Study on Proximate Composition of Rice (Oryza sativa) Bran and Chemical Properties with Fatty Acid Content of its' Extracted Oil Collected from the Northern Region of Bangladesh

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Highlights

- Rice bran of the northern region of Bangladesh is a prominent source of carbohydrates, proteins, and fats.
- The Bran oil is rich in important saturated and unsaturated fatty acids compared to other edible oils.
- Rice bran and bran oil of this area can be used as quality food resources for their nutritive standards.

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Abstract

Oryza sativa bran was analyzed for its proximate composition. The oil was extracted from the bran by the Soxhlet extraction method. Studies of some chemical properties and analysis of fatty acids by GC-MS of extracted oil were performed in this research work. The bran was collected from the Kurigram district of Bangladesh. The rice bran and the bran oil of this area are not much studied. The studies find out some significant properties and nutritional values of this area's rice bran and bran oil. The bran was rich in carbohydrates (38.78%), fats (20.30%), fiber (1.42%), and proteins (9.45%). Moisture and ash content were found 9.49% and 20.5% respectively. The important minerals were found by wet and dry tests like Fe, Zn, Ca, Mg, K, Na, etc. The higher saponification (110.13 mg KOH/g) value of the oil indicated that it could be used as raw materials in good quality soap. It was determined by refluxing the oil with alcoholic KOH solution followed by titration with standard acid. The low iodine (46.25 I_2/100g) value of the oil indicated that it could be used for cooking purposes and the oil was stable and consisted of polyunsaturated fatty acids mainly oleic and linoleic acid. From the fatty acid analysis by GC-MS, it was found that the oil had a high content of fatty acids such as oleic acid, linoleic acid, palmitic acid, stearic acid, etc. These acids have high biological importance for human health.

Keywords: Protein, Carbohydrate, Soxhlet, Minerals, Saponification, Fatty acids.

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Declarations and Statements

Ethical approval

Not applicable

Additional information

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Competing Interests

No, I declare that the authors have no competing interests, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

Author's contributions

Shabiba Parvin Shandhi the 1st and corresponding author has done all the lab works and writing, editing the manuscript related to the research work.

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Availability of data and materials

The different sets of data that have been generated and/or used and examined to write the present manuscript are available from the corresponding author upon reasonable request.

1. Introduction

The rice plant species *Oryza sativa* is from the family Gramineae (Grass family). *Oryza sativa* of the grass family Gramineae is extensively cultivated in warm climates, especially in East Asia producing seeds that are cooked and used as food. People in Asia and Africa consume it more than people in the European Union (Vlachos et al., 2008). Rice is cultivated in Bangladesh throughout the year. The rice kernel contains only a small amount of oil (2-3%), and this is found in the outer layers of the grain. From the processing of the paddy rice, 70% of endosperm (white rice) is obtained as the main product, and the rest are by-products (20% shell, 8% bran, and 2% germ).

Figure i: Rice Plant

Generally, the by-products are destined for animal feed; however, these could serve as raw materials for the production of oil and other products (pharmaceuticals, antioxidants, biofuels) (Krishna et al; 2003). Due to its chemical composition, rice bran is a unique raw material because of its high protein content and fiber, in addition to its oil content and natural antioxidant compounds (AL-Okbi et al.,2014; Lakkakula et al., 2004; Kong et al., 2015)

Rice is considered the queen among cereals because of its' nutritional quality and higher digestibility (Anjum et al., 2007). Rice bran contains protein, crude fiber, lipids, carbohydrates, oryzanols, phenolic compounds, polyunsaturated fatty acids, and monounsaturated fatty acids (Alauddin et al., 2019). Rice bran oil has a calorific value of approximately 41.1 MJ/kg and a high nutritional value, with 47% of its fats being monounsaturated, 33% polyunsaturated, and 20% saturated, making it an excellent option for nutraceutical applications (Gul et al., 2015; Sinha et al., 2008).

However, only a small portion of the total production of rice bran is used for edible oil production (Boulifi et al., 2013). This occurs as a consequence of cell disruption with the release of lipase enzymes and various other antinutritional factors (trypsin inhibitors, hemagglutinin-lectin, and phytates) when the bran layers are removed from the endosperm. The contact between such enzymes and oil causes its hydrolysis and the release of free fatty acids and glycerol, thus causing a drastic reduction in its quality and shelf life (Proctor et al., 1996; Amarasinghne et al., 2004). The degradation level of its oil can be so high that its usefulness for human or animal consumption is unsuitable, so its final destination can be as fuel for boilers. However, rice bran could be an excellent candidate for the development of other higher-added value products, in particular its oil. Nonedible rice bran oil has been used in products such as cosmetics, paints, soaps, and detergents.

2. Materials and Methods

2.1 Plant material

Bran of *Oryza sativa* was collected from a rice mill of Kurigram. The bran was air-dried and preserved in an air-tight container for investigation.

Figure ii: Rice Bran

2.2 Proximate Analysis

2.2.1 Determination of Moisture Content

The moisture content was determined by the oven-drying method. To determine the moisture content a crucible was taken and cleaned. Then the crucible was weighed and about 2.0g of bran was taken. After that, the crucible with bran was placed into an oven and heated at about 100ºC for three hours. Samples were then taken out from the oven and put in a desiccator with a partially covered lid for 30 minutes to allow for cooling to room temperature and then weighed.

(%) Moisture = (W1-W2/W1) x 100%

Where: W1 = weight (g) of sample before drying; and W2 = weight (g) of sample after drying

2.2.2 Determination of Ash Content

For ash content determination a crucible was taken and cleaned. Then the crucible was weighed and about 2g of bran was taken. Afterward, the crucible with bran was heated by burner at about 100ºC. The heat was applied continuously until the black color disappeared. Finally, the crucible was weighed again.

(%) Ash = (Weight of Ash/ Weight of Sample) x 100%

2.2.3 Determination of Fat

A 1000 mL round bottle flux was cleaned and washed. The bran (101.065g) was taken in a round bottom flask (1000 mL) and n-hexane (600 mL) was added to it. It was refluxed for an hour. The resulting mixture was cooled and filtered. The process was repeated twice. The extracted solution was then taken to a round bottom flask and concentrated into a rotary vacuum evaporator at a low temperature of (35-40 ºC).

2.2.4 Determination of Fiber

Exactly 2 g of the defatted bran was transferred into a 500 mL conical flask followed by 200 mL of boiled 1.25% H_2SO_4 solution. The mixture was boiled for 30 min under reflux. The acid-free residue was quantitatively transferred

into the refluxing flask followed by exactly 200 mL of 1.25% sodium hydroxide solution and refluxed for 30min. The digest was filtered, washed with boiling water, then alcohol, and lastly with diethyl ether before being dried at 100°C for 1 h. The dried residue was transferred into a porcelain crucible and incinerated for 1 h at 400-500°C using a muffle furnace. The crucibles were removed from the furnace left to cool in the desiccator and immediately transferred and weighed. Percentage fiber was calculated using the following equation:

(%) Fiber = (Weight of residues after oven drying/Weight of sample used) x 100%

2.2.5 Determination of Protein

The Macro Kjeldahl method was used to determine the nitrogen content (Protein) in the bran. The sample 0.4930g was weighed into a dry Kjeldahl flask. About 5g of digestion mixture and 20mL of pure conc. H_2SO_4 was added to the same sample and the mixture was digested by heating for 4 to 5hr. The contents of the Kjeldahl flask were cooled and diluted with distilled water and the mixture was made alkaline by adding 40% NaOH (about 75 mL). The ammonia liberated was distilled into a receiver containing 25mL of N/10 H_2SO_4. The excess of acid in the receiver was back titrated against N/10 NaOH using 3 drops of methyl red indicator.

Figure iii: Kjeldal (macro) method (digestion)

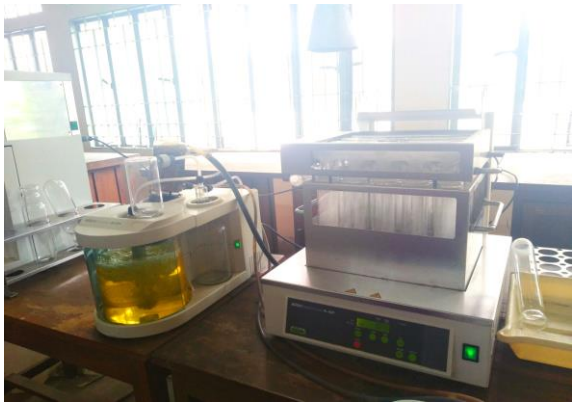

Figure iv: Kjeldal (macro) method distillation)

A reagent blank was similarly digested and distilled. This value was subtracted from the value obtained for the sample to get the true liter value "b". If "a" g of the sample was taken and if "b" and "c" mL of alkali of normality "d" are required for back-titration and to neutralize 25mL of N/10 H_2SO_4.

(%) Protein = [(c-b)*14 d*5.95/a*1000]*100

The amount of protein content was determined by multiplying the nitrogen content with a conversion factor of 5.95 which is comparable with the value 17% w/w reported previously.

2.2.6 Determination of Carbohydrates

The carbohydrate content was estimated by the difference method. It was calculated by subtracting the sum of the percentage of moisture, fat, protein, fiber, and ash contents from 100% as follows:

(%) Carbohydrates = 100% – [% crude protein + % fat content + % moisture content + % fiber + % ash]

2.3 Dry and Wet Tests

The presence of Minerals in ash was analyzed by qualitative inorganic analysis from the ash content of the bran through dry and wet tests.

2.4 Analysis of Fats (Oil)

2.4.1 Determination of Iodine Value

About 0.1027g of n-hexane extracted oil was dissolved in 10 mL carbon-tetrachloride solution and 10.00 mL Henus solution in a stopper bottle. The bottle was shaken occasionally for half an hour. Then 100 mL water and 10 mL 12% KI solution were added. Finally, the unreacted iodide was titrated with standard 0.1101M sodium thiosulfate solution.

2.4.2 Determination of Saponification Value

The saponification value is the number of milligrams of KOH required to completely saponify one gram of oil or fat. About 0.1512 g of n-hexane extracted oil was dissolved in excess standard alcoholic potash solution and refluxed the solution. The unused alkali was titrated with a standard 0.99 M HCl solution.

2.4.3 Analysis of Fatty Acids through GC-MS

Fatty acids were analyzed through the gas chromatography technique. n-Hexane extract (0.2 g) of bran was dissolved in n-hexane (50 mL) and extracted with 5% sodium bicarbonate solution (25mL× 2). The mixture was taken in a separatory funnel and shaken vigorously and allowed to stand overnight. Two layers were obtained. The lower layer (aqueous) was separated and taken to analyze free fatty acid (FFA). The upper layer was separated and taken to analyze bound fatty acids.

Figure v: Separation

Figure vi: Distillation

3. Result and discussion

3.1 Proximate composition

The nutritional value of rice bran constitutes carbohydrates, lipids, protein, fiber, moisture, and minerals respectively (Heredia- Olea et al., 2020). The proximate composition of the bran is shown in **Table ii,** which is compared with some previous studies (Manzoor et al., 2023; Faria et al., 2012; Park et al., 2017).

Table ii: Proximate composition of the Bangladeshi rice bran

Composition	Fats	Proteins	Carbohydrates	Fiber	Moisture content	Ash content
DW basis g/100g of rice bran	20.30%	9.45%	38.78%	1.42%	9.49%	20.56%

3.1.1 Moisture Content

The obtained moisture content of rice bran was 9.49% which is lower than the safe moisture content (14%) for the safe storage of processed rice (Brasil, 1988). The acceptable value is around 12% for long-term storage as well as to avoid insect attack and microbial growth (Adair et al., 1973; Cogburn, 1985). Moisture content invariably affects the quality and palatability of rice grains (Oko and Onyekwere, 2010), which plays a significant role in determining the shelf-life (Juliano, 1985).

3.1.2 Ash Content

The ash content of the rice bran in this study was 20.56%. Mineral element's presence in a food sample is reflected by the ash content of that sample (Bhat and Sridhar, 2008; Mbatchou and Dawda, 2013). The bran sample contains high ash content, so the probability of the mineral contents in this rice bran is high. The ash content differences in different rice brans may be due to the differences in the mineral content of the soils and the water used for irrigation (Shayo et al., 2006).

3.1.4 Fat Content

Through the Soxhlet extraction method, oil was extracted with n-hexane solvent. It was expressed as an n-hexane extract. The total percentage of it was 20.30%. The amount of oil present in the bran is quite good. In most cases, rice bran with high-fat content tends to be tastier and have less starch (Verma et al., 2017; Hirokadzu et al., 1979). The variations in fat value in different rice brans may be due to oxidation of fat.

3.1.5 Fiber Content

Fiber content was found 1.42%. Fiber possesses the ability to decrease blood cholesterol and sugar after meals in diabetics (Yeager, 1998). Fibre can reduce the risk of bowel disorders and fight against constipation (FAO/WHO, 1998). The presence of fiber in the diet increases the bulk of feces, which has a laxative effect on the gut (Mbatchou and Dawda, 2013)

3.1.6 Protein Content

On this rice bran, 9.45% protein was obtained. The result indicates its high protein content and can be employed as an important protein source. It can be used as a natural emulsifier in food because it can emulsify, jellify, and stabilize froth (Lee et al., 2004). Bran protein hydrolysates and extracts are used in several food products, including coffee whiteners, drinks, toppings, confectionery, bread, and meat, products, due to their emulsion qualities and solubility (Hamada et al., 1998; Jiamyangyuen et al., 2005; Watchararuji et al., 2008).

3.1.7 Carbohydrate Content

A high level of starch makes the individual grains stick to each other while low starch content prevents well from sticking of the grains together after cooking (Mbatchou and Dawda, 2013). The total carbohydrate content in this bran was found 38.78%. The bran has a good content of carbohydrates, so it can be used as a good source of carbohydrates.

3.2 Minerals

Results from different dry and wet tests reveal that- Ca, Na, K, Zn, and Fe ions were present. These minerals are very important for soil and also for animals.

3.3 Analysis of Extracted Oil

3.3.1 Saponification Value

The saponification value obtained in the rice bran was 110.13mg KOH/g. This indicates that the oil can be used in soap making. Saponification value indicates the nature of fatty acid constituents of the fat and thus depends on the average molecular weight of the fatty acid constituents. The smaller the molecular weight the greater the saponification value.

3.3.2 Iodine Value

The iodine value obtained in the rice bran was 46.25 I_2/100g (less than 100). That means the oil lacks unsaturation. Although the iodine value is used primarily in industry, it is of value to us because it indicates the oil's stability and health properties. The higher the iodine value, the greater the amount of unsaturation. The higher the iodine value, the less stable the oil and the more vulnerable it is to oxidation and free radical production. High iodine value oils are prone to oxidation and polymerization. During heating, such as when used in cooking, oils with a high iodine value readily oxidize and polymerize. Because they tended to harden when oxidized, polyunsaturated vegetables have been used extensively as bases for paints and varnishes. It makes a very safe cooking oil. These products of oxidation are associated with numerous health problems including cancer and atherosclerosis (hardening of the arteries)

3.3.3 Fatty Acids Analysis

A high percentage of the important unsaturated fatty acids e.g. oleic acid (BFA 35.69% & FFA 28.81%) and linoleic acid (BFA 15.95% & FFA 11.82%) respectively. Major saturated fatty acids present include palmitic acid (BFA 30.08% & FFA 47.76%). Another saturated fatty acid stearic acid was also obtained (BFA 5.12% & FFA 11.60%).

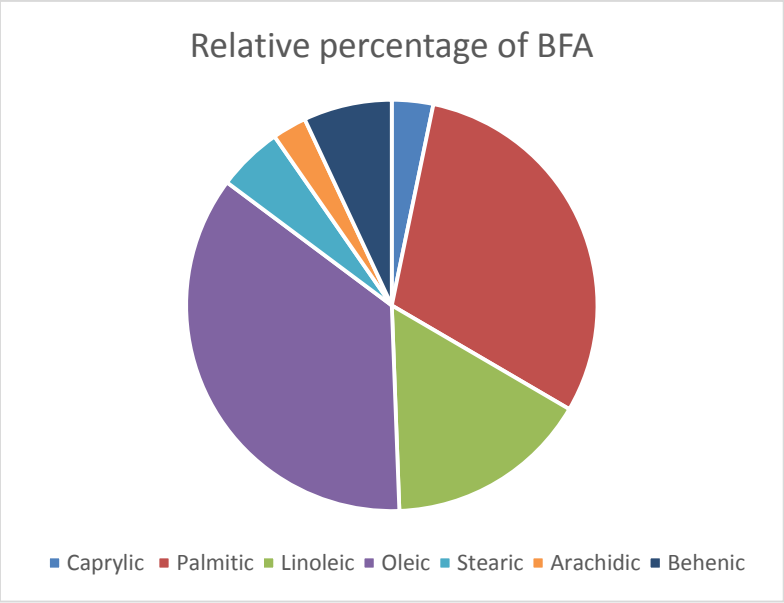

Figure vii: Relative percentage of BFA

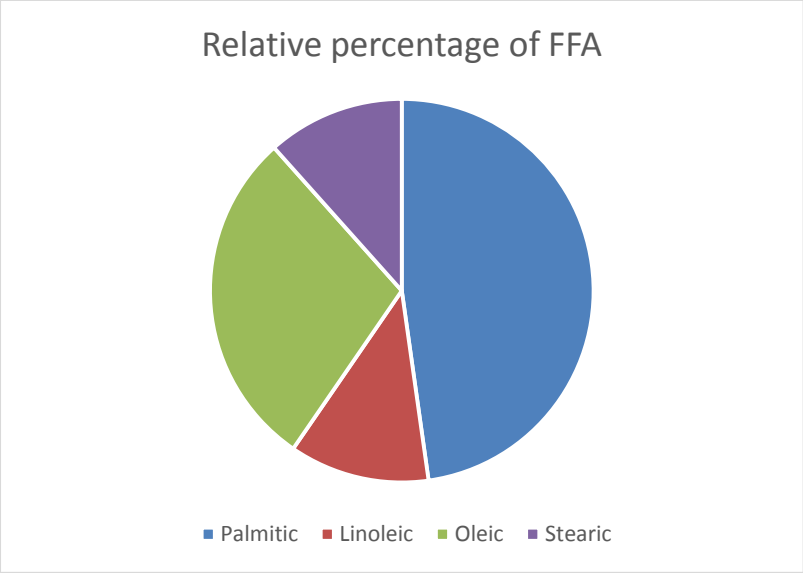

Figure viii: Relative percentage of FFA

This fatty acid profile of rice bran oil puts the oil at an advantage over conventional vegetable oils (e.g. palm oil) that are characterized by a high percentage of saturated fatty acids and therefore can pose health risks such as atherosclerosis, a disease associated with a heart attack. In contrast, the higher percentage of oleic acid obtained in rice bran oil (**35.69%**) in this work is higher than 24.7,7.4, 7.5, 22.9, 19.0, and 11.2% reported for palm oil, castor oil, coconut oil, cottonseed oil linseed, and melon seed oils respectively (Weast,1972-1973). The oil also contained a lower percentage of caprylic acid **(BFA 3.24%)** and arachidic acid **(BFA 2.69%),** behenic acid **(6.94%).** Oleic and linoleic acids are important essential fatty acids required for growth, physiological functions, and body maintenance.

4. Conclusion

From the research, it can be summarized that the rice bran of the northern region of Bangladesh has prominent nutritional value. Proximate composition analysis shows satisfactory results of moisture, ash, protein, fat, fiber, and carbohydrate content. The presence of important minerals is also seen. The extracted oil is also good quality. The higher saponification value of the oil indicated that it could be used as raw materials in good quality soap. The low iodine value of the oil indicated that it could be used for cooking purposes and the oil was stable and consisting of polyunsaturated fatty acids mainly oleic and linoleic acid. From the fatty acid analysis, it was found that the oil had a high content of fatty acids such as oleic acid, linoleic acid, palmitic acid, stearic acid, etc. The bran oil is rich in important fatty acids. These acids have high biological importance for the human body.

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