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Bioactive Phytochemicals in Rice Bran: Processing and Functional Properties

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Abstract

Rice bran is a by-product of rice milling industry and constitutes around 10% of the total weight of rough rice. It is primarily composed of aleurone, pericarp, subaleurone layer and germ. Rice bran is a rich source of vitamins, minerals, essential fatty acids, dietary fiber and other sterols. The quantification of γ-oryzanol in Rice bran can be performed by many methods that involve extraction of Rice bran oil from the bran, followed by analysis of the amount of γ-oryzanol in the Rice bran oil by HPLC. In order to determine the amount of γ-oryzanol in Rice bran oil it is very important to completely extract this fraction from the oil. Various extraction techniques have been used for the analysis of γ-oryzanol in Rice bran oil such as liquid–liquid extraction, solid phase extraction, supercritical fluid extraction (SFE) and direct solvent extraction. There is a widespread scientific agreement on various health benefits associated with consumption of dietary fiber. Consumer attitude towards health foods is promising and the scope of functional foods is growing in the world markets; rice bran is finding increased applications in food, nutraceuticals and pharmaceutical industries. In addition to the physiological benefits provided by high fiber foods, studies have shown that fiber components can give texture, gelling, thickening, emulsifying and stabilizing properties to certain foods. Considering the importance of rice bran, this review aims to focus on the functionalities of rice bran, its health benefits and potential applications in food industry.

Keywords: ***Rice bran; Stabilization; Functional food; Health benefits.***

Introduction

Rice bran is the by-product obtained during milling of rice grain which contains 12-15% protein [1]. Rice bran protein is a good source of well-balanced amino acid, bioactive phytochemicals such as γ-oryzanol, tocopherols, tocotrienols contains [2] and hypoallergenic protein which is desirable in infant food formulation [3]. Rice bran is an abundant by-product from rice

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production which can be served as a low cost attractive feedstock for the production of bioethanol [4,5]. Pure rice bran that was dilute acid pretreated enzyme saccharification has been reported as an effective substrate for ethanol production by Zymomonas mobilis biofilm. *Z. mobilis* biofilm has illustrated its potential for ethanol production from rice bran hydrolysate than free cells by representing higher survival, higher metabolic maintenance and higher ethanol yield when it is exposed to the toxic inhibitors [6]. Therefore, using biofilm as a biocatalyst represented its feasibility for ethanol production from lignocellulosic material which could lead to the reduction in the operating costs of bioethanol and minimizing the complexity of the process.

Rice bran protein has enormous health benefits and good potentials in food industry applications [7]. Rice bran protein concentrate and isolate are not commercially produced due to lack of commercially feasible extraction method [7]. Fabian et al. reported that several treatment methods (physical, heat, enzymatic and chemical) have been used in the extraction of protein from rice bran. The authors further reported that the use of enzyme and subcritical water treatment showed promising protein yield among all the available pretreatment methods, but the relative expensive cost of enzyme and the quality of protein extracted at relatively high temperatures for subcritical water treatment constitutes limitations to the use of the methods due to reduced protein functionality [7]. Shih found that treatment of rice bran by chemical, enzyme and heat methods prior to protein extraction could affect, often adversely, the protein functionality [8]. Rice bran is mostly burnt off at the rice mills and very little is used in animal feed. By understanding functional properties of dietary fiber, one can increase its use in food applications and aid in developing food products with high consumer acceptance. We systematically studied the chemical constituents and functional properties of dietary fiber from rice bran [9]. Rice bran hemicellulose B (RBHB) had been reported to have many biological activities including decreasing blood cholesterol and preventing colon cancer [10]. This review describes the functionalities and health benefits of rice bran. Supplementation of rice bran in various foods is also discussed.

Industrial processing of rice bran, in order to obtain phytochemical-rich fractions

γ-Oryzanol is an important value-added co-product of rice bran processing. Therefore, research to improve the recovery of γ-oryzanol and other phytochemicals in order to obtain fractions enriched in a particular compound or group of compounds, has been conducted. Within this concern, particular attention has been paid to brown rice milling and Rice bran oil extraction and refining by either physical or chemical techniques. Relatively novel procedures involve the use of SC-CO_2, subcritical water and enzymes [11].

Influence of milling

Rice bran has been traditionally processed as a homogenous material; however, it has been shown that high-value components in the rice bran layer vary according to kernel thickness, bran fraction, rice variety, and environmental conditions during the growing season [12]. Thus, current rice milling technology produces rice bran from different layers of the kernel caryopsis. The phytochemical contents of each of these fractions can vary widely, the γ-oryzanol concentration being higher in the outer bran layers [13].

Rice bran fractionation is advantageous for two reasons: (1) Some fractions contain higher concentrations of components of interest, with respect to the overall bran layer average, and (2) Less bran needs to be processed to obtain components of interest [12]. According to Ha et al., the lipid, tocols, γ-oryzanol, squalene and octacosanol contents of both brown and milled rice decreased as the degree of milling increased; however, the phytosterol profile remained the same, β-sitosterol being the most abundant (50-56% of total phytosterols). Milled and brown rice showed also differences in the relative percentages of α-tocopherol, and α and γ-tocotrienols [14].

Chemical and physical refining of rice bran oil

γ-Oryzanol and most other phytochemicals are largely lipophilic, and thus are extracted with the rice bran oil; however, differently from tocols, γ-oryzanol is transferred to soapstock during the neutralization step of chemical refining of RBO [15]. Alternatives to conventional chemical refining, including physical refining and the use of membrane technology [16] have been proposed. Zigoneanu et al., have investigated the microwave-assisted extraction of rice bran with isopropanol and n-hexane at increasing temperatures. The increase in tocols with temperature, from 40°C to 120°C, was 59.6% for isopropanol and 342% for n-hexane; however, isopropanol was better than n-hexane for the extraction of γ-tocopherol and γ-tocotrienol, also leading to higher oil yields at high temperatures. Further, fractions extracted with isopropanol at 120°C had the highest antioxidant activity [17]. Van Hoed et al. examined the effects of each individual step of chemical refining of RBO on its major and minor components. Large γ-oryzanol losses and a change in the individual phytosterol composition were produced by either alkalinisation or neutralization. After bleaching, some isomers of 24-methylenecycloartanol were detected. Due to their relatively high volatility, free phytosterols and tocotrienols were stripped off from RBO during deodorization, and thus concentrated in the deodorizer distillate, but the RBO γ-oryzanol concentration did not change upon deodorization [18].

Extraction with supercritical-CO_2

Owing to the low viscosity and high diffusivity of SC fluids, highly efficient extraction procedures can be developed. Further, from the environmental viewpoint, SC-CO_2 is much better than organic solvents. Xu et al. compared liquid organic solvents with SC-CO_2 relative to efficiency for extracting lipids and γ-oryzanol from rice bran. Among the solvents tested, a 50:50 n-hexane/isopropanol mixture at 60°C for 45-60 min produced the highest γ-oryzanol yield. Without previous saponification, the yield of γ-oryzanol was approximately two times higher than that with saponification. However, using SC-CO_2 the yield of γ-oryzanol was approximately four times higher than the highest yield obtained by extraction with liquid organic solvents [19].

Extraction with subcritical water

At temperatures and pressures close to that of its critical point (374°C, 22 MPa), water behaves like a highly hydrophobic solvent, thus being useful to extract lipophilic substances from solid and semi-solid matrices. After cooling and depressurizing, a lipophilic and a hydrophobic fraction can be obtained. High recoveries in extraction times much shorter than using SC-CO_2 can be achieved. Today, however, subcritical water extraction for rice bran processing has been still scarcely investigated, and as far as we know, scaled-up processes of industrial interest have not yet been described. Hata et al., have also treated defatted rice bran with subcritical water in the 180-280°C range. The total sugar concentration was the highest for the extracts at 200°C. The protein concentration and radical-scavenging activity increased at increasing temperatures. The extracts obtained below 200°C showed emulsifying and emulsion-stabilizing activities [20].

Enzymatic processing techniques

The use of enzymes in rice bran processing, including enzymes specifically modified by genetic engineering, is still today a new and relatively unexplored technology. Potential applications are the development of improved food products and novel products of pharmaceutical interest. A processing technology to polish rice in a selective way with the help of xylanases and cellulases has been developed [2,22]. Enzymes produced by *Aspergillus* sp. And *Trichoderma* sp. acted upon the non-starch polysaccharides of the bran layers of moistened brown rice, releasing their monomeric sugar constituents, as detected through HPLC. Surface degradation of the rice grain was also studied by scanning electron microscopy [22]. Selective degradation of bran layers facilitated the retention of phytochemicals. Antioxidant activity followed the order brown rice >enzyme-treated rice>milled rice. In comparison to mechanically-milled rice, bio-polished rice had better cooking attributes and higher antioxidant concentrations [21].

Stabilization of rice bran

Although being an excellent nutrient source, rice bran is not suitable for human consumption due to the rancidity caused by presence of lipases. While removing bran layers from the endosperm during milling, the individual cells are disrupted and lipase enzymes come into contact with fat causing hydrolysis to free fatty acids (FFA) and glycerol [23]. Additionally, various antinutritional factors present in rice bran limit its use as a food ingredient. The factors include trypsin inhibitors, heamagglutinin-lectin and phytates [24]. However, stabilization, an enzyme inactivation process, is widely employed to extend the shelf life of rice bran, enabling incorporation of rice bran back into our diet. Studies have shown that all undesirable factors except phytates present in bran are proteinaceous in nature, therefore, mild acid and alkali treatment and thermal cooking can denature these proteins [25]. Different techniques are employed for rice bran stabilization (TABLE 1).

TABLE 1. **Various techniques for stabilization of rice bran [26].**

Stabilization technique	Conditions
Hot air drying	100°C for 1h
Steaming	100°C for 30 min
Refrigeration	2°C
Sun drying	47°C (maximum) 7 h per day for 2 days
Fluidized bed drying	84°C for 1 h
Chemical stabilization Spraying	1000 ppm HCl solution
Microwave heating	2450 MHz for 2 min
Infrared	700 W for 30 min
Ohmic heating	20-40% moisture; 44-72 V/cm voltage gradients

Health benefits of rice bran

Natural products obtained from plants have been used as a prominent source of prophylactic agents for the prevention and treatment of diseases in humans and animals [27]. Nutraceuticals including phytochemicals are perceived as offering some of the greatest opportunities for improving human health [28]. Phytochemicals of dietary and non-dietary origin have been the focus of researchers in the recent past because of their potential to counter various diseases. Rice bran contains phytochemicals with promising health benefits [29]. Some of the important bioactive components presented in rice bran are presented in TABLE 2. Rice bran oil rich in natural antioxidants may play a role in reducing the risk of chronic diseases [30].

TABLE 2: **Bioactive compounds present in rice bran [31].**

Anthocyanins and flavonoids	**Polymeric carbohydrates**	**Phenolic and cinnamic acids**	**Steroidal compounds**
Anthocyanin monomers, dimmers and polymers	Arabinoxylans	Caffeic acid	Acetylated steyrl glucosides
Apigenin	Glucans	Coumaric acid	Cycloartenol ferulate
Cyaniding glucoside	Hemicellulose	Catechins	Campesterol ferulate
Cyanidin rutinoside	-	Ferulic acid	24-methylenecycloartenol ferulate
Epicatechins	-	Gallic acid	γ-oryzanol
Eriodtyol	-	Hydroxybenzoic acid	β-sitosterolferulate
Hermnetins	-	Methoxycinnamic acid	Tocopherols
Hesperetin	-	Sinapic acid	tocotrienol
Isohamnetins	-	Syringic acid	-
Luteolin	-	Vanillic acid	-
Peonidin glucoside	-	-	-
Tricin	-	-	-

The possible mechanisms for the hypocholesterolaemic effect of rice bran oil and its effect on aortic fatty streak (early atherosclerosis) formation was investigated. For this experiment, 30 hamsters were fed, for 8 weeks, chow-based diets plus 0.03-0.05% added cholesterol and 5-10% (w/w) physically-refined rice bran oil. Both plasma total cholesterol and LDL cholesterol were significantly reduced by the supplementation. A significant 30% increase in neutral sterol excretion was reported. The results suggested that lipid lowering is associated with attenuated cholesterol absorption and subdued fatty streak formation with this oil may be associated with its non-triacylglycerol components [32]. The effect of oryzanol (rice bran oil ferulic acid) on hamsters fed a chow-based high cholesterol diet was investigated. After 10 weeks on the diet, plasma total cholesterol and LDL concentrations were significantly lower and plasma HDL concentrations were elevated.

It was concluded that oryzanol and rice bran oil diets have significantly lower aortic cholesteryl ester accumulation compared to the control diet [33]. Cholesteryl ester is a highly hydrophobic ester with a role in atherosclerosis. Liquid-phase of this ester constitutes dominant component of atherosclerotic plaque and leads to atherothrombosis [34]. The effect of γ-oryzanol on lipid metabolism in Wistar rats with type 2 diabetes was evaluated. 15% palm oil added with 5.25 g γ-oryzanol markedly reversed the increase in plasma LDL cholesterol, plasma triacylglycerol and hepatic triacylglycerol levels [35]. The effect of substitution of cooking oil with a blend of 80% rice bran oil and 20% safflower oil on LDL concentration levels was studied. At the end of the 3 months consumption period, 82% of patients from the supplemented group had LDL levels less than that of the control group [36]. The effect of α-tocopherol, α-tocotrienol, or γ-tocotrienol-rich fraction on *in vivo platelet thrombosis* and *ex vivo* platelet aggregation was compared in canine model. Results showed that tocotrienols, given intravenously, could potentially prevent pathological platelet thrombus formation and thus provide therapeutic benefit to subjects vulnerable to stroke and myocardial infarction [37]. A randomized study was conducted to find the impact of phytosterol-containing spread manufactured from rice bran oil on cholesterol level. From blood sample analysis, it was gathered that rice bran oil significantly reduces total cholesterol. It was reported that consumption of 20 g rice bran oil spread consumed as normal diet ingredient is effective in lowering serum cholesterol [38]. The influence of rice bran oil consumption on plasma lipids and insulin resistance in patients with type 2 diabetes was investigated. In the group consuming 250 ml milk-fortified with 18 g rice bran oil daily for 5 weeks, total serum cholesterol and LDL cholesterol concentrations decreased significantly [39].

The mechanism of hypocholesterolaemic activity of γ-oryzanol was investigated through *in vitro* study. A 20-fold molar excess of γ-oryzanol significantly decreased apical uptake of cholesterol into human intestinal Caco-2 cells. Also, γ-oryzanol inhibited 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity. HMF-CoA reductase is the rate-controlling enzyme in the mevalonate pathway, responsible for synthesis of cholesterol and isoprenoids. Statin drugs administered to lower cholesterol target this enzyme; in this regard γ-oryzanol appears very promising [40]. The influence of rice bran and phytic acid on lipid metabolism and antioxidative status in high fat-fed mice was investigated. Supplementation of both components in the diet for 7 weeks counteracted the high fat induced hyperlipidemia and oxidative stress, by increment of faecal lipid excretion and regulation of antioxidant and lipogenic enzyme activities [41].

A randomized, clinical trial was conducted to determine the effect of rice bran oil (30 g/day), with a low-calorie diet, on lipid profiles of hyperlipidaemic patients. Metabolic parameters as serum triacylglycerol, total cholesterol, LDL, and HDL were measured before and after the intervention. At the end of the fourth week, total cholesterol, LDL, and atherogenic ratio of total cholesterol/ HDL were significantly decreased in the group treated with rice bran oil. The results confirm that rice bran oil, when consumed as part of a healthy diet, is effective in fighting the risk factors for cardiovascular disease [42]. Fractions of peptides were isolated and characterized from rice bran for possible inhibitory effects against obesity. Hydrolysis and gastrointestinal juices treatment followed by fractionation resulted in a pentapeptide with anti-obesity effects [43].

The antioxidant capabilities of anthocyanin and tocol extracts from black rice bran were evaluated. In an emulsion system containing cholesterol, anthocyanin proved superior in stabilizing cholesterol, while tocol showed better inhibition of fatty acid oxidation [44]. The effect of vitamin E analogues, especially γ-tocotrienol on hepatic triglyceride deposition in rat hepatocytes, was investigated and its inhibitory role was established. It was suggested that the tocotrienol might prevent hepatic steatosis and ameliorate endoplasmic reticulum stress and subsequent inflammation in the liver [45]. Whether rice bran enzymatic extract-supplemented diet is capable of attenuating microvascular alterations in obese rats was assessed. The extract (1% and 5%) administration for 20 weeks restored microvascular function in obese rats through a marked increase in nitric oxide and endothelial-derived hyperpolarizing factor contribution by up-regulation of eNOS and calcium-activated potassium channel expression, respectively. Also, the modulation caused substantial reduction of microvascular inflammation and superoxide anion formation [46].

Supplementation of rice bran for development of functional foods

Rice bran has a long history of use in livestock feed. The use of rice bran in chicken feed dates back to the early 1900s. This indicates that earlier rice bran, obtained during polishing, was primarily used as an animal feed but is gaining importance commercially due to the beneficial nutritive and biological effects. A number of studies have been carried out to evaluate rice bran as a functional ingredient in various foods to improve the nutritional quality. Rice bran being high in dietary fiber and in view of its therapeutic potential, its addition can contribute to the development of value-added foods or functional foods that currently are in high demand [47]. Rice bran hemicelluloses and preparations from defatted rice bran have great potential in food industry, especially in development of functional foods such as functional bakery products [9]. Enrichment of bakery products with rice bran and its effects are summarized in TABLE 3.

TABLE 3. **Enrichment of bakery products with rice bran [47].**

Product enriched	Purpose of addition	Inference	References
Gluten-free bread with dietary fiber fractions	Quality improvement	Acceptable structural and textural quality Sensory acceptance increased and shelf life extended	[48]
Cookies with rice bran	Effect on cooking quality	Average width, thickness and spread factor increased with increment in rice bran. Supplementation of heat stabilized rice bran at 10% is suitable for production of rice bran supplemented cookies	[24]
Pasta	Effect of enrichment on the color, cooking, sensory quality and shelf life of enriched pasta	Pasta with added brans had higher dietary fiber and protein contents Rice bran up to 15% level did not affect the Physico-chemical, cooking and sensory quality of pasta.	[49]

Conclusion

Rice bran is a good source of proteins, minerals, fatty acids, fiber and fatty acids. Considering the importance of rice bran, it can serve as an important raw material for the development of nutraceuticals and functional foods including bread, corn flakes, ice cream, pasta, noodles and zero-trans-fat shortening. Owing to numerous health benefits associated with the consumption of rice bran, detailed in vivo studies are recommended to create a strong data base. Also, the comparative analysis of the shelf life achieved by stabilization with different techniques has not been carried as of now which is an interesting area of research. This can help predict the best procedure for stabilization to enhance the supplementation of rice bran in various food systems.

REFERENCES

1. Zhang HJ, Zhang, H, Wang L, et al. Preparation and functional properties of rice bran proteins from heat-stabilized defatted rice bran. Food Research International. 2012;47:359-63.
2. Rohrer CA, Siebenmorgen TJ. Nutraceutical Concentrations within the bran of various rice kernel thickness fractions. J. Biosystems Engineering. 2004;88:453-60.
3. Xia N, Wang J, Yang X, et al. Preparation and characterization of protein from heatstabilized rice bran using hydrothermal cooking combined with amylase pretreatment. Journal of Food Engineering. 2012;110:95-101.
4. Okamoto K, Nitta Y, Maekawa N, et al. Direct ethanol production from starch, wheat bran and rice straw by the white rot fungus Trametes hirsuta. Enzyme Microb Technol. 2011;48:273-77.
5. Watanabe M, Takahashi M, Sasano K, et al. Bioethanol production from rice washing drainage and rice bran. J Biosci Bioeng. 2009;108:524-26.
6. Todhanakasem T, Sangsutthiseree A, Areerat K, et al. Biofilm production by Zymomonas mobilis enhances ethanol production and tolerance to toxic inhibitors from rice bran hydrolysate. New Biotechnol. 2014;31:451.
7. Fabian C, Ju YH. A review on rice bran protein: its properties and extraction methods. Crit Rev Food Sci Nutr. 2011;51:816-27.
8. Shih FF (2003) An update on the processing of high-protein rice products. Nahrung 47: 420-24.
9. Hu GH, Huang SH, Cao SW, et al. Effect of enrichment with hemicellulose from rice bran on chemical and functional properties of bread. Food Chemistry. 2009;115:839-42.
10. Hu G, Yu W. Binding of cholesterol and bile acid to hemicelluloses from rice bran. Int J Food Sci Nutr. 2013;64:461-66.
11. Lerma-García MJ, Herrero-Martínez JM, Simó-Alfonso EF, et al. Composition, industrial processing and applications of rice bran? Oryzanol. 2009;115:389-404.
12. Schramm R, Abadie A, Hua N, Xu Z, Lima M (2007) Fractionation of the rice bran layer and quantification of vitamin E, oryzanol, protein, and rice bran saccharide. J Biol Eng. 1:9.
13. Lloyd BJ, Siebenmorgen TJ, Beers KW. Effects of commercial processing on antioxidants in rice bran. Cereal Chemistry. 2000;77:551-55.
14. Ha T, Ko Y, Lee SN, Kim SM, et al. Changes in nutraceutical lipid components of rice at different degrees of milling. European Journal of Lipid Science and Technology. 2006;108:175-81.

15. Narayan AV, Barhate RS, Raghavarao KSMS. Extraction and purification of oryzanol from RBO and RBO soapstock. Journal of the American Oil Chemists' Society. 2006;83:663-70.
16. Manjula S, Subramanian R. Enriching oryzanol in rice bran oil using membranes. Appl Biochem Biotechnol. 2008;151:629-37.
17. Zigoneanu IG, Williams L, Xu Z, et al. Determination of antioxidant components in rice bran oil extracted by microwave-assisted method. Bioresour Technol. 2008;99:4910-18.
18. Van Hoed V, Depaemelaere G, Ayala JV, et al. Influence of chemical refining on the major and minor components of RBO. Journal of the American Oil Chemists' Society. 2006;83:315-21.
19. Xu ZM, Godber JS. Comparison of supercritical–fluid and solvent extraction methods in extracting oryzanol from rice bran. Journal of the American Oil Chemists' Society. 2000;77:547-51.
20. Hata S, Wiboonsirikul J, Maeda A, et al. Extraction of defatted rice bran by subcritical water treatment. Biochemical Engineering Journal. 2008;40:440-53.
21. Das M, Banerjee R, Bal S. Evaluation of physicochemical properties of enzyme treated brown rice (Part B). LWT-Food Science and Technology. 2008;41:2092-96.
22. Das M, Gupta S, Kapoor V, et al. Enzymatic polishing of rice – A new processing technology. LWT-Food Science and Technology. 2008;41:2079-84.
23. Malekian F, Rao RM, Prinyawiwatkul W, Marshall WE, Windhauser M, et al. (2000) Lipase and lipoxygenase activity, functionality, and nutrient losses in rice bran during storage (Bulletin Louisiana agricultural experiment station Louisiana state university agricultural center), p: 870.
24. Younas A, Bhatti MS, Ahmed A, et al. Effect of rice bran supplementation on cookie baking quality. Pakistan Journal of Agricultural Sciences. 2011;48:129-34.
25. Jiaxun T (2001) Method of stabilization of rice bran by acid treatment and composition of the same. United States [http://www.google.com.mx/patents/US6245377?]. Assessed on: 1st May 2014.
26. Dhingra D, Chopra S, Rai DR. Stabilization of raw rice bran using ohmic heating. Agricultural Research. 2012;4:392-98.
27. Bagchi D. Nutraceuticals and functional foods regulations in the United States and around the world. Toxicology. 2006;221:1-3.
28. Gul, K, Singh AK, Jabeen R. Nutraceuticals and functional foods: the foods for future world. Critical Reviews in Food Science & Nutrition. 2015.
29. Jariwalla RJ. Rice-bran products: phytonutrients with potential applications in preventive and clinical medicine. Drugs Under Experimental and Clinical Research. 2001;27:17-26.
30. Thanonkaewa A, Wongyai S, McClements DJ, et al. Effect of stabilization of rice bran by domestic heating on mechanical extraction yield, quality, and antioxidant properties of cold-pressed rice bran oil (*Oryza saltiva L.*). Food Science and Technology. 2012;4:231-36.
31. Friedman M. Rice brans, rice bran oils, and rice hulls: composition, food and industrial uses, and bioactivities in humans, animals, and cells. J Agric Food Chem. 2013;61:10626-41.
32. Ausman LM, Rong N, Nicolosi RJ. Hypocholesterolemic effect of physically refined rice bran oil: studies of cholesterol metabolism and early atherosclerosis in hypercholesterolemic hamsters. J Nutr Biochem. 2005;16:521-29.

33. Wilson TA, Nicolosi RJ, Woolfrey B, et al. Rice bran oil and oryzanol reduce plasma lipid and lipoprotein cholesterol concentrations and aortic cholesterolester accumulation to a greater extent than ferulic acid in hypercholesterolemic hamsters. J Nutr Biochem. 2007;18:105-12.
34. Duivenvoorden R, vanWijk D, Klimas M, et al. Detection of liquid phase cholesteryl ester in carotid atherosclerosis by 1H-MR spectroscopy in humans. JACC. Cardiovascular Imaging. 2013;6:1277-84.
35. Cheng HH, Ma CY, Chou TW, et al. Gamma-oryzanol ameliorates insulin resistance and hyperlipidemia in rats with streptozotocin/nicotinamideinduced type 2 diabetes. International Journal for Vitamin and Nutrition Research. Internationale Zeitschrift Für Vitamin- Und Ernährungsforschung. Int J Vitam Nutr Res. 2010;80:45-53.
36. Malve H, Kerkar P, Mishra N, et al. LDL-cholesterol lowering activity of a blend of rice bran oil and safflower oil (8:2) in patients with hyperlipidaemia: A proof of concept, double blind, controlled, randomised parallel group study. Journal of the Indian Medical Association. 2010;108:785-88.
37. Eady S, Wallace A, Willis J, et al. Consumption of a plant sterol-based spread derived from rice bran oil is effective at reducing plasma lipid levels in mildly hypercholesterolaemic individuals. The British Journal of Nutrition. 2011;105:1808-18.
38. Lai MH, Chen YT, Chen YY, et al. Effects of rice bran oil on the blood lipids profiles and insulin resistance in type 2 diabetes patients. Journal of Clinical Biochemistry and Nutrition. 2012;51:15-18.
39. Mäkynen K, Chitchumroonchokchai C, Adisakwattana S, et al. Effect of gamma-oryzanol on the bioaccessibility and synthesis of cholesterol. European Review for Medical and Pharmacological Sciences. 2012;16:49-56.
40. Kang MY, Kim SM, Rico CW, et al. Hypolipidemic and antioxidative effects of rice bran and phytic acid in high fat-fed mice. Food Science and Biotechnology. 2012;21:123-28.
41. Zavoshy R, Noroozi M, Jahanihashemi H. Effect of low calorie diet with rice bran oil on cardiovascular risk factors in hyperlipidemic patients. J Res Med Sci. 2012;17:626-31.
42. Kannan A, Hettiarachchy N, Mahadevan M. Peptides derived from rice bran protect cells from obesity and Alzheimer’s disease. International Journal of Biomedical Research. 2012;3:131-35.
43. Zhang X, Shen Y, Prinyawiwatkul W, et al. Comparison of the activities of hydrophilic anthocyanins and lipophilic tocols in black rice bran against lipid oxidation. Food Chem. 2013;141:111-16.
44. Muto C, Yachi R, Aoki Y, et al. Gamma-tocotrienol reduces the triacylglycerol level in rat primary hepatocytes through regulation of fatty acid metabolism. Journal of Clinical Biochemistry and Nutrition. 2013;52:32-7.
45. Justo ML, Claro C, Vila E, et al. Microvascular disorders in obese Zucker rats are restored by a rice bran diet. Nutrition, Metabolism, and Cardiovascular Diseases. 2014;24:524-31.
46. Gul K, Yousuf B, Singh AK, et al. Rice bran: Nutritional values and its emerging potential for development of functional food-A review. Bioactive Carbohydrates and Dietary Fibre. 2015;6:24-30.
47. Phimolsiripol Y, Mukprasirt A, Schoenlechner R. Quality improvement of rice-based gluten-free bread using different dietary fibre fractions of rice bran. Journal of Cereal Science. 2012;56:389-95.
48. Kaur G, Sharma S, Nagi HP, et al. Functional properties of pasta enriched with variable cereal brans. J Food Sci Technol. 2012;49:467-74.
49. Makar AB, McMartin KE, Palese M, et al. Formate assay in body fluids: application in methanol poisoning. Biochem Med. 1975;13:117-26.