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RESEARCH PAPER

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Studies on effect of stabilization methods on physico-chemical properties of rice bran

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SUMMARY :

Rice bran is a by-product of rice milling industry and constitutes around 8 per cent of the total weight of rough rice. It is primarily composed of aleurone, pericarp, subaleurone layer and germ. It is a good natural source of many vital nutrients but has some limitations in food application. It is highly susceptible to rancidity caused by the inherent enzyme lipase. In order to inactivate enzymatic deterioration, whole rice bran samples were subjected to stabilization methods. Stabilization of rice bran was carried out using hot air oven method and autoclave method. The rice bran was stabilized using hot air oven method at 130^0C for 20 min and autoclaved with commercial retort at 120°C for 20 min. The autoclave method resulted in better nutrient preservation than hot air oven method and appears to be a practical and rapid tool for heat stabilization of rice bran. Autoclaved rice bran has comparatively higher levels of protein, fat and ash contents than hot air stabilized rice bran. Ash and carbohydrate contents showed a significant changes on application of stabilization methods. Mineral composition of autoclave method is better than hot air oven stabilization method of rice bran. Thermal processing decreased the free fatty acids of rice bran after 8 weeks compared with unstabilized rice bran The rancidity in term of free fatty acid (FFA) was less increased 0.4 to 3.5 per cent in autoclave treated rice bran than untreated rice bran 1.2 to 35.5 per cent at the end of 2 months of storage period. The free fatty acid levels for both hot air oven and autoclaved rice bran were below the 4 per cent permissible level for 2 months for the product packed in polyethylene packs and stored in ambient room temperature. Results indicated that autoclave treatment might effectively improve the shelf-life of rice bran that contained a good amount of vital nutrients for health benefit and is useful in many food applications such as food supplement and edible oil extraction.

KEY WORDS : Rice bran, Nutritional composition, Stabilization methods, Free fatty acids, Enzyme inactivation

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Rice processing or milling produces several streams of material, including husks, milled rice, and bran. Rice bran, a byproduct of rice milling industry is an indispensable, less expensive abundantly available as soft and fluffy off-white powdery material, during the milling period. It constitutes 8 per cent of the weight of the whole grain that contains most of the nutrients (65 %). Around 60 million metric tons of rice bran is produced worldwide each year (Mohammed *et al*., 2014).

Recently, the use of rice bran is gaining importance in many studies due to the fact that, during the processing of whole rice, large amounts of the grain's outer layers are removed, raising the concentration of nutrients in the bran and rendering it an important source of nutrients for the food industry and human consumption (Renuka and Arumughan, 2007; Imsanguan *et al.,* 2008 and Lilitchan *et al.,* 2008).

Clean rice bran contains 20-22 per cent oil by weight (Mazza, 1998), but dilution of the rice bran during the milling process with other components such as hull and starch diminish the oil content to 15-20 per cent (Mazza, 1998). Approximately 95-98 per cent of the oil is extractable (Amarasinghe and Gangodavilage, 2004). The protein found in rice bran is reported approximately 12-15 per cent (Faiyaz *et al.,* 2007). The amino acid profile of rice bran has been generally reported to be superior to cereal grain proteins. The protein of rice bran has relatively high nutritional value. The interesting characteristic of rice bran protein is that it is composed of high amount of lysine, an essential amino acid (Sudarat *et al.,* 2005). Rice bran oil is an excellent cooking medium because of nutritional superiority, abundant micronutrients longer shelf-life as well as stability at higher temperature, better taste flavor to food items. The amount of rice bran components varies as a function of rice type, climatic conditions, storage conditions, rice bran stabilization, and processing methods (Amarasinghe and Gangodavilage, 2004 and Mazza, 1998), but it typically contains 88-89 per cent neutral lipids, 3-4 per cent waxes, 2-4 per cent free fatty acids and approximately 4 per cent unsaponifiables (Kim *et al*., 1999).

However, its utilization is limited due to enzymatic activity after rice dehulling. Rice bran is rich in lipids and intense lipase activity in the presence of endogenous lipoxygenase causes rapid deterioration of these lipids by rancification (Paucar-Menacho *et al.,* 2007). Because of lipid susceptibility, the commercial use of rice bran requires enzymatic inactivation immediately after bran separation to avoid fatty acid liberation, extend its shelf-life and allow its commercialization for human consumption (Wada, 2001). Enzymatic inactivation can be achieved by heating to high temperatures for a short period (Ramezanzadeh *et al.,* 1999). However, little is known about the influence of these processes on the nutritional value of rice bran. Therefore, this work was conducted to evaluate the effect of two different stabilization procedures on the level of preservation of the nutritional composition of rice bran, and to provide further information about a potential food ingredient for the human diet. From a commercial perspective, when there is a growing market for rice bran with added value and more scientific information available about its benefits, industries are expected to show more interest in processing the product for human consumption besides its current use as animal feed.

EXPERIMENTAL METHODS

The research work was carried out at Department of Food Engineering, College of Food Technology, Vasantarao Naik Marathwada Krishi Vidhyapeeth, Parbhani, Maharashtra in the year 2016. The rice bran was procured from the Shri Laxmi Narsinhma Rice Mill, Secunderabad.

Collection of the sample:

The rice bran, one of the valuable by products of rice milling, is normally finely granulated, light tan in colour and has a bland flavour. The sample namely full fatted raw rice bran was procured from the Shri laxmi Narsinhma Rice Mill, Secunderabad.

Processing of rice bran:

Different substances like husk, clay and wood may be present in the bran. Hence, the full fatted raw bran was sieved which removes husk, clay, wood and saw dust. The samples thus obtained were free from impurities.

Stabilization of rice bran:

The stabilization method reduces or inhibits the activity of lipase enzyme. This process increases the shelf-life of the rice bran and kills bacteria, moulds and insect eggs that cause further spoilage. The methods like hot air oven stabilization and autoclave stabilization were tried to get a well stabilized full fatted raw rice bran.

Hot air heating stabilization:

The rice bran was subjected to different heat treatments as described below. For hot air heating, a portion of rice bran (100 g) was transferred into shallow pans and spread uniformly in a layer of about 0.5 cm thickness. The pans were then placed in a preheated oven at 130^{0}C for 20 min (Bagchi *et al*., 2014). The treated rice bran was immediately cooled to room temperature and packed in polyethylene bags. The bran was then analyzed for free fatty acid content to ensure the stabilization.

Autoclave stabilization:

In autoclave stabilization, steam is used to stabilize the rice bran at high pressure. In autoclave stabilization, 100g of full fatted rice bran was weighed and packed in autoclave glass bottles and kept inside the autoclave at 120^{0}C for 20 minutes. The bottles were removed and cooled at room temperature. The bran was then analyzed for free fatty acid content to ensure the stabilization (Rosniyana *et al*., 2009).

Lipase activity:

Lipase activity was determined by estimating the amount of free fatty acids (FFA) in rice bran on days basis up to 8 weeks (AOAC, 2005). Increase in FFA (%) was taken as function of lipase activity in rice bran during storage.

Free fatty acids:

Rice bran samples were taken at 0, 2, 4, 6, and 8 weeks of storage, and the FFA content was determined using a standard titration method (AOCS, 1989). The rice bran oil (1 g) extracted, was dissolved in a mixture (100 ml) of ethanol and diethyl ether (1:1, v/v) and titrated with a KOH/ethanol solution (0.01 N). The FFA content was calculated as oleic acid equivalent, expressed as percentage of total lipids.

Physical properties of rice bran:

In physical properties of rice bran, colour of rice bran is measured using Hunter Lab's next generation colour Flex EZ spectrophotometer (Debabandya and Satish, 2014). The instrument was first calibrated using standard white tile provided by the manufacturer (L=91.10, a= -0.64, b= -0.43) as per the procedure given in the reference material. Hunter L [black (0)/white (100)], a [red (+)/green (-)] and b [yellow (+)/blue (-)] colour scale was selected for all measurements.

Rice bran samples were kept on the specimen port (95 mm diameter) so as to cover the full exposed area of the window (port) to the light with the sample. This was achieved by placing the rice bran samples (100 g), in a Glass Sample Cup provided by the manufacturer. The Sample Cup was covered by a light proof covering. Three measurements were made on each sample, after shaking the sample gently, and the average values of L, a, b were noted. All three values are required to completely describe an object's colour. The amount of variation, if any in the sample, was taken into account by shaking the samples every time the measurement was done, so that the effect of void space and orientation of the grains was nullified.

Proximate analysis of rice bran samples:

The proximate composition stabilized rice bran was analyzed using standard methods. Moisture was determined by drying the sample in hot air oven at 105°C for 4 h (AOAC, 2005). Protein was analyzed by the Kjeldahl method (AOAC, 2005). Crude fat was determined by the Soxhlet extraction technique followed by (AOAC, 2005). The carbohydrate content was estimated by the difference method. It was calculated by subtracting the sum of percentage of moisture, fat, protein and ash contents from 100 per cent according to (AOAC, 2005). The bulk of roughage in food is referred to as the fibre and is called crude fibre. Milled sample was dried, defatted with ethanol acetone mixture and then the crude fibre was determined using the standard method as described in (AOAC, 2005). Ash was analyzed by burning the sample in a muffle furnace at 525°C for 5 h (AOAC, 2005).

Mineral composition :

The minerals Ca, Mg, K, Na, Fe and Zn were determined after acid digestion by using atomic absorption spectrophotometer according to the method of (AACC, 2000).

Statistical analysis:

Triplicate data of the all experimental treatments were statistically analyzed by Completely Randomized Design (CRD) using analysis of variance (ANOVA) in

SAS statistical software. The analysis of variance revealed at the significance of S.E. and C.D. at 5 per cent level is mentioned wherever required (Panse and Sukhatame, 1967).

EXPERIMENTAL FINDINGS AND ANALYSIS

The rice bran had a characteristically uniform light brown colour. The colour values (L*, a* and b*) of the heat-treated rice bran samples are shown in Table 1. All color values (L*, a* and b*) were affected by heating temperature and time. The brightness (L*) of rice bran after hot air heating treatment decreased but redness (a*) and yellowness (b*) increased as temperature increased. Unstabilized bran had the lightest colour compared with stabilized rice bran. This was reflected by the higher L* values obtained (Table 1). Stabilized rice bran was also more red and more yellow and this was reflected by the higher a* and b* values.

According to (Symons and Dexter, 1991) the L* value of flour was highly related to pericarp and aleurone fluorescence. They also found that a* and b* values were sensitive to moisture content and flour particle size, whereas L* values were slightly sensitive to particles size only. Similarly, the decrease in brightness (L*) of rice bran with the increase in heating temperature has been reported (Aliva and Silva, 1999). This result could be due to the formation of some products from the Maillard reaction induced by the heat treatment. These results are in the same trend of those reported by Sung-Min Kim *et al.* (2014); Gopinger *et al*. (2015) and Tao *et al*. (1993).

The moisture content was lowest in hot air oven rice bran sample (5.6 %) which bagged in polyethylene

Table 1 : Effect of stabilization methods on colour values of stabilized rice bran

Sr. No.	Methods	L value	a value	b value
1.	Unstabilized rice bran	69.13	3.29	21.38
2.	Hot air oven method	63.12	3.72	23.94
3.	Autoclave method	66.53	3.51	22.36
	S.E. ±	0.021	0.008	0.004
	C.D. (P=0.05)	0.063	0.024	0.013

Table 2 : Effect of stabilization method on the moisture content (%) of rice bran stored at room temperature

Sr. No.	Storage period (Weeks)	Moisture content (%)		
		Unstabilized rice bran	Hot air oven rice bran	Autoclave rice bran
1.	0	8.2	6.3	6.7
2.	2	8.0	6.1	6.4
3.	4	7.9	5.9	6.3
4.	6	7.6	5.8	6.1
5.	8	7.4	5.6	5.9
	S.E. ±	0.125	0.146	0.126
	C.D. (P=0.05)	0.376	0.439	0.380

Table 3 : Effect of stabilization methods on proximate composition of stabilized rice bran

Sr. No.	Methods	Proximate composition (%)					
		Moisture	Fat	Protein	Carbohydrate	Crude fibre	Ash
1.	Unstabilized rice bran	8.21	17.23	12.58	45.44	7.4	9.1
2.	Hot air oven	6.32	16.88	12.84	47.82	6.7	9.4
3.	Autoclave method	6.74	17.14	13.11	46.08	7.0	9.9
	S.E. ±	0.021	0.010	0.005	0.013	0.023	0.016
	C.D. (P=0.05)	0.064	0.030	0.017	0.040	0.070	0.048

bags for 8 weeks of storage, while the highest value was found in untreated rice bran at the beginning of storage period (8.2 %).

These results are in the same trend of those reported by Sahar R. Abd El-Hady (2013), who reported that during storage moisture content of rice bran decreased, this reduction in moisture content could be attributed to the higher temperature and lower relative humidity (RH) during storage.

It can be inferred that the moisture content of rice bran samples varied from 6.32 to 8.21 per cent. In general, the unstabilized rice bran had maximum moisture content (8.21 %) compared to stabilized rice bran samples. Stabilization definitely reduced the moisture content of the rice bran, out of the different techniques used for rice bran stabilization, hot air oven stabilization was found to be effective in reducing the moisture content.

The ash content which is the measure of the inorganic residues ranged from 9.1 to 9.9 per cent. Regarding the carbohydrate content of rice bran, it was maximum in hot air oven stabilized rice bran (47.82 %) and the rest of the samples exhibited the values in the range of 45.44 to 46.08 per cent.

It was observed that the fat content was the lowest in the hot air oven stabilized rice bran (16.88 %) compared to all the other samples. Unstabilized and autoclave rice bran has high fat content. This finding is in agreement with the present finding that the stabilized rice bran had low fat content ranging from 16.88 to 17.14 per cent compared to the unstabilized (17.23 %) rice bran samples.

The protein content of the unstabilized rice bran was 12.58 per cent. The protein content of the stabilized rice bran varied from 12.84 to 13.11 per cent, the highest amount being present in autoclave stabilized rice bran (13.16 %). The crude fibre content ranged from 6.7 to 7.4 per cent. High crude fibre was found in unstabilized rice bran *i.e.* 7.4 per cent and low value is present in hot air oven stabilized rice bran *i.e.* 6.7 per cent. The present findings are found to be similar to the reports of Rosniyana *et al.* (2009); Premakumari *et al.* (2012); Sahar R. Abd El-Hady (2013); Priyanka *et al*. (2013) and Mohammed *et al*. (2014).

Rice bran contains lipases, primarily responsible for the hydrolysis of triglycerides into glycerol and free fatty acids; further oxidized by peroxidases, provoking bran's rancidity. In present study, increase in FFA was used as criterion of lipase activity. The highest FFA level (Table 4) was observed in unstabilized rice bran (35.5 %) compared to hot air oven stabilized rice bran (3.9 %) and autoclave stabilized rice bran (3.5 %) after two month storage at room temperature. After stabilization, there was less formation of FFA in all stabilized bran samples. These results are in the same trend of those reported by Devinder and Sangeeta (2014); Premakumari *et al.*

Table 4 : Effect of stabilization method on the free fatty acid (FFA) content (%) of rice bran stored at room temperature

Sr. No.	Storage period (Weeks)	Fatty acid (FFA) (%)		
		Unstabilized rice bran	Hot air oven rice bran	Autoclave rice bran
1.	0	1.2	0.7	0.4
2.	2	7.3	1.1	0.7
3.	4	9.8	1.6	1.4
4.	6	16.7	2.8	2.3
5.	8	35.5	3.9	3.5
	S.E. ±	0.042	0.018	0.159
	C.D. (P=0.05)	0.127	0.056	0.048

Table 5 : Effect of stabilization methods on mineral content of stabilized rice bran

Sr. No.	Methods	Minerals (mg/100g)					
		Ca	Mg	Na	K	Fe	Zn
1.	Unstabilized rice bran	81	852	16	1542	11	6
2.	Hot air oven	72	815	12	1502	12	4
3.	Autoclave method	76	876	9	1554	14	5
	S.E. ±	0.183	0.247	0.141	0.196	0.128	0.185
	C.D. (P=0.05)	0.552	0.744	0.427	0.591	0.385	0.558

(2012) and Sung-Min Kim *et al.* (2014).

Never the less, there was a gradual increase in FFA level in all bran samples during 8 weeks storage due to residual lipolytic activity that increased under favorable conditions. Whereas, the lowest FFA level after 8 weeks of storage was found in autoclave stabilized rice bran sample (3.5 %), However, the maximum increase was observed in unstabilized rice bran (35.5 %). Hydrolysis of triglycerides forms free fatty acids, the principal cause of deterioration occurring rapidly during the first few days or weeks after milling (Ramezanzadeh *et al.*, 1999 and Da Silva *et al.,* 2006). After bran separation, the oil is exposed to lipases, causing its rapid breakdown to free fatty acids

Free fatty acids concentration in rice bran is dependent on the changes in temperature and moisture content experienced, by the rice bran during storage (Fernando and Hewavitharana, 1993). The nutritional quality and palatability of rice bran deteriorate rapidly as the oil undergoes hydrolytic and oxidative rancidity (Tsai, 1982). Hence, stabilizing the bran just after milling can prevent oil deterioration.

Rice bran is a good source of minerals (Table 5) which are present in varied amounts. The mineral composition of rice grain depends considerably on the availability of soil nutrients during crop growth and generally present in higher levels in the bran layer of rice kernel (Juliano and Bechtel, 1985).

The major mineral in the samples was potassium which ranged from 1,542 to 1,554 mg/100g sample (Table 5). Potassium is the principle salts of phytin, and located in globoids in the aleurone protein bodies (Thompson and Weber, 1981). Autoclave stabilized rice bran had the highest amount of potassium 1554 mg/100g and showed a difference with unstabilized rice bran and hot air stabilized rice bran samples.

The iron levels varied within a range 11–14 mg/100 g rice bran sample. Autoclave rice bran significantly higher iron content than other unstabilized and hot air oven stabilized rice bran. The levels of calcium in the bran ranged from 72 to 81 mg/100g rice bran sample. The highest calcium content was observed in unstabilized rice bran 81 mg/100g. The magnesium content of rice bran samples was between 815 to 876 mg/100g. The highest magnesium content was observed in autoclave rice bran 876 mg/100g. The sodium levels varied within a range 9–16 mg/100 g rice bran sample. Unstabilized rice bran significantly higher sodium content (16 mg/100g) than other hot air oven stabilized rice bran and autoclave rice bran. The levels of zinc in the bran ranged from 4 to 6 mg/100g rice bran sample. The present findings are found to be similar to the reports (Rosniyana *et al.*, 2009 and Mohammed *et al*., 2014).

Conclusion :

Distinct rice bran stabilization procedures can change the content of some nutrients by varying degrees. In general, the nutritional quality was practically fully preserved in both treated rice bran samples, with a few exceptions for some components. Rice bran is readily oxidized by the residual enzyme such as lipase. Various thermal treatments including hot air heating and autoclaving were effective in stabilizing rice bran to oxidative rancidity. Especially, autoclaving appeared the most effective in stabilizing the rice bran for long term storage. Autoclave treatment represents a practical tool for rice bran heat stabilization and reaps more advantage of oil compounds considered beneficial to human health. Considering the importance of rice bran, it can serve as an important raw material for the development of nutraceuticals and functional foods including bread, corn flakes, ice cream, pasta, noodles.

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