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SHORT REVIEW

Exploring the Nutraceuticals and Biological Properties of Manipur Black Rice Protein and Significance after Modification: A Review

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Abstract

Background: Plants are considered nutritionally rich and cost-effective sources of proteins, thus, can be used as food ingredients. Proteins from plant sources are currently in great demand as plant-based proteins are considered healthy. Plant-based proteins are considered the best choice for the replacement of meat-based proteins for new product formulations. Plant protein functionality is highly important for designing new protein-based foods. This review paper explains importance of modified Manipuri black riceprotein for its Nutraceutical applications in food industry. **Aim:** Black rice, also known as forbidden rice, is a distinctive type of rice that is known for its rich nutritional profile and deep dark colour. This ancient grain, grown in Asia for millennia, was once reserved for royalty due to its rarity and health benefits. **Method:** Protein was isolated from Manipur black rice and then subjected to ultrasonication and enzyme hydrolysis treatment for determining its effect on various properties after modification. **Result:** The deep black color comes from anthocyanins, a powerful antioxidant also found in blueberries and blackberries that help reduce oxidative stress and inflammation in the body. In terms of nutrition, it contains more fiber, protein, and key minerals than white rice, so it's a good choice for those looking to eat healthier. This means it is gluten-free, so safe for those with gluten sensitivity. Their nutty flavour and chewy texture elevate salads, soups, and desserts in both taste and visual interest. Along with being extremely healthy, black rice is historically significant in many Asian cuisines. Its unique flavour and nutritional profile have made it increasingly popular in contemporary culinary trends and a widely accepted food choice in healthy diets. **Conclusion:** This review presents the medicinal activities and the other contents of black rice proteins and their possible usages in the food industry.
Major Findings: Black rice protein holds significant promise as a functional ingredient due to its rich nutritional profile, antioxidant activity, and potential health benefits such as anti-inflammatory and anti-obesity effects.

Keywords: Black Rice, Concentrate, Protein, Protein Modification

1. Introduction

As the staple diet of most Asians, rice (*Oryza sativa* L.) is the second most significant grain globally, with over 90% of the world's rice grown and consumed in Asia. India is the world's second-largest producer of rice, accounting for more than 42% of the nation's yearly production of food grains[1]. More than 65% of the nation's population eats it as a staple, and it is also the main source of calories and nutritional protein that the general population needs. Most people on the planet eat white or brown rice, but relatively few people in Asia occasionally eat black rice for special occasions. The precise gene that causes the plant to generate copious amounts of anthocyanin was discovered by Japanese researchers after they examined the genomes of 21 different types of black rice[2]. For a very long time, it has been integral to the socio-cultural customs of

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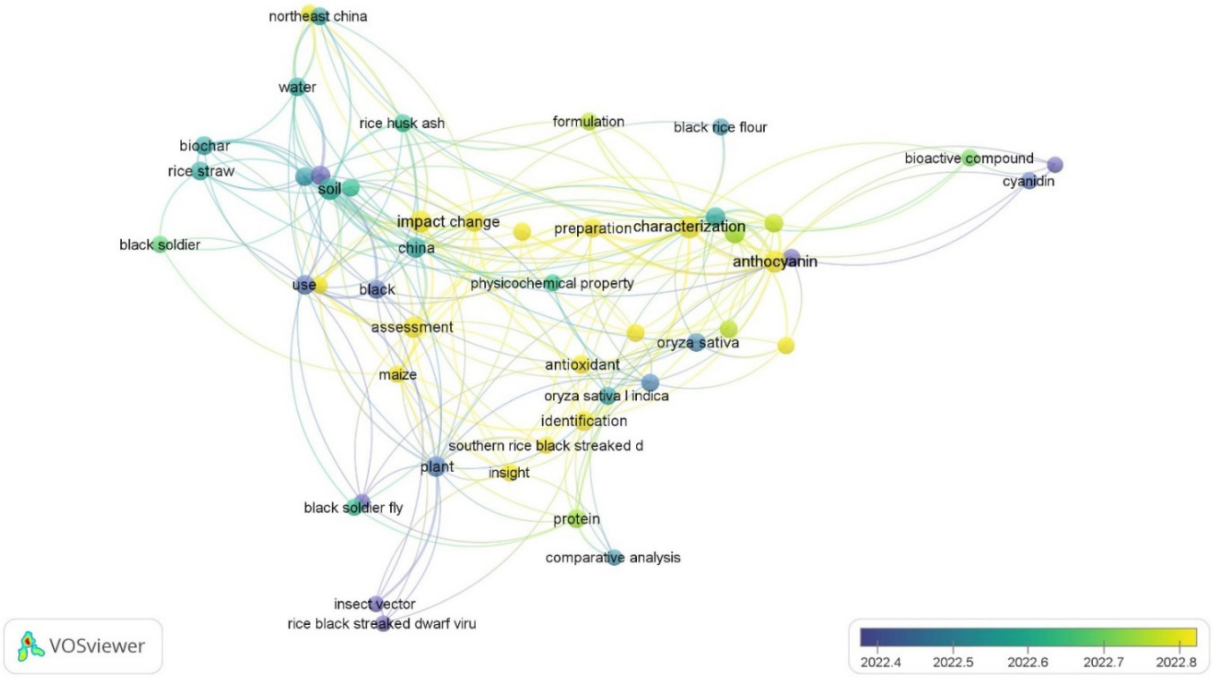


Figure 1. Keywords connection across the globe in last 5 years as per the data from WoS.

the Indigenous Meitei population in Manipur, India[3]. Manipur continues to be India's top producer of black rice. Due to its historical reservation for the Chinese Emperor, who was supposed to benefit from its health-promoting and longevity-promoting properties, black rice is also known as forbidden rice. In the past, black rice was outlawed for popular consumption and regarded as a royal luxury in Asian nations like China and Indonesia[4]. When cooked, black rice typically turns a deep purple tint from its original dark black color. It works well to make various foods, including kheer, noodles, cake, custard, porridge, and many more.

Plants can be utilized as nutritional elements since they are considered cheap and calorie-dense sources of proteins[5]. Since plant-based proteins are considered healthy, there is a great demand for plant-based proteins at present. Rendering these proteins is the best choice for replacing meat-based proteins in new product formulations[6]. The newest research conducted to substitute conservative proteins with plant-based proteins involves vegetable protein replacement with meat protein, egg proteins with mung bean proteins[7], and soymilk, almond milk, and vegetable cheese instead of milk and dairy[8]. When developing new protein-based foods, the working of plant proteins is paramount.

Due to its unique sensory qualities, high nutritional content, and, most importantly, its advantageous health qualities, black rice has drawn the most attention among the colored rice variants. Numerous research works have been carried out on various types of black rice concerning aspects like the physicochemical evaluation, phytochemical extraction, measurement, and identification of compounds with antioxidant capabilities (Figure 1).

Manipur black rice protein is used for preparing multiple food products like functional foods, plant-based milk substitutes, and protein supplements. Therefore, great nutrition and good functionality provided with creative approaches toward modifying can ensure the relevance for filling the gap of fulfilling world protein requirements9. Further research on perfecting these techniques and scaling them up for industrial use will open wider applications.

2. Black Rice Varieties

2.1 *Kalanamak*

Except for grain length, *Kalanamak* rice is claimed to surpass even the most expensive basmati rice in every aspect of quality. The rice is not basmati and has a short to medium grain length. More fragrant than

any basmati kind, *Kalanamak* rice is said to have been bestowed by Gautam Buddha. One of the most crucial quality characteristics in the global rice industry is elongation after cooking, which is 40% longer in rice than in Basmati.

2.1.1 Black Jasmine Rice

The term *Khaw-Hom-Nil*, which means "aromatic black onyx rice" in Thai, is used to refer to black jasmine rice. The rice releases a fragrant perfume when cooking that may be mistaken for a jasmine infusion.

2.1.2 Kalabhaat

Kalabhaat is firm, thin, and has flecks of black color. It creates luscious purple glutinous rice when cooked.

2.2 Manipur Black Rice

Manipur Black rice is unparalleled in its unique qualities, providing a plethora of health advantages. This sort of sticky black rice, known locally as "*ChakhaoAmubi*," is a glutinous rice variety from Manipur. The words "*Chakho*" and "*Ambui*" denote "tasty" and "black", respectively[10].

Starch, the primary ingredient in rice, gives us energy. Black rice also contains protein, found in the rice kernels. Rice protein may be categorized based on how soluble it is. Glutelins (soluble in alkaline solutions), prolamin (alcohol soluble; represent 25%), albumin (water soluble; represent 5%), and globulin (salt-water soluble; represent 10%) make up the majority (60%) of total protein. When it comes to providing the necessary amino acids for human metabolism, a protein's nutritional quality is determined by how much of these amino acids present and how bioavailable they are. Due to its greater amounts of lysine and sulfur-containing amino acids, rice (Amino Acid Score (AAS) of 68) has a more complete and balanced amino acid composition than wheat (AAS of 43) and maize (AAS of 35). In addition, rice has a greater protein efficiency quotient, biological properties, and digestibility than other cereals.

It has been referred to as a "superfood" recently, which it rightfully merits. Superfoods are dietary categories that offer several health advantages in addition to their intrinsic nutritional and caloric worth. The risk of several long-term ailments, such as cardiovascular disease, diabetes, and cancer, may be entirely eliminated by regularly consuming black rice. However, due to a lack of information, black rice is still far less popular in India than it is in the US and other Western nations where it is more popular than brown and white rice. Because of its nutritious qualities, black rice should thus be made more widely known so that people will desire it and learn about its advantages.

Plants are recognized as valuable sources of proteins due to their nutritional profile and low cost, so they can be applied as food ingredients. Plant-based proteins are healthy, making plant -source proteins highly sought after today. New product formulation also considers plant-based proteins the best alternative for the replacement of meat-based proteins. This shift includes the substitution of meat with veggie proteins, egg proteins with mung beans, and milk and dairy products with soymilk, almond milk, and veggie cheese. The plant-based food market is growing with no end in sight. However, the alarming growth of the human population and the resulting higher demand for food have forced the food industry to rely on plant sources for future food security. Undoubtedly, increasing niches like vegetarians, vegans, and flexitarians play an important part in the growth of plant-based products tagged "vegan" and "vegetarian". Ethical problems concerning animal welfare are one of the drivers to move towards more or only plant products[11]. The functionality of plant proteins is very important for the design of new protein-based foods. Protein functionality refers to the behaviour of protein in a polymer or food matrix. Physical, chemical, and enzymatic processing are the most general methods that can be used to improve the functionality of proteins from plant sources. Solubility, emulsification, and foaming properties are important for plant-based protein beverages design, and the same is applied in the design of coffee whiteners and whipped toppings. Ultrasound (US) is a non-invasive technique that is widely employed in food industries, which is more popular than other conventional methods because chemical modification includes certain toxic traits leading to the gradual development of food industries towards novel (ultrasonic) and biological modification methods. The most suitable technique to enhance protein functionality is high-intensity ultrasonication. Protein solutions can be sonicated in a wide range of ultrasound intensities (10-1000 W/cm^2) with time optimization using a probe

sonicator, which often modifies their physical and functional characteristics[12]. Recently, enzyme-assisted hydrolysis of proteins has gained considerable attention because enzymatic hydrolysis features considerable improvements in protein functionalities[13]. Similar findings have also been reported showing that enzyme treatment increases the functionality of plant proteins. In addition to functional properties, the antioxidant activity of protein hydrolysates can also be higher than its native protein[14]. Enzymatic modification can be employed to alter protein structure according to the desired functionality based on the functional property of the protein of interest. Proteolytic enzymes (pepsin, papain, trypsin, and alcalase) are used to alter or enhance the functionality of proteins.

2.3 Rice Protein Concentrate

The flesh quality and performances of *Pagellus bogaraveo* having diets containing rice protein concentrate (70% crude protein, 10% ether extract)[15]. Three isoenergetic and isoproteic diets (crude protein 47%, 22 MJ/kg DM) were prepared with increasing content of rice protein concentrate (0%, 20%, 35%). The fish with a mean weight of 75g were distributed in 12 tanks (4 replications of 3 diets, 10 fish/tank). This leads to differences in the fatty acid profile of dorsal muscles. The addition of rice protein concentrate as a 20% replacement of fish meal is possible without hindering the growth performance.

The Dried Rice Protein Concentrate (DRPC) was used to prepare the reduced lactose ice cream[16]. The standardized base ice cream mix had 8% fat, 11% solids not fat, 15% sugar, and 0.3% stabilizer. The Solid Not Fat (SNF) in the control ice cream mix was derived from skim milk powder, and DRPC was added in the following ratios: 20, 40, 60, 80, and 100% in place of SMP. Mixtures and finished products were evaluated for their physical, chemical, and organoleptic characteristics. The results demonstrated that when the ratio of additional DRPC in the mix increased, the amounts of ash and lactose were significantly decreased. When DRPC was used to prepare reduced lactose ice cream mixes, the flow curves improved in comparison to the control mix. According to sensory analysis, modified samples made by substituting 20, 40, and 60% were more appealing and acceptable. It was determined that skim milk powder may be replaced with DRPC up to 60% to provide reduced-lactose ice cream with better functional qualities and greater acceptance. It is an excellent method of introducing low-lactose ice cream products, particularly to those who have lactose sensitivity.

Wheat flour used in bakery goods is substandard, has a low protein content, and is absorbed poorly. The composition of baked goods may use sources of full proteins to address this issue. Animal proteins, however, may cause allergies. Protein isolates, hydrolysates, and concentrates of vegetable origin- especially those derived from rice- can serve as an alternative. Adding rice protein concentrate to wheat flour affects the dough's and bread's microbiological, biochemical, and structural alterations. It was compared by the researcher that the dough containing rice protein concentrate to the control sample, when the dose was increased, the dough's gas-forming capability dropped by 8.3-20.8%[17]. Because fermentation was slowed down by a reduction in food availability, gas production was less intense in the dough containing rice protein concentrate. The dough containing 4-8% rice protein concentrate showed the 1st peak on the gas generation graph after 65 minutes, whereas the control sample showed the same peak after 60 minutes. The initial peak of gas generation was not well defined when 16% of the additive was added. The second gas generation peak for the control sample appeared 150 minutes later, while the 2nd peak for the rice protein concentrate samples appeared somewhat later and was not well defined. Infrared spectra revealed that samples containing rice protein concentrate had a reduced relative reflection coefficient for both fermented and kneaded dough. The amount of produced sugars was unaffected by the addition of 4 and 8% rice protein concentrate, whereas the addition of 16% decreased this indication by 1.6%. Fermented sugar content dropped by 3.0% to 7.8%.

rice is regarded as a useful option because of its hypoallergenic properties, adequate amino acid balance, and high protein content. Therefore, protein concentrates were obtained from plain (SR-4, K-39The protein concentrates from plain (SR-4, K-39), pigmented (*Zhag*), and aromatic (*Mushqbudij*) rice cultivars of Kashmir were studied. Significant difference ($p<0.05$) were observed in ash (2.90-3.50g/100g) and protein (70.40-74.23g/100g) content of protein concentrates obtained from different rice cultivars. The "L" value was lowest for Zhag protein (40.20). However, it displayed

the highest emulsifying activity (47.29 m^2/g) as well as emulsion stability (13.38 min) at neutral pH. It also had the highest foaming stability and protein solubility in acidic conditions. Zhag protein was observed to have the highest denaturation enthalpy (9.55 Jg^{-1}) and temperature (81.70° C). The protein powder displayed the amorphous behavior as no sharp peaks were observed in X-ray diffractogram. A positive correlation was observed between protein solubility at pH 8 and Emulsifying activity indices at pH 7[18].

3. Protein Modification Techniques

3.1 Ultrasound

This modification approach uses rarefaction and compression to transport waves across exposed material molecules. High ultrasonic wave intensities result in a cavitation effect because the rarefaction cycle outweighs the molecular forces in the medium. Plant-based proteins techno functional qualities can be improved by using ultrasound.

Some inventors used a physical-enzymatic combination approach (ultrasound-alcalase) to hydrolyze Rice Bran Protein (RBP)[19]. An alkaline extraction procedure assisted by ultrasound was used to extract the RBP, and the extract was then hydrolyzed. In addition, Alcalase hydrolysis was preceded by ultrasonication (pH 8.5, 10 min, 55° C). To run the ultrasonication, two factors were taken into consideration: Treatment period (10, 25, and 40 minutes) and power (50, 100, and 150 W). The findings indicated that as power and time were increased, the degree of hydrolysis (DH%) rose as well. Alcalase and ultrasonication (150W, 40 min) produced the hydrolysates with the greatest DH (27.35 ± 0.45%). The Bioactive Peptides (BAPs) exhibited the maximum 2,2-Diphenyl-1-Picrylhydrazyl free radical (DPPH) scavenging activity (86.55 ± 1.17%) and lipase inhibitory activity (57.57 ± 0.91%) under the ideal ultrasonication setting of 50 w-10 min. Using High-Performance Liquid Chromatography (HPLC), the mass distribution of the materials was examined. All produced hydrolysates had lower molecular weight proteins and peptides than non-hydrolyzed samples. The findings matched those of sodium dodecyl sulfate polyacrylamide gel electrophoresis and DH analysis. By altering the protein structure, ultrasonication increased the impact of enzyme hydrolysis. The structural analysis employing ultraviolet-visible spectrophotometry and Fourier-transform infrared spectroscopy verified this.

There was the assessment of the impact of ultrasonic immersion power, duration, and temperature on parboiled rice head rice yield, texture, and nutritional value[20]. In comparison to untreated black rice, proper ultrasound-assisted immersion might lower the levels of arsenic and cadmium by up to 61% and 79%, respectively, and improve the head rice yield by around 20% and the GABA content by up to 133%. Despite a certain loss of anthocyanins, it may also somewhat raise the concentration of free and bound polyphenols as well as important minerals like calcium, iron, and zinc. It could also make cooked rice more palatable. Additionally, the ideal parameters of ultrasound-assisted immersion (540 W, 45 min, 57 °C) were obtained and validated using response surface studies based on the Box-Behnken design. Accordingly, the better head rice yield, nutrition, and texture of parboiled black rice over untreated black rice may be due in part to morphological changes, namely the creation of new cracks inside the rice and the removal of cracks close to the aleurone layer.

Some researchers used the Ultrasonic (US) pretreatments (10, 20, and 50 minutes) and hot-air drying (50, 60, and 70° C) to lock down the nutritional composition of Selenium-enriched Germinated Black Rice (SeGBR) and to study drying kinetics, mathematical modelling, thermodynamics, microstructure, bioactive profile, and volatile compounds[21]. Compared to control samples, those treated with ultrasond showed a 20.5% shorter drying time. US-SeGBR had specific energy consumption ranging from 6.45 to 12.32 kWh/kg, which was less than untreated, and activation energy values ranging from 3.97 to 13.90 kJ/mol. Fifty-five volatile chemicals were identified and measured by the HS-SPME-GC-MS analysis. The volatile chemicals in the US-treated SeGBR were higher, which might encourage the release of additional flavoring compounds. High water absorption through several micro-cavities was demonstrated by the scanning electron microscopy of the US-treated samples.

The combined effects of germination (96 hours), steaming (100° C for 10 and 20 minutes), and ultrasonication (50 KHz for 5 and 7 minutes) applied progressively on the colour, physicochemical, phytochemical, and antioxidant characteristics of the

black rice variety *Chakhao Poireiton*[22]. The amylose content and total starch of the treated rice dropped considerably ($p < 0.05$), while the sediment volume rose. The samples with the highest treatment severity had the greatest effects on the values. Treatments resulted in a decrease in lightness and an increase in redness and yellowness, which demonstrated the effects of steaming. *Chakhao Poireiton* had a significant level of monomeric anthocyanins (40 mg/100 g), flavonoids (77.31 mg/100 g), and phenolics (488.16 mg/100 g). The most heavily treated sample exhibited high DPPH scavenging activity (90.40%) and a total phenolic content of 167 mg/100 g, or 34.2% of the raw rice value. In this sample, the content of GABA (γ-aminobutyric acid) increased significantly to 334.98 mg/100 g. The 4 samples that were treated displayed surface morphological alterations that are typical of the treatment.

3.2 Enzymatic Hydrolysis

Among the many advantages of enzymatic hydrolysis as a biological modification method are its ease of handling and its gentler reaction conditions. It includes the use of proteases on the protein substrate, which cleave the bonds between peptides to break down proteins into tiny peptides and amino acids. This has a favorable impact on how proteins are absorbed and function (emulsification, foaming, and stability). Pressure, temperature, pH, enzyme type, substrate characteristics, and the ratio of enzyme to substrate can all have an impact on the hydrolysis of plant proteins using this modification technique.

The physicochemical and antioxidant properties of the peptide fractions and protein hydrolysatewere assessed by the researcher[23]. Response Surface Methodology (RSM) has been widely utilized to hydrolyze black bean protein using bromelain. The ideal conditions for hydrolysis were 52° C, pH 7, an E/S ratio of 2.2 (the ratio of enzyme to substrate), and 4 hours. The hydrolysate (BPH) demonstrated Fe2+ chelating activity and DPPH radical scavenging activity under these conditions, with IC50 values of 100.08 ± 2.42 and 71.49 ± 0.81 μg/mL, respectively. This might be explained by structural features that change depending on the distribution of molecular weights. Remarkably, peptides smaller than 3 kDa were found to have the greatest DPPH and ABTS radical scavenging activity among BPH and its peptide components. More interestingly, this peptide fraction (<3 kDa) was able to primarily extend the sunflower oil induction duration, increasing it by 1.31 times. High amounts of hydrophobic amino acids might be the cause of this. The peptide fraction ranging from 3 to 10 kDa exhibited the best Fe^{2+} chelating activity, which was surprisingly strongly positive for both histidine and arginine.

A study assessed how Flavourzyme protease's enzymatic hydrolysis affected the rice bran proteins encapsulation qualities in an effort to preserve the anthocyanins in grape juice microparticles. The micro encapsulation of grape juice by spray drying was accomplished by combining maltodextrin with rice Bran Protein Hydrolysates (RPH) with low (5%, LRPH), medium (10%, MRPH), and high (15%, HRPH) degrees of hydrolysis (DH) as carrier agents[24]. The ratio of 1 g of Carrier Agents (CA) to g of Soluble Solids from the juice (SS) and protein in the feed solutions was 15%. According to X-ray photoelectron spectroscopy, protein modification enhanced the protein's surface activity and its capacity to migrate to the microparticles surface and create a protective coating. When HRPH was used as a carrier agent in conjunction with maltodextrin, the antioxidant capacity as determined by the DPPH and ABTS+ tests, internal and total anthocyanin retention, and powder recovery were all enhanced in comparison to the control sample. Additionally, higher DH decreased particle size and powder stickiness. These particles lacked breaks and were coarser and more homogeneous. The effectiveness of microencapsulation exceeded 70%. Every powder showed minimal levels of caking and hygroscopicity. Thus, enzymatic hydrolysis turns out to be a viable substitute for enhancing the encapsulating qualities of rice bran protein.

3.3 Protein Emulsion Gels

Rice is one of the most staple grains all over the globe. The protein present in rice has great nutritional value because of its high levels of lysine, which is a limiting amino acid lacking in various other sources of plant proteins. The rice protein is also hypoallergenic in nature.

It was examinedhow the Degree of Hydrolysis (DH) affected the Rice Bran Protein's (RBP) solubility, structure, and emulsifying qualities[25]. The structural features were analyzed using methods such as surface hydrophobicity, intrinsic fluorescence, Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

(SDS-PAGE), and Circular Dichroism (CD). Proteins can undergo hydrolysis to change their molecular weight, enhance their fluidity and flexibility, and modify surface hydrophobicity. Rice Bran Protein Hydrolysates (RBPH) with varying DH values (1%, 3%, and 6%) was used to create oil-in-water emulsions. Thermal treatment (30-90° C, 30 min), ionic strength (0-300 mM NaCl), and pH (3-9) were the environmental pressures used to test the stabilities of the RBPH-stabilized emulsions. Measurements of particle size and surface charge (ζ-potential) were made to assess the stability of the emulsion. In comparison to emulsions made using RBPH and other DH values, 3% DH RBP emulsions were significantly more stable, and this RBP may find use as an emulsifier in the food sector.

The rice protein concentrate displays a poor emulsifying property (emulsifying activity index of 1.15m^2/g), which in turn causes the low encapsulation efficiency (less than 1%). The ϒ (12.9-90.6%) can be enhanced by the addition of modified starch content. Increased content of modified starch resulted in particles with porous structure and fewer oil droplets on the surface. The moisture content was also low (less than 5.5%) with an average bulk density of 0.2113g/cm^3 and hygroscopicity less than 0.1065 kg/kg. The packed bulk density was 0.3178g/cm^3 and the particle mean size was 42.36 µm. It was also hypoallergenic in nature and rich in essential amino acids as well as polyunsaturated fatty acids[26].

The enzymatic hydrolysis was used to enhance the functional characteristics of rice protein from 4 different rice varieties: RRF-127, RRF-105, *Swarna*, and *Protezin*. In the pH range of 4-10, the alcalase hydrolysis enhanced protein solubility to >80%. As the pH rose, Rice Protein Hydrolysates (RPHs) stability and foaming ability improved[27]. The Emulsification Activity Index (EAI) in the pH range of 5 to 9 was considerably enhanced by the hydrolysis. The hydrolysates demonstrated metal ion chelation, reducing power, and ABTS+ radical scavenging as antioxidant activities. Among the hydrolysates, RRF-105 was shown to have the strongest antioxidant activity. RPH had concentration-dependent protective effects and was able to reduce oxidative damage in erythrocytes. The ACE and DPP-IV enzymes were more strongly inhibited by the hydrolysates. Swarna had the most efficient DPP-IV inhibition (66%) and Protezin had the greatest ACE inhibition (63%). Alcalase hydrolysis enhanced the rice proteins bioactive and functional characteristics.

The alkaline deamination of rice protein concentrate can be carried out to modify its structure. It results in the production of a whole protein fraction as well as a soluble fraction. Both of them show higher solubility and were able to structure canola oil into a high internal phase emulsion 75% w/w canola oil and 25% of protein fractions {2, 4, and 6% w/w}. The emulsion also displayed smaller particle size and improved rheological measurements[28].

4. Conclusion

Rice is giving enough food for almost 50% of the world. So it is vital to educate such people regarding the nutritional value and health benefits that rice offers to persuade them to make it a part of their daily diet. Along with nutritious substances including vitamins, amino acids, minerals, flavanoids, phenolic compounds, α-tocopherol, γ-tocopherol, and γ-oryzanols, black rice also has the unique trace elements usually only found in these food products. The bran of black rice contains anthocyanin pigments such as cyanidin-3-glucoside and peonidin-3-glucoside, which is why it is called black rice, and it is a high antioxidant source. Black rice is a gluten-free and high-fiber meal too. Black rice therefore offers a number of health benefits. That is why black rice can be promising beneficial effects against constipation, carcinogenesis, tumour, coronary heart disease, atherosclerosis, inflammations, nephrological problems, and also against Type 2 diabetes.

5. Author Contribution

Irom Bipin Kumar Singh wrote the original manuscript and performed investigation; Sarvesh Rustagi reviewed the manuscript and did the conceptualization. All authors have read and approved the final version of the manuscript.

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