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PHYSICOCHEMICAL AND MICROBIAL STABILITY OF WHEAT BREAD AS INFLUENCED BY THE SUPPLEMENTATION OF RADIOFREQUENCY STABILIZED RICE BRAN

Ambreena Nazir, Monika Sood, Julie D. Bandral, Nadia Bashir* and Awsi Jan

Department of Food Science & Technology, S.K. University of Agricultural Sciences & Technology, Jammu – 180 009, Jammu & Kashmir (India)
*e-mail: *nadiabashir00@gmail.com*

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ABSTRACT

The present study was aimed to assess the suitability of radiofrequency-treated rice bran supplementation in wheat bread. The refined wheat flour was blended with radiofrequency stabilized rice bran in varying proportions (100:0, 95:5, 90:10, 85:15, 80:20, 75:25 and 70:30) and the formulated breads were assessed for their physicochemical and microbial characteristics. The bread with 30% stabilized rice bran supplementation showed highest crude protein (13.48%), crude fat (6.69%) and crude fiber (3.60%) contents, whereas control treatment (100% wheat flour) had lowest crude protein (11.64%), crude fat (2.43%) and crude fiber (1.56%) content. Loaf weight, redness (a*) and yellowness (b*) of the formulated breads increased with increase in stabilized rice bran; while loaf volume, specific volume, crust to crumb ratio and L* value decreased with the addition of stabilized rice bran. The supplementation of stabilized rice bran increased total bacterial and yeast and mould counts of bread from 2.84×10^4 to 3.19×10^4 and 1.49×10^4 to 1.84×10^4 cfu g^{-1}, respectively. During storage period of 4 days, microbial count and yeast and mould count of breads increased from 0.34×10^4 to 6.41×10^4 and 0.25×10^4 to 3.32×10^4 cfu g^{-1}, respectively. It may be concluded that supplementation of rice bran up 20% enhanced nutritional and overall acceptability scores.

Keywords: Microbial load, radiofrequency, redness, rice bran, yellowness

INTRODUCTION

Rice bran is the cuticle existing between the rice and husk of paddy and consists of embryo and endosperm of rice seeds (Sereewatthanawut *et al.*, 2008). It is usually generated as a byproduct during wheat milling. In India, approximately one million tonnes of bran are produced annually which are mostly used as animal feed (Faria *et al*., 2012). Being a rich source of proteins (14-16%), fat (12-23%), crude fiber (8-10%), vitamins and minerals such as iron, potassium, calcium, chlorine, magnesium and manganese, it has the potential to act as a food ingredient and oil source (Van *et al.*, 2006). However, high lipid content and intense lipase activity in presence of endogenous lipoxygenase rapidly deteriorate these lipids, thereby limit its food use (Faria *et al.*, 2012). Once the bran is removed from kernel, lipid substrate comes in contact with enzymes, catalyzes hydrolysis and causes rancidity that limits its use in food products. The inactivation of these deteriorative enzymes in freshly milled rice bran stabilizes the healthy food ingredient and enables its incorporation into diet (Tuncel *et al*., 2014a). Various stabilization methods like microwave heating, ohmic heating, refrigeration, extrusion, steaming and pH lowering can be applied to protect rice ban oil degradation (Lakkakula *et al.*, 2004; Mujahid *et al.*, 2005; Azrina *et al.*, 2008; Loypimai *et al*., 2009). However, radio frequency stabilization technique holds advantages over other methods because of having least residual enzyme activity and peroxide values thereby retaining the sensory qualities for longer periods (Ling *et al.*, 2018). The functional properties like water absorption capacity and emulsifying properties which play a vital role in maintaining the quality characteristics of bakery products are also better retained in radiofrequency stabilized rice bran than those subjected to conventional heating method (Ling *et al*., 2019).

Radiofrequency heating refers to the heating of materials with radiofrequency (RF) electromagnetic radiation. The radiofrequency portion of electromagnetic spectrum occupies a region between 1 and 300 MHz although the main frequencies used for industrial heating lie in the range of 10 to 50 MHz and are typically rated at 10-300 kW output power (Baskar *et al*., 2011). The mechanism underlying the radiofrequency heating is that the molecules within a product placed in a radiofrequency environment re-orient themselves (27 million times sec^{-1} at 27 MHz) continuously in response to the applied field. This response initiates volumetric heating within the entire product due to frictional interaction between the molecules, thus selectively heats only the product and not the air or equipment surrounding it (Jojo and Mahendran, 2013). This heating mechanism has advantage of uniform heating in homogeneous foods, high penetration depth that could be used to pasteurize or sterilize products, enzyme inactivation, drying and curing materials (Rao *et al*., 2005). It has a variety of industrial applications for heating, drying and curing materials and is often used for drying crackers, cookies and other snack foods. Baked goods are dried in a conveyor parallel plate system after baking to remove moisture (Baskar *et al*., 2011).

Bakery products have proven to be good vehicles for incorporation of bran from other cereals (Irakli *et al*., 2015). Bread is staple food in many countries worldwide and is mainly prepared from refined wheat flour. Nutritionally, the wide array of white breads provides energy, proteins, minerals and micronutrients (Eatrada *et al*., 2014). Bread with high nutritional value in diet is important, and the stabilized rice bran has greater potential as a supplementary source of many nutrients (Tuncel *et al*., 2014b). Stabilized rice bran or its components have been used in various food matrices such as milk powder (Nanua *et al*., 2000), ground beef (Shih and Daigle, 2003), pizza (Pacheco de Delahaye *et al*., 2005), cookies (Bhanger *et al*., 2008), tuna oil (Chotimarkorn *et al*., 2008), beverages (Faccin *et al*., 2009), etc. for functional and nutritional purposes. The present study was aimed to evaluate the potential of radiofrequency stabilized rice bran as a nutritious bread making ingredient in refined wheat flour.

MATERIALS AND METHODS

Raw materials

Freshly milled rice bran was purchased from Zamindara Rice Mills, R.S. Pura, Jammu (India) and was cleaned by passing through a 20-mesh sieve to remove broken pieces of rice, husk and other foreign materials. The sieved samples were then packed in airtight LDPE pouches (150 gauge) and stored at refrigeration temperature (4-5^{o}C) till further use. Refined wheat flour and other ingredients used for the preparation of bread *viz*., yeast, shortening, sugar, salt, glycerol monostearate, skimmed milk powder and calcium propionate were purchased from the local market.

Radiofrequency stabilization

Radiofrequency heating of rice bran was carried out using a 6 KW, 27.12 MHz radiofrequency system at Monga Strayfield Private Limited, Pune (Model PS-12T) as per the procedure described by Krittigamas *et al*. (2013). After moisture adjustment of rice bran to 18%, 100 g rice bran was transferred onto plates and spread uniformly in a layer of about 0.5 cm thickness. The plate was then put on a conveyor belt, placed in pilot-scale radiofrequency system and exposed at 80°C for predetermined exposure time of 240 sec. The treated rice bran after cooling to room temperature was packed in airtight LDPE bags (150 gauge) till further use.

Raw and radiofrequency stabilized rice barn

Preparation of supplemented breads

The following ingredients were used to prepare bread: refined wheat flour - 95 g, stabilized rice bran - 5 g, water - 60 g, sugar - 15 g, shortening - 5 g, salt - 1.5 g, yeast - 5 g, skimmed milk powder - 1 g, glycerol monostearate - 0.2 g, and calcium propionate - 0.15 g. For different treatment, rice flour was blended with radiofrequency stabilized rice bran in different proportions *viz*., 100:0, 95:5, 90:10, 85:15, 80:20, 75:25 and 70:30. The breads were prepared as per the flow sheet given in Fig. 1 following the method of Rodge *et al*. (2012). Three sets of breads (7 slices each) were formulated from each treatment. The experiment was conducted in a completely randomized design with each treatment replicated three times. The breads formulated were packed in LDPE pouches and were analyzed at an interval of 0 (fresh), 2 and 4 days. The bread pieces from each treatment were randomly chosen for physicochemical and other evaluations.

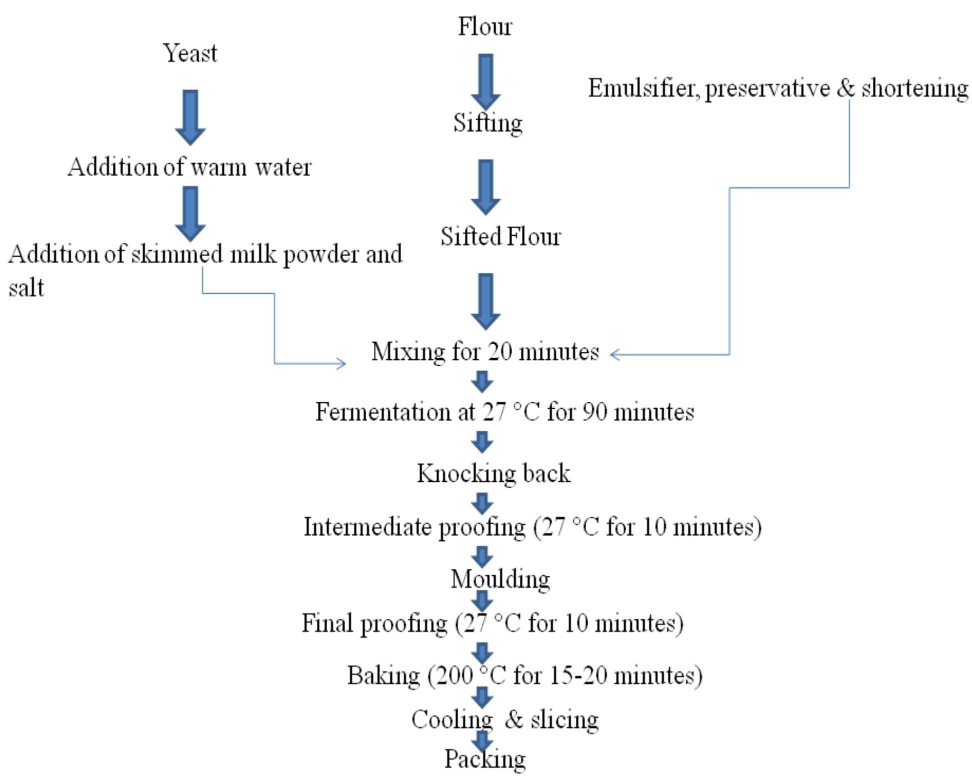

Fig. 2: Flow sheet for bread formulation

Proximate composition of rice bran supplemented bread

The moisture content in samples was determined after oven drying the material as per AOAC (2005). The crude protein content was determined by micro-Kjeldahl method, using the factor 5.95 for converting nitrogen content into crude protein (Sadasivam and Manickam, 2008). For crude fats, 5 g rice bran sample was extracted with petroleum ether at 120°C in Soxhlet extraction apparatus for 6 h (AOAC, 2002). The crude fiber and ash contents in rice bran were determined as per AOAC (2005). The carbohydrate content was estimated by difference method (by subtracting the sum of moisture, fat, protein and ash per cent from 100) as per AOAC (2005). The total energy value of bread was calculated as per the method of Bhat and Gupta (2015) using the following formula:

$$\text{Total energy (kcal)} = [(\%\ \text{available carbohydrates} \times 4) + (\%\ \text{protein} \times 4) + (\%\ \text{fat} \times 9)]$$

Physical analysis of rice bran supplemented bread

For colour analysis (L*, a*, b*), **t**he colour of rice bran and bread was measured using a Hunter's lab colour analyzer (Hunter Lab Color Flex Reston VA, USA S. No. CX 2013). The equipment was calibrated using white and black standard ceramic tiles. The loaf weight was measured directly using weighing balance. For loaf volume, the loaf was cooled for 3 h and loaf volume measured using rapeseed displacement method. Each loaf was put in a container and covered with rapeseed to fill the container. The volume of seed displaced by loaf was considered as loaf volume. The specific volume of bread was calculated as per AACC (2000):

$$\text{Loaf specific volume (mL g}^{-1}) = \frac{\text{Loaf volume (mL)}}{\text{Loaf weight (g)}}$$

Crumb to crust ratio was determined by separating crust and crumb using sharp blade and weighing each component (Barett *et al*., 2005).

Sensory evaluation

The samples were analyzed on the basis of colour, flavour, texture, taste and overall acceptability by semi-trained panel (9-10 judges) using 9-point hedonic scale assigning scores, 9 (like extremely) to 1 (dislike extremely). A score of 5.5 and above was considered acceptable (Sharif *et al*., 2009).

Microbial analysis of rice bran supplemented bread

Total microbial plate count: Total microbial count was determined by following spread plate technique as described by Ijah *et al*. (2014). One-gram sample was aseptically transferred to the test tube containing 9 mL sterile water and mixed vigorously. After mixing, 1 mL of this mixture was again transferred to a test tube containing 9 mL sterile water for further dilution. The process was continued until 4th diluents (10^4). Nutrient agar was inoculated with 0.1 mL of diluted sample (10^4), by spread plating technique and incubated at 37^{0}C for 48 h. Colonies were counted and multiplied by dilution factor.

Yeast and mold count: Yeast and mold count was determined using pour plate technique as per Ijah *et al*. (2014). Potato dextrose agar (PDA) medium was first sterilized and poured into petri-plates. The dilutions of samples were made up to 10^4 and 1 mL of each dilution transferred aseptically on Perti-plates separately. The plates were incubated at 37^{0}C for 48-72 h. The colonies developed on petri-plates were counted using a colony counter and the results expressed in cfu g^{-1}.

$$\text{Yeast and mold count (cfu g}^{-1}) = \text{No. of colonies} \times \text{dilution factor}$$

Statistical analysis

The data obtained was statistically analyzed using ANOVA and the differences between the treatments were compared at 5% level of significance (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Proximate composition of rice bran supplemented bread

The proximate composition of developed wheat bread revealed maximum moisture (35.58%), crude protein (13.48%), crude fat (6.69%), ash (3.92%) and crude fiber (3.60%) in 70:30::RWF:SRB

Table 1: Proximate composition of wheat bread supplemented with radiofrequency stabilized rice bran

Treatments	Moisture (%)	Crude protein (%)	Crude fat (%)	Ash (%)	Crude fiber (%)	Carbohydrate (%)	Total energy (k cal)
100:00::RWF:SRB	30.23	11.64	2.43	1.98	1.56	53.72	283.31
95:05::RWF:SRB	30.91	12.02	3.04	2.13	2.12	51.90	283.04
90:10::RWF:SRB	31.48	12.33	3.72	2.26	2.48	50.21	283.64
85:15::RWF:SRB	33.29	12.51	4.18	2.39	2.79	47.63	278.18
80:20::RWF:SRB	34.08	12.75	4.95	2.94	3.05	45.28	276.67
75:25::RWF:SRB	34.77	13.10	5.91	3.16	3.31	43.06	277.83
70:30::RWF:SRB	35.58	13.48	6.69	3.92	3.60	40.33	275.45
Mean	32.91	12.55	4.42	2.68	2.70	47.45	279.73
$CD_{0.05}$	0.41	0.32	0.51	0.11	0.10	1.98	0.37

RWF - Refined wheat flour SRB - Stabilized rice bran

treatment; whereas minimum values of 30.23% moisture, 11.64% crude protein, 2.43% crude fat, 1.98% ash and 1.56% crude fiber was observed in 100:00::RWF:SRB treatment (Table 1). The proximate composition of bread was significantly affected by supplementation of rice bran. The moisture content of bread samples increased significantly from 30.23 to 35.58% with increase in the level of stabilized rice bran supplementation. This might be due to higher insoluble dietary fiber content in rice bran which provides high capacity to retain water. With the incorporation of stabilized rice bran crude protein content increased from 11.64 to 13.48%. Nutritional profile of food products is uplifted specially with reference to protein content supplemented with rice bran (Ameh *et al*., 2013). With increase in rice bran supplementation, the crude fat, ash and crude fiber content showed increase while carbohydrate content showed decrease. Increase in fat content might be due to the presence more crude fat content in rice bran (Rosniyana *et al*., 2009). Decrease in carbohydrate content might be due to little carbohydrate left in rice bran after milling (Mumtaz *et al*., 2005). Our results are in agreement with Ameh *et al.* (2013) in wheat bread supplemented with stabilized undefatted rice bran. Irakli *et al*. (2015) in rice bran substituted wheat bread and Egbedike *et al.* (2016) in rice bran substituted water yam flour.

Physical properties of rice bran supplemented breads

The physical characteristics of breads were significantly affected by increase in the level of stabilized rice bran supplementation (Table 2). The loaf weight increased with enhanced supplementation of stabilized rice bran to refined wheat flour from 235.42 to 241.97 g. This might be attributed to the high water-absorption capacity of rice bran and reduction in air entrapment resulting in heavy dough(Ameh *et al*., 2013). Similar findings have also been reported by Jaimyangyuen *et al*. (2005) in bread supplemented with rice bran and Sangle *et al.* (2017) in functional wheat bread supplemented with stabilized rice bran. The loaf volume and specific loaf volume of developed bread decreased from 764.05 to 726.36 mL and 3.24 to 3.00 mL g^{-1} with increase in the level of rice bran. The maximum and minimum loaf volume of 764.05 and 726.36 mL, respectively, were observed

Table 2: Physical properties of wheat bread supplemented with radiofrequency stabilized rice bran

Treatments	Loaf weight (g)	Loaf volume (mL)	Specific loaf volume (mL g^{-1})	Crust to crumb ratio
100:00::RWF:SRB	235.42	764.05	3.24	0.271
95:05::RWF:SRB	237.30	758.46	3.19	0.267
90:10::RWF:SRB	238.23	753.55	3.16	0.258
85:15::RWF:SRB	239.19	745.89	3.11	0.250
80:20::RWF:SRB	240.09	739.35	3.07	0.248
75:25::RWF:SRB	241.02	733.48	3.04	0.241
70:30::RWF:SRB	241.97	726.36	3.00	0.233
Mean	239.03	745.88	3.12	0.253
$CD_{0.05}$	0.08	0.04	0.03	0.01

RWF - Refined wheat flour SRB - Stabilized rice bran

in control (100:00::RWF:SRB) and treatment 70:30:: RWF:SRB, respectively. The prepared bread had the highest specific loaf volume of 3.24 mL g^{-1} in control (100:0::RWF:SRB) and lowest value of 3.00 mL g^{-1} in treatment 70:30::RWF:SRB. This might be due to higher level of gluten present in refined wheat flour than composite flour which does not stretch properly during fermentation and proofing. The replacement of refined wheat flour by stabilized rice bran might have diluted the gluten content imparting the visco-elastic properties to dough (Ameh *et al.*, 2013). Similar findings have been reported by Maneju *et al.* (2011) while studying the effect of added brewer's dry grain on wheat bread quality. The findings are also in agreement with Feili *et al.* (2013) who studied high fiber bread incorporated with jackfruit rind; and Bhosale and Vijayalakshmi (2015) in case of processed rice bran and other food products.

Colour values

The hard outer portion of bread is known as crust. With increase in the percentage of the stabilized rice bran L* value decreased resulting in darker (less L*value), more reddish (higher a*value) and more yellowish (higher b*value) colour of the crust. This might be attributed to Maillard and caramelization reactions during baking of the dough resulting in the formation of brown colour of crust (Table 3). The main elements required for these reactions are amino acids and reducing sugars which are abundantly present in bran. Thus, the inclusion of bran can enhance these reactions resulting in darker colour of crust (Majzoobi *et al.*, 2013). Similar results have been reported by Irakli *et al*. (2015) in wheat bread substituted with rice bran.

Table 3: Hunter colour values of wheat bread supplemented with radiofrequency stabilized rice bran

Treatments	Crust colour			Crumb colour		
	L*	a*	b*	L*	a*	b*
100:00::RWF:SRB	46.54	12.22	25.05	71.77	1.37	16.21
95:05::RWF:SRB	45.87	12.97	26.20	68.28	1.98	18.95
90:10::RWF:SRB	44.89	13.53	26.91	64.53	2.46	18.23
85:15::RWF:SRB	44.00	14.66	27.84	61.65	3.01	18.76
80:20::RWF:SRB	42.12	15.31	28.65	57.40	3.64	19.14
75:25::RWF:SRB	41.22	16.15	29.57	53.72	4.13	19.68
70:30::RWF:SRB	40.23	17.04	30.42	50.06	4.76	20.07
Mean	43.55	14.55	27.81	61.06	3.05	18.72
$CD_{0.05}$	0.98	0.42	0.53	0.83	0.77	0.92

RWF = Refined wheat flour; SRB = Stabilized rice bran

Table 4: Sensory evaluation (hedonic score) of wheat bread supplemented with radiofrequency stabilized rice bran

Treatments	Crust colour	Crumb colour	Flavour	Taste	Texture
100:00::RWF:SRB	8.31	7.53	7.99	8.07	8.25
95:05::RWF:SRB	8.26	7.47	8.10	8.12	8.19
90:10::RWF:SRB	8.19	7.39	8.22	8.25	8.15
85:15::RWF:SRB	8.12	7.32	8.39	8.33	8.08
80:20::RWF:SRB	8.08	7.26	8.45	8.47	8.02
75:25::RWF:SRB	8.00	7.18	7.92	7.96	7.81
70:30::RWF:SRB	7.93	7.11	7.84	7.85	7.53
Mean	8.13	7.32	8.13	8.15	8.19
$CD_{0.05}$	0.09	0.11	0.16	0.10	0.09

RWF - Refined wheat flour SRB - Stabilized rice bran

Crumb is the soft inner portion of bread. With increase in the level of stabilized rice bran, the lightness (L*) of crumb of wheat bread decreased. However, the redness (a*) and yellowness (b*) increased with increase in the level of stabilized rice bran which might be due to Maillard and caremalization reactions occurring due to baking temperatures (Purlis, 2010). The results are similar to those reported by Feili *et al.* (2013) in high fiber bread incorporated with jackfruit rind flour and Tuncel *et al.* (2014a) in rice bran substituted pan breads.

Sensory evaluation

The sensory attributes of bread substituted with varying proportion of radiofrequency stabilized rice bran reflected that the crust, crumb colour and texture scores of bread gradually decreased with increase in the supplementation of stabilized rice bran from 8.31 to 7.93, 7.53 to 7.11 and 8.25 to 7.53, respectively (Table 4). However flavour, taste and overall acceptability scores increased significantly in treatment 80:20::RWF:SRB and thereafter showed a decreasing trend. The increase in taste score with the addition of stabilized rice bran might be due to rice bran which imparted good taste and a slightly toasted nutty flavour to the bread (Sharif *et al*., 2014). Similar results were reported by Irakli *et al.* (2015) in wheat bread substituted with rice bran and Tuncel *et al.* (2014a) while studying effect of infrared stabilized rice bran substitution in pan breads.

Microbial count

The microorganisms present in bread samples reflect the sanitary quality of the source of raw materials, processing and storage of samples (Ray and Bhunia, 2007). The results showed that the treatments significant differed in total plate count and yeast and mould count of functional wheat bread with the increase in the level of stabilized rice bran (Table 5 and 6). Total plate count increased from 2.84 10^4 to 3.19 $\times 10^4$ cfu g^{-1}, while as yeast and mold count increased from 1.49 x 10^4 to 1.84 $\times 10^4$ cfu g^{-1} with increase in supplementation of radiofrequency stabilized rice bran which might be due to the handling during baking and high moisture content of supplemented bread (Lucy *et al*., 2017). The increased moisture content of rice bran supplemented breads might have favoured the accelerated growth of microbes as microbial growth is dependent on moisture content of substrate (Bashir *et al*., 2019).

During 4 days of storage period, the microbial count of bread increased from 0.34 $\times 10^4$ to 6.41 $\times 10^4$ cfu g^{-1} while yeast and mould count increased significantly from 0.25 $\times 10^4$ to 3.32 $\times 10^4$ cfu g^{-1}. The increase in moisture content during storage might be the reason for increased microbial load (Nagi *et al*., 2012). Chakroborty and Chakroborty (2017) also reported an increase in microbial growth of salt-smoke-dried shoal in response to storage. The values obtained for bread samples were within the acceptable limits of International Commission on Microbiological Specification (ICMSF) and

Table 5: Total microbial plate count (× 10^4 cfu g^{-1}) of wheat bread supplemented with radiofrequency stabilized rice bran

Treatments	Storage (days)			
	0	2	4	Mean
100:00::RWF:SRB	0.11	2.14	6.27	2.84
95:05::RWF:SRB	0.20	2.18	6.32	2.90
90:10::RWF:SRB	0.28	2.21	6.36	2.95
85:15::RWF:SRB	0.33	2.25	6.40	2.99
80:20::RWF:SRB	0.41	2.30	6.46	3.06
75:25::RWF:SRB	0.49	2.36	6.52	3.12
70:30::RWF:SRB	0.57	2.41	6.58	3.19
Mean	0.34	2.26	6.41	
$CD_{0.05}$	Treatment = 0.03; Storage periods = 0.04; Treatment × storage = 0.07			

RWF - Refined wheat flour SRB - Stabilized rice bran

Table 6: Yeast and mold count (× 10^4 cfu g^{-1}) of wheat bread supplemented with radio-frequency stabilized rice bran

Treatments	Storage period (days)			
	0	2	4	Mean
100:00::RWF:SRB	0.06	1.24	3.16	1.49
95:05::RWF:SRB	0.13	1.30	3.21	1.55
90:10::RWF:SRB	0.19	1.37	3.27	1.61
85:15::RWF:SRB	0.25	1.42	3.32	1.66
80:20::RWF:SRB	0.32	1.49	3.38	1.73
75:25::RWF:SRB	0.36	1.55	3.43	1.78
70:30::RWF:SRB	0.41	1.61	3.49	1.84
Mean	0.25	1.43	3.32	
$CD_{0.05}$	Treatments = 0.02; Storage period = 0.03; Treatment × storage = 0.01			

RWF - Refined wheat flour SRB - Stabilized rice bran

recommendation for products of this nature (10^5 cfu mL^{-1}) in Good Manufactured Practices (ICMSF, 2009) for total plate count. The fungal counts were also within acceptable limits for bread by ICMSF for Good Manufacturing Practice (10^3 cfu mL^{-1}) (ICMSF, 2009).The results are in agreement with the results of Ogundare and Adetuyi (2003) in bread baked with wheat flour and Ezeocha *et al*. (2016) in bread made from living stone potato and wheat composite flour.

Conclusion: For the development of acceptable and nutritious bread, refined wheat flour can be substituted by radiofrequency stabilized rice bran up to 20% level. The supplementation of radio-frequency stabilized rice bran (up to 20%) resulted in higher crude protein (12.75%), crude fat (4.95%) and crude fiber (3.05%) as compared to the control having 11.64% crude protein, 2.43% crude fat and 1.56% crude fiber. Bread was microbiologically safe for consumption up to 4 days. With increase in the supplementation of radiofrequency rice bran the total microbial count and yeast and mold count showed increase. Overall, the supplementation of radiofrequency rice bran up to 20% can be used as a natural and inexpensive nutraceutical-rich material in the production of consumer acceptable and nutritionally improved bread. It is, therefore, advantageous to explore the possibility of rice bran composite flours for commercial production which may also solve the bye-product management.

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