Discovery, The Student Journal of Dale Bumpers College of Agricultural, Food and Life Sciences

Volume 12 Article 10

Fall 2011

Antioxidant and antihypertensive activities of rice bran peptides

Janika Hull
Alabama A&M University

Arvind Kannan
University of Arkansas, Fayetteville

Navam S. Hettiarachchy
University of Arkansas, Fayetteville

Antioxidant and antihypertensive activities of rice bran peptides

Janika N. Hull[*]*, Arvind Kannan*[†]*, and Navam S. Hettiarachchy*[§]

ABSTRACT

Protein isolates and peptide fractions from food sources (cereal grains), have been shown to exert bioactive properties including antiobesity, anticancer, antiangiogenic, etc. One such food source is rice bran, which is an underutilized co-product of rough rice milling. It contains 90% of the nutrients and nutraceuticals of value to health, including high quality protein. The high quality protein is a potential source to generate peptides that can reduce hypertension and oxidative stress, both being important risk factors for cardiovascular diseases. The objective of this study was to extract peptide hydrolysates from heat stabilized defatted rice bran by enzymatic hydrolysis, evaluate the hydrolysates for gastrointestinal (GI) resistance, fractionate the GI-resistant hydrolysates by ultrafiltration to obtain >50 and 10-50 kDa fractions, and determine antihypertensive and antioxidant activities in the fractions. For antihypertension activity, angiotension-1 converting enzyme (ACE) assay, and for antioxidant activity, the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay was conducted. We report that the ACE-I inhibition activity values for the unfractionated and unhydrolyzed (control), and fractions of >50 kDa, and 10-50 kDa were 6% (control), 78%, and 55%, respectively, clearly denoting antihypertensive activity for the peptide fractions. When tested for antioxidant activity, the >50 kDa fraction decreased from an initial DPPH of 95.48 to 78.99 mg/g, while the 10-50 kDa fraction decreased from an initial 110.35 to 76.53 mg/g, depicting reduction of radical-induced oxidant stress. The results demonstrated that the high molecular sized peptide hydrolysate fractions (>50 and 10-50 kDa) from rice bran bear antihypertensive and antioxidant properties and could possibly find a place as a health beneficial nutraceutical ingredient in food applications.

[*] Janika Hull is a 2011 graduate of Alabama A&M University with a major in food science and a minor in amimal science. This paper is based on research conducted with the protein chemistry group at the University of Arkansas during her summer internship in 2010 funded through the Masterfoods USA, IFT Foundation.

[†] Arvind Kannan is a Post-doctoral Associate in the Department of Food Science.

[§] Navam S. Hettiarachchy is a faculty mentor and a professor in the Department of Food Science.

MEET THE STUDENT-AUTHOR

Janika Hull

I am from Cuba, Ala. I attended Sumter County High school where I graduated valedictorian in May 2007. I began my college career in the fall of 2007 at Alabama A&M University on a Presidential Scholarship. I am majoring in food science with a minor in animal science.

I am involved in various activities in the food science department at Alabama A&M University such as Phi Tau Sigma Honor Society, Food Science Club, and I am also a part of the Institute of Food Technology. I have served as public relations officer and the junior class representative on the executive board of the Food Science Club.

I was awarded an internship through Masterfoods USA, IFT foundation to conduct exciting and challenging research in the protein chemistry group at the University of Arkansas in the summer of 2010. I plan to get my M.S. degree in nutritional biochemistry. I would like to thank Dr. Hettiarachchy, Dr. Kannan, and everyone that is a part of the protein chemistry group in the department of food science for all their advice, help, and guidance.

INTRODUCTION

Rice is the cereal grain with the second highest worldwide production of over 550 million tons annually. It is considered a staple food for a major part of the world. Asian countries like China and India top the overall production of rice, followed by the United States. Approximately 85% of the rice consumed in the United States is grown in the United States (USA Rice Federation, 2009). Rice is grown globally, but the bran and germ—the most nutritious and versatile parts of the rice kernel—traditionally have been underutilized as food sources.

Rice bran is a by-product of the rice milling process. Rice bran contains high quality proteins, which have unique nutritional value and properties (Tang et al., 2002). Rice bran is a hypoallergenic food ingredient and has been reported to have anti-cancer activity (Kannan et al., 2008, 2009).

Rice bran has become popular because of the complex number of nutrients contained within the bran. It contains phytosterols, polysaccharides, beta-sitosterol, fiber, Vitamin E complex and a large complement of B vitamins; including B15, a vital antioxidant. Rice bran also contains Coenzyme Q10, omega-3 and omega-6 fatty acids and oleic acid also (Good Nutrition Center, 2008). Antioxidants, which may also be found in rice bran, are nutrients that fight off free radicals in our bodies. These free radicals cause premature aging and many of the degenerative diseases of old age.

Hypertension (high blood pressure) affects 73 million adults in the United States alone (Crosta, 2009). Hypertension occurs when the blood pressure in arteries is too high. When this occurs, the heart has to work harder and this can lead to heart attack, stroke, heart failure, aneurysm, or renal failure. Rice bran fractions have been shown to possess the ability to inhibit angiotensin I-converting enzyme (ACE-I) which has the ability to control blood pressure (Bernstein, 2006). Although this has been found, not much work has been done with rice bran to characterize antihypertensive and antioxidant protein components.

Bioactive compounds are chemical compounds, such as vitamins, minerals, and electrolytes, that produce biological activity in the body. They possess the ability to impart health benefits or reduce the risk of disease. Bioactive compounds are being studied in nutrition and medicine for disease fighting properties. The positive health benefits of these bioactive compounds have been shown to come from plants and animals. It has been found that vegetarianism is on the rise; therefore, more plants are being pursued for bioactive compounds. Cereal grains and their components are being investigated for the presence of these components.

Hence the overall objective of this study was to utilize protein components for potential bioactive properties from a natural co-product such as rice bran. The specific objectives were 1) to produce peptides from heat-stabilized defatted rice bran (HDRB) by specific food-grade enzymatic

hydrolysis; 2) to produce gastrointestinal (GI)-resistant peptides; 3) to fractionate the peptides to obtain high molecular size (10-50, and >50 kDa) fractions; and 4) to evaluate them for antioxidant and antihypertensive activities.

MATERIALS AND METHODS

Heat-stabilized defatted rice bran was obtained from Riceland Foods (Stuttgart, Ark.), and the Romicon ultrafiltration system was purchased from Koch Membrane Systems, (St. Louis, Mo.). The food-grade alcalase enzyme from a bacterial strain was purchased from Novozyme (Cambridge, Mass.). All other chemicals were of reagent grade, and were obtained from BDH Chemicals (St. Louis, Mo.).

Preparation of Peptide Hydrolysates from Heat-Stabilized Defatted Rice Bran. Heat-stabilized defatted rice bran was ground and passed through a 60-mesh sieve. Fifty grams of HDRB was suspended in a total volume of 500 mL with deionized water. The mixture was homogenized (VWR Adaptable Homogenizer-VDI 25) on a setting of 3 for 3 min. The pH was then adjusted to 8.0 using 1N NaOH. Alcalase was added at 3.5 AU at 55 °C for 1 h in a shaker bath. The enzyme was inactivated at 85 °C for 5 min. The mixture was then centrifuged 10,000 x g for 15 min. The hydrolysate was separated as the supernatant.

Treatment of Peptide Hydrolysate with Simulated Gastric Juices. Sodium chloride (0.2 g) and 0.7 mL of concentrated hydrochloric acid were added to 100 mL of the hydrolysate. The combination was stirred for 30 min. The pH was adjusted to 2.0 using 3N HCl. Pepsin was added at 0.32 g/100 mL and the solution was allowed to shake incubate at 37 °C for 1 h. To inactivate the enzyme, the pH was adjusted to 7.2. The mixture was centrifuged at 3000 g for 20 min to obtain soluble peptide hydrolysates in the supernatant.

Simulated Intestinal Juice Preparation. Potassium phosphate monobasic (0.68 g) was prepared and stirred for 30 min. The pH of the solution was adjusted to 8.0, and the mixture was maintained at 37 °C. Pancreatin (Sigma-Aldrich, St. Louis, Mo.) at a final concentration of 0.1% was added and stirred. The simulated gastric juice treated hydrolysate was dissolved in this simulated intestinal juice and allowed to incubate at 37 °C with constant shaking. After 120 min, the enzyme was inactivated by heating at 85 °C for 10 min. The reaction mixture was centrifuged at 3000 g for 20 min to obtain soluble peptide hydrolysate in the supernatant. The hydrolysate was stored at 4 °C.

Fractionation of Gastrointestinal (GI)-Resistant Peptide Hydrolysate by Ultrafiltration. Fractionation was carried out with a Romicon ultrafiltration system (Koch Membrane Systems, St. Louis, Mo.) equipped with 2.54-cm diameter hollow-fiber polysulfone membrane cartridges. The filtered soluble GI-resistant peptide hydrolysate was run through sequential ultrafiltration columns with membrane cartridges having nominal molecular weight cutoffs (MWCO) of 50 and 10 kDa. The resulting retentates from each of the MWCO were freeze dried and stored at 4 °C until used for bioactivity assays.

ACE-1 Inhibition Activity. Fifty µL of the rice bran sample was added to 50 µL ACE-I (25 mU/mL). The mixture was then added to 150 µL H-H-L (Hippuryl-L-Histidyl-L-Leucine) in 0.1 M sodium borate buffer-pH 8.3 plus 0.5 M NaCl. The solution was incubated for 30 min at 37 °C, followed by 1N HCL addition at 250 µL along with 1 mL of ethyl acetate. The suspension was centrifuged for 10 min at 3,000 x g, and 0.75 mL was collected from the upper layer. The collected sample was evaporated at 95 °C for 30 min. After evaporation, 1 mL of deionized water was added to the residue. Absorbance of the sample was read at 228 nm on a spectrophotometer and percent inhibition was calculated using the equation:

$$\% \text{ Inhibition} = \left[\frac{1 - (\text{Abs}_{\text{peptide}} - \text{Abs}_{\text{blank}})}{(\text{Abs}_{\text{positive}} - \text{Abs}_{\text{blank}})}\right] \times 100$$

DPPH Assay to Evaluate Antioxidant Activity. One hundred microliters of the rice bran samples were added into 1.0 mL of 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) solution. The decrease in absorbance (mg/g of DPPH utilized) was determined at 515 nm using a spectrophotometer after 120 min, using the equation:

$$\text{Antioxidant activity} = \frac{\text{Abs}_{\text{control-sample}}}{\text{Abs}_{\text{control}}}$$

Data Analysis. All values obtained were from triplicate analyses with standard error, and significance of $P < 0.05$. Controls were unfractionated/unhydrolysed rice bran; positive control for antihypertensive study was Captopril at 25 µM.

RESULTS AND DISCUSSION

ACE-I Inhibition. The rice bran hydrolysates were obtained by the use of the alcalase enzyme treatment and treated with the gastrointestinal enzymes, pepsin and pancreatin, to obtain GI-resistant peptides. These GI-resistant peptides were fractioned into 10-50 kDa and >50 kDa by ultrafiltration and characterized for ACE-1 inhibitory activities.

Angiotensin II is a potent constrictor of blood vessels. It is derived from Angiotensin I by the action of an enzyme Angiotensin convertor enzyme-I (ACE-I). There are drugs available that prevent this enzyme's activity in order to prevent formation of Angiotensin II (Weir and Dzau, 2000).

We evaluated the ability of high molecular sized (>50 and 10-50 kDa) peptide fractions to inhibit the ACE-I enzyme for a possible antihypertensive effect. We report that the ACE-I inhibition activity values for the unfractionated and unhydrolyzed (control), and fractions of >50 kDa, and 10-50 kDa were 6% (control), 78% for the >50 kDa fraction, and 55% for the 10-50 kDa fraction, respectively (Fig. 1). When compared to the control, the rice bran extract showed significantly higher activity in ACE-I inhibition, indicating a possible antihypertensive activity for the peptide fractions. Ardiansyah et al. (2006) fractioned rice bran using Driselase and ethanol, and found that there were no significant differences between the two fractions compared to controls. No other study has evaluated these bioactive effects using GI-resistant peptides that were fractionated based on molecular sizes. In our study, the >50 kDa fraction had a significantly higher percent inhibition compared to the 10-50 kDa fraction. This study is the first to characterize rice bran extract based on molecular size for antihypertensive and antioxidant effects.

There are commercially available drugs for antihypertension such as Captopril and Enalapril, but these drugs have been reported to have adverse side effects (Ardiansyah et al., 2006). It is important to research rice bran for antihypertensive characteristics because rice bran is a natural food source having the potential to combat high blood pressure without side effects and inexpensively. A study done on ACE-I inhibition for soy protein suggested the potential for production of peptides with ACE-inhibitory activity upon physiological digestion of soy protein (Lo et al., 2006); and as with our study, a simulated digestion was used. Such studies, in complement with ours, can add value to natural food sources and co-products that can provide alternative potential as therapeutic agents for chronic diseases.

Antioxidant Activity. The DPPH radical assay is extensively used when assessing the radical scavenging activity of several natural compounds. The 2,2-Diphenyl-1-Picrylhydrazyl radical is scavenged by antioxidants through the donation of an electron. The color changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. Radical scavenging activity increases with increasing percentage of the free radical inhibition (Brand-Williams et al., 1995).

In our study we assessed the radical scavenging activity of the peptide fractions, demonstrating the amount (in mg/g) of remaining DPPH after reaction over time. We observed a decrease in DPPH levels, signifying reduction in free radical formation in the presence of peptide fractions (Fig. 2). It should be noted that both high molecular sized fractions (>50 and 10-50 kDa) caused an initial drop in DPPH levels in the first 30 min, and then remained until 90 min. We find a further reduction in DPPH level in the 10-50 kDa fraction from 90 to 120 min possibly signifying a delayed response. Nevertheless, both fractions were able to reduce the DPPH levels and maintain the observed effect for up to 90 min, signifying antioxidant property. The control slightly increased from 49.41 but later decreased back to 49.41, the >50 kDa fraction decreased from an initial DPPH of 95.48 to 78.99 mg/g after 120 min, and the 10-50 kDa fraction decreased steadily from an initial of 110.35 to 76.53mg/g.

Free radicals can cause many undesirable reactions leading to tissue damage. Lipids, proteins and DNA can all be victims of free radicals. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation. Recently much focus has been cast on natural antioxidants that serve to eliminate or reduce the risk of developing oxidative stress rather than synthetic ones, owing to reduced potential side effects and affordability. Several antioxidants have emerged from natural sources but very few from co-products. In our study, we not only establish anti-hypertensive action for peptides from rice bran but also suggest possible antioxidant activity too. Common peptides present in these fractions may function as key players in the reduction of chronic symptoms or manifestations occurring with cardiovascular diseases. In comparison to other natural cereal grains, such as corn, wheat, oat, and rice, the antioxidant levels were relatively high: corn had 87%, wheat had 90%, oat had 58% and rice had 71% (Horax et al., 2005, Horax, 2009). Rice bran had a higher antioxidant activity than rice and oat, but lower activity when compared to corn and wheat. Rice proteins are also hypoallergenic and hence are widely preferred over proteins derived from other sources. Thus it will be appropriate and valuable to characterize and use peptides derived from rice bran that show potential as antihypertensive and antioxidant agents.

In conclusion, the ACE-I inhibitions for the fractions were higher when compared to the negative control. The results show that the rice bran fractions (especially the >50 kDa fraction) have the ability to reduce high blood pressure. Antioxidant activity of rice bran extracts using DPPH resulted in a decrease in mg/g DPPH during a 120-minute time period. The decrease in DPPH signifies antioxidant activity promoting reduction in free radicals. These results show great promise and potential for rice bran being utilized as a nutraceutical to aid management of chronic illnesses like hypertension and oxidative stress.

LITERATURE CITED

Ardiansyah, H. Shirakawa, T. Koseki, K. Ohinata, K. Hashizume, and M. Komai. 2006. Rice Bran Fractions Improve Blood Pressure, Lipid Profile, and Glucose Me-

tabolism in Stroke-Prone Spontaneously Hypertensive Rats. J Agric Food Chem. 54:1914-1920.

Bernstein, M. 2006. How Nice, Brown Rice: Study Shows Rice Bran Lowers Blood Pressure In Rats. Med News Today: Health News. <http://www.medicalnewstoday.com/articles/38829.php>. Accessed 18 June 2010.

Brand-Williams, W., C.E. Cuvelier, and C. Berset. 1995. Use of free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol. 8, 25-30.

Crosta, P. 2009. Hypertension News from Medical News Today. Medical News Today: Health News. <http://www.medicalnewstoday.com/sections/hypertension/>. Accessed 10 June 2010.

Good Nutrition Center. 2008. What's All the Fuss about Rice Bran? The Center for Good Nutrition. Web. <http://www.goodnutritioncenter.com/ricebran.html>. Accessed 10 June 2010

Horax, R. 2009. Preparation, Physicochemical and Functional Properties of Protein and Extraction and Antioxidant Activities of Phenolics from Pericarp and Seeds of Bitter Melon (Momordica Charantia). Ph.D Dissertation, University of Arkansas, 200 pp.

Horax R., N. Hettiarachchy, and S. Islam. 2005. Total phenolic contents and phenolic acid constituents in 4 varieties of bitter melons (*Momordica charantia*) and antioxidant activities of their extracts. J Food Sci. 70:C275-C280.

Kannan A., N. Hettiarchchy, M.G. Johnson, and R. Nannapaneni. 2008. Human Colon and Liver Cancer Cell Proliferation Inhibition by Peptide Hydrolysates Derived from Heat-Stabilized Defatted Rice Bran. J. Agric. Food Chem. 56:11643-1647.

Kannan A., N. Hettiarchchy, and S. Narayan. 2009. Colon and Breast Anti-cancer Effects of Peptide Hydrolysates Derived from Rice Bran. The Open Bioactive Comp J. 2:17-20.

Lo, W.M.Y, E.R. Farnworth, and E.C.Y. Li-Chan. 2006. Angiotensin I-Converting Enzyme Inhibitory Activity of Soy Protein Digests in a Dynamic Model System Simulatingthe Upper Gastrointestinal Tract. J. Food Sci. 71:S231-237.

Tang, S., N. Hettiarchchy, T.H. Shellhammer. 2002. Protein Extraction from Heat-Stabilized Defatted Rice Bran. 1. Physical Processing and Enzyme Treatments. J. Agric. Food Chem. (50.20):7444-448.

USA Rice Federation. 2009. Domestic Usage Report for the Milling Year August 1, 2008-July 31, 2009. <http://www.usarice.com/index.php?option=com_content&view=article&id=1113:domestic-usage-report-consumption-increases-imports-decline&catid=104:usa-rice-dailyl> Accessed 10 June 2009.

Weir M.R. and V.J. Dzau. 2000. The renin-angiotensin-aldosterone system: a specific target for hypertension management. Am. J. Hypertens. 12: 205S–213S.

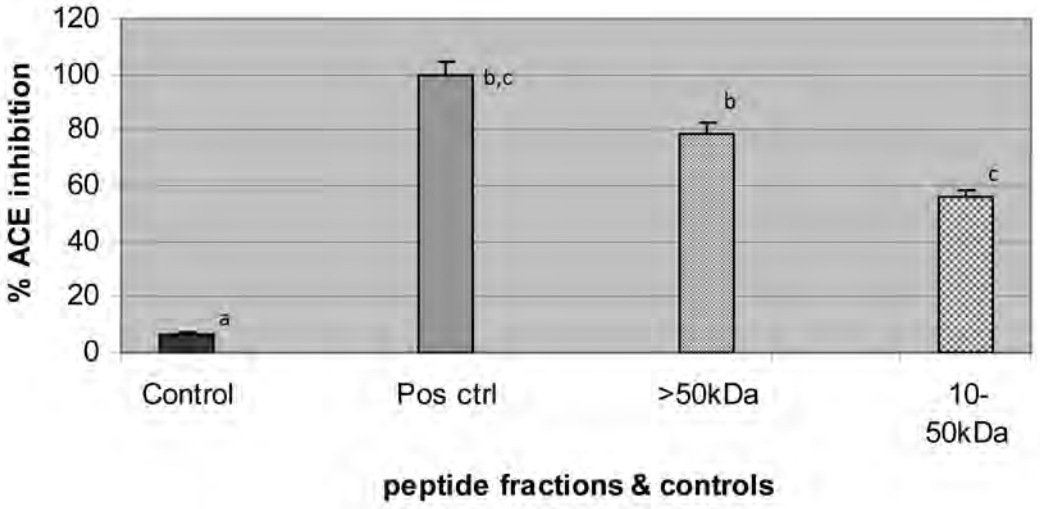

Fig. 1. ACE inhibitory activity of rice bran peptide hydrolysate fractions. (ACE-I) Angiotensin I Converting Enzyme inhibition was assayed using HHL (Hippuryl-LHistidyl-L-Leucine) substrate in presence and absence of the peptide fractions. All values are means of three replications ± Standard Deviation. Control: unfractionated and unhydrolyzed rice bran; pos ctrl; Captopril 25 μM.

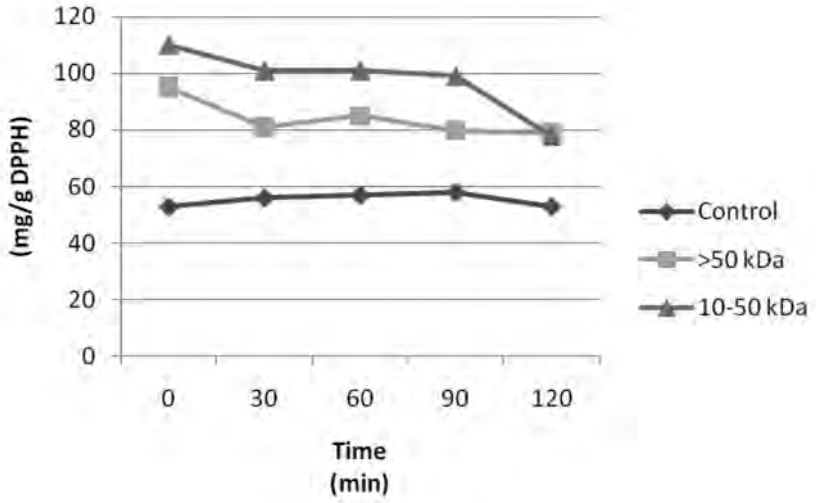

Fig. 2. Antioxidant activity of rice bran peptide hydrolysate fractions. Antioxidant activity was assayed using the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay. All values are means of three replications with standard error (too minimal to observe). Control: unfractionated and unhydrolyzed rice bran.