THE FUNCTIONAL PROPERTIES OF RICE PROTEIN ISOLATE EXTRACTED BY SUBCRITICAL WATER

Fatemeh Raeisi Ardali, Anousheh Sharifan, Seyed Mohammad Mousavi, Amir Mohammad Mortazavian, Behroz Jannat

Address(es): Dr. Anousheh Sharifan, Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran. Department of Food Science, Engineering and Technology, Faculty of Agricultural Engineering and Technology, University of Tehran, Iran. Faculty of Nutrition Sciences, Food Science Technology, National Nutrition and Food Technology Research Institute, Shahid Behesti University of Medical Sciences, Tehran, Iran.

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 °C). 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 °C in different contact time (15, 30, 45 min). The optimum subcritical water extraction condition was obtained at 120 °C 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 (Mihuc 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 outer 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 (Julliavndt 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 environmentally 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 (Yeam 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 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 produce 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 temperature 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 x 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 (Ghadamosi 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 filtrated 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:

\[
PS (\%) = \frac{S}{i} \times 100
\]

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

\(S\): 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:

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

Foaming stability (FS)

Foam stability (FS) was measured as the foam rested after 30 min. FS determined by the following equation:

\[
FS = \frac{(V_0 \times t)}{\Delta V}
\]

\(V_0\): The foam volume at 0 min.

\(\Delta V\): 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 protein solution were homogenized in a mechanical homogenizer at 10000 rpm for 1 min to produce the emulsion. The 50 µL 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:

\[
ES = \frac{(T_0 - \Delta T)}{\Delta T}
\]

\(\Delta T\): The change in turbidity of \(T_0\) in the \(\Delta t\) (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 8000-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:

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

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 BRR (combination of rice bran and rice in a proportion of 8:2) 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 BRR 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 | Moisture content | Ash content | Soluble protein | Fat content | Ash content | Crude fiber | Starch content | Total carbohydrate |
|-------|-----------------|-------------|-----------------|-------------|-------------|-------------|---------------|-------------------|
| RB    | 13.93±1.17      | 5.91±1.45   | 1.8±0.06        | 5.67±0.51   | 4.12±0.11   | 11.73±1.1   | 10.06±11.1    | 76.8±2.12        | 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    | 76.6±2.12     | 80.30             |
| BRR   | 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, BRR: rice bran and rice combinat |

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).
**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; Ruiz-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 exposing of the hydrophobic groups which made after SWE would be responsible for enhancing the emulsifying properties (EAI and ESI).
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.

REFERENCES
Alboofetileh, M., Rezaei, M., Tabarsa, M., You, S., Mariatti, F., & Cravotto, G. (2019). Subcritical water extraction as an efficient technique to isolate biologically-active fucoidans from Nizamuddinia zanardinii. International journal of biological macromolecules, 128, 244-253. https://doi.org/10.1016/j.ijbiomac.2019.01.119
AOAC (2005) Determination of Moisture, Ash, Protein and Fat. Official Method of Analysis of the Association of Analytical Chemists. 18th Edition, AOAC, Washington DC.
AOAC (2000) In Official Method of Analysis of AOAC Intl, (17th ed.). Association of Official Analytical Communities, Maryland, U.S.A.
Bandumula, N. (2018). Rice Production in Asia: Key to Global Food Security. Proceedings of the National Academy of Sciences. Biological Sciences, 1-6. https://doi.org/10.1074/jrst017.086777
Cao, X., Wen, H., Li, C., & Gu, Z. (2009). Differences in functional properties and biochemical characteristics of congegent rice proteins. Journal of Cereal Science, 50, 184-189 https://doi.org/10.1016/j.jcs.2009.04.009
Chen, H. M., Fu, X., & Luo, Z. G. (2015). Properties and extraction of pectin-enriched materials from sugar beet pulp by ultrasonic-assisted treatment combined with subcritical water. Food chemistry, 168, 302-310. https://doi.org/10.1016/j.foodchem.2014.07.078
Chen, L., Chen, J., Ren, J., & Zhao, M. (2011). Modifications of soy protein isolates using combined extrusion pre-treatment and controlled enzymatic hydrolysis for improved emulsifying properties. Food Hydrocolloid, 25(5), 887-897. https://doi.org/10.1016/j.foodhyd.2010.08.013
David, B.A., Rogelio, M.K., Alma, C.C., Arturo, C.R., Ma Eugenia, J.F., & Luis, C.G. (2009). Functional properties of hydrolysates from Phaseolus lunatus seeds. Journal Food Science and Technology, 44, 128-137. https://doi.org/10.1111/j.1365-2621.2007.01690.x
Espinoza, A.D., & Morawicki, R.O. (2012). Effect of Additives on Subcritical Water Hydrolysis of Whey Protein Isolate. Journal of Agricultural Food Chemistry, 60(20), 5250-5256. https://doi.org/10.1021/jf300581r
Ghadamosi, S., Abiøse, S., & Aluko, R. (2012). Amino acid profile, protein digestibility, thermal and functional properties of Conophor nut (Tetracarpidium conophorum) defatted flour, protein concentrate and isolates. International Journal of Food Science, 47, 731-739. https://doi.org/10.1111/j.1365-2621.2011.02921.x
Jallivan, M., Kamali, H., & Nematollahi, A. (2013). Pressurized fluid extraction of rice bran oil using a modified supercritical fluid extractor and a central composite design for optimization. Journal of Liquid Chromatography and Related Technologies, 36(11), 1562-1574. https://doi.org/10.1002/jlc.2012.092152
Kahlón, T. S. (2009). Rice bran: production, composition, functionality and food applications, physiological benefits. CRC Press, Taylor and Francis Group: Boca Raton, FL, USA.
Manoi, K., & Rizvi, S.S.H. (2009). Emulsification mechanisms and characterizations of cold, gel-like emulsions produced from texturized whey protein concentrate. Food Hydrocolloids, 23(7), 1837-1847. https://doi.org/10.1016/j.foodhyd.2009.02.011
Martínez, K.D., Ganesan, V., Pilosof, A.M.R. & Harte, F.M. (2011). Effect of dynamic high-pressure treatment on the interfacial and foaming properties of soy protein isolate–hydroxypropylmethylcelluloses systems. Food Hydrocolloids, 25 (6), 1640-1645. https://doi.org/10.1016/j.foodhyd.2011.02.013
Mihuze, V. G., Silversmit, G., Szaloki, I., De Sambier, B., Schoonjans, T., Tatár, E., & Záravy, G. (2010). Removal of some elements from washed and cooked rice studied by inductively coupled plasma mass spectrometry and synchrotron based confocal micro-X-ray fluorescence. Food Chemistry, 121(1), 290-297. https://doi.org/10.1016/j.foodchem.2009.11.060
Nair, Y., & Iouye, K. (2012). High antioxidant activity of coffee silverskin extracts obtained by the treatment of coffee silverskin with subcritical water. Food Chemistry, 135(3), 943-949. https://doi.org/10.1016/j.foodchem.2012.05.078
Piotrowicz, I.B.B., & Salas-mellado, M.M. (2017). Protein concentrates from defatted rice bran: preparation and characterization. Food and Technology, 37, 165-172. DOI: http://dx.doi.org/10.1590/1678-457X.34816
Ruiz-Henestrosa, V.P., Sánchez, C.C., Pedroche, J.J., Millán, F., & Rodríguez, J.M. (2009). Improving the functional properties of soy glycinin by enzymatic treatment Adsorption and foaming characteristics. Food Hydrocolloid, 23(2), 377-386. https://doi.org/10.1016/j.foodhyd.2008.03.011
Sanni, T.A., Gbolagade, O.H., Ogunbusola, E.M., Araoye, K.T. (2020). Quality assessment of cookies made from composite flour of wheat, sorrel seed protein

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).

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).

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 word 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.
isolate and yellow cassava flours. *Journal of Microbiology, Biotechnology and Food Science*, 9 (6), 1073-1079. [https://doi.org/10.15414/jmbfs.2020.9.6.1073-1079](https://doi.org/10.15414/jmbfs.2020.9.6.1073-1079)

Teo, C.C., Tan, S.N., Yong, J.W.H., Hew, C.S, & Ong, E.S. (2010). Pressurized hot water extraction (PHWE). *Journal of Chromatography, 121*(16), 2484-2494. [https://doi.org/10.1016/j.chroma.2009.12.050](https://doi.org/10.1016/j.chroma.2009.12.050)

Wang, Y., Luan, G., Zhou, W., Meng, J., Wang, H., Hu, N., & Suo, Y. (2018). Subcritical water extraction, UPLC-Triple-TOF/MS analysis and antioxidant activity of anthocyanins from Lycium ruthenicum Murr. *Food chemistry*, 249, 119-126.

Wang, X.S., Tang, C.H., Li, B.S., Yang, X.Q., Li, L., & Ma, C.Y. (2008). Effects of high-pressure treatment on some physicochemical and functional properties of soy protein isolates. *Food Hydrocolloid*, 22(4), 560–567. [https://doi.org/10.1016/j.foodhyd.2007.01.027](https://doi.org/10.1016/j.foodhyd.2007.01.027)

Yılmaz, N. (2016). Middle infrared stabilization of individual rice bran milling fractions. *Food chemistry*, 190, 179-185.

Yeom, H.J., Lee, H.E., Ha, M.S., Ha, S.D., & Bae, B.D. (2010). Production and Physicochemical Properties of Rice Bran Protein Isolates Prepared with Autoclaving and Enzymatic Hydrolysis. *Journal of the Korean Society for Applied Bio Chemistry*, 55(1), 62–70. [https://doi.org/10.3839/jkabc.2010.011](https://doi.org/10.3839/jkabc.2010.011)

Yoon, J.H., Jung, D.C., Lee, E.H., Kang, Y.S., Lee, S.V., Park, S.R., Yeom, H.J., Ha, M.S., Park, S.K., Lee, Y.S., Ha, S.D., Kim, G.H., & Bae, D.H. (2009). Characteristics of a black soybean (Glycine max L. Merril) protein isolate partially hydrolyzed by alcalase. *Korean Journal of Food Science and Technologyology*, 38, 488–493.

Yuan, B., Ren, J., Zhao, M., Luo, D., & Gu, L., (2012). Effects of limited enzymatic hydrolysis with pepsin and high-pressure homogenization on the functional properties of soybean protein isolate. *LWT - Food Science and Technology*, 46(2), 453–459. [https://doi.org/10.1016/j.lwt.2011.12.001](https://doi.org/10.1016/j.lwt.2011.12.001)

Zubaidah, E., Nurcholis, M., Wulan, S. N., & Kusuma, A. (2012). Comparative study on Lactobacillus casei and newly isolated Lactobacillus plantarum B2 in Wistar Rats. *APCJEE Procedia*, 2, 170–177.

Zhang, Q.T., Tu, Z., Wang, H., Huang, Z.X., Fan, L.L., Bao, Z.Y. & Xiao, H. (2014). Functional properties and structure changes of soybean protein isolate after subcritical water treatment. *Journal of Food Science and Technology*, 22, 130-140. [https://doi.org/10.1007/s13197-014-1392-9](https://doi.org/10.1007/s13197-014-1392-9)