Rice Bran Water Extraction through Autoclaving and Sonication: Protein Content and Amino Acid Profile

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INTRODUCTION

Rice Bran, or *Oryza sativa*, is one of the by-products of paddy milling process in producing polished white rice (Parrado et al., 2006). Rice bran with its main components; protein, carbohydrate, fat, fiber, and ash, is also rich in nutrients with different types of vitamins, minerals and antioxidants (Abdul-Hamid et al., 2007; Parrado et al., 2006)

Generally, rice bran possesses considerably high content of protein ranging from 9 to 38% depending on the method of rice bran preparation and extraction (Ali et al., 2010; Chittapalo and Noomhorm, 2009; Juliano, 1985; Parrado et al., 2006; Prakash and Ramanathan, 1994; Sharma et al. 2004; Shih et al., 1999; Xia et al., 2012). Protein of rice bran is appraised as a high-grade protein due to variation of amino acids and types of protein in the crude extract (Madsen, 2008; Saunders, 1990). Proteins and its building blocks, amino acids, are one of the crucial components in human diet due to its numerous functions in many parts of human body system including other amino acids, proteins and enzymes production, cell growth, immune system, tissues and organs supports and many others. (Chaitow et al., 1991). In addition, proteins of rice bran has high hypoallergenicity (Ali et al., 2010; Chand and Sogi, 2007; Tsuji et al., 2001) besides showing a great potential as an anti-cancer agent in preventing and controlling cancers (Ali et al., 2010, Fan et al., 2000; Kannan et al., 2010). Prakash (1996) also has valued the wide-ranged potentials of rice bran protein to be used in various kinds of food.

Protein is widely known as a sensitive macronutrient that can easily be denatured and hydrolyzed under various situations when exposed to certain conditions of temperature, pH and salt concentration. There are many different methods of hydrolyzing proteins including usage of heat, pressure, solvents and enzymes. For years protein has been extracted using alkali solvents that are not only harmful to the environment but also for people. This is due to composition changes of extracted materials under high pH condition, which cause reduced nutrients, benefits and functionalities (Kinsella, 1981) as well as create toxic compounds which is
unsafe for consumption (Ali et al., 2010). As for enzymatic extraction, although there are many studies that have proven its ability to extract high amount of high-quality protein (Hamada, 1998; Hamada, 2000; Hannoumgaï et al., 2001; Hannoumgaï et al., 2002; Tang et al., 2003), this process is not economically efficient due to the high cost of enzymes.

Nevertheless, there are a few novel methods that uses water have been developed for protein extraction such as subcritical water extraction (Khuiwijitjaru et al., 2007; Pourali et al., 2009; Sreeewatthanawut et al., 2008; Sunphorka at, there, 2002-2013) or determine the effectiveness of the 320W, 20kHz. 2003-2012) de an. 32 kHz. 2012) and hydrothermal cooking (Wang and Johnson, 2001; Xia et al., 2012), which both are performed under high temperature and pressure. These methods have gained lots of interest due to uses of water that is harmless and more economical in comparison to other methods. Besides that, there are also uses pf physical treatment such as blending (Tang et al., 2002) and sonication (Chittapalo and Noomhorm, 2009; Tang et al., 2002; Zhu and Fu, 2012) in order to improve protein extraction yield.

Therefore, this study is proposing to combine different techniques into one protein extraction process from full-fat unstabilized rice bran that is environmentally friendly, practical and economical as well as comparable to other methods. The process involves cooking of the rice bran in water inside an autoclave under moderately high temperature (140 °C) and pressure (0.26 MPa) of various durations, combined by sonication before or/and autoclaving. In addition, the high temperature during autoclaving is believed to simultaneously stabilize the rice bran while extracting protein from it (Pourali et al., 2009). The total soluble protein content and amino profile are analyzed to determine the effectiveness of the proposed extraction method.

1. Methodology:

Rice Bran. Rice bran used is from paddy variety MR 219. Fresh rice bran is collected immediately from polishing within 1 hour from BERNAS factory in Sekinchan, Selangor, Malaysia and stored in a freezer under approximately -20 °C.

Chemicals and Standard. Concentrated Sulphuric acid (H₂SO₄)-free/low nitrogen for protein analysis (MERCK); Kjeldahl catalyst, high selenium (Ajax FineChem); 20% (w/v) sodium hydroxide, NaOH in distilled water (RCI Labscan); 35% (w/v) sodium hydroxide, NaOH in distilled water (RCI Labscan); 4% (w/v) boric acid, H₃BO₃ (MERCK) in distilled water with bromocresol green (MERCK) and methyl red (MERCK) indicator solution; 0.2N 37% hydrochloric acid, HCl (MERCK); 2N sodium hydroxide, NaOH (QREC); 1% (w/v) copper sulfate, CuSO₄·5H₂O in distilled water (Fisher Chemical); 2% (w/v) sodium potassium tartrate in distilled water (QREC); 2% (w/v) sodium carbonate, Na₂CO₃ in distilled water (QREC); Folin-Ciocalteu reagent (Sigma Aldrich); bovine serum albumin, BSA (Sigma Aldrich). 6N hydrochloric acid, HCl (MERCK); 12N sodium hydroxide, NaOH in distilled water (RCI Labscan); AccQ-Fluor Borate Buffer (Waters); Reconstituted AccQ-Fluor reagent (Waters); Acetonitrile (MERCK); Formic Acid (MERCK); Eluent Al (Waters).

Protein hydrolysis. Raw rice bran (5%, w/v) in distilled water is autoclaved (Autoclave ALP Model CL-32L, Japan) in a shott bottle at 140 °C (0.26 MPa) for various time (15, 30, 45 and 60 min). Each autoclaving time is run in duplicates. Then the rice bran extract is let to cool down to room temperature for around 15 min in ice water. After that, the extracts are again let to cool down to room temperature in water bath and then centrifuged at 8000 rpm for 15 min. The supernatant is collected.

Autoclaving (AC) time that produce extract with highest soluble protein content and best essential amino acid profile will be combined with sonication (Sonic Dismembrator Fisher Scientific Model 500, USA) at 80% output (320W, 20kHz). The sonication is fixed at 5 min before (S-AC), after (AC-S) and both before and after (S-AC-S) autoclaving. Each combination is run in duplicates. After that, the extracts are again let to cool down to room temperature in water bath and then centrifuged at 8000 rpm for 15 min. The supernatant is collected.

Protein Content Analysis. Total protein content of raw rice bran is analyzed in triplicates using Kjeldahl method (AOAC 981.10). 0.5 g sample is added into a 300 ml test tube. The samples are digested (Heating Digestor VELP Scientifica Model DK 20, Italy) and then analysed using an Automatic Steam Distillation & titration Unit (VELP Scientifica Model UDK 152, Italy) to determine the amount of nitrogen. 0.2M HCl is used as the titrant. The nitrogen converting factor used for rice bran is 5.95.

Soluble protein content is the amount of protein extracted and soluble in the water. Soluble protein content of the collected supernatants is analyzed using Lowry assay (Lowry et al., 1951) and BSA as the protein standard. Each sample is analyzed in duplicates. All simples’ absorbance value are read at 750 nm (UV-VIS Spectrophotometer Scinco Model 2-1300, Korea). Protein recovery is calculated based on equation below:

\[
\text{Protein recovery (\%)} = \left(\frac{\text{soluble protein}}{\text{total protein}}\right) \times 100
\]

Amino Acid Profile. Each sample is analyzed in triplicates. For sample preparation, 0.5 g (raw rice bran) or 1.0 g (extract supernatant) is weighted into a test tube, 5 ml of 6N HCl is added to each tube and then digested under 110 °C for 24 hours. After the samples have cooled down to room temperature, pH of each sample is adjusted between 4.8 to 5.2 and then filled up to 50 ml (solid) or 20 ml (water extract) with distilled water. Then, around 1 to 2 ml of each sample is filtered through 0.22μm syringe filter. After that, the samples are further processed.
and analyzed by ultra-performance liquid chromatography (UPLC, Waters) and a C18 column (Waters) using a method developed by Koh et al. (2012).

Results:
Total protein content of raw rice bran powder obtained is 13.09 ± 0.0g/100g. This is in the range of 10-38 % protein obtained from other work on rice bran using various methods (Ali et al., 2010; Juliano, 1985; Parrado et al., 2006; Shih et al., 1999).

Protein Hydrolysis through Autoclaving. Based on Table 1, Soluble protein content of autoclaved mixture of rice bran and water at 15, 30, 45 and 60 min were 6.65 ± 0.035 g/100g, 7.19 ± 0.023 g/100g, 7.66 ± 0.014 g/100g and 8.41 ± 0.022 g/100g, giving protein recovery 50.77%, 54.95%, 58.55% and 64.21%, respectively. From this data, the highest soluble protein was obtained in the 60 min water extract. Through autoclaving for 60 min also, highest amino acid content was obtained (5.30 ± 0.001 g/100g) (Table 2). Among the highest essential amino acids extracted were leucine (0.31 ± 0.001 g/100g), lysine (0.27 ± 0.001 g/100g) and valine (0.27 ± 0.001 g/100g) and their recovery rate were 29.81%, 38.57%, and 34.62%, respectively (Table 2).

The highest conditionally essential amino acids obtained were arginine (0.39 ± 0.001) and glycine (0.33 ± 0.001) and the recovery rate were 39.40% and 43.42% respectively. On the other hand, methionine was the lowest extracted amino acid (0.05 ± 0.042 g/100g, 26.32%) while cysteine was not obtained at all.

Protein Hydrolysis through Autoclaving and Sonication. Since water extract from 60 min autoclaving had the highest soluble protein and amino acid content, this treatment was combined with 5 min sonication in order to investigate the effect of sonication in improving protein extraction. From fig. 3, it is shown that combination with sonication has negatively affected protein hydrolysis. Soluble protein content for S-AC, AC-S, and S-AC-S extracts were 7.82 ± 0.059, 8.10 ± 0.035 and 8.33 ± 0.015 g/100g, respectively while their protein recovery were 59.71%, 61.87% and 63.62%, respectively.

### Table 1: Soluble protein content and protein recovery water extracts of varied autoclaving time.

<table>
<thead>
<tr>
<th>Autoclaving time (min)</th>
<th>Soluble protein content (g/100g)</th>
<th>Protein recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Stir 60 min</td>
<td>5.39 ± 0.069</td>
<td>41.15</td>
</tr>
<tr>
<td>AC 15 min</td>
<td>6.65 ± 0.035</td>
<td>50.77</td>
</tr>
<tr>
<td>AC 30 min</td>
<td>7.19 ± 0.023</td>
<td>54.95</td>
</tr>
<tr>
<td>AC 45 min</td>
<td>7.66 ± 0.014</td>
<td>58.55</td>
</tr>
<tr>
<td>AC 60 min</td>
<td>8.41 ± 0.022</td>
<td>64.21</td>
</tr>
</tbody>
</table>

Discussion:
Protein hydrolysis through autoclaving. The results of soluble protein content of autoclaved samples are comparable to the conventional alkali extraction which managed to recover 30-80% protein at pH ranging from 7.0 to 12.0 (Fabian & Ju, 2011) and 52.5-58.9% protein concentrate from rice bran protein concentrate at pH 9.0 (Chandi & Sogi et al., 2007). However, it is shown that lysinoalanine, a possible toxic product (Cheftel et al., 1985), is identified during strong alkali extraction between pH 10.0 to 12.2 (de Groot & Slump, 1969). Other green methods such as hydrothermal and subcritical water extraction have managed to hydrolyze protein by using only water or steam at high pressure and temperature. Xia et al. (2012) has recovered 37.4 and 50.0% protein out of heat-stabilized rice bran at 120 and 150 °C, respectively, which is comparable to the highest protein recovery yield in this study, 64.21% at 60 min autoclaving. However, some studies on subcritical water extraction reported higher recovery yield of 84% at 220 °C for 30 min (Watchararuji et al., 2008) and 100% at 250 °C for 60 min (Sunphorka et al., 2012).

A pattern of increasing soluble protein content with increasing treatment time is shown in Fig. 1. This pattern is in agreement with Wang and Johnson (2001) that claimed during hydrothermal process, protein solubility is improving with increasing treatment time. Besides that, studies on rice bran extraction through subcritical water extraction also have demonstrated enhancing protein solubility as the temperature increases, until to a certain time or temperature limit (Khuwijitjaru et al., 2007; Sereewatthanawut et al. 2008; Sunphorka et al., 2012; Watchararuji et al., 2008). Moreover, an increasing amount of total amino acids with increasing time (Fig. 2) is in agreement with subcritical water treatment of deoiled rice bran at its optimum temperature (200 °C) when the amino acid content rises with time (Sereewatthanawut et al., 2008).

Furthermore, eight essential amino acids have managed to be extracted during autoclaving. Essential amino acids are important because they are not naturally synthesized by our body and therefore must be consumed from outside sources, but on the other hand non-essential amino acids are the readily produced amino acid in our body system. This is enough (Chaitow et al., 1991) such as food and supplements. As for the conditionally essential amino acids, four amino acids were obtained with arginine and glycine as the highest. This class of amino acid is needed because of its deficiency under certain age group or situation such as illness. For instance, arginine is essential for children besides help to cure wound faster while glycine is a major amino acid in the non-essential amino acids production in our...
body, as well as an instrument of detoxification particularly for liver (Chaitow et al., 1991). However, the necessity of conditionally essential amino acids varies to each individual based on his physiological, metabolic, pathological and nutritional conditions (Chaitow et al., 1991; Fürst & Stehle, 2004; Jaksic et al., 1991).

Table 2: Amino acids profile of rice bran water extracts at different autoclaving time.

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Soluble protein content (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw Rice Bran</td>
</tr>
<tr>
<td>Essential</td>
<td></td>
</tr>
<tr>
<td>Histidine (His)</td>
<td>0.39 ± 0.001</td>
</tr>
<tr>
<td>Isoleucine (Ile)</td>
<td>0.52 ± 0.001</td>
</tr>
<tr>
<td>Leucine (Leu)</td>
<td>1.04 ± 0.001</td>
</tr>
<tr>
<td>Lysine (Lys)</td>
<td>0.70 ± 0.003</td>
</tr>
<tr>
<td>Methionine (Met)</td>
<td>0.19 ± 0.001</td>
</tr>
<tr>
<td>Phenylalanine (Phe)</td>
<td>0.62 ± 0.004</td>
</tr>
<tr>
<td>Threonine (Thr)</td>
<td>0.56 ± 0.002</td>
</tr>
<tr>
<td>Valine (Val)</td>
<td>0.78 ± 0.001</td>
</tr>
<tr>
<td>Sum</td>
<td>4.79 ± 0.001</td>
</tr>
<tr>
<td>Non-essential</td>
<td></td>
</tr>
<tr>
<td>Alanine (Ala)</td>
<td>0.93 ± 0.001</td>
</tr>
<tr>
<td>Aspartic acid (Asp)</td>
<td>1.38 ± 0.001</td>
</tr>
<tr>
<td>Glutamic acid (Glu)</td>
<td>2.16 ± 0.001</td>
</tr>
<tr>
<td>Serine (Ser)</td>
<td>0.72 ± 0.001</td>
</tr>
<tr>
<td>Sum</td>
<td>5.19 ± 0.001</td>
</tr>
<tr>
<td>Conditional</td>
<td></td>
</tr>
<tr>
<td>Arginine (Arg)</td>
<td>0.99 ± 0.003</td>
</tr>
<tr>
<td>Cysteine (Cys)</td>
<td>0.06 ± 0.005</td>
</tr>
<tr>
<td>Tyrosine (Tyr)</td>
<td>0.29 ± 0.001</td>
</tr>
<tr>
<td>Glycine (Gly)</td>
<td>0.76 ± 0.002</td>
</tr>
<tr>
<td>Proline (Pro)</td>
<td>0.65 ± 0.001</td>
</tr>
<tr>
<td>Sum</td>
<td>2.75 ± 0.002</td>
</tr>
<tr>
<td>Total amino acids</td>
<td>12.73 ± 0.001</td>
</tr>
</tbody>
</table>

Fig. 1: Protein recovery for rice bran water extracts autoclaved at varied time durations.

Fig. 2: Soluble protein content from amino acid (a.a.) profile analysis using UPLC at different autoclaving time.

In case of methionine that was least extracted and cysteine which was not extracted at all, this might be mainly caused by their originally low amounts in the raw rice bran which are 0.19 ± 0.001 and 0.06 ± 0.005 g/100g, respectively. Besides that, both methionine and cysteine are among amino acids...
that are commonly reacting to reducing sugars through maillard reaction, also known as browning reaction (Belitz et al., 2009). Based on Sereewatthanawut et al. (2008), reducing sugars is present as well as increasing with temperature (120-220 °C) and time (5-30 min) in the water extract treated under subcritical water condition. Furthermore, in the presence of the reducing sugars as reducing agent, water can act as oxidizing agent thus possibly oxidizes methionine and cysteine that are readily to be transformed to sulfoxide and cystine, respectively (Belitz et al., 2009). Moreover, methionine and cysteine also can form acrylamide in the presence of glucose at temperature more than 100 °C or prolonged heating. These reactions may be the causes for reduced amount of methionine and cysteine extracted (Belitz et al., 2009).

![Fig. 3: Protein recovery of rice bran water extracts resulted from combinations between autoclaving (60 min) and sonication (5min).](image)

AC: 60 min autoclaving; S-AC: Sonication before 60 min AC; AC-S: Sonication after 60 min AC; S-AC-S: Sonication before and after 60 min AC.

As for the sonication treatment, Fig. 3 demonstrates negative effect of sonication on the protein content. Based on a study done by Tang et al. (2002) on protein extractability from heat-stabilized defatted rice bran in water by sonication, the protein recovery increases with increasing power output but not significant enough. However, sonication that is applied simultaneously with alkali (Chittapalo and Noomhorm, 2009; Zhu and Fu, 2012) or buffer extraction (Messman and Weiss, 1993; Singh et al., 1990) has been proven to improve extraction efficiency. Therefore, it can be resolved that a substantial protein extraction cannot be accomplished by performing sole or separated sonication. Simultaneous process of extraction and sonication may be further investigated as this is more relevant to be practiced in industrial scale. This is due to high loss of production through multiple steps which reduce yield and efficiency of the extraction process (Fabian & Ju, 2011).

4. Conclusion:

The protein extraction through autoclaving has successfully extract protein from rice bran with relatively high soluble protein content and good profile of amino acids. The highest protein is extracted by autoclaving for 60 min that is 8.41 ± 0.022 g/100g with 64.21% recovery. Under the same treatment, highest amino acid content of each essential, conditional and non-essential is also obtained giving the total amino acid of 5.30 ± 0.001 g/100g. However, combination with sonication does not have positive effect on protein extraction yield.

REFERENCES


