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Optimization of microwave-assisted extraction of rice bran protein and its hydrolysates properties

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ABSTRACT

Microwave-assisted extraction (MAE) was applied for extracting rice bran protein with a response surface methodology (RSM). The optimal condition was 1000 W of microwave power, 90 s of extraction time, and a solid to liquid ratio of 0.89 g rice bran/10 mL of distilled water. The protein yield of MAE was higher than that of alkaline extraction (ALK) by about 1.54-fold ($P < 0.05$), while the protein digestibility was similar. The protein hydrolysates (PHs) with at different degrees of hydrolysis (DH) (5.04, 10.37 and 15.04%) were produced by alcalase. The molecular weight (MW) of the rice bran protein concentrates (RBPC) and the PHs ranged between <11 kDa and 100 kDa. The excessive enzymatic hydrolysis resulted in a negative effect on water and oil absorption capacities. The PHs with DH15.04% acted as the strongest DPPH radical scavenger, ferric reducing agent, and also metal ion chelator ($P < 0.05$). However, a DH of 5.04% was sufficient for improving the functional properties of RBPC, especially foam ability and the emulsion activity index. This study suggests that the desirable properties of rice bran protein can be controlled with enzymatic modification.

1. Introduction

Alkaline extraction is the most common method for extracting protein from plant materials due to its simplicity and low cost. Rice bran is a trendy plant material for protein production because it is an inexpensive raw material, and it is a source abundant with essential amino acids (Xia et al., 2012). However, severe alkaline conditions negatively affect the nutritional and functional properties of the protein. This process also requires a long time for extraction and consumes large volumes of buffer. Therefore, other methods such as physical and enzymatic treatments are increasingly being considered as alternative methods.

Nowadays, microwave treatment is one of the most commonly used methods for solid-liquid extraction due to its power, convenience, and reasonable cost. Many studies have reported about the benefits of microwaves for extracting some active compounds from plant materials such as triterpene, saponins, and antioxidant components etc. (Li et al., 2010; Zhao et al., 2013). These results highlight the ability of microwaves to disrupt hydrogen bond networks. The microwave-induced dipole rotation of molecules, and the migration of ions that enhance the penetration of solvent in to matrix, disrupts the cell wall and releases the intracellular product, allowing for the extraction of different components (Li et al., 2010). Due to all of these reported benefits, the authors hypothesize that microwaves can be used for improving the extractability of protein in rice bran than other methods. Several variables affect extraction efficiency, including extraction time, solid-liquid ratio, temperature, the type of solvent, particle size, pH, etc. To study the effect of independent variables, the traditional method of testing one variable at a time is inefficient and not cost effective. Thus, response surface methodology (RSM) was applied in order to optimize the process, and to evaluate whether or not it has a more desirable response and could be used as a better alternative method.

Enzymatic treatment has been used to improve functional properties such as solubility, foaming, and emulsifying properties of protein concentrate from various sources. Several studies have reported about the function of enzymes to enhance the functional properties of different protein sources such as the use of alcalase 2.4 L for rapeseed protein (Chabanon et al., 2007) and trypsin for oat bran protein (Guan et al., 2007). Moreover, the degree of hydrolysis also affects to the antioxidative properties including free radical scavenging activity, metal chelating, or the reducing power of protein hydrolysates (Chanput et al., 2009).

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The objective of this study was to optimize the conditions for microwave-assisted extraction of protein from defatted rice bran with a response surface methodology. Three variables were studied. They include microwave power, extraction time, and solid-liquid ratio. Furthermore, the overall extraction efficiency was compared to an alkaline extraction method. The obtained protein concentrates were also investigated for *in vitro* digestibility, and they were hydrolyzed by alcalase® at different degrees of hydrolysis. Finally, the protein concentrates and its hydrolysates were determined for their physical, chemical, and functional properties.

2. Materials and methods

2.1. Materials and chemicals

Raw organic rice bran was supplied by Urmatt Ltd. (Chiang Rai, Thailand). Alcalase® Enzyme from *Bacillus licheniformis* (2.990 U/mL) was purchased from EMD Chemicals, Inc. (San Diego, CA, USA). 1,1-diphenyl-2-picryhydrazyl (DPPH), ferrozine, pepsin and trypsin were purchased from the Sigma-Aldrich Company (St.Louis, MO, USA).

2.2. Rice bran preparation and alkaline extraction (ALK)

Raw organic rice bran was defatted with 95% ethanol (1:5, w/v) by stirring at room temperature for 1 h. The slurry was centrifuged at 1020 g for 5 min. The precipitate was collected and re-extracted twice. Defatted rice bran (DFRB, 14.13 ± 0.07% protein content) was kept in a plastic zip lock bag at −18 °C before use in further experiments.

For alkaline extraction, DFRB was dispersed in distilled water (1:10, w/v), and then the pH was adjusted to 10 using 3 M sodium carbonate. The mixture was stirred (200 rpm) at room temperature (30–35 °C) for 1 h. After centrifugation at 10,000 g, at a temperature of 4 °C for 10 min, the supernatant was collected and the pH was adjusted to 4.5 using 3 M citric acid. It was then centrifuged in the same condition described above. The precipitate was adjusted to a pH of 7.0 and then freeze dried. The obtained powder was referred to "rice bran protein concentrate: RBPC".

2.3. Experimental design and optimization

The experimental design and statistical analysis were performed using Stat-Ease software (Design-Expert 6.0.10 Trial). The response surface methodology (RSM) and a three-level three-factor Box–Behnken design were chosen to evaluate the effect of microwave power (X_1; 600–1000 W), extraction time (X_2; 60–120 s), and solid–liquid ratio (X_3; 0.5–1.5 g/10 mL distilled water). The complete design consists of 17 combinations including five replicates of the center point (Table 1). The response value was protein yield.

The extraction was carried out in a microwave machine with multimode cavities and a frequency of 2450 MHz (LG MC8088HRC, LG Electronics Co., Ltd., Thailand). The amount of DFRB of each run was mixed with a precise volume of distilled water (200 mL) in 1000 mL-beaker. The mixtures were adjusted to attain a pH of 10 by using 3 M sodium carbonate, and they were then placed on a turning plate in a microwave machine. Afterwards, the slurry of each treatment was cooled down and centrifuged at 10,000 g at a temperature of 4 °C for 10 min. The supernatant was collected, filtered, and then subjected to a precipitation of protein by adjusting the pH to 4.5. The precipitate obtained after centrifugation was adjusted to a pH of 7.0 and then dried using a freeze dryer. The dry matter was referred to as the rice bran protein concentrate (RBPC). The RBPC was kept in a plastic zip lock bag at −18 °C. The protein yield was calculated by the following equation;

Table 1
Protein extraction yields of 17-treatment combinations, optimal condition and conventional alkaline extraction.

Run	Factors			Response value	Predict value	Residual
	X_1	X_2	X_3			
1	600	90	0.5	4.36 ± 0.01	4.29	0.07
2	800	60	1.0	4.20 ± 0.07	4.21	−0.01
3	800	60	1.0	3.55 ± 0.04	3.52	0.03
4	1000	120	1.0	4.17 ± 0.02	4.10	0.07
5	800	90	1.0	4.26 ± 0.02	4.21	0.05
6	800	120	1.0	4.08 ± 0.00	4.11	−0.03
7	600	60	1.5	3.57 ± 0.06	3.63	−0.06
8	1000	90	1.5	4.25 ± 0.02	4.29	−0.04
9	600	90	0.5	3.74 ± 0.02	3.70	0.04
10	600	120	1.0	4.25 ± 0.14	4.29	−0.04
11	800	90	1.0	4.18 ± 0.04	4.21	−0.03
12	800	90	0.5	4.27 ± 0.03	4.21	0.06
13	1000	90	0.5	4.10 ± 0.01	4.17	−0.06
14	800	90	1.5	4.19 ± 0.01	4.21	−0.02
15	1000	60	1.0	4.33 ± 0.04	4.29	0.04
16	800	120	1.5	4.10 ± 0.04	4.09	0.01
17	800	90	1.0	4.15 ± 0.03	4.21	−0.06
Optimal	1000	90	0.89	4.37 ± 0.05	4.36	0.01
Conventional alkaline extraction				2.92 ± 0.03	–	–

$$\text{Protein yield}(\%) = (\text{Gram of protein powder obtained}/\text{DFRB used}) \times 100 \quad (1)$$

The experimental data was fitted to a second-order polynomial model and regression coefficients were obtained. The generalized second-order polynomial model used in the response surface analysis was as follows;

$$Y = \beta 0 + \sum_{i=1}^{3} \beta iXi + \sum_{i=1}^{3} \beta iiXi^2 + \sum_{i-1}^{2} \sum_{j=2}^{3} \beta ijXiXj \quad (2)$$

where β_0 was defined as the constant, β_i the linear coefficient, β_{ii} the quadratic coefficient, and β_{ij} the interaction coefficient. X_i and X_j are levels of the independent variables.

The analysis of variance (ANOVA) table was generated and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The significance of all terms in the polynomial was evaluated statistically by computing the *F*-value at a probability (*P*) of 0.05. The regression coefficients were used to make statistical calculations to generate 3-dimension contour plots from the regression models.

2.4. In vitro digestibility

In vitro digestibility was assayed according to the method of Xia et al. (2012) with some modification. The RBPC (1%, w/v) was mixed with distilled water, and the pH was adjusted to 1.5. The mixture was incubated at 37 °C for 5 min and then pepsin (enzyme: protein of 1:100, w/w) was added. The digestion was continued to 120 min. After that, the mixture was neutralized with 1.0 M NaOH to stop the pepsin digestion. The trypsin digestion was next performed by adding trypsin (enzyme: protein of 1:100, w/w) into the neutralized pepsin-digested mixture. After incubation at 37 °C for 120 min, the mixture was heated at 95 °C for 10 min to terminate the trypsin activity. An equal volume of 10% (w/v) Trichloroacetic acid (TCA) was added to the mixture and centrifuged at 5500 g for 10 min. The TCA-precipitate was collected and freeze dried, and then the protein content was determined by using the Kjeldahl method