IMPROVEMENT OF OXIDATIVE STABILITY OF RICE BRAN OIL EMULSION BY CONTROLLING DROPLET SIZE

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ABSTRACT

Well-planned emulsification studies are needed to improve oxidative stability of rice bran oil. Factors that affect the droplet size during emulsification of rice bran oil include the amount of rice bran oil content, types of emulsifiers, power intensity and duration of sonication. In this study, the emulsions were prepared at different oil concentrations (10, 15 and 20%), using different emulsifiers (Tween 80 and its mixtures with Span 80), and subsequently sonicated at different durations (5–50 min). Longer sonication time resulted in smaller-sized emulsion droplets. The emulsions with the mixture of Tween 80 and Span 80 formed smaller droplets and better oxidative stability than those with Tween 80 only. In conclusion, rice bran oil emulsion with smaller droplets improves oxidative stability. This study may be helpful for tailoring oxidative-stable emulsions with well-defined droplet size distribution.

PRACTICAL APPLICATIONS

Recently, food manufacturers have been interested in utilization of the polishing by-products such as rice bran. Rice bran oil contains well-balanced saturated and unsaturated fats and provides a good source of vitamin E, antioxidants, oryzanol and other micronutrients. However, food application of rice bran oil is limited because of lipid oxidation. One of the promising technologies is nanoemulsion fabrication, which is being applied to enhance the oral bioavailability and oxidative stability of the poorly water-soluble bran oils. Herein, attention is focused on improving oxidative stability by controlling droplet size through adding mixture of surfactants in the oil-in-water emulsion. Narrow size distribution of droplets is a challenge for improved oxidative stability. In this study, proper formulations such as a mixture of emulsifiers provide a resolution to overcome the oxidative stability limitations in utilization of rice bran oils.

INTRODUCTION

Consumers have recently become aware that plant oils including rice bran oil have important health benefits, and new research in this field is attracting immense public interest. Rice bran oil contains well-balanced saturated and unsaturated oils and provides an excellent source of vitamin E, antioxidants, oryzanol and other micronutrients (Monsoor et al. 2003). Rice bran oil has a number of potential uses as functional emulsions in many fields such as the cosmetic, pharmaceutical and food industries (Hasenhuttl and Hartel 2008). One of the promising technologies is nanoemulsion fabrication, which is being applied to enhance the oral bioavailability and gravitic stability of poorly soluble components. Nanoemulsions because of small droplet size have a higher solubilization capacity than simple micellar solutions.

Food applications of rice bran oil can be expanded when its health benefits are improved by proper processing techniques. Droplet size usually relates to functionality of food emulsions (Raikar et al. 2009). An emulsion with smaller-size droplets has a larger surface area and is desirable for processes such as improved water absorption, flavor release and bioavailability (Aungst 1993). However, food application of rice
Bran oil is limited because of lipid oxidation during storage; lipid oxidation is a major cause of quality deterioration in rice bran oil products by altering odor and nutritional quality. The droplets’ characteristics may affect the oxidation kinetics in oil-in-water (o/w) emulsion, depending on their size and physical state (Osborn and Akoh 2004). Lipid oxidation is accelerated by reactions that take place at the surface of emulsion droplets; smaller droplets have an increased specific surface area and are more prone to oxidation than larger droplets (Lethuaut et al. 2002). Nanoemulsion, which has increased specific surface area, may not be considered a good system to retard lipid oxidation. However, surface characteristics of the droplets are more important than their size. Several authors have found that surface charge can influence the oxidative stability of lipid droplets in emulsion systems (Yoshida and Niki 1992; Mei et al. 1998). Interfacial properties of the lipid droplets could be the important determinants of the degree of oxidation in an emulsion. The physical characteristics of the droplets may also affect the oxidative stability of o/w emulsion depending on their concentration, size, physical state and charge (Mancuso et al. 1999). However, determination of the optimal emulsion preparation condition has been difficult. Regression analysis allows for an approximate fit by minimizing the gap between the data points and the fitted curve. Thus, the regression analysis of experimental data may help determine the process conditions to obtain an emulsion with the desired physicochemical characteristics. Herein, we hypothesized that a smaller-size droplet with proper surface characteristics may be a favorable structure to enhance gravitic aqueous stability and retard oxidation of rice bran oil.

Uniform and narrow size distribution of droplets is required for better control of functionality and product quality. Emulsion droplet size depends on the nature of the emulsifiers, the oil and aqueous phase, temperature, mechanical energy (high shear, homogenizer, centrifugation, ultrasound generators) and emulsification conditions (Dalglish et al. 1995; Dickinson 2009). Emulsifiers also play a role in the oxidative stability of oil droplets (Fomuso et al. 2002). The primary role of the emulsifier is to adsorb at the surface of the freshly formed droplets and concomitantly prevent them from coalescing with their neighboring droplets (Lee et al. 2009). Tween 80 (hydrophilic-lipophilic balance [HLB] 15) and Span 80 (HLB 4.3) are the most commonly used emulsifiers that improve the stability of emulsions by forming a protective layer around the droplets. Tween 80 is also used as an emulsifier in foods (Goff 2000). Tween 80 and Span 80 are nonionic surface active agents, which are generally considered to be safe. Emulsifiers control interdroplet forces, thereby either preventing or retarding the rate of coalescence of colloidal droplets during emulsion formation (Wulff-Pérez et al. 2009). Tween 80 is soluble in water and is therefore used to form o/w emulsions, while Span 80 can be used to form o/w emulsions. Combinations of these emulsifiers may aid in the formation of well-stabilized emulsions. Because of the closely related chemical characteristics of Tween 80 and Span 80, they complement each other in terms of both hydrophilic and hydrophobic interactions. When used together, the mixture of Tween 80 and Span 80 can pack more densely along the interface between the oil and aqueous phase (Ingrid et al. 2007). Because it is usually desired to obtain emulsions having maximum oxidative stability and well-defined droplet characteristics, proper formulations such as a mixture of Tween 80 and Span 80 may have the potential for approaching optimal conditions during emulsification.

Herein, attention is focused on improving oxidative stability by controlling droplet size simply through adding a mixture of surfactants in the o/w emulsion. In addition, droplet size versus sonication time is modeled to predict the optimal emulsion preparation conditions for obtaining controlled droplet characteristics and oxidative stability in bran oil emulsions.

**Materials and Methods**

**Bran Oil Emulsion Preparation**

An emulsion was prepared by adding slowly distilled water to a mixture of rice bran oil (Cosmetic Aromanara Co., Seoul, Korea) and emulsifier under a magnetic stirrer. HCl (0.1 N, Deajung Chemical & Metals Co., Shiheung, Korea) was added to adjust the pH of the emulsion to 7.0 in a phosphate (5 mM; pH 7.0) buffer solution. The temperature was kept at 25°C. The varying parameters of the emulsion preparations were bran oil contents, type of emulsifiers, and sonication durations. Different concentrations of bran oil at 10, 15 and 20% wt were studied. For o/w emulsion system preparation, three combinations of the emulsifiers’ compositions containing 6% wt Tween 80 (polyoxyethylene sorbitan monooleate, HLB 15, Tokyo Chemical Industry Co., Tokyo, Japan), mixture of 4% wt Tween 80 and 2% wt Span 80 (sorbitan monooleate, HLB 4.3, Tokyo Chemical Industry Co.), and mixture of 3% wt Tween 80 and 3% wt Span 80 were investigated.

Ultrasonication experiments employed a Sonics VCX 750 (Sonics & Materials Inc., Newtown, CT) with a 6-mm diameter tip, which was placed in a glass tube with an internal diameter of 50 mm with an in-built cooling jacket. An ultrasound having a power of 562 W was applied for 5, 15, 30, 40 and 50 min, and corresponding accumulative powers were 46.8, 140.5, 281.0, 374.6 and 468.3 Wh, respectively. Chilled water was passed continuously through the jacket to avoid temperature increase during the sonication process. The levels of independent parameters are presented in Table 1.
TABLE 1. LEVELS OF INDEPENDENT PARAMETERS OF BRAN OIL EMULSION PREPARATION

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Values of levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil content (%)</td>
<td>10; 15; 20</td>
</tr>
<tr>
<td>Ratio of Tween 80 to</td>
<td>6.0 (HLB 15); 4.2 (HLB 11.4); 3.3 (HLB 9.6)</td>
</tr>
<tr>
<td>Span 80</td>
<td></td>
</tr>
<tr>
<td>Time (min)/ultrasonication</td>
<td>5/46.8; 15/140.5; 30/281.0; 40/374.6; 50/468.3</td>
</tr>
<tr>
<td>power (Wh)</td>
<td></td>
</tr>
</tbody>
</table>

Size Distribution and Zeta-Potential of Droplets

Droplet size distribution and zeta-potential were determined to observe the emulsion stability using particle size analyzer (Delsa nano C, Beckman Coulter, Inc., Brea, CA). Samples were diluted with distilled water according to instructions given in the service manual and 3 mL of diluted samples were placed in a glass cell. All samples were measured three times at fixed temperature and angles of 25° and 165°, respectively. The particle size analyzer provided mean particle size distribution. The samples mentioned earlier were further diluted and adjusted to pH 4.0 to measure zeta-potential. The sample was injected into a flow cell in which the zeta-potential was measured.

Nonlinear Regression

Regression analysis of the experimental data was performed based on nonlinear models. A dependent variable (droplet size) and one or more independent variables (sonication time) were set as the numerical data. The droplet size in the regression equation was modeled as a function of the sonication time. The parameters were estimated to give the best fit of the data. The Sigma Plot (Ver. 10.0, Systat Software Inc., San Jose, CA) was used for nonlinear regression analysis. The data was analyzed to determine the best predictive model between the emulsion preparation condition and the droplet size. In addition, Duncan’s multiple tests were used to compare the minimum droplet sizes predicted by the nonlinear models.

Oxidative Stability

Lipid hydroperoxides were measured to evaluate oxidative stability of the rice bran oil emulsions (Shantha and Decker 1994). In brief, all of the samples were stored at 50°C to accelerate the lipid oxidation process. Lipid hydroperoxides were measured by mixing 3.4 mL of an emulsion sample with 25 mL of isooctane/2-propanol (3:1, v/v) mixture by vortexing for 30 s and subsequently isolating the organic solvent phase by centrifugation at 10,000 rpm for 10 min. The organic solvent phase (200 µL) was added to 2.8 mL of methanol/butanol (2:1, v/v) mixture, followed by 15 µL of 3.97 M ammonium thiocyanate and 15 µL of ferrous iron solution, which were prepared by mixing 0.132 M BaCl₂ and 0.144 M FeSO₄. Then, the solution was placed into a test tube and allowed to oxidize at 37°C in the dark for 30 min. The absorbance of the solution was measured at 510 nm (DU 730, Life science UV/Vis Spectrophotometer, Beckman Coulter, Inc., Brea, CA). Hydroperoxide concentrations were determined using a standard curve made from cumene hydroperoxide.

Statistical Analysis

Data in triplicate were analyzed by one-way analysis of variance using a SAS program (SAS Institute Inc., Cary, NC). Statistical significance was considered at *P < 0.05.*

RESULTS AND DISCUSSION

Effect of Emulsifiers on Droplet Size in Bran Oil Emulsion

The dynamic light scattering particle size analyzer measured the droplet size of the bran oil emulsion prepared as shown in Fig. 1. The size distribution of the droplets in the emulsion was obtained and corresponding mean size was calculated from the particle size analyzer. The average droplet size of the bran oil emulsion depended on the types and the compositions of the emulsifiers as shown in Fig. 2. The results indicate that the mixture prepared with Tween 80 and Span 80 provided smaller droplet sizes than that emulsified with only Tween 80. When 10% oil concentration was used, the droplet size of the emulsion prepared with mixture of 4% Tween 80 and 2% Span 80 was the smallest among the three emulsifier combinations tested at the early sonication stage, but the rate of droplet size reduction decelerated after 20 min. When the sonication time increased, the droplet size decreased after increasing the ratio of Span 80 in the emulsifier used. Results using the 15% oil concentration showed a similar pattern to that of the 20% oil.

As shown in Fig. 2, increasing the oil concentration from 10 to 20% led to an increase of the droplet size in the emulsion. The emulsions with 10% oil content and the mixture of the emulsifiers were stabilized quickly over time under the ultrasonication treatment because proper combination of hydrophilic and hydrophobic emulsifiers prevented separation of the oil from water phases. The emulsion system with high oil concentration (20%) required an increased quantity of hydrophobic emulsifier to disperse the oil completely and for further stabilization. Moreover, the mixture of Tween 80 and Span 80 packed more densely along the interface between water and oil phases, and the emulsion stabilization capacity of the mixture was relatively stronger than individual emulsifiers tested. As shown in Fig. 1, it can also be observed that emulsion with 3% Tween 80 and 3% Span 80 has narrow droplet size distribution compared with other
emulsions. The droplet size in all samples decreased with the increasing sonication time for the emulsions prepared at all oil concentrations tested.

The HLB values of the emulsifier mixtures (HLB<sub>mix</sub>) were calculated using weight fraction of the corresponding emulsifiers as shown in the following:

$$\text{HLB}_{\text{mix}} = f_A \times \text{HLB}_A + f_B \times \text{HLB}_B$$

where HLB<sub>A</sub> and HLB<sub>B</sub> are the HLB values of components A and B, respectively, and \(f_A\) and \(f_B\) are the weight fractions of components A and B, respectively (Wang et al. 2009). The HLB<sub>mix</sub> values of Tween 80 only, mixture of 4% Tween 80 and 2% Span 80, and mixture of 3% Tween 80 and 2% Span 80 were 15, 11.4 and 9.6, respectively (see Table 1).

The effect of mixed emulsifiers has also been studied by other researchers. The effect of weight ratios of different hydrophobic emulsifiers (Span 20, 40, 60 and 80) to a hydrophilic emulsifier (Tweem 80) has been studied during microemulsion preparation (Cho et al. 2008). The mixtures of emulsifiers provided better microemulsion storage stability than Tween 80 alone. The stability of the emulsions has been shown to improve by using a combination of emulsifiers, because the hydrophilic-lipophilic properties of an emulsifier are balanced by using a mixture (optimal HLB). The optimal HLB provides only a minimal interfacial tension between the oil and aqueous phase (Forster et al. 1992, 1994). Hence, at the optimal HLB of the emulsion system, the smallest droplet can be formed.

**FIG. 1. DROPLET SIZE DISTRIBUTION OF THE BRAN OIL EMULSIONS PREPARED AT (A) 6% TWEEN 80, (B) 4% TWEEN 80 – 2% SPAN 80 AND (C) 3% TWEEN 80 – 3% SPAN 80**
**Zeta-Potential**

The result of the zeta-potential measurement at pH 4 is shown in Fig. 3. The zeta-potentials of all samples displayed a negative value and were dependent on the surfactant type. The emulsion stabilized by the surfactant mixture of Tween 80 and Span 80 indicated larger negative zeta-potential value compared with either emulsion without surfactant or Tween 80-added emulsion at all oil contents. In the dispersion system, particles or droplets have charges on their surface because of the selective adsorption of ions, including protons (Lin and Chaudhury 2008). Zeta-potential is a useful indicator to understand the behavior and interaction of particles and droplets in an aqueous solution, which is affected by factors such as pH, electrolytes and surfactant (Karraker and Radke 2002). In this study, the emulsion formed with Tween 80 only showed the zeta-potential value close to zero, ranging $-4$ to $-9$ mV. The result resembled those of an earlier study (Kato et al. 2009), in which it was reported that the stability of a dispersion stabilized by Tween 80 increases in spite of displaying a low zeta-potential value because the surfactant adsorbed on the surface of dispersion has a sterical interaction and small electrostatic interaction between their molecules; the sterical repulsive power of surfactant molecules could prevent the agglomeration between droplets or particles.

**Nonlinear Regression of Droplet Size as a Function of Sonication Time in Bran Oil Emulsion**

From Fig. 4, it can be seen that the droplet size decreases as the sonication time increases. In the method used here, the

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**FIG. 2. EFFECT OF EMULSIFIERS ON DROPLET SIZE OF BRAN OIL EMULSIONS PREPARED AT (A) 10, (B) 15 AND (C) 20% OIL CONTENT**

![Graph showing droplet size as a function of sonication time for different emulsifiers at 10%, 15%, and 20% oil content.](image-url)
intensive ultrasound supplied the power needed to disperse small droplets of the bran oil phase into the water phase. In the dispersing zone, the imploding cavitations caused intensive shock waves through the bubbles in the surrounding liquid, resulting in the formation of liquid jets with high liquid velocity (Gohtani et al. 1999).

Several mathematical models were used to fit the curve of the emulsion droplet size versus sonication time. Because the droplet size decreased nonlinearly with the sonication time during emulsification, three mathematical models were chosen to present the decay shape (Jena and Das 2006; Ko and Gunasekaran 2008):

- **Model I**
  \[ y = ae^{-bx} \]
- **Model II**
  \[ y = y_0 + ae^{-bx} \]
- **Model III**
  \[ y = ae^{cx} \]

Where parameters \( x \) (the sonication time) and \( y \) (the droplet size) are independent and dependent variables, respectively; \( a, b, c \) and \( y_0 \) are coefficients.

The goodness-of-fit of the chosen nonlinear models was tested by \( R^2 \), which showed values from 0.0 to 1.0. An \( R^2 \) value of 0.0 means that knowing \( x \) is not useful to predict \( y \). There was no linear relationship between \( x \) and \( y \). When \( R^2 \) equals 1.0, all points lie exactly on a straight line with no scatter, and thus
knowing $x$ predicts $y$. The sum of squares (SS) and mean square (MS) of the models were compared, and results are listed in Table 2. We observed that $R^2$ of Model II is larger than the $R^2$ of Model I but similar to that of Model III. Because the $R^2$ of Model I was not acceptable for the curve fitting, Model I was rejected. At infinite $x$ (long-time sonication), the nonlinear regression from Model III has a horizontal asymptote $y = a$, which means that coefficient $a$ is the minimum droplet size. Minimum droplet sizes calculated from Models II (coefficient $y_0$) and III (coefficient $a$) were compared with those of the experimental data, and the results are listed in Table 3. Duncan’s multiple tests were used to compare the minimum droplet sizes. The minimum droplet size $y_0$ of Model II is comparable with the results of the experimental data. Simultaneously, compared with Model III, SS and MS values of Model II were low. In conclusion, Model II was chosen to fit the nonlinear curve of droplet size as a function of sonication time.

The coefficients were calculated using Model II ($y = y_0 + ae^{-bx}$) as shown in Fig. 5. The mathematical meanings for coefficients $y_0$, $a$, and $b$ of Model II can be easily interpreted. For Model II, at a larger $x$ value (i.e., infinite $x$), $e^{-bx} = 0$, at which the curve has a horizontal asymptote $y = y_0$. Thus, with a larger $x$ value, $y_0$ is the minimum droplet size. The initial droplet size before sonication can be represented using coefficients $y_0$ and $a$. The initial droplet size is $y_0 + a$, because $y = y_0 + a$ when $x = 0$. In general, for fine emulsion preparations, the minimum droplet size is small (i.e., $y_0 << a$, $y_0 + a = a$), so the initial droplet size can be defined as coefficient $a$. In addition, coefficient $b$ of Model II means the stiffness of the curve.

The effect of the types and the compositions of the emulsifiers at different bran oil contents can be easily explained by using the coefficients $y_0$, $a$, and $b$ of Model II. Coefficient $a$ (initial droplet size) and coefficient $y_0$ (minimum droplet size) were the smallest when the Span 80 concentration was increased to 3%. This model fits well for the emulsion preparation conditions (10, 15 and 20% oil content) and the combinations of emulsifier compositions. Based on the above
results, $y = y_0 + ae^{-bx}$ was the best model to fit the nonlinear curve of droplet size in terms of sonication time.

### Effect of Sonication Time on Droplet Size in Bran Oil Emulsion

The effect of sonication time on droplet size was prominent. From the initial stage of emulsification, droplet size decreased steeply as shown in Fig. 4. Even after 30 min, droplet size continued to decline but at a slower rate. At the last emulsification stage, the effect of sonication was not as strong as in the initial stage. Droplet size did not decrease significantly when sonication time was over 40 min.

As the ratio of Span 80 to Tween 80 increased, the final droplet size of the emulsion decreased. A similar trend was observed with all emulsifier ratios in the emulsions prepared with all tested oil concentrations (10, 15 and 20%). The emulsion with 15% oil content especially provided the best correlation of the model and the experimental data in terms of sonication time and droplet size. On the other hand, when the oil content was 20%, the correlation of the model and the experimental data was not as good as that of 10 or 15%. In the case of 20% oil content using Tween 80 and Span 80, the mixture could not disperse completely in the aqueous phase because, in general, a larger amount of hydrophilic emulsifier was needed for stabilization with a larger oil content (Dickinson 2009).

Coefficient $b$ was calculated using Model II means the stiffness of the curve of the emulsion droplet size versus sonication time. The coefficient $b$ is shown in Fig. 5. In other words, the stiffness of the plots of droplet size versus sonication time depended significantly on the type of emulsifier and the ratio of emulsifier composition. The coefficient $a$ of the model has the largest value for the emulsion prepared with the mixture of 4% Tween 80 and 2% Span 80, but smallest value obtained with the mixture of 3% Tween 80 and 3% Span 80.

### Table 2. The Fitness ($R^2$, SS, MS) of the Models I, II, and III at Different Oil Contents and Types of Emulsifiers

<table>
<thead>
<tr>
<th>Model</th>
<th>Fitness</th>
<th>Oil content (%)</th>
<th>T6</th>
<th>T4-S2</th>
<th>T3-S3</th>
<th>T6</th>
<th>T4-S2</th>
<th>T3-S3</th>
<th>T6</th>
<th>T4-S2</th>
<th>T3-S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>$R^2$</td>
<td>0.96 ± 0.02</td>
<td>0.94 ± 0.01</td>
<td>0.98 ± 0.01</td>
<td>0.92 ± 0.00</td>
<td>0.90 ± 0.01</td>
<td>0.96 ± 0.02</td>
<td>0.61 ± 0.02</td>
<td>0.95 ± 0.02</td>
<td>0.87 ± 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>10,712.8</td>
<td>6,681.7</td>
<td>27,509.2</td>
<td>19,050.6</td>
<td>22,000.1</td>
<td>3,031.9</td>
<td>12,161.9</td>
<td>5,684.2</td>
<td>6,620.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>824.1</td>
<td>513.9</td>
<td>1,528.3</td>
<td>1,465.4</td>
<td>1,692.3</td>
<td>233.2</td>
<td>935.4</td>
<td>437.2</td>
<td>509.3</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>$R^2$</td>
<td>1.00 ± 0.00</td>
<td>0.99 ± 0.00</td>
<td>0.98 ± 0.07</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
<td>0.96 ± 0.02</td>
<td>0.88 ± 0.03</td>
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<tr>
<td></td>
<td>SS</td>
<td>303.15</td>
<td>683.74</td>
<td>1,368.87</td>
<td>246.6</td>
<td>968.4</td>
<td>373.6</td>
<td>9,133.4</td>
<td>817.5</td>
<td>722.6</td>
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<tr>
<td></td>
<td>MS</td>
<td>151.6</td>
<td>56.9</td>
<td>693.4</td>
<td>20.5</td>
<td>80.7</td>
<td>31.1</td>
<td>761.1</td>
<td>69.8</td>
<td>80.2</td>
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</tr>
<tr>
<td>III</td>
<td>$R^2$</td>
<td>1.00 ± 0.00</td>
<td>0.99 ± 0.01</td>
<td>0.98 ± 0.01</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
<td>0.96 ± 0.02</td>
<td>0.87 ± 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>1,665.7</td>
<td>1,102.4</td>
<td>N/A</td>
<td>618.4</td>
<td>718.8</td>
<td>388.1</td>
<td>9,820.7</td>
<td>1,267.9</td>
<td>1,120.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>138.8</td>
<td>91.8</td>
<td>N/A</td>
<td>51.3</td>
<td>59.9</td>
<td>32.3</td>
<td>818.4</td>
<td>105.6</td>
<td>93.4</td>
<td></td>
</tr>
</tbody>
</table>

- T6: 6% Tween 80 only.
- T4-S2: 4% Tween 80 and 2% Span 80.
- T3-S3: 3% Tween 80 and 3% Span 80.
- N/A: the model was not converged but exceeded maximum number of iterations.

MS = mean of squares; SS = sum of squares.

### Table 3. Minimum Droplet Size of the Bran Oil Emulsion Predicted by the Models II and III at Different Types of the Emulsifiers, and 10 15 and 20% Oil Content

<table>
<thead>
<tr>
<th>Minimum droplet size (nm)</th>
<th>Oil content (%)</th>
<th>T6</th>
<th>T4-S2</th>
<th>T3-S3</th>
<th>T6</th>
<th>T4-S2</th>
<th>T3-S3</th>
<th>T6</th>
<th>T4-S2</th>
<th>T3-S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimentally measured</td>
<td>250</td>
<td>172</td>
<td>141</td>
<td>336</td>
<td>262</td>
<td>207</td>
<td>170</td>
<td>260</td>
<td>329</td>
<td></td>
</tr>
<tr>
<td>Model II</td>
<td>219</td>
<td>179</td>
<td>64</td>
<td>321</td>
<td>253</td>
<td>175</td>
<td>170</td>
<td>200</td>
<td>304</td>
<td></td>
</tr>
<tr>
<td>Model III</td>
<td>115</td>
<td>89</td>
<td>N/A</td>
<td>228</td>
<td>184</td>
<td>99</td>
<td>168</td>
<td>84</td>
<td>216</td>
<td></td>
</tr>
</tbody>
</table>

- A, B and C are Duncan’s groupings of the minimum droplet sizes at the same oil content and emulsifier.
- N/A: the model was not converged but exceeded maximum number of iterations.
sonication time decreased droplet size during emulsification of an o/w emulsion of d-limonene. Reducing the amount of dispersed phase (d-limonene) decreased the size of emulsions.

Our predictive model showed a good fit for the bran oil emulsions prepared with different oil concentrations, the types of emulsifiers and the ratio of emulsifiers. The predictive model is also useful to determine required sonication time for formulation of emulsions with defined droplet size.

**Effect of Engineering Parameters on Oxidative Stability**

Oxidative stability determined by hydroperoxide concentration is shown in Fig. 6. Hydroperoxide concentration increased with increasing oil content in the emulsions. It is also interesting to note that there was no significant increase in the hydroperoxide levels until 20 days, and thereafter a drastic increase was observed in all samples. Emulsions with the emulsifiers such as Tween 80 or mixture of emulsifiers were more stable from lipid oxidation than non-emulsifier-added emulsions. The emulsifiers surrounding oil droplets act as a radical scavenger and a protective barrier that prevents oxidation-inducers from penetration and diffusion inside the droplets (Porter et al. 1995).

In the case of emulsions without an emulsifier, the effect of high oil content on hydroperoxide increase was conspicuous on long-time storage. After 30 days of storage, the hydroperoxide values of the emulsions with 10 and 15% oil content increased, but the difference compared with the emulsion without emulsifier was not large. However, the difference was relatively large in the emulsions with 20% oil content.

As mentioned previously, droplet size of the emulsion with only Tween 80 was larger than that of the mixture of Tween 80 and Span 80. As expected, the hydroperoxide values were low in the emulsions prepared with 6% Tween 80. The emulsion
with Tween 80 (only hydrophilic emulsifier) could obtain good oxidative stability because a higher concentration of Tween 80 would form a thicker interfacial membrane on the surface of the droplet and the packing of the surfactant molecules at interface would be tightened (Tadros et al. 2004); hence, this membrane acts as an efficient barrier to the diffusion of oxidative initiators into the oil droplets (McClements and Decker 2000). However, beyond a 20-day storage period, hydroperoxide values of emulsion prepared with 6% Tween 80 increased steeply. Different types and concentrations of emulsifiers give different droplet sizes (Ko and Gunasekaran 2006) that might also affect the oxidation stability.

The oxidative stability of the emulsions prepared with the mixture of Tween 80 and Span 80 (mixture of hydrophilic and hydrophobic emulsifiers), may be better or worse than those prepared with Tween 80 only. In this study, the smallest droplets formed at 10% oil content and mixture of 4% Tween 80 and 2% Span 80 showed higher hydroperoxide levels. On the contrary, small droplets were prepared with the mixture of Tween 80 and Span 80, but showed low hydroperoxide levels (Fig. 7). A few studies have reported that decrease in droplet size increases specific surface area, which is more prone to oxidation (Gohtani et al. 1999; Lethuaut et al. 2002); the mixed emulsifiers decrease droplet size, but can increase lipid oxidation. However, the mixture of emulsifiers can provide a positive effect on the prevention of lipid oxidation. Addition of the hydrophobic component in the mixed emulsifiers would contribute to prevention of lipid oxidation (Abismail et al. 1999). Span 80 can prevent movement of water-soluble oxidant such as lipoxygenase. Herein, the droplet size decreased by increasing the ratio of Span 80 concentration in the mixture of emulsifiers used, but there was no significant difference in oxidative stability during short-term storage. Moreover, the relatively small droplets prepared at 3% Tween 80 and 3% Span 80 mixture condition showed the best antioxidant efficiency (Fig. 7).
There are several reports that the effect of droplet size on lipid oxidation is not uniform; some studies indicated that smaller droplet sizes led to higher oxidation rates because of increased surface area (Lee et al. 2009), but another reported no dependence of lipid oxidation rate on droplet size (Roozen et al. 1994). Because limited amounts of hydroperoxides were available in the systems, they might have all been present at the droplet surface in every o/w emulsion system studied.

Effect of emulsifier type on lipid oxidation is relatively significant. In this study, we used the nonionic emulsifiers with the HLB values 15, 11.4, and 9.6, respectively. It was observed that hydroperoxide value decreased with increasing HLB value of emulsifiers. Therefore, the effect of emulsifier composition was more pronounced than droplet size on oxidation stability of bran o/w emulsion. The use of emulsifier mixture not only improves gravitic aqueous stability through droplet size reduction, but also controlled oxidation stability of the emulsion system. The result was in close agreement with previous studies (Kubouchi et al. 2002). Generally, at higher emulsifier concentrations, the packing of emulsifier molecules at the oil–water interface is tighter; hence, the membrane acts as an efficient barrier to the diffusion of lipid oxidation initiators into the oil droplets (Coupland and McClements 1996). In another report, lower emulsifier levels (0.25%) showed significantly higher oxidation levels than emulsions prepared with 1% emulsifier (P < 0.05) (Fomuso et al. 2002). It may be deduced that emulsifier concentration rather than droplet size distribution caused changes in oxidation properties.

CONCLUSION

This study investigates the effects of emulsifier type and ratio, oil content, and sonication time on droplet size and oxidative stability. The mathematical model $y = y_0 + ae^{-bx}$ is proposed for the bran o/w emulsion based on the experimental observation. The effect of emulsion preparation conditions on droplet size distribution can be used to predict the sonication time required for a bran o/w emulsion with different ratios of emulsifiers. Under the same circumstances, the droplet size can be deduced because of the sonication time. This model can be expanded to predict formulations for preparing the emulsions with defined droplet sizes for various food applications. In addition, oxidative stability of emulsion was mainly affected by oil content, and emulsifier conditions with a small amount of oil addition induced lower hydroperoxide values. In general, small size is not good for oxidative stability, but it increases bioavailability of the system. Therefore, we overcome the problem by controlling emulsifier conditions, which give a good choice for both oxidative stability and bioavailability properties. For future applications, the droplet size distribution could be used as an evaluation criterion for the quality and stability of emulsions prepared under different preparation conditions.

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