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Original research article

Enhanced Physical Stability of Rice Bran Oil-in-Water Emulsion by Heat and Alkaline Treated Proteins from Rice Bran and Soybean

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ABSTRACT

The aim of this work was to improve physical stability of rice bran oil-in-water emulsion by heat and alkaline treated proteins from rice bran and soybean. Rice bran protein (RBP) was extracted from defatted rice bran by alkaline extraction and isoelectric precipitation. RBP and soy protein (SP) were modified by heat and alkaline treatment (pH 9 at 60 °C for 60 min). The ability of modified rice bran protein (MRBP) and modified soy bean protein (MSP) to stabilize rice bran-oil-in-water emulsion was investigated. Results showed that the MRBP and MSP to form and stabilize oil-in-water emulsions were better than those of RBP and SP. Emulsions with small particle sizes diameter and creaming stability could be produced at pH 6.5 for 0.4-1.0 %wt MRBP and 0.6-1.0 %wt MSP. Improved physical stability of rice bran oil-in-water emulsion by heat and alkaline treated will enhance the utilization RBP and SP as food ingredient in the food industry.

Keywords: Rice bran protein; Soy protein; Modified protein; Emulsifying properties

1. Introduction

Rice (*Oryza sativa* L.) is one of the most staple all over the world. In 2015, about 27.42 million tons of paddies were produced in the Thailand, which generate 2-3 million tons of rice bran as a by-product each year [1]. Rice bran has high nutritional value with 15% protein content [2]. RBP is higher in lysine content than rice endosperm protein or any other cereal bran protein [3]. Protein digestibility of rice bran is greater than 90%. It is considered a good source of hypoallergenic protein [4]. However, emulsifying properties of RBP could not form and stabilize emulsion. Therefore, emulsifying properties of RBP could be improved by heat and alkaline treatment. Soy protein (SP) has been widely applied in

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the food industry as an important ingredient due to their highly nutritious and desirable functional properties [5]. The proximate composition of soybeans around 40% protein, 35% carbohydrate, 20% lipids and 5% ash [6]. However, in the application of the SP is limited due to incompatibility between their solubility and other properties e.g. emulsifying activities. Thus, soy proteins were hydrolyzed may be provide improved functional properties. The objective of this study was to improve physical stability of rice bran oil-in-water emulsion by heat and alkaline treated proteins from rice bran and soybean.

2. Materials and Methods

2.1 Materials

Rice bran (RB) was obtained from Sin Rungrueang Phokhaphan Co., Ltd. RB had a composition of 12.49 %wt protein, 14.99 %wt fat, 6.81 %wt ash, 5.41 %wt fiber and 62.36 %wt carbohydrate. Soy protein (SP) was purchased from Thai Food and Chemical Co., Ltd.

2.2 Preparation of rice bran protein

The RB was defatted using hexane (1:9 %w/v) by stirring at room temperature for 1 h. The defatted rice bran was dispersed in distilled water (1:10). The mixture was adjusted to pH 9.0 with 4.0 N NaOH, stirred for 1 h to extract the protein, and then centrifuged for 15 min at 28,313 x g at 25 ºC to remove the insoluble materials. The protein in the supernatant was adjusted to pH 4.5 with 4.0 N HCl for 30 min and centrifuged for 15 min at 28,313 x g at 25 ºC in order to precipitate the protein. The protein slurry was neutralized to pH 7.0. The RBP sample was cooled and dialyzed against distilled water at 4 °C and freeze-dried. The freeze-dried RBP and SP were analyzed for the content of protein, fat, moisture, ash and fiber using AOAC (1999) [7].

2.3 Preparation of modified rice bran protein (MRBP) and modified soy bean protein (MSP)

RBP and SP were dispersed in distilled water (10 %w/v) and adjusted to pH 9 with 1 N NaOH. The proteins dispersion was stirred for 30 min at room temperature, heated at 60 °C for 60 min and then cooled to 30 °C with tap water. The MRBP and MSP were neutralized to pH 7 with 1 N HCl.

2.4 Degree of hydrolysis (DH)

The DH was determined by measuring the soluble protein content in 10% trichloroacetic acid (TCA) according to the modified method of Qi et al. (1997) [8]. An aliquot (15 ml) of an aqueous dispersion of protein hydrolyte was mixed with 15 ml of 20% TCA and centrifuged for 15 min at 7,600 x g at 25ºC. The protein content of supernatant was determined by Biuret method [9]. The DH was calculated as follow: DH (%) = (protein content of hydrolyte after TCA precipitation / total protein) x 100.

2.5 Protein solubility (PS)

Protein solubility of RBP and SP were prepared according to the modified method of Tsumura et al. (2005) [10]. Protein dispersion (0.2 %w/v) was made with distilled water, stirred for 1 h with either 2 N HCl or 2 N NaOH to adjust the various pH values ranging from 3 to 9. The sample solutions were centrifuged at 21,677 x g for 10 min. The protein content of the filtered supernatant was determined by the Biuret method [9]. The PS was calculated using the following expression: PS = 100 x PS/PT, where PS is the protein content remaining in the supernatant after centrifugation and filtration, and PT is the total protein content present in the original solution.

2.6 Emulsion preparation

Protein solutions of RBP, SP, MRBP and MSP were prepared according to the modified method of Onsaard et al.

(2006) [11]. Aliquots (0.2-1.0 %w/v) of the protein samples were dispersed into buffer solution (5 mM phosphate buffer, pH 6.5, 0.02 %wt sodium azide), and the stirring for 60 min at room temperature. Oil-in-water emulsion samples were prepared by blending 10 %wt rice bran oil and 90% protein solution of various concentration using a high-speed blender for 2 min (T25 digital ultra turrax, IKA, Germany) These emulsion were homogenized twice through a high-pressure homogenizer (SPX Lab Homogenizer APV-2000, UK) at 3,000 psi at room temperature for 5 min.

2.7 Creaming stability measurements

Creaming stability measurement was prepared according to the method Onsaard et al. (2006) [11]. Emulsion samples (10 g) were transferred into a test tube (15 mm diameter and 125 mm height) and sealed with aluminum foil to stand at room temperature for 24 h. A number of emulsions separated into an opaque layer "cream" at the top and a slightly turbid or transparent layer "serum" at the bottom. The creaming was calculated from creaming index = 100 x (HS/HE) where HS is the height of the lower serum layer and HE is the total height of the emulsions in the tube.

2.8 Particle size determination

The particle size of emulsion oil droplet was determined using a laser light scattering instrument (Zetasizer, ZEN3600, Malvern Instruments, Worcestershire, UK.) Sample emulsions were appropriately 1,000-fold diluted with phosphate buffer solution (pH 6.5) to avoid multiple scattering. A refractive index ratio of 1.467 was used in the calculation of the particle size. Particle sizes of the emulsions were reported as the average diameter of particles. All measurements were made on at least two freshly prepared samples, and the results were reported as the mean and standard deviation.

2.9 Zeta-potential measurements

Zeta-potential of oil droplet in the emulsion sample was measured using the dynamic light scattering (Zetasizer, ZEN3600, Malvern Instruments, Worcestershire, UK.). The sample emulsions were 1000-fold diluted with phosphate buffer solution (pH 6.5) for preventing multiple scattering effects. All measurements were made on at least two freshly prepared samples, and the results were reported as the mean and standard deviation.

2.10 Optical microscopy

The sample emulsions structure was observed using a conventional optical microscope (Nikon microscope Eclipse E-400, Nikon Corporation, Japan) and recorded by a digital camera linked to imaging software installed on a computer.

2.11 Statistical analysis

All experiments were conducted in three replicates. Averages and standard deviations were reported. Data were analyzed by the analysis of variance (ANOVA) at $P < 0.05$ using SPSS software version 10.

3. Results and Discussion

3.1 Proximate composition

The proximate composition of RBP and SP is presented in Table 1. The protein and moisture content were higher in SP than in RBP, but the fat, ash, crude fiber and carbohydrate content were lower in SP than in RBP. These differences in composition could partly account for differences in the observed emulsifying properties and stability of O/W emulsions, and which also affect their functional properties.

Table 1. Proximate composition of rice bran protein (RBP) and soy protein (SP)

Chemical composition	RBP	SP
Moisture	1.48±0.52	14.14±0.04
Protein[a]	76.09±0.80	80.52±0.26
Fat	7.92±0.70	0.10±0.02
Ash	5.25±0.76	4.25±0.03
Crude fiber	0.63±0.14	0.48±0.18
Carbohydrate[b]	8.63±0.27	0.51±0.11

Note: [a]6.25 was use as the nitrogen conversion factor. [b]estimated by difference.

3.2 Degree of hydrolysis (DH)

The DH of the MRBP and MSP was examined by using 10% TCA. After modified by heat and alkaline treatment, it was clearly observed that the hydrolysis of MRBP and MSP slightly increased (range of 0.43-2.16% for MRBP and 0.22-0.64% for MSP) (Table 2). This result indicated a decrease in peptide bonds available for heat and alkaline treatment. The DH of MRBP at all protein concentrations was higher than those of MSP. This result may be related to the different protein solubility of RBP and SP (Fig.1). The solubility of RBP was higher than SP so that the higher solubility of RBP may be easier hydrolysis of susceptible amide and peptide bonds. The alkali can cause changes such as the hydrolysis of such as the hydrolysis of susceptible amide and peptide bonds, racemization of amino acid, splitting of disulfides [12].

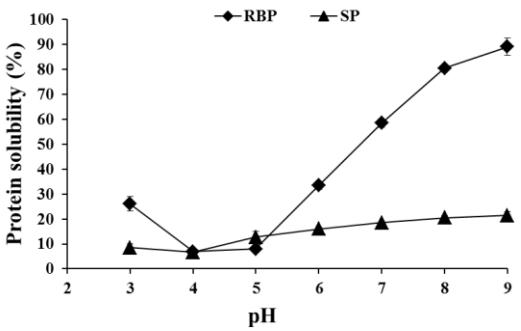

Fig. 1. Protein solubility profiles of rice bran protein (RBP) and soy protein (SP) at different pH values.

3.3 Protein solubility

The protein solubility of RBP and SP increased with an increase in alkalinity and acidity is shown in Fig 1. RBP and SP solubility showed a minimum solubility at around pH 4-5, which is close to isoelectric points (pI) of this protein [2, 10]. The lower solubility (pH = pI) may have arisen from an increase in exposed hydrophobic residues, leading to hydrophobic interactions between surface nonpolar patches are maximum and the electrostatic repulsion and ionic hydration are minimum [13]. On the other hand, when the pH value was over 5 or below 4 had protein solubility increased. The increase of protein solubility could be at extremely acidic and alkaline, proteins carry net positive and negative charges, respectively, and thus electrostatic repulsion and ionic hydration promoted the solubilization of the protein [14]. The solubility of RBP at pH>5 or pH<4 showed higher solubility than SP. Due to different structures of these proteins decides the different emulsifying ability. The solubility is one of the most important characteristic of proteins, as it influences on the emulsion properties [15].

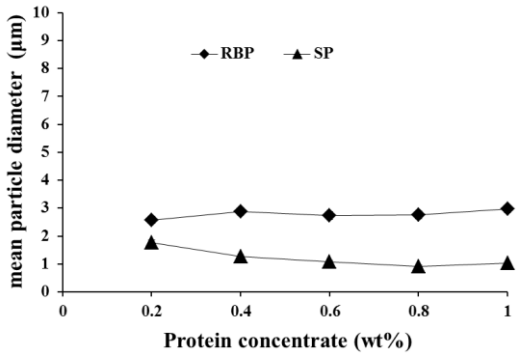

Fig. 2. Particle size diameter of rice bran protein (RBP) and soy protein (SP) prepared by 10 %wt rice bran oil-in-water emulsion stabilized at various concentration values at pH 6.5 measured after 24 h storage at room temperature.

3.4 Influence of protein concentration on emulsion formation and stability

The influence of protein concentration on mean particle diameter, zeta-potential and creaming stability of 10 %wt rice bran oil- in-water emulsion stabilized by 0.1-1.0 %wt RBP and SP at pH 6.5 was measured 24 h after homogenization. The mean particle of the SP emulsions were smaller (<2 μm) than that of the RBP emulsions (>2 μm) at all protein concentrations. This result indicated that emulsion with smaller droplet size could be produced using SP than by using RBP (Fig.2). The zeta-potential of the SP emulsions (-51 to -43 mV) were less negative charges than that of the RBP emulsions (-34 to -24 mV) at all protein concentrations (Fig. 3).

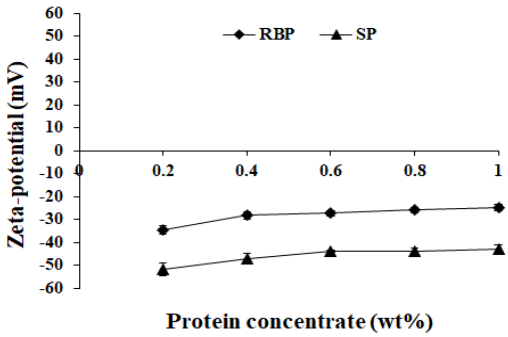

Fig. 3. Zeta-potential of rice bran protein (RBP) and soy protein (SP) prepared by 10 %wt rice bran oil-in-water emulsion stabilized at various concentration values, pH 6.5 measured after 24 h storage at room temperature.

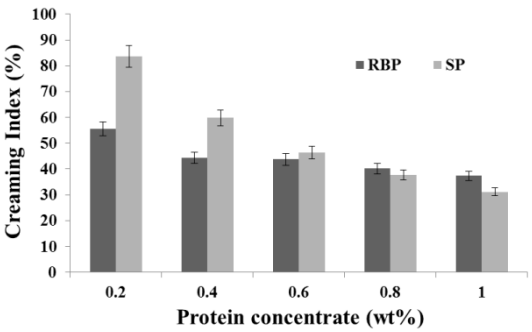

Fig. 4. Creaming stability of rice bran protein (RBP) and soy protein (SP) prepared by 10 %wt rice bran oil-in-water emulsion stabilized at various concentration values, pH 6.5 measured after 24 h storage at room temperature.

The emulsion stability of RBP and SP emulsions was determined using creaming index and visual observation (Fig. 4 and 5). The creaming index of the emulsions stabilized by RBP was less than stabilized by SP at 0.2-0.4 %wt, and non-significant with protein concentration increase (Fig 4). The visual observation showed that the RBP and SP emulsions were unstable to creaming, forming a turbid serum layer at the bottom and an opaque creamed layer at the top (Fig 5). This result suggests that the emulsions stabilized by all protein are unable to prevent droplets aggregation, because the both RBP and SP emulsions have large particle size with all protein concentrations. The observed differences in the stability of RBP and SP emulsions to droplet sizes, charges and creaming could be attributed to the influence of the proteins on the colloidal interactions between the droplets. The SP emulsions seem that the electrical charges on the emulsion droplets are better prevent droplet flocculation than the RBP emulsions, which may be attributed to the fact that there is high electrical charge on the droplets compared to RBP.

3.5 Influence of modified proteins by heat and alkaline treatment on emulsion stability

The influence of modified proteins (60ºC for 60 min at pH 9) on mean particle diameter, zeta-potential, creaming stability and microstructure of 10 %wt rice bran oil-in-water emulsion stabilized by 0.1-1.0 %wt MRBP and MSP at pH 6.5 was measured after stored overnight at room temperature. The mean particle diameter of MRBP was relatively high (>2 μm) at protein concentration rate during the 0.2 %wt to 0.6 wt% and decreased when the protein concentration increase, while MSP was relatively high (>2 μm) at protein

concentration is 0.2 %wt, 0.8 %wt and 1.0 %wt respectively and decreased (<2 μm) at protein concentration rate between 0.4%wt to 0.6 %wt, which indicated that mean particle diameter of the both proteins were influence by heat and alkaline treatment caused to decrease in molecular size due to the peptide bond is destroyed and in accordance with the DH of the data (Table. 2).

The protein concentration of the zeta-potential of the droplet in the MRBP emulsion was compared with that of a MSP emulsion (Table. 2). The zeta-potential of the both droplets in the MRBP and MSP emulsion was highly negative at all protein concentration, become less negative with increasing protein concentration (pH 6.5). This indicates that the zeta-potential of the droplets in the both emulsion were influence of electrical charges between the droplets.

In the MRBP emulsion, there was some droplet aggregation and creaming at relatively low protein concentration (0.2 %wt), which was relatively stable to droplet aggregation and creaming at higher protein concentration (>0.2 %wt). On the other hand, the MSP emulsion were relatively stable to droplet aggregation and creaming at higher protein concentration than MRBP (>0.4 %wt) (Fig.6) which indicate that the stable MRBP and MSP emulsion was found when proteins were used in range of 0.4-1.0 %wt and 0.6-1.0 %wt, respectively.

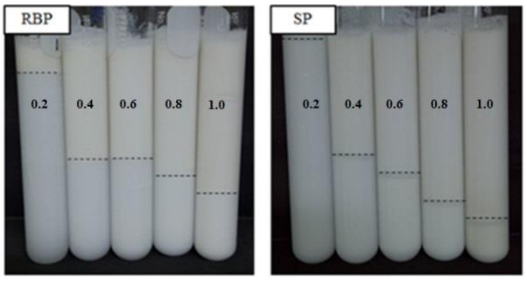

Fig. 5. Visual observation of rice bran protein (RBP) and soy protein (SP) prepared by 10 %wt rice bran oil-in-water emulsion stabilized at various concentration values, pH 6.5 measured after 24 h storage at room temperature.

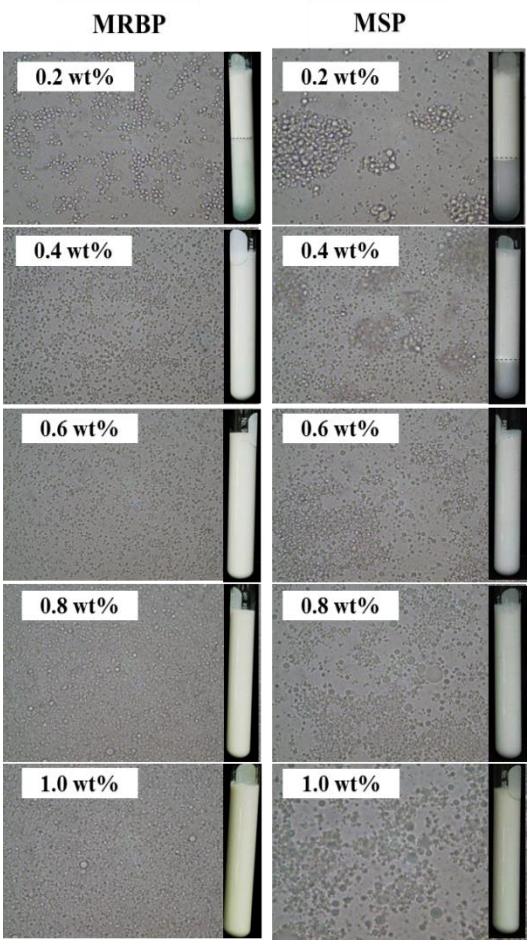

Fig. 6. Photomicrographs of modified rice bran protein (MRBP) and modified soy protein (MSP) prepared by 10 %wt rice bran oil-in-water emulsion stabilized at various concentration values, pH 6.5 measured after 24 h storage at room temperature.

Table 2. Changes in the degree of hydrolysis (DH), particle size diameter and zeta-potential of modified rice bran protein (MRBP) and modified soy protein (MSP) prepared by 10 %wt rice bran oil-in-water emulsion stabilized at various concentration values at pH 6.5

Conc. Protein (%wt)	DH (%)		particle size diameter (μm)		zeta- potential (mV)	
	MRBP	MSP	MRBP	MSP	MRBP	MSP
0.2	0.43±0.17^{b}	0.22±0.17^{a}	5.30±1.16^{a}	2.59±0.10^{ab}	-36.60±3.68^{ab}	-40.77±1.37^{c}
0.4	0.63±0.30^{b}	0.42±0.34^{a}	3.42±0.65^{a}	1.55±0.20^{b}	-37.23±1.89^{b}	-38.43±1.93^{bc}
0.6	0.92±0.52^{b}	0.64±0.21^{a}	2.77±1.06^{b}	1.53±0.12^{b}	-33.88±2.99^{ab}	-34.05±1.11^{ab}
0.8	2.16±0.96^{a}	0.53±0.18^{a}	1.72±0.08^{b}	2.38±1.03^{ab}	-29.93±0.66^{a}	-33.15±1.58^{a}
1.0	1.98±0.25^{a}	0.24±0.15^{a}	1.17±0.08^{b}	3.36±0.42^{a}	-30.53±2.12^{a}	-31.50±2.40^{a}

Note: The results are expressed as mean ± SD. (n=15)

This result suggests that there was sufficient protein to cover the surface of the droplets. The physical stability of MRBP and MSP was higher than that of RBP and SP, indicating that heat and alkaline treatment improved the stability of MRBP and MSP. The thermal treatment improved stability of emulsion because the protein unfolded and increased the surface hydrophobicity of the droplets [11, 16-18].

4. Conclusion

Emulsifying properties of RBP and SP can be improved by heat and alkaline treatments. This work has shown that MRBP and MSP could be used to prepare oil-in-water (o/w) emulsions using homogenization because these proteins enabled us to produce emulsions containing relatively small droplets and creaming stability. MRBP and MSP may be most effective at stabilizing emulsions that are fairly viscous e.g., deserts, yogurts and dressings.

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