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Functionality and Application of Rice Bran Protein Hydrolysates in Oil in Water Emulsions: Their Stabilities to Environmental Stresses

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***Abstract*—**Rice bran protein hydrolysates (RBPH) were prepared from defatted rice bran of two different Thai rice cultivars (Plai-Ngahm-Prachinburi; PNP and Khao Dok Mali 105; KDM105) using an enzymatic method. This research aimed to optimize enzyme-assisted protein extraction. In addition, the functional properties of RBPH and their stabilities to environmental stresses including pH (3 to 8), ionic strength (0 mM to 500 mM) and the thermal treatment (30 °C to 90 °C) were investigated. Results showed that enzymatic process for protein extraction of defatted rice bran was as follows: enzyme concentration 0.075 g/ 5 g of protein, extraction temperature 50 °C and extraction time 4 h. The obtained protein hydrolysate powders had a degree of hydrolysis (%) of 21.05% in PNP and 19.92% in KDM105. The solubility of protein hydrolysates at pH 4-6 was ranged from 27.28-38.57% and 27.60-43.00% in PNP and KDM105, respectively. In general, antioxidant activities indicated by total phenolic content, FRAP, ferrous ion-chelating (FIC), and 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) of KDM105 had higher than PNP. In terms of functional properties, the emulsifying activity index (EAI) was was 8.78 m^2/g protein in KDM105, whereas PNP was 5.05 m^2/g protein. The foaming capacity at 5 minutes (%) was 47.33 and 52.98 in PNP and KDM105, respectively. Glutamine, Alanine, Valine, and Leucine are the major amino acid in protein hydrolysates where the total amino acid of KDM105 gave higher than PNP. Furthermore, we investigated environmental stresses on the stability of 5% oil in water emulsion (5% oil, 10 mM citrate buffer) stabilized by RBPH (3.5%). The droplet diameter of emulsion stabilized by KDM105 was smaller (d < 250 nm) than produced by PNP. For environmental stresses, RBPH stabilized emulsions were stable at pH around 3 and 5-6, at high salt (< 400 mM, pH 7) and at temperatures range between 30-50°C.

***Keywords*—**Functional properties, oil in water emulsion, protein hydrolysates, rice bran protein.

I. Introduction

RICE bran, a byproduct of rice milling with low value but high in bioactive compounds and nutraceuticals [1], [2]. In general, rice bran has been used for oil extraction, in animal feed and as a food [3]. Rice bran is a good source of γ-oryzanol, Inositol, campesterol, β-Sitosterol, and p-Coumaric acid [4]-[6] that attribute to the hypocholesterolemic effects [2]. The main proteins in rice bran are albumins (37%), globulins (36%), prolamins (5%) and glutelins (22%) [5], [7]. The recovery rice bran proteins have been reported using physical, chemical and enzymetic method. The size of the protein and amino acid arrangement of peptide molecules may have shown a variety of protein functionalities [5], [8].

Enzymatic extraction method have been used for recover protein from rice bran including improve the functional properties of protein such as emulsifying ability, foaming capacity, viscosity, gelatinization, and water absorption capacity [5], [8], [10]. Reference [9] reported that proteases were used to hydrolyze rice bran protein for better solubility and greater extractability. Two classes of protease, exoprotease and endoprotease can be utilized of rice bran protein yield about 60-93% and obtain a wide range of hydrolysates [9]. The use of endoprotease and exopeptidases has been studied in reference [8], [9], [11].

Khao Dok Mali 105 (KDM105) is one of the recommended commercial rice cultivars from Thailand. It has been distinctive aroma. Therefore it has been consumed by cooking. Whereas PNP is the traditional rice cultivars obtained from Prachinburi province, Thailand. It is commonly processed as a noodle for consume. In Thailand rice bran usually used as a source of proteins for animal feed.

To increase the value of rice bran and extend its application in food, this research aimed to optimize an enzymatic extraction condition in rice bran. The obtained hydrolyzed rice bran protein extracts was investigated on some physicochemical and functional properties including apply the RBPH in O/W emulsions and observed their stabilities to the pH, ionic strength and thermal stresses.

II. Materials and Methods

A. Raw Material and Chemicals

Full-fat rice bran, Khao Dok Mali 105 (KDM105) and PNP were obtained from Suan Dusit Rajabhat, Rice mill factory, Co Ltd. (Prachinburi, Thailand) and passed through a sieve (60 mesh).The defatted rice bran was prepared by solvent extraction in a Soxhlet apparatus using hexane. After completed removal of hexane, the defatted rice bran was stored in LDPE plastic bag under -18°C for further experiment. Chemicals and reagents of analytical grade were purchased from Univar (USA Inc., USA) and Merck

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(Darmstadt, Germany). Alcalase 2.4 L and Flavourzyme were donated by Novozymes (Novozymes A/S, Bagsvaerd, Denmark).

B. Preparation of RBPH

The protein extraction from rice bran was described as in [9]. Rice bran suspension (5.0 g proteins in 250 ml water) heated to 50°C, the pH adjusted to 8 then 0.025 g of Alcalase 2.4 L and Flavourzyme were added for proteolysis and inactivated by heating at 85°C for 10 minutes. The extraction conditions consisted of enzyme concentrations (0.025-0.075 gram/ 5 gram of protein), extraction times (2-4 h) and temperatures (40-60°C). The enzyme was added to rice bran suspension, then the mixture was stirred for 30 minutes and the pH was controlled during that extraction. The suspensions of rice bran were centrifuged (20°C at 5,000 rpm, 15 minutes) by refrigerated centrifuge (Rotana A35R, Hettich, Germany). Protein in the bran was recovered and washed three times with 100 ml water during the second and third centrifugation. Then evaporated (70°C, 120 mbar for 45 minutes) using a rotary evaporator (R-205, Buchi, Switzerland) and followed by freeze drying (-50°C and 0.11 mbar for 10-12 h.) using freeze dryer (Alpha 1-4 LSC, Christ, Germany). The obtained rice bran protein was packed in laminated aluminium bags and stored below 4°C prior to use for analysis.

C. Physical, Chemical and Functional Properties of RBPH

RBPH was measured for *protein* by the Kjeldahl method [12] and crude protein was calculated using the 5.95 conversion factor, *moisture content* according to the standard method of AOAC [12]; a_w was measured by Aqua lab (CX3TE, England) under 25°C, *color* using Hunter LAB (Color Flex 45/0, Color global, USA).

Bulk density was determined by the method as in [13]. RBPH were added into the graduated measuring cylinders. The bulk densities were calculated as weight per unit volume (g/ml).

Solubility of protein was measured the method as in [14]. Dispersed RBPH in deionized water 0.5 grams: 50 ml, and then shaken and centrifuged at 5000 X G for 15 minutes. The nitrogen content of the supernatant was determined by the Kjeldahl method [12] and solubility (%) was calculated as follows:

Solubility (%) = (protein content in supernatant/total protein content in sample) x 100

Degree of hydrolysis: Reactions were observed by measuring the amount of α-amino acid using a modified method [15], [16]. RBPH 100 µl was mixed with 2 ml of phosphate buffer 0.2125 M and 1 ml of 0.02% of 2,4,6-trinitrobenzenesulfonic acid (TNBS) in screw-cap test tube with adjusted pH 8.2 using NaOH/ or HCl. The suspension was heated at 50 °C for 30 minutes under dark condition, then 2 ml of 0.1 M sodium sulfite was add into the suspension and vortexed for 5 second to stop a reaction. Absorbance of the suspension was read at 420 mm using a UV-visible spectrometer (Thermo Electron Corporation, Waltham, MA). Alpha amino acid was expressed in terms of L-leucine. The DH (%) defined as follows:

DH (%) = (Number of peptide bonds cleaved after hydrolysis/ Total number of peptide bonds in sample before hydrolysis) x 100

Total phenolic content: The total phenolic content was determined using Folin-Ciocalteu method [17]. The reaction mixture contained 200 µl of RBPH solution, 150 µL of Folin-Ciocalteu reagent (1:10 v/v) and 1.2 ml of 7.5% $NaCO_3$. The mixture was incubated in the dark at room temperature for 30 minutes. Then measured at 765 nm. Distilled water was used as the control and gallic acid at 0-10 mg/ 100 ml.

FRAP: The assay was performed according to method of [18] with slightly modification. An amount of 30 µl extracted samples were mixed with 270 µl FRAP reagent in test tubes and undergoes vortex. Blank samples were prepared for both methanol and deionized water extracted samples. Samples and blank were incubated in dark room for 4 minutes at 37°C and determined against blank at 593 nm. Series of stock solution at 0 mg/100 ml, 1.0 mg/100 ml, 2.0 mg/100 ml, 3.0 mg/100 ml, 4.0 mg/100 ml, and 5.0 mg/100 ml were prepared using aqueous solution of ascorbic acid as standard curve. The values obtained were expressed as mg ASC/ g/ gram dried sample.

ABTS: The ABTS radical scavenging capacity of RBPH was evaluated described by [19]. Diluted sample with distill water 5 mg RBPH/ 1 ml of distill water. Pipette 20 µl of sample or standard solution (ascorbic acid) and mixed with ABRS solution 280 µL (7 mM of 2,2 Azino-bis (3-ethyl Benzothiazoline-6 sulfonic acid, K_2SO_4 140mM 179 µl kept dark at room temperature before used). The mixture was incubated in the dark at room temperature for 24 h. Then measured at 734 nm. The inhibition (%) was calculated below;

$$\text{Inhibition (\%)} = [(A_0-A_1)/A_0]x100$$

where A_0 is the sample absorbance or standard reagent at 0 mg/100 ml, A_1 is the sample absorbance or standard reagent at different concentrations.

FIC: Ferrous Ion-Chelating or FIC assay was determined followed [20]. The solution contained 5 mg of RBPH/ 1 ml distilled water. Pipette sample 100 µL, $FeSO_4$ 0.1 mM 100 µl, and ferric chloride 25 mM 100 µl. The mixture was shaken and incubated for 10 minutes in the dark at room temperature. The absorbance was measured at 562 nm. Distilled water was used as the control and EDTA was used as standard. The inhibition (%) was calculated as the equation below:

$$\text{Inhibition (\%)} = [1-(A_{sample1}-A_{control\ blank1})/(A_{sample0}-A_{control\ blank0})]x100$$

where $A_{sample0}$ is absorbance of sample/$FeSO_4$/Ferrozine at low conc.; $A_{control\ blank0}$ is absorbance of sample/$FeSO_4$/distill water at low conc., $A_{sample1}$ is absorbance of sample/$FeSO_4$/Ferrozine at low conc.; $A_{control\ blank1}$ is absorbance of sample/$FeSO_4$/distill water at low conc.

Foaming: Foaming capacity was measured by the method as in [21]. RBPH 2.0% w/v was dispersed in deionized water then adjusted pH to 7. The 20 ml of solution was homogenized in a mechanical homogenizer (ULTRA-TURRAX® T25 basic, IKA® WERKE, Germany) at 13,500 rpm for 3 minutes. The foaming capacity was calculated as follows:

Foaming capacity (%) = (Volume of foam after whipping/ Volume of foam before whipping) x 100

Emulsifying properties: The emulsifying properties of RBPH were determined as in [22]. Ratio between soybean oil: protein solution was 10:30 gram. The solution was mixed using homogenizer (ULTRA-TURRAX® T25 basic, IKA® WERKE, Germany) at 13,500 rpm for 1 minute. 50 µl of the emulsion was collected and mixed with 5 ml of 0.1% SDS solution using a vortex mixer. The absorbance at 0 (A_0) and 10 (A_{10}) was measured at 500 nm using a spectrophotometer (SP300, Optima, Japan). The EAI was calculated as;

EAI (m^2/ g protein) = (2 x 2.203 x A_0 x dilution factor)/(L x φ x C x 10,000)

where L = the patch length of cuvette (cm), φ = oil volume fraction of emulsion, C = weight of protein/ unit volume (g/ml) of aqueous phase before emulsion formation

Amino acid profiles: RBPH was analyzed for the amino acid profile by using in house method based on AOAC Official Method 994.12.988.15 (2000) detected by GC-MS.

D.Preparation of Oil in Water Emulsion and Measurement

Aqueous phases were prepared by dispersing RBPH 3.5% wt/wt of KDM105 and PNP in aqueous buffer solutions (10.0 mM sodium citrate) followed by stirring at room temperature overnight to ensure complete dispersion and hydration. Rice bran oil-in-water emulsions were prepared by homogenizing 5.0 wt% oil phase with 95.0 wt% aqueous phase at ambient temperature. An emulsion pre-mix was prepared using a high-speed blender (2 minutes, BioSpec Products Inc., Bartlesville, USA), which was then passed through a high pressure homogenizer (Model 101, Microfluidics, Newton, Massachusetts, USA) three times at 9,320 psi.

The stability of the emulsion to pH, ionic strength and temperature was tested. 5% wt oil-in-water emulsions were prepared using RBPH. *Stability to pH*: Emulsion samples were prepared in aqueous buffer solutions and then the pH was adjusted to pH 3 to pH 8 using NaOH and/or HCl solution, and then transferred into glass test tubes (160 x 15 mm). *Stability to ionic strength*: Emulsions (pH 7) were diluted with different amounts of NaCl and buffer solution to form a series of samples with same droplet concentration but different salt concentrations (0-500 mM NaCl). The emulsions were stirred for 30 minutes and transferred into glass test tubes (160 x 15 mm). *Stability to heating:* Emulsions (pH 7) were prepared and then 10 ml sample were transferred into glass test tubes, which were stored in a water bath for 30 minutes at fixed temperature ranging from 30°C to 90°C. The samples were stored at ambient temperature overnight prior to analysis.

Particle size and charge measurement: The particle size distribution (PSD) of the emulsions was measured using a laser light scattering instrument (MalvernSizer2000, Malvern Instruments Ltd., Worcestershire, UK). *ζ-potential:* The electrical charge (ζ-potential) of lipid droplets in the emulsions was determined using a particle electrophoresis instrument (ZEN3600, Nano-series, Zetasizer, Malvern Instruments, Worcestershire, UK). The particle size and zeta-potential were observed during the course of the experiments.

E. Statistical Analysis

This research was conducted using 3 x 3 factorial in a completely randomized design (CRD). The study of the interaction between variables and the optimization extraction conditions for rice bran protein extracted measuring by the analysis of variance (ANOVA). All qualities were performed in triplicate and presented as mean±standard deviation. The differences between means were measured by independent t-test and DMRT by using SPSS statistic program version 18.0. The probability value of less than 0.05 was considered significant.

III. Results and Discussion

A. Optimization of Extraction Conditions

Rice bran protein extracts from two cultivars had slightly yellowish powder. KDM105 RBPH had slightly light color and a unique aroma than PNP RBPH. The percentage of yield and protein from rice bran protein are presented in Table I. The treatments under low enzyme concentration, low temperature and time for extracted had lowest the percentage of yield, whereas increasing the concentration, temperature and time had more yield. The enzyme concentration, temperature and time were strong influence on extracted yield. These factors resulted in higher %DH. Reference [23] reported that when increased concentration of enzyme the primary amine in solution increased resulting in increased protein solubility due to the formation of small peptides.

Therefore, the optimal conditions for rice bran protein extraction were 0.075 g of enzyme concentration per 5 g of protein, 50°C of extraction temperature, and 4 h of extraction time.

B. Physicochemical and Functional Properties of RBPH

The physicochemical properties of two cultivars of RBPH were showed in Table II. The percentages of moisture content and water activity of RBPH were 11.16-11.58% and 0.27-0.29% for PNP and KDM105, respectively. Lightness and yellowness was 81.71-83.06% and 11.81-13.67%. For bulk density (g/ml) of RBPH was 0.43-0.45 g/ml. Under controlled extraction condition, degree of hydrolysis (%) was 19.92% to 21.05% for KDM105 and PNP, respectively, which contribute to the enzyme hydrolyzed peptide bonds in rice bran. Then the concentration of amine group was increased from the protein hydrolysates.

TABLE I
THE PERCENTAGE OF YIELD AND PROTEIN AT DIFFERENT EXTRACTION CONDITIONS OF RBPH

Treatments	Extraction conditions			% Yield		%Protein (g/100 g.dry RBPH)	
	Enzyme (gram/5 gram of protein)	Temperature (Celsius)	Time (h)	PNP	KDM105	PNP	KDM105
1	0.025	40	2	21.3±2.2^{e}	23.0±1.5^{e}	30.8±3.2^{cd}	30.9±2.2^{d}
2	0.025	50	2	24.5±1.7^{d}	27.6±2.3^{c}	33.5±4.6^{bc}	32.8±3.1^{bc}
3	0.025	60	2	22.3±2.0^{e}	24.4±2.0^{de}	31.1±2.6^{cd}	32.9±3.4^{bc}
4	0.050	40	3	25.8±3.1^{c}	25.2±2.3^{d}	34.6±2.6^{b}	33.5±2.6^{bc}
5	0.050	50	3	26.2±1.9^{bc}	27.1±1.7^{c}	33.8±2.3^{bc}	31.1±1.6^{cd}
6	0.055	60	3	27.4±2.0^{b}	28.3±2.4^{bc}	35.3±2.4^{a}	34.6±2.6^{ab}
7	0.075	40	4	25.7±1.4^{cd}	29.5±1.8^{ab}	28.2±4.1^{e}	33.8±2.3^{bc}
8	0.075	50	4	29.6±1.7^{a}	31.1±2.3^{a}	35.4±2.0^{a}	35.8±1.4^{a}
9	0.075	60	4	26.0±1.9^{bc}	27.0±1.6^{c}	30.2±2.3^{d}	32.5±2.2^{c}

Means (±SD) with different superscript letters in the same column (a-f) indicate significant differences ($P < 0.05$).

TABLE II
PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF THE OBTAINED RBPH

RBPH properties	PNP	KDM105
Physicochemical properties		
Moisture content (%)^{ns}	11.16±0.83	11.58±0.56
Water activity	0.27±0.00^{b}	0.29±0.01^{a}
Color		
L* (lightness)	81.71±1.02^{b}	83.06±0.58^{a}
a* (redness)^{ns}	0.95±0.64	0.72±0.28
b* (yellowness)	13.67±0.73^{a}	11.81±0.42^{b}
Bulk density(g/ml)	0.43±0.03^{b}	0.45±0.01^{a}
Degree of hydrolysis (%)	21.05±0.84^{a}	19.92±0.53^{b}
Solubility (%)		
pH 4^{ns}	27.28±1.34	27.60±0.56
pH 5	30.19±2.36^{b}	41.00±2.16^{a}
pH 6	38.57±0.84^{b}	43.00±1.43^{a}
Functional properties		
Total phenolic content (mg Gallic/g sample)	6.77±0.07^{b}	7.48±0.08^{a}
FRAP(mg ASC/g sample)^{ns}	0.78±0.006	0.86±0.005
FIC (mg EDTA/g sample)^{ns}	0.02±0.003	0.04±0.007
ABTS(mg ASC/g sample)	1,605.03±62.25^{b}	2,495.26±287.10^{a}
Emulsifying properties		
EAI (m^2/g protein)	5.05±0.31^{b}	8.78±0.70^{a}
Foaming capacity (%)		
At 5 minutes	47.33±4.65^{b}	52.98±8.78^{a}

Means (±SD) with different superscript letters in the same row (a-b) indicate significant differences ($P < 0.05$). The superscript "ns" indicates no significant differences among the means in the same row.

There are several factors involved in protein extracted by enzymatic method such as enzymatic concentration and extraction time. Reference [23] reported that the increasing DH (%) was related to enzymatic concentration and time to decompose the substrate in the seed which increased the protein hydrolysates. Whereas, the different extraction methods as in [6] reported that lightness value (83.88) of rice bran protein extracted using microwave method was dark and more reddish than enzymatic method. Reference [24] reported that 0.3 g/mL of bulk density was found in microwave rice bran isolated.

The solubility (%) of KDM105 at pH 4-6 ranged from 27.60% to 43.00%. The solubility depends on the size of the protein peptide. In general, short chain peptides have higher solubility than long chain peptides protein, which is directly related to –COOH and $–NH_2$ groups of amino acids of the protein. Therefore, the solubility of amino acids, peptides and proteins is strongly dependent upon their net charge due to the zwitterion form of amino acids [25], [26]. The result was consistent with previous reports that the pH and extraction time affected on the solubility of protein extracted from mung bean, when the extraction time at 60 minutes was highest solubility. However the solubility was decrease when adding to 90 minutes [27].

The functional properties of RBPH are presented in Table II. Total phenolic content (mg gallic/ g sample) in RBPH were 7.48 and 6.77 for KDM105 and PNP, antioxidant activity of the RBPH was evaluated using FRAP, FIC and ABTS radical scavenging activity and indicated that the antioxidant activity of KDM105 had higher than PNP. The result also showed that FRAP (mg ASC/g sample) were 0.86 and 0.78 for KDM105 and PNP, respectively. FIC (mg EDTA/g sample) were 0.04 and 0.02 for KDM105 and PNP, respectively. For ABTS (mg ascorbic acid/ g sample) were 2,495.26 and 1,605 for KDM105 and

PNP, respectively. FRAP is a measure of "ferric reducing antioxidant power", which is the ability to convert Fe(III) to Fe(II). ABTS is a measure of "antioxidants" to scavenge free radical or donate electrons to quench the radical. ABTS method is carry out in aqueous system. The high-pigmented and hydrophilic antioxidant was better reacted by ABTS method [19]. Reference [26] reported that the formation of short chain peptides may contribute to increasing antioxidant activity of the sample. Not only does the higher antioxidant activity of short chain peptides contribute to the ability to donate electrons to radicals, but it also contributes to the ability to chelate metal ions. It was well-known that metal ions can act as a pro-oxidant that accelerates lipid oxidation, which can be inhibited using metal chelators [27]. FIC assay has the ability to chelate transition metal ions, especially Fe^{2+} and Cu^{2+} in the system. These transition metal's act as pro-oxidant that induce oxidative stress [20].

Emulsifying and foam properties of the rice bran protein were expressed in terms of the EAI and foam capacity (%). Results showed that EAI was 8.78±0.70%. There were several reports suggesting that emulsifying properties of proteins may be influenced by degree of hydrolysis, protein conformation, molecular weight or size, and net charge of proteins [28]. Peptides and protein with high emulsifying properties could be due to their ability to arrange their structures on the oil/ water interfacial region resulting in decreases in surface tensions between oil and water phases [27]-[30]. For foam capacity (%) of RBPH at 1-5 minutes was 80.19±6.30 to 35.96±3.26. Reference [31] reported that foam capacity of rapeseed proteins treated with Alcalase at 1 minute was 44%. However, foam capacity of the rice bran protein decreased over time. Furthermore, It was suggested that stability of air/ liquid foam structures was strongly influenced by several factors such as ability to form thick layers between air and liquid interfaces, ability to reduce surface tension, and the viscosity of the liquid solution at the air/ liquid interfaces [32], [33].

C.Amino Acid Profile of RBPH

The amino acid composition of KDM105 RBPH is shown in Fig. 1. Glutamine was the highest amino acid content (1.74±0.62 g/100 g) followed with aspartic acid (0.80±0.12 g/100 g), valine (0.80±0.05 g/100 g), alanine (0.77±0.08 g/100 g) and Leucine (0.53±0.03 g/100 g), respectively. Some essential amino acid existed in a large amount (glutamine, aspartic, valine, alanine), whereas the remaining was ranges in the level lower than 0.1 g/100 g. of RBPH (hydroxyproline, hydroxylamine and cystine). Total amino acid content of KDM105 was higher than PNP 1.63 fold, which was 7.41±0.43 g/100 g in KDM105 whereas it was 4.55 ±0.67g/100 g in PNP. Reference [34] reported the content of lysine and histamine in rice bran protein was 5.4 g/g and 3.3 g/g, respectively.

D.Stability of O/W Emulsion Stabilized by RBPH

All RBPH emulsifiers were capable of forming emulsions containing small droplets, with droplets being <1000 nm. The mean particle diameters depended on pH (Fig. 2 (a)). Under pH 3 to pH 7 had mean particle lower than pH 8.

The electrical characteristics of the emulsion are important because they determine the droplets stability to aggregation, as well as their interactions with other charged group. The ζ-potential of the droplets depend on pH: lowest -0.5 mV at pH 4 and highest -4.7 at pH 7 for KDM105 (Fig. 2 (b))The ζ-potential of the oil droplets coated by RBPH were a negative at all pH values.

The influence of ionic strength (100-500 mM NaCl) on the stability of emulsions: In the presence of salt, the mean droplet diameter of emulsion was 224-254 nm. Furthermore, the results showed slightly increase in droplet at higher salt concentration. For ζ-potential, there was a decrease in the negative charge with increasing salt concentration (Figs. 3 (a) and (b)).

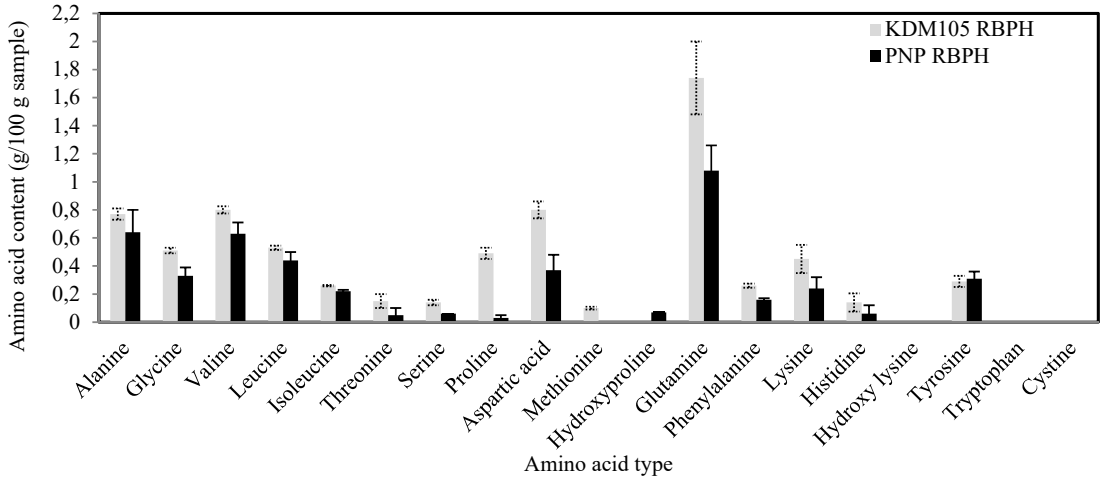

Fig. 1 Amino acid profile of RBPH using GC-MS

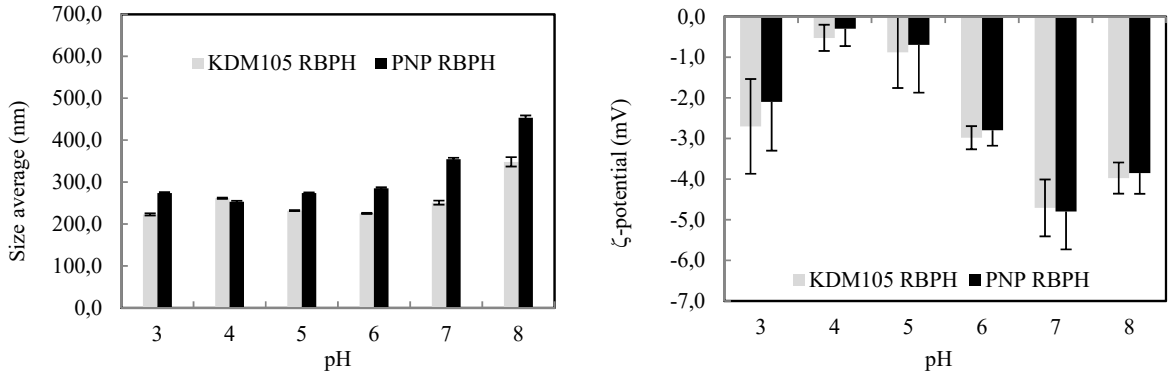

Fig. 2 pH dependence of the (a) mean droplet diameter and (b) particle electrical charge (ζ-potential) of diluted 5% O/W emulsions stabilized by RBPH

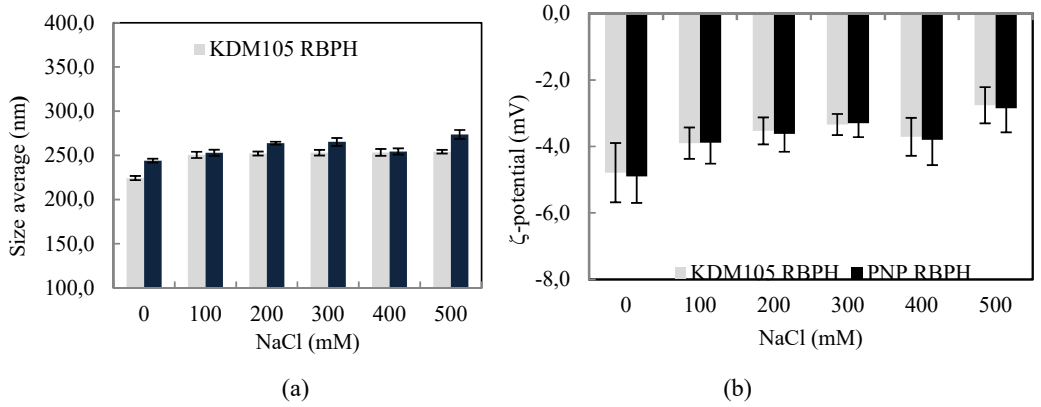

Fig. 3 Ionic strength dependence of the (a) mean droplet diameter and (b) particle electrical charge (ζ-potential) of diluted 5% O/W emulsions stabilized by RBPH

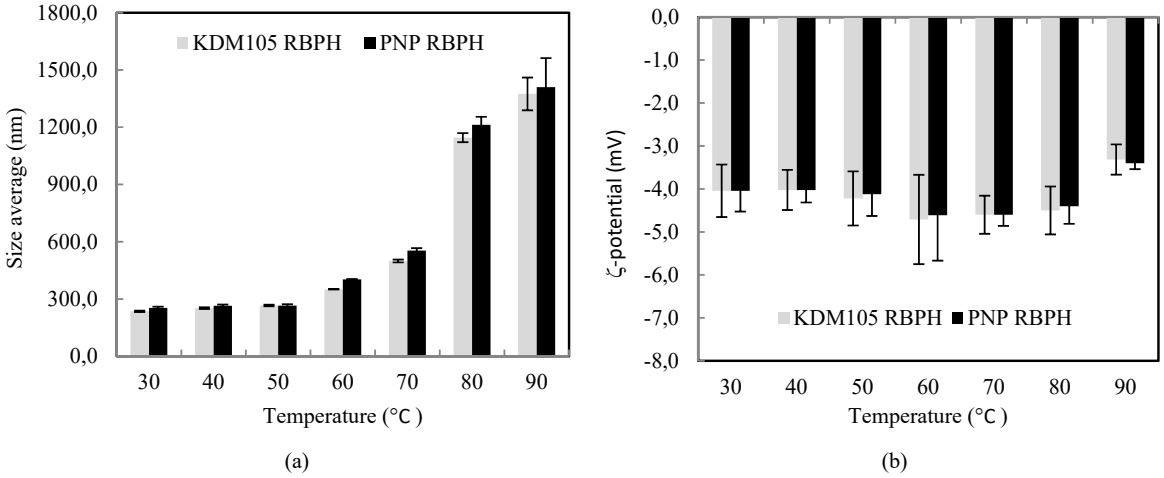

Fig. 4 Heat treatment dependence of the (a) mean droplet diameter and (b) particle electrical charge (ζ-potential) of diluted 5% O/W emulsions stabilized by RBPH

The influence of thermal process on emulsions stability (30-90°C for 30 minutes): The mean droplet diameter of emulsion was increase when the thermal increased. The mean droplet showed higher than 1000 nm after heat above about 70°C. The electrical characteristics of all emulsions was the negative charge (-3 mV to -5 mV) (Figs. 4 (a) and (b)).

IV. Conclusion

The findings of this study showed that the extraction conditions affected the physicochemical and functional properties of hydrolyzed protein, which occurred from protein fractions and differed among rice varieties. Moreover, the investigation of the stability of RBPH in O/W emulsion under different environmental stress during processing showed that RBPH was able to produce the small droplet of O/W emulsion. The emulsions were stable to a wide range of environment stresses: pH below 7, all salt concentration and thermal below 60 °C. These results have important implications for the development of commercial RBPH as a food ingredient and apply to food products.

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References

[1] Elizabeth, P. R. "Bioactive food components and health properties of rice bran," J AmVetMed Assoc, 238(5), 2011, pp. 593-600.
[2] Khalid G., Basharat, Y., Singh, A. K., Singh, P. and Wani A. A. "Rice bran: Nutritional values and its emerging potential for development of development of functional food-Review," Bioact Carbohydr Dietary Fibre, 6, 2015, pp.24-30.
[3] Hanmoungjai, P., Pyle, D. L. and Niranjan, K. "Enzyme-assisted water-extraction of oil and protein from rice bran," J ChemTechnol Biotechnol, 77, 2002, pp. 771-776.
[4] Saunders, R. M. "The properties of rice bran as a food stuff," Cereal Food World 35, 1990, pp. 632-662.
[5] Fabian, C. and Ju, Y. "A Review on rice bran protein: Its properties and extraction methods, "Cri Rev Food SciNutr, 51, 2011, pp. 816-827.
[6] Patsanguan, S., Hisaranusorn, N., Phongthai, S. and Rawdkuen, S. "Riec bran protein isolates: Preparation and their physico-chemical and functional properties," Food App Biosci J, 2(3), 2015, pp. 169-182.
[7] Juliano, B. O. 1985. Rice: Chemistry and technology. 2nd ed., St. Paul, Minnesota: American Association of cereal chemists.
[8] Hamada, J. S. "Characterization of protein fractions of rice bran to devise effective methods of protein solubilization," Cereal Chem, 74, 1997, pp. 662-668.
[9] Hamada, J. S. "Characterization and functional properties of rice bran proteins modified by commercialexoproteases and endoproteases," JFood Sci, 62, 2000, pp. 305-310.
[10] Ansharullah, H. J. A. and Chesteman, C. F. "Application of carbohydrates in extracting protein from rice bran," J Sci Food Agri, 4, 1997, pp.141-146.
[11] Pommer, K. "New proteolytic enzymes for the production of savory ingredients," Cereal Food World, 40, 1995, pp.745-748.
[12] AOAC. Official Method of Analysis Vol.2. 17th ed., 2000, pp. 684. Association of Official Analytical Chemists: Washington, D. C.
[13] Matthew, P. M., Lauren, E. B., Rajesh, N. D., Beth, A. L., Mark, P. and Daniel, O. B. "Applying dry powder coating to pharmaceutical powders using a comill for improving powder flow and bulk density," Powder Tech, 212, 2011, pp.397-402.
[14] Klompong, V., Benjakul, S., Kantachot, D. and Shahidi, F. "Antioxidative activity and functional properties of protein hydrolysate of yellow trevally (*Selaroides leptolepis*) as influenced by the degree of hydrolysis and enzyme type," FoodChem, 102, 2009, pp. 1317-1327.
[15] Adler-Nissen J. "Determination of the degree of hydrolysis of food protein by trinitrobenzenesulfonic acid," JAgrFood Chem, 27, 1979, pp. 1258-1262.
[16] Benjakul, S. and Morrissey, M. T. "Protein hydrolysates from pacific whiting solid wastes," JAgrFood Chem, 45, 1997, pp. 3423-3430.
[17] Chan, E. W. C., Lim, Y. Y., Wong, S. K, Lim, K. K., Tan, S. P. and Lianto, F. S. "Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species,"FoodChem, 113(1), 2009, pp. 166-172.
[18] Wang, Y., Zhang, M., Ruan, D., Shashkov A.S., Kilcoyne, M., Savage A.V., et al. "Chemical components and molecular mass of six polysaccharide isolated from the sclerotium of *Poriacocos*," Carbohydr Res, 339, 2004, pp. 327-334.
[19] Floegel, A., Kim, Dae-Ok., Chung, Sang-Jin., Koo, Sung I and Chun, Ock K. "Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods," JFood ComposAnal, 24, 2011, pp. 1043-1048.
[20] Adjimani, J. P. and Asare, P. "Antioxidant and free radical scavenging activity iron chelators," Toxico Rep, 2, 2015, pp. 721-728.
[21] Bandyopadhyay, K., Charaboty, C. and Barman, A. K. "Effect of microwave and enzymatic treatment on the recovery of protein from Indian defatted rice bran meal." J Oleo Sci, 61, 2012, pp. 525-529.
[22] Pearce, K. N. and Kinsella, J. E. "Emulsifying properties of protein: evaluation of a turbidimentric technique," JAgrFood Chem, 26, 1978, pp. 716-723.
[23] Sari, Y. W., Bruins, M. E. and Sanders, J. P. M. "Enzyme assisted protein extraction fromrapeseed, soybean and microalgae meals," Ind Crops Prod, 43(9),2012, pp. 78– 83.
[24] Khan, S. H., Butt, M. S., Sharif, M. K., Semeen, A., Mumtaz, S. and Sultan, M. T. "Functional properties of protein isolates extracted from stabilized rice bran by microwave, dry heat and parboiling," JAgrFood Chem, 59, 2011, pp. 2416-2420.
[25] Mao, X. and Hua, Y. "Composition, structure and functional properties of protein concentrates and isolates produced from walnut (Juglans regia L.)," Int J Mol Sci, 13, 2012, pp. 1561-1581.
[26] He, R., Abaham, T. G., Malomo, S. A., Ju, X. and Aluko, R. E. "Antioxidant activities of enzymatic rapeseed protein hydrolysate and the Membrane ultrafiltration fraction," JFunctFood, 5, 2013, pp. 219-227.
[27] Kaewumporn, T. "Effects of pH and coagulant of functional properties of mung bean protein products," Bangkok, Thailand: Kasetsart University, MSc thesis, 2006, pp. 67-72.
[28] Chobert, J. M., Bertrand-Harb, C. and Nicolas, M. G. "Solubility and emulsifying properties of caseins and whey proteins modified enzymatically by trypsin," JAgrFood Chem, 36, 1988, pp. 883-886.
[29] Kinsella, J. E. "Functional properties of food proteins in foods: a survey," CritRev Food SciNutri, 8(4),1976, pp. 219-280.
[30] McClements, D. J. Food Emulsions: principles, practice, and techniques. New York: CRC Press LLC.1999.
[31] Chabanon, G. and Chevalot, L. "Hydrolysis of rapeseed protein isolates: Kinetics, characterization and functional properties of hydrolysates," Process Biochem, 42, 2007, pp. 1419-1428.
[32] Phillips, R. D. and Beuchat, L. R. "Enzyme modification of proteins,". In Cherry, J. P. (Ed). Protein functionality in foods, Washington, D.C.: American Chemical Society.1981, pp. 275.
[33] Damodaran, S. "Function properties," In Nakai, S. and Modler, H. W. (Eds). Food proteins properties and characterization, New York: Wiley.1996, pp. 167-234.
[34] Tang, S., Hettiararchy, N. S., Eswaranandam, S. and Chandall, P. "Protein extraction from heat-stabilized defatted rice bran. 2. The role of amylase celluclast, and viscozyme,"JFood Sci, 68, 2003, pp.471-475.