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Evaluation of the physicochemical and structural properties of rice bran oil and proteins

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ABSTRACT

Rice (*Oryza sativa L.*) is one of the major staple cereals for human nutrition, particularly in Asia. Rice bran, the outer layer accounting for about 10% of the brown rice kernel, is a by-product of the milling process. Rice bran contains 10–16% protein, 12–22% fat, dietary fiber, and bioactive compounds such as B vitamins, vitamin E, and γ-oryzanol, which possess significant antioxidant and nutritional properties. Oil extracted from rice bran, with a balanced composition of fatty acids—approximately 43% oleic acid (monounsaturated), 32% linoleic acid (polyunsaturated), and about 15% palmitic acid (saturated)—plays an important role in promoting cardiovascular health and reducing oxidative stress. The ratio of saturated to unsaturated fatty acids in this oil is approximately 20:80. In addition, the presence of a minor amount of linolenic acid (0.8%) is one of its distinctive characteristics. The unsaponifiable compounds, including tocopherols, phytosterols, polyphenols, and γ-oryzanol (approximately 1.76%), enhance the antioxidant activity and cholesterol-lowering properties of rice bran oil. Rice bran proteins, mainly composed of albumin, globulin, glutelin, and prolamin, exhibit high digestibility (over 90%) and a protein efficiency ratio of 2.0–2.5, making them a valuable nutritional protein source. The genetic diversity of rice leads to variations in the amino acid composition of rice bran. The ratio of essential amino acids to total amino acids in different fractions of the bran ranges from approximately 31.35% to 34.8%, indicating the consistent protein quality across its layers. Despite its richness in valuable compounds, the direct consumption of rice bran is limited, and it is mainly utilized in animal feed, fertilizers, and fuel. Nevertheless, owing to its balanced amino acid profile, beneficial fatty acids, and antioxidant components, rice bran is considered a valuable material for food, pharmaceutical, and health-related applications.

Introduction

Rice (*Oryza sativa* L.) is a staple food consumed by nearly 3 billion people—more than half of the world's population—particularly in Asian countries. Since rice provides about 20% of global calorie intake, it is among the most widely cultivated cereals worldwide and holds great economic and social importance. Rice is mostly consumed as polished grain after the removal of the husk, bran, and germ. Although regional differences in consumer preferences exist, most people favor fully milled white rice or rice with only a small portion of bran remaining on the endosperm. Its primary use is in household consumption, where rice is typically prepared by boiling, frying, or serving alongside stews, as commonly practiced in Bangladesh and several other countries [1–4].

Rice bran is a by-product of rice processing obtained during the milling process and comprises parts of the grain such as the pericarp, tegman, and aleurone layer. It accounts for approximately 11% of the total milled rice, equivalent to about 83 million tons of solid waste generated annually worldwide. Among rice-producing countries, China ranks first globally, producing at least 14 million tons of rice bran annually. Rice bran is rich in bioactive and nutritional compounds, including dietary fiber, proteins, lipids, carbohydrates, vitamins, minerals, and valuable antioxidant components such as γ-oryzanol, tocopherols, tocotrienols, polyphenols, flavonoids, and peptides. It also contains significant amounts of phytic acid, ranging from 6 to 10%, making it an excellent source for phytic acid extraction [5,6].

Despite its high nutritional and bioactive value, rice bran has not yet been effectively or widely utilized in the food industry. Currently, a large portion of rice bran is allocated solely to animal feed, representing a limited use and a form of natural resource wastage. To address this issue and achieve sustainable development, this by-product should be utilized as an alternative resource across various industries. Some of potential applications include the production of edible oils, dietary supplements, biofertilizers, microbial culture media, prebiotic carbohydrates, bioethanol, antioxidants, and protein sources, which can be both economically profitable and environmentally valuable [7].

Although rice bran proteins have been reported to possess high nutritional value, rice bran protein concentrates and isolates are not commercially available due to the lack of suitable processing technologies. In particular, their structural complexity, low solubility, and tendency to aggregate make it difficult to separate these proteins from other cell wall components, limiting their use as a food ingredient [8,9].

In recent years, a variety of approaches have been

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developed to facilitate the extraction of valuable compounds from rice bran, such as oil and proteins, including physical, chemical, and enzymatic treatments. Chemical processes, such as alkaline or acid hydrolysis, are difficult to control and may lead to protein denaturation and alterations in amino acid structure. In contrast, enzymatic methods based on carbohydrases and proteases are considered among the most effective approaches, as they significantly enhance protein recovery. Carbohydrases degrade the cell wall components and facilitate the release of proteins from the polysaccharide matrix of rice bran, whereas proteases hydrolyze peptide bonds, converting proteins into a mixture of soluble peptides and amino acids [10–12].

Enzymatic hydrolysis is carried out under mild conditions, which prevents side reactions and preserves the nutritional value of the protein. Moreover, depending on the enzyme specificity and the degree of hydrolysis, hydrolysates with functional, biological, and nutritional properties distinct from those of the native protein can be produced. Specifically, it is now well recognized that the biological properties of protein hydrolysates are associated with their molecular weight and constituent peptides. Low-molecular-weight peptides generally exhibit the highest biological activity, as they can readily cross the intestinal barrier and exert their biological functions. In this context, ultrafiltration using membranes with defined molecular weight cut-off ranges is one of the most common strategies for the separation of protein hydrolysates, as it offers advantages such as high yield, low cost, and preservation of product purity under ambient conditions [13–15]. Such processes also offer advantages for oil extraction. By employing cell wall–degrading enzymes, oil-bearing rice bran can be treated to enable the extraction of oil and other compounds under milder conditions; for example, these methods operate at lower extraction temperatures, eliminate the need for flammable and toxic solvents such as hexane, and do not generate harmful residues [16,17].

Description of rice

Rice (Oryza sativa L.) is one of the most important cereal crops worldwide, particularly in Asia and Africa. According to statistics reported by the Food and Agriculture Organization (FAO), China and India are the largest exporters of rice, accounting for nearly 50% of the global rice trade. Rice is one of the most important cereal crops for human nutrition, particularly in Asian countries. According to statistics reported by the Food and Agriculture Organization of the United Nations (FAO, 2024), its large-scale production (526 million tons in 2022–2023) has resulted in the generation of substantial amounts of rice by-products and residues. The global rice production forecast for 2024–2025 has been slightly revised to 543.3 million tons, reflecting an annual increase of 1.6%, mainly attributed to the expansion of cultivation. The global rice production forecast for 2024–2025 has been slightly revised to 543.3 million tons, reflecting an annual increase of 1.6%, mainly attributed to the expansion of cultivation.

Compared with maize, wheat, and potato, rice is considered a good source of carbohydrates, minerals such as calcium and iron, as well as vitamins including thiamine, pantothenic acid, folate, and vitamin E. In addition to white rice, certain specialty varieties, such as colored and aromatic rice, are also cultivated [19,20].

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Structure and composition of rice

The rice grain is composed of three main parts: the endosperm or white rice (approximately 70%), the husk (approximately 20%), and the bran (approximately 10%) [21]. As shown in Fig. 1. [22], rice bran consists of the pericarp (husk), seed coat, nucellus, aleurone layer, germ, as well as some portions of the husk and fragments of the endosperm. Fig. 1. illustrates the different parts of the rice grain, consisting of the outer layers (husk and bran) and the internal components, including the endosperm and germ [23–25].

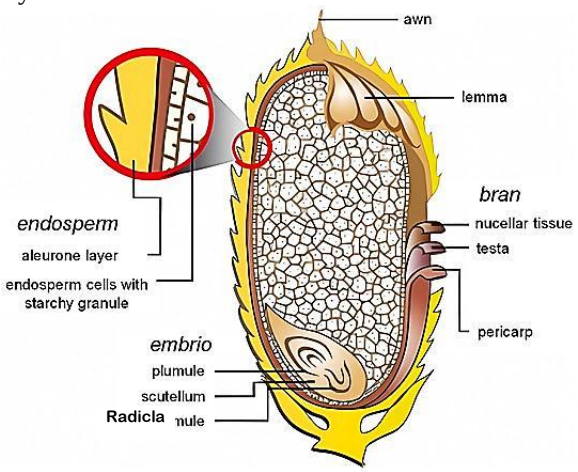

Fig 1. Structure of the rice grain

As shown in Fig. 2., rice bran constitutes approximately 10% of the outermost layer of brown rice, which is obtained during the milling process that converts brown rice into white rice. During the rice milling process, 70% of the grain (endosperm) is obtained as the main product, while the by-products include 20% husk, 8% bran, and 2% germ. According to FAO, rice bran is a by-product of polishing brown rice, consisting of the pericarp, aleurone layer, germ, and a portion of the endosperm [26,27]. This by-product contains approximately 10–23% fat, 37–60% carbohydrates, 9–14% ash, and 13–19% protein [28].

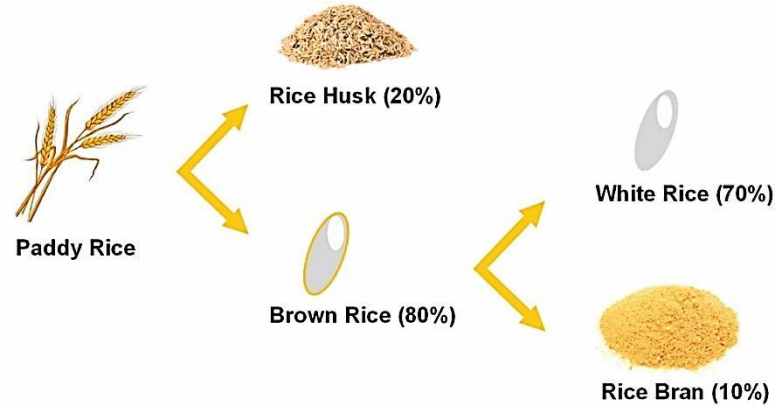

Fig 2. Fractions obtained from brown rice milling

The components obtained from rice milling differ in their protein composition. Reported data in the literature regarding the solubility of rice protein fractions are highly variable, and these variations depend on the rice cultivar and the extraction methods employed. Rice proteins are classified based on solubility according to the system proposed by Osborne in 1924. The four main rice protein fractions include albumin (water-soluble), globulin (salt-soluble), glutelin (soluble in acidic or alkaline solutions), and prolamin (alcohol-soluble) [29,30]. Analysis of the protein content of rice protein fractions indicates that glutelin constitutes the dominant portion of rice protein, accounting for approximately 75% of the total protein and exhibiting the highest protein concentration (around 95%). Following glutelin, globulin accounts for approximately 15%, while albumin (6%) and prolamin (2.7%) occupy the subsequent ranks. Using Osborne's extraction method, albumin exhibits the lowest protein content (around 78%) compared to the other fractions—globulin, prolamin, and glutelin—all of which have protein contents exceeding 90% [31,32].

The protein content of rice is typically determined by multiplying the nitrogen content, measured through the Kjeldahl method, by a nitrogen-to-protein conversion factor of 5.95. This factor has been established based on the nitrogen content (16.8%) of glutelin, the principal protein in rice. However, in nutritional studies, a conversion factor of 6.25 is commonly used to standardize the calculation of nitrogen across all proteins. It should also be noted that the protein content of rice is influenced by factors such as management and cultivation practices, climatic conditions, and genotype (the plant's genetic composition) [25].

Rice proteins are predominantly found in storage organelles known as protein bodies (PBs), which accumulate in the starchy endosperm of the grain. In general, proteins are distributed across different parts of the rice grain, including the endosperm, aleurone layer, and bran; however, the majority are located within the endosperm cells as storage proteins, which are contained in protein bodies situated among the starch granules. Detailed studies on seed storage proteins have demonstrated that cereal proteins are present in the form of these organelles. In the rice endosperm, two types of protein bodies with distinct morphological structures have been identified: Type I (PB-I), which is spherical with a layered structure and rich in prolamins, and Type II (PB-II), which exhibits a crystalline, irregular structure and predominantly contains glutelins. Prolamin is stored in protein body type I (PB-I) and consists of subunits with molecular weights ranging from 10 to 16 kDa, accounting for approximately 20–30% of the total storage protein. In contrast, glutelin is initially synthesized as a 55–60 kDa proglutelin, which is subsequently processed into acidic and basic subunits. It enters the vesicular transport system and accumulates in protein body type II (PB-II), which contains 70–80% of the total storage protein. In addition to glutelin, PB-II also contains a certain proportion of globulin. Prolamin and glutelin are the major storage proteins present in the rice endosperm. The storage proteins of the endosperm consist of 60–65% PB-II, 20–25% PB-I, and 10–15% albumin and globulin, which are distributed within the cytoplasm [33–35].

Albumin and a portion of globulin are located in the outer layers and the embryo of the rice grain, and are lost during the milling and polishing processes. Together, these two protein types account for only 10–15% of the total grain protein. From a nutritional perspective, glutelin is of greater importance due to its higher content of the amino acid lysine—the limiting amino acid in cereals—compared to prolamin, as well as its superior digestibility in the gastrointestinal tract. In fact, the primary limitation in the nutritional value of rice proteins is their deficiency in lysine. In this regard, glutelin plays a more significant role in enhancing the nutritional quality of the rice grain [36].

In the Osborne extraction method, which is employed for the separation of different rice proteins, the milled rice flour is first subjected to defatting, and then extracted with water to obtain the albumin fraction. Subsequently, the sample is sequentially treated with dilute salt solution, dilute alkali, and 70% ethanol to extract the globulin, glutelin, and prolamin fractions, respectively. Recent studies have shown that enzymatic removal of starch from rice flour can be an effective method for producing protein-rich isolates containing up to 76% protein. However, it remains unclear how starch removal—either through enzymatic hydrolysis or modification via gelatinization—prior to Osborne extraction affects the protein recovery, structural properties, or functional characteristics [1,35].

Rice proteins are valued for their colorlessness, richness in essential amino acids, mild flavor, hypoallergenic properties, and cholesterol-lowering effects. However, for food applications, it is desirable that they also possess additional functional properties such as foaming [37,38], gelling [39], and emulsifying [40] capacities. Information regarding these functional properties indicates that they depend on the rice variety. It has also been demonstrated that the method employed for protein isolation can markedly influence these properties [32,37].

The protein and amino acid of rice is influenced by various factors. For instance, previous research has indicated that the levels of 17 amino acids and total protein in rice significantly increase under nitrogen fertilizer treatment, while the eating quality of the rice is markedly reduced [41,42]. This is likely attributable to the increase in protein content induced by nitrogen fertilizer, which results in the formation of more protein bodies. These protein bodies attach to the surface of starch granules, thereby restricting water absorption and complete swelling, and ultimately inhibiting the gelatinization of the rice. In essence, higher protein content under nitrogen treatment corresponds to a greater number of protein bodies, which interfere with starch gelatinization [43]. Furthermore, different rice varieties exhibit varying capacities for protein accumulation, and consequently, the extent of protein increase differs among them [44,45].

Physicochemical properties of rice bran

Rice bran is considered a by-product of the rice industry, accounting for approximately 10 g per 100 g of whole rice grain. Owing to its richness in protein, fat, minerals, and antioxidant compounds, rice bran is used for oil extraction, animal feed production, and as a raw material in the formulation of various food products [46].

In recent years, rice bran has attracted considerable attention due to its potential bioactive properties. These include antioxidant activity [47,48], anti-inflammatory effects [49,50], cancer risk reduction [51,52], prevention of cardiovascular diseases [53,54], and cholesterol-lowering effects [55,56]. Many people assume that the polished white rice they consume is highly nutritious. In reality, however, approximately 65% of the nutrients and bioactive compounds (phytochemicals) are concentrated in the rice bran. For example, the anthocyanins present in the bran layer are responsible for the color of these rice varieties, and these colored types are rich in antioxidants and bioactive compounds [57–59].

Depending on the variety, rice bran typically contains 12–22% oil, 10–16% protein [60], 18% total dietary fiber, 22% starch, and nearly 4% of other components. It is noteworthy that the composition of rice bran varies depending on the variety and cultivation conditions. Rice bran oil is pale yellow, odorless, clear, and has a mild flavor. It contains γ-oryzanol and vitamin E, both of which are considered potent antioxidants. In addition, rice bran is rich in B-complex vitamins [12,61].

Table 1 presents the percentage composition of the chemical constituents of rice milling fractions at 14% moisture. The data indicate that, compared to brown and white rice, rice bran is substantially richer in protein, fat, fiber, and minerals (crude ash), whereas its starch and available carbohydrate content are markedly lower. In contrast, white rice contains the highest levels of starch and carbohydrates but is comparatively poorer in nutrients such as protein, fat, and fiber. Brown rice falls between the two, offering a balance of nutritional value and carbohydrates. Overall, rice bran represents a concentrated source of nutrients, whereas milling results in a substantial reduction in nutritional quality [25].

Table 1. Percentage of chemical composition of rice grain milling fractions at 14% moisture content

Chemical Composition	Brown Rice	White Rice (Milled)	Rice Bran
Protein (Nitrogen × 5.95)	7.1 – 8.3	6.3 – 7.1	11 – 15
Crude Fat	1.6 – 2.8	0.3 – 0.5	15 – 20
Available Carbohydrates	73 – 76	77 – 78	34 – 52
Starch	66	78	14
Crude Fiber	0.6 – 1.0	0.2 – 0.5	7 – 11
Crude Ash	1.0 – 1.5	0.3 – 0.8	6.6 – 9.9

Rice bran oil is a rich source of free fatty acids, waxes, unsaponifiable compounds (3–4%), and polar lipids, and its extraction process is challenging [19]. This oil also contains unsaturated fatty acids, comprising 38–42% oleic acid and 32–35% linoleic acid [21]. Rice bran oil can be obtained using conventional solvent-based methods as well as modern extraction techniques, such as microwave-assisted extraction (MAE) and aqueous enzymatic extraction (AEE). One of the negative effects associated with the use of solvents is their toxicity to humans and the environment. Aqueous enzymatic extraction (AEE) is one of the latest extraction

techniques, in which enzymes are employed in an aqueous medium to hydrolyze and break down cell walls, thereby releasing oil from rice bran [62].

The chemical composition of rice bran reflects the high diversity of its nutritional and bioactive components. As shown in Table 2, important compounds such as γ-oryzanol and tocopherols are listed as potent antioxidants that play a role in promoting cardiovascular health and reducing oxidative stress. Additionally, its fatty acid composition includes saturated and monounsaturated fatty acids, with the highest proportion being oleic acid (42.6%), which has been recognized for its beneficial effects on improving blood lipid profiles. Polyunsaturated fatty acids also make a significant contribution, including linoleic acid and smaller amounts of linolenic acid (0.8%) as well as omega-3 and omega-6 fatty acids. This diverse and balanced fatty acid profile, together with its antioxidant compounds, makes rice bran a valuable food ingredient for cardiovascular health [63–66].

Rice bran contains a wide range of bioactive compounds, including oryzanol, phytosterols, tocotrienols, squalene, policosanols, phytic acid, ferulic acid, and inositol hexaphosphate. Ferulic acid is one of the predominant phenolic acids present in rice bran, followed by p-hydroxycinnamic acid, sinapic, gallic, protocatechuic, hydroxybenzoic, and vanillic. As previously mentioned, rice bran oil contains a wide spectrum of fatty acids, of which 47% are monounsaturated fatty acids, 33% are polyunsaturated fatty acids, and 20% are saturated fatty acids. These bioactive compounds exhibit multiple biological activities and function as antioxidants, antidiabetic agents, and anticancer agents [67,68].

Table 2. Nutritional components and fatty acid composition of rice bran oil

Compound	Percentage Composition	Source
γ-Oryzanol	0.9 – 2.9	Lloyd et al., 2000; Patel and Naik, 2004 [63,64]
Tocopherol	0.01 – 0.14	Lloyd et al., 2000; Patel and Naik, 2004 [63,64]
Saturated Fatty Acids	22.5	Orsavova et al., 2015 [65]
Palmitic Acid	21.6	Krishna et al., 2006 [66]
Stearic Acid	2.1 – 4.7	Orsavova et al., 2015 [65]
Arachidic Acid	0.1	Krishna et al., 2006 [66]
Myristic Acid	0.03 – 0.39	Krishna et al., 2006 [66]
Monounsaturated Fatty Acids (MUFA)	44.0	Orsavova et al., 2015 [65]
Oleic Acid	42.6	Krishna et al., 2006 [66]
Palmitoleic Acid	0.19	Orsavova et al., 2015 [65]
Polyunsaturated Fatty Acids (PUFA)	33.6	Orsavova et al., 2015 [65]
Linoleic Acid	28.0	Krishna et al., 2006 [66]
Linolenic Acid	0.8	Krishna et al., 2006 [66]
n-3 Polyunsaturated Fatty Acids (PUFA n-3)	0.5	Orsavova et al., 2015 [65]
n-6 Polyunsaturated Fatty Acids (PUFA n-6)	33.1	Orsavova et al., 2015 [65]

Due to its high phytic acid content, rice bran is considered a valuable source for the extraction and production of phytic acid. Phytic acid, or myo-inositol hexaphosphate, is an organic phosphorus compound widely found in seeds and cereal grains, with its primary role being the storage of phosphorus in plant seeds. Owing to its strong chelating capacity, this compound exhibits a high affinity for binding multivalent metal ions, particularly iron, zinc, and calcium. This property has long led to its classification as an antinutritional factor, as its binding to these minerals reduces their bioavailability in the human body, thereby potentially exerting a negative effect on the absorption of essential micronutrients. Since the 1990s, however, scientific interest in phytic acid has grown, as numerous studies have demonstrated that its medicinal and biological benefits may outweigh its negative effects [69–71].

Among the different parts of the rice grain, phytic acid is unevenly distributed. The highest concentration is found in the rice germ, with approximately 7.6 g per 100 g, whereas the rice endosperm contains only about 1.2 g per 100 g of phytic acid. Rice bran, which consists of the pericarp, aleurone layer, and germ, contains high concentrations of phytic acid, reported to range from 5.94 to 6.09 g per 100 g. However, the exact content may vary depending on the climatic and agricultural conditions of the rice cultivation area [72–74].

One of the most important biological properties of phytic acid is its ability to activate the SIRT-1 enzyme in various body tissues. SIRT-1 is a NAD^+-dependent deacetylase (nicotinamide adenine dinucleotide) that plays a key role in regulating various cellular processes, including metabolism, aging, and inflammation. By activating this pathway, phytic acid can contribute to the regulation of iron metabolism, reduction of oxidative stress, and attenuation of inflammation. This property makes phytic acid a promising compound for the prevention or management of chronic diseases [75,76].

Numerous studies have investigated the beneficial effects of phytic acid on human health. The results of these studies suggest that phytic acid can contribute to the prevention of a wide spectrum of diseases, including

kidney stones, certain types of cancer, type 2 diabetes, and Parkinson's disease, in addition to exerting beneficial effects on lowering blood lipid levels. Thus, phytic acid should not be regarded merely as a nutritional concern; rather, it may be recognized as a potential health-promoting agent [77–79].

In this context, several studies have been conducted to optimize the extraction and purification methods of phytic acid from rice bran. One study was specifically designed to introduce an effective analytical approach for the extraction and purification of phytic acid, employing rice bran as a model system. The utilization of rice bran in this context not only enhances the added value of this by-product but also enables the exploitation of its bioactive compounds in the pharmaceutical and food industries [80].

As shown in Table 3, phytic acid extracted from rice bran contains significantly higher levels of mineral elements—including manganese, iron, zinc, cobalt, calcium, and magnesium—compared to standard phytic acid. In addition, its protein and nitrogen content is also markedly greater. In contrast, the levels of total phosphorus and phytate phosphorus are higher in the standard sample, indicating that purification reduces phytate phosphorus while enhancing the bioavailability of mineral elements. This, in turn, may increase the nutritional significance of rice bran–derived phytic acid compared to its standard counterpart [20].

Table 3. Comparison of the composition of phytic acid extracted from rice bran with standard phytic acid

Compound / Element	Unit	Standard Phytic Acid	Purified Rice Bran Phytic Acid
Phytate Phosphorus	mg/g	124.86 ± 2.78^{a}	64.63 ± 2.77^{b}
Inorganic Phosphorus	mg/g	0.01 ± 0.00^{b}	0.11 ± 0.00^{a}
Total Phosphorus	mg/g	132.36 ± 1.75^{a}	103.88 ± 1.17^{b}
Total Nitrogen	g/100g	0.03 ± 0.00^{b}	0.15 ± 0.00^{a}
Soluble Proteins	g/100g	0.00 ± 0.00^{b}	0.06 ± 0.01^{a}
Total Proteins	g/100g	0.19 ± 0.00^{b}	0.94 ± 0.00^{a}
Copper	mg/kg	0.00 ± 0.00^{b}	3.00 ± 0.00^{a}
Zinc	mg/kg	1.27 ± 0.00^{b}	742.19 ± 0.01^{a}
Nickel	mg/kg	0.95 ± 0.07^{a}	1.00 ± 0.28^{a}
Cobalt	mg/kg	1.05 ± 0.07^{b}	4.15 ± 0.07^{a}
Manganese	mg/kg	2.45 ± 0.00^{b}	820.30 ± 0.02^{a}
Iron	mg/kg	1.36 ± 0.00^{b}	732.45 ± 5.45^{a}
Calcium	g/kg	0.01 ± 0.00^{b}	1.39 ± 0.01^{a}
Sodium	g/kg	11.00 ± 0.00^{a}	11.00 ± 0.00^{a}
Magnesium	g/kg	0.25 ± 0.00^{b}	32.73 ± 0.00^{b}
Potassium	g/kg	0.00 ± 0.00^{b}	1.83 ± 0.00^{a}

Data in the above table are presented as "mean ± standard deviation" from three replicates. Different lowercase letters (^{a} and ^{b}) in the same row indicate statistically significant differences based on Tukey's test at a significance level of $p \leq 0.05$.

Moreover, rice bran contains CoQ10H2 or ubiquinone-10, and epidemiological as well as biochemical evidence indicates that this compound functions as an important intracellular antioxidant. It plays a crucial role in inhibiting lipid peroxidation and in regenerating other antioxidants, such as α-tocopherol. The hypocholesterolemic activity of rice bran is attributed to the presence of γ-oryzanol and phytosterols. Additionally, the tocotrienols in rice bran inhibit HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis [28,81].

Various studies have demonstrated that the parboiling process can significantly affect the properties of rice bran. Parboiling refers to a process in which paddy rice (rice with hull) is soaked in water, steamed, and then dried prior to milling into white rice. The lipase enzymes in rice bran hinders the optimal utilization of its valuable and nutritious compounds. These enzymes hydrolyze triacylglycerols into free fatty acids and glycerol, and during this process, the free fatty acids generate peroxides through two different pathways, namely the non-enzymatic pathway or the enzymatic oxidative pathway mediated by lipoxygenase, which ultimately leads to rancidity and the development of undesirable flavors and odors in the oil. Various methods for preventing lipase activity have been proposed by researchers, including physical methods (dry/wet heating, microwave irradiation, ohmic heating, infrared treatment, and extrusion), biological methods (addition of protease enzymes for lipase inactivation, development of high-PUFA production lines, and enhancement of antioxidant levels), and chemical methods (acidification or alkalization) [20]. However, different methods of lipase inactivation vary in their effectiveness [8,82]. Among the various approaches, parboiling at 100 ± 2 °C for 30 minutes is considered a simple and highly efficient method for lipase inactivation [83]. In one study, the effect of the parboiling process on the enzymatic extract of rice bran was investigated. Two types of extracts were prepared: one from raw bran and the other from parboiled bran. The results showed that parboiled rice bran contained higher fat and lower carbohydrate levels, while

no difference was observed in protein content. In terms of bioactive compounds, both extracts were rich in phytosterols, tocopherols, tocotrienols, and γ-oryzanol. However, parboiled rice bran contained higher levels of tocopherols and phytosterols, whereas the enzymatic extract of raw rice bran exhibited the highest amount of hydrophilic phenols. Evaluation of antioxidant activity revealed that the enzymatic extract of rice bran exhibited a greater capacity for radical scavenging and protection against lipid and protein oxidation. Therefore, it can be concluded that the enzymatic extract of rice bran possesses stronger bioactive properties compared to parboiled rice bran. Overall, parboiling alters the bioactive composition of rice bran, and these extracts may have potential applications in the pharmaceutical and cosmetic industries [84].

Regrettably, despite being a rich source of valuable nutrients and bioactive compounds, rice bran has a very limited dietary utilization, leading to the frequent loss of these beneficial components. As a result, rice bran is predominantly utilized as animal feed, or employed in the production of fertilizers and biofuels, while a considerable portion is discarded in landfills [61].

Rice bran oil: fatty acid profile and constituent components

Due to its unique properties, high therapeutic potential, and richness in valuable compounds, rice bran oil is widely applied in the food, pharmaceutical, cosmetic, personal care, and chemical industries. Among edible vegetable oils, rice bran oil is distinctive, providing a healthy source of polyunsaturated fatty acids and exhibiting beneficial nutritional attributes. In Japan, it is known as the 'heart oil' due to its positive effects on cardiovascular health. Moreover, owing to its favorable fatty acid composition, high cooking quality, and desirable shelf life, it is regarded as one of the highest-quality vegetable oils. Its high smoke point (254 °C), mild flavor, and 15% lower oil absorption during cooking make it an ideal choice for frying and high-temperature cooking [85,86].

As noted earlier, the distinctive properties of rice bran oil—such as antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and anticancer activities—render it a valuable resource for a wide range of applications in both food sectors (including cooking oils, dairy products, and meat products) and non-food industries (such as polymers, lubricants, biofuels, structural lipids, and cosmetic formulations). Compared to other vegetable oils, rice bran oil is characterized by higher free fatty acid content, coupled with unusually elevated levels of waxes, unsaponifiable matter, polar lipids, and pigments [19,87].

Rice bran oil possesses unsaturated fat content consistent with World Health Organization (WHO) standards. Accordingly, the Indian Council of Medical Research, WHO, the American Heart Association, and the China Cereals and Oils Association have endorsed rice bran oil as a 'healthy oil' based on its fatty acid composition and associated bioactive compounds. According to the 2010 Codex Alimentarius international standard (CXS 210-1999), rice bran oil is one of the healthiest edible oils and serves as a balanced source of saturated fatty acids (20% palmitic acid), monounsaturated fatty acids (42% oleic acid), and polyunsaturated fatty acids (32% linoleic acid). Linoleic acid is widely recognized as an essential fatty acid, contributing to lowering blood cholesterol and preventing atherosclerosis as well as other adverse health effects [19,21,62].

In a study conducted by Yoshida et al. (2011), the fatty acid composition of rice bran lipids was examined across several varieties, with palmitic acid (C16:0), stearic acid (C18:0), oleic acid (18:1n-9), and linoleic acid (18:2n-6) identified as the major components. As shown in Table 4, despite differences in lipid type, the fatty acid distribution pattern across total lipids, TAGs, FFAs, and PLs was comparable across the different varieties. The results indicated that the major portion of the lipid composition consisted of unsaturated fatty acids, particularly linoleic and oleic acids, which accounted for over 70–80% of the total lipids. The fatty acid composition of the three rice varieties—Koshihikari, Haenuki, and Akitakomachi—showed that oleic acid (C18:1) and linoleic acid (C18:2) were the predominant fatty acids in all lipid classes, accounting for approximately 40–44% and 35–42% of the total fatty acids, respectively. In contrast, palmitic acid (C16:0) accounted for 18–26%, while stearic acid (C18:0) represented only 1–2% of the total fatty acids. A comparable distribution pattern was noted in triacylglycerols (TAGs), yet at the sn-2 position, unsaturated fatty acids—especially linoleic (50–52%) and oleic (43–44%) acids—were present at markedly higher concentrations, while palmitic and stearic acids were virtually absent. This characteristic enhances the nutritional value, as unsaturated fatty acids at the sn-2 position demonstrate increased bioavailability. In contrast, the sn-1,3 positions were characterized by higher palmitic acid content (23–26%). In free fatty acids (FFAs), the proportion of palmitic acid increased while linoleic acid decreased, likely due to the hydrolysis of triacylglycerols during storage. Furthermore, in phospholipids, linoleic acid predominated over oleic acid, reflecting a higher content of polyunsaturated fatty acids in this lipid class. Overall, inter-varietal differences were minimal, with variations primarily reflecting shifts in fatty acid proportions rather than alterations in the overall lipid composition profile [88].

Table 4. Fatty acid distribution in major lipid classes extracted from rice bran cultivars

Lipid Class	Cultivar	Fatty acid (wt-%)					
		16:0	18:0	18:1	18:2	18:3	Others
Total Lipids	*Koshihikari*	20.0 ± 1.0^{c}	1.3 ± 0.1^{b}	40.5 ± 1.8^{b}	36.1 ± 1.4^{b}	1.2 ± 0.1^{b}	**0.9 ± 0.1^{b}**
	Haenuki	20.2 ± 0.1^{c}	1.2 ± 0.1^{b}	40.4 ± 1.8^{b}	36.3 ± 1.2^{b}	1.3 ± 0.1^{b}	**0.6 ± 0.1^{a}**
	Akitakomachi	19.8 ± 0.8^{c}	1.5 ± 0.1^{b}	41.7 ± 2.0^{c}	35.2 ± 1.5^{b}	1.2 ± 0.1^{b}	**0.6 ± 0.1^{a}**
Triacylglycerols	*Koshihikari*	17.7 ± 0.8^{b}	1.5 ± 0.1^{b}	40.9 ± 2.0^{b}	37.0 ± 1.6^{b}	1.4 ± 0.1^{b}	**1.5 ± 0.1^{c}**
	Haenuki	17.3 ± 0.8^{b}	1.3 ± 0.1^{b}	40.3 ± 2.0^{b}	38.3 ± 1.6^{b}	1.6 ± 0.1^{c}	**1.2 ± 0.1^{b}**
	Akitakomachi	16.2 ± 0.7^{b}	1.6 ± 0.1^{b}	43.3 ± 2.1^{c}	36.3 ± 1.5^{b}	1.4 ± 0.1^{b}	**1.2 ± 0.1^{b}**
sn-2 position of TAG	*Koshihikari*	2.4 ± 0.1^{b}	1.0 ± 0.1^{a}	43.6 ± 2.0^{c}	50.2 ± 2.0^{d}	1.6 ± 0.1^{c}	**1.2 ± 0.1^{b}**
	Haenuki	1.6 ± 0.1^{a}	1.3 ± 0.1^{a}	43.4 ± 2.0^{c}	51.3 ± 2.1^{d}	1.2 ± 0.1^{b}	**1.2 ± 0.1^{b}**
	Akitakomachi	1.5 ± 0.1^{a}	0.8 ± 0.1^{a}	43.8 ± 2.0^{c}	52.3 ± 2.2^{d}	1.0 ± 0.1^{a}	**0.6 ± 0.1^{a}**
sn-1,3 position of TAG	*Koshihikari*	25.4 ± 1.0^{d}	1.7 ± 0.1^{b}	39.6 ± 1.6^{b}	30.4 ± 1.3^{b}	1.0 ± 0.1^{a}	**1.9 ± 0.1^{d}**
	Haenuki	25.2 ± 1.0^{d}	1.3 ± 0.1^{b}	38.8 ± 1.5^{b}	31.8 ± 1.3^{b}	1.8 ± 0.1^{c}	**1.1 ± 0.1^{b}**
	Akitakomachi	23.6 ± 1.0^{d}	2.0 ± 0.1^{c}	42.9 ± 1.8^{c}	28.4 ± 1.2^{b}	1.6 ± 0.1^{c}	**1.5 ± 0.1^{c}**
Free fatty acids	*Koshihikari*	26.5 ± 1.2^{d}	1.6 ± 0.1^{b}	44.1 ± 1.8^{c}	25.8 ± 1.2^{a}	0.6 ± 0.1^{a}	**1.4 ± 0.1^{b}**
	Haenuki	26.0 ± 1.2^{d}	1.6 ± 0.1^{b}	43.0 ± 1.8^{c}	27.1 ± 1.2^{b}	0.7 ± 0.1^{a}	**1.6 ± 0.1^{c}**
	Akitakomachi	25.0 ± 1.2^{d}	1.7 ± 0.1^{b}	45.0 ± 1.8^{c}	26.3 ± 1.2^{a}	0.8 ± 0.1^{a}	**1.2 ± 0.1^{b}**
Phospholipids	*Koshihikari*	20.3 ± 1.0^{c}	1.1 ± 0.1^{a}	35.7 ± 1.2^{a}	40.9 ± 2.0^{c}	1.7 ± 0.1^{b}	**0.3 ± 0.1^{a}**
	Haenuki	20.1 ± 1.0^{c}	1.0 ± 0.1^{a}	34.0 ± 1.2^{a}	42.1 ± 2.0^{c}	1.5 ± 0.1^{b}	**1.3 ± 0.1^{b}**
	Akitakomachi	19.6 ± 0.9^{c}	1.0 ± 0.1^{a}	35.7 ± 1.2^{a}	41.1 ± 2.0^{c}	1.2 ± 0.1^{b}	**1.4 ± 0.1^{b}**

The fatty acid distribution at the sn-1,3 positions was calculated using the following formula:
[3 × (fatty acid composition in TAG) − (fatty acid composition at the sn-2 position)] ÷ 2
Each value represents the mean of three measurements and is expressed as the relative weight percentage (% wt) of total fatty acids. The term "Others" includes minor fatty acids such as C14:0, C16:1, C20:0, and C22:0.

Given the unique bioactive constituents of this valuable food source, rice bran oil is enriched with specific fatty acids, phenolic compounds, γ-oryzanol, ferulic acid, and vitamin E, including both tocopherols and tocotrienols. Overall, it contains more than 80% oleic, linoleic, and linolenic acids. Due to its very high smoke point, neutral taste, and delicate aroma, this oil has become an ideal choice for cooking. Oleic acid can prevent atherosclerosis and reduce the risk of cardiovascular diseases by lowering low-density lipoproteins (LDL) or 'bad' cholesterol. Moreover, linoleic acid, an essential fatty acid, has been shown to reduce blood cholesterol levels. Reports also indicate that linoleic acid contributes to decreased body fat and plays a significant role in lowering the risk of atherosclerosis. Therefore, the presence of these two fatty acids in rice bran oil classifies it as a functional, health-promoting oil [21,89,90].

Fig 3. illustrates the overall fatty acid profile of rice bran oil, demonstrating that the levels of individual fatty acids fall within defined ranges. It should be noted that the content of each fatty acid may vary slightly among varieties and under different environmental conditions. The results indicate that the fatty acid composition of the samples examined was predominantly composed of unsaturated fatty acids. Oleic acid (C18:1) with 43% and linoleic acid (C18:2) with 32% represent the major components, reflecting the high proportion of mono- and polyunsaturated fatty acids and, consequently, the favorable nutritional and health-promoting potential of rice bran oil. Among the saturated fatty acids, palmitic acid (C16:0) accounts for the highest proportion at 15%. The presence of a minor amount of linolenic acid (C18:3) is also noteworthy, as this fatty acid is found in certain plant sources. Overall, the lipid profile indicates favorable quality and potential functional properties for applications in the food and pharmaceutical industries [61,89].

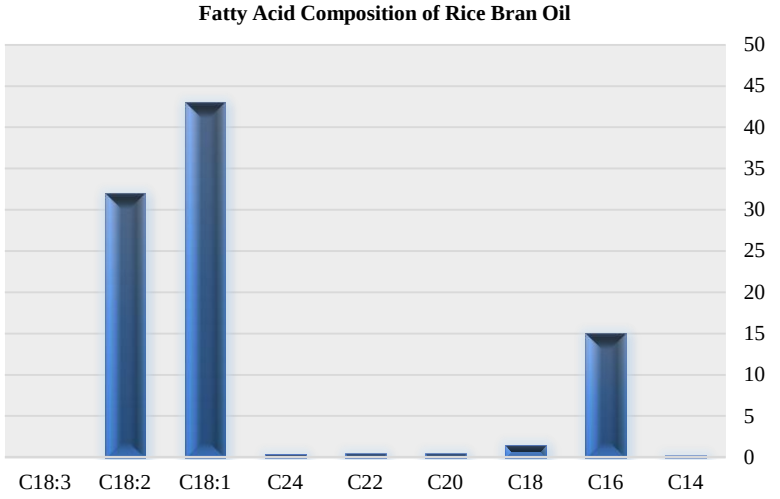

Fig 3. Characterization of the fatty acid composition of rice bran oil

Rice bran oil consists of two fractions: the saponifiable and the unsaponifiable. The unsaponifiable fraction contains valuable compounds with notable biological and nutritional properties. Among these compounds are tocopherols and tocotrienols, both of which are forms of vitamin E with potent antioxidant properties. Gamma-oryzanol is another compound that, in addition to its antioxidant role, has been recognized as effective in lowering blood cholesterol. Phytosterols, due to their structural similarity to cholesterol, contribute to reducing cholesterol absorption in the intestine. Polyphenols, as plant-derived compounds, exhibit anti-inflammatory and antioxidant activities. Lastly, squalene, a natural lipid compound, plays an important role in skin health and immune function. These compounds have made rice bran oil a unique vegetable oil with functional and pharmaceutical properties. Crude rice bran oil, compared to other vegetable oils, contains higher levels of triacylglycerols. Fig. 4. presents the composition of the saponifiable fraction of rice bran oil [19].

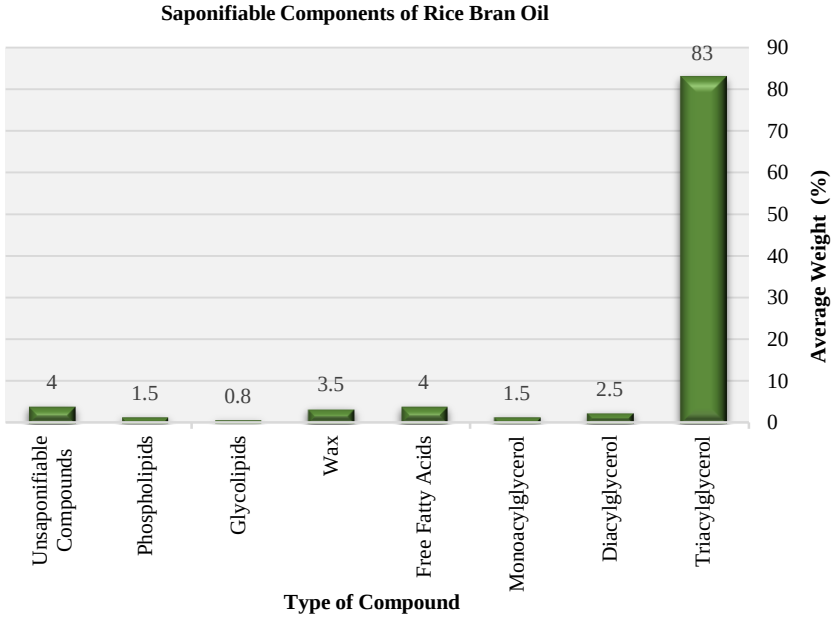

Fig 4. Quantitative composition of saponifiable fractions in rice bran oil

Gamma-oryzanol is one of the unsaponifiable compounds present in rice bran oil. It is considered one of the most important micronutrients responsible for many of its beneficial effects on human health. This bioactive compound was first isolated from rice bran oil in 1945 by Kaneko and Tsuchiya. Initially, γ-oryzanol was regarded as a single compound; however, subsequent studies revealed that it is in fact a mixture of ferulic acid esters and phytosterols. Although there is some disagreement regarding the exact number of constituents in this bioactive mixture, recent studies have succeeded in identifying, isolating, and characterizing twenty-three distinct

compounds of γ-oryzanol. Nevertheless, four major esters—cycloartenyl ferulate, 24-methylene cycloartenyl ferulate, campesteryl ferulate, and β-sitosteryl ferulate—account for approximately 80% of the γ-oryzanol present in rice bran oil. A shared characteristic of these compounds is the ferulic acid moiety, which contains hydroxyl groups that enhance their polarity and, as a result, make γ-oryzanol soluble in both polar and nonpolar solvents. Other antioxidants present in rice bran, including tocopherols, tocotrienols, and γ-oryzanol, have demonstrated significant antioxidant effects in cholesterol and linoleic acid oxidation models. Research indicates that γ-oryzanol exhibits greater antioxidant activity in preventing cholesterol oxidation compared to tocopherols and tocotrienols [91–93].

Some valuable compounds, such as rice bran, are widely produced on a large scale; however, several tons of it are discarded as waste each year without being properly utilized. This is despite the fact that rice bran can serve as a rich raw material for the production of bioactive and value-added compounds. Proper utilization of these resources not only prevents their wastage but also offers considerable nutritional and economic benefits. The optimal utilization of these resources is not only economically cost-effective but also contributes to the reduction of environmental pollution. Hence, the selection of an appropriate method for extracting valuable compounds is of particular importance [94].

The recovery method for high value-added compounds from food wastes such as rice bran should be carefully selected to ensure maximum extraction yield and purity, prevent loss of compound activity, and obtain a final product of suitable quality for food applications. To achieve these objectives, the so-called 'universal five-stage recovery process' can be employed which involves steps at both macroscopic and microscopic levels. The steps are as follows:

1. Macroscopic-scale pretreatment,
2. Separation of macromolecules and micromolecules,
3. Extraction,
4. Separation and purification,
5. Product formation.

Among these stages, extraction is considered the most critical step, as it is a mass transfer phenomenon in which the solute moves from the matrix into the solvent, thereby directly influencing the yield of the final product [95].

Modern extraction methods for this oil are more efficient and environmentally compatible compared to traditional approaches. Advances in extraction using innovative techniques such as super/subcritical carbon dioxide extraction, microwave-assisted extraction, enzyme-assisted aqueous extraction, ultrasound-assisted aqueous extraction, and subcritical water extraction have demonstrated that these methods not only enhance the extraction yield but also improve the nutritional profile of rice bran oil [19,86,96].

In commercial production of rice bran oil, hexane is commonly used as the solvent. Since hexane is recognized as an air pollutant, studies have shown that the use of aqueous and enzyme-assisted extraction processes can mitigate some of the problems associated with solvent use [12].

Aqueous enzyme-assisted oil extraction offers several advantages over traditional extraction methods. For instance, this approach eliminates the use of organic solvents, which may also lead to reduced capital costs. A recent study compared the extraction of rice bran oil using enzyme-assisted methods and hexane solvent extraction with commercially available rice bran oil on the market. Moreover, enzymatic pre-treatment of oilseeds prior to extraction increases the oil yield, as specific enzymes break down the cell walls. efficient method due to its satisfactory yield. In this process, incubation parameters such as temperature and time significantly affect the oil yield [62].

Table 5 presents a comparison of the quality of rice bran oil obtained from different extraction methods (commercial, hexane solvent, and enzymatic). The results of the table indicate that the extraction method has a significant impact on the quality of rice bran oil. Commercial oil and hexane-extracted oil exhibited much lower free fatty acid contents compared to the enzymatically extracted oil (0.1% and 2.36% vs. 7.4%, respectively), indicating better stability and higher quality of these oils. The results indicate that commercial oil has the lowest free fatty acid content, peroxide value, and acceptable saponification number, reflecting its high quality in terms of oxidative stability. Nevertheless, the enzymatically extracted oil contained a high amount of γ-oryzanol (1.76%) and a relatively substantial level of tocopherols (0.9%), indicating its greater richness in bioactive and antioxidant compounds. However, this oil also exhibited the highest peroxide value (12.01), which may indicate an increased susceptibility to oxidation. The enzymatically extracted oil exhibited the highest iodine value and γ-oryzanol content compared to commercial oil, indicating a greater richness in bioactive compounds. Moreover, the enzymatic method produced lower levels of acetone-insoluble matter and peroxide compared to the hexane method, indicating relatively higher purity and stability of the oil. These results indicate that the extraction method can significantly influence the nutritional and chemical quality of the oil. Overall, despite some limitations in stability, the enzymatic extraction method outperforms other methods in terms of nutritional value and beneficial compounds [12].

Table 5. Comparative analysis of rice bran oil quality extracted via commercial, hexane-based, and enzymatic methods

Analytical Feature	Industrial Specification	Commercial Rice Bran Oil	Hexane Extracted Oil	Enzyme Extracted Oil	Codex standard range (Refined rice bran oil)
Free Fatty Acids (% as oleic acid)	≤ 0.3	0.1	7.4	2.36	0.3
Iodine Value	92 - 115	95.9	95.40	97.18	-
Peroxide Value (meq/kg oil)	≤ 10	5.5	8.2	12.01	Up to 15

Saponification Value	180 - 195	188.3	187.60	188.72	
Insoluble Matter in Acetone (%)	—	1.32	10.23	5.45	
Gamma Oryzanol (%)	—	0.07	2.04	1.76	
Tocopherols (%)	—	0.07	0.10	0.09	
Refractive index		1.4681			1.4600–1.4700

The reported values represent the mean of results obtained from three experimental replicates. The enzymatic extraction was conducted by adding 1 gram of Alcalase enzyme per 100 grams of rice bran, and the mixture was maintained at 50 °C and pH 9 for 2 hours.

One of the main challenges in the effective utilization of this nutrient is the presence of active lipase enzymes in the outer layer of rice bran. These enzymes are naturally inactive, but once brown rice undergoes milling, they become activated and immediately react with the lipids present in the rice bran structure. The result of this reaction is the hydrolysis of fats into free fatty acids, which not only leads to a decline in oil quality and nutritional value but also causes rice bran spoilage within a few hours after milling. This issue becomes particularly critical for long-term storage of rice bran, as reports indicate that the free fatty acid content in oil obtained from crude, untreated bran can increase by up to 47.5% within 30 days—a substantial rise that limits the industrial applicability of this oil. Therefore, to prevent the degradation of beneficial compounds and enhance the stability of rice bran, researchers have developed various methods to stabilize or inactivate lipolytic enzymes in freshly milled bran. These methods serve as a crucial prerequisite step in rice bran oil extraction, playing a key role in preserving the quality of the final product [89].

Hydroperoxides, as primary products of spontaneous oil oxidation, are tasteless, colorless, and odorless compounds. Upon decomposition, these compounds generate a wide range of carbonyl compounds, hydrocarbons, furans, and other products. The peroxide value is commonly used as an indicator to assess the extent of oil deterioration. The formation of hydroperoxides indicates the initiation of oil oxidation or degradation, ultimately leading to rancidity, spoilage, and the development of unpleasant odors [89].

Several studies have investigated various methods for rice bran oil extraction and their effects on the quality and quantity of the final product. The following section reviews a selection of these studies:

In a study conducted by Hanmoungjai et al. (2001), the fatty acid composition of rice bran oil obtained using three different extraction methods—commercial, hexane, and enzymatic—was investigated. It should be noted that the reported values represent the average of two measurements, and enzymatic extraction was performed using 1 g of Alcalase per 100 g of bran at 50 °C and pH 9 for 2 hours. These findings indicate that the enzymatic method can be a suitable option for producing oil with higher nutritional quality. The results are presented in Table 5,6 [12].

Table 6. Comparative analysis of individual fatty acid content in different rice bran oils (commercial, hexane, and enzymatic extraction)

Percentage of total fatty acids (%)	Commercial Rice Bran Oil	Hexane-Extracted Oil	Enzymatically Extracted Oil	Codex Standard range (%)
C14:0	3.24	2.99	3.22	ND-1.0
C16:0	13.19	11.88	11.44	14-23
C18:0	1.47	1.40	1.29	0.9-4.0
C18:1	26.16	30.13	29.85	38-48
C18:2	55.06	53.30	53.75	21-42
C18:3	0.89	0.31	0.45	0.1-2.9

Oleic acid and linoleic acid have been reported as the predominant unsaturated fatty acids in rice bran oil, with a saturated-to-unsaturated fatty acid ratio of approximately 20:80. Overall, the thermal stabilization process does not appear to affect the fatty acid profile [97]. The data in the table indicate that the fatty acid composition of rice bran oil varies depending on the extraction method. Oils extracted using hexane and the enzymatic method contained higher amounts of oleic acid (beneficial unsaturated fat) and lower percentages of palmitic acid (saturated fat) compared to commercial oil, which may suggest better nutritional quality of these oils. Furthermore, the enzymatic method demonstrated superior performance in retaining higher amounts of linolenic acid (omega-3) compared to the hexane method, suggesting that enzymatic extraction may be a more favorable approach for preserving beneficial compounds [12,98].

In a previous study conducted by Mourad et al. (2009), rice bran was stabilized using microwave treatment to improve the stability and quality of the extracted oil. This method resulted in the production of oil with notable stability for up to 48 weeks, demonstrating the effectiveness of the stabilization process in inhibiting lipolytic enzyme activity and preventing lipid degradation. Subsequently, the stabilized rice bran underwent enzymatic hydrolysis using three different enzymes, including protease, macroenzyme, and alpha-amylase. The results showed that the use of all three enzymes significantly enhanced the oil extraction yield. However, significant differences in the quality of the resulting oil were observed. Protease and macroenzyme treatments produced oils with acidity within an acceptable range, whereas the use of alpha-amylase resulted in oils with high free fatty acid content (15–19%), which is considered undesirable in terms of quality [99].

In some studies, various pretreatment methods, including the enzymatic approach, have been employed. This method involves the use of proteolytic, cellulolytic,

and pectinolytic enzymes to break down the cellular structures of oilseeds [61,100]. These enzymes have been applied as a pretreatment prior to mechanical extraction to disrupt cell walls and oleosome membranes, thereby facilitating oil release. Research findings indicate that such enzymatic treatments effectively enhance oil extraction yield, particularly when a combination of multiple enzymes is used. As shown in Table 7, all the oils examined contained small amounts of myristic acid (0.09–0.31%). The palmitic acid content was higher than previously reported values (20–26.67%), whereas stearic acid ranged from 2.06–2.48%, which is consistent with earlier reports. On the other hand, oleic acid was considerably lower than the reported range (31.97–51.01%), whereas linoleic acid was higher than previously reported values [61].

Table 7. Comparative analysis of fatty acid profiles in rice bran oil obtained from various extraction treatments

Fatty Acid	OC (Commercial)	OS (Supercritical CO_2)	OH (Hexane)	OM (Mechanical)	Rice Bran Oil (Reported Range)
Myristic Acid	0.13	0	0.09	0.30	0.2
Palmitic Acid	20.63	20.00	21.27	26.67	15.74 – 16.74
Palmitoleic Acid	0	0.34	0.13	0	–
Stearic Acid	2.06	2.41	2.30	2.48	1.9
Oleic Acid	31.97	51.01	24.98	27.30	42.5 – 42.8
Linoleic Acid	45.02	72.24	51.20	43.25	34.7 – 39.1
Linolenic Acid	0.01	Trace	0.03	Trace	0.19 – 1.1

In this study, OC refers to the oil obtained from stabilized rice bran under similar processing conditions. The parameter OS denotes the oil obtained via solvent extraction from rice bran stabilized with a mixed enzyme treatment.

The parameter OH refers to oil obtained through hydraulic pressing after enzymatic treatment, whereas OM represents oil derived from rice bran treated in a hexane environment. All reported values are averages of two analyses [101,102].

Rice bran proteins and amino acid profile

Rice bran contains crude protein in the range of 12–22%, which is higher than that of wheat and corn; however, the amount varies depending on the rice variety, cultivation conditions, and processing methods [36,103]. Protein extracted from rice bran, with a balanced amino acid profile, high quality, good digestibility, and very low allergenicity, is considered a valuable nutrient for the production of plant-based foods.

This protein exhibits multiple functional properties, including antioxidant [104,105], antidiabetic [106,107], and anticancer activities [108,109], and therefore holds high potential for the production of value-added products, particularly in the food industry. Its concentrate form can be utilized in the development of a wide range of food products and is particularly applicable in formulations for infant foods and products designed for individuals with allergies. Rice, which is considered a good source of energy, ranks high among plant proteins in terms of nutritional quality, although its protein content is relatively lower compared to other cereals [7,110,111].

The protein efficiency ratio of rice bran protein ranges from 2 to 2.5, which is comparable to that of casein (2.5), and its protein digestibility exceeds 90% [9,112]. Rice bran is also rich in dietary fiber and, compared to the bran of other major cereals, contains higher amounts of minerals (particularly iron, magnesium, phosphorus, potassium, and manganese) as well as B and E vitamins [113].

To obtain isolated protein from rice bran, two main steps—extraction and protein separation—are involved, and their effective execution requires precise knowledge of protein characteristics such as solubility and molecular weight. Rice bran primarily contains storage proteins, including albumin, globulin, glutelin, and prolamin, which, according to the Osborne classification, are grouped based on their solubility. These proteins vary in structure, solubility, and nutritional quality, and a thorough understanding of these properties is crucial for selecting the most appropriate extraction method. In addition to their nutritional role, these compounds are highly important in industrial applications, particularly in the extraction of hydrolyzed proteins. During the rice polishing process, albumin and part of the globulin proteins are removed along with the bran. The protein composition of rice bran typically includes albumin, globulin, glutelin, and prolamin, accounting for approximately 37%, 36%, 22%, and 5%, respectively. This product not only exhibits high digestibility but also has a low allergenic potential. The protein of rice bran varies depending on the rice variety. For example, in Iranian cultivars, it has been measured at approximately 6.24% using the Kjeldahl method. Compared to other plant protein sources, rice bran is considered a valuable resource, particularly due to its high content of essential amino acids such as lysine [60,114–116].

Albumin, a water-soluble protein, contains fewer disulfide bonds than other proteins and is also soluble in saline solutions. It exhibits a broad molecular weight range (10–200 kDa) and includes glycoproteins of approximately 60 kDa. Albumin is easily digestible and absorbable, and its antioxidant properties make it highly suitable for human consumption. This diverse amino acid composition and functional characteristics render rice

bran proteins a nutritious and valuable source for both nutritional and industrial applications.

Globulin is generally soluble in saline solutions and, due to the natural salts in the plant, is often extracted alongside albumin. This protein is composed of polypeptides with molecular weights of 16 and 25 kDa and is rich in sulfur-containing amino acids, such as cysteine and methionine, which are important for protein synthesis and cellular function [7].

The 19-kDa globulin is one of the seed storage proteins in *Oryza sativa* (rice), with a molecular weight of approximately 19 kDa. According to UniProt (ID: P29835), this protein is primarily found in rice seeds and plays a crucial role in providing the necessary nutrient reserves for germination. The 19-kDa globulin is localized in the vacuoles of cells and belongs to the cupin superfamily, which is characterized by its protected β-barrel structure. This protein consists of 173 amino acids, and its gene expression is predominantly observed in the developing endosperm. The crystallographic structure is shown in Fig. 5. [117].

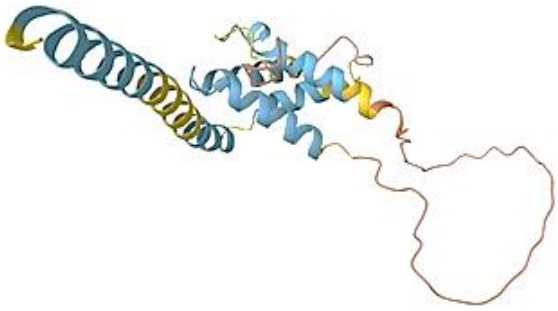

Fig 5. Crystallographic structure of rice globulin protein

Glutelin is soluble only in alkaline environments, and the disulfide bonds and glycosylation complicates its extraction and solubility. Its molecular weight ranges from 45 to 150 kDa. This protein is rich in lysine, an essential amino acid, and can enhance the quality of dietary protein [7]. Glutelin type-B2 (UniProt ID: Q02897) is one of the major storage proteins in *Oryza sativa* (rice) seeds, playing a fundamental role in supplying nitrogen and energy reserves to the developing embryo post-germination. This protein, with a molecular weight of approximately 52.2 kDa and a chain of 462 amino acids, is predominantly expressed in the seed endosperm and stored in the vacuoles of cells. Glutelin type B2 is processed into light and heavy subunits post-translation and belongs to the glutelin family, which plays a significant role in enhancing the nutritional quality of rice. The crystallographic structure of this protein is shown in Fig. 6. [117].

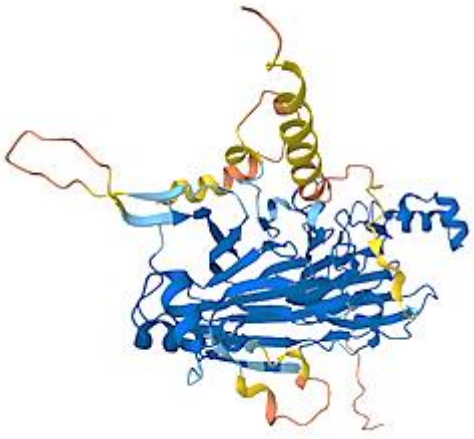

Fig 6. Crystallographic structure of rice glutelin type B2 protein

Prolamin is soluble in alcohol, requiring 60–70% ethanol for dissolution. This protein has a molecular weight ranging from approximately 12 to 17 kDa and is rich in amino acids such as glutamic acid, alanine, glycine, and arginine, which are involved in key metabolic functions of the body [7]. Among the storage proteins of *Oryza sativa*, prolamins comprise several types that differ primarily in their molecular weight and genetic structure. The 14-kDa prolamins, including the 14P and 14E isoforms, are the predominant storage proteins, playing a prominent role in both embryo nutrition and grain structure. These two isoforms likely

differ in their regulatory gene regions, which may influence their expression patterns. Prolamin D17, with a larger molecular weight, exhibits a more complex structure, whereas the 10-kDa prolamin, being the smallest, has the simplest structure and is typically involved in the early stages of storage protein synthesis. This structural and functional diversity reflects the complexity of genetic regulation and underscores the importance of these proteins in rice grains. Among the various types of prolamins, the 14-kDa class—particularly the 14P and 14E isoforms—represents the most predominant form. This protein accounts for nearly 70–80% of the total grain prolamins and is predominantly expressed in the endosperm. Furthermore, this type plays a key role in the protein structure of the grain and its nutritional quality, and it exhibits considerable genetic diversity and regulatory expression. Its crystallographic structure is presented in Fig. 7. [117].

Fig 7. Crystallographic structure of 14 kDa prolamin in rice

As previously mentioned, and as shown in Fig. 8., rice proteins are predominantly observed in the form of storage organelles known as protein bodies (PBs). Two structurally distinct types of protein bodies have been identified in the rice endosperm, namely type I (PB-I) and type II (PB-II). Type I protein bodies have a layered structure, a spherical shape, and are rich in prolamins, whereas type II protein bodies display a crystalline structure, an irregular shape, and contain a high proportion of glutelins. Reports also indicate that endosperm storage proteins consist of approximately 60–65% type II protein bodies, about 20–25% type I protein bodies, and 10–15% albumins and globulins in the cytoplasm.

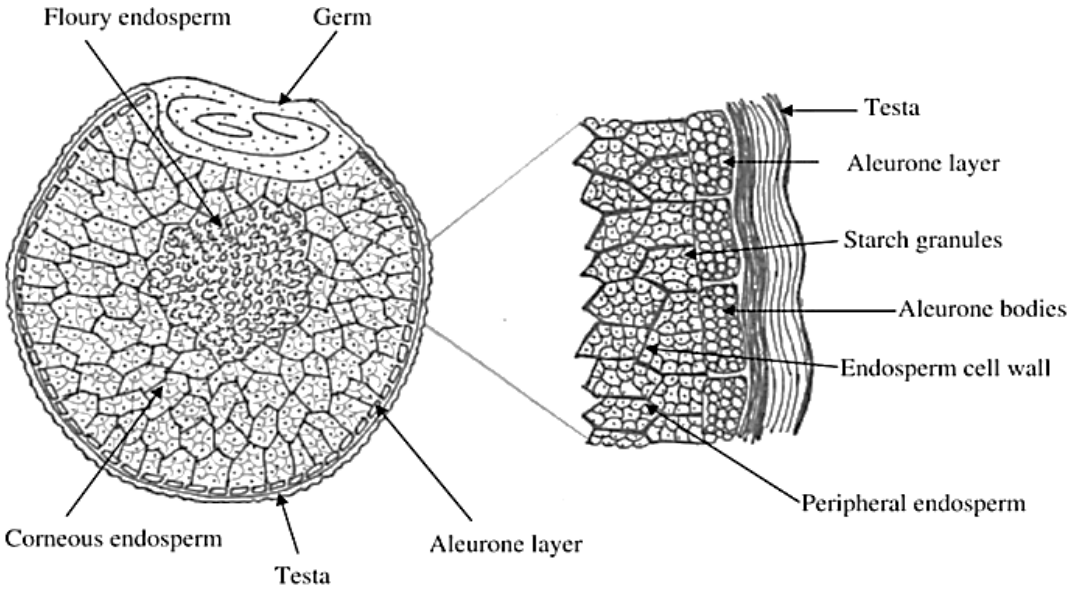

Fig 8. Schematic representation of rice protein bodies and compound starch granules located in the sub-aleurone layer of the endosperm

Despite their high nutritional value, rice bran proteins are not yet commercially available in the form of concentrates or isolates due to the lack of appropriate processing technologies. A major reason for this is the complex structure, low solubility, and tendency of these proteins to aggregate, which complicates their separation from other cell wall components and limits their application in the food industry. In recent years, various approaches, including physical, chemical, and enzymatic treatments, have been developed to improve protein extraction from rice bran. Although chemical processes such as alkaline or acid hydrolysis may alter protein structures and damage amino acids, enzymatic methods (particularly the application of carbohydrases and proteases) are considered more effective due to their substantial enhancement of protein recovery. Carbohydrases degrade the cell wall to release proteins from the polysaccharide matrix, while proteases hydrolyze peptide bonds, converting them into soluble peptides and amino acids [8–10].

Extraction methods can influence the functional and nutritional properties of proteins. The performance of rice

bran protein is affected by surface properties, hydration characteristics, as well as its molecular structure and size. In contrast, the nutritional properties of rice bran protein are associated with protein quality, digestibility, hypoallergenicity, and the health-promoting benefits of its hydrolysates and peptides. Furthermore, studies indicate that rice bran protein represents a potential ingredient in the production of plant-based meat, beverages, biofilms, and delivery carriers [7].

Enzymatic hydrolysis preserves the nutritional value of proteins, as it is carried out under mild conditions and prevents the occurrence of side reactions. Besides, depending on the enzyme specificity and the degree of hydrolysis, it is possible to generate hydrolysates with functional, biological, and nutritional properties distinct from those of the native protein. Specifically, it is now widely accepted that the biological properties of protein hydrolysates are closely related to the molecular weight of their constituent peptides. Low-molecular-weight peptides are generally considered to exhibit the highest biological activity, as they can readily cross the intestinal barrier and exert their biological functions. In this context, ultrafiltration using membranes with defined molecular weight cut-off ranges is one of the most common strategies for separating protein hydrolysates, owing to advantages such as high yield, low cost, and preservation of product purity under ambient conditions [8,13–15].

Numerous studies have demonstrated that the bioactivity of peptides depends on their chain length, amino acid composition, and sequence. For instance, the hydrophobic amino acids such as valine (V), leucine (L), and isoleucine (I), particularly near the C-terminal end, has a significant influence on angiotensin-converting enzyme (ACE) inhibitory activity. In addition, the proline (P) within peptide structures enhances the stability and effectiveness of ACE-inhibitory peptides, owing to its unique cyclic structure. Similarly, the sequence RGQLLIVPQHYV contains hydrophobic residues (L, I, V, and P — leucine, isoleucine, valine, and proline), aligning it with the features of peptides effective in ACE inhibition [28,118,119].

Han et al. (2015) demonstrated that rice bran protein exhibits superior nutritional quality compared to rice endosperm protein. Rice bran protein presented a true digestibility (TD) of 94.8%, a biological value (BV) of 72.6%, and a protein digestibility-corrected amino acid score (PDCAAS) of 0.9, while the corresponding values for rice endosperm protein were reported as 90.8%, 66.7%, and 0.63, respectively [120].

The composition of essential amino acids (g per 100 g of protein) in rice bran protein and rice endosperm protein is presented in Table 8. As the table indicates, rice bran protein and rice endosperm protein are nearly equal in total essential amino acids, although differences exist in their specific compositions. Rice bran protein is richer in lysine and tryptophan, indicating a higher nutritional quality, particularly for diets deficient in these amino acids. In contrast, rice endosperm protein contains higher amounts of methionine + cysteine and phenylalanine + tyrosine, which play important roles in cellular function and the synthesis of neurotransmitters. Consequently, both types of protein possess distinct nutritional advantages, and their combination may contribute to a more balanced amino acid profile in the diet [121, 1,120].

Table 8. Composition of essential amino acids (g/100 g protein) in rice bran protein and rice endosperm protein

Amino Acid	Rice Bran	Rice Endosperm
Histidine	4.48	2.46
Isoleucine	3.61	3.80
Leucine	7.69	8.15
Lysine	4.55	3.31
Methionine + Cysteine	2.70	3.88
Phenylalanine + Tyrosine	8.24	10.10
Threonine	3.68	3.46
Tryptophan	1.17	0.82
Valine	5.53	5.12
Total Essential Amino Acids	**41.7**	**41.1**

In another study, Yu et al. (2022) divided rice bran into four layers—from the outermost to the innermost regions, designated as layers 1, 2, 3, and 4—to investigate its amino acid composition. Structurally, rice bran consists of the pericarp (~10 µm), testa (~2 µm), nucellus (~1–2 µm), and aleurone (~20 µm) layers from the outer to the inner region. Thus, layers 1 and 2 primarily comprise the pericarp, testa, and nucellus, whereas layers 3 and 4 are mainly composed of the aleurone layer [122].

Table 9 compares the amino acid composition across four distinct layers of rice bran, from the outermost to the innermost regions. The data indicate that the levels of certain amino acids, such as glutamic acid, glycine, alanine, and tyrosine, increase from the outer to the inner layers. These amino acids not only contribute to protein structure but may also influence the palatability and functional properties of the product. Furthermore, the nutritional ratios, such as the ratio of essential to total amino acids and the ratio of essential to non-essential amino acids, are slightly higher in the outer layers. This indicates that, in terms of protein quality, the outer layers of rice bran exhibit a more balanced essential amino acid profile and superior nutritional value, while the inner layers may be preferred for their more desirable taste

characteristics. Overall, these differences may have significant implications for food processing, the selection of rice bran types for dietary supplements, or the development of specific rice-based products [122,123].

Table 9. Amino acid composition of different microstructural fractions of rice bran

Amino Acid (mmol/kg)	Fraction 1	Fraction 2	Fraction 3	Fraction 4
Aspartic acid	107.5	107	101	98
Threonine	49	49	46.5	45.5
Serine	65	67	67	69.5
Glutamic acid	140	148.5	160.5	176.5
Glycine	97.5	99	90.5	84.5
Alanine	91.5	95	89.5	87.5
Cysteine	90	90	90	90
Valine	73.5	74	73	75.5
Methionine	9	7.5	9	9
Isoleucine	38.5	39	39	41
Leucine	96	97.5	98	103
Tyrosine	27.5	28.5	30.5	34.5
Phenylalanine	37	38	39	42
Lysine	46	45	39	34.5
Histidine	23	24	22.5	22
Arginine	62.5	65.5	66	67
Total Essential AAs / Total AAs (%)	31.35	34.80	34.50	34.49
Total Non-Essential AAs / Total AAs (%)	58.54	53.39	52.66	52.65

A review of various studies on the characteristics of rice bran protein and its extraction methods has shown that several treatment approaches, including physical, thermal, enzymatic, and chemical methods, have been applied for protein extraction from rice bran. The results indicated that among all available pretreatment methods, enzymatic approaches demonstrated the most promising protein yields. However, the relatively high cost of enzymes, along with their sensitivity to specific industrial conditions, has been identified as a limitation for the use of these methods. Furthermore, studies have shown that pretreating rice bran with chemical, enzymatic, or thermal methods prior to protein extraction can adversely affect the functional properties of the proteins [1,124].

In conclusion, rice bran protein, owing to its unique properties, holds significant potential for diverse applications across various industries.

In conclusion, due to its unique properties, rice bran protein holds significant potential for diverse applications across various industries. For example, its high water- and oil-binding capacity, which aids in moisture retention and the development of a soft mouthfeel, makes it a suitable ingredient for products such as bread, pastry creams, sausages, and other food formulations. Moreover, this protein can serve as an excellent base for high-sugar products, such as cake batters, frozen desserts, and various confections. Hydrolyzed rice bran protein also possesses a wide range of applications, including use in dietary supplements, functional ingredients, flavor enhancers, coffee whiteners, cosmetics, and personal care products. In addition, it is employed to fortify beverages such as soft drinks and fruit juices, as well as in the production of soups, sauces, meat products, and other food items [60].

Different rice varieties and bran types

There are over 40,000 rice cultivars worldwide, of which more than 15,000 have been cultivated across China to date. Asian cultivated rice has undergone significant genetic differentiation as a result of adaptation to diverse ecological conditions, influenced by both natural and human selection. This genetic differentiation has generated extensive genetic diversity in rice, including the indica and japonica ecotypes. During the domestication process, indica and japonica rice have diverged in terms of morphological, agronomic, physiological, and biochemical traits, as well as in yield, quality, and stress resistance. These ecotypes can be further divided into two subgroups: inbred lines and hybrids. Consequently, the four main rice types include indica inbred lines (II), indica hybrids (IH), japonica inbred lines (JI), and japonica hybrids (JH) [125].

Indica rice is predominantly cultivated in tropical and subtropical lowland regions, including the Yangtze River area and southern coastal zones, accounting for nearly 70% of the total rice cultivation area in China. Japonica rice exhibits greater cold tolerance compared to indica, and is therefore cultivated in regions with wider temperature fluctuations and often at higher latitudes or elevations, such as northeastern China and the southwestern Chinese plateau. The cultivation area of

japonica rice has increased over time and now covers approximately 9 million hectares, accounting for 30% of the total rice-growing area in China. Hybrid rice is also widely cultivated, covering more than 53% of China's total rice-growing area, of which 51.5% corresponds to indica hybrids (IH) and 1.5% to japonica hybrids (JH) [126,127].

In China, new rice cultivars must be registered with provincial or national plant variety committees before being released to the market. To identify superior cultivars, the Chinese government established an evaluation system for new rice varieties in the 1960s. In the first stage, new improved lines are evaluated by breeders over a 1- to 2-year period through comparative trials conducted at research stations. In these trials, agronomic traits such as yield, yield components, growth duration, plant height, grain length and width, adaptability, stability, resistance to major diseases and pests, and grain quality are assessed for each line. After 2 to 3 years of regional trials, new cultivars are submitted for official registration to the provincial or national plant variety committees [128].

In a study conducted by Zarei et al. (2018), the metabolite profiles of rice bran from 17 different varieties were analyzed using principal component analysis (PCA), as shown in Fig. 9. For this purpose, the relative abundances of metabolites were normalized and then analyzed to identify patterns of variation and similarity among the varieties. The results indicated that the first (PC1) and second (PC2) principal components accounted for 20.3% and 16% of the metabolic variation, respectively, suggesting a core metabolome in rice bran. However, substantial differences were observed in the range of individual metabolites (approximately 60 to 90 compounds) across various chemical classes, including amino acids, fatty acids, sugars, and phenolic compounds. Furthermore, comparison of the varieties based on their country of origin revealed distinct differences in metabolite distribution [129].

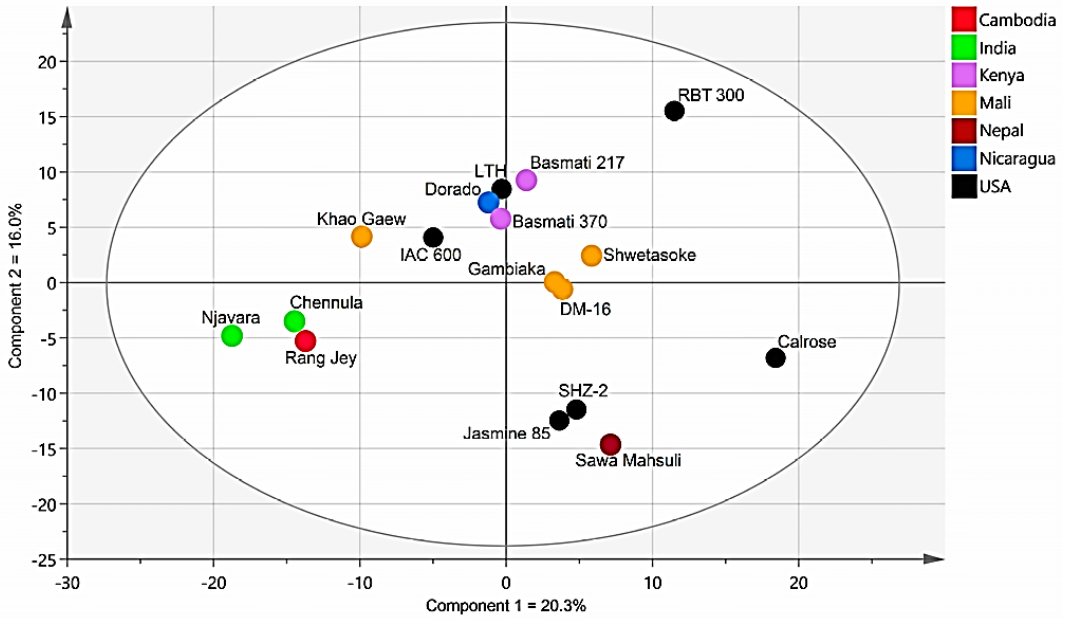

Fig 9. Principal component analysis (PCA) of rice bran metabolome in 17 rice cultivars. PCA was performed using median-scaled relative abundance of all bran samples in these 17 cultivars. The first principal component (PC1) explained 20.3% and the second principal component (PC2) explained 16% of the variation in the metabolite profiles. Colored dots indicate the country where the rice was produced.

In another study by Hamada (1997), the effect of rice bran variety on the extraction of proteins—including albumin, globulin, prolamin, and glutelin—was evaluated using ANOVA. A significant difference among varieties was observed in the contents of albumin, prolamin, and acid-soluble glutelin, while the globulin content showed no notable variation. The results are presented in Table 10 [130].

Table 10. Effect of cultivar on rice bran proteins

Cultivar	Albumin	Globulin	Prolamine	Glutelin
Bengal	34.7^{b}	17.4^{a}	$6.7^{a,b}$	$\mathbf{11.9^{b,c}}$
Cypress	33.0^{b}	13.7^{a}	7.8^{b}	$\mathbf{9.7^{a,b}}$
Della	32.2^{b}	13.5^{a}	5.7^{a}	$\mathbf{10.4^{b}}$
Mars	39.5^{c}	17.0^{a}	5.3^{a}	$\mathbf{12.1^{b,c}}$
Maybelle	33.4^{b}	12.8^{a}	5.9^{a}	$\mathbf{14.7^{c}}$
Toro-2	30.2^{a}	14.3^{a}	5.3^{a}	$\mathbf{6.6^{a}}$

In another study conducted on 17 different rice varieties, 21 amino acids were found to differ significantly, many of which are known to play important roles in human and animal health. Fig. 10. illustrates the Z-score distribution of amino acids, indicating the extent to which the level of each amino acid in different rice varieties is above or below the mean. A positive Z-score represents a value higher than the mean, while a negative Z-score indicates a value lower than the mean. This approach helps to identify the relative differences in amino acids among the varieties. For instance, DM-16 rice bran contained lower levels of quinic acid and higher levels of serotonin. In the Rang Jey variety, the levels of tryptophan and tyrosine were lower, whereas Chennula rice bran exhibited reduced levels of four amino acids: lysine, trimethyllysine, threonine, and arginine. In contrast, Gambiaka rice bran contained higher abundances of methylproline, stachydrine, and trans-4-hydroxyproline compared to the other varieties. The Njavara variety exhibited lower levels of serotonin, asparagine, glutamate, glutamine, pyroglutamine, and taurine, but a higher content of N-acetylglutamate. Similarly, Sawa Mahsuli rice bran showed elevated levels of pipecolate and glycine. In addition, the Khao Gaew and IAC 600 varieties showed lower abundances of methionine sulfoxide and aspartate, respectively. Finally, the varieties Basmati 217, Basmati 370, Shwetasoke, Dorado, Calrose, RBT 300, Jasmine 85, LTH, and SHZ-2 exhibited no significant changes in amino acid levels [129].

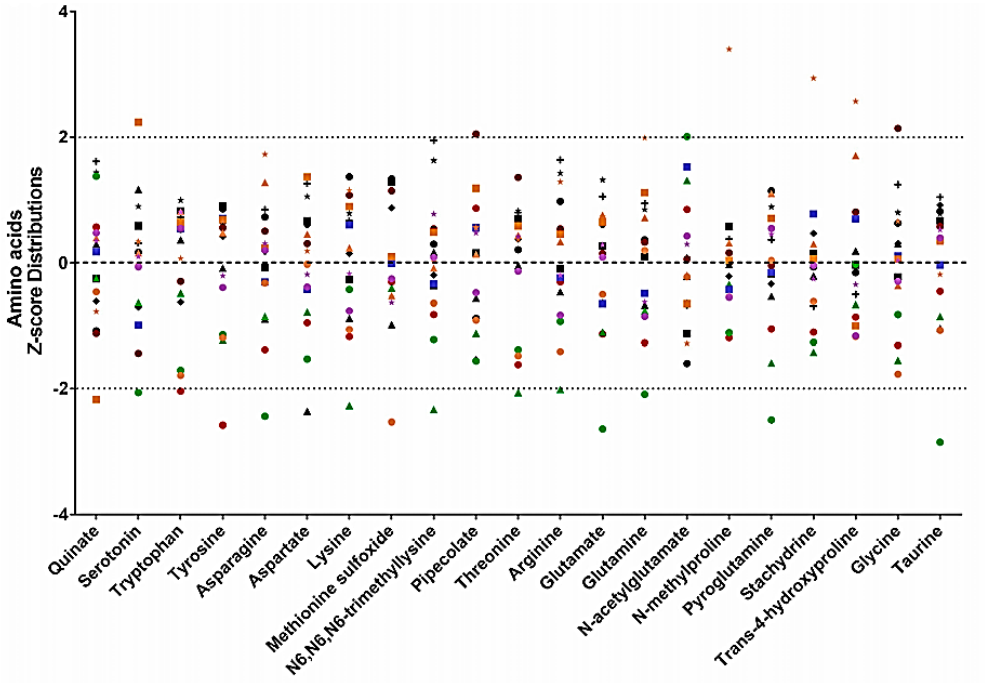

Fig 10. Analysis of Z-score distribution of amino acids in 17 rice bran cultivars

Furthermore, a study on Iranian rice cultivars showed, as illustrated in Fig. 11., that the rice bran oils of the Tarom and Khazar varieties differed significantly in their fatty acid profiles ($p < 0.05$). Specifically, Tarom bran oil contained higher levels of oleic, palmitic, and linolenic acids, whereas Khazar bran oil was richer in myristic, stearic, linoleic, arachidic, and gadoleic acids compared to Tarom. These findings indicate that rice cultivar can significantly influence the fatty acid composition of rice bran oil [89].

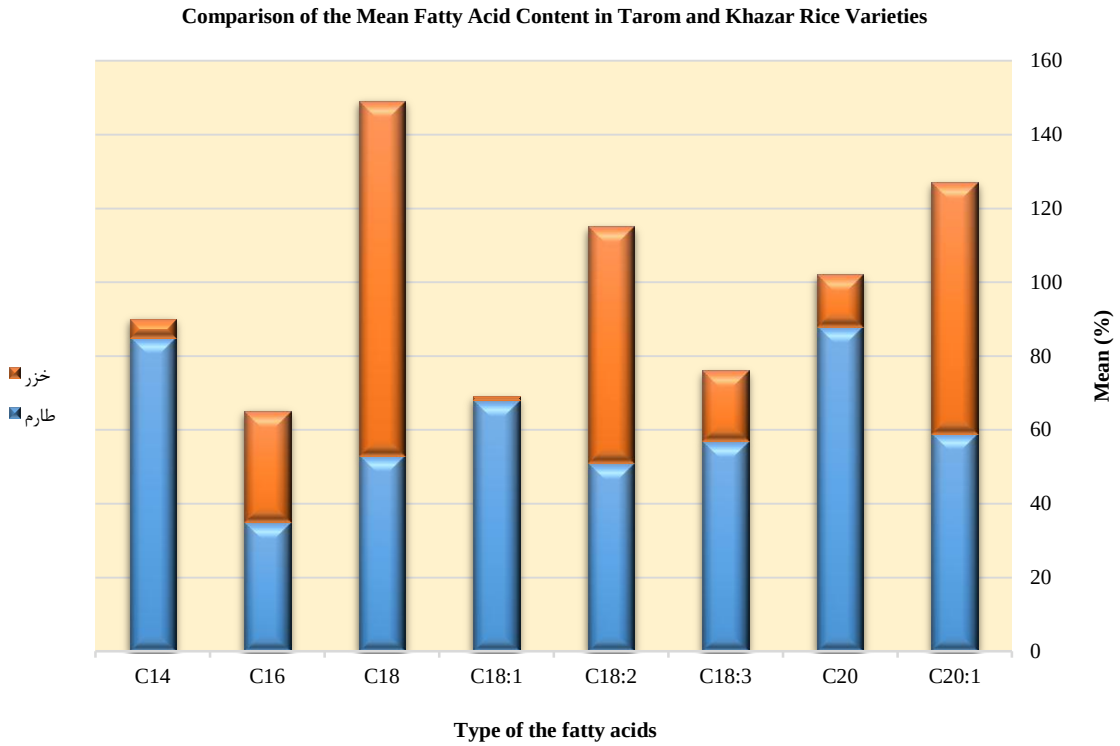

Fig 11. Comparison of mean fatty acid profiles of rice bran oil among different varieties

Another study which examined Iranian rice cultivars, demonstrated significant differences in the levels of storage proteins among different rice genotypes. Specifically, the Sang Tarom and Roshan cultivars exhibited the highest albumin protein contents, measuring 19.53 mg/g and 24.40 mg/g, respectively. In contrast, the Brown Grain variety demonstrated the lowest albumin and globulin contents but had the highest levels of glutelin and prolamin, resulting in the highest total protein content at 160.43 mg/g. Evaluation of the banding patterns of storage proteins also revealed that, except for the Roshan mutant (with a band around 60 kDa) and Shahriar (with a band around 13 kDa), the patterns were similar and consistent across the other genotypes. Densitometric analysis of protein subunits further revealed that the highest glutelin subunit levels were observed in the Sang Tarom cultivar, while the lowest levels were found in the Shahriar cultivar and the Brown Grain variety. Additionally, the highest prolamin subunit levels were observed in the Sang Tarom cultivar and the Brown Grain variety, while the lowest levels were found in the Shahriar cultivar, which consequently showed the highest glutelin-to-prolamin protein ratio. Ultimately, the study results indicated that although the seed protein profiles of different rice varieties are largely similar in both quantity and quality, notable differences were observed in the Roshan mutant and Shahriar cultivars [36].

Conclusions

Rice (Oryza sativa L.) is one of the main staple cereals in Asia and holds great significance for human nutrition. Rice bran, which constitutes approximately 10% of the brown rice grain, is a by-product of the milling process. Rice bran contains protein (10–16%), fat (12–22%), dietary fiber, and bioactive compounds such as B vitamins, vitamin E, and γ-oryzanol, which contribute to its notable antioxidant and nutritional properties. Rice bran oil, with a balanced fatty acid composition comprising 43% oleic acid, 32% linoleic acid, and 15% palmitic acid, plays an important role in cardiovascular health and the reduction of oxidative stress. The ratio of saturated to unsaturated fatty acids in this oil is approximately 20:80, and the presence of linolenic acid is another distinctive feature. Non-saponifiable compounds, such as tocopherols, phytosterols, and polyphenols, enhance the antioxidant and cholesterol-lowering properties of rice bran oil. Rice bran proteins include albumin, globulin, glutelin, and prolamin, which are considered a valuable protein source due to their high digestibility (>90%) and a protein efficiency ratio of 2–2.5. Genetic diversity in rice leads to variations in the amino acid composition of rice bran, with the ratio of essential to total amino acids in different bran fractions ranging from 31.35% to 34.8%, indicating stable protein quality. Despite containing such valuable components, direct human consumption of rice bran remains somewhat limited, and it is mainly used in animal feed, fertilizers, and biofuel. However, as mentioned, rice bran—with its balanced amino acid profile, beneficial lipids, and antioxidant compounds—has high potential for applications in food, pharmaceutical, and health-related industries.

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