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Characterizations of different varieties of rice bran samples available in West Bengal, India for their proximate composition, oil composition and bioactive compounds present in oil

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Rice is available in different varieties in West Bengal. Rice bran is obtained in ample amounts during milling of rice. Now-a-days oil is generally extracted from the discarded rice bran which is also known to be rich in different nutrients. Therefore the aim of our study is to compare the proximate composition, oil composition and different bioactive compounds present in the oil of the twelve different varieties of rice bran available in West Bengal, India. The proximate composition of rice bran samples were in the order of moisture (10–12%), protein (8–12%), ash (3–6%) and fat (12–15%). Different types of trace elements are present in all varieties of rice bran. The fatty acid composition of rice bran oil showed that all the varieties of oil contain appreciable amounts of polyunsaturated fatty acids. All the varieties of oil extracted from rice bran have been shown to possess different types of phytosterols and oryzanol.

Keywords: Rice bran, rice bran oil, oryzanol, proximate composition, fatty acid composition.

Introduction

For many years human beings are enjoying grain foods specially cereals which are dietary staples for many populations worldwide and India too is no exception. Among the cereals rice (*Oryza sativa*) is the staple food for two-thirds of the world's population. In response to the expected world population growth, the International Rice Research Institute predicted that 800 million tons of rice will be required in 2025. As worldwide rice production currently is less than the population growth rate, a significant consumption of different varieties, genetically modified high yield rice is expected.

Rice bran is the cuticle existing between the rice and the husk of the paddy. Much of the nutritional value of rice lies in its germ and bran which is normally separated during milling process. Thus rice bran is one of the valuable by-products of rice processing industries. Rice bran is rich in essential nutrients like oryzanols, tocopherols, tocotrienols and phytosterols which are known to be potent antioxidants. The composition of rice bran also includes 20% oil, 15% protein, 50% carbohydrates and dietary fibres like pectin, beta-glucan and gum[1,2]. Previously rice bran was used as fertilizer or animal feed due to lack of knowledge about its importance, but now-a-days it is used mainly for extraction of oil namely rice bran oil (RBO). The major acids in RBO are palmitic (12±28%, typically 20%), oleic (35±50%, typically 42%) and linoleic acid (29±45%, typically 32%). The oil contains phospholipids (~5%), a wax which may be removed for industrial use and unsaponifiable material. It also contains phytosterols, triterpene, tocols and oryzanol. Bran obtained from different varieties of rice may vary in different components. Both glyceride and non-glyceride components present in the bran have nutritional aspects. Modification or processing may render them more valuable. Detailed characterization along with different agronomic data may provide us more useful data for further study in future.

To meet up the ever growing demand of rice, several type of high yielding, iron rich, arsenic resistant, genetically modified hybrid rice have been made by the scientists. Bran samples were collected from some of such varieties from Rice Research Station (Chinsura, West Bengal) and local Rice mills. Among those were IR64, IET 1029, IET 4094, Miniket (high yielding), Swarna P07, and CN 1646-6 (Aro-

matic high yield variety), CN 1794-2 and CN 1646-2 (Arsenic resistant) and Gobindobhog, Chapakhushi, Seetabhog, Jayasilet (iron and zinc rich) which were collected and studied with respect to their proximate composition, ultimate composition, trace elements content, fatty acid profile of oil extracted from these brans, phytosterols and higher alcohol content in unsaponifiable matters and oryzanol content. Indica rice variety IR64 is a genetically modified rice and was transformed with three genes, namely *psy* (phytoene synthase), *crtI* (phytoene desaturase), and *lcy* (lycopene cyclase), that are involved in the biosynthetic pathway of Beta-carotene in the endosperm[3]. This is an International check varities. IET 1029, IET 4094, Miniket are high yielding variety cultivated in West Bengal. Gobindobhog, Seetabhog are indigenous non-basmati aromatic rice from West Bengal[4]. Chapakhushi, Seetabhog, Jayasilet are three iron-enriched varieties collected from RRS, Chinsura, West Bengal. Previously a study was conducted to check the effect of these iron-enriched rice varieties upon consumption on 6 persons who were suffering from iron-deficiency in Dharamshala, India. From the study it was revealed that a significant improvement in blood iron level was achieved after consumption of this iron-enriched rice in these persons[5].

Characterization of oils and fats has mainly been focused on the principal components, which constitute the saponifiable fraction and amounts about 95% of the total. However, it is now generally recognized that the minor components, which are generally present in the unsaponifiable (unsap) matter, have important bioactive, nutritional, and characteristic compositional properties that affect the quality of individual oils and fats. The unsaponifiable matters present in vegetable oils and fats are usually composed of sterols, fatty alcohols, tocopherols, triterpene alcohols, and hydrocarbon (squalene) which have individual biological importance. Some of the above constituents may exert a pronounced effect on stability of the oils and their nutritional properties. The defatted meals are usually sent to poultry or cattle feed.

The present study aims to determine and compare the different compositions of the different varieties of rice bran available in West Bengal, India and also to compare the fatty acid composition and composition of unsaponifiable matter of RBO obtained from the different varieties of rice bran. The conventional thin layer chromatography (TLC), gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS) were adopted for compositional analysis.

Experimental

Chemicals:

HPLC grade methanol, acetonitrile, hexane, ethyl acetate and ethanol were purchased from Merck India. γ-Oryzanol standard was purchased from Merck, India. All chemicals and reagents were of analytical grade.

Rice bran samples:

Bran samples were collected from Rice Research Station, Chinsura, West Bengal, India and from local rice mills. Total twelve number of bran samples were studied like Gobindobhog, Seetabhog, CN 1794-2, CN 1646-6, CN 1646-2, Chapakhusi, Jayasilett, IR-64, IET 4094, IET 1029, Miniket and Swarna P07.

Preparation of rice bran samples:

Rice bran samples were stabilized by heating in a Microwave oven taken in microwave safe bags, followed by heating for 3 min at 120°C in a pre-heated oven and cooled down at room temperature[6]. Then the samples were kept at –20°C until analyses started.

Proximate analysis of rice bran samples:

Proximate components of rice bran samples were analyzed following AOAC methods[7].

Analysis of trace elements in rice bran samples:

Samples were prepared by microwave digestion in Anton Parr Microwave Digester. Trace elements were analyzed in all the twelve rice bran samples using ICP-OES (Perkin-Elmer Optima 7000).

Extraction of RBO:

Extraction of oil was carried out using Soxhlet method. Rice bran was placed in a thimble with hexane as solvent in Soxhlet extractor and extracted for 4 h. The oils were collected after evaporation of n-hexane using rotary evaporator at 40°C and stored at –20°C.

Determination of fatty acid composition:

Analysis of methyl esters of corresponding fatty acids (FAME) was carried out using Gas Chromatograph (GC : Agilent 6890) to determine the relative amount of fatty acids present in the oil[8,9].

Extraction of unsaponifiable matter from extracted oils:

Unsaponifiable matters were extracted from the oils by standard AOCS method[10]. Preparative TLC was done with the unsaponifiable matters for fractionation and qualitative analysis was done by GC/MS after extracting them from TLC plates[11].

GC/MS analysis:

The GC/MS analysis was carried out using Varian CP-3800 GC couples with Varian Saturn 2200 MS under electron impact ionization of 70 eV. Full scans were performed in 40–650 AMU mass range using chromatographic column with fused silica WCOT capillary column (30 m × 2.5 mm id; VF – 5MS, 0.25 mm). The GC method followed was: Injector temperature at 250°C in splitless mode. Helium was used as carrier gas maintaining a flow of 1 ml/min. Column oven temperature program was started at 75°C for 1.5 min to 200°C at 10°C/min; followed by 280°C at 10°C/min with 20 min hold time. Identification had been done of different components based on NIST mass spectral library.

Determination of γ-Oryzanol:

RBO contains Oryzanol which is a group of ferulic acid esters triterpene alcohols. These esters are bioactive compounds having antioxidant property and also have physiological and biological effects. Freshly extracted oils (approx. 0.1 g) were dissolved in 20 ml of petroleum ether and absorbance was measured at 315 nm using UV/Vis spectrophotometer (Perkin-Elmer Lamda 25).

Statistical analysis:

All the data are presented as means with their standard errors. Statistical comparisons between groups were performed using one way ANOVA.

Results and discussion

Proximate composition of rice bran samples:

Proximate composition analysis included moisture content, crude protein, crude fat, ash, silica and volatile matter as illustrated in Table 1. The proximate composition of rice bran samples were in the order of moisture (10–12%), protein (8–12%), ash (3–6%) and fat (12–15%). The moisture content was found to be highest in IET 1029 variety (11.92%) and lowest in Jaya Silet variety (10.78) which proved that the shelf life of IET 1029 variety was best and Jaya Silet variety was least. The crude protein content was found to be highest in Sheeta Bhog variety (11.66%) and lowest in IET 1029 variety (8.93%). The crude fat content was seen to be highest in IET 1029 (14.74%) and lowest in Chapa Khusi variety (12.14%). Proximate composition varies from study to study as there are several factors upon which plant composition depends. Such factors may be genetics, environment, soil type, type of fertilizer used, climate, microbial type and load present in soil, cultivation practices, post-harvest practices, handling and storage etc.[12,13].

Table 1. Proximate composition of rice bran samples

Sl. no.	Bran samples	Moisture (% w/w)	Ash (% w/w)	Crude protein (% w/w)	Crude fat (% w/w)
1.	Gobindo Bhog	10.85±0.11	5.47±0.06	11.47±0.43	13.57±0.36
2.	CN 1794-2	11.36±0.16	3.44±0.09	10.37±0.25	14.24±0.20
3.	CN 1646-6	11.75±0.11	3.98±0.11	10.60±0.20	14.22±0.13
4.	Chapa Khushi	10.92±0.09	4.20±0.09	11.33±0.14	12.28±0.17
5.	CN 1646-2	11.16±0.10	4.08±0.06	10.75±0.35	14.34±0.09
6.	Sheeta Bhog	11.22±0.12	5.26±0.06	11.34±0.30	13.17±0.06
7.	Jaya Silet	10.78±0.08	5.26±0.06	11.16±0.46	13.77±0.09
8.	IET 1029	11.92±0.06	5.30±0.04	8.96±0.24	14.63±0.10
9.	IET 4094	11.65±0.12	4.71±0.04	9.59±0.15	13.33±0.03
10.	IR-64	11.22±0.10	4.07±0.06	10.53±0.18	13.80±0.21
11.	Miniket	10.96±0.09	5.12±0.04	10.12±0.12	12.94±0.19
12.	Swarna	11.82±0.10	4.13±0.02	9.21±0.18	12.72±0.09

Values are Mean±S.D.

Analysis of trace elements in rice bran samples:

Digestion was done of the rice bran samples using an Anton Parr microwave digester. Analysis was carried out in Perkin-Elmer Optima 7000 ICP-OES for calcium, zinc, chromium, sodium, potassium, phosphorus, manganese, iron and arsenic. There was no significant difference between the trace element contents in different varieties of rice bran samples as depicted in Table 2.

Fatty acid profile of extracted RBOs:

RBO is mainly composed of saturated fatty acids like palmitic acid (around 22%) and stearic acid (around 3%), and unsaturated fatty acids like oleic acid (around 38%), linoleic acid (around 34%) and α-linolenic acid (around 2%). Saturated fatty acids like arachidic acid (20:0) and behenic acid (22:0) are also found in trace amounts in RBOs. Fatty acid profiles of the twelve RBO samples are shown in Table 3. Palmitic acid content varies from 16–30%, stearic acid var-

Table 2. Amount of trace elements in rice bran samples

Sl. no.	Bran samples	Ca (ppm)	Zn (ppm)	Cr (ppm)	Na (ppm)	Fe (ppm)	As (ppm)
1.	Gobindo Bhog	122.5±0.6	5.8±0.3	0.4±0.02	14.0±0.2	44.0±0.4	0.5±0.01
2.	CN 1794-2	108.6±0.7	6.0±0.5	0.8±0.01	15.0±0.5	32.0±0.1	0.5±0.01
3.	CN 1646-6	116.2±0.2	7.0±1.1	0.7±0.01	12.0±0.6	28.0±0.1	0.5±0.01
4.	Chapa Khushi	98.4±1.8	8.3±0.1	2.2±0.03	15.0±0.2	44.0±0.1	1.0±0.01
5.	CN 1646-2	107.3±0.7	6.4±0.1	0.6±0.01	22.0±0.4	36.0±0.4	1.0±0.02
6.	Sheeta Bhog	110.4±0.5	5.0±0.1	0.8±0.01	15.0±0.6	46.0±0.1	0.5±0.01
7.	Jaya Silet	121.8±0.4	6.0±0.4	0.6±0.01	13.0±0.2	42.0±0.1	0.5±0.02
8.	IET 1029	104.0±0.2	6.0±0.1	2.0±0.02	15.0±0.3	31.0±0.2	1.0±0.01
9.	IET 4094	131.0±1.5	7.0±0.1	0.8±0.01	14.0±0.2	25.0±0.2	1.0±0.03
10.	IR-64	129.0±3.5	6.0±0.7	0.6±0.01	20.0±0.3	31.0±0.1	1.0±0.01
11.	Miniket	140.0±1.5	8.0±0.3	4.0±0.01	15.0±0.5	33.0±0.3	0.5±0.01
12.	Swarna	93.0±0.8	5.0±0.3	0.6±0.01	12.0±0.6	42.0±0.1	0.5±0.01

Values are Mean±S.D.

Table 3. Fatty acid profile of rice bran oil extracted from different rice bran samples

Fatty acids (% w/w) → / Rice varieties ↓	C16:0	C18:0	C18:1	C18:2	C18:3
Gobindo Bhog	21.64±0.83	2.06±0.09	35.94±0.06	24.44±0.31	1.67±0.07
CN 1794-2	23.99±0.45	3.08±0.04	34.25±0.08	22.51±0.18	1.48±0.06
CN 1646-6	23.13±0.12	3.08±0.12	38.22±0.07	30.43±0.23	2.05±0.09
Chapa Khushi	29.22±0.47	3.15±0.04	32.64±0.23	32.36±0.25	1.25±0.05
CN 1646-2	30.32±0.17	3.06±0.06	33.33±0.19	27.51±0.31	1.98±0.09
Sheeta Bhog	20.35±0.11	1.24±0.05	32.05±0.06	22.60±0.11	1.46±0.06
Jaya Silet	22.22±0.12	0.94±0.09	37.46±0.06	22.10±0.12	1.38±0.07
IET 1029	26.14±0.04	1.91±0.03	32.40±0.28	23.13±0.11	1.42±0.06
IET 4094	23.45±0.08	3.04±0.04	33.25±0.22	20.87±0.12	1.45±0.09
IR-64	25.45±0.27	2.34±0.04	34.01±0.12	23.92±0.18	1.18±0.04
Miniket	27.64±0.14	2.85±0.05	38.98±0.18	23.72±0.09	1.23±0.05
Swarna	16.40±0.20	1.41±0.04	26.27±0.11	19.80±0.31	1.11±0.10

Values are Mean±S.D.

ies in the range of 1.2–3%, oleic acid was present in the range of 26–39%, linoleic acid varies from 20–30% and α-linolenic acid was present in the range of 1–2%. There was not much difference in the fatty acid profile of the 12 varieties of rice bran. However Chapa Khushi variety showed to possess highest amount of saturated fatty acids while CN 1646-6 variety showed to possess highest amount of polyunsaturated fatty acids in comparison to the other varieties.

Unsaponifiable matter content and Oryzanol content in extracted oils:

RBO usually contains 1–2% oryzanol. Oryzanol content depends on various factors like genotype and degree of ripening of rice, degree of milling, storage etc. Table 4 shows that the oryzanol content and amount of unsaponifiable matters in the different varieties of bran samples. This study shows that oryzanol content varies between 0.8 to 1.46% in different varieties of bran samples with the highest amount in IET 4094.

Table 4. Amount of bioactive components in extracted rice bran oils

Sl. no.	Bran samples	Unsaponifiable matter content (% w/w)	Oryzanol content (% w/w)
1.	Gobindo Bhog	2.73±0.02	1.24±0.02
2.	CN 1794-2	2.11±0.06	1.15±0.01
3.	CN 1646-6	2.11±0.02	0.98±0.05
4.	Chapa Khushi	2.65±0.08	1.14±0.02
5.	CN 1646-2	3.05±0.03	0.81±0.05
6.	Sheeta Bhog	2.69±0.12	1.24±0.02
7.	Jaya Silet	3.14±0.02	1.12±0.02
8.	IET 1029	3.58±0.08	1.22±0.12
9.	IET 4094	3.92±0.54	1.43±0.01
10.	IR-64	2.46±0.25	1.06±0.08
11.	Miniket	2.84±0.11	1.30±0.02
12.	Swarna	3.41±0.13	1.21±0.01

Values are Mean±S.D.

Table 5. Composition of unsaponifiable matters obtained from different rice bran oil samples

Sl. no.	Name of sterol	r/t (min)	Gobino Bhog	CN 1794-2	CN 1646-6	Chapa Khushi	CN 1646-2	Seeta Bhog	Jaya Silet	Swarna P07	Miniket	IR-64	4094	1029
1.	1-Hexadecanol	8.5	√	√	√	√	√	√	√	√	√	√	√	√
2.	Squalene	18.41	√	√	√	√	√	√	√	√	√	√	√	√
3.	Campesterol	25.85	√	√	√	√	√	√	√	√	√	√	√	√
4.	Ergostanol	26.2	×	√	√	√	√	√	×	×	√	√	×	√
5.	Stigmasterol	26.54	√	√	√	√	√	√	√	√	√	√	√	√
6.	β-Sitosterol	28.31	√	√	√	√	√	√	√	√	√	√	√	√
7.	Stigmastanol	28.75	√	×	√	√	√	√	√	√	√	√	√	√
8.	γ-Eargostanol	30.18	√	×	√	√	√	√	√	√	√	√	√	×
9.	Lanostarol	30.86	√	√	√	x	√	√	×	√	√	×	×	×

Unsaponifiable matter composition of RBOs:

Among the various vegetable oils, RBO contains highest amount of phytosterols. Among these α-sitosterol, stigmasterol and campesterol are the major components. Detailed phytosterol composition is presented in Table 5. It is observed from the table that the sterol composition of these varieties under study is somewhat similar in all the varieties.

Conclusion

During rice milling process rice bran is obtained as a byproduct. In this study twelve varieties of rice bran obtained from all over India was taken and compared. From the proximate composition of rice bran it revealed that all the brans were good source of protein and fat. The amount of trace elements was also found to be present in appreciable amounts in the different varieties of rice bran. In the study RBO was extracted from the different varieties of rice bran and the fatty acid composition and unsaponifiable matter composition of the extracted oils was analyzed. From this study it could be concluded that the RBOs are rich source of polyunsaturated fatty acids which has immense health benefits. RBOs also contain ample amounts of phytosterol and oryzanol both of which has been proved beneficial in ameliorating different lifestyle diseases. Therefore it can be concluded that all the twelve varieties of rice bran obtained from

all over India possess health beneficial effects due to the presence of above mentioned components like phytosterol, oryzanol and polyunsaturated fatty acids.

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