

Application of ultrasound to protein extraction from defatted rice bran

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Abstract

In this study, water was used as a cheap and eco-friendly solvent in ultrasound-assisted extraction of protein from defatted rice bran meal. The effects of sonication variables on the protein yield were firstly investigated. The first-order kinetic model was then used to describe the extraction. The initial extraction rate and extraction constant in the ultrasonic extraction were 3.48 times and 2.20 times, respectively higher than those of the conventional extraction. In addition, the ultrasonic extraction resulted in significantly higher protein yield than the conventional extraction. The rice bran protein concentrates from both ultrasound-assisted and conventional extraction had similar protein profile, water and oil absorption capacity, emulsifying capacity and emulsion stability. Use of ultrasound in the protein extraction increased gelation capacity but decreased foaming capacity and stability of the protein concentrate.

Keywords

Extraction

Functional properties

Rice bran

Protein

Ultrasound

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Introduction

Rice is the second largest cereal crop worldwide. This cereal is mostly grown and consumed in Asian countries. Rice bran is an important by-product from rice milling. The main chemical composition of rice bran is as follows: 15.0 – 20.0% lipid, 10.0 – 16.0% protein, 31.4 – 52.3% carbohydrate, 7.0 – 14.1% fibers, 6.6 – 9.9% ash and 8.0-12.0% moisture (Fabian and Ju, 2011). Although rice bran is rich in valuable components for human diet, it is usually not consumed as food due to possible hull contamination. In addition, activation of lipase in the bran is observed during rice milling and that leads to rancidity and off-flavor development (Saunders, 1990). Rice bran has been used as main material in the production of rice bran oil (Shahidi, 2005) as well as rice protein concentrate for food industry (Day, 2013).

Protein concentrates have been used as nutritional supplement and functional ingredient in food processing. Among cereal proteins, rice protein has the highest nutritional value due to its high content of limiting essential amino acids such as lysine and threonine (Juliano, 1985). Moreover, rice protein also has nutraceutical properties including hypoallergenicity and anti-cancer activity (Saunders, 1990). It was reported that emulsifying and foaming properties of rice protein concentrate and isolate were quite good in formulation of various food products. As a result, rice protein has been recognized as a

potential protein in food industry (Fabian and Ju, 2011).

Extraction is a key operation in the production of protein concentrate from vegetable source. The nature of solvent could affect strongly protein composition in the extract (Yada, 2004). Rice bran proteins consisted of four fractions: 37% water-soluble albumin, 31% salt-soluble globulin, 27% alkali-soluble glutelin and 2% alcohol-soluble prolamin. In the extraction of rice bran protein, the use of alkali as solvent is the most common method since sodium hydroxide can break hydrogen, amide and disulfide bonds in protein for improvement in extraction yield (Fabian and Ju, 2011). However, severe alkaline conditions changed the nutritional characteristics of protein and produced toxic compounds such as lysinoalanine (Cheftel *et al.*, 1985). It can be noted that the main fractions of rice bran protein are albumin and globulin. Albumin can be extracted with water which is a cheap and eco-friendly solvent. In addition, during albumin extraction, some mineral compounds can be dissolved in the extract and some globulin fractions can be extracted from the material.

From the last decade, application of ultrasound to extraction has attracted great attention. Ultrasound-assisted extraction delivers various advantages including better penetration of the solvent into cellular material, improvement in mass transfer, better release of the extract due to the disruption of cell wall (Feng *et al.*, 2011). Ultrasound was

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proved to improve protein extraction from different vegetable sources (Karki *et al.*, 2010; Zhu and Fu, 2012; Tu *et al.*, 2015), including defatted rice bran (Chittapalo and Noomhorm, 2009). Ultrasound-assisted extraction of protein from defatted rice bran was performed under alkaline condition (pH 11) and Chittapalo and Noomhorm (2009) reported that increase in ultrasonic power significantly reduced the extraction time and augmented the reaction rate constant.

In this study, for the first time, water was used as solvent in protein extraction from defatted rice bran meal. The objective of this study was to clarify the effects of ultrasound-assisted extraction variables on the protein yield; the extraction kinetic parameters as well as the functional properties of the rice bran protein concentrates obtained from the ultrasound-assisted extraction and conventional extraction were then compared.

Materials and Methods

Materials

Rice bran was supplied from a rice processing plant in Long an, Vietnam. The cultivar *Oryza sativa* OM488 was used in this study. Hexane was used for lipid extraction from rice bran meal. The rice bran/hexane ratio, extraction temperature and time were 1/10 (w/w), 40°C and 36 h, respectively. After extraction, the solid phase was separated by centrifugation at 5,000×g and dried at 40°C to a moisture content less than 10%. The obtained defatted rice bran meal was stored at 4°C until use for protein extraction. Chemical composition of the defatted rice bran was as follows (% w/w): moisture content: 8.5±0.1, protein: 13.6±0.3, carbohydrate: 64.3±0.1, lipid: 2.5±0.6 and ash 11.5±0.7.

De-ionized water was used as solvent for protein extraction. Protein standards and all chemicals used in electrophoretic analysis were originated from GeneOn (Germany). Other chemicals used in this study were of analytical grade and purchased from Sigma-Aldrich (The United States).

Ultrasound-assisted extraction of protein from defatted rice bran meal

The protein extraction was carried out in 1 L Erlenmeyer flasks containing 50 g defatted rice bran meal and 500 mL de-ionized water. The pH of the mixture was nearly 7.0. The ultrasound-assisted extraction consisted of two steps: ultrasonic treatment and additional extraction. For the first step, the ultrasonic treatment was performed using a horn-type ultrasonic probe with frequency of 20

kHz (model VC 750, Sonics and Materials Inc, The United States). During the ultrasonic treatment, all Erlenmeyer flasks were put in a cooling water bath (model SC100-A28; Thermo Fisher Scientific, The United States) and the sample temperature was adjusted to be lower than 30°C. For the second step, all Erlenmeyer flasks were transferred into a thermostatic shaker (model 30157BI-MaxQ 2000, Thermo Fisher Scientific, The United States) and the additional extraction was conducted at 30°C and 200 rpm.

First series: The sonication power was changed: 0 (control), 5, 10, 15, 20 and 25 W/g of material dry mass. The sonication time was 3 min. After the ultrasonic treatment, the time of the additional extraction was 30 min.

Second series: The sonication time was varied: 0, 1, 2, 3, 4 and 5 min. The selected ultrasonic power was 15 W/g. After the ultrasonic treatment, the time of the additional extraction was fixed at 30 min.

Third series: The sonication power and time were set at 15 W/g and 2 min, respectively. After the ultrasonic treatment, the time of the additional extraction was changed: 0, 5, 10, 20, 30, 40, 50 and 60 min.

At the end of the extraction, all samples were centrifuged (model Sigma 3K30, Sartorius, Switzerland) at 5,000×g and 20°C for 30 min to remove the solid phase and the supernatant was used for protein quantification.

Comparison of kinetic extraction parameters of the conventional and ultrasound-assisted method

The time when defatted rice bran meal was mixed with solvent was considered as the beginning of the extraction. Conventional extraction: 50 g defatted rice bran meal and 500 mL de-ionized water were added to 1 L Erlenmeyer flasks and the pH of the mixture was nearly 7.0. Extraction was performed at 30°C and 200 rpm in a thermostatic shaker (model 30157BI-MaxQ 2000, Thermo Fisher Scientific, The United States) for 60 min.

Ultrasound-assisted extraction: Firstly, the ultrasonic treatment was performed at the sonication power of 15 W/g for 2 min and the sample temperature was kept lower than 30°C. The additional extraction was then conducted at 30°C, 200 rpm for 58 min.

In both methods, during the extraction, samples were taken for centrifugation at 5,000×g and 20°C for 30 min to remove the solid phase and the obtained supernatant was used for protein quantification. At the end of the extraction, the samples were treated in the similar way and the supernatant was used for electrophoretic analysis.

The first-order kinetic model was used for determination of the extraction rate constant of protein (Aguilera and Garcia, 1989). The general first-order model was as follow:

$$(C_{\infty} - C_t)/(C_{\infty} - C_w) = e^{-kt} \quad [1]$$

where, C_{∞} is maximal protein concentration in the extract (g/L), C_t is protein concentration in the extract at a given extraction time t (g/L), C_w was initial protein concentration in the extract (g/L), k was extraction rate constant (g/L.min).

Due to $C_w = 0$ when $t = 0$, the first-order model can be written as Equation [2]:

$$(C_{\infty} - C_t)/C_{\infty} = e^{-kt} \quad [2]$$

The integrated rate law for a first-order extraction under the boundary conditions $t = 0$ to t and $C_t = 0$ to C_t can be written as Equation [3]:

$$\begin{aligned} d(C_t)/dt &= d(C_{\infty} \times (1 - e^{-kt}))/dt \\ d(C_t)/dt &= k \times C_{\infty} \times e^{-kt} \end{aligned} \quad [3]$$

When $t = 0$, initial extraction rate h (g/L.min) can be defined as:

$$h = k \times C_{\infty}$$

The maximal protein concentration in the extract C_{∞} (g/L), initial extraction rate h (g/L.min) and extraction rate constant k (g/L.min) were determined by using R software (version 3.1.0).

Preparation of protein concentrate from defatted rice bran meal

The protein extracts at the end of the conventional and ultrasound-assisted extraction in the previous section were used for protein concentrate preparation. The protein extract was adjusted to pH 4.2 using 0.1 M HCl for protein coagulation. The solid phase was then separated by centrifugation at 5,000×g, 20°C and re-dissolved in de-ionized water. The procedure of protein coagulation at pH value was repeated 2 times for increase in protein ratio in the concentrate. The solid phase at the end of the final centrifugation was freeze-dried (Floor model, Labconco, The United States) to a moisture content less than 8%; the vacuum pressure was 0.1 mbar and the maximum freeze-drying temperature was 40°C. The obtained protein concentrates were used for determination of proximate composition and functional properties including water absorption capacity, oil absorption capacity, emulsifying capacity and emulsion stability,

foaming capacity and foam stability, gelation capacity.

Analytical methods

Total protein content in the defatted rice bran meal and the extract was determined by Kjeldahl method (Latimer, 2012). Protein profile in the extract was analyzed by electrophoresis on sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) under reducing conditions according to the procedure of Laemmli (1970). Moisture, carbohydrate, lipid and ash contents were analyzed using AOAC official methods (Latimer, 2012). Total phenolic content was measured by spectrophotometric method using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). Surface hydrophobicity of soluble proteins was measured by fluorescence spectrometric method described by Kato and Nakai (1980) using 8-anilino-1-naphthalene sulphonate. Water and oil absorption capacity was evaluated by the method described by Lawal *et al.* (2005). Emulsifying capacity and emulsion stability were determined according to the method reported by Pearce and Kinsella (1978). Foaming capacity and foam stability were evaluated by the method reported by Deng *et al.* (2011). Gelation capacity was evaluated using the method described by Lawal *et al.* (2005).

Calculation formula

The protein yield of the extraction was calculated by the following formula:

$$Y = (P_a - P_t)/P_t$$

Where: Y (%) was the protein yield, P_a (g) was the total protein content in the extract, P_t (g) was the total protein content in defatted rice bran meal used in protein extraction.

Statistical treatment

All experiments were performed in triplicate. The experimental results were expressed as means \pm standards deviation. Mean values were considered significantly different when $P < 0.05$. One-way analysis of variance was performed using the software Statgraphics Centurion XV.

Results and Discussion

Effects of ultrasound-assisted extraction variables on the protein yield

Figure 1A presents the effects of sonication power on the protein yield. Increase in sonication power from 0 to 15 W/g enhanced the protein yield by 2.2 times.

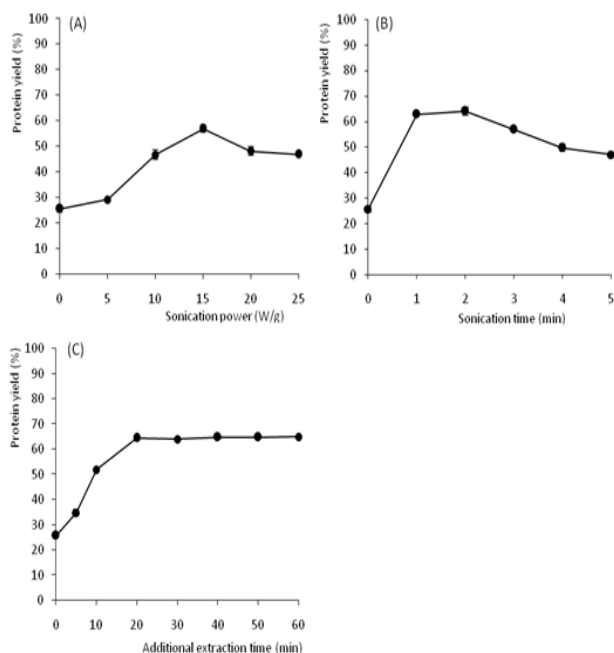


Figure 1. Effects of (A) sonication power, (B) sonication time and (C) additional extraction time on the protein yield from defatted rice bran

However, when the sonication power augmented from 15 to 25 W/g, the protein yield slightly decreased. In all cases, the use of ultrasound significantly enhanced the protein yield in comparison with the conventional extraction. In solid-liquid system, ultrasound generated cavitation which resulted in disintegration of the material particles and improvement in mass transfer; the higher the sonication power, the more intensive the acoustic cavitation (Feng *et al.*, 2011). As a result, the extraction yield was enhanced. Nevertheless, high ultrasonic power produced strong shear forces which promoted the aggregation of protein molecules (Sotomayor and Schulten, 2007). This phenomenon decreased the content of soluble proteins in the extract. Improvement in protein yield was also noted when ultrasound was applied to protein extraction from defatted pumpkin seed meal (Tu *et al.*, 2015) and defatted soy flake (Karki *et al.*, 2010). When alkali was used in ultrasound-assisted extraction of protein from defatted rice bran, Chittapalo and Noomhorm (2009) also reported an increase in protein concentration in the extract with the increase in sonication power. However, the ultrasonic power range (0-5 W/g) used in the previous study was much narrower than that used in our study (0-25 W/g).

The effects of sonication time on the protein yield are visualized on Figure 1B. The highest protein yield was noted at the sonication time of 2 min. When the sonication time increased from 2 to 5 min, the protein yield was slightly reduced. Similar observation was

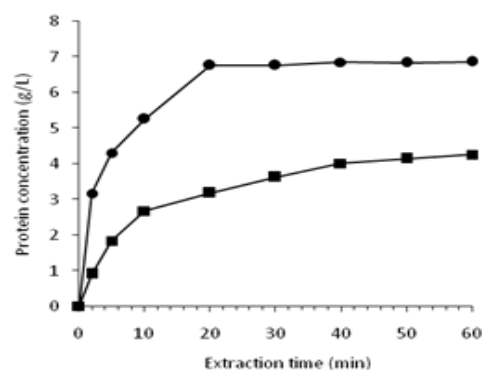


Figure 2. Change in protein concentration in the extract during the (■) conventional and (●) ultrasound-assisted extraction.

reported in ultrasound-assisted extraction of protein from defatted pumpkin seed meal (Tu *et al.*, 2015). However, our results were different from the findings of Chittapalo and Noomhorm (2009). According to these authors, protein concentration in the extract did not decrease during the ultrasonic treatment although the sonication time lasted 40 min. It was due to much lower ultrasonic power (5 W/g) in comparison with that used in our study (15 W/g) and low shear forces did not lead to protein denaturation.

Figure 1C shows that the protein yield achieved 25.6% at the end of the ultrasonic treatment. Additional extraction was therefore essential for improvement in protein yield. During the additional extraction, the protein yield gradually increased and achieved maximum of 64.5% when the additional extraction time was 20 min. Longer extraction time did not change the protein concentration in the extract.

Comparison of kinetic extraction parameters of the conventional and ultrasound-assisted method

Figure 2 shows the change in protein concentration of the extract during the conventional and ultrasound-assisted extraction. It can be noted that the use of de-ionized water as solvent in protein extraction from defatted rice bran in our study resulted in significantly higher protein content in the extract in comparison with the use of alkali in the previous study of Chittapalo and Noomhorm (2009). That was due to high level of albumin and globulin in rice bran protein (Fabian and Ju, 2011).

Based on the obtained results, the maximal protein concentration in the extract C_{∞} (g/L), initial extraction rate h (g/L.min), extraction rate constant k (g/L.min) and coefficient of determination R^2 were determined and shown in Table 1. The coefficient of determination R^2 for both conventional and ultrasound-assisted extraction was very high. It can be concluded that the first order kinetic model describes

Table 1. Comparison of the first-order kinetic parameters of the conventional and ultrasound-assisted extraction of protein from defatted rice bran

Methods	Maximum protein content in the extract, C= (g/L)	Extraction rate constant, k (g/L.min)	Initial extraction rate, h (1/min)	Coefficient of determination, R^2
Conventional extraction	4.04 ^a	0.10 ^a	0.42 ^a	0.990
Ultrasound-assisted extraction	6.74 ^b	0.22 ^b	1.46 ^b	0.995

Values with different lower case letters in the same column are significantly different ($p < 0.05$)

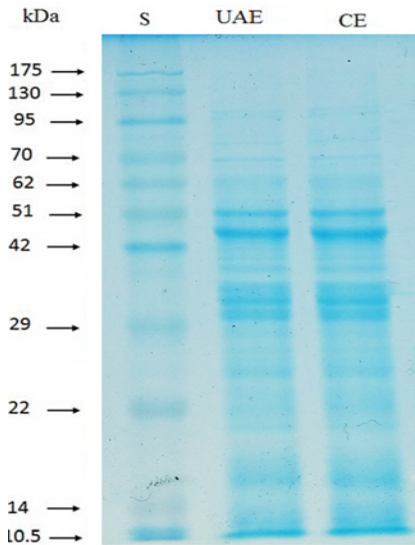


Figure 3. Electrophoresis of protein extracts using different extraction methods; UAE: ultrasound-assisted extraction, CE: conventional extraction, S: Protein standards

well the experimental results in our study. Previously, first order model was also used to calculate extraction kinetic parameters in protein extraction (Aguilera and Garcia, 1989; Chittapalo and Noomhorm, 2009; Tu *et al.*, 2015).

According to the model, the maximum protein concentration in the extract in the ultrasound-assisted method was 1.67 times higher than that in the conventional method. In addition, the initial extraction rate (h) and extraction rate constant (k) of the ultrasound-assisted extraction were 3.48 times and 2.20 times, respectively higher than those of the conventional extraction. That was due to an improved mass transfer in ultrasonic extraction in comparison with that in conventional extraction (Feng *et al.*, 2011). Higher extraction rate led to a shorter extraction time. When alkali was used in protein extraction from defatted rice bran, the extraction rate constant of the ultrasonic method was 1.1-15.9 times higher than that of the conventional method (Chittapalo and Noomhorm, 2009). Difference in extraction rate constant in protein extraction from defatted rice bran can be explained by difference in the use of various

solvents and extraction conditions.

The protein profile of the extract from both conventional and ultrasound-assisted methods was similar (Figure 3). Application ultrasound to the extraction did not change the protein composition of the extract. This observation was recently noted in ultrasonic extraction of protein from defatted pumpkin seed meal (Tu *et al.*, 2015). In this study, the molecular weight of the extracted proteins varied from 10.5 to 95 KDa while that of the albumin and globulin fractions of Langi cultivar brown rice from Australian ranged from 13.9 to 53.6 KDa (Agboola *et al.*, 2005). Different protein profile of different rice cultivars was due to difference in genetics.

Proximate composition and functional properties of rice bran protein concentrate from conventional and ultrasound-assisted extraction

The proximate composition of the rice bran protein concentrates from the two extraction methods was similar (Table 2). However, ultrasound-assisted extraction decreased protein surface hydrophobicity in comparison with the conventional extraction. According to Feng *et al.* (2011), shear forces from acoustic cavitation could result in conformation change of some protein molecules in the extract and that would change protein surface hydrophobicity.

Table 2 shows that the two protein concentrates from defatted rice bran had similar water absorption capacity, oil absorption capacity, emulsifying capacity and emulsion stability. The same observation was recently reported in ultrasound-assisted extraction of protein from defatted rambutan seed meal (Co *et al.*, 2016). Nevertheless, our results were different to the findings of Chittapalo and Noomhorm (2009). When alkali was applied to protein extraction from defatted rice bran, the use of ultrasound increased both water and oil absorption capacity but the values obtained in that study were significantly lower than those in our study. That was due to difference in protein composition in the extract.

Ultrasonic extraction reduced the foaming

Table 2. Chemical composition and functional properties of rice bran protein concentrates from conventional and ultrasound-assisted extraction

Chemical composition	Conventional extraction	Ultrasound-assisted extraction	Functional properties	Conventional extraction	Ultrasound-assisted extraction
Moisture (%)	4.67 ^a ±0.29	4.40 ^a ±0.17	Water absorption capacity (mL/g)	4.5 ^a ±0.1	4.2 ^a ±0.3
Protein (%)	73.27 ^a ±0.66	72.64 ^a ±0.75	Oil absorption capacity (mL/g)	8.9 ^a ±0.1	9.0 ^a ±0.3
Carbohydrate (%)	14.64 ^a ±0.89	15.30 ^a ±1.03	Emulsifying capacity (Absorbance)	0.8 ^a ±0.0	0.8 ^a ±0.0
Lipid (%)	0.20 ^a ±0.02	0.23 ^a ±0.02	Emulsion stability (min)	11.6 ^a ±0.2	11.7 ^a ±0.3
Ash (%)	7.22 ^a ±0.10	7.43 ^a ±0.11	Foaming capacity (%)	93.0 ^a ±2.3	40.7 ^b ±0.1
Phenolics (mg/100g)	34.76 ^a ±0.84	37.68 ^a ±0.42	Foaming stability (%)	79.3 ^a ±0.1	64.1 ^b ±4.3
Surface hydrophobicity (S ₀)	11481 ^a ±463	8494 ^a ±251	Least gelation concentration (% w/v)	10 ^a	9 ^b

Values with different lower case letters in the same row are significantly different (p<0.05)

capacity and stability of the rice bran protein concentrate. It should be noted that foaming stability depends on surface hydrophobicity of protein. Protein molecules with low surface hydrophobicity could not stabilize cohesive film around the gas bubbles (Belitz *et al.*, 2009) and that leads to reduced foaming stability. In addition, use of ultrasound improved the gelation capacity of the protein concentrate. It can be explained that change in protein conformation during the ultrasonic extraction could change the interaction between protein molecules for gelation (Belitz *et al.*, 2009).

Conclusion

In this study, water was demonstrated as a potential solvent for protein extraction from defatted rice bran meal. Application of ultrasound significantly improved the protein yield as well as reduced the extraction time. Both ultrasound-assisted and conventional extraction resulted in similar protein profile of the extract. Ultrasonic extraction did not change water and oil absorption capacity, emulsifying capacity and emulsion stability of the rice bran protein concentrate. However, the use of ultrasound increased gelation capacity but decreased foaming capacity and stability of the protein concentrate. Further study on pilot scale is essential for application of the ultrasonic extraction of protein in food processing.

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