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Physico-chemical and nutritional properties of germ from different types of rice bran

K.A. Asma Nazreen, Manisha Guha, B. V.Sathyendra Rao and Vasudeva Singh*

Central Food Technological Research Institute, Mysore– 570020

ABSTRACT

Size separation, pneumatic air classification and saline separation procedures employed to separate germ from different types of rice bran (raw, steamed and parboiled) were collected from rice mills and were analyzed individually for physico-chemical and nutritional properties. The porosity of germ from steamed and parboiled rice bran were 51.42 and 54.05%, respectively where as that of raw rice germ exhibited higher value (65.81%). The protein, fat, and oryzanol content of steamed and parboiled germ showed an average increase of 5.28, 2.15, and 0.08%, respectively compared to raw rice germ. The free fatty acid content was < 6.2% for pure germ. The thiamine content was highest in raw germ (1.61mg/100g) and least for parboiled germ (0.26mg/100g). Phosphorus content of parboiled germ was least (32.47%) compared to raw germ (72.1%). Rice germ could be utilized for food formulations, like geriatric food, mother food, and specialty foods.

Key words : *rice germ-bran mixture, thiamine, oryzanol content, fatty acid profile*

Rice is one of the major sources of nutrition for world's population. Rice provides 23% of global human per capita energy and 16% per capita protein (IRRI, 2002). India contributes about 150 M T of paddy to the world paddy production basket. From this 10% goes for the production of rice products (expanded, popped and flaked rice). About 5% for seed purpose. From the balance stock (~128 MT), 50% goes for the production of raw rice and another 50% for the production of parboiled rice. All these rice produced are used for human consumption. The paddy grain consist of brown rice (caryopsis ~ 72-75%) and husk (woody, inedible ~ 23 - 25%). The husk comprises of two specialized thick leaves lemma (covering dorsal part of the seed) and palea (covering the ventral portion). The caryopsis (brown rice) consists of the endosperm, embryo, and several thin layers of different tissues i.e. pericarp (the ovary wall), the seed coat and nucleolus. The seed coat consists of six layers, the innermost being aleurone layer. Rice germ (embryo) is small and contains embryonic leaves (plumule) enclosed by a sheath (coleoptiles), embryonic primary root (radical) enclosed by coleorhiza, and joining part (mesocotyl). Rice endosperm consists of starch granules in protein matrix, sugars, fat, crude fiber, and inorganic matter. By-products of rice milling are husk, bran, brokens and germ. Depending on the pre and post harvest practices and the type of milling machinery, the extent of breakage of rice varies. Nutritionally, brokens are comparable to head (whole) rice but have lower economic value, due to higher surface area and uneven shapes of the endosperm compared to whole grain. Rice bran is the most valuable byproduct of rice industry. It is obtained from the outer layers of brown rice and consists of pericarp, aleurone layers, germ, and a part of endosperm. Rice bran can be utilized as a potential source of edible oil, which consists of oryzanol, an antioxidant, which is absent in any other types of edible oil. Rice germ is extremely small and is located on the ventral side of the caryopsis or endosperm. It consists of five different parts; epiblast, coleorhiza, plumule, radicle, and scutellum. Scutellum is an important part which contains highest amount of vitamin. Rice germ accounts for 20-25% of the rice bran (w/w), hence germ separation and purification becomes difficult (Luh 1991). Rice germ is characteristically rich in protein, fat, and vitamins mainly thiamine along with the antioxidant oryzanol. So it is a good source of nutrients and nutraceuticals. Commercially available rice bran is a mixture of bran, brokens (coarse and fine), germ,

pulverized husk and is not suitable for human consumption. Although much detailed work are not available for germ purification in literature was attempted by Manjunath and Desikachar (1974).

Separation of germ from bran fraction has been practiced by countries like Italy and Spain and purified germ is marketed as Morret . Retention of germ in rice to maintain its nutritive value has been practiced in Japan (Kawabata *et al*, 1999). A laboratory scale batch degermer was used to separate germ, based on the friction caused between the grains (Lu 1991) wherein germ was recovered up to 90-95%. A process was developed by Institute of Agricultural Chemistry and Food Technology (IACFT), Valencia, Spain, to separate the germ successfully, by placing bran on a screen and passing air through it upwards, separating lighter impurities at lower air current, followed by germ, whereas the heavy brokens and impurities are retained on the screens resulting in germ separation (Barber, 1981). Of late, work on rice germ is directed towards the extraction of oil, along with rice bran oil from commercial rice bran-germ mixture. Rice bran oil and rice germ oil are found to be rich (about 40%) in monounsaturated fatty acid (MUFA), which can lower low density lipoprotein cholesterol (LDL-C), and increase the high density lipoprotein cholesterol (HDL-C). Rice bran and germ oil are known for the advantages of lowering the cholesterol and triglycerides, preventing heart and arterial disease, protecting against cancer, maintaining nervous system and brain capability, adjusting hormone system for the elderly, and complexion care. Vitamin E and γ-oryzanol, the important health promoting compounds have been found in rice bran (Shanggong, 2007). It was reported that the content of vitamin E in rice germ was 5 times greater than in rice bran and the level of γ-oryzanol in rice germ was 5 times lower than in rice bran. The major vitamin E component was á-tocopherol in rice germ and γ-tocotrienol in rice bran, suggesting that the rice bran and germ have significantly different profiles of vitamin E and γ-oryzanol components. The study by Kawabata et al., (1999) suggested that the constituents of rice-germ could be possible dietary components preventive against human colon cancers. Rice germ being a very good source of nutrients and nutraceuticals, has a high potential for the development of specialty food formulations. However, non availability of pure rice germ or a process for purification of rice germ is a major bottleneck for its utilization for human consumption. An attempt was therefore made to process the commercial bran-germ mixture to fractionate into the pure germ. In the present study the steps employed for purification of rice germ from commercial rice bran, its physico-chemical and nutritional properties of rice germ are reported.

MATERIALS AND METHODS

Rice bran-germ mixtures of raw, steamed (Sona mahsuri) and parboiled (Jyothi), were collected from local rice mills in Mysore District, Karnataka, India. About 10 kg of each of different rice bran samples (with germ mixture, as generally rice bran formation during milling detaches germ portion from the endosperm, invariably) were brought from different rice mills. From each type of bran, representative samples were taken by dividing each of them several times by a Sample divider.

The bran-germ mixture from raw rice sample was stabilized in the laboratory by steaming it for 20 min. in open atmosphere. This stabilized bran and parboiled bran (already stabilized while parboiling process) were used as such. All the samples were stored in the cold room which was maintained at 8 ± 1º C, before further analysis. Samples were brought to ambient temperature (29±1°C) before further processing. Size, pneumatic and saline separation methods were employed to separate germ from the commercial rice bran-germ mixtures, employing the differences in particle size, aerodynamic properties and density of the mixture constituents.

Rice bran-germ mixtures were sieved using standard laboratory sieves with varying mesh size. The fraction retained on the sieve was referred with (+) sign, followed by the sieve number (+16, +18, etc.), whereas the fraction passing through sieve was referred with a (–) sign, followed by the sieve number (-16, -18, etc.). The germ-rich fraction obtained after sieving was further processed to remove impurities such as fine husk particles, fine brokens and to enhance the purity of the germ by air classification. In case of steamed rice bran fraction, the germ–rich portion obtained after sieving was directly subjected to saline separation, as air separation was not effective due to very similar aerodynamic properties of the constituents.

Germ–rich fraction obtained after sieving was subjected to air separation in a laboratory air classifier (Petkus, Germany). Volumetric flow rate of air was adjusted to bring about the separation. Husk and other lighter particles were separated at lower air volumes of $10cm^3$, and majority of like small broken were separated at air volumes of $25cm^3$. This step was followed by the saline separation.

Germ–rich fraction obtained after air classification was subjected to saline separation by dipping it in brine solutions of specific concentrations and stirring. Germ with lower density floats on the surface of the solution, whereas the brokens settle at the bottom. Germ is scooped out and washed with plain water to remove adhering salt and then dried in hot air dryer. Pure germ so obtained was used for further analysis.

Differences in the physical properties of mixture constituents are utilized for their classification. Some of the physical properties determined, are the particle size distribution of the mix, gravimetric properties (standard weight (1000 speck weight), specific gravity, bulk density, and porosity), aerodynamic properties (terminal velocity), and standard methods are used for their determination (Guener, 2007)

The terminal velocities of germ fractions were measured using an air column. An air stream was directed through a vertically positioned transparent tube of 53.9 mm inside diameter. A roots blower (rotary positive displacement blower) was used to develop air velocities. Air flow was regulated by infinitesimally adjusting the blower speed using frequency inverter or by varying the blower inlet cross section. For each test, the sample was dropped into the air stream from the top of the air column. Air was blown upward to suspend the germ in the air stream. The air velocity near the location of the germ suspension was measured by electronic anemometer (make: Delta Model: 2103:1) having a least count of 0.1 m s^{-1} (Calisir, Ozcan *et al*., 2005). The terminal velocity for the germ particles was measured ten times and the average terminal velocity was determined.

Color values of samples were determined by using hunter lab scan XE model (M/S Hunter associate laboratory Inc., Reston-V.A., USA. view angle of 2^0). The colour values of the samples were determined by the hunter system L, a, b values. In the hunter system "L" indicates redness and negative value indicates greenness. Positive 'b' value indicates yellowness and negative 'b' indicates blueness, "E indicates the overall average colour (Hameeda and Singh, 2011).

Proximate compositional analysis for fraction obtained by the separation process such as moisture, fat, protein, ash were carried out as per AOAC method (1995). The phosphorus content was estimated as per the method of Singh and Ali (1987). The free fatty acid (FFA) content in the germ samples was estimated according to AOAC method (1995). The amount of thiamine content present in the sample was estimated according to the method of Subba Rao and Bhattacharya (1966). The Oryzanol content was estimated using the method of Seetharamiah and Prabhakar (1986).

The in-vitro starch digestibility was determined using the method of NgoSom *et al* (1992). 100mg of defatted samples were incubated with 0.1ml of termamyl enzyme (a amylase) in a water bath for 20mins. To this 30ml of glycine-HCl buffer and 10mg pepsin was added and incubated for 2h at 37^0 C. After incubation, it was neutralized to pH 6.8 with 0.2M NaOH, 15ml phosphate buffer and 15mg pancreatin was added and incubated for 2hrs at 37^0 C. Later, 15ml of acetate buffer and 15mg amylo- glucosidase was added and incubated for 2h at 55^0 C. After incubation the volume was made up, filtered through Whatman No. 4 and sample was estimated for sugar using dinitro salicylic acid method (Bernfeld ,1955).

The fatty acid profile of raw and processed rice germ was determined by gas liquid chromatography. The methyl esters of fatty acid were prepared for GLC analysis (Deepa *et al*, 2008). In brief, the test portion of the sample was accurately weighed into a stoppered glass centrifuge vial. 2ml of hexane followed by 0.1ml of 2N methanolic potassium hydroxide were added. The vial was closed and shaken well for 30 sec before centrifugation. After centrifugation, two drops of the upper layer was removed and diluted with 2ml of hexane. 1µL of sample was injected for capillary column GLC analysis using split injection.

RESULTS AND DISCUSSION

The bran fractions were subjected to various stages of separation and purification and a cumulative index was

plotted to obtain the average particle size at 80% through. The cumulative index of sieved fractions gives a comparison between the particle size of different commercial rice bran mixtures used. Also, the cumulative index of sieved fractions gives a comparison between the different types of commercial rice bran used (Table 1).

Table 1. Cumulative Index of Different Rice Bran Fractions

Mesh No.	Mesh Size (μm)	Raw rice germ			Steamed rice germ			Parboiled rice Germ		
		Weight Retained	Cumulative throughs	% throughs	Weight Retained	Cumulative throughs	% throughs	Weight Retained	Cumulative throughs	% throughs
14	1200	-	-	-	0	400	100	0	404	100
16	1000	0	1000	100	256.5	143.5	35.875	124.5	279.5	69
18	850	877	123	12.3	035.5	108.0	27.000	037.0	242.5	60
28	600	101	22	02.2	071.0	037.0	09.250	127.5	115.0	28
44	355	-	-	-	-	-	-	061.5	053.5	13
pan	0	22	0	0	37	0	0	053.5	0	0
		1000			400			404		

In commercial raw (lab stabilized) rice bran fractions, high moisture content was observed in (+18) fraction (which had more brokens), whereas (+28b) fraction had low moisture content (Table 2). Moreover, (–28) fraction showed high ash content and (+18) had low ash content, which may be due to the presence of silica in the fraction showing high ash content. In case of Fat content, (+28b) fraction showed high fat, whereas (+18) showed low fat. The high fat content in (+28b) fraction may be due to higher concentration of germ. In bran fractions, of commercial steamed type, high moisture content was seen in (+16) fraction (rich in rice brokens), and least moisture in (+28) mesh sieve (Table 2). The variation in moisture content may be due to difference in drying period and drying conditions and this fraction (rich in bran and pulverised husk particles) showed higher ash content, whereas ash

Table 2. Fractional Analysis of Commercial germ-bran mixture from Raw Rice, Steamed Rice and Parboiled Rice

Fractions	Commercial germ bran mixture											
	Raw Rice				Steamed Rice				Parboiled Rice			
	Weight (%)	Moisture (%)	Ash (%)	Fat (%)	Weight (%)	Moisture (%)	Ash (%)	Fat (%)	Weight (%)	Moisture (%)	Ash (%)	Fat (%)
+16 mesh	-	-	-	-	64.12	10.44	1.24	3.12	30.71	12.18	8.40	3.09
+18 mesh	87.7	13.33	01.41	02.22	8.87	9.95	1.87	3.25	9.31	6.59	9.52	7.80
+28 mesh	-	-	-	-	17.75	7.44	7.34	23.99	31.48	10.38	8.84	4.11
+28 mesh (a*)	10.1	11.39	05.35	07.55	-	-	-	-		8.33	6.73	21.91
+28 mesh (b*)		09.73	06.32	15.66	-	-	-	-		8.45	14.46	15.07
+28 mesh (c)	-	-	-	-	-	-	-	-				
–28 mesh	02.2	11.68	11.22	08.46	9.25	8.25	8.84	17.42	-	-	-	-
+44 mesh (d)	-	-	-	-	-	-	-	-	15.18	9.01	10.08	11.15
+44 mesh (e)	-	-	-	-	-	-	-	-		8.68	8.54	25.47
-44 mesh	-	-	-	-	-	-	-	-	13.21	8.75	11.34	21.68

[a]Air separated at 25cm^3, [b]Air separated at 10cm^3(coarse particles),
[c] Air separated at 10cm^3(fine particles), [d]Air separated at 9cm^3(fine particles),
[e] Air separated at 9cm^3(coarse particle

content was seen to be the least in (+16) fraction. The high ash content of +28 fraction may be due to high silica from the husk particles. (+28) fraction showed high fat content, and +16 fraction had low fat content. The (+28) fraction was found to be rich in germ.In Commercial Parboiled rice bran fractions, (+28c) fraction had highest ash content and (+28b) fraction had the least (Table 2). The high ash content may be due to the high amount of silica present in the fraction (+28c) (comprising of bran and little amount of husk). And in case of fat content, (+44e) fraction showed high fat content, with (+16) fraction (with coarse husk) having least fat content. High fat content in +44e fraction may be due to higher concentration of germ in this fraction.

The cumulative index of sieved fractions gives a comparison between the particle size of different commercial rice bran mixtures (Table 1). Among the three types ofd bran, the percentage of raw bran passing through the 14 # sieve was 100% in the case of steamed and parboiled rice bran, but could did not pass through in the case of raw rice bran. In 16 # sieve the pass through was 100% in raw rice bran but it reduced with different method of processing , highest reduction was noticed in the case of steamed rice bran. With 18 and 28# sieve, the percent pass through increased with severity o f processing which is clearly seen from the Table 1.

1 000 speck weight of purified germ from raw, steamed and parboiled rice are shown in Table 3. Highest weight was observed in raw rice germ and least in parboiled rice germ indicating the effect of processing of paddy i.e raw, steaming, parboiling. Terminal velocity was almost same among 3 types of germ, however the terminal velocity of parboiled germ was least (1.87 ms^{-1}) whereas bulk density of raw germ showed least value (0.40 gcm^{-3}) that of steamed rice bran germ was high and in between was shown by parboiled rice germ. Porosity of steamed germ exhibited least value (51.4 %), whereas raw germ showed the highest value (65.8 %) indicating compactness was high in steamed paddy germ. Highest fluffyness was existed in raw rice germ. The differences in physical properties of raw, steamed and parboiled germ compared to admixture like husk and brokens facilitates the purification of germ by different methodologies adopted in the present study.

The colour value as recorded in Schimadzu hunter colour system indicated that raw and steamed germ are lighter in ur compared to parboiled germ (Table 3). The L value of parboiled germ was lower compared to others, indicating that it was darker in colour, compared to raw, and steamed which were lighter. The ÄE values for the steamed and raw germ were not different significantly, indicating that they were almost of same colour whereas parboiled germ was darker in colour and hence the value was highest.

For proximate compositional analysis, (+28b) portion in parboiled rice bran-germ mixture, (+28) in steamed rice bran-germ mixture, and (+28a) portion of the raw stabilized rice bran-germ mixture were considered. The protein content in raw rice germ was least compared to that of steamed and parboiled rice germ. The protein content of steamed and parboiled germ were higher by 4.9% and 6.4 % respectively compared to raw germ. This information is quite interesting as rice endosperm consists of 6 to 9% protein content depending on the degree of polish of the rice. Highest being present in the zero percent polished rice i.e dehusked or brown rice. Similarly the fat content in the brown rice is always 2 to 3%, whereas that of germ being highest to the extent of 46 to 50% depending on

Table 3. Physical Properties and colour measurement of Raw, Steamed and Parboiled rice Germ

Parameters		1000 Speck Weight (g)	Terminal Velocity (ms^{-1})	Bulk Density (gcm^{-3})	True Density (gcm^{-3})	Porosity (%)	Colour			
							L	a	b	ΔE
Purified Germ	Raw Rice Germ	0.21	1.95	0.40	1.17	65.81	53.90	2.29	16.84	40.33
	Steamed Rice Germ	0.19	1.94	0.51	1.05	51.42	54.25	1.57	19.03	40.90
	Parboiled Rice Germ	0.15	1.87	0.46	1.11	54.05	43.51	2.59	13.21	48.95

the type of processing of paddy. Fat content of steamed and parboiled germ were higher by 3.3 and 3.8% respectively compared to raw germ (Table 4). Thus the importance of the rice germ with respect to the protein and fat contents.

The nutritional composition of purified germ from raw, steamed and parboiled rice bran are presented in Table 4. Thiamine content of 3 types of germ could be seen, where we find that raw rice germ had highest thiamine content and least was I the case of parboiled rice germ, indicating the fact that hydrothermal treatment makes the this vitamin to migrate inside the endosperm from the germ side. Hence there is reduction in thiamine content in the germ of hydrothermally processed rice or paddy grain. Also it can be seen that thiamine content of steamed and raw germ was 4.5 and 6.2 times higher than parboiled germ indicating the transfer of thiamine to the endosperm from germ, hence always milled parboiled rice will have highest amount of thiamine compared to that of milled raw rice. Oryzanol content of raw rice germ was least and highest was found in the germ of parboiled one. It is also observed that the oryzanol of steamed and parboiled germ was almost 2 and 5 times higher than raw germ. The free fatty acid from steamed germ was very high (6.2%) compared to parboiled (2.5%) and raw germ (2.2%). This may be due to insufficient heat treatment to inactivate the lipase enzyme in steamed germ. In-vitro starch digestibility of raw germ showed higher value (15.4%) compared to steamed and parboiled germ. This may be due to the formation of resistant starch during heat treatment and subsequent drying in case of steamed and parboiled germ, which may also be due to the retrogradation of starch during steaming and parboiling of rice. Fatty acid profile of these 3 types of bran are presented in Table 5. The purified germ from raw, steamed and parboiled rice contained oleic, linoleic and linolenic acids which ranged between 35.0 and 35.9%; 32.7 and 34.0%; and 1.5 and 2.0% respectively, indicating that purified germs are good source of poly unsaturated fatty acids, which is well known for their health benefits.

Germ rich fraction obtained after step wise separation by air classification at different air velocity in Petkus air separator can be used for per germ separation by saline separation method, wherein the rice germ was successfully purified from commercially available bran-germ mixture from various processed rices. This process has potential for commercial

Table 4. Proximate and nutritional Composition of Raw Rice Germ, Steamed Rice Germ and Parboiled rice Germ

Parameters	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate Content (%)	Thiamine (mg/100g)	Oryzanol (%)	Phosphorus (%)	*IV*SD (%)
Raw Rice Germ	6.3	21.2±0.16	46.6±2.7	6.2±0.3	22.69	1.61	0.05±0.01	72.1±9.18	15.44
Steamed Rice Germ	6.8	26.1±0.24	49.9±4.4	6.7±0.05	14.94	1.14	0.13±0.02	73.1±10.25	12.49
Parboiled Rice Germ	4.1	27.6±1.00	50.4±3.7	3.8±0.05	18.59	0.26	0.24±0.01	32.5±4.65	12.95

[1]*IV*SD: *In vitro* starch digestibility

Table 5. Percentage of free fatty acids and Fatty Acid Composition of germ from Raw Rice, Steamed Rice and Parboiled Rice

parameters	Free fatty acid (%)	Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)
Raw germ oil	2.25	27.13	1.40	35.94	34.03	1.50
Steamed germ oil	6.20	29.17	1.11	35.02	32.69	2.01
Parboiled germ oil	2.47	28.41	1.52	35.31	32.76	2.02

exploitation. The purified germ is a good source of protein and fat. The oryzanol content of the parboiled germ was 5 time more than raw rice germ. Thiamine content in the parboiled rice germ was least indicating the transfer of this vitamin to the endosperm while hydrothermal processing. These germs are good source of unsaturated fatty acids. Value added food formulation can be developed using purified rice germ as base material which will be more suitable for children and lactating mothers due to its high protein and fat content.

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