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Process standardization for rice bran stabilization and its' nutritive value

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ABSTRACT

Considering the nutritional importance of rice bran (RB), a study was undertaken to observe the effect of various treatments on stability of RB and to identify the method which would safely preserve the bran for at least six months. The RB samples from the organically grown aromatic rice cultivar Ketakijoha were processed immediately after milling for stabilization. The rice bran samples were subjected to eight treatments including hot air oven heating, micro wave heating and ethanol to study their effect on RB stability. Different parameters including rancidity of rice bran oil and its' nutritive values were determined on zero, 30 days and 180 days of storage at room temperature (32°C) after applying these treatments. Near Infra Red spectrometer was used to determine crude fiber and ash content. Micro wave heating (900W) for 2.5 min was found to be best among the treatments as it provided higher stability in respect of antioxidant activity, ã-oryzanol, crude oil and total protein content. The rancidity indicators like free fatty acids accumulation, peroxide value, moisture content and pH value changed only marginally during the storage period. The ash and crude fiber content of rice bran remained nearly constant in the treated samples whereas the control sample became completely rancid, discoloured with formation of clumps and emitted unpleasant odour which increased during the study period.

***Keywords*:** *Antioxidative capacity, ã-oryzanol, HPLC, NIR spectroscopy, protein, rancidity, rice bran, stabilization*

Rice bran (RB) is the pericarp and germ of *Oryza sativa* L. seeds and constitutes nearly 10% of rough rice grain (Juliano, 1985). The mature rice grain is harvested from the field as covered grain *i.e.* rough rice, in which the caryopsis (brown rice) is enclosed in a tough siliceous hull (husk). The husk consists of lemma and Palea and it is highly lignified and brittle. Under the husk there is a very thin layer, surrounding the endosperm, is called bran. It consists of three fused layers –Pericarp, seed caot and nucellus and little aleurone layer. The colour of the bran is varied with varieties or land races; it may be whitish, brownish, reddish or blackish. The flavonoid compound, anthocianin is mainly responsible for pigmentation in RB. It is a byproduct in the milling process and has been used as a feedstock and has the potential to be used as a food ingredient and oil source. As compared to other portion of a whole grain, bran contain highest amount of protein, crude fat, crude ash, crude fiber, total diatary fibre, phenolics, ã-oryzanol, vitamin-E, anthocyanin pigment and some essential minerals (Fe, Zn). Coloured RB have high antioxidant properties (Yawadio, *et al.*, 2007) and contains anthocyanine, which reduces cholesterol (Lee, *et al.*, 2008) and also have anticancer effect (Chen, *et al.*, 2006). But unfortunately, the bran cannot be preserved as it is for long period of time due to hydrolytic and oxidative rancidity of the oil (12-19%), present in it. Therefore, it must be stabilized immediately upon production due primarily to the presence of lipase, an enzyme that rapidly hydrolyzes oil to free fatty acids (FFA) and glycerol, resulting drastic quality reduction of the RB.

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These free fatty acids produced by the hydrolysis reaction are harmful compounds which make RB unsuitable for edible use (Orthoefer, 2005). The other enzymes like lipoxygenase and peroxidase also play a key role for oxidative rancidity (the oxidation of double bond present in fatty acids). The primary means for rice bran stabilization include deactivating the enzymes through heat treatment through hot air oven or microwave heating (Zigoneanu, *et al*, 2008), ohmic heating (Loypimai, *et al*, 2009), steaming (Juliano, 1985), extrusion (Zhu and Yao, 2006), refrigeration and pH lowering by HCl application (Amarasinghe, *et al*, 2009). Finally, this stabilized bran can be used as raw materials of different food products.

Rice is the main staple food of India and on an average 105 million ton of milled rice and consequently 10.5 million ton rice bran are produced every year (USDA).According to World data from Oil world 2012 & SEA Data Bank for India, india produced 0.90 million ton of rice bran oil in 2012-13, which consumes only 7.5 million ton of rice bran and rest three million ton are being underutilized every year; these are either used as cattle feed or become rancid without any utility. Therefore, it is an holistic effort to stabilize RB for longer period (at least for six months), so that it can be utilized for value added product development. Simultaneously, it is also important to evaluate the nutritive values at the time of preservation.

MATERIALS AND METHODS

The rice bran was prepared by milling of brown rice (var. *Ketakijoha*) with the Satake miller machine, Japan. Immediately after milling the bran was

collected and primarily sieved with market available wheat flour sieving tools to separate husk and broken rice grain. RB was further sieved (with 100 mesh size) to separate coarse husk particles and finely broken endosperm. 100g fresh finely powder RB was taken in each nine microwavable air tight plastic containers (diameter-6.5 cm and length-11cm) and treatments were imposed after 14 hours of preservation in the freezing temperature (-20°C). The treatments were (1) Control, (2) Autoclave and subsequently drying at 80°C overnight, (3) Hot Air Oven (HAO) at 130°C for 20 min, (4) HAO at 180°C for 5 min, (5) Micro wave (MW) at 900 Watt for 1min, (6) MW at 900 Watt for 1.5 min, (7) MW for 2.5 min, (8)Soaking with 80% Et-OH for 6 hours and after filtration, drying of the RB in the oven at 80°C and subsequently grinding into powdered form. After completion of the treatments, all the containers were kept in room temperature (32°C) and the following biochemical parameters were estimated at 1 day, 30 days and 180 days interval.

Biochemical analysis

Free fatty acids

Free fatty acid content was determined by titration method according to method as AOAC, 1999 with some modification. Accurate weigh of RB oil (1.4 g) was dissolved in 10 ml of ethyl alcohol in presence of 1% phenolphthalein as an indicator. The mixture was titrated with 0.1 N of NaOH with vigorous shaking. The FFA content was calculated following the formula % FFA (as oleic acid) = [V × N × 28.2]/m; where, V = volume of NaOH (ml), N = normality of NaOH and m = accurate weight of oil (g).

Peroxide

Peroxide value was evaluated according to AOCS Official Method Cd 8-53 (1998).

pH

It was measured by Digital PH meter (mixing the bran with double distilled water at 1:5 (w/v) ratio with 4 hours of shaking at 30 rpm by a shaker).

Total Antioxidants capacity by DPPH

The DPPH radical scavenging assay was conducted according to the method of Zhu, *et al.* (2002) with little modification. 1g finely powdered RB and 10ml ethyl alcohol were mixed for 10 mins with mortar and pestle and vortexed. After centrifugation at 10000g for 20 min, the supernatant was collected and volume was made upto 10 ml with ethanol. With 1 ml of extracts, 2ml ethyl alcohol and 1 ml DPPH (10 ìM *i.e.*2 mg 50 ml^{-1}) solution was added. After 30 min at room temperature, the absorbance was measured at 517nm against blank. The perent inhibition was calculated.

Total Protein and Crude Oil

RB (2g) was defatted with hexane by Soxhlet extraction for 8 h and air-dried in 24 h at room temperature. Crude protein content (N × 5.95) in the samples was determined by measuring the nitrogen (N) content by Kjeldahl digestion method (Juliano,1985). Crude oil content was determined by the Soxhlet method using hexane.

NIR spectroscopy analysis of rice bran

RB samples was scanned on an NIR Systems III Rapid Content Analyser (Model XDS monochromator type XM-1000, Foss NIR SDS2500, Sweden) equipped with the Win ISI III Project Manager Software version 1.50 (FOSS NIR Systems, Silver Springs, MD, USA) and MOSAIC Solo (configuration software) to obtain the reflectance spectra and perform calibration and validation. Individual samples weighing 3.0 g each were loaded in small cup (with an internal diameter of 38 mm and a depth of 10 mm). The reflectance spectra were recorded between 424 and 2498 nm. The NIRS absorption data were expressed as Log (1/R), where R is the relative reflectance. Each sample was subsequently scanned 64 times, and the average spectrum was recorded and used for analysis. The Protein, Crude oil, Ash, Fiber and Moisture content of RB after 180 days of treatments were measures by calibrated NIR analyzer (Foss NIR SDS2500).

ã-oryzanols content of bran by RP-HPLC

Extraction was performed according to Chen and Bergman (2005) with some modifications and simplification. Briefly, 0.5 grams of RB were mixed with 5 ml of HPLC-grade isopropanol in a falcon tube, vortexed for 2 min at 25°C, centrifuged at 4500 $grpm^{-1}$ for 10 min and the supernatant was collected. The resulting residue was further extracted twice with 5 ml of isopropanol under the same conditions, and the combined supernatant fractions were evaporated under hot water bath. The concentrates of the samples were then dissolved in 5 ml of HPLC-grade isopropanol. The extracts were filtered through a 0.45 ìm membrane and 20 ìL aliquots were transferred directly into a HPLC vial for the concomitant HPLC analysis of ã-oryzanol. It was separated by an analytical Shimadzu High Performance Liquid Chromatography (RP-HPLC) system equipped with an LC-20AT pump, SPD-M10AVP photodiode array detector (Shimadzu, Kyoto, Japan) using the method described by Cristina de Simone Carlos Iglesias

Pascual et al (2011) with some modification. Samples were injected onto a 250mm × 4.60mm (5µ) (C18) Phenomenex Column. Elution was performed at 25°C and at a flow rate of 1 ml min^{-1}. The composition of the mobile phase was 35% acetonitrile, 55% methanol and 10% isopropanol and operated in low pressure gradient mode. Total runtime was 35 min including column equilibration. The ã-oryzanol standard (obtained from Oryza oil and fat company ltd., Japan), monitored with PDA detector at 325 nm, was separated into 4 main peaks between 13 and 18 min.

Two way analysis of variance (ANOVA) was performed to obtain significant differences between number of days of preservation using PROC GLM. All the analysis was performed using SAS 9.2, SAS Enterprise Guide 4.2 and MS Excel.

RESULTS AND DISCUSSION

The nutritional quality of rice bran (RB) differs significantly at different days of preservation at room temperature (32°C) (Table 1). The antioxidative activity (AA) of crude ethanolic extracts was determined by DPPH assay methodology. At the first day of treatment, the highest AA (87.53% inhibition) was found from treatment 6 (microwave heating for 1.5 minutes) followed by treatment 7 *i.e.* microwave heating for 2.5 minutes (85.39% inhibition) and after 180 days of preservation, both of the above treatments performed better (highest AA: 83.4 and 83.53% inhibition respectively) as compared to others (Table 1). Actually, tocols and oryzanols are the main antioxidants present in the rice bran (Shahid Iqbal, 2005). The other compounds like phenolics, flavonoids are also responsible for its' AA.

Table1: Properties of rice bran at different duration of preservation at room temperature (32°C)

Treatments	Antioxidative capacity(% inhibition)			Total protein (% w/w)			Crude oil (% w/w)			ã-oryzanol (mg g^{-1})		
	Days after treatment											
	1	30	180	1	30	180	1	30	180	1	30	180
1	84.46	63.17	53.2	12.6	5.07	3.43	13.35	12.4	0.12	2.26	1.05	0.24
2	80.57	75.77	66.22	13.6	11.83	12.5	15.39	15.17	15.03	2.51	1.95	1.21
3	84.57	82.37	82.67	13.38	11.7	12.5	15.29	15.22	14.33	2.45	1.87	1.32
4	84.76	84.38	84.61	13.32	11.9	12.03	15.72	15.33	14.47	2.15	1.78	1.52
5	84.45	83.27	83.07	13.57	12.07	12.43	15.07	14.4	14.44	2.15	1.65	1.42
6	87.53	85.37	83.4	13.44	11.8	12.7	16.14	15.37	14.43	2.34	1.89	1.71
7	85.39	83.53	83.53	13.22	11.63	12.7	16.81	16.35	16.26	2.63	2.16	1.98
8	82.43	78.87	71.47	12.02	10.73	10.57	13.1	12.62	12.41	2.41	1.48	1.27
LSD (0.05)		**0.71**			**0.33**			**0.04**			**0.65**	
CV%		**1.114**			**3.522**			**0.374**			**0.125**	

(1) Control (2) Autoclave and subsequently drying at 80°C overnight (3) Hot Air Oven (HAO) at 130°C for 20 min (4) HAO at 180°C for 5 min (5) Micro wave (MW) at 900 Watt for 1min (6) MW at 900 Watt for 1.5 min (7) MW for 2.5 min. (8)Soaking with 80% Et-OH for 6 hours

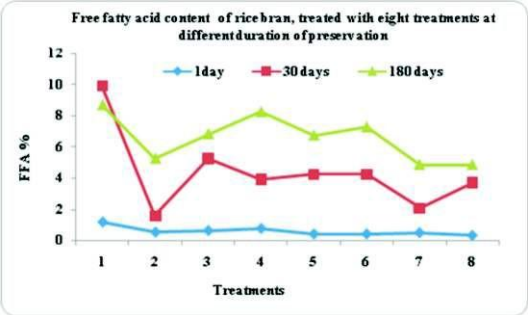
The protein content of RB was varied from 12.02 to 13.6% immediately after treatments and highest was recorded from autoclaved sample which was at par with treatment 5,6 and 7 but interestingly after 180 days of preservation, treatment 6 and 7 showed the highest protein content (12.7%). In fact, RB has high nutritional value with 12–15% protein than whole grain (8.65-9.22%) (Mahesh *et al.* 2012) and its' protein digestibility is greater than 90% (Wang, *et al*., 1999). Therefore, RB is considered as a good source of hypoallergenic protein and suitable ingredient for infant food formulation. In this study, the protein content of RB did not decrease so much after 180 days of treatment.

The crude oil content of RB was varied from 12.4 to 16.26% at first day of treatment. This was in agreement with those reported by Amissah, Ellis, Oduro, and Manful (2003, 13.3– 19.8%), using hexane as solvent. The highest oil content was recorded from T_7 (16.81%) followed by T_6 (16.14%) while the lowest was found from T_8 (13.1%). In T_7, the highest amount of crude oil was also found after 180 days (16.26%) and observed that this treatment maintain almost same concentration of RB oil throughout the preservation period (Table 1). But, in control, the crude oil content

depleted drastically after 180 days of preservation (0.12%).

ã-oryzanol is an important component of rice and mainly found in the RB. Structurally, it is ferulic acid and associated with antioxidative activity of rice. We observed that it was varied from 2.15 to 2.63 mg g-1 in RB at first day of treatment but its concentration decreases significantly after progress of preservation. However, in treatment 7 the depletion of concentration of ã-oryzanol was minimum *i.e.*1.98 mg g^{-1} after 180 days (Table 1).

In fact, rice bran's high lipid content limits its use as a whole, particularly if the grain has not been parboiled, and rancidity starts soon after production of raw bran. An endogenous enzyme (lipase) activates during milling, resulting in rapid deterioration of the oil (due to hydrolytic and oxidative rancidity), rendering it unsuitable for consumption. Therefore, the aim of this experiment was to minimize the rancidity as well as maintenance of nutritional quality throughout the preservation. Actually, thermal treatment denatures the enzyme lipase and simultaneously reduces the moisture content, which is responsible for rancidity of RB. Here we found that free fatty acid concentration and peroxide value did not increase so much with progress of preservation in treatment 7 as compared to others (Fig. 1). The PH and moisture content also was not varied significantly in treatment 7 after 180 days of preservation. This observation depicts that treatment7 (microwave heating for 2.5 minutes at 900W) was the best in respect of minimum rancidity of oil as well as higher nutrition value. The NIR spectroscopy measurement of RB after 180 days of preservation showed that in this treatment, ash and crude fiber content did not varied significantly as compared to others except control (Fig. 2). The picture of RB (Fig. 3) after 180 days of preservation, revealed that the texture and colour of RB was very good in treatment 7 but in control, clumping and unpleasant smell was found *i.e.* this sample was totally rancid after 180 days of preservation.



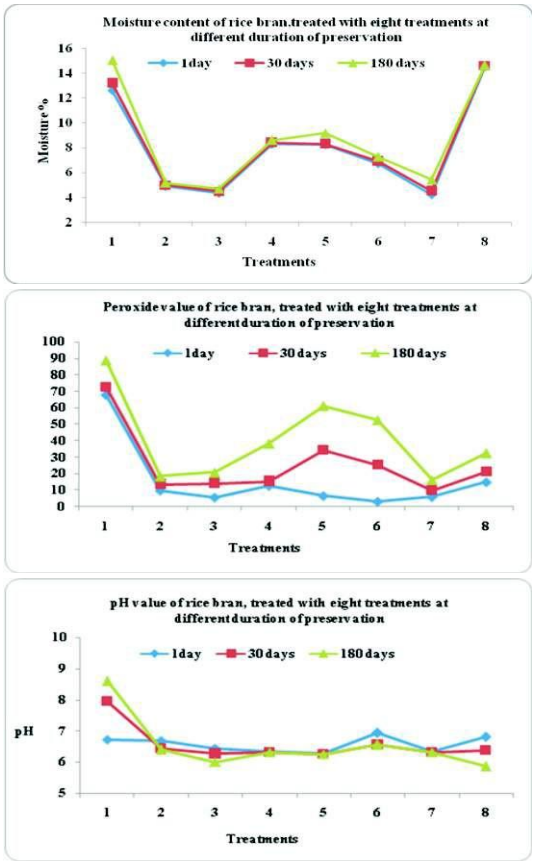
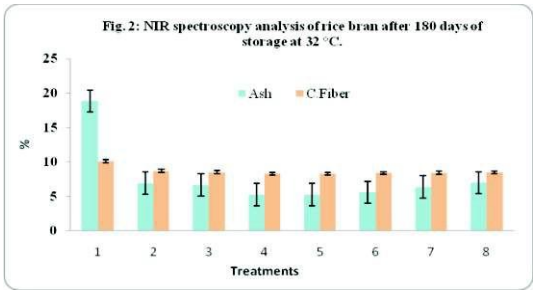


Fig.1: Components of rice bran, responsible for its rancidity; free fatty acid content (FFA), moisture content of bran, peroxide value of bran and pH



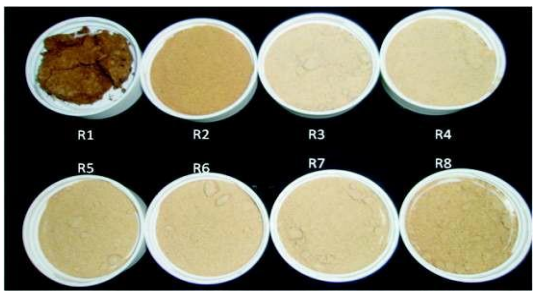


Fig. 3: Texture, colour and adhesiveness of rice bran after 180 days of preservation

From the present work, it could be concluded that preservation of RB is dependent on temperature, humidity and treatment period to inactivate lipase enzyme. The specific temperature and period is necessary because deviation from these specific treatments produces undesirable characters of RB *i.e.* either burning of samples or rancidity. Therefore, Microwave treatment for 2.5 minutes gave very good results in respect of minimum rancidity and higher nutritional value, when we preserved it for 180 days. Actually, preservation of RB is necessary because the value added product like RB cake, biscuit can be prepared by this stabilized bran and these products are very good for our health especially for cardiovascular diseases. In this study, we only focused on rancidity and nutritional value of RB but in future the microbiological as well as enzymatic changes associated with these treatments is necessary to get a clear picture of RB stabilization.

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