X_4) had a negligible effect on the MEE ($p > 0.1$). The optimized composition for the MESO by response optimizing process was 23.65% of SO, 19.02 of MPI, 54.83% of dextrin, 2.5% of soy lecithin. In addition to seed oil concentration, the significance of coating materials (MPI) and emulsifier (soy lecithin) in the microencapsulation process has been reported (Rosenberg et al., 1985; Rosenberg & Lee, 1993; Rosenberg & Young, 1993). Most importantly, it was found from this statistical analysis that seed oil concentration and coating materials in the present study was a critical factor for MESO with high MEE, compared to emulsifier or homogenizing pressure. The measured MEE of 31 different MESO was determined in the range of 70.2–94.6%. In accordance with maximal MEE (94.6%) observed in Table 2, the MESO under optimized conditions displayed about 96.6% MEE. This result represents that the regression equation was a suitable model to describe the response of the experimented parameters to the MEE of MESO.

The surface morphology of MESO under optimized conditions was verified by using a scanning electron

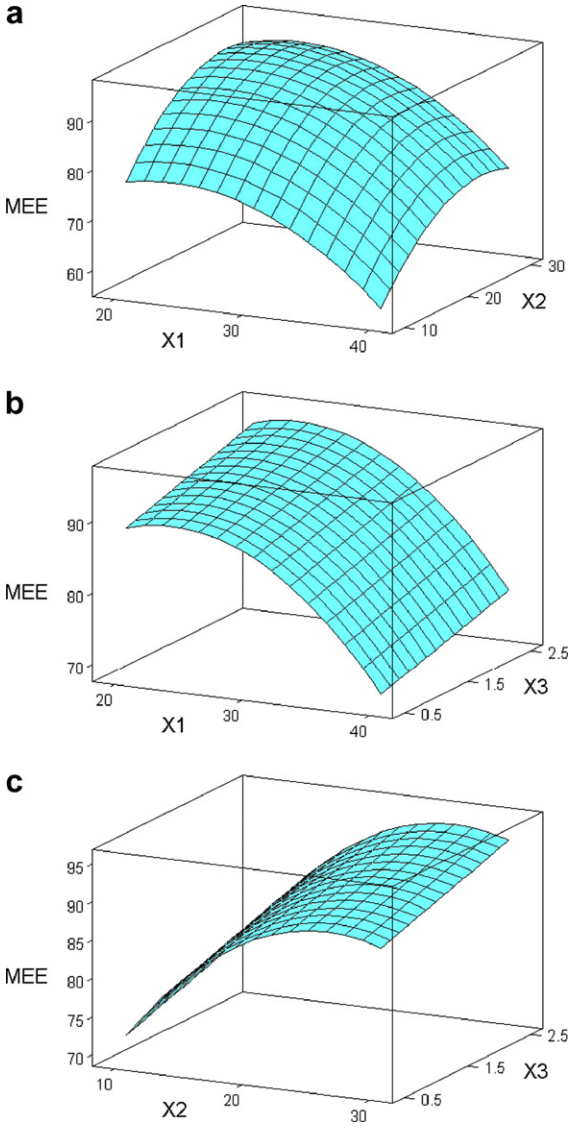

Fig. 1. Response surface for MEE of the MESO with respect to (a) SO concentration (X_1) and proportion of MPI to the coating wall (X_2) (b) SO concentration (X_1) and soy lecithin concentration (X_3) (c) proportion of MPI to the coating wall (X_2) and soy lecithin concentration (X_3).

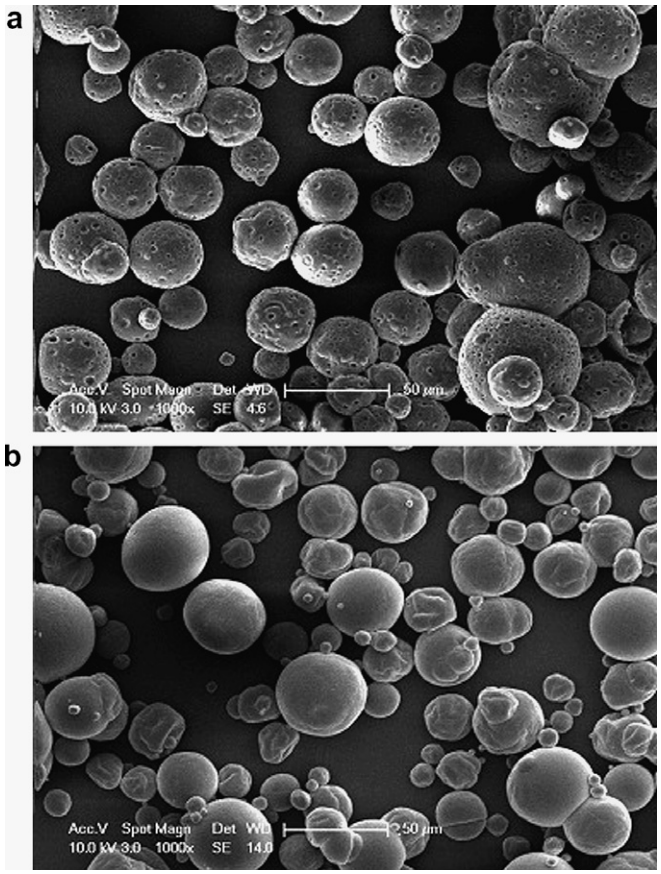

Fig. 2. Scanning electron micrographs of MESO fabricated at the control and optimized conditions (a) MESO with 70.2% MEE (average particle size 41.5 μm) and (b) MESO with 96.6% MEE (average particle size 37.3 μm).

microscopy (SEM). As shown in Fig. 2, there were many holes and cracks on the surface of MESO showing 70.2% of MEE (Fig. 2a), while MESO at the optimized condition (96.6% in MEE) exhibited smooth and free of pores, cracks and surface indentation (Fig. 2b). The morphology of the MESO in Fig. 2b was similar to microcapsules manufactured by the spray-dried microencapsulation (Rosenberg & Lee, 1993; Rosenberg & Young, 1993), implying that MEE can be maximized through optimization of microencapsulation conditions.

3.2. Lipid oxidation and antioxidant stability of MESO

Changes of the POV both in outer free oil and inner encapsulated oil of MESO were measured under accelerated storage conditions at 60 ± 1 °C for 30 days in order to check lipid oxidation in MESO. As shown in Fig. 3, initial POV of outer free fat shortly after microencapsulation under optimized conditions was as low as 8.7 meq/kg, while the POV of MESO showing 70.2% of MEE (run number 18 in Table 2) was 15.2 meq/kg. There was no significant difference in POV from inner encapsulated oil. Generally, variation of POV between the outer free and inner encapsulated oil in MESO can occur due to the different oxidation rates. Although SO has been known to be vulnerable to lipid oxidation in various foods (Bou, Codony, Baucells, & Guardiola, 2005), it has been reported that heterogeneous change (i.e. different oxidative patterns) between outer and inner lipid layers was clearly observed in the study of freeze dried MESO (Velasco et al., 2006).

To check the oxidative stability of MESO under optimized conditions, the POV of the MESO was measured at the accelerated storage condition at 60 ± 1 °C for 30 days. In contrast to MESO with a low MEE (70.2%), the POV of MESO at the optimized condition (96.6% of MEE) increased over time (Fig. 3). In particular, the MESO with 70.2% MEE showed a significant change both in outer free oil and inner encapsulated oil (539.3 meq/kg and 78.0 meq/kg, respectively), while the

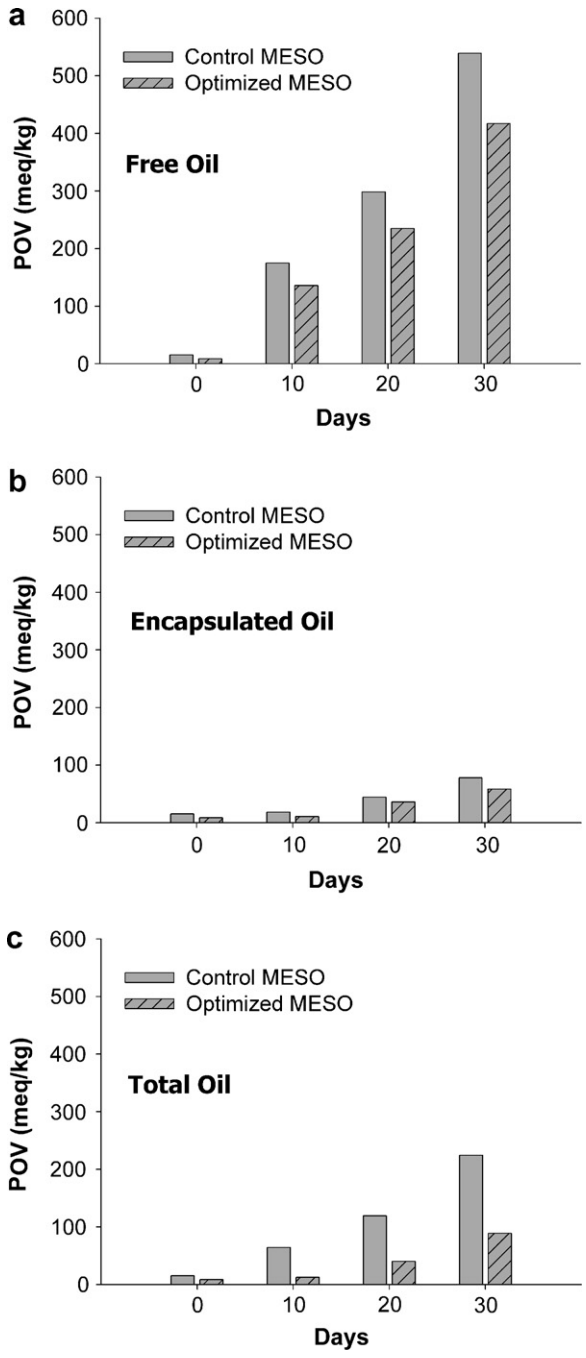

Fig. 3. Changes in the POV caused by oxidation of (a) free, (b) encapsulated and (c) total oils from MESOs showing different microencapsulation efficiencies. The microcapsules were exposed at 60 °C, and the POV was determined as a function of time.

POV of MESO under optimized conditions increased up to 417.0 meq/kg for outer free oil and 58.5 meq/kg for inner encapsulated oil after 30 days, respectively. Consequently, the POV from total oil in MESO under optimized conditions remained at a lower level (88.8 meq/kg) than that in the control MESO (232.8 meq/kg). Since efficient microencapsulation can lead to the decrease of the lipid oxidation through the reduction of free oil content, this difference between two MESOs showing distinct MEE seems to be mainly caused by oxidation level from free fat content on the surface of MESO, rather than from the encapsulated oil content. Thus, low free oil in MESO at the optimized condition may contribute to less lipid oxidation. Furthermore, when the oxidative degree of the MESO was also investigated by an active oxygen method (AOM) based on Rancimat, the resultant induction time of the MESO was prolonged to 20.8 h compared to 12.7 h in MESO showing 70.2% MEE. This result indicates that optimization of microencapsulation by using RSM can effectively prevent lipid oxidation.

In conclusion, we have demonstrated the optimization of microencapsulation conditions for SO by using response surface methodology (RSM). The microencapsulation efficiency of MESO was significantly affected by SO concentration, proportion of MPI to coating wall, and soy lecithin concentration. From the RSM, the optical conditions for microencapsulation of SO were determined to be 23.6% SO, 19.0% MPI, 2.5% soy lecithin, and 54.8% dextrin. MESO under optimized conditions showed 96.6% MEE, showing a good coincidence with the predicted value. In addition, lipid oxidation on the surface of MESO could be effectively reduced through optimization of the microencapsulation conditions. The approach presented in this study can provide a very useful guideline to optimize other oil systems very efficiently.

Acknowledgement

The authors thank K.M. Yeo, B.J. Kim, and S.Y. Ahn for their skillful technical assistance for microencapsulation.

References

AOCS Official Method Vols. 1 & 2 (1983). In W. E. Link (Ed.), *Official and tentative methods*. Champaign, IL: American Oil Chemists' Society.

AOCS Official Method Cd 8-53 (1993). In F. Gunstone (Ed.), *Official methods and recommended practices of the American oil chemists' society method Cd 8-53. Peroxide value acetic acid-chloroform method* (4th ed.). Champaign, IL: AOCS Press.

Bou, R., Codony, R., Baucells, M. D., & Guardiola, F. (2005). Effect of heated sunflower oil and dietary supplements on the composition, oxidative stability, and sensory quality of dark chicken meat. *Journal of Agricultural and Food Chemistry, 53*, 7792–7801.

Esterbauer, H., Schaur, R. F., & Zollner, H. (1991). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biology and Medicine, 11*, 81–128.

Frankel, E. N. (1998). *Lipid oxidation*. Dundee, UK: The Oily Press.

Gordon, M. H. (1991). Oils and fats: Taints or flavor. *Chemistry in Britain*(November), 1020–1022.

Guardiola, F., Dutta, P. C., Codony, R., & Savage, G. P. (2002). *Cholesterol and phytosterol oxidation products: Analysis, occurrence, and biological effects*. Champaign, IL: AOCS Press.

Guilleän, M. D., Cabo, N., Ibargoitia, M. L., & Ruiz, A. (2005). Study of both sunflower oil and its headspace throughout the oxidation Process. Occurrence in the headspace of toxic oxygenated aldehydes. *Journal of Agricultural and Food Chemistry, 53*, 1093–1101.

Hraś, A. R., Hadolin, M., Knez, Ź., & Bauman, D. (2000). Comparison of antioxidative and synergistic effects of rosemary extract with α-

tocopherol, ascobyl palmitate and citric acid in sunflower oil. *Food Chemistry, 71*, 229–233.

Iqbal, C., & Bhanger, M. I. (2007). Stabilization of sunflower oil by garlic extract during accelerated storage. *Food Chemistry, 100*, 246–254.

Int. Dairy Fed. Stand. No. 9: (1993). Determination of fat content (Röse-Gottlib Reference Method). In IDF-FIL, International Dairy Federation, Brussels, Belgium.

Keogh, M. K., & O'Kennedy, B. T. (1999). Milk fat microencapsulation using whey proteins. *International Dairy Journal, 9*, 657–663.

Läubi, M. W., & Bruttel, P. A. (1986). Determination of the oxidative stability of fats and oils: Comparison between the active oxygen method (AOSC Cd 12-57) and Rancimat method. *Journal of the American Oil Chemists Society, 63*, 792–795.

Liyana-Pathirana, C., & Shahidi, F. (2005). Optimization of extraction of phenolic compounds from wheat using response surface methodology. *Food Chemistry, 93*, 47–56.

Makhoul, H., Ghaddar, T., & Toufeili, I. (2006). Identification of some rancidity measures at the end of the shelf life of sunflower oil. *European Journal of Lipid Science and Technology, 108*, 143–148.

Newstead, D. F., & Headifen, J. M. (1981). A reappraisal of the method for estimation of the peroxide value of fat in whole milk powder. *New Zealand Journal of Dairy Science and Technology, 16*, 13–18.

Pauletti, M. S., & Amestoy, P. (1999). Butter microencapsulation as affected by composition of wall material and fat. *Journal of Food Science, 64*, 279–282.

Pont, E. G. (1955). A de-emulsification technique for use in the peroxide test on the fat of milk, cream, concentrated and dried milks. *Australian Journal of Dairy Technology, 10*, 72–75.

Rodríguez-Nogales, J. M., Ortega, N., Perez-Mateos, M., & Busto, M. D. (2007). Experimental design and response surface modeling applied for the optimisation of pectin hydrolysis by enzymes from A. niger CECT 2088. *Food Chemistry, 101*, 634–642.

Rosenberg, M., Kopelman, I. J., & Talmon, Y. (1985). A scanning electron microscopy study of microencapsulation. *Journal of Food Science, 50*, 139–144.

Rosenberg, M., & Lee, S. L. (1993). Microstructure of whey protein/anhydrous milkfat emulsions. *Food Structure, 12*, 267–274.

Rosenberg, M., & Young, S. L. (1993). Whey proteins as microencapsulating agents. Microencapsulation of anhydrous milk fat-structure evaluation. *Food Structure, 12*, 31–41.

Sanders, T. A. B. (1983). Nutritional significance of rancidity. In J. C. Alllen & R. J. Hamilton (Eds.), *Rancidity in foods* (pp. 59–66). London, UK: Elsevier Applied Science Publisher.

Sankarikutty, B., Sreekumar, M. M., Narayanan, C. S., & Mathew, A. G. (1998). Studies on microencapsulation of cardamon oil by spray-drying technique. *Journal of Food Science and Technology, 25*, 352–356.

Shahidi, F., & Han, X. Q. (1993). Encapsulation of food ingredients. *Critical Reviews in Food Science and Nutrition, 33*, 501–547.

Twu, Y.-K., Shih, I.-L., Yen, Y.-H., Ling, Y.-F., & Shieh, C.-J. (2005). Optimization of lipase-catalyzed Synthesis of octyl hydroxyphenyl-propionate by response surface methodology. *Journal of Food Science, 53*, 1012–1016.

Wanasundara, U. N., & Shahidi, F. (1998). Stabilization of marine oils with flavonoids. *Journal of Food Lipids, 5*, 183–196.

Velasco, J., Marmesat, S., Dobarganes, C., & Márquez-Ruiz, G. (2006). Heterogeneous aspects of lipid oxidation in dried microencapsulated oils. *Journal of Agricultural and Food Chemistry, 54*, 1722–1729.