

Effects of rice hull phenolic extract on the stability of emulsions stabilized by rice bran protein hydrolysate

*Cheetangdee, N. and Benjakul, S.

Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112 Thailand

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Abstract

Effect of rice hull phenolic extract (RHPE) on stability of oil-in-water (O/W) emulsion stabilized by rice bran protein hydrolysate (RBPH) was investigated. Dispersibility of the RBPH stabilized emulsions was improved by incorporating RHPE, mainly due to the increased electrical charge on the surfaces of oil droplets. RHPE could also improve oxidative stability of emulsions. Higher dispersibility and oxidative stability of the RBPH stabilized emulsions was observed when rice bran oil was employed as a dispersed phase, compared to soybean oil and palm olein. Nevertheless, RHPE impaired the dispersibility of palm olein emulsion. When RHPE was incorporated, the highest oxidative stability was obtained in rice bran oil emulsion. Therefore, bran and hull, by-products from rice milling process, could be promisingly used to improve colloidal and oxidative stability of O/W emulsion.

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Introduction

Emulsions are crucial and associated with quality and acceptability of several foods. Phase separation and lipid oxidation are the major drawbacks in food emulsion. Ingredients employed for emulsion preparation have been known to affect the characteristics of system. The intense emulsification process was required to produce the emulsion with small sized oil drops, when the oil with high viscosity was employed as a dispersed phase (Jumaa and Müller, 1998). Fatty acid composition of oils affected the susceptibility of the emulsion against lipid oxidation (McClements and Decker, 2000). With the presence of some constituents in the oils, e.g., phenolics and sterols, the colloidal and oxidative stability of emulsion were varied (Di Mattia *et al.*, 2010, 2011; Maldonado-Valderrama *et al.*, 2010; Piotrowski *et al.*, 2012; Wan *et al.*, 2014; Wojciechowski *et al.*, 2014).

Synthetic agents have been introduced widely to enhance stability of emulsion. Nonetheless, there is a continuously increasing interest in using natural additives in food processing, because of a low toxicity and intrinsic biodegradability of natural compounds (Wan *et al.*, 2014). Protein hydrolysates could be a promising candidate to be used as a natural emulsifier, attributed to their amphiphilic characteristic

(Cheetangdee, 2014). Phenolic rich extracts with antioxidative capacity prepared from various plants could improve oxidative stability of emulsions (Hu *et al.*, 2004; Viljanen *et al.*, 2005). The ability of grape seed extracts in prohibiting hydroperoxide and propanol formation in whey protein stabilized emulsions was reported (Hu *et al.*, 2004). Interaction between phenolics and the employed emulsifiers has been considered to affect the characteristic of emulsion. Due to their different chemical structures, catechin and quercetin exhibited dissimilar phase partitioning behavior, thereby influencing the physicochemical stability of olive oil emulsions in different manners (Di Mattia *et al.*, 2010). Catechin could reduce interfacial tension effectively but failed to improve oxidative stability, whereas quercetin could potently delay lipid oxidation and improve drop dispersibility (Di Mattia *et al.*, 2010).

Rice is one of the most important agricultural products of Thailand, bringing the income more than 4.9 billion USD in 2014 (Official of Agricultural Economics, Ministry of Agriculture and Cooperative, Thailand, 2015). Bran and hull are the abundant by-products from rice milling process. Rice bran could be used to produce rice bran protein hydrolysate (RBPH) with emulsifying and antioxidative activities (Cheetangdee, 2014; Cheetangdee and Benjakul, 2015). Preparation of rice hull phenolic extract

*Corresponding author.
Email: nopparat.ch@psu.ac.th

(RHPE) with an effective free radical scavenging ability and reducing power was achieved via methanolic extraction (Cheetangdee and Benjakul, 2016). Nevertheless, the incorporation of RHPE in emulsion stabilized by RBPH might affect colloidal and oxidative stability of the resulting emulsion. Furthermore, types of oil used as a dispersed phase might be oxidized in different manners when RHPE was employed. The present study aimed to elucidate the effects of RHPE on the characteristics of soybean oil (SBO), rice bran oil (RBO), and palm olein (PO) emulsions using RBPH as an emulsifier.

Materials and Methods

Chemicals

The defatted bran and hull of rice (*Oryza sativa* L.) were provided by Thai Edible Oil Co., Ltd (Bangkok, Thailand) and rice milling community enterprise (Phattalung, Thailand), respectively. SBO, RBO, and PO were purchased from a local market in Hat Yai (Songkhla, Thailand) and used without further treatment. Viscozyme-L, α -amylase, Alcalase, and thiobarbituric acid (TBA) were bought from Sigma-Aldrich (St. Louis, MO, USA). Viscozyme-L, the multi-enzyme complex consisting of arabanase, cellulase, β -glucanase, hemicellulase, and xylanase, possessed the activity of 120 Fungal β -glucanase units (FBG)/mL, in which 1 FBG is the amount of enzyme required under the standard conditions, i.e., 30°C, pH 5.0, and 30 min reaction time). α -amylase from *Aspergillus oryzae* had the activity of 1.5 U/mg, in which 1 U corresponds to the amount of enzyme that liberates 1 μ mol maltose/min at pH 6.0 and 25°C, using starch as a substrate. Alcalase, the protease from *Bacillus licheniformis*, had the activity of ≥ 5 U/g (1 U corresponds to the amount of enzyme that sets free 1 μ mol Folin-positive amino acids and peptide (as tyrosine) per min at pH 7.0 and 37°C, using casein as a substrate). Trichloroacetic acid was obtained from Carlo Erba Reagenti (Rodano, Italy). HCl, isooctane, 2-propanol, methanol, and 1-butanol were purchased from J.T. Baker (Deventer, Netherland). All chemicals are of analytical grade.

Preparation of RBPH

Protein from rice bran was isolated via the dual enzyme-aided extraction following the method of Cheetangdee (2014). Briefly, the defatted bran was mixed with deionized (DI) water with a ratio of 1:10 (w/v) at room temperature for 1 h. The mixture was adjusted to pH 4.1 and added with Viscozyme-L (0.5%). Extraction was performed at 45°C (water bath, Memmert, Schwabach, Germany) for 1 h. Then,

the mixture was adjusted to pH 6.25 and α -amylase (0.5%) was added, followed by incubation at 45°C for 1 h. After terminating the enzyme reaction by elevating pH to 11, the mixture was stirred for 15 min. Thereafter, the protein was recovered by adjusting pH of the mixture to pH 4. The pellet was washed with DI water and neutralized to pH 7 using 0.1 N HCl. The obtained pellet was lyophilized by a freeze dryer (FTS system Flex-Dry™, SP Scientific, NY, USA).

To prepare RBPH, the isolated protein was mixed with DI water adjusted to pH 9 with a ratio of 1:10 (w/v) and pre-incubated at 60°C for 15 min. Alcalase (1% by weight of the isolated protein) was added to the mixture, and the hydrolysis reaction was carried out at 60°C for 45 min. Enzymatic reaction was terminated by heating the mixture at 90°C for 5 min and immediately cooled in ice bath. The suspension was neutralized using 0.1 N HCl, lyophilized, and kept as powder at 4°C for less than 2 months before use.

Preparation of RHPE

RHPE was prepared following the method described by Cheetangdee and Benjakul (2016). Briefly, the hull was mixed with a mixture of methanol-water (3:1, v/v) with a ratio of 1:10 (w/v), before homogenizing at 11,000 rpm for 2 min (Ultra Turrax T25, Ika, Staufen, Germany). The extraction was conducted at 50°C for 180 min. The mixture was then filtered, lyophilized, and kept as powder at 4°C for less than 2 months before use.

Preparation of RBPH stabilized emulsions added with RHPE

Oil-in-water (O/W) emulsions stabilized by RBPH were prepared using different oils, i.e., SBO, RBO, and PO, as the dispersed phases. Firstly, RBPH was dissolved in 10 mM Na-phosphate buffer (pH 7) in the presence of 0.02% NaN₃ to obtain the concentration of 0.75%. RHPE was added to the protein solution at different concentrations (1, 2 and 3% w/v). The mixture was then homogenized at 19,000 rpm for 3 min. The selected oil was added to the mixture, before further homogenizing at 19,000 rpm for 5 min. The final emulsion contained oil fraction of 0.1. Two batches of the emulsion were prepared separately and subjected to analyses.

Colloidal stability of the emulsions

The emulsions were kept in a screw capped bottle at room temperature and their colloidal stability was measured.

Particle size and ζ -potential

Diameter and ζ -potential of dispersed oil droplets were determined using a laser diffraction particle size analyzer (ZetaPALS, Brookhaven Instrument, Holtville, NY, USA). The size of oil droplets was expressed as a volume-weighted average mean diameter, $d_v = \sum n_i d_i^3 / \sum n_i d_i^2$, where n_i is the number of droplets of the diameter (d_i). To evaluate a change in droplet size with storage time, percentage of increased d_{43} was calculated as follows:

$$\% \text{ increased } d_{43} = 100 * [d_{43}(t) - d_{43}(0)] / d_{43}(0)$$

where $d_{43}(0)$ and $d_{43}(t)$ are the mean diameters of oil droplets measured at the initial time and at time t , respectively (Rao and McClements, 2012).

Emulsion ability index (EAI) and emulsion stability index (ESI)

EAI and ESI were measured following the method of Pearce and Kinsella (1978). The emulsion was diluted with sodium dodecyl sulfate solution (0.1%, w/v) and the absorbance at 500 nm was read (UV-1700, Shimadzu, Kyoto, Japan). EAI and ESI were calculated by the following equations:

$$EAI(m^2/g) = \frac{2 \times 2.303 \times A_0}{c \times \phi \times (1 - \theta)}$$

$$ESI(\%) = A_0 \times \frac{\Delta t}{(A_0 - A_{10})}$$

where A_0 and A_{10} are the absorbance measured immediately and after 10 min of emulsification; c is the concentration of RBPH; ϕ is the optical path length; θ is the fraction of oil in the initial emulsion, and Δt is 10.

Oxidative stability of the emulsions

The emulsions were kept in a screw capped bottle at a controlled temperature of $37 \pm 2^\circ\text{C}$ in the dark. Butylated hydroxyl anisole (BHA) was employed at the legal permission level of 0.02% as a positive control. The emulsion samples were kept for 18 days and periodically taken for analyses.

Peroxide value (PV)

PV was determined following the method of Hu *et al.* (2004). The emulsion was mixed with a mixture of isooctane–propanol (3:1) with a ratio of 1:5 (v/v), before centrifuging at $4,000 \times g$ for 2 min. The solvent phase (200 μL) was added with 2.8 mL

Table 1. Characteristics of SBO emulsions stabilized by RBPH as affected by RHPE at different concentrations

| RHPE (%) | EAI (m ² /g) | d_{43} (nm) | ESI (%) | ζ -potential (mV) |
|----------|--------------------------------|------------------------------|--------------------------------|--------------------------------|
| 0 | 0.636 \pm 0.017 ^b | 2.77 \pm 0.43 ^A | 59.76 \pm 1.02 ^B | -20.34 \pm 1.52 ^A |
| 1 | 0.640 \pm 0.002 ^b | 2.37 \pm 0.29 ^A | 61.60 \pm 0.88 ^{AB} | -21.73 \pm 2.86 ^A |
| 2 | 0.639 \pm 0.022 ^b | 2.35 \pm 0.29 ^A | 60.70 \pm 1.40 ^{AB} | -21.72 \pm 2.84 ^A |
| 3 | 0.659 \pm 0.017 ^A | 2.45 \pm 0.11 ^A | 62.28 \pm 0.61 ^A | -22.29 \pm 1.72 ^B |

Means \pm standard deviation (n=3) were shown. Different letters in the same column indicate significant different between means (p<0.05).

of a mixture of methanol and 1-butanol (2:1), 15 μL of 3.97 M ammonium thiocyanate, and 15 μL of ferrous iron solution containing an equal volume of 0.132 M BaCl₂ and 0.144 M FeSO₄·7H₂O. The mixture was incubated at room temperature for 20 min, before measuring an absorbance at 510 nm. PV was quantified using cumene hydroperoxide as a standard and reported as mg hydroperoxide Equiv/L of sample.

Thiobarbituric reactive substances (TBARS)

The emulsions were mixed with TBA solution at a ratio of 1:5 (v/v). The TBA solution consisted of 0.375% TBA, 15% trichloroacetic acid, and 0.25 N HCl. The mixture was then heated at 100°C for 30 min. After cooling to room temperature, an absorbance at 532 nm was read. TBARS was estimated using malonaldehyde (MDA) as a standard, and reported as mg MDA Equiv/L of sample as described by Aewsiri *et al.* (2009).

Statistical analysis

The experiments were carried out in triplicate, and mean values with standard deviations were reported. Statistical analysis was performed by analysis of variance (ANOVA) using Duncan's multiple range tests (SPSS for windows: SPSS Inc., Chicago, IL, USA) at a 95% confident level.

Results and Discussion

Stability of SBO emulsions stabilized by RBPH as affected by RHPE

The emulsions made from SBO and incorporated with RHPE at different levels (1–3%) were prepared, and their characteristics were examined as shown in Table 1. EAI of the emulsions increased when 3% RHPE was added (p<0.05). However, RHPE at levels of 1 and 2% had no effect on EAI (p>0.05). The result suggested that RHPE at an adequate level

Table 2. Effect of 3% RHPE on characteristics of RBPH based emulsion made from different oil types

| Parameters | Dispersed phase | Emulsions | |
|-----------------------|-----------------|-----------------------------|-----------------------------|
| | | Control (no RHPE) | RHPE adding (3%) |
| EAI | SBO | 0.636 ± 0.017 ^{Bb} | 0.659 ± 0.017 ^{Ba} |
| | RBO | 0.657 ± 0.007 ^{Ab} | 0.677 ± 0.006 ^{Aa} |
| | PO | 0.643 ± 0.006 ^{Ab} | 0.662 ± 0.011 ^{Aa} |
| Initial d_{43} (nm) | SBO | 2.77 ± 0.43 ^{Aa} | 2.45 ± 0.11 ^{Aa} |
| | RBO | 2.86 ± 0.39 ^{Aa} | 2.89 ± 0.52 ^{Aa} |
| | PO | 2.86 ± 0.44 ^{Aa} | 2.42 ± 0.69 ^{Aa} |
| ESI | SBO | 59.76 ± 1.02 ^{Bb} | 62.28 ± 0.61 ^{Ba} |
| | RBO | 61.19 ± 0.25 ^{Ab} | 65.19 ± 1.18 ^{Aa} |
| | PO | 60.37 ± 2.64 ^{Bb} | 61.21 ± 0.18 ^{Bab} |
| ζ -potential | SBO | -20.34 ± 1.52 ^{Aa} | -22.29 ± 1.72 ^{Ab} |
| | RBO | -19.39 ± 1.07 ^{Aa} | -23.07 ± 2.06 ^{Ab} |
| | PO | -20.37 ± 1.60 ^{Aa} | -21.44 ± 2.68 ^{Ab} |

Means ± standard deviation (n=3) were shown.

In each tested parameter, different capital letters in the same column indicate significant difference between means ($P < 0.05$). Different small letters in the same row indicate significant difference between means ($p < 0.05$).

could improve emulsion formability of the system. Interaction between RBPH and phenolic compounds present in RHPE might take place when RHPE at sufficiently high level was present. The phenolics predominantly existed in RHPE were p-coumaric and vanillic acids (Cheetangdee and Benjakul, 2016). In the presence of phenolics, the protein films surrounding oil droplets might be formed at a faster rate. Improvement on interfacial activity of β -lactoglobulin could be achieved by interacting with phenolics, i.e., syringic acid, tyrosol, and oleuropein (Maldonado-Valderrama *et al.*, 2010). With amphiphilic characteristic, some phenolic compounds, e.g., gallic acid, catechin, quercetin and oleuropein, could be accumulated at the oil-water interfaces and facilitated a reduction of interfacial tension, thereby enhancing emulsion formation (Di Mattia *et al.*, 2010, 2011). RHPE addition at different concentrations had no significant effect on droplet size of the emulsions ($p > 0.05$). Nonetheless, the improved colloidal stability could be observed for the emulsions incorporated with RHPE at the level of 3%, as indicated by the increased ESI ($p < 0.05$). However, there was no difference in ESI when varying RHPE levels (1–3%) were used ($p > 0.05$). Thus, phenolics in RHPE were likely strengthened the films of RBPH surrounding the oil droplets, regardless of levels of phenolics used in the present study. Increased net charge on drop surfaces of the

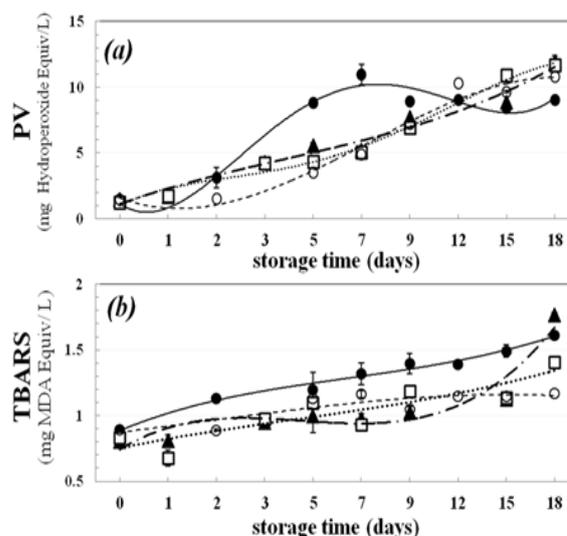


Figure 1. PV (a) and TBARS (b) of the RBPH stabilized SBO emulsions during the storage of 18 days. The emulsions contained no antioxidant (—●—) and RHPE at 1% (---▲---), 2% (---□---), and 3% (---○---). Bars represent the standard deviation (n=3).

emulsion was found, when 3% RHPE was added ($p < 0.05$). Increased surface charge of oil droplets could facilitate emulsion dispersibility through electrostatic repulsive force (Jumaa and Müller, 1998; Cheetangdee *et al.*, 2011). Colloidal stability of whey protein stabilized emulsions could be improved by adding phenolic rich-black berry and raspberry extracts (Viljanen *et al.*, 2005). Increasing negative charge of emulsion implied that the phenolics might modify RBPH structure, in which negatively charged domains were more exposed. Phenolics could interact with nucleophilic amino groups via both covalent and non-covalent bonds, resulting in a structural alteration of proteins (Kroll *et al.*, 2003; Aewsiri *et al.*, 2009). It was noted that the increased negative charge of the emulsions added with 3% RHPE was coincidental with the significantly higher ESI of the emulsions.

Effect of RHPE at different concentrations on the oxidative stability of SBO emulsions stabilized by RBPH was investigated by measuring PV and TBARS at various storage times (Figure 1). For the control (emulsion without RHPE), PV increased continuously and reached to the plateau at day 7, and then slightly declined. Reduce in PV after reaching to a maximum content could be attributed to a decomposition of hydroperoxides into the secondary products of the oxidative reaction (Osborn and Akoh, 2004). For the RHPE added emulsions, PV continuously increased through 18 days. Incorporation of RHPE at 2 and 3% led to the lowered TBARS, compared to the control emulsion, whereas the emulsion added with 1%

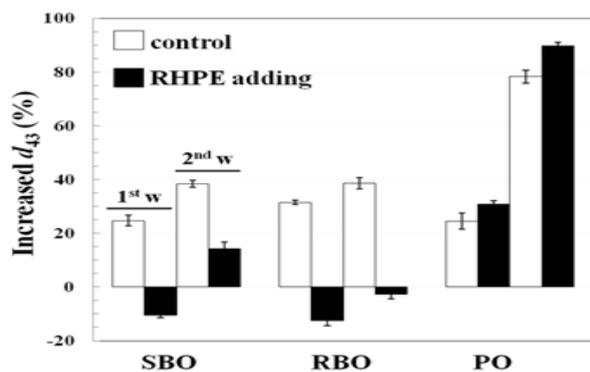


Figure 2. Percentage of droplet size increase of the emulsions after keeping for 1 and 2 weeks. The emulsions were stabilized by RBPH and made from different types of oils. Bars represent the standard deviation ($n=3$)

RHPE showed a comparable TBARS to the control at the end of storage. The results suggested the higher oxidative stability of the emulsions in the presence of RHPE at 2 and 3%, which was more likely due to free radical scavenging and reducing abilities of RHPE (Cheetangdee and Benjakul, 2016). Efficiency of RHPE in prevention of lipid oxidation in emulsion model was demonstrated (Cheetangdee and Benjakul, 2016).

In the present study, RHPE at 3% led to the improved colloidal and oxidative stability of the SBO emulsions. Therefore, physicochemical stability of the emulsions prepared using different types of oil was further investigated in the presence of 3% RHPE.

Effects of RHPE on stability of RBPH based emulsions made from different oils

Table 2 shows colloidal properties of the emulsions made from different types of oils as affected by 3% RHPE addition. Emulsions made from different oils exhibited dissimilar characteristics. Higher EAI was observed when the emulsions were prepared using RBO and PO, compared to that of SBO ($p<0.05$). The presence of some surface active constituents in RBO and PO might promote emulsion formability. Increasing EAI was obtained when RHPE was incorporated, irrespective of oil types ($p<0.05$). Properties of oils employed as a dispersed phase had a crucial role in characteristics of emulsion. Chung *et al.* (2001) suggested that the oil with higher interfacial tension could provide the emulsion with initially smaller sized oil droplets and greater stability, compared to the lower ones. In the present study, a similar initial droplet size of the emulsions made from different kinds of oil was noticeable ($p>0.05$), but the colloidal stability was varied. Higher ESI was found for the RBO emulsions than those observed for the SBO and PO counterparts

($p<0.05$). RHPE significantly increased ESI of SBO and RBO emulsions ($p<0.05$), which was coincidental with the increasing negative charge on surfaces of SBO and RBO droplets ($p<0.05$). Nonetheless, RHPE had no effect on the ESI and the charge of PO emulsions ($p>0.05$). No difference in ζ -potential values of the emulsions made from different kinds of oils was observed ($p>0.05$), regardless of RHPE incorporation.

Colloidal stability of the emulsions was further evaluated by measuring the change in droplet size as a function of storage time. The increases in d_{43} of the emulsions after storage for 1 and 2 weeks are shown in Figure 2. For the emulsions made from SBO and RBO, improvement on drop dispersibility could be achieved by adding RHPE, as indicated by the markedly lowered % increased d_{43} , compared to the control over 2 weeks of storage. However, RHPE incorporation led to obviously increased droplet size for the PO emulsion. Variation in properties of the oils employed as a dispersed phase could influence the characteristic of emulsion. Considering on the predominant fatty acid composition of the used oils, linoleic acid with the content of 53.7% of total fatty acids (TFA) was reported for SBO (Kamal-Eldin and Andersson, 1997). Oleic and linoleic acids with the contents of 40% and 40% TFA were found in RBO (Rukmini and Raghuram, 1991), and palmitic and stearic acids with the contents of 44.8% and 38.9% TFA were constituted in PO (Kamal-Eldin and Andersson, 1997). Among all oils, PO showed the highest saturation degree. It has been previously suggested that larger sized and irregularly shaped oil droplets tended to be formed in the emulsions made from the oil with higher saturation degree (Ahmad *et al.*, 1996). In the presence of RHPE, the emulsions made from RBO showed a higher stability, compared to those of SBO and PO, as indicated by no droplet size increment throughout 2 weeks (Figure 2) and higher ESI (Table 2). RBO contains phytosterols, especially for γ -oryzanol (Lilitchan *et al.*, 2008). γ -oryzanol consists of ferulic acid esterified to a sterol backbone, and is amphiphilic in nature (Nyström *et al.*, 2005). Sterol backbone of γ -oryzanol could bind to proteins via hydrophobic interaction (Wan *et al.*, 2014) and might enhance emulsion stability. Synergistic effect between soy protein isolate (SPI) and stevioside, the monosaccharide moieties esterified to a steviol backbone, to lower oil–water interfacial tension was reported and attributed to a formation of the SPI–stevioside complex at the interfaces (Wan *et al.*, 2014). Moreover, binding of stevioside to SPI could promote partial dissociation and loosen the rigidity of protein structure, leading to improve emulsifying

ability of SPI (Wan *et al.*, 2014). Interaction between stevioside (0.25–1%) and SPI resulted in a formation of viscoelastic interfacial films of the SPI–stevioside complex (Ruíz-Henestrosa *et al.*, 2008), and enhanced emulsion dispersibility throughout 120 days (Wan *et al.*, 2014). Interfacial and emulsifying activities of β -lactoglobulin (Piotrowski *et al.*, 2012) and β -casein (Wojciechowski *et al.*, 2014) could also be improved by interacting with saponin.

Degree of lipid oxidation occurring in the emulsions made from different oils was monitored by measuring PV and TBARS at various storage times (Figure 3), in comparison with that containing BHA. Irrespective of oil types, oxidative stability of the emulsions could be improved by incorporating RHPE. No change in PV was found for the RHPE incorporated emulsions within the first 2 days, whereas PV increased initially for the control (emulsion without antioxidants). Moreover, the lowered TBARS could be observed for the RHPE added emulsions, compared to the control emulsion during 18 days of storage ($p < 0.05$). It was noted that the emulsion added with BHA showed the lowest PV throughout the storage of 18 days.

Without RHPE addition, the maximum PV was observed after keeping for 7, 9, and 12 days for the SBO, PO, and RBO emulsions, respectively. Higher TBARS was observed for the SBO emulsions (ca. 1.75 mg MDA Equiv/L), compared to those of RBO and PO emulsions (ca. 1.5 mg MDA Equiv/L). The results suggested the highest oxidation of SBO emulsion, associated with the polyunsaturated fatty acids abundantly present in SBO (McClements and Decker, 2000; Chotimakorn and Silalai, 2008). Greater oxidative stability of RBO might be expected due to the presence of γ -oryzanol. Effective antioxidant activity of γ -oryzanol occurred through hydrogen donating ability of the phenolic group of ferulic acid (Nyström *et al.*, 2005). The effective capacity of γ -oryzanol to inhibit lipid oxidation was confirmed in several oil containing models (Xu *et al.*, 2001; Nyström *et al.*, 2005). At a final stage of storage, the lowest TBARS was found for the RHPE added emulsions. The secondary products of lipid oxidation, especially for aldehydes, have low thresholds, and are thus largely responsible for detectable off-odor (Martín *et al.*, 2000). RHPE, therefore, might be potently used to improve oxidative stability and extend shelf-life of emulsified products. Due to its lipophilicity, BHA prefers to situate in oil phase, and thus might not be able to act as antioxidant properly in emulsion system (Payato *et al.*, 2013). With several constituents, RHPE might localize in different phases of emulsion, thereby exhibiting efficient antioxidant

capacity in emulsion model (Tan and White, 1994). Higher ability of plant extracts, compared to BHA, to retard lipid oxidation in dispersion models was previously reported (Duh, 1999; Payato *et al.*, 2013; Cheetangdee and Benjakul, 2016).

Conclusion

Stability of the emulsions stabilized by RBPH was affected by RHPE and depended on the types of oil used as a dispersed phase. Generally, RHPE could enhance emulsion dispersibility by increasing negative charge on droplet surfaces. The emulsions made from RBO exhibited the highest colloidal and oxidative stability, when RHPE was added. Nonetheless, the dispersibility of PO emulsion was inferior in the presence of RHPE. The present work suggested that RBPH and RHPE, which were prepared from byproducts of rice milling process, might be a potential candidate to be used as a natural additive to enhance the stability of emulsified food products with the selected oil.

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References

- Aewsiri, T., Benjakul, S., Visessanguan, W., Eun, J. B., Wierenga, P. A. and Gruppen, H. 2009. Antioxidative activity and emulsifying properties of cuttlefish skin gelatin modified by oxidized phenolic compounds. *Food Chemistry* 117(1): 160–168.
- Ahmad, K., Ho, C. C., Fong, W. K. and Toji, D. 1996. Properties of palm oil-in-water emulsions stabilized by nonionic emulsifiers. *Journal of Colloid and Interface Science* 181(2): 595–604.
- Cheetangdee, N. 2014. Effects of rice bran protein hydrolysates on the physicochemical stability of oil-in-water emulsions. *Journal of Oleo Science* 63(12): 1231–1241.
- Cheetangdee, N. and Benjakul, S. 2015. Antioxidant activities of rice bran protein hydrolysates in bulk oil and oil-in-water emulsion. *Journal of the Science of Food and Agriculture* 95(7): 1461–1468.
- Cheetangdee, N. and Benjakul, S. 2016. Oxidation and colloidal stability of oil-in-water emulsion as affected by pigmented rice hull extracts. *Journal of the American Oil Chemists' Society* 93: 519–529.
- Cheetangdee, N., Oki, M. and Fukada, K. 2011. The coalescence stability of protein-stabilized emulsions estimated by analytical photo-centrifugation. *Journal of Oleo Science* 60(8): 419–427.
- Chotimarkorn, C. and Silalai, N. 2008. Addition of rice bran oil to soybean oil during frying increases the oxidative

- stability of the fried dough from rice flour during storage. *Food Research International* 41(3): 308–317.
- Chung, H., Kim, T. W., Kwon, I. C. and Jeong, S. Y. 2001. Oil components modulate physical characteristics and function of the natural oil emulsions as drug or gene delivery system. *Journal of Control Release* 71(3): 339–350.
- Di Mattia, C. D., Sacchetti, G., Mastrocola, D., Sarker, D. K. and Pittia, P. 2010. Surface properties of phenolic compounds and their influence on the dispersion degree and oxidative stability of olive oil O/W emulsions. *Food Hydrocolloids* 24(6–7): 652–658.
- Di Mattia, C., Sacchetti, G. and Pittia, P. 2011. Interfacial behavior and antioxidant efficiency of olive phenolic compounds in O/W emulsions as affected by surface active agent type. *Food Biophysics* 6(2): 295–302.
- Duh, P. D. 1999. Antioxidant activity of water extract of four Harnng Jyur (*Chrysanthemum morifolium Ramat*) varieties in soybean oil emulsion. *Food Chemistry* 66(4): 471–476.
- Hu, M., McClements, D. J. and Decker, E. A. 2004. Antioxidant activity of a proanthocyanidin-rich extract from grape seed in whey protein isolate stabilized algae oil-in-water emulsion. *Journal of Agricultural and Food Chemistry* 52(16): 5272–5276.
- Jumaa, M. and Müller, B. W. 1998. The effect of oil components and homogenization conditions on the physicochemical properties and stability of parenteral fat emulsions. *International Journal of Pharmaceutics* 163(1–2): 81–89.
- Kamal-Eldin, A. and Andersson, R. 1997. A multivariate study of the correlation between tocopherol content and fatty acid composition in vegetable oils. *Journal of the American Oil Chemists' Society* 74(4): 375–380.
- Kroll, J., Rawel, H. M. and Rohn, S. 2003. Reactions of plant phenolics with food proteins and enzymes under special consideration of covalent bonds. *Food Science and Technology Research* 9(3): 205–218.
- Lilitchan, S., Tangprawat, C., Aryasuk, K., Krisnangkura, S., Chokmoh, S. and Krisnangkul, K. 2008. Partial extraction method for the rapid analysis of total lipids and γ -oryzanol contents in rice bran. *Food Chemistry* 106(2): 752–759.
- Maldonado-Valderrama, J. and Rodríguez Patino, J. M. 2010. Interfacial rheology of protein-surfactant mixtures. *Current Opinion in Colloid and Interface Science* 15(4): 271–282.
- Martín, L., Timón, M. L., Petrón, M. J., Ventanas J. and Antequera T. 2000. Evolution of volatile aldehydes in Iberian ham matured under different processing conditions. *Meat Science* 54: 333–337.
- McClements, D. J. and Decker, E. A. 2000. Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. *Journal of Food Science* 65(8): 1270–1282.
- Official of Agricultural Economics (OAE), Ministry of Agriculture and Cooperative, Thailand. (June 2015) Thailand Foreign Agricultural Trade Statistics. Retrieved on February 15, 2016 from OAE Website: www.oae.go.th/download/journal/2558/thailandtradestat2557.pdf
- Nyström, L., Mäkinen, M., Lampi, A. M. and Piironen, V. 2005. Antioxidant activity of steryl ferulate extracts from rye and wheat bran. *Journal of Agricultural and Food Chemistry* 53(7): 2503–2510.
- Osborn, H. T. and Akoh, C. C. 2004. Effect of emulsifier type, droplet size, and oil concentration on lipid oxidation in structured lipid-based oil-in-water emulsions. *Food Chemistry* 84(3): 451–456.
- Payato, C., Navarro-Blasco, I., Calvo, M. I., Cavero, R. Y., Astiasarán, I. and Ansorena, D. 2013. Oxidative stability of O/W and W/O/W emulsions: Effects of lipid composition and antioxidant polarity. *Food Research International* 51(1): 132–140.
- Pearce, K. N. and Kinsella, J. E. 1978. Emulsifying properties of proteins: evaluation of a turbidimetric technique. *Journal of Agricultural and Food Chemistry* 26(3): 716–723.
- Piotrowski, M., Lewandowska, J. and Wojciechowski, K. 2012. Biosurfactant-protein mixtures: Quillaja bark saponin at water/air and water/oil interface in presence of β -lactoglobulin. *The Journal of Physical Chemistry B* 116(16): 4843–4850.
- Rao, J. and McClements, D. J. 2012. Impact of lemon oil composition on formation and stability of model food and beverage emulsions. *Food Chemistry* 134(2): 749–757.
- Ruíz-Henestrosa, V. P., Carrera Sánchez, C. and Rodríguez Patino, J. M. 2008. Adsorption and foaming characteristics of soy globulin and Tween 20 mixed systems. *Industrial and Engineering Chemistry Research* 47(9): 2876–2885.
- Rukmini, C. and Raghuram, T. C. 1991. Nutritional and biochemical aspects of the hypolipidemic action of rice bran oil: A review. *Journal of the American College of Nutrition* 10(6): 593–601.
- Tian, L. L. and White, P. J. 1994. Antioxidant activity of oat extract in soybean and cotton seed oils. *Journal of the American Oil Chemists' Society* 71(10): 1079–1086.
- Viljanen, K., Halmos, A. L., Sinclair, A. and Heinonen, M. 2005. Effect of blackberry and raspberry juice on whey protein emulsion stability. *European Food Research and Technology* 221: 602–609.
- Wan, Z. L., Wang, L. Y., Wang, J. M., Zhou, Q., Yuan, Y. and Yang, X. Q. 2014. Synergistic interfacial properties of soy protein–stevioside mixtures: Relationship to emulsion stability. *Food Hydrocolloids* 39: 127–135.
- Wojciechowski, K., Kezwon, A., Lewandowska, J. and Marcinkowski, K. 2014. Effect of β -casein on surface activity of Quillaja bark saponin at fluid/fluid interfaces. *Food Hydrocolloids* 34: 208–216.
- Xu, Z., Hua, N. and Godber, S. 2001. Antioxidant activity of tocopherols, tocotrienols, and γ -oryzanol components from rice bran against cholesterol oxidation accelerated by 2'-azobis (2-methylpropionamide) dichloride. *Journal of Agricultural and Food Chemistry* 49(4): 2077–2081.