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Impact on the nutritional attributes of rice bran following various stabilization procedures

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Running Title: Rice Bran, Stabilization and Nutrition

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ABSTRACT

Rice bran, a valuable byproduct of the rice milling process, has limitations in food industrial applications due to its instability during storage. This review summaries the methodology for stabilization and its impact on the nutritional properties of rice bran. A variety of treatments have been used and these include heat treatment, low-temperature storage, biological and chemical approaches and these will be discussed in terms of their ability to destroy/inhibit enzyme activity and improve storage performance of rice bran. More importantly, changes in the nutritional value of rice bran in terms of vitamins, polyphenols, tocopherols, flavonoids, free fatty acids caused by stabilization of rice bran will also be discussed. This review highlights the importance of appropriate design of processes for stabilization and controlling storage conditions to ensure quality of the rice bran and enhancing levels of phytochemicals in the bran for novel applications in functional foods.

KEYWORDS: Rice bran; Phytochemicals; Lipase activity; Stabilization mechanism; Antioxidant; Nutrition

Introduction

Rice bran which is present on the outer layer of the rice kernel is an important byproduct resulting from the rice milling process (Delahaye, Jiménez, and Pérez 2005). Rice bran has been reported to be an excellent source of nutrients including protein (14–16%), crude fiber (8–10%), fat (12–23%), carbohydrates, minerals and vitamins (Kahlon 2009; Malekian et al. 2000). In addition, rice bran is also a rich source of phytochemicals such as phytosteroids, γ-oryzanol, tocopherol and tocotrienols. Other compounds such as lipoic acid and co-enzymes are also present as minor constituents (Rashid et al. 2015; Riaz et al. 2010; Loypimai, Moonggarm, and Chottanom 2009; Ausman, Rong, and Nicolosi 2005; Prakash 1996). The components of rice bran provide the potential for it to be used as a raw material for food, medicine and health care products, with enhanced benefits compared to other bran types. For example, certain antioxidant components which are present in rice bran such as tocopherols, tocotrienols and γ-oryzanol have been demonstrated to have free radical scavenging ability and antioxidant activity (Butsat and Siriamornpun 2010) which can contribute to lowering oxidative-stress in diseases such as cancer, cardiovascular disorders, inflammation, aging and obesity (Amarasinghe, Kumarasiri, and Gangodavilage 2009). It has also been reported that these phytochemicals are also associated with beneficial effects associated with nerve imbalance, menopausal problems, serum hypercholesterolemia, coronary heart disease and cancer. Cicero and Derosa (2005) and Roberts (2000) have reported that dietary fibers can decrease the incidences of gastrointestinal disease and colon cancer. In addition, the components of natural fibers in rice bran can also meet the recommended dietary intake for an adult (approximately 27 g/d) (Diet 1990). Moreover, rice bran has a higher protein content rich in essential amino acids, such as lysine. Rice bran can also be used as an ingredient in food recipes (Wang et al. 1999).

However, utilization of rice bran is limited due to its instability during storage and in order to enhance the utilization of rice bran in the food industry, it is essential to stabilize rice bran, which prolongs its shelf life and increases the added value of rice bran. This article reviews different procedures for stabilizing rice bran in particular the influences of these processes on the status of the nutritional composition of rice bran, and also provides further understanding of the chemical changes in rice bran during the stabilization process, and maintenance of rice bran nutritional value. From a commercial perspective, there is a growing market for rice bran with added value and supportive scientific information of these benefits will promote food industry interest in processing novel rice bran based products for human consumption besides its current use as an animal feed (Faria and Bassinello 2012).

The instability mechanism of rice bran

The restrictive factor for limited utilization of bioactive components in rice bran is instability due to hydrolytic and oxidative rancidity (Tao, Rao, and Liuzzo 1993). When bran layers are removed from the endosperm during the rice milling process, the individual cells are disrupted and lipids in rice bran come in contact with highly reactive enzymes such as lipases (Kim, Chung, and Lim 2014). These naturally occurring lipases in rice bran hydrolyze lipids to free fatty acids (FFA), resulting in a rapid increase in acidity of rice bran together with an unpleasant flavor (Ramezanzadeh et al. 1999). The content of lipid in rice bran is about 20%, and the main components are neutral lipids and phospholipids in addition to certain glycolipids (Takano 1993). The neutral lipid component is mainly composed of triglycerols whilst lecithin, phosphatidyl inositol and phosphatidyl inositols are the most abundant phospholipids. Rice bran also contains several types of lipases, including lipolytic enzyme and 3 types of phospholipases, including phospholipase A (both A1 and A2), each acting on fatty acid esters, and phospholipase C and phospholipase D (Takano 1993). The comparative activity ratio of these four enzymes is 100: 24: 35: 39, with lipolytic enzyme being the most active lipase (Takano 1993). Rice bran lipase has an optimum activity at pH 7.5-8.0, and at a temperature of 37 ^{o}C (Oliveira et al. 2012). During the decomposition of triglycerols, the enzyme cleaves fatty acid ester bonds at the 1,3-site, releasing the fatty acids. Neutral triglycerols are decomposed into acid fatty acids and monoglyceride and diacylglycerol, which increases the acidic value. Due to steric hindrance, lipases have a relatively weaker effect on the ester bond of triglycerols and under acidic conditions, the monoglyceride is broken down by lipolytic enzymes producing the final products glycerol and fatty acids (Takano 1993; Aizono et al. 1971). Furthermore, rice bran also contains many types of phospholipases, which can decompose phospholipids in rice bran under optimum conditions. The ester bond of the first (or second) fatty acid in phospholipids is first hydrolyzed by phospholipase A1 (or A2) into a lysophospholipid with a free hydroxyl, which can be further modified by phospholipase B to produce fatty acids. The activity site of phospholipase C and D is specific for the ester bond of phospholipids and glycerol and phospholipids and choline (Takano 1993).

Secondly, a high content of crude fiber in rice bran can affect the mouth feel of rice bran based food, which is also a restrictive factor and as a result the product is mainly used as animal feed (Pradeep et al. 2014; Paucarmenacho et al. 2007). Therefore, enzymatic inactivation to avoid FFA formation and to promote its utilization after milling is essential for development of new food based products (Faria and Bassinello 2012).

Rice bran stabilization

Current approaches to prevent the rice bran from developing rancidity is through the stabilization of rice bran to inactivate lipase activity, and reducing lipid hydrolysis, which delays the deterioration of rice bran and improves storage performance (Faria and Bassinello 2012;

Ramezanzadeh et al. 1999). The stabilization methods can be summarized to include a range of physical methods (low temperature refrigeration, heat treatment), biological and chemical methods as shown in Table 1 (Patil and Mohapatra 2016; Mada, Sanches, and Amante 2006).

Physical stabilization methods

Low temperature storage or refrigeration

Rice bran can be stored at low temperatures to arrest the activity of lipase (Prakash 1996), achieving a state of stabilization. As early as 1949, Loeb et al. placed rice bran samples in temperatures of 3, 25, and 31 ^{o}C, and found that the samples incubated at 3^{o}C showed a reduced increase in FFAs. Amarasinghe et al. (2009) used refrigeration (0 ^{o}C) to stabilize rice bran and found that the activity of lipase in rice bran was successfully controlled and resulted in only a 6% reduction in oil extraction after 50 days suggesting that low temperature (0 ^{o}C) storage or refrigeration can effectively control lipase activity. However, this method has limitations due to restoration of enzyme activity at room temperature resulting in incomplete inhibition of fat decomposition and oxidative rancidity of rice bran (Tao, Rao, and Liuzzo 1993). This method is only suitable for small-scale storage purposes and the desired results would not be achieved in a large-scale industrialized production setting due to changeable environmental conditions. Additionally, economic considerations include the high energy consumption involved in maintaining the low temperature (Amarasinghe, Kumarasiri, and Gangodavilage 2009).

Heat treatment

Heat treatment can also inactivate the lipase of rice bran, which can effectively destroy microorganisms, and maintain nutrient content in rice bran (Thanonkaew et al. 2012). Depending on the type of heat treatment, lipase activity can be either reversibly inhibited or permanently denatured. This practical approach allows the use of different methodologies to treat rice bran such as heating in open pans (Ahmed et al. 2007), microwave oven (Ramezanzadeh et al. 1999), and twin-screw extruder equipment (Riaz et al. 2010). There are different types of heat stabilization procedures: dry heating, moisture heating, microwave heating, ohmic heating, radiation heating and extrusion (Bagchi, Adak, and Chattopadhyay 2014; Thanonkaew et al. 2012).

Dry heat method

Hot air is used to reduce the moisture content of rice bran which results in inactivation of lipase and stabilization of rice bran (Bagchi, Adak, and Chattopadhyay 2014; Thanonkaew et al. 2012). However, increased storage time, the re-absorption of water in rice bran will reduce the inhibitory effect, with renewed lipase activity on fat and decomposition of a large number of FFAs. Therefore, control of temperature and moisture content is an important parameter, of which Viraktamath and Desikchar (1971) reported optimum conditions to stabilize rice bran at a temperature of 100-130 ^{o}C by dry heating. The factors of uniform heating, heating duration and energy consumption should also be considered in large-scale industrialized dry heating (Wang et al. 2017).

A previous study has highlighted that hot air drying is an effective method for stabilizing rice bran, but the process is not economical and not suitable for use in a small-scale (Amarasinghe, Kumarasiri, and Gangodavilage 2009). Simple heat treatment procedures using pan roasting and microwave heating have been used to arrest lipase action and increase the shelf-life of rice bran (Ahmed et al. 2007). In the heat-treated rice bran, the increase in FFA content at the end of a 3-month storage period has been shown to 1.6 to 2.5-fold, compared to 12- to 23-fold in the absence of heat treatment. Moreover, the nutritional profile of the rice bran was not altered by the heat treatment methods used in this study.

Specifically, the protein and fat content were both increased as heat treatment also reduced the moisture content of the rice bran, while the total ash content remained the same with thiamine content slightly increased. More importantly, rats fed with heat-treated rice bran showed increased food intake, body weight gain, organ weight, and increased hemoglobin and serum protein levels compared to controls, suggesting a suitable safety profile for possible animal/human consumption and (Ahmed et al. 2007).

Further investigation of the influence of domestic heating (hot air heating, microwave heating, roasting, and steaming) on the stabilization of rice bran showed that hot air and microwave heating are effective in controlling the activity of lipase enzyme in rice bran, reducing levels of FFA and peroxide compared to unstabilized rice bran. In addition, hot air heating and microwave stabilized rice bran oil (RBO) contained a higher level of total phenolic compounds with associated increase of antioxidant activities for RBO compared to the unstabilized RBO. Hot air heating stabilized RBO showed the highest content of γ-oryzanol. These results indicated that the stabilization of rice bran by domestic heating could be an effective method for RBO extraction with improved oil extraction yield, quality and antioxidant properties compared to unstabilized RBO. Hot air heating is an efficient and economical method, suitable for use in small and medium scale operations and more importantly, domestic heating can be employed without deleterious changes to major nutrient components in the bran (Thanonkaew et al. 2012).

Moisture heating

By adding hot steam to the rice bran, the temperature of rice bran is increased, and, lipase is inactivated resulting in stabilized rice bran. Compared with the dry heating method, the main advantages of moisture heating are uniform heating, short heating time and efficient enzyme inactivation. In one study the superheated steam was applied to treat oat that had been milled out of the hull and lipase activity was completely inactivated and could be stored for 24 weeks at 21 °C, or 12 weeks at 38 °C with low levels of FFA, demonstrating that the moisture heating method can inactivate lipase and prolong the storage time of rice bran (Head et al. 2011). Amarasinghe et al. (2009) compared several methods including steam treatment to stabilize rice bran and also investigated the effect of stabilization methods on rice bran oil extraction. The results showed that steam treatment was a very efficient method at stabilization, successfully controlling the lipolytic activity of rice bran, resulting in lower FFA levels when compared with unstabilized rice bran. In addition, steam treatment resulted in only a 3% reduction in oil extraction in comparison with 89% unstabilized bran. Stabilization through steaming, roasting, hot air and microwave heating has been shown to retard the generation of an acidic environment and production of increased FFA and peroxide levels compared with unstabilized rice bran. Furthermore, the γ-oryzanol content of rice bran stabilized with steaming (at 130 ^{o}C for 2 min), hot air, roasting, and microwave was significantly higher than that of crude bran (Thanonkaew et al. 2012). Thanonkaew et al. (2012) has reported the process induced disintegration of cell wall and consequent increase in fat soluble components. Heat processing reduced the polyphenolic components (Randhir, Kwon, and Shetty 2008). However, study from Pradeep et al. (2014) showed that the contents of oryzanols, tocotrienols and tocopherols in steam-stabilized bran were higher than in native and without significant loss of health beneficial components in rice bran. This may imply that the steaming treatment not only effectively denatures lipases activity, but also significantly promotes the level of above bioactive compounds due to the shift from bound to free status induced by the effect of heat-moisture treatment. This study indicated that steaming is one of ideal methods for stabilization and retention of these nutraceuticals.

Microwave heating

In recent years, microwave heating has gained increasing attention in rice bran stabilization. Microwave heating is based on ultra high frequency electromagnetic waves with strong penetrating power (Abdul-Hamid et al. 2007; Malekian et al. 2000). When microwave passes through rice bran, the energy is converted into heat energy by intermolecular friction which is transferred to the rice bran quickly and uniformly. With the rise in treatment temperature, the lipase in rice bran is denatured and inactivated (Malekian et al. 2000). Advantages of microwave heating for rice bran stabilization include penetration into material and simultaneous heating of the whole material without heat conduction from outside to inside. In summary, microwave heating is time-saving, providing uniform heating with small thermal inertia and high thermal conversion rates (Nordin et al. 2014; Zigoneanu et al. 2008; Ramezanzadeh et al. 1999). During heating process, the initial moisture content is a critical factor in the microwave stabilization of rice bran, which should be controlled reasonably to optimize the treatment process. In addition to enzyme inactivation, microwave heating also has beneficial bactericidal and insecticidal effects, which has little effect on nutritional quality or functional properties of the rice bran (Patil and Mohapatra 2016).

Various heat treatments, including dry heating, microwave heating, have also been effective in stabilizing rice bran and reducing oxidative rancidity (Kim, Chung, and Lim 2014). Moreover, the bio-functional components of rice bran such as tocols, phytosterols and policosanols have been shown to be increased in comparison with those in untreated samples due to the disassociation of those compounds with proteins or lipids caused by the applied thermal energy (Moreau, Hicks, and Powell 1999). This also recently confirmed that the dry heating and microwave heating under the appropriate conditions improve the availability of bioactive components in rice bran in addition to stabilization effect (Kim, Chung, and Lim 2014).

In one study in order to inactivate lipase, two heat treatment strategies were applied: roasting on a conventional stove and heating in a microwave oven. The results showed that the levels of some nutrients of treated samples showed significant changes compared to corresponding raw samples, highlighting the preservation of some nutrients during microwave treatment together. The microwave oven procedure had a better nutrient preservation effect compared with whole rice bran. The total lipids, proteins and insoluble and soluble fiber content as well as some fatty acids (palmitic acid, stearic acid and oleic acid) and minerals (calcium and zinc) levels in microwave treated were higher than that in whole rice bran. These results indicated that microwave heating is a practical and rapid stabilization method of rice bran (Faria and Bassinello 2012).

A recent study (Patil and Mohapatra 2016) indicated that fiber content of rice bran stabilized by microwave and parboiling was significantly lower than untreated one, which was possibly due to thermal degradation of fiber during the process. Loss of fiber was not significantly different between the two treatments. Furthermore, the analysis also found that the nitrogen free extracts, which mainly constitute carbohydrates and soluble fiber, were also reduced following the two treatments. However, microwave treatment retained a relative higher level of nitrogen free extracts than parboiling treatment. Meanwhile, other components, such as minerals, proteins and crude fat, were significantly ($p < 0.05$) higher in the stabilized rice bran compared to the untreated samples. The higher crude fat content in treated samples might be due to the formation of agglomeration in the bran for enhancing its oil extractability. In contrast, parboiled rice bran had a higher oil content compared to microwave-treated sample. Another study may consistently interpret this phenomenon, in which parboiling makes the bran surface more likely to be permeable for enhancing the flow of oil and decreases the affinity of oil for solid surfaces on bran which ensures an increased yield of oil (Amarasinghe and Gangodavilage 2004).

Ohmic heating

Ohmic heating is based on the passage of alternating electric current through a conductor resulting in the production of heat. During this process, rice bran serves as an electrical resistance and heat energy is generated instantly inside the rice bran (Dhingra, Chopra, and Rai 2012; Reznick 1996), resulting in lipase inactivation and stabilization of rice bran. This inside-out heating pattern is much faster than conventional outside-in heating. The levels of heat generated are proportional to the current induced by the applied voltage gradient and the inherent electrical conductivity of the food (Sastry and Li 1996). This technology results in uniform heating and the absence of a hot surface in ohmic heating reduces fouling problems and thermal damage to the products (Sastry and Barach 2015). Ohmic heating can be considered as a value-added processing approach and has great potential for use in a wide variety of food processing operations involving heat and mass transfer (Dung 2014).

Ohmic heating has previously been reported as an effective method for stabilization of rice bran with moisture addition (Loypimai, Moonggarm, and Chottanom 2009). In this study, electrical field strengths at 150-225 Vcm^{-1} and a moisture content of rice bran of 30-40 % were the optimum conditions to retard the increasing FFA content, lipase activity and lipid oxidation during storage. The presence of an electric field in ohmic heating can influence biochemical reactions by changing molecular spacing and increase the interchains reaction. Alternatively, the electric field may remove the metallic prosthetic groups present in the lipase structure thus causing the activity loss (Castro, Macedo, Teixeira, and Vicente 2004). Moreover, the levels of the phenolic compounds, α-tocopherol, γ-oryzanol, and antioxidant activity in stabilized rice bran using ohmic heating were enhanced. Furthermore, Dung (2014) have also used ohmic and microwave heating to achieve effective stabilization of rice bran with the addition of moisture. Both the ohmic and microwave-treated samples showed lower levels of lipase activity compared with the untreated samples, accompanied by reduced FFA in treated samples and greater phenolic content and antioxidant activity.

Infrared radiation heating

Infrared radiation (IR) based heating, is a nonchemical and emerging technology, which can achieve efficient drying and simultaneously inactivate lipase in rice bran without compromising quality. When IR is used to heat or dry moist materials, the radiation penetrates exposed material, and radiation energy is converted into heat (Wang et al. 2017). Even though penetration is limited, it can provide more uniform heating and may reduce the moisture gradient during heating and drying. Compared to convective heating, radiation heating is fundamentally different due to the fact that the material is dried directly by absorption of radiation because of the high heat transfer rate (Bal et al. 1970). Radiation does not heat up the medium, and the temperature is not limited by the wet bulb temperature of the surrounding air which results in the rice bran being quickly heated to a higher temperature in a short time (Wang et al. 2017). Moreover, radiation based heating processes generate less dust because less air flows across the product (Sharma, Verma, and Pathare 2005). Additional advantages include versatility, simplicity of the required equipment, and the fast response of heating (Chua and Chou 2003).

Previous research has shown that IR stabilization effectively prevented hydrolytic rancidity of rice bran and when compared with raw bran, the γ-oryzanol content and fatty acid composition of IR-stabilized rice bran showed no significant change. However, tocopherol content decreased significantly by up to 50%. Stabilization at a high IR power for a short time period resulted in a relatively lower loss of tocopherol content. This study suggests that optimal IR stabilization of the nutritious by-products of rice milling could provide a potential additional commercial use and value-added commodity in the food industry (Yılmaz, Tuncel, and Kocabıyık 2014). Moreover, a study by Yılmaz in 2016. demonstrated that stabilization at 700W middle infrared power for 7 min provided 3 months of shelf life for rice bran without a notable change in FFA content. Total contents of tocopherol and γ-oryzanol in the stabilized rice bran fractions were also higher than their crude counterparts. The impact of treatment method of far-infrared radiation (FIR), hot-air and cellulase on the changes of antioxidant capacity and levels of bioactive compounds in bran was also investigated (Wanyo et al., 2014), and the data demonstrated that the FIR-treated group showed higher antioxidant activity and total phenolic content (TPC) than hot-air and cellulase treated samples. In particular, FIR irradiated treatment led to a significantly increased α- and γ-tocopherols compared to its corresponding raw sample, while those in the hot-air and cellulase treated ones remained unchanged. The plausible interpretation is that the FIR process may have the capability to cleave covalent bonds and liberate antioxidants such as flavonoids, carotene, tannin, ascorbate, flavoprotein or polyphenols from repeating polymers (Niwa et al, 1988). More interestingly, σ-tocopherols were only detected in FIR irradiated rice bran, indicating that an appropriate process could improve the properties of naturally occurring antioxidants or potentially induce the formation of new compounds with novel antioxidant functionality (Wanyo, Meeso, and Siriamornpun 2014; Tomaino et al. 2005).

Extrusion

Extrusion is reported to be one of the most suitable methods to stabilize rice bran (Zhu and Yao 2002) which is characterized with a multi-functional thermal process. The high temperature, high pressure and high shearing force produced during the extrusion process can inactivate enzymes such as lipase and peroxidase in rice bran, leading to stabilization of rice bran (Shivendra, Shirani, and Lara 2007; Kim, Tanhehco, and Ng 2006). Studies have shown that when compared with the non-extruded rice bran, the growth rate of FFA in rice bran is decreased after extrusion, and the rice bran preservation period is extended. Meanwhile, other nutrients and natural antioxidants in rice bran could be highly retained following the extrusion. In general, extrusion demonstrates advantages over other stabilization methods such as high productivity, short processing time, simple operation, generation of unique product shapes, and ultimately good product stability (Kim, Tanhehco, and Ng 2006).

Rice bran stabilized by extrusion and dry heat has shown a better improvement in shelf life after extrusion treatment. Additional increased amounts of fiber, gum fiber and total fiber in dry heat treated rice bran did not differ significantly from raw rice bran. The functional properties of stabilized rice bran such as water absorption, water solubility, bulk density, and enzyme susceptible starch of rice bran are also improved in extrusion-stabilized rice bran compared to dry heat treatment (Sharma, Chauhan, and Agrawal 2004). A recent study described the use of extrusion for stabilizing rice bran with the results of improved color and other physicochemical property (Rafe and Sadeghian 2017). In comparison with raw rice bran, the levels of phytic acid, protein and vitamin E in stabilized rice bran are also significantly reduced whilst lipids, vitamins B2, B3, B5 and folic acid remained statistically unchanged, and the dietary fiber content was enhanced which benefits potential functionality. Based on the improved nutritional properties, the extruded bran could facilitate its use as an ingredient in a wide rage of food varieties (Rafe and Sadeghian 2017).

However, the extrusion method requires strict control of operating conditions to maintain nutrient values. In general, mild process conditions, such as a higher moisture content, a lower residence time and a lower temperature, are essential to achieve higher nutritional aspects (e.g. greater retention of amino acids, protein and vitamins, improved starch digestibility, increased soluble dietary fiber and reduced lipid oxidation). On the other hands, harsh extrusion conditions can cause nutrients great destruction (Shivendra, Shirani, and Lara 2007).

Sub-critical water technique

Considering that sub-critical water (Sub-CW) technique is an environmentally friendly approach, it was also being used for inactivating lipase activity and extracting bioactive compounds (Herrero, Cifuentes, and Ibanez 2006). In one study lipase was denatured without an increase in total FFA concentration in the Sub-CW treated RBO, benefiting for obtaining stabilized edible RBO. In addition, the oil extraction yield was generally increased following increased treatment temperature and residence time. Moreover, in addition to its extraction efficiency, the RBO extracted by Sub-CW also demonstrated a better stability during storage (Pourali, Asghari, and Yoshida 2009).

Biological method

Stabilization of rice bran by biological methods is also referred to as enzymatic treatment stabilization, which utilizes protease to decompose lipase. Protease catalyzed biological based decomposition of lipase can be achieved through mixing rice bran with water, and is maintained at a constant temperature to achieve irreversible inactivation of lipase (Vallabha et al. 2015; Laokuldilok and Rattanathanan 2014). This method results in selective targeting of enzyme action with mild reaction conditions and no limiting reagent residue and preservation of rice bran nutrients. Advantages of this method include simple operation and low cost (Mourad et al. 2009). Novel approaches have been developed based on enzymatic stabilization of rice bran. A US patent describes the use of an antilipase enzyme, which would preferably be a nonspecific protease of plant or fungal origin (Hammond 1998). However, exact conditions of enzymic stabilization are not clear from the published document.

Both protease and endoglucanase have been previously used to process rice bran using enzymatic treatment, where the lipase was completely inactivated along with a 50 % reduction in lipoxygenase activity in addition to maintaining viable nutrients and nutraceuticals derived from rice bran. For example, γ-oryzanol, α-tocopherol and other polyphenols were retained within a range of 68 to 110 % with improved total antioxidant activity. The enzyme treated rice bran also showed a significant increase in prebiotic activity enriched with short chain fatty acids such as acetate and propionate. The important cellular antioxidant ubiquinol-10 was also retained in the enzyme treated rice bran following inactivation of lipase and lipoxygenase activities, and this helped to retain the reduced form of ubiquinol (Vallabha et al. 2015). It is reported that ubiquinol-10 can inhibit lipid peroxidation as well as regenerate other antioxidants such as α-tocopherol (Sunesen, Weber, and Holmer 2001), thus the preservation of ubiquinol-10 in rice bran can provide a similar protection effect to antioxidants. Enzymes offer the potential for many exciting applications to improve the design of functional foods, such as papain, a proteolytic enzyme from the cysteine proteinase family. Mangal et al. (2014) successfully applied papain to stabilize rice bran when treated with a 50 mg (per 100 g of rice bran) enzyme concentration at 60℃ for 30 min. The resulting bran showed FFA levels of less than 10% even after 15 days of storage when compared with unstabilized status.

Chemical stabilization

There are differences in the inactivation of purified lipase and the lipase in rice bran. When purified lipase is inactivated, the conditions can be focused on destroying the enzyme, while in inactivation of lipase of rice bran, it is also necessary to avoid destruction of other important components in rice bran. Chemical stabilization of rice bran is mainly achieved through modification of the acidity or alkalinity of rice bran, with the use of hydrochloric acid, acetic acid and propanol or sodium metabisulfite (Gopinger et al. 2015; Prakash 1996; Azeemoddin et al. 1979). The optimum pH for lipase activity in rice bran is known to be 7.5-8.0 (Oliveira et al. 2012; Takano 1993), and lipase activity will decline with either an increase or decrease in pH value. A simple chemical method for the stabilization of rice bran was developed by Prabakar et al. (1986), which is based on the principle of reducing lipase activity by lowering the pH of the bran. In their study, hydrochloric acid at 40 L/ton of bran was used which lowered the pH of rice bran from 6.9-6.0 to 4.0. Results showed that the acid stabilized bran was not easily infected by microorganisms, such as fungi and appeared to facilitate extraction of crude bran oil. A sprinkling or spraying system can be applied for dispersion of hydrochloric acid providing a practical method which is low cost and applicable for developing countries with numerous small rice mills.

When an HCl solution (0.1%) was applied through spraying, results exhibited control of lipase activity with only a 7% reduction in oil extraction (Amarasinghe, Kumarasiri, and Gangodavilage 2009). Other chemical reagents, such as a mixture of acetic and propionic acids have also been employed to evaluate their effects on rice bran during storage. The whole rice bran treated with 2% of an acetic and propionic acid mixture and stored over 120 days showed an improvement in preservation quality demonstrated by higher gross energy values, and decreased lipid acidity, less lipid oxidation product formation, and yellow color maintenance. However, the use of chemicals may contaminate the bran oil, and chemical stabilization maybe more useful for treating rice bran which is ultimately used as animal feed (Gopinger et al. 2015).

The stabilization of rice bran with the combination of various methods

In general, the combination of several stabilization methods has a better effect. Ramezanzadeh et al. combined microwave heating, packing and storage temperature to stabilize rice bran. The rice bran was first microwave-heated and then packed in zipper-top bags or vacuum-sealed bags and stored at 4-5 or 25 °C for 16 weeks. Results showed that hydrolytic rancidity of rice bran was prevented by microwave heating and that the recommended storage temperature for microwaved rice bran was 4-5 °C in zipper-top bags (Ramezanzadeh et al. 1999). The stabilization of rice bran was carried out by using microwave heating (2450MHz for 3min) then inoculated with a Lactic Acid Bacillus culture, resulting in reduced lipase activity and increased shelf life of rice bran and accompanied by increases in the protein, fat, phosphorous and iron content (Bhosale and Vijayalakshmi 2015). In a recent study, Wang et al. achieved a high heating rate, drying efficiency and milling quality for rough rice by using infrared based heating followed by tempering treatment and natural cooling under a one- and two-pass drying processes. The storage time of stabilized rice bran with an FFA concentration less than 10% could be extended up to 38 days compared to 7 days for the control. These results suggest that IR heating combined with other treatments achieves simultaneous drying and effective stabilization for rice bran and provides a more effective way for the utilization of rice bran without affecting the quality of rice bran oil (Wang et al. 2017).

The influence of multi-treatment on rice bran property was also recently performed through the steaming plus individual or cocktail enzyme and lactic acid bacterial fermentation (Liu et al. 2017). Results showed pre-treatment of rice bran using α-amylase led to an enhanced total phenolics and flavonoids following the multi-treatment of fermentation and complex enzyme hydrolysis, and ferulic acid showed the largest increase in the compounds profile. It is proposed that phenolic esterase and carbohydrase are more produced from lactic acid bacteria for hydrolyzing the ester bond between phenolics and cell wall components to release greater phenolic compounds. Meanwhile, the applied complex enzymes (glucoamylase, cellulase and protease) convert macromolecules to small molecules for promoting bacterial fermentation and softening cell wall structure, which further stimulates the formation of free phenolic acids (Liu et al. 2017).

Conclusion and outlook

There is growing trend in the application of rice bran in functional foods, and consistent stabilization of rice bran is a prerequisite for its effective use. Rice bran stabilization is typically performed after rice milling. In comparing stabilization methods, low temperature storage and refrigeration are poor cost-efficiency (Malekian et al. 2000). Hot air heating may be limited in terms of how quickly the desired temperature can be reached and potential exposure of the bran to non-uniform heating could damage valuable components (Delahaye, Jiménez, and Pérez 2005; Kim et al. 1987). Microwave-based heating is also uneconomical and not suitable for use in rural areas (Thanonkaew et al. 2012). There are a number of technical restrictions associated with radiation treatment, including optimization of process conditions, etc. Moreover, certain radiation types may have deleterious effects on the stability of nutritional components in rice bran, such as γ-irradiation (Shin and Godber 1996). The addition of chemicals to achieve stabilization may contaminate the bran and bran oil, which may influence their utilization and subsequent refining of the oil together with safety concerns. The ideal stabilization method should meet the following requirements: (1) lipase activity should be effectively suppressed so as to prolong the storage period of rice bran; (2) the effect on nutritional components such as protein, starch, and polyphenols should be minimized as far as possible in rice bran, so that functional properties can be maintained; (3) the chosen stabilization method must be economically feasible and easy to apply. Combination of stabilization methods such as microwave and/or enzyme treatment can effectively inhibit the activity of lipase in rice bran, and improve the longevity of rice bran storage together with nutrient content. In addition, current research on the stabilization of rice bran highlights relatively few studies on the influence of stabilization methods on nutrient content, flavor and smell of rice bran. Therefore, it will be necessary to assess dietary rice bran in animal models in terms of its nutritional effect and safety. The influence of various treatments on rice bran nutritional attributes was summarized in Table 1.

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CAPTIONS

Tables

Table 1 Method for stabilizing rice bran and its impact on nutritional attributes

Stabilization method	Treatment	Stabilization mechanism	Influence on nutritional composition

Physical methods	Low temperature storage, refrigeration	Lipase activity is inhibited through low temperature management	The degradation of nutrients was delayed (Amarasinghe, Kumarasiri, and Gangodavilage 2009).

	Heat treatment	Dry heating	Loss of moisture in bran achieves the inactivation of lipase	Protein and oil content increased; total mineral content remained unchanged; thiamine content was slightly increased (Ahmed et al. 2007); formation of acidic compounds, such as FFA and peroxides was delayed; high content of γ-oryzanol achieved (Thanonkaew et al. 2012).

		Moisture heating	Co-existence of heat & moisture destroys lipase activity	Lower FFA and higher oil yield achieved (Amarasinghe, Kumarasiri, and Gangodavilage 2009); the γ-oryzanol content significantly increased (Thanonkaew et al. 2012); the contents of oryzanol, tocotrienol and tocopherol in steam-stabilized bran were higher than in native (Pradeep et al. 2014).

		Microwave heating	Heat energy is transferred to rice bran quickly and the lipase is denatured and inactivate by the increased temperature	Content of mineral and protein slightly increased and the extractability of the oil was enhanced; dietary fiber content & nitrogen free extracts were reduced (Patil and Mohapatra 2016); functional components such as tocols, phytosterols and policosanols were greatly increased (Kim, Chung, and Lim 2014).

		Ohmic heating	Lipase is inactivated by the generated heat energy	Formation FFA and lipid oxidation were reduced; the levels of phenolic compounds, α-tocopherol, γ-oryzanol and antioxidant activity were promoted (Loypimai, Moonggarm, and Chottanom 2009); level of FFA of treated samples increased more slowly with greater phenolic content and antioxidant activity (Dung 2014).

		Infrared radiation heating	Radiation energy is converted into heat to dry the bran and inactivate the lipase	Total contents of tocopherol and γ-oryzanol were higher than their crude counterparts (Yılmaz 2016); content of α-, γ-tocopherols and total phenolic compounds was increased accompanied by a greater antioxidant activity; individual phenolic compounds, such as hydrocinnamic and hydroxybenzoic acids, were enhanced due to cleavage of covalent bonds for liberating them into free statues (Wanyo, Meeso, and Siriamornpun 2014).

		Extrusion	High temperature/pressure/shear force inactivates enzymes such as lipase and peroxidase	Total dietary fiber increased significantly (Sharma, Chauhan, and Agrawal 2004); protein content was reduced due to the denaturation and degeneration of protein; level of phytic acid and vitamin E significantly reduced; Vitamins B2, B3, B5 and folic acid were remained unchanged (Rafe and Sadeghian 2017).

		Sub-critical water technique	Temperature (120-240^{o}C) quickly inactivates lipase and other enzymes activity	No increase in total FFAs concentration observed; yield of oil extraction increased (Pourali, Asghari, and Yoshida 2009).
Biological methods	Enzymatic treatment	The applied enzymes bring destruction onto bran matrix		Soluble fiber content increased; γ-oryzanol, α-tocopherol and polyphenols were retained in the range of 68 to 110 % with improved total antioxidant activity; prebiotic functionality was improved by production of

			short-chain fatty acids in the gut; ubiquinol-10 was retained (Vallabha et al. 2015).
Chemical methods		Acidity or alkalinity of rice bran is adjusted by exterior chemicals for inhibiting the activity of lipase	A lower level of lipid acidity was achieved, and less lipid oxidation was obtained (Gopinger et al. 2015).
Combination of various methods	Combined microwave packing and storage temperature	Multi-influence is achieved by the combined treatments for contributing to either inhibition or destruction of the activity of lipase	Content of extractable protein, fat, phosphorous and iron was increased,

	Combined microwave and probiotic treatment		facilitating a better availability of these nutrients (Bhosale and Vijayalakshmi 2015).

	Steamed with a-amylase, fermented with lactic acid bacteria, and hydrolyzed with complex enzymes		Total content of phenolics and flavonoids was increased with enhanced antioxidant activity due to the formation of phenolic esterase and carbohydrase from lactic acid bacterial fermentation. Addition of complex enzymes (glucoamylase, cellulase and protease) destructed cell wall matrix, benefiting the conversion of bound phenolic compounds into free status (Liu et al. 2017).