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Antioxidative activity and structural stability of microencapsulated γ-oryzanol in heat-treated lards

Mun-Hee Suh [a], Sang-Ho Yoo [b], Hyeon Gyu Lee [a,*]

[a] Department of Food and Nutrition, Hanyang University, 17 Haengdang-dong, Seongdong-gu, Seoul 133-791, South Korea
[b] Department of Food Science and Technology, Sejong University, 98 Gunja-dong, Gwangjin-gu, Seoul 143-747, South Korea

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Abstract

Using the central composite design, we have designed the experimental platform for optimizing γ-oryzanol (GO) microencapsulation. Response surface methodology (RSM) was applied to explore maximal microencapsulation yield (MY) using three independent variables; the ratio of core materials to coating materials (X_1), temperature of dispersion fluid (X_2), and emulsifier concentration (X_3). As a result of least-square regression (RSREG) analysis, the regression model equation for the MY (%) to the change of independent variables could be predicted as follows; $YM = 102.71 - 2.88X_1^2 - 2.97X_3^2$. Applying this model equation to the surface plot and canonical analysis, the optimal conditions for the GO microencapsulation were determined to be 4.8:5.2 (X_1), 24.99 (X_2) and 0.38% (X_3). The resulting MY, with statistically optimized parameters, was 95.7%. The thiobarbituric acid-reactive substances (TBARs) values of heated lard were determined to evaluate the effect of microencapsulation on the stability against heat-induced lipid oxidation. The MGO (microencapsulated γ-oryzanol)-treated lard displayed significantly greater oxidative stability than did the GO-treated lard up to a 10-day heating period. During the heating process, a substantially larger amount of GO remained in the MGO-treated lard as well. Apparently, microencapsulation could be used as a good potential technique to protect GO from the heat-induced loss of its antioxidant effect.

Keywords: γ-oryzanol (GO); Microencapsulation; Response surface methodology (RSM); Thiobarbituric acid-reactive substances (TBARs)

1. Introduction

Gamma-oryzanol extracted from rice bran has substantial commercial significance in Japan as a food and medical antioxidant. In recent years, there has been a trend to use natural antioxidant compounds rather than synthetic antioxidants in the food industry (Rankin et al., 1993). γ-Oryzanol (GO) is known to be a powerful inhibitor of iron-driven hydroxyl radical formation, and it was also reported to possess antioxidant activity in stabilizing lipids (Duve & White, 1991; Kim, Godber, & Prinaywiwatkul, 2000). The GO, that is a mixture of ferulic acid esters of triterpene alcohols and sterols, has been investigated for its hypocholesterolemic activity in rats (Seetharamaiah, Krishmakantha, & Chandrasekhara, 1990). This plant-derived sterol has been used to alleviate menopausal disorders, accelerate growth, and to stimulate sexual glands of animals (Nakayama, Manabe, Suzuki, Sakamoto, & Inagaki, 1987; Uehara, Murase, Hirota, & Shirafuji, 1963).

Microencapsulation is a technique by which solid, lipid droplets consisting of core materials are packaged in continuous films that can release their microencapsulated materials at controlled rates under the desired conditions (Dziezak, 1988). The microencapsulating method has been widely applied in the food industry for encapsulating vitamins, minerals and other sensitive ingredients. Microencapsulation provides several advantages in the food industry by protecting the core materials from their environments. The microcapsules offer the food processors a means to protect sensitive food components. In this study, a microencapsulating technique was applied to stabilize

* Corresponding author. Tel.: +82 2 2220 1202; fax: +82 2 2292 1226.
E-mail address: hyeonlee@hanyang.ac.kr (H.G. Lee).

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chemically unstable GO against heating, and to improve its solubility. The aim of this study was to establish the optimal microencapsulating conditions of GO by means of response surface methodology. The effectiveness of microencapsulated γ-oryzanol (MGO) as an antioxidant was evaluated during the heat treatment of animal fat, lard.

2. Materials and methods

2.1. Materials

Waxy corn starch and γ-oryzanol (GO) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan), respectively. Agar was obtained from Duksan Pure Chemical Co. (Seoul, Korea). Emulsifiers, such as Tween 20, Tween 80 and Span # 20 (Sorbitan Monolaurate), were obtained from DaeJung Chemicals & Metals Co. Ltd. (Seoul, Korea).

2.2. Microencapsulation of γ-oryzanol

Emulsions, consisting of agar and waxy corn starch mixture as a coating material and one of Tween 20, Tween 80 or Span # 20 as an emulsifier, were prepared to determine the emulsion stability. The preparation of the emulsion was completed by heating the mixture in an 80 °C water bath for 30 min and cooling to 15 °C, followed by centrifugation at 1000*g* for 5 min. The emulsion stability was calculated by an index, which was based on the following equation:

$$\text{Emulsion stability index (ESI, \%)} = \frac{\text{Height of emulsion after heating and centrifugation}}{\text{Total height of contents in tube}} \times 100$$

The ESI values of the O/W emulsions were determined to optimize the concentration and composition of the coating materials, and hydrophilic–lipophilic balance (HLB) of emulsifier. Based on the evaluation of the emulsion's ESI values, the mixtures of agar and waxy corn starch, and Tween 20 were selected, respectively, as coating material and emulsifier.

With the 2:1 (w/w) mixture of agar and waxy corn starch as a coating material, γ-oryzanol (GO) was emulsified and microencapsulated, using an extrusion spraying technique. The method reported by Chang, Ha, Roh, and Choi (2000) was used with some modifications for microencapsulation. The operating variables for producing GO microcapsules were investigated in terms of the ratio of GO to the coating material, the temperature of dispersion fluid, and the concentration of emulsifier. The mixing ratio of core material:coating material was varied from 3:7 to 7:3 (w/w), and the temperature of dispersion fluid ranged was from 5 to 45 °C. Agar and waxy corn starch were mixed and dissolved in 1 l of distilled water at 78 °C. Then, Tween 20 (sorbitan laurate + ethylene oxide, HLB = 16) and GO were added as a mixture from 0% to 0.8% (v/v) and 100 mg, respectively, to the coating material solution, and the whole mixture was dispersed with a homogenizer (T25 basic, IKA. Labortechnik Staufen, Germany) for 30 s at 9000*g*. The O/W emulsion droplets were generated and injected into 200 ml of distilled water (dispersion fluid) using a spray gun (Wagner 300, Wagner Spraytech (UK) Ltd., Germany). The resulting MGO suspension was used for further study.

2.3. Encapsulation yield of γ-oryzanol

The encapsulation yield of GO was determined, using an HPLC, by quantifying the amount of the released GO from the encapsulating matrix. The GO entrapped in the microcapsules was extracted with hexane for this analysis. An analytical C_{18}-column (5 μm, 100 Å) was used, and the mobile phase consisted of methanol, acetonitrile, dichloromethane, and acetic acid (50:44:3:3) (Kim, Suh, Yang, & Lee, 2003). The detection wavelength of the UV detector was 330 nm for the GO. The released amount of GO in the sample was quantified, based on the standard curve generated by calculating HPLC peak area of highly pure GO. Each quantified datum was averaged from triplicate analysis.

$$\text{Yield } (\%) = 1 - \frac{(\text{The amount of the released } \gamma\text{-oryzanol from the encapsulating matrix})}{(\text{The total amount of } \gamma\text{-oryzanol added in the emulsion})} \times 100$$

2.4. Experimental designs for response surface methodology (RSM)

The process optimization of GO encapsulation was designed by central composite design. The response surface methodology (RSM) was applied to optimize the encapsulation yield of GO by means of three independent variables: the ratio of core material to coating material (X_1), ranging from 3:7 to 7:3 (w/w), the temperature of dispersion fluid (X_2), from 5 to 45 °C, and the concentration of emulsifier (X_3) from 0% to 0.8% (v/v). The actual variable was coded to facilitate multiple regression analysis (Table 1). The least-square regression model was adequately fitted into the responses taken from the experimental data and to define an optimization process of the yield.

Table 1
Coded levels for independent variables used in experimental design for microencapsulation of γ-oryzanol

Variables	Coded X_i	Coded level					ΔX[a]
		−2	−1	0	1	2	
Ratio of [C_1m] to [C_2m][b] (w/w)	X_1	3:7	4:6	5:5	6:4	7:3	1:−1
Temperature of dispersion fluid (°C)	X_2	5	15	25	35	45	10
Concentration of emulsifier (%)	X_3	0	0.2	0.4	0.6	0.8	0.2

[a] ΔX is the increment of the experiment factor values corresponding to one unit of the coded variable.
[b] The [C_1m] and [C_2m] are core and coating materials, respectively.

The optimization conditions were studied at the centre of the design to find the accurate results of statistical experimentation. At the beginning of the studies, the second-order composite design, with fractional 2^4 factorial points (16 points), star points (6 points) and central point (1 point), was effective in searching for the direction of the optimum domain. The regression model equation for the microencapsulation yield of GO could be predicted as follows:

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

where, MY (%) was the microencapsulation yield; 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 coefficients; the b_{ij} values were quadratic coefficients; X_i and X_j were the level of the independent variables. The analysis of data was carried out using the Statistical Analysis System (SAS) programme (Cary, 1990). Response surface plots were generated by the Statistical™ software.

2.5. Storage stability of microencapsulated γ-oryzanol

One hundred millilitres of MGO dispersion, which contained 100 mg of GO, were added to 1 kg of lard, and the lipid oxidation of the lard sample was induced or accelerated by heating it at 180 ± 5 °C for up to 10 days. The oxidized lard sample was taken at different time intervals during 10 days of the heat-induced oxidation process. Thiobarbituric acid-reactive substances (TBARs) were determined by the distillation method of Tarladgis, Watts, Younathan, and Dungan (1960), which was modified by Ockerman (1980) to measure the level of lipid autoxidation. The results were expressed as milligrams of TBARs per 1 kg of lard. The residual amount of intact GO after heat-induced oxidation treatment was analyzed by the aforementioned HPLC method. Residual GO was extracted by dissolving microspheres in acetone, followed by hexane extraction. The extracted GO was directly injected in to the HPLC system (Kim et al., 2003). Both analyses above were triplicated.

2.6. Statistical analysis

A randomized complete block design with 4 × 4 factorial arrangements was used. The number of replications ($n = 3$) was blocked and the additive treatment and the incubation time were the main treatment factors. The general linear model (GLM) procedure was applied to the data with a level of $p < 0.05$ for statistical analysis (Cary, 1990) and least significance difference (LSD) was used to compare the mean differences among the treated samples.

3. Results and discussion

3.1. Optimization of γ-oryzanol microencapsulation by response surface methodology

From results of the emulation stability test, 2:1 of agar to waxy corn starch was found to be optimal ratio, and Tween 20 was selected for our following experiment. Based on the central composite design, the encapsulation yield of each experimental group (total 23 data points, $n = 3$) was determined and the obtained data were analyzed by SAS. The encapsulation yield of GO was in the range 88.2–99.1% (Table 2). The results on the regression coefficients calculated for the degree of yield of microencapsulation by RSREG are shown in Table 3. The quadratic regression was significant at the level of $p < 0.01$, but linear regression ($p > 0.74$) and cross product regression ($p > 0.78$) were not significant. As for the microencapsulation of GO, the regression model equation for the yield of microencapsulation (YM, %) to the change of an independent variable could be predicted as follows: $\mathrm{YM} = 102.71 - 2.88X_1^2 - 2.97X_3^2$. According to the model equation, the ratio of core materials ([C_1m]) to coating materials ([C_2m]) (X_1) and concentration of emulsifier (X_3) appear to be the major factors affecting the microencapsulation yield among the variables studied. The temperature of dispersion fluid was shown to have little effect on the microencapsulation. This revealed that the above regression equation was a suitable model to describe the response of the experimental parameters (independent variables) to microencapsulation yield of GO.

3.2. Response surface graph of microencapsulation yield

The model equation was applied to obtain optimal conditions of GO encapsulation by the three-dimensional graphical methodology. The resulting three-dimensional surface plots of encapsulation yield versus two process parameters (X_i) are presented in Figs. 1–3. In this analytical process, the coded level of third parameter not used for the

Table 2
Central composite design for the optimization of γ-oryzanol microencapsulation

Run number	Coded variable[a]			Process variable[a]			Experimental point[b] (%)
	X_1	X_2	X_3	X_1	X_2	X_3	
1	−1	−1	−1	4:6	15	0.2	98.52
2	1	−1	−1	6:4	15	0.2	95.25
3	−1	1	−1	4:6	35	0.2	96.56
4	1	1	−1	6:4	35	0.2	97.12
5	−1	−1	1	4:6	15	0.6	98.15
6	1	−1	1	6:4	15	0.6	95.89
7	−1	1	1	4:6	35	0.6	99.10
8	1	1	1	6:4	35	0.6	96.56
9	−1	−1	−1	4:6	15	0.2	95.74
10	1	−1	−1	6:4	15	0.2	99.03
11	−1	1	−1	4:6	35	0.2	98.52
12	1	1	−1	6:4	35	0.2	97.45
13	−1	−1	1	4:6	15	0.6	98.12
14	1	−1	1	6:4	15	0.6	93.12
15	−1	1	1	4:6	35	0.6	95.99
16	1	1	1	6:4	35	0.6	96.26
17	−2	0	0	3:7	25	0.4	89.45
18	2	0	0	7:3	25	0.4	89.15
19	0	−2	0	5:5	5	0.4	98.44
20	0	2	0	5:5	45	0.4	96.54
21	0	0	−2	5:5	25	0.0	89.74
22	0	0	2	5:5	25	0.8	88.15
23	0	0	0	5:5	25	0.4	98.95

[a] The X_1, X_2, and X_3 were the ratio of core material to coating material (w/w), the temperature of dispersion fluid (°C), and the concentration of emulsifier (%), respectively.
[b] The experimental point (%) is equivalent to the encapsulation yield of γ-oryzanol.

Table 3
Values of regression coefficients calculated for the microencapsulation of γ-oryzanol

Independent variable[a]	Coefficient	Standard error	*t*-Value	Significance level (*p*)
Constant	102.71000	2.062564	49.80	<0.1
X_1	−0.4425000	0.498156	−0.89	0.3905
X_1^2	−2.8825000	0.498156	−3.95	0.0017
X_2	−0.0025000	0.498156	−0.01	0.9916
X_1X_2	0.2787500	0.729226	0.46	0.6553
X_2^2	−0.8350000	0.610115	−1.15	0.2728
X_3	−0.3408333	0.729226	−0.68	0.5059
X_1X_3	−0.5650000	0.610115	−0.93	0.3713
X_2X_3	0.0950000	0.610115	0.16	0.8787
X_3^2	−2.9712500	0.729226	−4.07	0.0013
r^2				0.6993
F				3.36
Probability of *F*				0.0237

[a] The X_1, X_2, and X_3 were the ratio of core material to coating material (w/w), the temperature of dispersion fluid (°C) and the concentration of emulsifier (%), respectively.

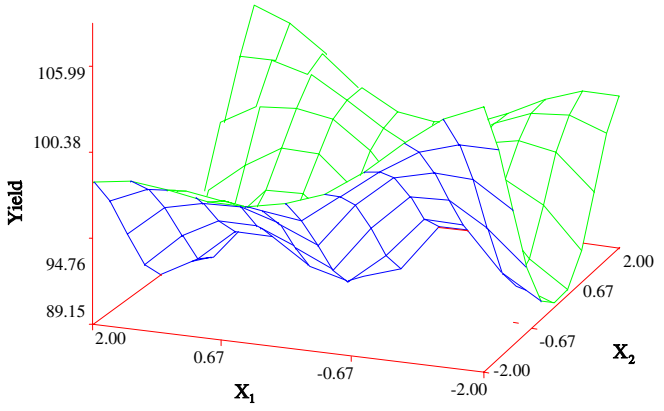

Fig. 1. Response surface plot for the effect of core to coating material ratio and dispersion fluid temperature on microencapsulation yield. X_1, the ratio of core to coating material (w/w) and X_2, the temperature of dispersion fluid (°C).

plotting was placed at zero level to interpret the effect of two independent variables on microencapsulation yield. As a result, it was unreasonable to predict the optimal point from the plots of X_1, X_2 vs. yield (Fig. 1) and X_2, X_3 vs. yield (Fig. 3). On the other hand, the stationary ridge shape was observed in the surface plot of X_1, X_3 vs. yield (Fig. 2). Thus, it was confirmed that the ratio of core materials to coating materials (X_1) and concentration of emulsifier (X_3) were the crucial factors for the yield of microencapsulation among the variables studied. From the analysis of three-dimensional surface plot, the maximal MY (%) point could be deduced at the ratio of [C_1m] to [C_2m] of 4.8:5.2 (w/w) and the concentration of emulsifier of 0.38% (Fig. 2).

3.3. Canonical analysis

Finally, the microencapsulation yield was determined at the optimum conditions calculated from the canonical analysis. As the eigenvalues were all negative, the station-

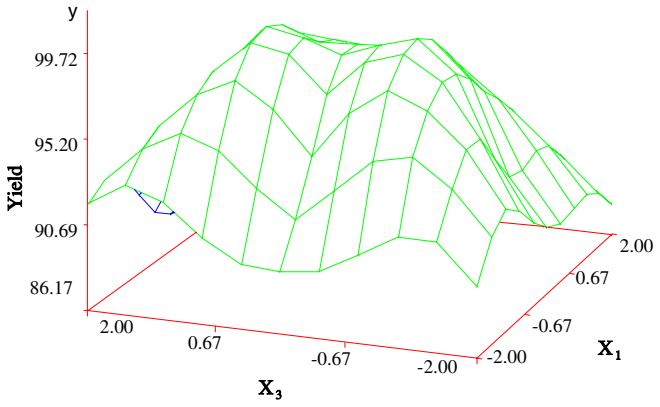

Fig. 2. Response surface plot for the effect of core to coating material ratio and emulsifier concentration on microencapsulation yield. X_1, the ratio of core to coating material (w/w) and X_3, the concentration of emulsifier (%).

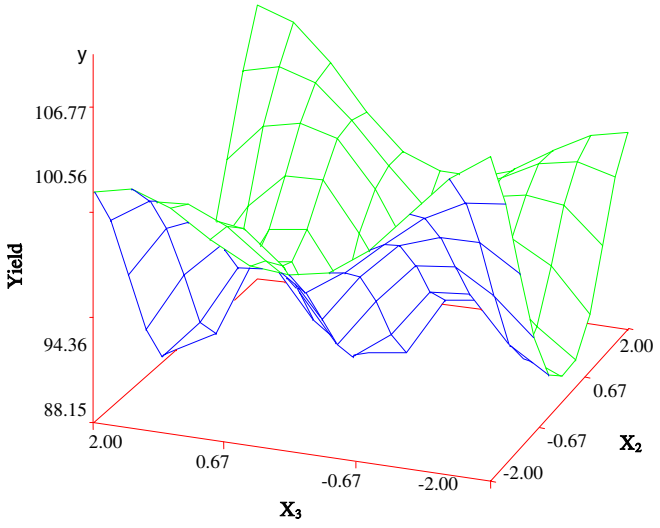

Fig. 3. Response surface plot for the effect of dispersion fluid temperature and emulsifier concentration on microencapsulation yield. X_2, the temperature of dispersion fluid (°C) and X_3, the concentration of emulsifier (%).

Table 4
Ridge of maximum response from coded radius 0.1 for optimal condition of response surface

Variable[a]	Coded factor value	Uncoded factor value
X_1	−0.1351	4.8:5.2
X_2	−0.0008	24.99
X_3	−0.1041	0.38

[a] The X_1, X_2 and X_3 were the ratio of core material to coating material (w/w), the temperature of dispersion fluid (°C) and the concentration of emulsifier (%), respectively.

ary point was the maximal one. Therefore, critical value reflected optimal conditions for the microencapsulation of the GO. The critical value is shown in Table 3. The optimal conditions for the microencapsulation of the GO were determined to be the ratio of [C_1m] to [C_2m] of 4.8:5.2 (w/w), the dispersion fluid temperature of 24.99 °C and the emulsifier concentration of 0.38% (Table 4). From these optimum compositions, 95.73% of microencapsulation yield was obtained. These results revealed that the most stable microcapsule of GO would be formed with the greatest yield of microencapsulation. This value was close to 93.6% yield, as counted from actual experimental observations, which indicated that the predicted maximum value of the degree of the yield was about the same as that of the experimental result within ±5% of error range. The overall yield of microencapsulation was somewhat less than predicted. Since not all the factors on their own, or simple interactions between them, could explain the variation in the data, there may be more complex interactions to be considered.

3.4. Thiobarbituric acid-reactive substances (TBARs)

The microcapsules produced under the optimal conditions were applied for the analysis of storage stability. The effects of GO and MGO were evaluated on oxidative stability of heat-treated lard. It was clear that GO had significant protection activity against heat-induced lipid oxidation, regardless of whether it was encapsulated or not (Table 5). The TBARs values for the control, group A, was kept larger than those of groups B (containing GO) and C (containing MGO) until 10 days of heating at 180 °C. After 5 days of heating, the TBARs values increased by more than 10-fold in the group A but not as drastically as in the groups B and C. There was no significant difference in TBARs values between group B and C up to 7 days of heating, but it was shown, in the 10-day heating treatment, that microencapsulation of GO significantly retarded the lipid oxidation (Table 5). As a result, the oxidative stability of lipid compounds in heated lard was clearly improved by the treatment of MGO.

3.5. Effect of heat treatment on the stability of γ-oryzanol

The mechanism of cholesterol oxidation was known to be similar to that of other lipid oxidation (Chien, Wang, & Chen, 1998; Maerker, 1987; Smith, 1987; Yan & White,

Table 5
Thiobarbituric acid-reactive substances (TBARs) in lard heated at 180 °C oven for 0, 2, 5, 7 and 10 days

Heating time (day)	TBARs (mg/kg)		
	A[a]	B[a]	C[a]
0	0.04 ± 0.01[b]a[c]	0.05 ± 0.01a	0.05 ± 0.00a
2	0.25 ± 0.01b	0.17 ± 0.00c	0.16 ± 0.00c
5	0.42 ± 0.02d	0.22 ± 0.01b	0.20 ± 0.01b
7	0.44 ± 0.01e	0.27 ± 0.02b	0.22 ± 0.01b
10	0.68 ± 0.02f	0.37 ± 0.01d	0.23 ± 0.00b

[a] A, B and C were the fresh lard, lard plus 100 ppm of γ-oryzanol and lard plus 100 ppm of microencapsulated γ-oryzanol, respectively.
[b] Mean ± SD, $n = 3$.
[c] Values with different letters were significantly different among the samples at $p = 0.05$ level by least significance difference (LSD).

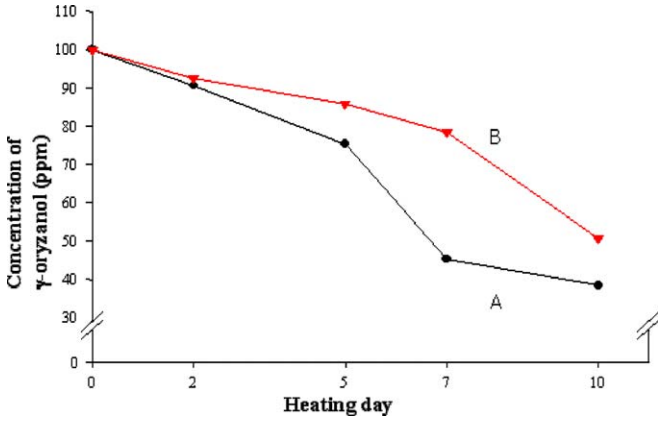

Fig. 4. Effect of heating at 180 °C on the stability of γ-oryzanol in lard. (A) Lard containing 100 ppm of γ-oryzanol (●) and (B) Lard containing 100 ppm of microencapsulated γ-oryzanol (▽).

1990). Because GO has a sterol, moiety in its chemical structure, it can be degraded by heat through a mechanism similar to that of cholesterol oxidation. Thus, the protecting effect of microencapsulation was evaluated by determining the remaining GO after heat treatment (Fig. 4). Up to 2 days of heating at 180 °C, the difference in residual amount of GO was not clear. However, significantly more GO was detected in the heat-treated lard sample containing MGO as the heat-treatment time went up to 7 days. During the next 3 days of heat treatment, sample B (containing MGO) displayed a higher level of GO than did sample A, but the degradation rate of GO in sample B was much faster than that in sample A. This result suggested that the microencapsulation provided GO with a physical barrier against heat-induced destruction. Microencapsulation increased the solubility of GO as well, since hydrophilic coating materials, starch and agar, were used to improve the adaptability of GO in the aqueous environment. Another advantage associated with their use is their extended shelf life, because the protective microcapsules are able to enhance the stability of MGO against temperature and moisture, and are likely to reduce the reactivity of targeted core materials with other ingredients.

4. Conclusion

The TBARs of lards, after heat-treatment, indicated that MGO had greater antioxidative activity than did the original γ-oryzanol (GO). The level of GO remaining in the heat-treated lard sample also showed that microencapsulation provided GO with an effective physical barrier against heat-induced destruction. These results suggested that this microencapsulating technique could be utilized for increasing stability of functional nutrients in heat-treated processed foods.

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