

Mini Review

Preparation, properties and application of rice bran protein: A review

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Abstract

A plenty of rice bran are produced as by-product during rice milling process nowadays. It is increasingly considered as an alternative and economic source of plant-based hypoallergenic and high quality protein. Several methods have been used to convert this by-product into other useful substances such as bioactive compounds, food ingredients or food processing aids. The physical processes (homogenization, colloid milling) and some novel technologies (microwave and ultrasonics) are used to extract protein instead of alkaline extraction which provides a low yield and consumes a lot of solvent. Furthermore, enzymatic treatment is also used for assisting protein extraction and improving its bio-functions and functional properties. From this literature review, the information could be useful to create or generate the new idea to extract or produce other innovative protein substances from rice and/or other plant materials. In addition, rice protein may be increasingly applied in food or other products such as nutraceutical or cosmetics.

Keywords

Rice bran

Protein extraction

Functional propertie, Bio-
functions

Rice protein-enriched
products

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Introduction

Rice is widely grown and consumed as staple food for more than half the world's population. According to world rice production, paddy rice was produced about 678 million metric tons (MMT) in 2014 (USDA, 2015). Generally, the composition of whole rice grain is comprised of 63.6-73.2% carbohydrate, 1.5-2.3% fat, 5.8-7.7% protein, 7.2-10.4% fiber and 2.9-5.2% ash (Juliano, 1985). Rice grain contains 3 main parts including endosperm or white rice (~70%), hull/husk (~20%) and bran (~10%) (Figure 1). The white rice is commonly eaten by humans, due to its soft texture and gorgeous appearance. This part is comprised of carbohydrate up to 76.7%, which is the main source of energy while protein was found about 7.4% (Cao *et al.*, 2009). Whereas, hull fraction is not suitable for consuming according to it contains high fiber content (34.5-45.9%) making its hard texture. However, there were some researchers reported about health benefits of rice bran, resulting in increasing of brown rice consuming and generating some ideas to use rice bran as food ingredients etc.

Rice bran is an abundant source of antioxidant compounds such as tocopherols, γ -oryzanol and other phenolics (Aguilar-Garcia *et al.*, 2007), which could help in health effects including lowering of blood cholesterol, decreasing platelet aggregation and anti-inflammation (Chotimakorn *et al.*, 2008 and Lai *et al.*, 2009); in addition, it has also been

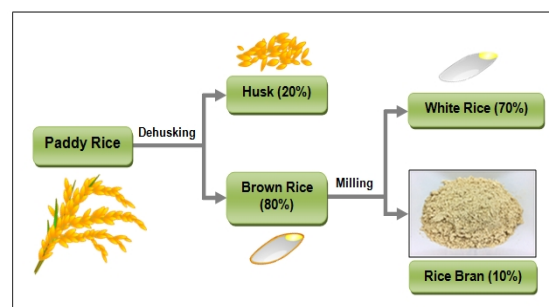


Figure 1. Rice milling process

reported as a source of 12.6-15.4% hypoallergenic and high quality protein (Jiamyangyuen *et al.*, 2004; Huang *et al.*, 2005; Cao *et al.*, 2009) with protein efficiency ratio (PER) of 1.6-1.9 when compared with casein (2.5) (Wang *et al.*, 1999). Furthermore, the chemical, functional and bio-function properties of rice protein seems superior to other proteins such as soya flake, potato starch, peanut, sorghum, kidney bean, and groundnut (Lawal *et al.*, 2007; Londhe *et al.*, 2011; Mesa-Stonestreet *et al.*, 2012; Waglay *et al.*, 2014; Sun and Xiong, 2014). Then, the several methods to extract protein from rice grain especially bran fraction are interested and developed including physical, chemical and enzymatic treatments. Rice protein is suggested as one of important plant-based protein that can be applied or used as ingredient in many products such as infant food, gluten-free products and also cosmetic goods. In this review, an

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overview on rice protein, production, properties and its application were mentioned completely.

Rice protein

Protein is the second most abundant constituent of milled rice, following starch. The main source of protein in rice grain is bran fraction. The protein content ranges from 10% to 16% (Kulp and Ponte, 2000; Cao *et al.*, 2009; Faria *et al.*, 2012) depending on its cultivars. Normally, it can be grouped into four fractions according to their solubility by Osborne fractionation, including glutelin (32.5%), albumin (30.9%), globulin (24.9%) and prolamin (11.6%) (Chanput *et al.*, 2009). The proportion of each protein also differs in different rice cultivars.

Many processes have been developed for rice protein extraction (Figure 2). Thus, the basic and necessary information such as solubility and molecular weight of each protein is important for considering or choosing the suitable method of isolation. The characteristics and properties of each protein fraction are as follows;

Glutelin is the major fraction of rice protein and its content ranges about 22.7-40.25% of the total protein in rice bran (Cao *et al.*, 2009; Chanput *et al.*, 2009). This protein has limited solubility in water due to its molecules are linked by extensive disulfide bond and hydrophobic interactions, which may partly account for the difficulty in protein extraction (Xia *et al.*, 2012). It is soluble below pH 3 and above pH 10 (Juliano, 1985). It has the highest molecular weight (MW) among the rice protein fractions (Houston, 1972). Rice glutelin was composed of acidic subunits (30-39 kDa) and basic subunits (19-25 kDa) which came from a 57 kDa polypeptide precursor. Two subunits were covalently linked to each other by an intermolecular disulfide bond resulting in glutelin molecules with MWs ranging from 64 to 500 kDa (Lasztity, 1996; Cao *et al.*, 2009). Hamada (1997) found that rice glutelin composed of high MW protein ranging from 45 to 150 kDa; while, Chanput *et al.* (2009) and Xia *et al.* (2012) observed MWs of glutelin at 10-60 kDa.

Albumin is normally known as water-soluble protein. The extraction of albumin with water always results in globulin contamination because of minerals present in the rice grain that dissolve in water solvent (Juliano, 1985). Albumin in rice grain ranges about 6.24-9.73% and about 30.9-42.7% (Cao *et al.*, 2009; Chanput *et al.*, 2009). Rice albumins were separated into three to four sub-fractions using gel filtration chromatography (Lasztity, 1996). It has MWs ~100 kDa or less when analyzed by size-exclusion HPLC (Hamada, 1997). Furthermore, Cao *et al.* (2009)

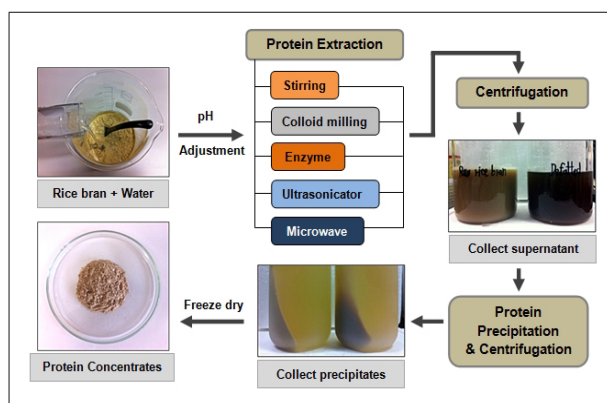


Figure 2. Production of rice bran protein concentrates

reported that albumins have major polypeptides with MWs of 18 to 20 kDa, while Chanput *et al.* (2009) observed two main bands of albumin between 30 to 50 kDa using SDS-PAGE.

Globulin is salt-soluble and sulfur-rich proteins. It was found about 12.5-24.9% of the storage protein in the bran (Cao *et al.*, 2009 and Chanput *et al.*, 2009). Rice globulin contains the different polypeptide chains which were stabilized by disulfide linkages (Hamada, 1997). The globulin of rice endosperm could be separated by gel filtration chromatography into four subfraction; MWs ranged from 16 to 130 kDa (Lasztity, 1996). Xia *et al.* (2012) reported that MWs of globulin in broken rice was 26 kDa. Furthermore, the globulin of Thai rice variety also had the MWs at about 15, 30 and 50 kDa (Chanput *et al.*, 2009).

Prolamin is an alcohol-soluble protein in rice grain. It is usually extracted after the removal of albumin and globulin (Juliano, 1985). This protein contains only about 3.24-11.6% of total protein in rice bran (Cao *et al.*, 2009; Chanput *et al.*, 2009). It may be extracted with 70% ethanol or 50% propanol. According to its very low content and ability to be co-extracted in alkaline extraction, prolamin is usually not focused on consideration the solvent or condition of protein extraction. The major component of rice prolamin had MWs at 23 kDa (Lasztity, 1996). Chanput *et al.* (2009) reported that the major bands of prolamin fraction were observed at 10, 15 and 25 kDa. Meanwhile, the similar result by Cao *et al.* (2009) also confirmed that prolamin consisted of three polypeptide subunits with apparent molecular weight of 10, 13 and 16 kDa.

Preparation of rice protein

Protein are not a major component in rice grain, it is often aggregated or linked to other components such as cell wall material or starch; for example, rice bran contains high phytate (1.7%) and fiber content (12%), and thus make their separation from other

Table 1. Protein extraction efficiency of different methods/conditions

Methods / Raw materials	Conditions	Protein recovery /yield (%)	References
1.) Alkaline extraction			
Full fat rice bran	pH 9.0 at temperature of 24°C	14-20	Connor <i>et al.</i> (1976)
Defatted rice bran	pH 2.0-12.0, extraction time of 32 to 58 min	1.36-12.20	Jiamyangyuen <i>et al.</i> (2004)
Defatted rice bran	pH 8.0, extraction temperature of 63 °C for 340 min	31.27	Silpradit <i>et al.</i> (2010)
Defatted rice bran	pH 9.0, stir at room temperature for 2 hrs	37.6-46.2	Yeom <i>et al.</i> (2010)
Rice bran	pH 9.5, shake (300 rpm) at 50°C for 2 hrs	32.9	Zhang <i>et al.</i> (2012)
Broken rice	0.1% NaOH solution, stir at room temperature for 1 hr, and then left overnight	77.30	Xia <i>et al.</i> (2012)
Rice bran	pH 11.0, solid-liquid ratio of 1:7.5 (w/v), stir for 1 hr	25	Cheetangdee (2014)
2.) Physical extraction			
Defatted rice bran	Blending + Alcalase (enzyme)	81	Hamada <i>et al.</i> (2000)
Defatted rice bran	Freezing 16 hrs + thawing	12-56	Tang <i>et al.</i> (2002)
	Blending + Amylase & Protease	66	
	High pressure + Amylase & Protease	67	
Rice bran	Colloid milling + homogenizing	37.8-67.5	Anderson and Guraya (2001)
Broken rice	Colloid milling	63.8	Xia <i>et al.</i> (2012)
3.) Enzyme-assisted extraction			
Defatted rice bran	Phytase & xylanase, stir at 55°C for 2 hrs	34.0-74.6	Wang <i>et al.</i> (1999)
Defatted rice bran	Amylase, ratio of rice bran to water of 1:17, extraction temperature of 50.90°C	58.4	Tang <i>et al.</i> (2003)
Defatted rice bran	0.1% (w/w) Papain & 5% (v/w) viscozyme, at 37°C for 1 hr	54-82.6	Bandyopadhyay <i>et al.</i> (2012)
Rice bran	Cellulase & hemicellulase, pH 4.0, at 65°C	35-46	Shih <i>et al.</i> (1999)
Rice bran	0.5% w/w α -amylase & viscozyme, pH 4.1-6.25, stir for 60 min	30-35	Cheetangdee (2014)
4.) Novel technology techniques			
Defatted rice bran	Ultrasonic at 100 W for 5 min, pH 11.0	~25.86/4.45	Chittapalo <i>et al.</i> (2009)
Defatted rice bran meal	Microwave at 800 W for 20-90 s, pH 8.0	67-70	Bandyopadhyay <i>et al.</i> (2012)
Defatted wheat gem	Ultrasonic at 363 W for 24 min	37-57%	Zhu <i>et al.</i> (2009)

components difficult (Wang *et al.*, 1999; Fabian and Ju, 2011). Rice proteins are contained in protein bodies inside cell walls so cell disruption such as homogenization and colloid milling is required before they can be totally solubilized and extracted. Several classical and novel techniques including alkaline extraction, microwave- and ultrasonic-assisted extraction are also used to extract proteins.

Alkaline extraction is a most common method by dissolving the soluble rice proteins in dilute alkaline solution. It is considered to break some of the hydrogen, amide, and disulfide bonds in rice glutelin, leading to the reduction in molecular size of protein and the improvement in protein extraction (Xia *et al.*, 2012). There were researchers reported that the use of alkaline-solvent (pH 8-12) improved the extraction yield (30-80%). However, severe alkaline condition changes the nutritional characteristics of protein and produced toxic products. Cystein and serine residues of protein can be converted to dehydroalanine which later on transforms into lysinoalanine which corresponded with decreased contents of cystine and lysine and lower net protein utilization of the protein isolates (Shewry and Mifflin, 1985; Fabian and Ju, 2011). Moreover, high alkaline condition could cause denaturation and hydrolysis of proteins, increasing of maillard reaction which provides the dark color products, and increasing of non-protein components extraction which co-precipitate with protein and lower the protein quality (Wang *et al.*, 1999). The other chemicals such as 0.5 M NaCl and 70% ethanol have also been used to recover the globulin

and prolamin fractions, respectively (Chanput *et al.*, 2009). Many studies have reported about the application of chemical extraction of protein from rice bran following in Table 1.

Generally, physical techniques are easier to be adapted and utilized in industry and can be more economical than other techniques. It is often more desirable than chemical or enzymatic methods in the processing of foods because they induce lesser alterations in foods and lesser health concerns. Common physical methods such as colloid milling, homogenizing, high speed blending, freeze-thaw and high pressure have been employed for protein extraction. The shear forces involved in colloid milling, homogenization, and high speed blending provide the means in disrupting cells for the subsequent releasing of proteins and thus enhance its extraction. For the freeze-thaw process, cell lysis occurs due to the formation of ice crystals that ruptures intracellular membrane structure, whereas when cells are subjected to high pressure, cell breakage can occur (Fabian and Ju, 2011). It has been reported that colloid milling and microfluidization can break protein-starch agglomerates under high shear force in the wet process, leading to better density-based separation of two ingredients (Xia *et al.*, 2012). Some physical methods that have been applied to extract protein from rice are summarized in Table 1.

Alkaline extraction affects to denature of protein, and change its nutritional and functional properties (Tang *et al.*, 2003). Then, enzymatic treatment has been attempted for rice protein production to avoid

these unfavorable consequences. It is an alternative method that allows the extraction of rice bran protein at neutral and slightly basic pH levels. Carbohydrases which can attack the cell wall components including cellulase, hemicellulase, xylanase, or its combination may increase protein yield by liberating more protein from the polysaccharide matrix of bran (Fabian and Ju, 2011; Xia *et al.*, 2012). Other enzymes such as α -amylase and phytase could enhance protein extraction by attacking the interaction of proteins with starch and phytate in the bran. Enzymatic hydrolysis does not affect the nutritional value of the proteins. Additionally, it can improve the physicochemical, functional, and sensory properties of native proteins (Yeom *et al.*, 2010). However, protease has been used to enhance solubility of rice protein (Silpradit *et al.*, 2010). Although the enzymatic extraction produces non-toxic chemicals, it shows some disadvantages such as the long time required and very high cost. The extraction efficiencies of enzyme-assisted extractions are listed in Table 1.

Microwave- (MAE) and ultrasonic-assisted extraction (UAE) are increasingly focused nowadays due to its advantages including higher yield, reduce treatment time, non-toxic, and lower cost of operation. In the case of extraction, the advantage of microwave heating is the disruption of weak hydrogen bonds promoted by the dipole rotation of the molecules. A higher viscosity of the medium lowers this mechanism by affecting molecular rotation. Furthermore, the migration of dissolved ions increases solvent penetration into the matrix and thus facilitates the solvation of the analyte or solute (Kaufmann and Christen, 2002).

Sonication or ultrasonics can break cell walls and molecular bonds due to the effects of high temperature and shock waves causing cavitations collapse of bubbles generated from ultrasound (Tang *et al.*, 2002). Many researchers assured the advantages of ultrasonic-assisted extraction compared with the conventional method (Bean *et al.*, 2006; Zhu *et al.*, 2009). Chittapalo and Noomhorm (2009) stated that the mechanical effects of ultrasound can disrupt rice bran cell wall which was observed under scanning electron microscopy (SEM).

The application of these methods to extract protein from rice is concluded in Table 1.

Precipitation techniques

Protein solubilization and precipitation are considered as the key factor to choose the proper method for protein production. The general methods have been used to isolate protein as following;

The isoelectric point (pI) of protein is the pH

where the net charge on the protein is zero. Since there is no electrostatic repulsion keeping them apart, proteins tend to aggregate and precipitate at their pI (Fennema, 1996). Thus, they can be separated from other components by adjusting the pH of solution. Conner *et al.* (1976) reported that pI precipitation can recover protein from crude rice bran extract about 88.47%.

Among the precipitants, the most widespread substance is ammonium sulphate (Rawdkuen *et al.*, 2010). The addition of high amounts of salt into a protein solution provokes an increase of protein interactions followed by protein aggregation and finally precipitation. Anionic polysaccharide such as alginate and carrageenan were used for recovering of rice bran protein. Both alginate and carrageenan were found to be very effective in precipitating protein (Fabian and Ju, 2010).

Three-phase partitioning or TPP is the use of ammonium sulphate to precipitate the protein, and t-butanol to make three-phase layers and to remove some interference compounds. Protein is recovered in a purified form at the interphase, while the contaminants mostly partition in t-butanol (top phase) and aqueous phase (bottom phase) (Rawdkuen *et al.*, 2010). It provides high protein recovery from rice bran protein extraction about 66-78% (Patsanguan *et al.*, 2013).

Properties of rice protein and its hydrolysates

Rice protein is gaining a lot of interest in the food industry due to its unique properties. Moreover, hydrolysis of protein with proteases could produce many potential peptide sequences; provide a numerous functional and antioxidative properties (Hwang *et al.*, 2010). It could also enhance the antioxidative properties of native protein by attacking the peptide bonds in the interior of polypeptide chain, producing a range of polypeptides, which differ in molecular weight or amino acid sequences (Hamada, 2000). However, a very high degree of hydrolysis can have negative effects on the functional properties, especially the interfacial properties (Intarasirisawat *et al.*, 2012).

Utilization of rice protein as food ingredients greatly depends on the favorable characteristics they impart on food. Protein functionality can be modified by forming intramolecular or intermolecular crosslinks such as Transglutaminase (TG) catalyses the reaction between α -amino group on protein bound lysine residues and a γ -carboxamide group on protein bound glutamine residues, leading to the covalent crosslinking of the proteins (Marco and Rosell, 2008). The protein solubility, emulsifying

and foaming properties can be improved with a limited degree of hydrolysis by protease and the use of autoclaving (Cao *et al.*, 2009; Yeom *et al.*, 2010).

Water solubility is one of the most important characteristics of proteins, as it influences other properties including emulsion, foaming, and gel forming ability (Lawal *et al.*, 2007; Yeom *et al.*, 2010). The increase of protein solubility could be due to smaller molecular peptides, and the unfolding of the protein molecule. At extremely acidic and alkaline pHs, proteins carry net positive and negative charges, respectively, and thus electrostatic repulsion and ionic hydration promoted the solubilization of the protein (Yeom *et al.*, 2010). The solubility of rice protein isolates in water is minimum at pH 4.0 and increased gradually below pH 4.0 and above pH 6.0. The maximum solubility of rice protein was observed at pH 10 (Wang *et al.*, 1999).

Oil (OAC) and water absorption capacity (WAC) directly influence the important functions of rice protein in food systems. The mechanism of OAC is attributed to the combination of physical entrapment of oil and the hydrophobicity of the material (He *et al.*, 2013). OAC of proteins could be explained and more accurately predicted using surface hydrophobicity and solubility together. The unfolding of protein structure, as well as exposure of more hydrophobic groups allows the physical entrapment of oil. The low hydrophobicity of rice protein would not facilitate the interaction between proteins and oil, resulting in the decrease of oil absorption capacity (Zhang *et al.*, 2012). In addition, hydrophobicity of protein hydrolysates develops because hydrolysis cleaves the protein chain so more internal hydrophobic groups are exposed (Fabian and Ju, 2011). High OAC is essential in the formulation of food systems such as sausages, or cake batters; whereas, rice protein that has good WAC could be used in products requiring high water absorption (Zhang *et al.*, 2012). High water absorption of proteins helps to reduce moisture loss in packed bakery goods. Also it is required to maintain freshness and moist mouth feel of baked foods.

Moreover, the difference in the bulk density may affect to the surface area of the protein particles in those protein samples, resulting in the difference amount of water and oil absorbed (Guan *et al.*, 2007). Further, these capacities are closely related to its amino acid profile, number of charged residues and conformation of protein in each sample (Moure *et al.*, 2006).

Foaming ability is a basic functional property of proteins. To have high foaming capacity; proteins should be solubilized and rapid absorbed at the air-

water interface during whipping before undergo rapid conformation changing and rearrangement or form cohesieve layer at the interface, and then reduce the interface tension suddenly (Yeom *et al.*, 2010). This requires flexible protein molecules with few secondary and tertiary structures (Chittapalo and Noomhorm, 2009) since intermolecular cohesiveness and elasticity are important to produce stable foams (Tang *et al.*, 2003). Thus, rice protein concentrate or isolate could retain its foaming stability better than its hydrolysate form. The increase in protein solubility by proteolysis would increase foaming capacity; extensive hydrolysis may lower foaming due to excessive charge that prevents the formation of stable foam (Zhang *et al.*, 2012). Lawal *et al.* (2007) stated that protein adsorption and viscoelasticity at an air-water interface in maximal could be observed near or at isoelectric pH because of protein possesses low net charge, lack of repulsive interactions of protein molecules enhancing favorable protein-protein interactions and formation of a viscous film at the interface. More protein molecules absorbed at air-water interface facilitated the formation of stable molecular layers in the interface, which imparted stability to the foam (Guan *et al.*, 2007).

Emulsions are normally formed due to the presence of hydrophobic and hydrophilic groups in protein structures. The mechanisms to generate the emulsion system are attributed to (1) the diffusion of peptides at oil-water interfaces, (2) and adsorption of peptides on the surface of freshly formed oil droplets, and (3) then formation of a protective membrane that inhibits coalescence of the oil droplet (Klompong *et al.*, 2007). Increase of pH after isoelectric pH has been attributed to formation of charged layers around oil droplets, which caused mutual repulsion and/or by forming a hydrated layer around interfacial material, which lower interfacial energy and retarded droplet coalescence (Lawal *et al.*, 2007). A poor emulsifying property of rice protein (mainly glutelin) is affected by its low solubility and high molecular weight. In addition, proteins that are not soluble in aqueous systems and possess high amount of disulphide bonding make poor emulsifiers (Romero *et al.*, 2012). Xia *et al.* (2012) reported that the use of hydrothermal cooking can improve physicochemical and emulsifying properties of rice protein. Moreover, enzymatic hydrolysis makes protein denaturation resulting in an increase hydrophobic surface and flexibility (Moure *et al.*, 2006) and it might also uncover buried hydrophobic groups, which could improve the hydrophilic-lipophilic balance for better emulsification (Guan *et al.*, 2007). A smaller molecular size would facilitate that diffusion and

Table 2. Functional and bio-function properties of rice protein

Properties	Results/ Benefits	References
1.) Functional properties		
1.1) Solubility	-Maximum nitrogen solubility at pH 10 and 12 were 82 and 80% -Maximum solubility of 60% at pH 11 -Maximum solubility of 72.5% and 84.56% at pH 11.0 -Maximum solubility of 97.4% at pH 10.0	Wang <i>et al.</i> (1999) Pinciroli <i>et al.</i> (2009) Zhang <i>et al.</i> (2012) Yeom <i>et al.</i> (2010)
1.2) Foaming ability	-Maximum foam capacities of 98% and 115% and maximum foam stabilities of 30.6 and 26.9 mL at 30 min -Foaming capacities ranged between 40-90%, foam stabilities of 30-90 min -Foaming capacities was more than 60% at pH 11	Zhang <i>et al.</i> (2012) Yeom <i>et al.</i> (2010) Cao <i>et al.</i> (2009)
1.3) Emulsifying capacity	-Maximum emulsifying capacities of 0.149 and 0.634 and maximum emulsion stabilities of 24.26 and 25.96 min -Emulsifying capacities ranged between 0.6-0.8 and emulsion stabilities were 20-30 min	Zhang <i>et al.</i> (2012) Yeom <i>et al.</i> (2010)
1.4) Water absorption and oil binding capacity	-Water absorption of 3.71 and 4.4 g/g and oil absorption of 4.24 and 5.13 g/g. -Highest water absorption of 3.83 mL/g -Water binding capacity was in the range of 3.87–5.60 (g/g), oil absorption capacity ranged between 3.74 and 9.18 (g/g)	Zhang <i>et al.</i> (2012) Cao <i>et al.</i> (2009) Chandi and Sogi (2007)
2.) Bio-function properties		
2.1) Free radical scavenging activity	-ABTS; IC ₅₀ of 0.19-0.74 mg/mL -ABTS; 10.8-28.3 mmol Trolox equivalents (TE)/g dry weight -DPPH; %Inhibition of 31.2-49.7% -ORAC; 34.2-87.3 mmol TE/g dry weight	Watchararujij <i>et al.</i> (2008) Zhou <i>et al.</i> (2012) Zhou <i>et al.</i> (2012)
2.2) Reducing power activity	6964, 2904, 2017 and 1809 mmol of Fe ²⁺ for albumin, globulin, prolamin and glutelin fractions, respectively	Chanput <i>et al.</i> (2009)
2.3) Inhibit lipid peroxidation	Able to inhibit lipid oxidation, reducing the formation of lipid hydroperoxides and TBARS	Zhao <i>et al.</i> (2012)
2.4) ACE-inhibitory activity	-IC ₅₀ of 0.46 mg/mL (hydrolysate) -10 mg/mL, could inhibit 68.51-96.83% (hydrolysate)	Chen <i>et al.</i> (2013) Li <i>et al.</i> (2005)
2.5) Other bio-functions	-Reduces serum cholesterol concentrations in rats (α -globulin) -Potential to potentiate anti-leukaemia immunity (prolamin) -Prevent hypercholesterolemia -Cholesterol-lowering action in rats -Possess cancer growth inhibitory on colon, breast, lung and liver cancer cells	Tong <i>et al.</i> (2012) Chen <i>et al.</i> (2010) Yang <i>et al.</i> (2013) Yang <i>et al.</i> (2011) Kannan <i>et al.</i> (2010)

enhance the interaction between protein and lipid (Zhang *et al.*, 2012). However, the lower degree of hydrolysis of protein exhibited higher emulsifying properties because the peptides with too low molecular weight may not be amphiphilic enough to exhibit good emulsifying properties, and small peptides migrate rapidly and adsorb at the interface but show less efficiency in decreasing the interface tension since they cannot unfold and reorient at the interface like large peptides to stabilize emulsions (Klompong *et al.*, 2007).

The functional properties of rice bran protein, including solubility, water and oil binding capacity and emulsifying capacity are shown in Table 2.

Rice protein has been reported to have antioxidative properties including free radical scavenging activity, reducing power, and inhibition of lipid peroxidation. For the past few years, protein hydrolysates from various plant materials have been found to possess antioxidant activities, and have been used as antioxidants in foods. Their uses are becoming more popular due to safety concerns, high nutritional values, and low costs (Zhao *et al.*, 2012). Rice protein hydrolysates obtained from hydrolysis of pepsin and trypsin exhibited much greater antioxidative activities than those from before digestion (Chanput *et al.*, 2009). Phongthai and Rawdkuen (2015) prepared rice bran protein hydrolysate by using crude papain. The obtained result was high in recovery and bioavailability properties. Furthermore, the peptides having tyrosine at the N-terminal exhibited the highest antioxidative activity (Fabian and Ju, 2011). Antioxidative properties of rice protein are concluded in Table 2 and described as following data.

DPPH radical scavenging assay has been widely used to evaluate antioxidative properties of compounds as free radical scavengers or hydrogen donors. The radical would then be scavenged, reducing the absorbance at 517 nm (Klompong *et al.*, 2007). A difference activity of protein hydrolysates may be governed by the distinction of amino acid composition especially aromatic amino acids including tyrosine, histidine, methionine and phenylalanine which was reported as stronger proton donor than other amino acids (Bernardini *et al.*, 2012; Fang *et al.*, 2012). Moreover, it may depend on specificity of enzyme to cleavage and release hydrophobic amino acid such as valine, alanine, proline and leucine that showed higher ability to scavenge the oil-soluble free radicals (hydrophobic) (Intarasirisawat *et al.*, 2012). Apart from that, the EC₅₀ of rice protein hydrolysates from the study of Zhao *et al.* (2012) were imparted in the range of 8.73-14.04 mg/mL. The scavenging activities of rice protein hydrolysates prepared by other enzymes also have been confirmed by the reports of Chanput *et al.* (2009), Zhang *et al.* (2010), and Zhao *et al.* (2012).

Lipid peroxidation is a chain reaction initiated by the hydrogen abstraction or addition of an oxygen radical, resulting in the oxidative damage of polyunsaturated fatty acids (PUFA) (Halliwell and Chirico, 1993) because they contain multiple double bonds in between which lie methylene bridges (-CH₂-) that possess especially reactive hydrogens. The reaction consists of three major steps: initiation, propagation, and termination. Natural and synthetic antioxidants are able to break/inhibit this chain reaction by donating its hydrogen atom to free

Table 3. The usage, type of product and function of rice protein

Samples	Products	Usage	Function/ Results	References
Rice bran protein concentrate	Bread	1-5%	% Weight loss and microbial load less than control sample	Jiamyangyuen et al. (2005)
Rice bran protein isolate	Rice noodles	10%	Improving the nutritional quality, dough properties, cooking quality, and microstructure of rice noodle	Kim et al. (2014)
Rice bran protein concentrate	Biscuit	5, 10 and 15%	Adding of 15% rice bran protein concentrate increased protein content	Yadav et al. (2011)
Rice bran protein	Films-containing	4%	Most desirable with regard to the physical property of films and it was suggested to apply in food packaging	Shin et al. (2011)
Rice bran protein hydrolysate	Yeast-culture media	2%	Supporting the growth of yeast same as the use of glucose	Sereewatthanawut et al. (2008)

radicals. Zhao *et al.* (2012) found that rice protein is able to inhibit lipid oxidation, reducing the formation of lipid hydroperoxides and TBARS.

The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron or hydrogen. Antioxidative peptides were able to reduce the Fe^{3+} /ferric cyanide complex to the ferrous form. Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Klompong *et al.*, 2007). Chanput *et al.* (2009) reported that the albumin fraction of rice bran protein showed the highest reducing activity (6964 μ mol of Fe^{2+}) followed by globulin, prolamin, glutelin with activities of 2904, 2017, 1809 μ mol of Fe^{2+} , respectively. The reducing power of rice dreg protein hydrolysate obtained by five different proteases showed different reducing power activity, due to its degree of hydrolysis (Zhao *et al.*, 2012). The difference in reducing power might be caused by different amino acid sequence and length of the resultant peptides from different hydrolysis condition (Thiansilakul *et al.*, 2007). Furthermore, the reducing potential of protein hydrolysates are dependent on the presence of cysteine due to the sulfhydryl group in its structure can act as a strong reducing agent (Udenigwe and Aluko, 2011).

Blood pressure regulation is based on the fact that renin can convert angiotensinogen to angiotensin I, which in turn is converted by ACE to angiotensin II (Ang-II). Ang-II is a potent vasoconstrictor that also induces the release of aldosterone and therefore, increases sodium concentration and blood pressure. Besides, ACE is also known to hydrolyze bradykinin, which is a potent vasodilator, thus leading to the inability of the blood vessels to relax following contraction. Therefore, by inhibiting ACE activity, formation of angiotensin II and destruction of bradykinin will be reduced, which can contribute to lowering of blood pressure (He *et al.*, 2013). There were some studies reported about the ability on ACE-inhibition of rice protein hydrolysate. It has an IC_{50} for ACE inhibitory activity about 0.46 mg/mL (Chen *et al.*, 2013). Li *et al.* (2005) found that rice protein hydrolysates (10 mg/mL) could exhibit ACE inhibitory activity about 68.51-96.83%. The differences in ACE-inhibitory activities of the protein

or its hydrolysates might be due to the different molecular weights, peptides level and amino acids sequences of ACE-inhibitory peptides generated by proteins hydrolysis (Li *et al.*, 2005; Nasri *et al.*, 2013). The most potent ACE inhibitors contain hydrophobic amino acid (tryptophan, phenylalanine, tyrosine, or proline) residues at each of their C-terminus and branched aliphatic amino acids at the N-terminus (Li *et al.*, 2014).

Besides the antioxidative and ACE-inhibitory properties, rice protein can also act as other bioactive substances in living systems. Usually the amino acids in bioactive peptides range from 3 to 16 amino acids depending upon the nature of their activities amino acids like valine, leucine, proline, histidine, tyrosine, glutamic acid, or aspartic acid have been shown to predominate (Kannan *et al.*, 2010). Morita *et al.* (1997) reported that the cholesterol-lowering effect of rice protein depended on its methionine content. The details of the bio-functions of rice bran are concluded in Table 2.

Applications of rice protein

Rice protein has high potential as functional food ingredients and nutritional supplements (Tang *et al.*, 2003). The rice proteins not only serve as basic nutritional supplements but are also suitable for a broad range of industrial food applications. It can be used for bakery goods, whipped toppings, and sausages etc. owing to its high water and oil binding capacities, which helped to reduce moisture loss and maintain soft mouth feel. In addition, they can form an excellent base for the high sugar food systems like cake batters, frozen desserts and confections (Cao *et al.*, 2009). Being known to be hypoallergenic, it is a suitable ingredient for infant food formulations and for the restricted diets of children with food allergies. Furthermore, protease treatment has been used to improve the solubility of protein hydrolysates and to modify its physicochemical and functional properties (Xia *et al.*, 2012) before applying to suitable products. Rice protein hydrolysates may be used as nutritional supplements, functional ingredients, and flavor enhancers in foods, coffee whiteners, cosmetics, personal care products, and confectionary, and in the fortification of soft drinks and juices. It is

also used in soups, sauces, gravies, meat products, and other savory applications (Fabian and Ju, 2011). Rice protein has been widely applied in many food products. The usage, type of product and its function are summarized in Table 3.

Conclusion

Rice bran is an economic source of high quality plant-based protein that can exhibit an excellent functional properties and interesting bio-functions. Rice protein and bran have been used as food ingredients in many food products such as meat ball, noodles, biscuit, breads and gluten-free products. Moreover, its hydrolysates form has a potential to apply in nutraceutical products.

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