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Production of Rice-By Product Protein Isolate Using the Subcritical Water Extraction Method

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ABSTRACT

Background: Subcritical water extraction (SWE) is a friendly technique applied to produce rice by-product extract. The aim of this study is using SWE as a new friendly extraction method for producing protein isolates (PI) of rice bran and rice combination as a byproduct of Iranian agriculture product. **Methods:** D-optimal combined design was used to optimize bran rice ratio, solid/water ratio, extraction time, and particle size to obtain maximum protein. Bran (8-12%) and rice ratio (88-92%) solid water (0.04 -0.12%), time (15-45 min), and particle sizes (420 µm and 710 µm) were selected as independent variables, and protein as dependent variable. After optimization, the effects of extraction parameters on functional properties were investigated. **Results:** Increasing bran/rice ratio has a significant effect on the protein extraction. In this study, the optimum SWE conditions were solid water (0.12), bran rice ratio (8:92), time (45 min), particle size (420 µm), and the optimum SWE temperature was obtained at 120 C°. SWE also could significantly enhance functional properties, such as the solubility, emulsifying activity index (EAI), and degree of hydrolysis of by-product of rice milling (BRM) PI. **Conclusion:** According to the positive impact of SWE on protein extraction and the functional properties of BRM PI, this method could be applied as a novel extraction technique to modify the properties of rice protein isolate for functional purposes in the future. However, more investigation is required to study the quantity and quality of the extracted valuable materials to scale it up for industrial means.

Keywords: *Rice; Bran; Subcritical water extraction; Protein isolate; Functional properties*

Introduction

Rice protein is one of the commonly used proteins in the world (Costa and Garcia, 2016). Rice is the main part of meals in Asian countries (Bandumula, 2018). Rice protein has been applied in many food formulations due to its excellent functional properties, such as solubility, emulsifying activity index (EAI) , and foaming properties (Mihucz *et al.*, 2010).

Rice bran (RB) is a by-product of rice milling (BRM) that is obtained by removing the rice seeds hull. It is very nutritious because of has protein, essential fatty acids, fiber, vitamin B group , and

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minerals (Yılmaz, 2016). Also, bran has some antioxidants like tocopherol, oryzanol, tocotrienol, and ferulic acid (Zubaidah *et al.*, 2012). The lysine content of RB is nearly four times higher than rice as it is located in proteins of the out layer of cereals legumes (Sanni *et al.*, 2020).

Considering all these advantages, there is a need for a practical procedure for the production of rice and RB extract. There are some conventional methods for extraction like Soxhlet, alkaline extraction, and direct solid-liquid extraction (Jalilvand *et al.*, 2013). There are environmental problems with these methods, such as the side effects on humans' health due to emitting hazardous contaminants. Therefore, a novel eco-friendly method is needed for extraction in the food industry (Chen *et al.*, 2015).

Given that common methods of extraction, such as enzymatic hydrolysis or modification, high-temperature treatments are so costly and may affect the functional properties of proteins, having a better alternative seems necessary (Yeom *et al.*, 2010). Subcritical water extraction (SWE) is a novel method to extract proteins, bioactive components, and essential oils (Narita and Inouye, 2012). It is applied to process the food protein to modify the functional properties, such as solubility, EAI, degree of hydrolysis (DH%), and protein content (Espinoza and Morawicki, 2012) It can increase the extraction yield, decrease the extraction time, and be cost-effective)Alboofetileh *et al.*, 2019, Wang *et al.*, 2018) There is limited knowledge about the functional properties and structure changes of BRM protein isolate after SWE. The optimal SWE conditions would increase the effectiveness of the extraction. This study aims to increase the functional properties of rice protein isolate. In this regard, the SWE method was used to produce the bran and RB isolate. The optimized SWE condition for rice protein isolate was discovered. SWE parameters, such as extraction time and temperature were optimized to obtain the optimal condition of rice milk production from a BRM. The BRM was determined for its physico-chemical properties. The aim of this study is using SWE as a new friendly extraction method for producing protein isolates (PI) of rice bran and rice combination as a byproduct of Iranian agriculture product.

Materials and Methods

All the chemicals were Sigma-Aldrich Chemicals Ltd. (St. Louis, MO, USA) and Merck Company (Germany). All chemicals were of analytical grade. High-quality rice and RB were purchased from Golestan Company.

Establishing the combined design for optimization of protein extraction: Combined design consisted of mixture design and response surface methodology (RSM) (Luga and Atis, 2018). The successful application of mixture models to predict the behavior of solvent mixtures has been reported previously for the crude extract and fractions from plant materials. According to mixture models, the extracted material seemed to depend on the amount of the solvent in the extracting medium (Costa and Garcia, 2016). In the present study, the mixture components were the rice (A) and bran (B), with ratios ranging between 8-12 and 88-92, respectively. The process factors in RSM included the SWE time (C) and within the ranges of 15-45 min the particle size (D) type (F = 420 µm and G = 710 µm). The factor's levels and constraints in the experimental design are presented in **Table 1**. The response under the experimental conditions was designated using the D-optimal design. Overall, 25 experimental points were obtained. The Design-Expert software (version 11.1.2, Stat-Ease Inc., Minneapolis, USA) was applied for modeling the adjacent composition of RBM as a function of the mixture component concentration (i.e., RB and broken rice) and levels of process factors (i.e., time and particle size). Equation (1) was used for fitting the second-order model to the independent variables in RSM.

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{i=1}^{k} \beta_{jj} X_j^2 + \sum_{i=1}^{j-1} \sum_{j=1}^{k} \beta_{ij} X_i X_j + \varepsilon \qquad (1)$$

Where Y (protein) is the predicted response; *i* and *j* are the linear quadric coefficient; X_i and X_j are the encoded independent variables; *k* is the number of optimized studied factors; β_0 is a

constant coefficient; β_j, β_{jj}, and β_{ij} are the interaction coefficient of linear, quadratic, and second-order terms, respectively; and ε represents the error. **Equation (2)** exhibits the canonical form of the *quadrati*c model for the mixture design that corresponds to a linear model if only the first part is used for the fitting:

$$Y = \sum_{i=1}^{q} \beta_i X_i + \sum_{i<j}^{q} \sum \beta_{ij} X_i X_j \qquad (2)$$

Where, β_i is the expected response to the pure mixture ($X_i = 1, X_j = 0, j \neq i$). The first part of **Equation (2)** refers to the response as long as the mixture contains exactly additives, and no interactions occur among the components of the mixture (i.e., linear models). The term $\beta_{ij} X_i X_j$ indicates the different response to the linear model due to interactive effects between two components. This interaction may be antagonism ($\beta_{ij} < 0$) or synergism ($\beta_{ij} > 0$) (SantafÃ©-Moros *et al.*, 2005). Under the predefined conditions, the software was used to draw the contour and 3D surface plots. Optimization was performed by maximizing the protein of the BRM extract.

Subcritical water extraction: During the extraction process, the laboratory mill (IKA 1603600 M 20 Universal Mill, 230V, USA) was used for grinding broken rice into flour to obtain homogenous particles. Then, sieves of different mesh sizes were used to separate two fractions of < 420 microns and < 710 microns (mesh N° (ASTM E11) 40, sieve size 420 µm and N° (ASTM E11) 25, sieve size 710 µm, Sarv azma co, Iran). The resultant products were individually packed in a sealed plastic bag and stored at -18 °C until the subsequent use. SWE was performed using synthwave apparatus (Milestone, Bergamo Italy) with some modification. For the preparation of the samples according to runs determined by the combined design, broken rice and RB were mixed in water for 15 min by an industrial blender (IKA MYP2011100) until a homogeneous mixture was obtained. Subsequently, the substrates were subjected to SWE (120 ºC for 15-45 min). After that, the BRM extract was allowed to cool down to room temperature for around 15 min and sifted in a previously sterilized cotton fabric and through a two-mm mesh strainer (Costa and Garcia, 2016).

Protein isolate (PI) production: For preparing the PI, after SWE, the sample (The optimum sample of SWE) was mixed in a blender (IKA 1100, Germany) at room temperature for 10 min. The pH of the mixture was then adjusted to 9.0 with 1 N NaOH, stirred for two h at room temperature to extract the protein, and centrifuged at 12000 rpm for 20 min at 4 °C to remove the insoluble materials. The supernatant was collected and adjusted to pH 4.0 with 1 N HCl and centrifuged at 12000 rpm for 15 min at 4 °C to recover protein precipitate. Then the precipitate was washed twice with distilled water for 30 min to remove all soluble materials. The precipitate was then suspended in distilled water (1:1, w/v) and neutralized by adjusting the pH to 6 and then freeze-dried for later evaluations (Gbadamosi *et al.*, 2012)

Physicochemical analysis: The moisture, protein, fat, fiber, ash, starch, and carbohydrates contents were determined by standard AOAC Methods (AOAC., 2005). Total nitrogen content of the samples was measured according to the Kjeldahl method and crude protein content using the 6.25 conversion factor (AOAC., 2005). Soluble protein was determined by the Bradford procedure using Coomassie Brilliant G-250 dye binding and bovine serum albumin as the standard (Chen *et al.*, 2011).

Solubility: The protein solubility (PS) of the samples was determined as the following: first, the protein sample was dispersed in deionized water, and the pH was adjusted to a range of 3 to 10 using 0.1 mol/l HCl or NaOH, magnetically stirred at room temperature for 30 min. After pH adjustment, the samples were centrifuged at 10000 rpm for 20 min at 20 °C. Then each supernatant was filtered with Whatman filter paper (No. 1) (Chen *et al.*, 2011). The soluble protein was measured using the Kjeldahl method according to AOAC Official Method 930.29 (AOAC., 2005). Protein solubility was calculated as follows.

$$\text{PS (\%)} = \frac{C_s}{C_i} \times 100$$

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C_S: The protein concentration in the supernatant (mg/ml)

C_i: The protein concentration in the initial suspension (mg/ml)

Foaming capacity (FC): To measure the foaming capacity and foaming stability, 20 ml of protein solution were whipped in a mechanical homogenizer (Kinematic PT1200E, Swiss) at 10000 rpm for 3 min (Ogunwolu *et al.*, 2009) Foaming capacity was calculated with the following equation.

$$\text{FC (\%)} = \frac{\text{volume after whipping} - \text{volume before whipping}}{\text{volume before whipping}} \times 100$$

Foaming stability: Foam stability (FS) was measured when the foam rested for 30 min. FS was determined from the following equation.

$$\text{FS} = \frac{(V_0 \times t)}{\Delta V}$$

$V_\circ$: The foam volume at 0 min

Δv: The change of the foam volume during the time interval

t: 30 min

Emulsifying properties: Emulsifying activity (EA) and emulsion stability (ES) were determined using the Klompong and Benjakul method (Khajenoori *et al.*, 2009) Moreover, 24 ml of proteins solution was homogenized in a mechanical homogenizer at 10000 rpm for 1 min to produce the emulsion. The 50 µl of the emulsion was taken out of the bottom of the container at 0 and 10 min after homogenization and then mixed with 5 ml sodium dodecyl sulfate solution (0.1 %). The absorbance of emulsions was measured at 500 nm with the UV–VIS-Spectrophotometer (UV-2800, China). The absorbance that measured immediately after the emulsion formation was called the emulsifying activity of the protein, and emulsion stability was determined using the following formula.

$$\text{ES} = \frac{(T_0 \times \Delta t)}{\Delta T}$$

ΔT: The change in turbidity of T_0 in the Δt (time interval).

T_0: The absorbance of the emulsion after homogenization.

Degree of hydrolysis (DH): DH of the PI was determined by determining the soluble nitrogen content. An aqueous dispersion of PI (10 ml) was mixed with trichloroacetic acid (TCA) (20%) and then centrifuged for 20 min in 10000 rpm at 4 °C (Yoon *et al.*, 2009). The soluble nitrogen of supernatant was measured by the Kjeldahl method (AOAC., 2005). The DH (%) was calculated based on the following formula.

$$\text{DH (\%)} = \frac{\text{Soluble nitrogen in 10\% TCA solution (mg)}}{\text{Total nitrogen (mg)}} \times 100$$

Data analysis: Data analysis of the variance was performed with the Statistical Analysis System software 8.2 (SAS, USA). All experiments were tested three times, and all data were reported as means ± SD for functional properties. Differences among means were evaluated using Duncan's multiple range tests at a significance level of P-value < 0.05). In all statistical analyses, P-value < 0.05 was considered to be significant. Plots were plotted in Excel 2016.

Results

Physicochemical properties: The approximate composition of RB, rice, and RBR (combination of RB and rice in a proportion of 8:92) are shown in **Table 2**. According to this table, the RBR was found to contain greater nutrients, such as protein, soluble protein, fat, crude fiber, ash, starch, and total carbohydrate than in the RB and rice. **Table 2** also indicates that carbohydrates, mainly starch, are the major components of both the RBR and rice. Protein is the second major component of rice after starch. There are greater amounts of protein and crude fiber in bran because they are primarily concentrated in the outer bran layer of rice grain.

Model analysis: The Design-Expert software was applied to develop a good model of protein, and the optimal values were estimated based on the model equation. Analysis of variance (ANOVA) was conducted to investigate the adequacy of the final model to describe the data and quality of the model fit. The significant level of the model was expressed as the p-value of <0.05.

Model analysis for protein content: Protein content fit to a reduced quadratic x 2-FI (2-factor interaction) model. The ANOVA results and model

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summary statistics for protein are shown in **Table 3**. The p-value of the model was less than 0.05. Generally, p-value < 0.05 shows the statistical significance of the model, whereas p-value > 0.05 represents the lack of fit (i.e., it is insignificant relative to the pure error) (Yang *et al.*, 2014). In the model analysis for protein responses, there was no p-value for lack of fit, since the pure error of the estimate was zero. The model f-value of 30519.71 demonstrated that the model was significant too, and there was only a 0.01% chance and the f-value could be obtained because of noise. In this case, A, B, AC, AD, AE, BC, BD, BE, CD, ABC, ABD, ABE, ACD, ACE, ADE, BCE, BDE, ABCD, and ABDE were significant model terms. The coefficient of variation (CV%), which expresses the standard deviation as a percentage of the mean, was 0.23 for this response. The low CV values mean better reproducibility. CV deals with the extent to which the data were dispersed. High CV values imply that there are significant variations in mean values, and an adequate response model is satisfactorily developed (Liyana-Pathirana and Shahidi, 2005). High values of the coefficient of determination (R^2 = 0.9997) were observed for protein, suggesting a high degree of correlation between the predicted and experimental values (Martínez *et al.*, 2011) Moreover, the predicted R^2 value of 0.9995 was in reasonable agreement with the adjusted R^2 value of 0.9996. In other words, the difference was less than 0.2. The signal-to-noise ratio expressed by Adeq-Precision is desirable when it is > 4. In this study, the Adeq-Precision value of 643.788 revealed adequate model discrimination used for the design space navigation.

Final equation generated for protein content (%): According to the experimental results, the regression model (**Equation 3**) was developed to predict the percentage of protein.

Protein= 0.602 * A + 0.695 * B - 0.0042 * AB + 0.061 * AC + 0.250 * AD -0.003 * AE + 0.03989* BC + 0.1499 * BD + 0.0048* BE + 0.0097 * CD - 0.053 * ABC + 0.0390* ABD + 0.165 * ABE - 0.010* ACD + 0.0234 * ACE + 0.004 * ADE + 0 * BCD + 0.019 * BCE + 0.060 * BDE + 0.038 * ABCD + 0.002 * ABCE -0.069 * ABDE (**3**)

The effect of rice (A) bran (B) ratio and the effect of extraction time (C) and solid/water ratio (D) on protein values at optimal particle size (E) are indicated in **Figures** (**Figure 1a and 1b**), respectively. It was found out that the percentage of protein increased as the broken rice/ bran ratio increased. In addition, the increase in the percentage of protein was remarkable for the samples with higher rice content. The maximum protein was achieved when the rice/ bran ratio was 92:8. **Figure 1b** also shows that by increasing extraction time and solid/water ratio, protein content extract increased. The maximum protein was achieved when the solid/water ratio was 0.12% and time 45 min was the highest. Despite the evidence that increased time and temperature of SWE have led to the enhanced yield of extracted protein and produced peptides or amino acids, extraction may denature proteins and alter their functionality features. Therefore, it is essential to select SWE parameters for the best results.

Functional properties: After optimization of the sample, and selecting rice/ bran ratio (92:8), time (45 min) and particle size F (420 microns), the sample was prepared from optimized parameter. Then, the effect of extraction temperature on solubility, and extraction time on other functional properties of the sample were compared with the optimized sample.

Solubility: Solubility is an essential factor that affects the structure and also functional properties of proteins. The PIs act as functional ingredients in the food system (Cao *et al.*, 2009). There are some factors affecting the PI's solubility. In this study, the effect of extraction time and temperature on the solubility of the samples was studied.

Figure 2(a) shows the effect of SWE temperature (110–130 °C) on the solubility of PI. The highest PI solubility was at 120°C. After discovering the optimum temperature (120 °C) for SWE, the effect of time on PI solubility was evaluated. **Figure 2(b)** reveals that as time passes from 15 to 45 min, the PI solubility increased significantly (P < 0.05).

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Foaming capacity and foaming stability): Based on **Figure 3**, foaming properties were changed. Foaming capacity (FC) and foaming stability (FS) showed a similar trend of increase during time. Both FC and FS of PI were higher than other samples in 45 min. The formation of foam is affected by three factors, including penetration, transportation, and rearrangements of the molecule under the air-water surface.

Emulsifying activity index and emulsion stability index: The emulsifying properties of PI, EAI and ESI under different time intervals are shown in **Figure 3**.

Degree of hydrolysis (DH): As shown in **Figure 5**, DH of the PI increased during time and reached the highest amount in 45 min.

Table 1. Factors, factor levels, and constraints considered for the experimental design.

				Variable level		
Variables	**Symbol**	**Units**	**Type**	**-1**	**0**	**+1**
Bran	A	-	Mixture	8	10	12
Broken rice	B	-	Mixture	88	90	92
Time	C	Minutes	Numeric	15	30	45
Solid/water ratio	D	-	Numeric	0.04	0.08	0.12
					Levels	
Particle size	E	Micron	Categoric	F (410)		G(720)

Table 2. The composition of RB, Rice and a combination of RB, and rice (g/100 g).

	Content							
Variables	**Moisture content**	**Protein**	**Soluble protein**	**Fat**	**Crude fiber**	**Ash**	**Starch**	**Total carbohydrate**
RB	13.93±1.17$_a$	15.91±1.45	1.81±0.06	5.67±0.51	4.12±0.11	11.73±1.1	18.16±2.74	47.34
Rice	11.23±0.72	6.82±0.23	0.58±1.02	0.55±0.34	0.4±0.45	0.7±0.21	76.68±2.12	80.30
RBR	11.52±0.93	7.61±0.34	1.69±0.09	0.98±0.54	0.73±0.60	1.45±0.34	70.95±2.12	76.80

RB: rice bran, RBR: rice bran and rice combination; [a] : Mean ± SD

Table 3. Model summary statistics for protein (%).

P-value	<0.0001	R^2	0.9997
F-value	30519.71	Adjusted R^2	0.9996
P-value of lack of Fit	-	Predicted R^2	0.9995
CV%	0.23	Adequate Precision	643.7880

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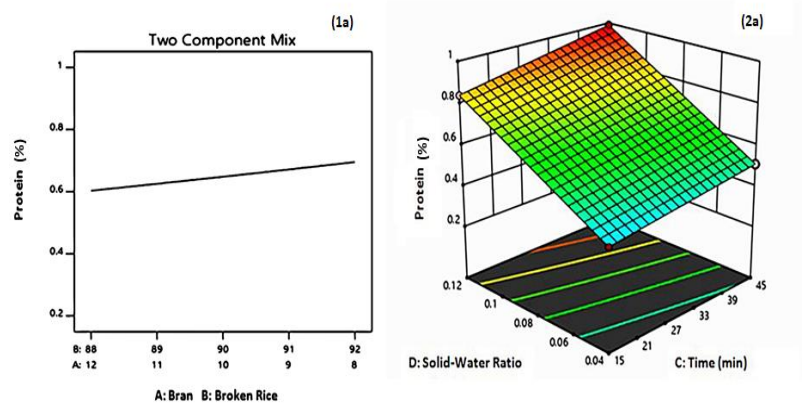


Figure 1. The effect of rice (A) bran (B) ratio (Figure 1a**),** and the effect of extraction time (C) and solid/water ratio (D) on protein values at optimal particle size (E).

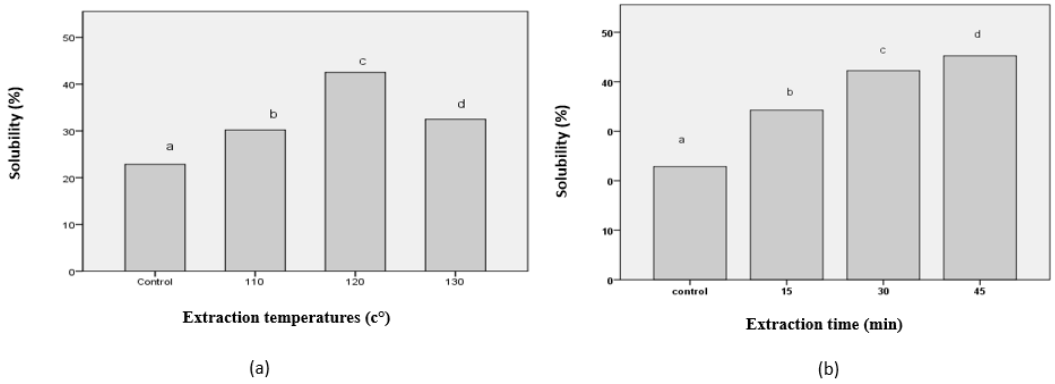


Figure 2: Solubility of PI at different temperatures (**a)**, different time **(b)**. Different letters on the top of the bars denote significant differences ($P < 0.05$). Control: PI of RBR before extraction.

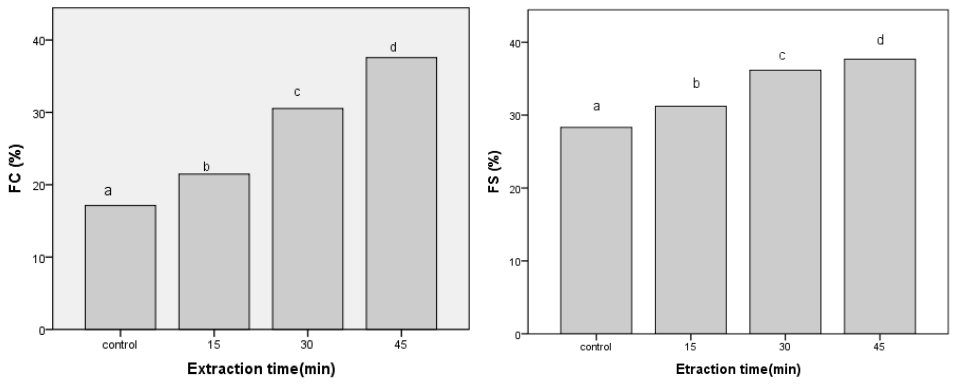
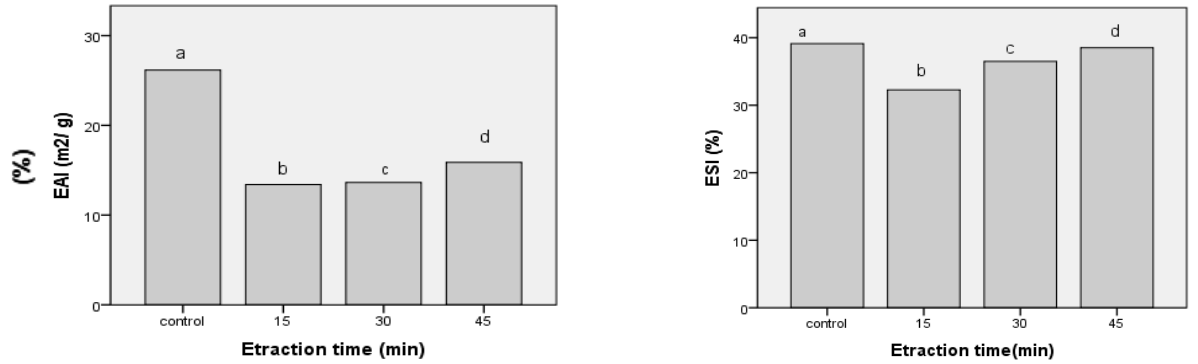


Figure 3: Foaming capacity **(a)** and foaming stability **(b)** of PI at different times (15, 30, 45 min) at 120 °C. Different letters on the top of the bars denote significant differences ($P < 0.05$). Control: PI of RBR before extraction.



(a) (b)

Figure 4. Emulsifying activity index (EAI) **(a)**, emulsifying stability index (ESI) **(b)** of PI at different times (15, 30, 45 min) at 120°C. Different letters on the top of the bars denote significant differences ($P < 0.05$). Control: PI of RBR before extraction.

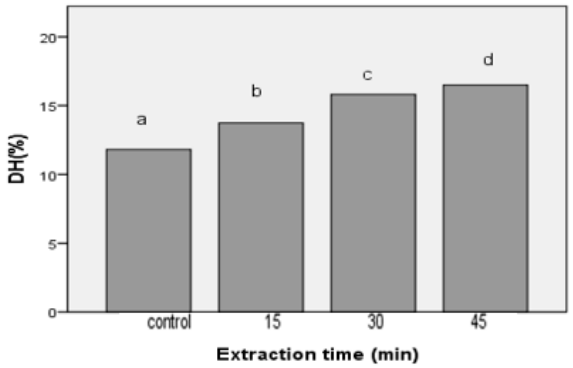


Figure 5. The DH of PI at different times (15, 30, 45 min) at 120 °C. Different letters on the top of the bars denote significant differences ($P < 0.05$). Control: PI of RBR before extraction.

Discussion

According to results, by increasing rice/broken rice ratio, protein content increased, solubility in water of the RB protein was limited compared to that of the white rice protein. It was due to the fact that there was a strong aggregation through hydrophobic interactions, and its association with the cell wall was extensive. Nevertheless, subcritical water may improve its solubility due to the hydrolysis of the RB protein and cell wall (Fabian and Ju, 2011). Additionally, SWE parameters have a positive effect on the protein content. In the present study, increasing solid/water ratio and extraction parameters, such as extraction time lead to an increase in the protein. There are some studies elucidating the influence of extraction factors, such as time and SWE temperature on the compounds of the BRM extract. For instance, Seçmeler *et al.* examined the kinetic properties of the RB protein hydrolysis in subcritical water. Their findings exhibited that amino acids were on the rise by increasing temperature and reaction time. They also reported that the optimized SW condition could help obtain amino acid recovery during the RB extraction process (Seçmeler *et al.*, 2018).

The effect of the temperature and extraction time on the production of milk analogues from RB protein hydrolysate was investigated using the subcritical water technique by Wongthaweewatana. In their study, temperature and reaction time were shown to have direct impacts on both protein extraction and the molecular size of RB protein, which significantly increased the production of milk analogues (Wongthaweewatana *et al.*, 2021)

Extraction parameters, such as time and temperature, also affect functional properties. In the present study, the solubility increased by rising

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temperature to 120 °C due to the hydrolysis reaction, but at 130 °C decreased. It is due to the fact that in higher temperature (more than 120 °C), the aggregation was started and affected the solubility which is in accordance with previous studies (Cao *et al.*, 2009, Teo *et al.*, 2010). The investigation of extraction temperature also showed that, solubility increased by increasing extraction temperature from 15 to 30 min. The effect of temperature on PI solubility in the SWE method is also reported to be the result of the average particle size of PI. The particle size was decreased gradually up to 120 °C, and after that, it increased to 160 °C, so the solubility decreased (Martínez and Pilosof, 2012). SWE parameters also affect FC and FS. SWE increased flexibility and hydrophobicity of protein's surface (Yeom *et al.*, 2010). At high temperatures (more than 100 °C), with passage of time up to 1 hour, a severe degradation and disaggregation in proteins cause an increase in FC and FS (Yuan *et al.*, 2012). First, the proteins started to unfold, and then the particle accumulations made higher foaming properties (Zhang *et al.*, 2015). In this study, the aggregation of proteins would be the main factor of rising FC and FS by increasing time at 120 °C, which is in accordance with the previous studies (Yuan *et al.*, 2012). The protein's emulsifying properties are affected by some factors like surface charge, hydrophobicity, hydrophilicity, and solubility of proteins (Piotrowicz and Salas-Mellado, 2017). The SWE influenced the EAI and ESI. SWE treatment enhanced EAI significantly with the increasing SWE time (**Figure 4a**) and slightly influenced the ESI (**Figure 4b**). The samples with higher extraction time showed higher EAI and ESI. Wang *et* al. reported similar results (Wang *et al.*, 2008). In the emulsification process, the hydrophobic and also aggregation interactions are the main factors that affect the emulsifying properties of proteins (Manoi and Rizvi, 2009). It is stated that the protein unfolding state and the exposure of the hydrophobic groups made after SWE would be responsible for enhancing the emulsifying properties (EAI and ESI). Furthermore, regarding DH%, as another functional characteristic of the sample, by increasing SWE time this parameter also increased. The same trend of DH was reported by Yeom and Yoon. The hydrolysis increases the number of available hydrophilic groups and reduces the protein molecular weight, which results in changes in the functional properties and increases the DH (Betancur- Ancona *et al.*, 2009, Yeom *et al.*, 2010, Yoon *et al.*, 2009).

Conclusion

SWE, as a novel extraction method, improved the solubility, emulsifying, and foaming properties and degree of hydrolysis of rice protein isolate. Rice is a great source of nutrients like proteins, carbohydrates, and minerals used around the world, especially in Asian countries. Also, rice products can be used as the primary raw material for many other functional food and beverages. SWE is an environmentally friendly and also economical method to produce protein isolate from different raw cereals. In this study, the optimal SWE conditions for producing protein from the combination of rice 92 and RB 8 was at 120°C for 45 min. Furthermore, the suitable properties of PI are obtained at 45 min. SWE could significantly enhance functional properties, such as solubility, EAI, and DH% of BRM PI, so it can be used as a great alternative technique to modify the properties of various proteins isolate for specific purposes in the food industry.

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Author's contribution

Raiesi Ardali F, Ebrahimzadeh Mousavi MA and Sharifan A involved in conceptualization, data curation, formal analysis, and writing the original draft. Mohammad Mortazavian A and Jannat B involved in validation, visualization, funding acquisition, formal analysis, project administration, supervision and writing the draft of manuscript. All authors approved the final the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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