Amino Acid Composition of Rice Bran Concentrate from Several Local Rice Varieties as a Potential Source of Bio-emulsifier Material

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ABSTRACT

Research on determination of amino acid composition of rice bran concentrate (RBC) of three local rice varieties (Ciherang, Ciliwung and IR-66) as a potential source of bio-emulsifier material has been carried out. The research aim is to determine the content of protein, composition and weight percent of amino acids from rice bran concentrate. The rice bran concentrate was obtained by defatting of rice bran with n-hexane liquid. The content of protein is determined using Kjeldahl method and hydrolyzed with HCl. Amino acid analyses were carried out by High Performance Liquid Chromatography with the mobile phase of sodium acetate pH 6,5, methanol 95% and OPA reagent. This research results in protein content of 1,78% (yield 1,4%), 2,42% (yield 1,2%), and 8,31% (yield 1,4%) for Ciherang, IR66, and Ciliwung varieties, respectively. The amino acid content (mg/g of protein) of rice bran concentrate of the three local rice varieties generally have higher levels in polar amino acids of histidine, arginine, aspartic acid, glutamic acid, serine, threonine and lysine. Proteins contain both polar and non polar amino acids. One of the ways that proteins minimize their energy is by folding into structures of low free energy. These structures generally result when the interactions of polar groups with water are maximized and the interactions of non polar groups with water are minimized. The mechanisms will influence its emulsifying and foaming properties. Thus, the rice bran concentrate can be proposed as a potential source of natural emulsifier.

Keywords:
Amino acid composition, rice bran concentrate, local rice varieties, bio-emulsifier material

1. INTRODUCTION

Rice bran is an inexpensive, underutilized milling co-product of rough rice. In 2006, about 54 million tons of rice was produced in Indonesia [1]. If rice bran recovery is 0,065 ton rice bran/ton rice [2], Indonesian rice will result in about 3.51 million tons of bran each year. A large amount of them has been discarded and only a small amount has been used as animal feed.

Demand of relatively inexpensive sources of proteins that can be incorporated to value-added food products is increasing. Worldwide, much of the research is going on various sources of plant proteins ([3], [4], [5], [6]) that may help in increasing the nutritional value of food products at low cost.

Rice bran is the major by product generated during milling, which is further extracted for oil. Proteins extracted from heat stabilized defatted rice bran could be used as a neutraceutical food ingredient [7]. The development of new food items from rice by products calls for the most precise information obtainable on desirable and undesirable components. In addition, a group of researchers ([8], [9], [10]) proposes potential use of RBC as a food protein ingredient due to its emulsifying and foaming properties. Even though rice bran is a by-product produced in large quantity in Indonesia yearly, its application, for example, in foods is very limited, as well as its application of such ingredient to food products have not been widely studied and published.

Rice bran has high nutritional value [11], and appears to have a beneficial dietary component that improves hypertension, hyperlipidemia, and hyperglycemia [12]. Rice bran protein is higher in lysine content than rice endosperm protein or any other cereal bran proteins [13]. The protein efficiency ratio (PER) has been widely used as an indicator of protein nutritional quality. The PER values for rice bran concentrates (RBC) range from 2.0 to 2.5, compared to 2.5 for casein. Protein digestibility of rice bran is greater than 90%. Rice bran is considered a good source of hypoallergenic proteins, and as such, rice bran protein may serve also as a suitable ingredient for infant food formulations ([14].

Although the nutritional and pharmaceutical potential of rice bran has been recognized ([11, 12]), at present, rice bran concentrates (RBC) and isolates are not commercially available. This lack of availability could be due to the following: (1) The proteins in rice bran are of a complex nature. Rice bran proteins contain 37% albumin, 36% globulin, 22% glutelin, and 5% prolamin [15], (2) The poor solubility of rice bran protein includes its strong aggregation and/ or extensive disulfide bond cross-linking [16], (3) Rice bran contains high phytate (1.7%) and fiber content (12%) [13]; these two components could bind with proteins, making the protein bodies very hard to separate from other components.

It has been widely accepted that differences in functional properties and structure of protein result from differences in amino acid composition and sequences. Knowledge of the sequence of amino acid in a protein can offer insights into its three-dimensional structure and its function. Many protein structural studies, for instance, in mixing and baking studies, have postulated that disulfide bonds are present in the gluten structure and contribute to the process of dough formation through the process of disulfidesulfhydryl exchange [17]. Gluten is the most important protein that is responsible to rheological properties of wheat flour dough. Another evidence indicates that tyrosine bonds form in wheat doughs during the processes of mixing and baking, contributing to the structure of the gluten network [18].

Therefore, the purposes of this study, as an initial step to utilize the bulk production of rice bran from local rice varieties, especially in South Sulawesi Province, were to determine the protein content of rice bran concentrate and its amino acid composition. We discuss the possibility of RBC from local rice varieties as a potential source of bio-emulsifier material in the future and the possible application in food product, especially for infant foods.

2. MATERIALS AND METHODS

Rice Bran Samples: Dried rough rice (three local rice varieties: Ciherang, Ciliwung, IR-66) were obtained from local rice companies in Kabupaten Takalar, Kabupaten Sinjai and Kabupaten Bulukumba in South Sulawesi Province. Brans were obtained by milling the grains of these samples in a rice machine with a capacity of 50.5 kg/hr.

Chemicals: Chemicals used in this research are n-hexane, HCl (0,01 – 0,02 M), NaOH (0,01 and 0,05 M), aquadest and aquabidest, filter paper (Whattman 41), universal pH paper, selenium nitrate, sulfuric acid (concentrated), bromcresol green and methyl red, nitrogen gas, sodium acetate pH 6,5, potassium borate pH 10,4, methanol 95%, standard amino acid solution and OPA reagent. All of chemicals used were reagent grade or the highest purity obtainable.

Preparation of Defatted Rice Bran: Rice bran obtained from a local milling factory was processed into two steps: defatting and protein extraction. The defatting procedures employed are according to Wang *et al.* (1999). Briefly, rice bran is defatted twice using hexane in bran to solvent ratio of 1:3 at a setting of 250 rpm in a lab stirrer for 30 min and centrifuge at 5000 g for 10 min. at room temperature (RT). The defatted rice bran (DRB) is air-dried overnight, sieved through a 100 mesh screen, packed in a bag and stored at 5ºC. The extraction step is a modification of Gnanasambandam and Hettiarachchy, 1995: alkaline extraction followed by isoelectric precipitation are used to prepare rice bran extract. The process is described as follows; defatted rice bran sample and distilled deionized water (1:4) was pH adjusted to 9.5 and stirred 30 min at room temperature (RT). The slurry was centrifuged (Sorvall® RC 28 S) at 5000 g for 30 min. (RT). The pH of supernatant was adjusted to 4.5 and centrifuged again at 5000 g for 30 min. (RT). Precipitate was washed using water (pH 4.5). The residue was suspended in distilled deionized water (pH 7.0) and frozen overnight. The final product, which is called rice bran concentrate (RBC), was then frozen dried (Freeze dryer, Flexi-Dry FTS ™system) and stored at -5 ºC.

Protein Content and Yield Determination. The protein content of RBC was determined by the Kjeldahl method ([19]). The Kjeldahl Digestion System 6 (Tecator Co., Sweden) was used to digest the protein, and Distilling Unit 1026 (Tecator Co., Sweden) with setting at 2.0, 0.2, and 3.6 for alkali, delay, and steam, respectively, was used to the determine nitrogen content of the protein samples. The value of 5.95 was used as protein conversion factor. Protein yields were calculated as:

$$\text{Yield (\%)} = \frac{\text{weight (g) of RBC}}{\text{weight (g) of DRB}} \times 100$$

Amino Acid Analysis: For cystein and methionine determination, RBC was first oxidized with performic acid for 16 h in an ice bath and then neutralized with hydrogen bromide [19]. Oxidized and un-oxidized RBC samples were hydrolyzed at 121 °C with 6 N HCl for 18 h. For tryptophan determination, RBC was hydrolyzed with 4.2 N NaOH at 110 °C for 20 h [19]. After hydrolysis, amino acids of RBPI samples were separated by HPLC. Post column modification was performed with ninhydrin for detection at 570 nm.

3. RESULTS

Table 1. Percent protein and percent yield of rice bran concentrates

No	South Sulawesi Rice Varieties	Protein content (%)	Yield (% b/b)
1	IR 66	79,9	1,2
2	Ciliwung	82,8	1,4
3	Ciherang	76,9	1,4

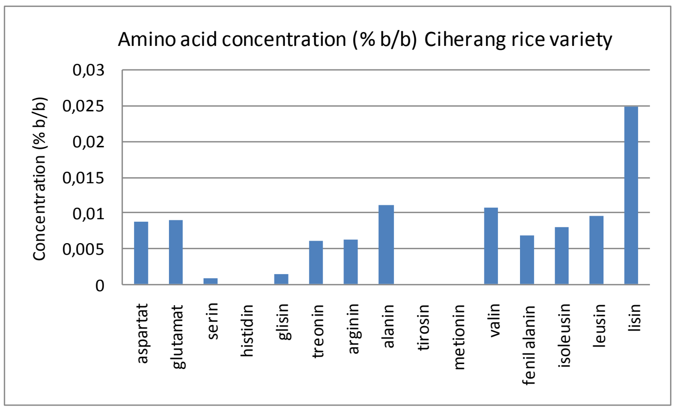

Figure 1. Amino acid concentration (% b/b) in Ciherang rice variety

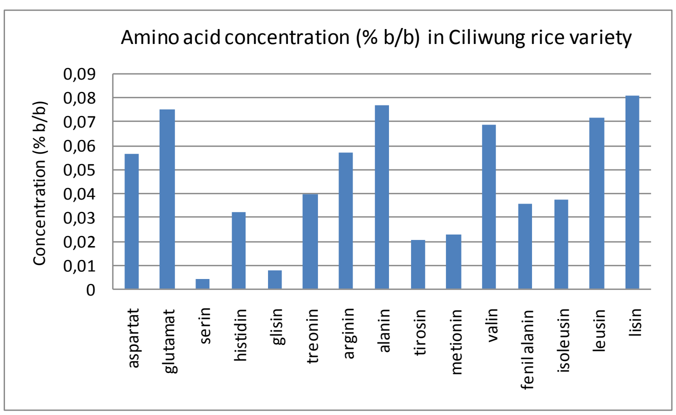

Figure 2. Amino acid concentration (% b/b) in Ciliwung rice variety

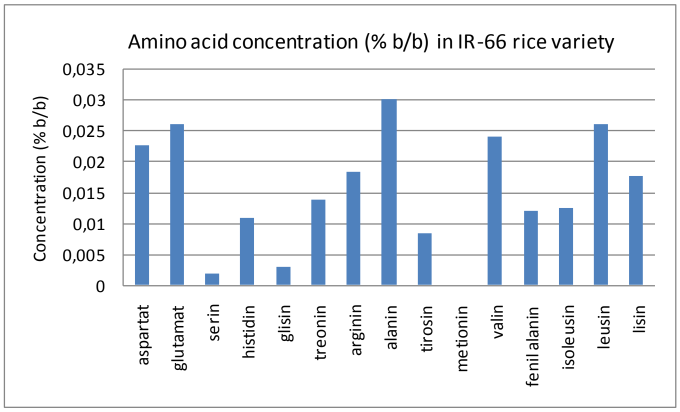

Figure 3. Amino acid concentration (% b/b) in IR-66 rice variety

4. DISCUSSION

Protein content and yield. Table 1 shows experimental results of protein contents and percent yield of RBC of three local rice varieties in South Sulawesi Province. The RBC with the highest protein content (8.31%) was extracted from Ciliwung rice variety. The lowest one (1,78%) was obtained from Ciherang rice variety. This is, however, a crude protein content determined with Kjeldahl method through alkali extraction at pH 4,5. It is generally known that pH 4.5 is a protein isoelectric point (pI) indicating that protein has minimal solubility at this point. Jiamyangyuen *et al*. [20] reported that even at lower pH than pI, (pH 2.0), the protein content of RBC will increased compared to that obtained at isoelectric point. It was also reported that at low pH, phytate, a component in rice bran, can interact with protein leading to decreased solubility of protein (Bera and Mukherjee, 1989). The protein content of RBC extracted at higher pH than isoelectric point will result in higher content of protein ([21]Chen and Houston (1970). At high pH, some non-protein nitrogen could solubilize and contribute to protein quality and purity. It should be tried to vary the effect of extraction pH to the protein content of RBC of three local rice varieties in the future experiment.

The reason why alkali extraction was adopted in this study is because alkali is the most commonly used solvent to extract proteins from rice bran ([9], [15]). High alkaline conditions, however, could cause undesirable side reactions and potential toxicity, such as lysinoalanine, thus losing the nutritive values of protein. In addition, high alkaline conditions could cause the following: (1) denaturation and hydrolysis of proteins; (2) increased Maillard reaction which causes darkcolored products; (3) increased extraction of nonprotein components which coprecipitate with protein and lower the isolate quality and quantity [22]. Enzyme extraction should be considered as a better technique for protein extraction in RBC [8]. The latter technique, however, might be a little bit expensive.

The percent yield of different RBC of three local rice varieties varied from 1.20-1.40%. The percent yield of RBC of three local rice varieties is not significantly different compared to their protein content.

Amino acid content. The amino acid content (mg/g of protein) of RBC is given in Fig. 1 – 3. The amino acid content (mg/g of protein) of rice bran concentrate of the three local rice varieties generally have higher levels in polar amino acids of histidine, arginine, aspartic acid, glutamic acid, serine, threonine and lysine. Lysine has the highest concentration in RBC of local rice varieties except for IR-66. Histidine is found in every variety except in Ciherang variety. Proteins contain both polar and non polar amino acids. One of the ways that proteins minimize their energy is by folding into structures of low free energy. These structures generally result when the interactions of polar groups with water are maximized and the interactions of non polar groups with water are minimized. This mechanisms will influence its emulsifying and foaming properties. Thus, from this stand point, the rice bran concentrate can be proposed as a potential emulsifier.

Methionine fraction is only found in Ciliwung variety. Methionine is an amino acid which determines foam volume especially in baking process. High-methionine fractions had higher foam volume, affected emulsifying capacity and hydrophobicity. Modified hydrolysates with a high-methionine fractions have a potential for use in soluble high nutritional products. [23]

All of rice varieties have a quite high histidine concentration, except in Ciherang rice variety. Chen et al. (1998) demonstrated that histidine containing peptides can act as a metal-ion chelator, an active-oxygen quencher, and a hydroxyradical scavenger. These properties seem to be important in explaining how the peptides possess their antioxidative activity. The addition of leucine and/or proline residues will increase the hydrophobicity of histidine-containing peptides. The hydrophobicity of the antioxidant is an important factor for increasing the accessibility of lipophilic fatty acids or oxidants and hence in expression of its antioxidative activity. However, such a correlation was not observed with the His containing peptides.

RBC had similar or higher levels of histidine, arginine, isoleucine, valine, methionine, tyrosine, and tryptophan in comparison to those amino acids reported by Juliano (1985). Other amino acids in RBC were lower than the report (Juliano, 1985). In comparison to casein, RBC had similar or higher levels in valine, cystine, phenylalanine, threonine, histidine, arginine, alanine, asparatic acid, and glycine contents. Leucine, valine, methinonine, cystine, phenylalanine, tyrosine, threonine, histidine, arginine, alanine, glycine, and tryptophan contents of RBC were similar or higher than those of soy protein isolate reported by Wang et al. (1999).

Infants have very critical nutritional requirements due to rapid growth and immaturity of gastrointestinal function (Behrman and Vaughan, 1983). Nine amino acids have been identified to be essential for infants (threonine, valine, leucine, isoleucine, lysine, tryptophan, phenylalanine, methionine, and histidine). Arginine and cystine are also essential for low birth weight infants (Behrman and Vaughan, 1983). When compared to the essential amino acid requirement (FAO/WHO/UNU, 1985) for infants, RBC had high valine, histidine, and tyrosine contents. Casein had a lower level of tyrosine (14 mg/g of protein) in comparison to that for infant requirements but higher levels in other essential amino acids [8]. For all other age groups except infants, the Joint FAO/WHO Expert Consultation recommended the essential amino acid requirement for 2-5-year-old children as a suitable pattern to evaluate the protein quality (Joint FAO/WHO, 1990).

5. CONCLUSION

This research results in protein content of 1,78% (yield 1,4%), 2,42% (yield 1,2%), and 8,31% (yield 1,4%) for Ciherang, IR66, and Ciliwung varieties, respectively. Extraction methods for increasing protein recovery should be adopted. In general, the amino acid content (mg/g of protein) of rice bran concentrate of the three local rice varieties have higher levels in polar amino acids of histidine, arginine, aspartic acid, glutamic acid, serine, threonine and lysine. Proteins contain both polar and non polar amino acids. One of the ways that proteins minimize their energy is by folding into structures of low free energy. These structures generally result when the interactions of polar groups with water are maximized and the interactions of non polar groups with water are minimized. This mechanisms will influence its emulsifying and foaming properties. Thus, the rice bran concentrate can be proposed as a potential emulsifier, especially the application for infant food products.

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