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Production of Protein Hydrolysate from Broken Rice by Crude Bromelain Enzyme in Different Conditions

Zalniati Fonna Rozali
Department of Agricultural Product Technology
Universitas Syiah Kuala
Banda Aceh, Indonesia

Nurheni Sri Palupi
Department of Food Science and Technology,
Bogor Agricultural University
Bogor, Indonesia

Endang Yuli Purwani
Indonesian Center for Agricultural
Postharvest Research and Development
Bogor, Indonesia

Maggy Thenawidjaja Suhartono
Department of Food Science and Technology,
Bogor Agricultural University
Bogor, Indonesia

Abstract:- Broken rice is a by-product of low-value rice mills in Indonesia, a country where pineapples are also produced abundantly. The proteins derived from broken rice, along with bromelin enzymes from pineapples can potentially be used in the production of their vegetable hydrolyzates, the demand of which has been increasing lately. This study was designed to determine the conditions suitable for the production of rice protein hydrolyzates from broken rice using crude bromelain enzyme. This study used flour from broken rice and its protein isolates as substrate in the hydrolysis process while using the crude extracts of bromelain enzyme as a source of protease. In the initial stage, bromelain enzyme activity was measured at pHs of 3, 4, 5, 6, 7 and 8. Furthermore, rice flour and its protein isolate were hydrolyzed by crude bromelin enzymes at a selected pH for 1 and 3 hours with the changes in protein profile later being analyzed at 1 and 3 hours after hydrolysis. The result indicated that the protein content of rice flour was 6.45% and the bromelain enzyme activity at 50 ºC was 3.28 U/mL. The intensity of all proteins decreases after 1 hours of hydrolysis, with the exception of proteins with a molecular weight of 65 kDa, which is still visible after 3 hours hydrolysis. The best condition for protein hydrolysis in rice flour and rice protein isolates by crude bromelain enzyme is in pH 8 for a duration of 1 hour.

Keywords:- *Rice; Bromelain, Hydrolysis, Protein; Intensity.*

I. INTRODUCTION

Rice is one of the cereals which are rich in protein and calories, providing nutrition for millions of people in the world, including some Asian and European countries[1]. The component of protein in rice, at 7–9% by weight, is not high, nevertheless, it is a major source of the nutrient for its consumers. Rice protein possesses unique nutritional properties, in general hypoallergenic, rich in essential amino acids[2]. It also serves as a suitable source of protein for vegetarians as it is a suitable substitute for animal proteins such as milk and can be processed into a variety of food products[3].

Rice has been studied for its disease-preventative properties such as antioxidants, anti-carcinogenesis, anti-mutagenesis, and anti-atherosclerosis[4-5]. In addition to providing good nutrition, bioactive proteins in rice also improve the health of its consumers and prevent diseases[6]. These proteins, such as short chain peptides are produced from hydrolysis by protease enzyme. Rice protein hydrolyzate obtained from alkalase hydrolysis showed anti-hypersensitive effects both in vitro and in vivo studies[7-8], and induces apoptosis in cell myocardiocytes H9C2[9].

The enzymatic process, which is perceived to be cheaper and more appropriate is used in making the protein hydrolyzate in industries as the process is faster and provide protein hydrolyzate without losing a lot of essential amino acids. Bromelain is a cysteine protease enzyme of plant origin pineapple (*Ananas comosus* L), non-toxic and known for its therapeutic properties[10]. This study was designed to study the right condition for production of rice protein hydrolyzates from broken rice using crude bromelain enzyme.

II. MATERIALS AND METHODS

A. Materials

Broken rice of the IR 42 variety were obtained from the Central Rice Research Center, Sukamandi, Indonesia, then milled, dried and sifted at 80 mesh. Ripe pineapples of the Palembang variety were purchased from a local market at Bogor, then peeled and the crude bromelain enzyme extracted with an extractor.

B. Rice Protein Isolate

Rice flour was prepared as sources of protein isolate according to de Souza et al. [11]. A part of rice flour was extracted with 15 part of 0.18% NaOH for 30 min with stirrer 1000 rpm at a temperature of 25 ºC. The suspension was centrifuged at 4000 rpm for a duration of 5 minutes. The supernatant was collected and the precipitate washed with 5 part 0.18% NaOH after which the suspension obtained was centrifuged again at 4000 rpm for 5 min. The supernatant was collected and the pH value was adjusted to 4.8 then the protein precipitate was separated by centrifugation at 7000 rpm for a duration of 5 minutes and the precipitate was collected and stored at a temperature of -20 ºC.

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C. Chemical Analysis

Moisture, ash, fat, and protein contents of samples were determined according to the standard methods of AOAC (1990). The crude protein was determined by multiplying the total nitrogen by 6.25 while the carbohydrate was obtained by difference.

D. Determination of Enzyme Activity and Specific Activities

Measuring the activity of bromelain enzyme was carried out according to Bergmeyer et al. [12] with modifications. 0.05 mL of crude bromelain enzyme was added to 0.25 mL phosphate buffer saline 0.05 M (pH 3, 4, 5, 6, 7 or 8) and 0.25 mL 2% casein. Then the mixture was incubated at 50 ºC for 10 min and centrifuged at 1500 rpm for the same duration. 0.375 mL of supernatant were taken and 0.125 mL **of** 0.4 M Na2CO3 and 0.25 mL of Folin Ciocateau reagent (1: 2 dilution) were added and incubated at 37 ºC for 20 min while absorbance was measured at a wavelength of 578 nM. Aquades were used as controls and 5 mM tyosine was used as a standard. One unit of enzyme activity shows the amount of enzyme that can hydrolyze 1 µmol tyrosine per min under test conditions.

E. Enzymatic Hydrolysis

The 25 mL of crude bromelain enzyme was added to the 5 gr of rice flour and 1.6 gr rice protein isolates (adjusted to make similar protein concentration). Hydrolysis was carried out for 1 and 3 hours at 50 ºC, and at selected pH and normal pH of crude bromelain enzyme then it was terminated by increasing the temperature of the mixture to 90 ºC for 15 min.

F. Electrophoresis and Protein Profile Determination

Preparation of rice protein samples was carried out using the SDS-PAGE method according to Laemmli [13]. The buffer sample was carried out with 4 mL aquabidest; 1 mL of 0.5 M Tris – HCl solution pH 6.8; 0.8 mL glycerol; 1.6 mL of 10% SDS solution; 0.4 mL solution α-mercaptoetanol; 0.2 mL blue bromophenol 0.05%. The 15 µg of sample was mixed with 45 µL of the buffer sample, then the mixture heated at a temperature of 95 ºC. After cooling, each 15 µL sample was loaded to the electrophoresis gel. The 4% stacking gel and 16% separating gel were used to visualize the protein profile derived from the rice flour, rice protein isolate, and protein hydrolysate by a Mini-Protean Kit instrument (BioRad, USA, Model 1000/500). The gel was stained with Coomasie Brilliant blue-staining solution and gently shaken overnight using an electric shaker then followed by destaining solution for 90 min and replicated twice.

III. RESULT AND DISCUSSION

The chemical composition of the rice flour from IR 42 variety is shown in Table 1. The table indicates that carbohydrates are the main component, showing that the chemical composition of the rice flour is slightly different from that reported by Alonso-Miravalles et al. [14]. While the protein content of rice flour according to their study was 8.2%, in this study, it is 6.45%. The protein level of IR 42 rice flour is lower but is still sufficient to be hydrolyzed using bromelain in order to produce protein hydrolyzate. The use of vegetable protein such as the one derived from rice, is gradually gaining popularity because of its contribution to environmental sustainability, economic sources of the nutrient and a challenge for food security[14].

Component	Value (%)
Water	14.26 ± 0.37
Protein	6.45 ± 0.24
Lipid	0.31 ± 0.25
Ash	0.34 ± 0.03
Carbohydrate	78.64 ± 0.37

Table 1:- Macronutrient composition of broken rice flour

The temperature, pH, enzyme activity and time for the hydrolysis of rice flour can vary, therefore, it was necessary to determine the perfect conditions for the production of rice protein hydrolysate. Table 2 shows the temperature optimization process of bromelain hydrolysis in terms of the rice flour's physical appearance. To apply bromelain optimally for the production of rice hydrolysate protein, the right physical structure of the rice flour is required because when enzyme hydrolysis is too long or the temperature is too high, the rice flour at 20% solution becomes pasta which will potentially inhibit the production of rice protein hydrolysate. According to data from Table 2, temperatures of 50–60 ºC for 1 and 3 h bromelain treatment is favorable for the production of rice protein hydrolysate.

The bromelain enzyme used in this study is originally from Palembang variety which is actual at pH 8 and 80 ºC[15]. However, at this temperature the suspension of rice flour and bromelain extract appears in pasta form. The formation of the pasta shows that there has been an increase in viscosity and gelatinization of starch in this suspension. Rice flour is easier to gelatinize at high temperatures, which start from 67.7-71.5 ºC while it is perfectly gelatinized at a temperature of 86.6-94.9 ºC[16]. The increase in viscosity and gelatinization temperature in rice starch varies depending on the variety tested. In medium with high viscosity there will be limitation of the bromelain action during the protein hydrolysis[17]. Bromelain from the fruit axis (core) of pineapple has an optimum temperature of 50-60 ºC, while that from pineapple meat has an optimum temperature of 37-70 ºC[18].

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Temp. (°C)	Hydrolysis time (hours)		
	1	2	3
50	Pasta Not Formed	Pasta Not Formed	Pasta Not Formed
60	Pasta Not Formed	Formed Pasta	Formed Pasta
70	Formed Pasta	Formed Pasta	Formed Pasta
80	Formed Pasta	Formed Pasta	Formed Pasta

Table 2:- Physically appearance of rice flour after bromelain treatment

pH	Enzyme activity (U/ml)	Specific activity (U/mg)
3	1.75 ± 0.10	11.11 ± 0.63
4	1.77 ± 0.14	11.22 ± 0.10
5	2.21 ± 0.19	13.97 ± 1.19
6	2.13 ± 0.37	13.49 ± 2.32
7	2.06 ± 0.11	13.03± 0.70
8	2.38 ± 0.28	15.07 ± 1.77

Table 3:- Enzyme activity and specific activity of crude bromelain enzyme extracts

Table 3 shows the activity of bromelain enzyme extract at a temperature of 50 °C. The highest activity of bromelain was obtained at pH 8, which was 2.38 U/ml or 15.07 U/mg. Bromelain activity at pH 3, 4, 5, 6 and 7 at 50 °C were lower. So, in the next experience we then appearance bromelain at pH 8.

The electrophoresis result in Fig. 1 shows that all rice protein was hydrolyzed using crude bromelain enzyme. This experiment indicate that bromelain is effective used as protease sources as the protein profile of the rice is similar to those reported earlier by some researchers. The protein bands at 13 – 16 kDa indicated the presence of albumin[19-20], while those at 20 – 22 kDa pointed to glutelin which is the basic subunit of the seed storage protein[21]. Glyoxalase I (33 kDa) present in rice is one of the allergenic protein alongside several proteins with lower molecular weight (13 - 22 kD)[20]. The globulin (65 kDa) and vicilin like-protein (133 kDa)[20-21]. Whereas the crude bromelain enzyme shows protein bands at 25 to 28 kDa which is in agreement with that reported by others at 23 - 35.75 kDa[18].

Rice flour treated for 1 and 3 hours with the crude bromelain enzyme show a reduction in the number of protein bands. Those of less than 25 kD were degraded by the enzyme and this degradation was completed by 1 hour incubation. As some of these proteins were known to be allergenic, this increased the utilization of rice peptides or hydrolysate for health purposes. The protein bands of 65 kD in the rice flour was more resistant to proteolysis, while that of 33 kD was digested into smaller proteins. The 65 kD protein only appeared in the rice flour but was present in small quantities in the protein isolate; this protein might be degraded or solubilised during the isolation procedure. Bromelain seems to be more effective for the degradation of rice protein when compared with the subtilisin element used in earlier experiment[21].

IV. CONCLUSION

This study revealed that is possible to use crude bromelain enzyme to produces rice protein hydrolysate. The optimum condition for production is at temperature 50 °C, pH 8 for 1 hour incubation. Both rice protein in flour and its isolate could be used to make rice protein hydrolysate which will be an alternate choice for vegetarians and potential for functional food.

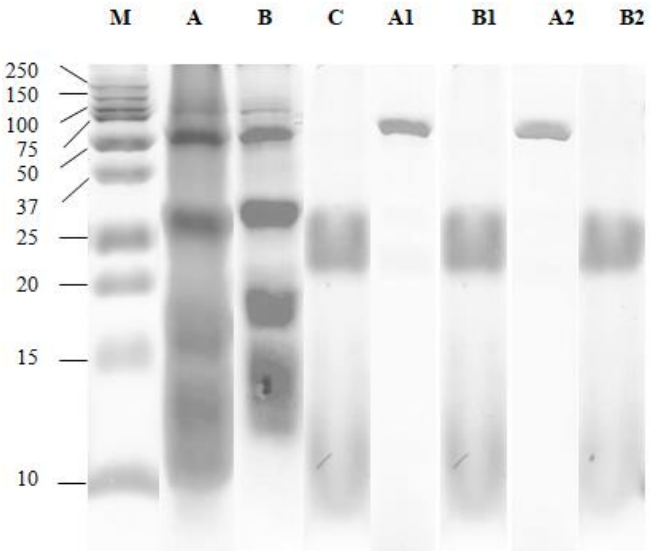

Fig 1:- Electroforesis of protein of the rice flour (A), rice protein isolate (B), crude bromelain enzyme (C), rice flour and rice protein isolate following hydrolysis with crude bromelain enzyme at 1 hour (A1, B1) and 3 hours (A2, B2), respectively. M is protein molecular marker

REFERENCES

[1]. Sasaki, T and B Burr, *International Rice Genome Sequencing Project: the effort to completely sequence the rice genome.* Current opinion in plant biology, 2000. **3**(2): p. 138-142.

[2]. Agboola, S, D Ng, and D Mills, *Characterisation and functional properties of Australian rice protein isolates.* Journal of cereal science, 2005. **41**(3): p. 283-290.

[3]. Al-Doury, MK, NS Hettiarachchy, and R Horax, *Rice-Endosperm and Rice-Bran Proteins: A Review.* Journal of the American Oil Chemists' Society, 2018. **95**(8): p. 943-956.

[4]. Burris, RL, et al., *Dietary rice protein isolate attenuates atherosclerosis in apoE-deficient mice by upregulating antioxidant enzymes.* Atherosclerosis, 2010. **212**(1): p. 107-115.

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[5]. Nam, S, et al., *Bran extracts from pigmented rice seeds inhibit tumor promotion in lymphoblastoid B cells by phorbol ester.* Food and chemical toxicology, 2005. **43**(5): p. 741-745.
[6]. Kannan, A, et al., *Human colon and liver cancer cell proliferation inhibition by peptide hydrolysates derived from heat-stabilized defatted rice bran.* Journal of agricultural and food chemistry, 2008. **56**(24): p. 11643-11647.
[7]. Boonla, O, et al., *Peptides-derived from Thai rice bran improves endothelial function in 2K-1C renovascular hypertensive rats.* Nutrients, 2015. **7**(7): p. 5783-5799.
[8]. Wang, X, et al., *A novel antioxidant and ACE inhibitory peptide from rice bran protein: Biochemical characterization and molecular docking study*. LWT, 2017. **75**: p. 93-99.
[9]. Yang, T, et al., *Rice protein hydrolysate attenuates hydrogen peroxide induced apoptosis of myocardiocytes H9c2 through the Bcl-2/Bax pathway*. Food research international, 2012. **48**(2): p. 736-741.
[10]. Kumar, L and SK Jain, *Proteases: A Beneficial Degradative Enzyme in Therapeutic Applications.* Int. J. Sci. Res. in Biological Sciences Vol, 2018. **5**: p. 4.
[11]. de Souza, D, et al., *Characterization of rice starch and protein obtained by a fast alkaline extraction method.* Food Chemistry, 2016. **191**: p. 36-44.
[12]. Bergmeyer, H and M Grassel, *Reagents for enzymatic analysis: enzymes-a-amylase*. Methods of enzymatic analysis, 1983. **2**: p. 151-152.
[13]. Laemmli, U, *Slab gel electrophoresis: SDS–PAGE with discontinuous buffers.* Nature, 1979. **227**: p. 680-5.
[14]. Alonso-Miravalles, L and JA O'Mahony, *Composition, Protein Profile and Rheological Properties of Pseudocereal-Based Protein-Rich Ingredients.* Foods, 2018. **7**(5): p. 73.
[15]. Mulyono, N, et al., *Quantity and quality of bromelain in some Indonesian pineapple fruits.* IJABPT, 2013. **4**: p. 235-240.
[16]. Chun, A, et al., *Effects of ripening temperature on starch structure and gelatinization, pasting, and cooking properties in rice (Oryza sativa).* Journal of agricultural and food chemistry, 2015. **63**(12): p. 3085-3093.
[17]. Feng, X, et al., *Bromelain Kinetics and Mechanism on Myofibril from Golden Pomfret (Trachinotus blochii).* Journal of food science, 2018. **83**(8): p. 2148-2158.
[18]. Arshad, ZIM, et al., *Bromelain: an overview of industrial application and purification strategies.* Applied microbiology and biotechnology, 2014. **98**(17): p. 7283-7297.
[19]. Chen, M, A Shelton, and G-y Ye, *Insect-resistant genetically modified rice in China: from research to commercialization.* Annual review of entomology, 2011. **56**: p. 81-101.
[20]. Nakase, M, S Kudo, and S Matsutomo, *Reduction of proteins in rice flour by proteases in kiwifruit and pineapple.* Bull. Minamikyushu Univ. 44A, 2014. **43**: p. 49.
[21]. Dorglamud, S, et al., *Physicochemical properties of protease-treated rice flour.* Starch-Stärke, 2013. **65**(7-8): p. 613-620.