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Black rice flour: proximate composition, physicochemical properties and phytochemical screening in sequential extracts

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Abstract
Introduction: Black rice has been eaten throughout Asia for thousands of years and has a significant history of use in China, India and Thailand. In India black rice is grown in Manipur, Assam, west Bengal, Jharkhand and Orissa. Among which, Manipur grows about 10% of Black Rice of total cultivation are of rice.
Objective: To analyze the proximate composition, physicochemical properties and phytochemical screening in sequential extracts of BRF.
Materials & Method: Black rice flour collected from Imphal, Manipur, India was analyzed for proximate composition, physicochemical properties, phytochemical screening in sequential extracts (hexane, petroleum ether, chloroform, methanol, ethanol and water) and comparison of bulk density, water absorption, fat absorption, foam capacity, foam stability, degree of rehydration with brown rice and white rice.
Result & Conclusion: The BRF proximate composition was- Moisture- 6.37%, protein-2.275%, fat-3.7%, ash -4.92%. The physicochemical properties were compared with brown and white rice, where it was observed that the bulk density, water and fat absorption, emulsifying capacity were less than the brown and white rice. In sequential extracts, polyphenol content was high in water extract (0.568g/100g) followed by ethanol> Methanol > acetone> chloroform > Pet. Ether. Qualitative analysis of phytochemical using different extract showed rich source of triterpinoids, terpinoids followed by alkaloids and quinines. Sequential extract of different solvents was higher in water extract followed by ethanol, methanol, acetone, chloroform least amount of sequential extract seen in pet ether and hexane. Black Rice has a potential to be used as functional food ingredient due to its very low fat, salt, sugar, gluten, cholesterol and high protein as reported. There are limited studies reported on the food product development and functional properties. This data can be further utilized to find out the efficiency of Black Rice when used in functional food formulations.

Keywords: black rice, physicochemical properties, polyphenols, phytochemicals

Introduction
Black Rice is a type of the rice species Oryza sativa L. which is glutinous, packed with high level of nutrients and mainly cultivated in Asia. The pericarp (outer part) of kernel of this rice color is black due to a pigment known as anthocyanin, an antioxidant. Many people assume this rice as a panacea of many culinary diseases because of its high nutritive value and curative effect. This rice is supposed to enhance the longevity of life; hence it is also known as long life rice. This rice is free of gluten, free of cholesterol, low in sugar, salt and fat. Black rice is a whole grain, super nutritious type of rice that is high in fiber, anthocyanin, antioxidants, vitamins B and E, iron, thiamine, magnesium, niacin and phosphorous. (Shen *et al*. 2009) [1]
There are more than 200 types of black rice varieties in the world. Demand for this rice is this rice includes several varieties with a long history of cultivation in Southeast growing fast in the USA and European countries due to its value as a healthy food and its attractive organic food color (Kong *et al*. 2008) [2].
China cultivates maximum black rice followed by Sri Lanka, Indonesia, India and Philippines etc. Thailand occupies the ninth position to black rice cultivation Chinese black rice is a food, eaten frequently by many Chinese peoples and people groups, particularly those in and around the Yunnan Province where it is grown mostly. Black rice is grown about 10 per cent of the total cultivated rice area in Manipur. (Ichikawa *et al*. 2001; Sompong *et al*. 2011) [3, 4].
Black rice has long been consumed in Japan and China and is considered to be a healthy food because of its antioxidant content that are able to prevent oxidative stress. Oxidative modification of low-density lipoprotein (LDL) may play an important role in the development of atherosclerosis. This rice is claimed to be good for the kidney, stomach and liver in China Black rice has high nutritional value and it contains the highest levels of anthocyanin (Ling *et al*, 2001) [5]

Objectives
With this background the present study was carried with the objectives, to analyze the proximate composition, to screen phytochemical composition qualitatively in sequential extracts and analyze polyphenol content in sequential extract of Black Rice Flour

Materials and Methods
Chemicals
All chemicals were used for analytical grade.

Sample collection
Black Rice was purchased from agriculture farm of Imphal, Manipur, India. The black rice was powdered finely in a blender. It was packed in air tight pouches and stored in

ambient condition until further analysis.

Proximate composition (dry basis)
Moisture content was determined by using Moisture analyser (Metler Toledo MJ33, Lab systems Bangalore, India). Fat, protein, ash, total fiber (soluble and insoluble fiber) was estimated as per the AOAC standard methods. Iron, Calcium, Phosphorus, Copper, Manganese, Zinc, Sodium, Potassium, Magnesium and chromium were analyzed using Atomic Absorption spectroscopy (ELICO SL-168, range 160-900nm).

Phytochemical composition (dry basis)
Total phenols were extracted from a weighed portion (50-500 mg) of dried sample with 5ml of 1.2M HCl in 50% aqueous methanol for 2 h and analyzed by Folin-Ciocalteu micro method. Results are expressed as µmol Gallic acid equivalent g-1 dry weight (Reddy & Urooj, 2011)

Sequential extraction and of phytochemical screening in in Black Rice Powder
Sequential extraction of phytochemicals in Black Rice Powder *Black Rice* was evaluated as per the method of Faraz Mojab phytochemicals alkaloids, flavonoids, saponins, tannins and phenolic compounds, Terpinoids,, steroids and triterpenoids, quinones, glycosides, coumarins, steroids and phytosteroids, phlobalamins, anthraquinones (Mojab *et al*, 2003) [7].

physicochemical properties: Bulk density, Water absorption capacity, Fat absorption capacity, foam capacity and foam stability, Emulsification capacity
Weigh 1g of sample. Add 25ml of water. Blend in a mixer for 30 secs at low speed. Add5ml refined oil and blend again for 1min. observe for formation of emulsion. Continue addition of 5ml oil and blending for 1min till separation of oil is observed. Record ml of oil which can be emulsified per g of sample. Repeat twice more using the last but one measure of oil with increment of 1ml. for confirmation. (Beuchal *et al*, 1975)

Results and Discussion
The results of the present work in Black Rice flour are discussed as follows:

Proximate composition
The proximate composition of black rice is presented in fig.no.1. Moisture was 6.37% as the black rice is exposed to dry heating during processing. Black rice had protein content of about 2.275%. The fat content of black rice was (3.7%). The ash content was found to be 4.92% shown in

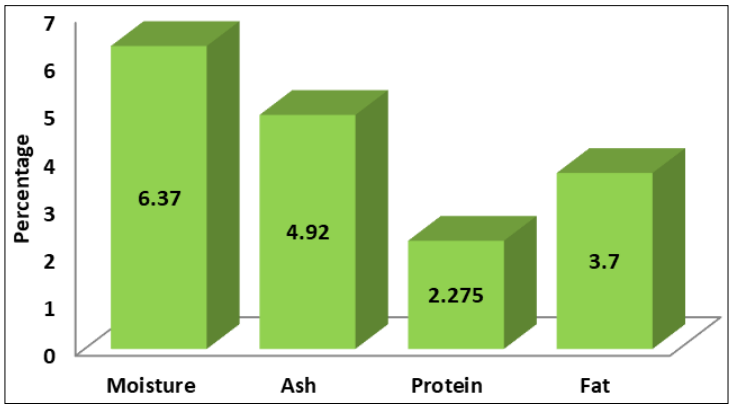

Fig 1: Proximate composition of black rice

Mineral composition
The composition of selected minerals in black rice flour ash solution was assessed by Atomic Absorption Spectrometry (AAS). Among the minerals analyzed phosphorous was found in higher quantity followed by potassium, calcium, zinc, and sodium. Iron, copper and cobalt were seen in very low amounts (table no.1)

Table 1: Mineral composition of the Black rice flour

SL. No.	Minerals	In mg/l
1	Calcium	12.94
2	Phosphorus	196.49
3	Iron	0.813
4	Sodium	1.00
5	Zinc	1.308
6	Potassium	119.39
7	Cobalt	BDL (DL 0.01)
8	Copper	0.154

BDL: Below Detection Limit, DL: Detection Limit

Physicochemical properties
The physicochemical properties of Black rice flour were compared with brown rice and white rice, shown in Table no. 2. The Bulk density, water and fat absorption, foaming capacity and emulsifying capacity of the samples were in the order of white rice > Brown rice> Black rice. Although the BRF, physicochemical properties were less than the white rice and Brown rice, the values were comparable. The properties were was less than both brown and white rice whereas, water absorption capacity was less than white rice and higher than brown rice is shown in table no 2 and fig. no. 2a-2e.

Table 2: Comparison of Functional properties of Black Rice with Brown and White Rice

Characteristic	Black rice	Brown rice	White rice
Bulk density(gm/100ml)	68 ±0	83 ±0	86±0
Water absorption capacity (ml/100)	120±0	110±0	150±0
Fat absorption(gm/100ml)	120±0	200±0	200±0
Foam formation (%)	5±0	7±0	10±0
Emulsification capacity(ml/g)	2±0	3±0	3±0

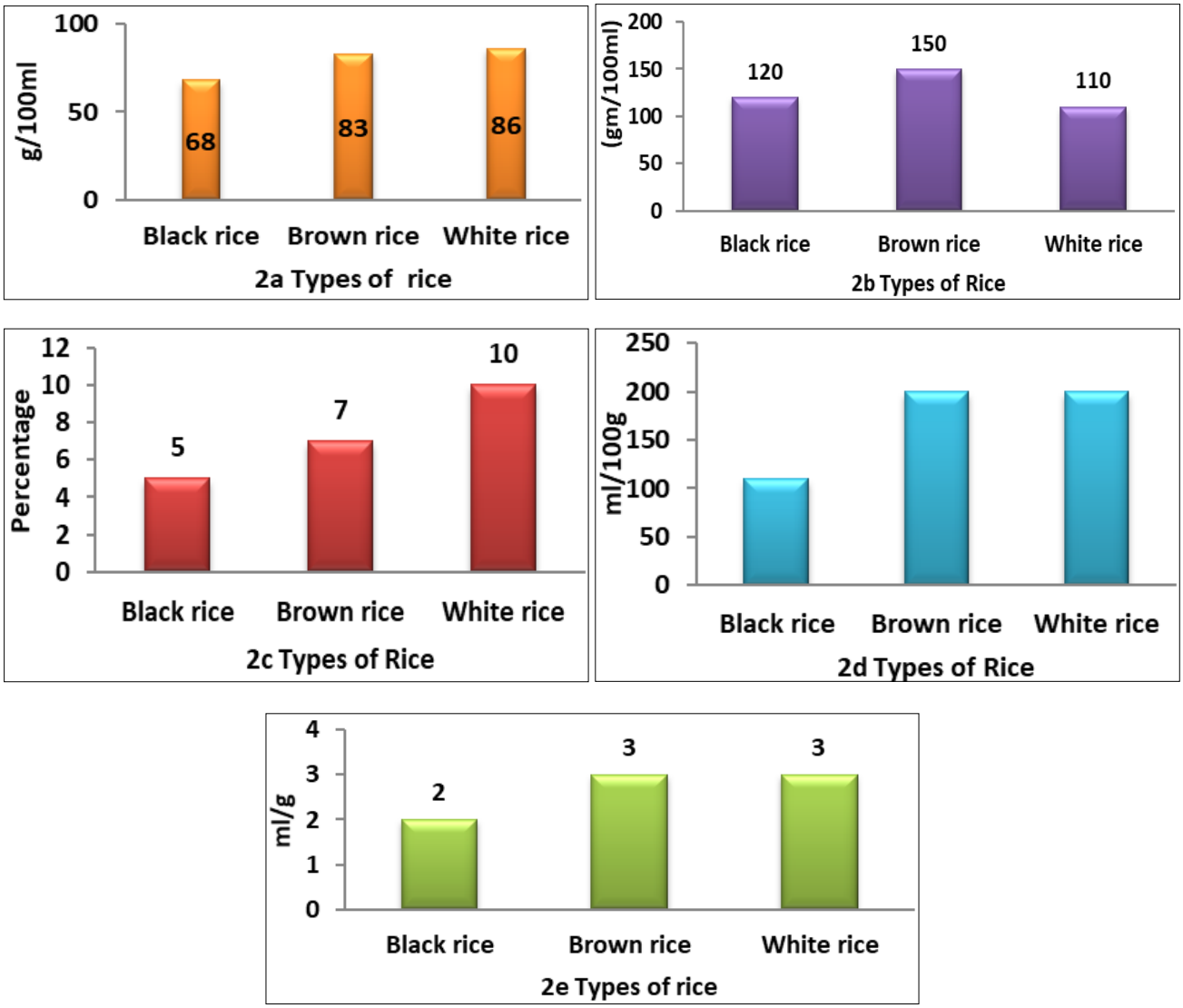

Fig 2a: Bulk density; 2b-Water absorption capacity, 2c- Foam formation; 2d- Fat absorption capacity; 2e- Emulsification capacity of the black rice in comparison to brown & white rice flour.

Table 3: Degree of rehydration, water solubility and comparative evaluation physicochemical properties.

	Degree of rehydration (%)	Water solubility (%)	Comparative evaluation (%)	
			WAI	WSI
Black rice	106	0.07	2.66	266
Brown rice	103	9.16	2.46	246
White rice	131	8.2	2.425	242.5

Polyphenol content in sequential extracts of black rice flour

The content of Sequential yield was more in water extract followed by ethanol, methanol, acetone, chloroform and less sequential yield seen in petroleum ether and hexane (fig. no. 4)

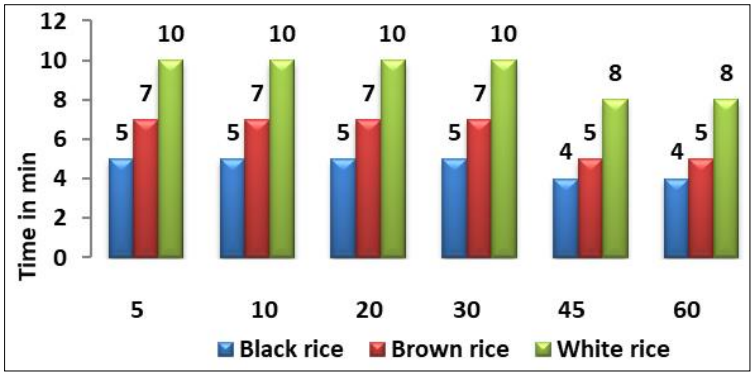

Fig 3: Foam stability of different types of rice

Phytochemical composition in sequential extracts

The qualitative analysis of Black Rice revealed the following data as shown in table no 4a and 4b. Black rice found to be rich in terpinoids, triterpinoids and quinones. Petroleum ether, ethanol, acetone were found to be rich in terpinoids and triterpinoids. Alkaloids are found in all the solvents except hexane. Steroids and phytosteroids are found in hexane, petroleum ether and chloroform. Cardiac glycosides, coumarins, tannins, phenolic compounds and phlobalamins are seen in lesser amount followed by saponins. Flavonoids, glycosides and anthraquinones are not found in respective solvents. Overall petroleum ether, hexane, chloroform and acetone extracts seemed to be the preferred solvents for extraction as evidenced by presence of most of the antioxidants found in black rice.

Table 4a: Qualitative analysis of phytochemical screening of Black Rice

solvent	Alkaloids		Flavonoids	Saponins	Tannins and phenolic compound	Terpinoids	Steroids
	Mayer's test	Wagner's test					
Hexane	-	-	-	-	+	+	+++
Pet. Ether	++	+	-	-	-	+++	+++
Chloroform	+	+	-	-	-	++	++
Acetone	+	+	-	+	-	+	-
Methanol	++	+	-	-	-	-	-
Ethanol	++	++	-	-	-	+++	-
Water	+	++	-	-	+	++	-

*Sample taken for -1mg/ml solvent

Table 4b: Qualitative analysis of phytochemical screening of Black Rice

Sample	Triterpinoids	Quinones	Glycosides	Cardiac glycosides	Coumarins	Phytosteroids	Phlobalamins	Anthraquinones
Hexane	++	+	-	-	+	+++	-	-
Pet. Ether	++	+	-	++	+	+++	-	-
Chloroform	-	+	-	+	-	++	-	-
Acetone	+++	+	-	-	+	-	-	-
Methanol	++	-	-	-	-	-	++	-
Ethanol	++	+	-	-	-	-	-	-
Water	+	+	-	-	-	-	-	-

Discussion

Moisture content plays a vital role in regulating the shelf life of a food. The ash content was high in black rice (4.92%). The amount of ash content indicates the levels of minerals present in the food sample. The protein content of black rice reported in another study was high compared to present result. (Kang MY *et al* 2011) [9]. According to Lee *et al* (2006) study the major fatty acids content in the black rice were oleic, linoleic and palmitic acids which was accounted for total 90% of fatty acids. The study conducted by Thomas R *et al* 2013 the fat content of black rice was 0.7% but in the present study the fat content was 3.7%. The difference in fat content may be due to the cultivation, regional difference or laboratory errors (Kang MY *et al* 2011) [9]. Phosphorus and potassium are found in higher quantity. Cobalt and copper are seen in lower quantities. Reported mineral content of brown rice was zinc (0.6mg-2.8mg/100g), calcium (10-15mg/100g), phosphorous (0.17-0.43g/100g) Mineral contents of black rice were found to be less when compared to brown rice (Juliano BO and Hicks PA 1996) [11]. Although few studies have been conducted on proximate composition of BRF some variations in ash, protein and fat was observed which may be due to the geographical variation or varietal difference.

The physicochemical properties of Black, brown and white rice flour analyzed. The bulk density of black rice was less when compared to brown and white rice which will be an advantage in the preparation of weaning food formulation. Water absorption capacity is low in brown rice when compared to black and white rice. Fat absorption capacity, foam formation and emulsification capacity of black rice is low when compared to brown and white rice. The bulk density and water uptake ratio was varied depending on rice variety. Black rice water absorption uptake was moderate when compared to white rice, brown rice. High water absorption uptake can contribute to longer cooking time and not beneficial to fuel consumption. Foam capacity and foam stability is low in black rice due to less protein content. Fat absorption capacity is less due to the decrease in the availability of amino acids by masking the non-polar residues from the interior protein molecules. Emulsification capacity is increased by the quality

The polyphenols were present at some quantity in all the solvent extracts, however maximum concentration was observed in water due to high polarity. As per the literature the best solvent for extracting the pigments and phenolic compounds is water followed by ethanol, methanol, acetone and chloroform. In case of sequential extracts, all the phytochemicals viz were present at some concentration as the components screened were soluble in either polar or non-polar solvents (Laware 2015) [12].

Conclusion

The Black rice is a type of rice species *Oryza sativa L* highly nutrient dense. Black color of this is due to anthocyanin an important antioxidant help to protect arteries and DNA damage. It is mainly cultivated in Asian countries (Ichikawa *et al* 2001) [3]. Antioxidant activity is twofold stronger with respective to antioxidant activities of blue berries this can be utilized as a functional food with high antioxidant an low fat sugar salt fat gluten and cholesterol. Black rice can be used in desserts or dressing or condiment because the black color turns to shiny indigo or purple when cooked (Smolkova K. Black Rice–Super food for any dish). The black rice needs traditional knowledge and awareness as a unique food source. It has wide varieties of therapeutic

benefits as reported. It has a potential to be used as a functional ingredient owing to its very low fat, salt, sugar, gluten, cholesterol and high protein as reported in studies. There are limited studies reported on the food product development and functional properties. Data on nutritional composition, therapeutic effects and antioxidant activity of black rice are essential. This data can be further utilized to find out the efficiency of black rice when used in food system. Evidence reveals the potency of black rice to be utilized as a part of the food system and also as a functional ingredient owing to its strong therapeutic nutritional value.

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