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THE FUNCTIONAL PROPERTIES OF RICE PROTEIN ISOLATE EXTRACTED BY SUBCRITICAL WATER

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ABSTRACT

Subcritical water extraction is a unique technique to produce protein isolate from foodstuffs. In this study, the protein isolate from rice bran and rice (in a proportion of 8:92) was treated by subcritical water extraction. The main independent variables in extraction process were: the extraction time (15, 30, 45 min) and the extraction temperature (110, 120, 130 ºC). The Solubility, foaming capacity and foaming stability, emulsifying activity and emulsion stability and the degree of hydrolysis of the rice protein isolate were determined at 120 ºC in different contact time (15, 30, 45 min). The optimum subcritical water extraction condition was obtained at 120 ºC in 45 min. Better functional properties were observed in subcritical water extraction products that indicated this method can be applied as a novel alternative technique to modify the properties of rice proteins isolate for the functional purposes in future.

Keywords: Rice; bran; subcritical water; extraction, Functional

INTRODUCTION

Rice protein is one of the common used proteins in the world. Rice is the main part of meals in Asia countries that nearly used everyday (**Bandumula, 2017**). Rice protein has been applied in many food formulations due to the excellent functional properties such as solubility, emulsifying and foaming properties (**Mihucz *et al.*, 2010**).
Rice bran is a rice milling by- product that is obtained by removing the rice seeds hull (**Kahlon, 2009**). It is very nutritious because of having protein, essential fatty acids, fiber, vitamin B group and minerals (**Yılmaz, 2016**). Also bran has some antioxidants like tocopherol, oryzanol, tocotrienol, and ferrulic acid (**Zubaidah *et* al., 2012**). The lysine content of rice bran is nearly 4 times higher than rice as it is located in proteins of the out layer of cereals legumes (**Sanni *et* al., 2020**).
Having all these advantages, there is a need of a practical procedure for the production of rice and rice bran 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 so, a novel environmentall friendly method is needed for extraction in food industry. (**Chen *et* al., 2015**).
As the common methods of extraction such as enzymatic hydrolysis or modification, high temperature treatments are so costly and they 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 used to extract proteins, bioactive components and essential oils (**Narita and Inouye, 2012**). It is applied to process the foodstuffs' protein to modify the functional properties (**Espinoza and Morawicki, 2012**). Also it would increase the extraction yield, decrease the extraction time and be cost effective as well (**Wang *et* al.,2018; Alboofetileh *et* al.,2019**).
There are limited knowledge about the functional properties and structure changes of PI after SWE. The investigation of optimal SWE conditions would increase the effectiveness of the extraction. The main objective of this study were to increase the functional properties of rice protein isolate. In this regard the SWE method was used to producet the bran and rice bran isolate. The optimised SWE conditions for rice protein isolate was discovered. In our study, therefore, subcritical water extraction parameters such as extraction time and temprature were optimized in order to obtain the optimal condition of rice milk production from RBR. The RBR was determined for their physico chemical properties.

MATERIAL AND METHODS

Chemicals

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 rice bran were purchased from golestan Company (Iran).

Rice and rice bran preparation

Rice and rice bran (ratio of 92:8) were ground into powder by the help of a laboratory mill (Universal Mill, U.S.A). Then the powder was sieved (the mesh size < 710 micron).

Protein isolate (PI) production

For preparing the PI, the above combination was mixed with distilled water for 5 min using an industrial blender (IKA 1100, Germany) and then autoclaved (ALP CL-32L, Japan). After subcritical water extraction in autoclave, the sample mixed in a blender (IKA 1100, Germany) at for 10 min. The pH of the mixture was then adjusted to 9.0 with 1 N NaOH, stirred for 2 h at the room temperature to extract the protein, and then centrifuged at 9000×g 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 8000×g 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 estimated by standard AOAC Methods (**AOAC, 2005**). Total nitrogen content of samples was measured according to the Kjeldahl method and crude protein content by 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**).

The functional properties of PI

Solubility

The protein solubility (PS) of 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 the pH adjustments the samples were centrifuged at 10000 g 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 Kjeldahl method according to AOAC Official Method 930.29 (**AOAC, 2005**) protein solubility calculated as the following:

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

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 by the following equation:

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

Foaming stability (FS)

Foam stability (FS) was measured as the foam rested after 30 min. FS 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 by the help of Klompong and Benjakul method (**Khuwijitjaru *et al.*, 2011**). 24 mL of proteins solution were homogenized in a mechanical homogenizer at 10000 rpm for 1 min to produce the emulsion. The 50µL of emulsion were taken out of the bottom of the container at 0 and 10 min after homogenization and then mixed with 5 mL sodium dodecyl sulphate 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 as the emulsifying activity of protein, and emulsion stability was determined as:

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

$\triangle$T: The change in turbidity of T_0 in the $\triangle$t (time interval).
T_0: The absorbance of 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 8900×g at 4°C (**Yoon *et al.*, 2009**). The soluble nitrogen of supernatant was measured by the Kjeldahl method (AOAC, 2000). The DH (%) was calculated as follows:

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

Statistical analysis

Statistical 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. Differences among means were evaluated using Duncan's multiple range tests at a significance level of $P<0.05$.

RESULTS AND DISCUSSION

Physicochemical properties

The approximate composition of rice bran (RB), rice and RBR (combination of rice bran and rice in a proportion of 8:92) are shown in Table 1. According to this table, the BRR was found to contain greater content of nutrients such as protein, soluble protein, fat, crude fiber, ash, starch, and total carbohydrate than that in the RB and rice. Table 1 also indicate that carbohydrates, mainly starch, are the major components of both the RBR and rice. Protein is the second major component of rice after starch. Protein and crude fiber are in more amount in bran because they are mostly concentrated in the outer bran layer of rice grain.

Table 1 The composition of Rice bran, Rice and combination of rice bran and rice (g/100 g)

index	content							
	Moisture content	**protein**	**Soluble protein**	**Fat**	**Crude fiber**	**Ash**	**Starch**	**Total carbohydrate**
RB	13.93±1.17	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	76.8

RB: rice bran, RBR: rice bran and rice combinatin
Mean values ± SD of triplicate replicant.

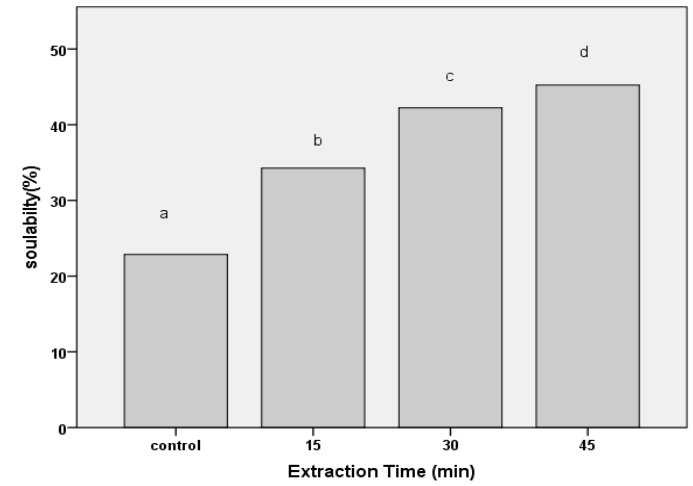
PI properties

Solubility

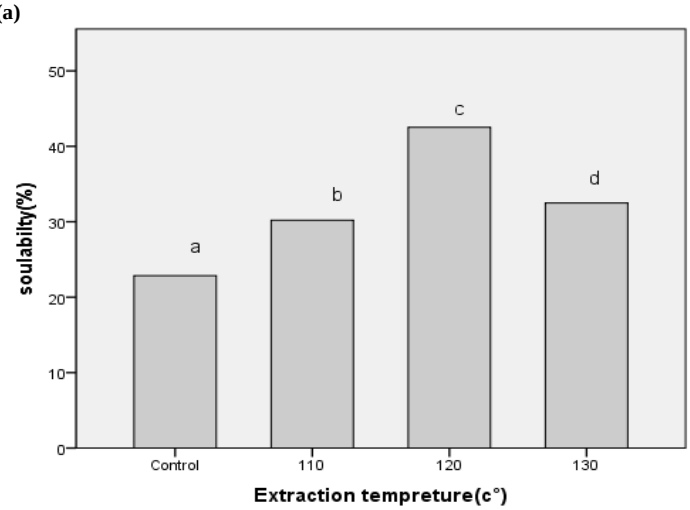
Solubility is an important factor that affects the structure and also functional properties of proteins. The PIs act as functional ingredients in food system (**Cao *et al.*, 2009**). There are some factors that affects the PI's solubility. In this study the effect of extraction time and temperature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

Fig. 1(a) shows the effect of SWE temperature (110–130°C) on the solubility of PI. The highest PI solubility was at 120°C. The solubility of PI increased by temperature rising up to 120°C due to the hydrolysis reaction, but at 130 °C it decreased, because the in further temperature (more than 120°C) the aggregation was started and affected the solubility which is in accordance with previous studies (**Teo *et al.*, 2010; Cao *et al.*, 2009**). The effect of temperature on PI solubility in SWE method is also reported to be as 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 *et al.*, 2011**).

After discovering the optimum temperature (120°C) for SWE, the effect of time on PI solubility was evaluated. As shown in Fig. 1(b) by passing time from 15 to 45 min, the PI solubility increased significantly ($p<0.05$).

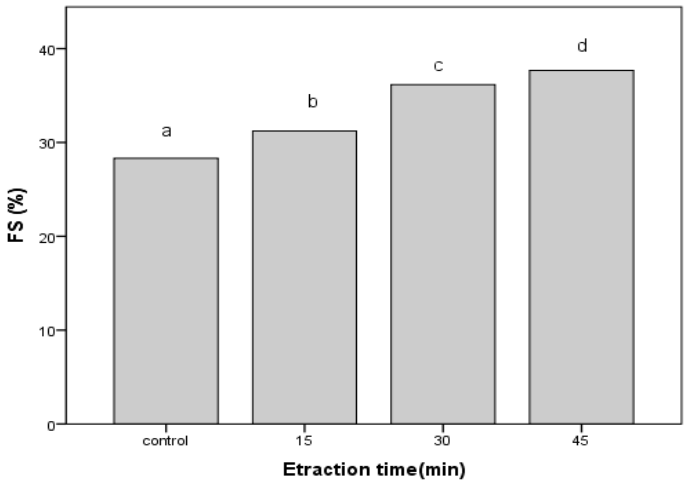


(a)

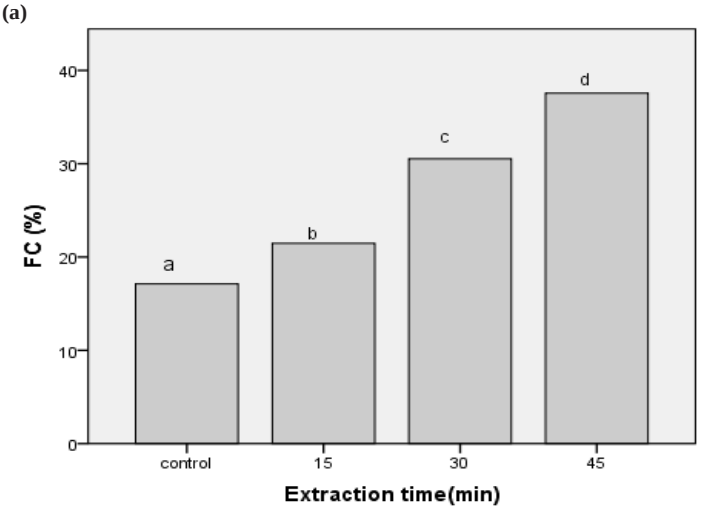


(b)

Figure 1 Solubility of PI at different temperatures **(a)**, different time **(b)**. Different letters on the top of the bars denote significant difference ($p<0.05$). Control: PI of RBR before extraction.



(a)



(b)

Figure 2 Foaming capacity **(a)** and foaming stability **(b)** of PI at different time (15, 30, 45 min) at 120°C. Different letters on the top of the bars denote significant difference ($p<0.05$)
Control: PI of RBR before extraction.

Foaming properties of PI

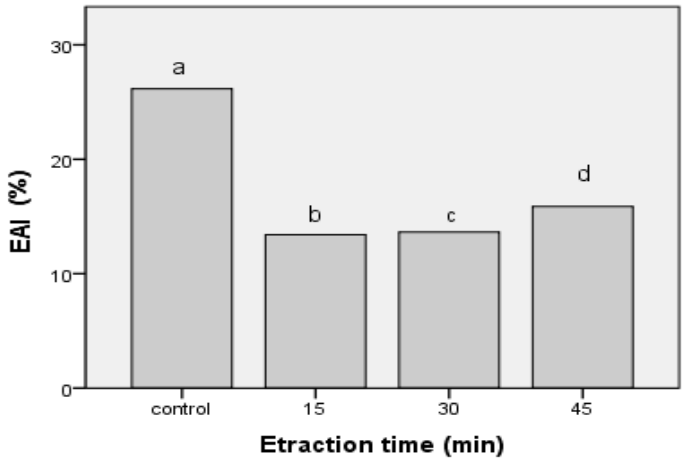
Foaming capacity (FC) and Foaming stability (FS)

As shown in Fig. 2, foam properties are changed. Forming capacity (FC) and forming stability (FS) showed similar trend of increase during the time period. Both FC and FS of PI were higher other samples in 45 min. The formation of foam is affected by 3 factors; penetration, transportation and rearrangements of the molecule under the air-water surface. SWE increased flexibility and hydrophobicity of protein's surface (**Yeom *et al.*, 2010**). At high temperatures (more than 100 °C) by passing 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 particles accumulations make higher foaming properties (**Zhang *et al.*, 2014**). In this study, the aggregation of proteins would be the main factor of rising FC and FS by increasing time at 120 °C, that is in accordance with the previous studies (**Yuan et al., 2012; Ruíz-Henestrosa *et al.*, 2009; Martínez *et al.*, 2009**).

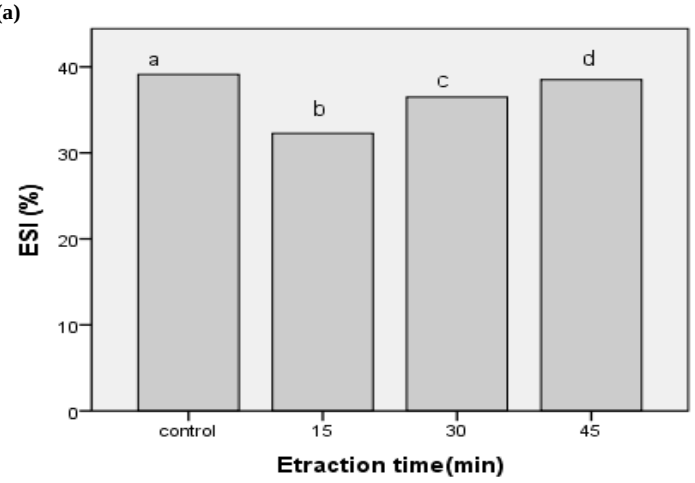
Emulsifying properties of PI

Emulsifying Activity (EA) and Emulsion Stability (ES)

The emulsifying properties of PI, the emulsion activity (EAI) and emulsion stability index (ESI) under different time intervals are shown in Fig. 3. The protein's emulsifying properties are affected by some factors like surface charge, the hydrophobicity, hydrophilicity and solubility of proteins (**Piotrowicz and Salas-mellado, 2017**). The SWE influenced the EAI and ESI. SBW treatment enhanced the EAI significantly with the increasing SWE time (Fig. 3a) and also slightly influenced the ESI (Fig. 3b). The samples with higher extraction time showed higher the EAI and ESI. Similar results were observed by **Wang *et al.* (2008).**
In 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's unfolding state and exposuring of the hydrophobic groups which made after SWE would be responsible for enhancing the emulsifying properties (EAI and ESI).



(a)



(b)

Figure 3 Emulsifying activity index (EAI) **(a)**, emulsifying stability index (ESI) **(b)** of PI at different time (15, 30, 45 min) at 120°C. Different letters on the top of the bars denote significant difference ($p<0.05$)
Control: PI of RBR before extraction.

Degree of hydrolysis (DH)

As shown in Fig. 4, DH of the PI increased during the time period and reached to the highest amount in 45 min. The same trend of DH was reported by **Yeom *et al.* (2010) and Yoon *et al.* (2009)**. The hydrolysis increases the number of available hydrophilic groups and reduces the protein's molecular weight, which results in the changes in the functional properties and increasing the DH (**David *et al.*, 2009**).

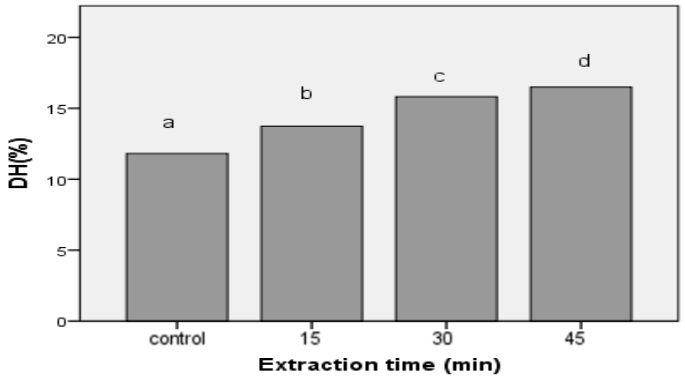


Figure 4 The degree of hydrolysis of of PI at different time (15, 30, 45 min) at 120°C. Different letters on the top of the bars denote significant difference ($p<0.05$)
Control: PI of RBR before extraction.

CONCLUSION

SWE as a novel extraction method, improved the solubility, emulsifying and foaming properties and degree of hydrolysis of rice protein isolate. Rice is the great sources of nutrients like proteins, carbohydrates and minerals used around the world especially in asian countries as their main course. Also rice products can be used as the main raw material for many other functional foods 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 PI from the combination of rice 92% and rice bran 8% was at 120°C for 45 min. SWE can degrade the protein's structure to make better functional properties so it can be used as a great alternative technique to modify the properties of various proteins isolate for specific purposes in food industry.

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