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Chapter 2

Effect of Yeast Fermented Brown Rice Flour Substitution on Nutritional, Rheological and Textural Properties of Steamed Brown Rice Bread

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Additional information is available at the end of the chapter

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Abstract

The current study investigated effect of fermented brown rice (*Oryza sativa*) flour (FBRF) at moderate acidity (pH 5.5) on the nutritional, rheological and textural properties of steamed brown rice bread (SBRB). Brown rice flour was substituted with 40% FBRF and its batter and steamed bread characteristics were evaluated. The results revealed that incorporation of 40% FBRF decreased breakdown, setback and final viscosity of brown rice flour, while its peak viscosity significantly increased. The batter system containing 40% FBRF had softer structure than the control, which was reflected by lower storage module (G′) and loss module (G″). Furthermore, the crumb texture of its bread was also significantly ($p < 0.05$) improved, since it had higher chewiness, cohesiveness and springiness, as well as higher specific volume than the control. Incorporation of 40% FBRF significantly increased protein, zinc, nicotinic acid and pyridoxine contents of SBRB. However, its content of antioxidant activity, total γ-oryzanol and phytic acid significantly decreased. This investigation approved that FBRF can be used as a valuable ingredient to modify technological and nutritional properties of steamed brown rice bread.

Keywords: fermented brown rice flour, steamed brown rice bread, nutritional value, rheological properties, textural properties

1. Introduction

Regular consumption of whole grain cereals was approved to have several health benefits since, whole grain cereals are cereals are the appropriate source of fiber and many bioactive components. The bioactive components, which were reported to have positive health influ-

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ences, include fiber, phenolics and vitamins [1]. Moreover, recent researches have pointed out that cereal fiber could be a functional constituent that helps to deliver antioxidant substances to the gut [2, 3]. Brown rice (*Oryza sativa*) is one of the most important whole grain cereals and a rich source of several bioactive substances, such as vitamin E, vitamin B, fiber, phenolics and γ-oryzanol. Its consumption as a whole grain (brown rice) is not popular due to its hard texture and dark color. Thus, it can be ground into flour and utilized to produce numerous kinds of gluten-free foods, such as cakes and breads [4, 5].

Brown rice bread is one of the most common non-gluten products, which are suitable for celiac patients, where the demand is increasing. Currently, the only effective way to deal with celiac disease is to avoid consumption of gluten-containing cereals such as wheat, barley and rye [6]. Indeed, development of gluten-free products is a difficult task for food technologists. They are nutritionally poor and are subjected to short shelf life and poor texture [7]. The gluten-free dough show lesser cohesive and elastic cake batter-like compared to wheat dough due to the absence of gluten, which makes them greatly sticky and challenging to handle. In addition, their gluten-like protein network is extremely weak, when untreated non-gluten flours are used as a major constituent of the mixture [8]. As a result, the volume of their end product is relatively low with dense crumb, because of low ability to hold CO_2 released through proofing [9, 10]. For that reason, some food additives or biotechnological pretreatments of the flour would be taken into consideration to improve its baked product qualities [11].

A variety of additives and different nutritive ingredients were investigated in order to improve their technological and nutritional characteristics [12]. Utilization of whole grain flour such as millet, brown rice and sorghum to produce these kinds of formulations could enhance their nutritional value [13, 14]. On the other hand, the incorporated amount of cereal bran or the usage of whole flour in which their health benefits can be predictable causes several detrimental effects on product quality due to the bran fraction [15]. Addition of enzymes and usage of gelatinized starch and sourdough are also attempts which have been investigated to overcome gluten-free product disadvantages and to relief the negative effects of the bran [4, 5].

Technologically, pre-fermented flour or sourdough was pointed to significantly modify rheological properties of non-gluten batters. In our previous investigation, incorporation of yeast fermented brown rice flour significantly improved texture and volume of steamed white rice bread [16]. A study also indicated that addition of amaranth sourdough to amaranth batters positively affected their viscoelastic properties [17]. Sorghum bread quality significantly improved with addition of sorghum sourdough compared to that supplemented with hydroxypropyl methyl cellulose (HPMC) [18]. A number of suggestions have been reported to explain the influence of sourdough on batter and bread qualities including the direct impact of pH on batter structure, in addition to enzymatic and microorganism activities [19].

Pre-fermented flour or sourdough could also modulate the nutritional properties of gluten-free and whole grain products in several ways, like increasing content or bioavailability of

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bioactive substances, hindering starch digestibility and reducing anti-nutritional factors [19, 20]. However, there is a noticeable gap between the fundamental basis for gluten-free bread building up structure and their nutritional significance. Investigations regarding non-gluten-free foods, specifically bread, have been focused on improving technological parameters that include volume and crumb hardness, as well as sensorial aspects. However, their nutritional concept has not been well addressed.

Cereal-based fermented foods are attracting both technologists and consumers because of higher content of phytochemicals, minerals and dietary fiber, in addition to low fat content [19, 20]. Certainly, fermentation is an ancient, inexpensive and simple technique that can be applied at home and an important technique in the third world countries for enrichment and preservation of a food material [21]. However, investigations concerning influence of food processes, such as fermentation on the fate of nutritive components and the rheological properties of steamed brown rice bread as an important non-gluten product, are still limited. These knowledge is required when development of whole grain functional foods is considered. Accordingly, the objective of this study was to investigate the effect of yeast fermented brown rice flour substitution on flour, batter and steamed brown rice bread qualities. The obtained outcomes may allow development of gluten-free functional foods with high qualities.

2. Materials and methods

2.1. Materials

Baker's yeast (Eagle, CY 1266, China) and Eco-brown rice grains (MR219) were purchased from a local supermarket in Selangor, Malaysia. Brown rice flour (BRF) was prepared by grinding brown rice grains in a FOSS Tecator (Cyclotech™ 1093, Hoganas, Sweden) to attain a particle size of 500 μm, whereas fermented brown rice flour (FBRF) with moderate acidity (pH 5.5) was prepared as described in our previous study [20]. Flour samples were packaged in polyethylene plastic and stored at 4°C till further analysis. The used chemicals for analysis were of analytical or HPLC grade and were supplied by Merck (Darmstadt, Germany) and Sigma-Aldrich (USA).

2.2. Methods

2.2.1. Bread making process

A preliminary study was firstly conducted, where BRF was substituted with 0, 10, 20, 30, 40 and 50% of FBRF to evaluate the sensory properties and the volume of their steamed brown rice bread (SBRB). According to the results, SBRB with 40% FBRF recorded the highest overall acceptability and bread volume among the others (data not presented). Therefore, SBRB with 40% FBRF was selected for further analysis to be compared with the control sample (SBRB without FBRF). Bread samples were prepared based on batter formula of 100 g of BRF, which consisted of 2% sugar, 2% salt, 3% baker's yeast and 93% volume of water based on the flour weight. During preparation of the batter, instant yeast was dissolved in a solution of water and

sugar and then pre-fermented in a fermenting chamber (Binder 10-01536, Germany) at 32°C for 10 min. Afterwards, dry ingredients, which consisted of BRF, salt and FBRF were thoroughly mixed, then all the ingredients were mixed manually in a beaker for 3 min. After mixing, the batter samples were located in bread pans and fermented in the fermenting chamber at 32°C for 30 min. After fermentation, the samples were steamed for 15 min, cooled at room temperature (25°C) for 1 h before further analysis. Bread samples were made in five replicates.

2.2.2. Determination of batter acidity

Titratable acidity of brown rice batter and brown rice batter with 40% FBRF was determined following the method described by Kati et al. [22], whereas, a pH meter (DELTA 320, shanghai, China) was utilized to measure the pH values.

2.2.3. Determination of pasting properties

Pasting properties of BRF and BRF with 40% FBRF were determined using Rapid Visco Analyser (RVA) (Newport Scientific Pty. Ltd., Warriewood NSW 2102, Australia) according to AACC [23].

2.2.4. Determination of dynamic rheological properties

The rheostress (HAAKE Rheowin 600, Germany) at 30°C using parallel plate geometry (35 mm diameter and 1 mm gap) was utilized to measure the dynamic rheological properties of batter samples as detailed in the early study [16].

2.2.5. Bread volume measurement

Bread volume was determined according to the seed displacement method described by Hallén et al. [24] using sago pearls after 1 h from steaming as reported in our previous study [16].

2.2.6. Texture profile analysis

Crumb texture properties of bread samples were measured using Texture analyser (TA-XT2, UK) equipped with a 30 N load cell and compression plate with a diameter of 75 mm as previously performed [16].

2.2.7. Microscopic analysis

Microstructure of bread samples was examined using scanning electron microscope (JEOL-JSM-6400 SEM, Japan). Bread samples were freeze-dried, ground and then attached on circular aluminum stubs, coated with gold and scanned at an accelerating potential of 15 KV.

2.2.8. Determination of proximate composition and nutritional value

Proximate composition of SBRBs, which included moisture, crude protein, total lipid, total fiber, soluble fiber and insoluble fiber contents, was determined according to the methods of AOAC [25]. Concerning total ash, it was measured according to ISO method [26]. Mineral contents, which include calcium, magnesium, iron and zinc contents, were estimated following the method of AOAC [25]. Phosphorus content quantification was carried out subsequent to

the yellow method with the ammonium-vanadomolybdate reagent according to AOAC [25]. The concentration of phytic acid was determined following the method described by Wu et al. [27] with some modifications [20].

Total phenolic content (TPC) was evaluated using Folin-ciocalteau method according to Beta et al. [28] with certain modifications [20]. The extraction procedure used to determine TPC was employed to measure the ferric reducing ability power of the bread sample as performed in the previous research [20].

The extraction of tocopherols, tocotrienols and total γ-oryzanol were carried out according to the described method by Aguilar-Garcia et al. [29]. Their quantification was conducted by high-performance liquid chromatography (HPLC) fitted with fluorescence detector (Agilent Technologies 1200 Series, Germany). Vitamin E standards were prepared according to Ye et al. [30]. Determination of vitamin B2, B3 and B6 contents was conducted using HPLC fitted with UV detector (Waters 2489 UV/visible Detector and Empower software, USA) following the method of AACC [31] with some modifications [20].

2.2.9. Statically analysis

One-way analysis of variance (ANOVA) and Tukey's multiple range tests with p-value >0.05 were used to report the significant differences between data obtained.

3. Results and discussion

3.1. Batter acidity

The initial pH of FBRF (5.7) was in the acidity range of sourdough prepared with yeast (4.7–5.8) that was previously reported [19]. Substitution of BRF with 40% FBRF resulted in a moderate acidification of brown rice batter. The pH value of the treated batter (6.13) was significantly ($p < 0.05$) lower than the control (6.50), and the same trend was observed for TTA values (**Table 1**). A possible explanation for this result could be related to the effect of the initial pH of FBRF. Additionally, the reached pH (5.5) of FBRF is close to the optimum pH of some enzymes, such as α-amylase, protease, phytase, β-glucanase and pentosanase [19], that could activated and allowed them to breakdown the macro-components of FBRF and produce some organic acids like lactic and acetic acids that led to a reduction in the pH value of the batter, in addition to the effect of microbial metabolism products.

Batter	pH	TTA (mL)
BRB	6.50 ± 0.00[a]	2.23 ± 0.10[a]
BRB + 40% FBRF	6.13 ± 0.02[b]	3.78 ± 0.46[b]

[a] Represented values are the means ± standard deviations of three replicates.

[b] Values with the same superscript letter in a column are not significantly different ($p > 0.05$).

Table 1. The pH and TTA values of brown rice batters (BRBs).

3.2. Rheological properties

The rheological measurements of cereal flour are a significant indication to the bakery industry, where they assist to predict dough handling and processing characteristics as well as final backed product quality [32, 33]. The variation in storage modulus (G′ elastic component) and loss modulus (G″ viscous component) with frequency sweep of brown rice batters is presented in **Figure 1**. The moduli were greater for control batter than the batter containing FBRF. This may be an indication that control batter had more rigid structure than the batter containing FBRF, since it had higher G′ and G″. The G′ and G″ of the batter containing FBRF became less independent of frequency compared to the control. Thus, the structure of the batter having FBRF became softer and stronger than the control as indicated by lower (G′) and (G″). Complex modulus (G^*) was also lower for the batter containing FBRF (**Figure 1**), which indicates a decrease in resistance to deformation. These changes may be related to the effect of protease supplemented by FBRF, where it was reported that addition of protease to brown rice batter reduced resistance to deformation [5]. It also indicated that the elastic (G′) and viscous (G″) moduli values of white rice batter having 40% FBRF were lower than the control at all the tested frequency ranges, and they were independent of the frequency [16]. The increase in protein content of bread containing FBRF [20] might also have an effect on the rheological properties of the batter [15]. From the current results, there was no significant change in tan delta (δ) between the samples (**Figure 1**). The tan δ is an indication of liquid to solid state, and this may demonstrate that water absorption of the batter was not significantly affected by FBRF. Thus, it can be suggested that substitution of BRF with 40% FBRF did not affect water holding capacity of the flour, but could alter protein interactions and functionality, such as changes in sulfhydryls (-SH) and disulphides (-S-S-) bonds, which play an essential role in developing protein network as reported by Elkalifa et al. [34]. It could be expected that, these observed alterations in the viscoelastic properties of the treated batter would be also attributed to the degradation of macro-components (starch, protein and fiber) as affected by the active enzymes supplemented by FBRF. According to Rieder et al. [35], pre-fermented barley flour degraded β-glucan in composite wheat bread as indicated by a reduction in its molecular weight.

The pasting parameters of BRF were also significantly ($p < 0.05$) influenced by its substitution with 40% FBRF (**Figure 2**). It caused significant increase in hot paste viscosity, while cold paste viscosity, breakdown and setback were significantly reduced. Chinma et al. [36] also indicated a decline in pasting parameters of wheat flour when it was substituted by 15% of natural and yeast fermented rice bran protein concentrations. According to Renzetti and Arendt [4], addition of protease to BRF during bread making decreased peak viscosity, final viscosity and breakdown without a significant effect on setback. Similarly, another study reported that addition of α-amylase to wheat dough decreased setback [12]. As mentioned earlier fermented brown rice flour could be a source of these enzymes that caused a reduction in pasting parameters of BRF. These observations might give indication about the modification of starch and/or protein interactions due to enzyme actions [7]. Moreover, the decrease in carbohydrate and the variation in protein content might lead to a reduction in pasting parameters [37]. During RVA determinations, starch granules could not swell

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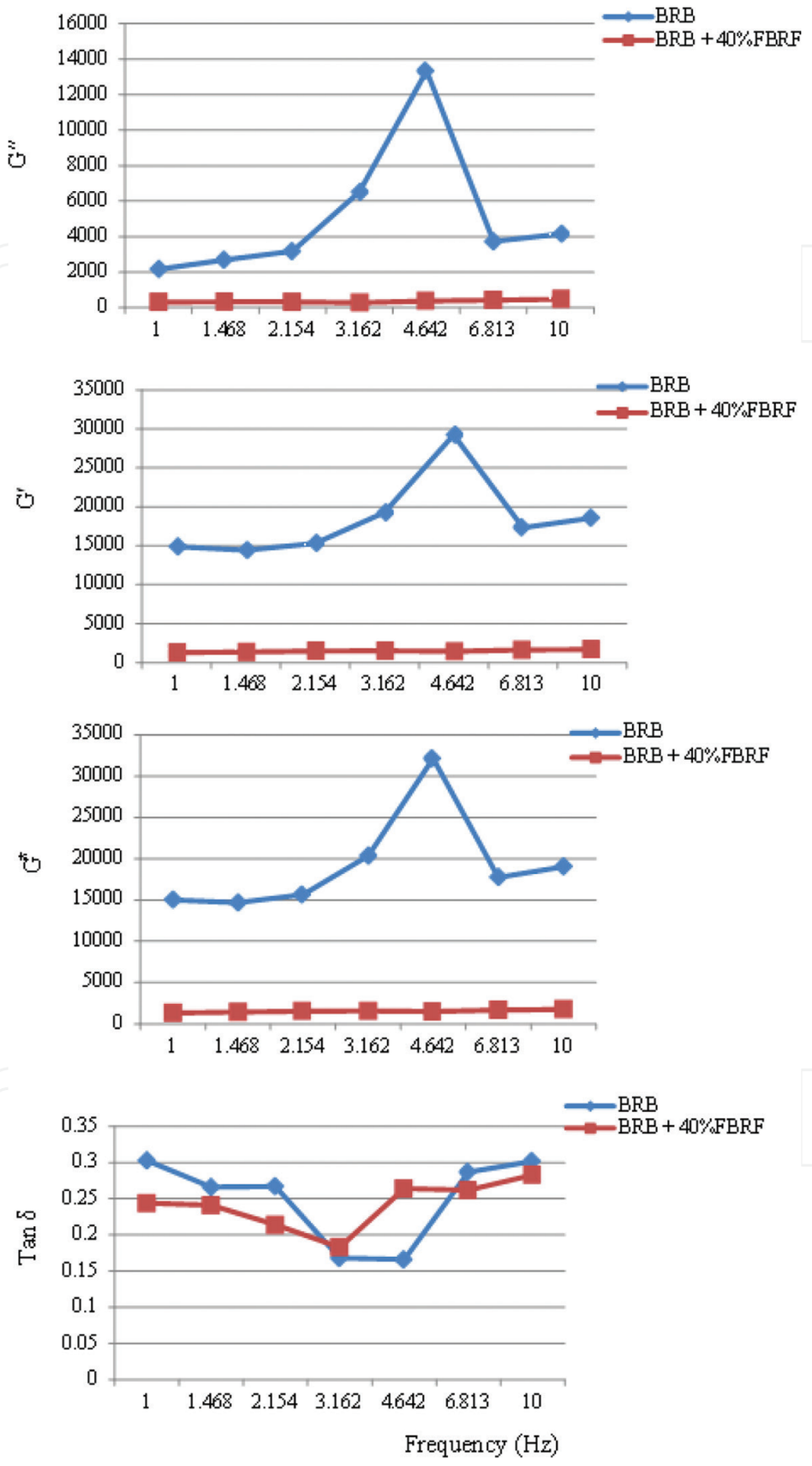


Figure 1. Viscoelastic properties of brown rice batters (BRBs).

to their maximum size due to the limited amount of water. In such environment, protein structure surrounded starch granules causing rigid paste [38] and elevated viscosity as observed for the control flour. Protein and starch hydrolysis, which occurred during pre-fermentation process to produce FBRF could disrupt the rigidity of the paste, as a result, reduced viscosity of the treated flour [39]. The decrease in breakdown value might explain the increase in resistance to deformation and higher stability of the paste. A study reported that the positive effect of sourdough on sorghum bread quality was due to formation of strong starch paste during baking [18]. Furthermore, the decrease in setback delays the retrogradation process (lower degree of amylose polymerization) in the final product and increased its shelf life.

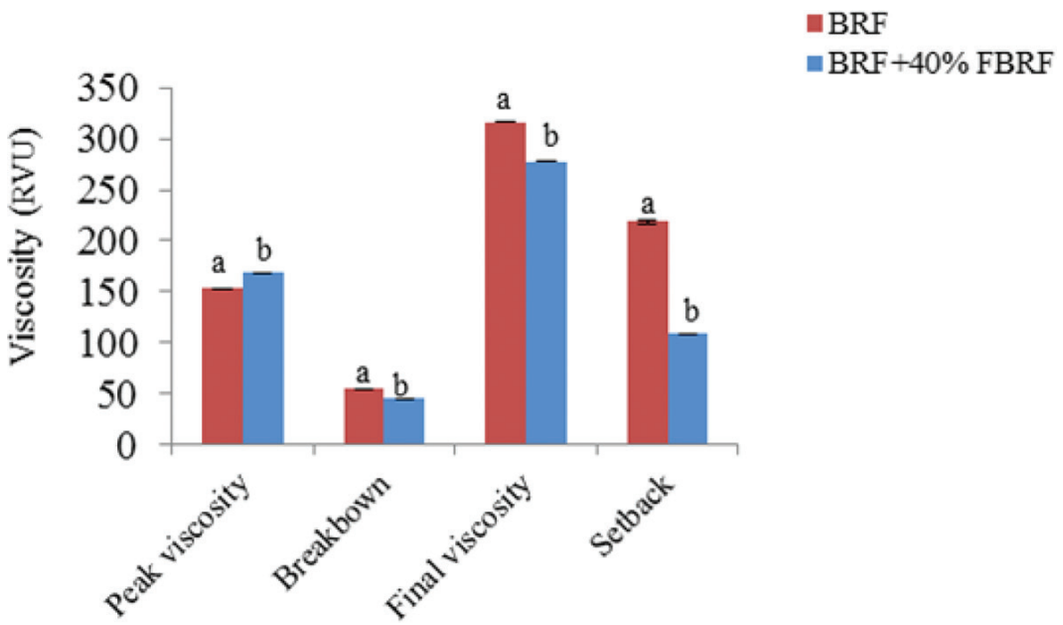


Figure 2. Pasting properties of brown rice flours (BRFs). Error bars: standard deviations of three replicates. Each different small letters above columns means statistically significant ($p > 0.05$) difference.

3.3. Steamed bread volume

Specific volume of bread is a significant quality consideration as it is associated with ability of dough-inflating and oven spring and could not be too large or too small as it influences the crumb structure and determine the overall bread quality [40]. **Figure 3** shows that specific volume of bread significantly increased from 2.2 to 2.75 cm^3/g with usage of FBRF. Also, our previous study indicated that steamed white rice bread containing 40% FBRF had higher specific volume (2.46 cm^3/g) compared to the control sample (2.06 cm^3/g) [16]. In this study, the increase in specific volume of the bread could be due to the reduction in viscoelastic properties and the viscosity of its batter and improve protein network that could make it softer and enhanced its ability to hold more CO_2 [41, 42]. Furthermore,

FBRF could have more damaged starch due to pre-fermentation which perhaps increased the yeast activity leading to higher gas production.

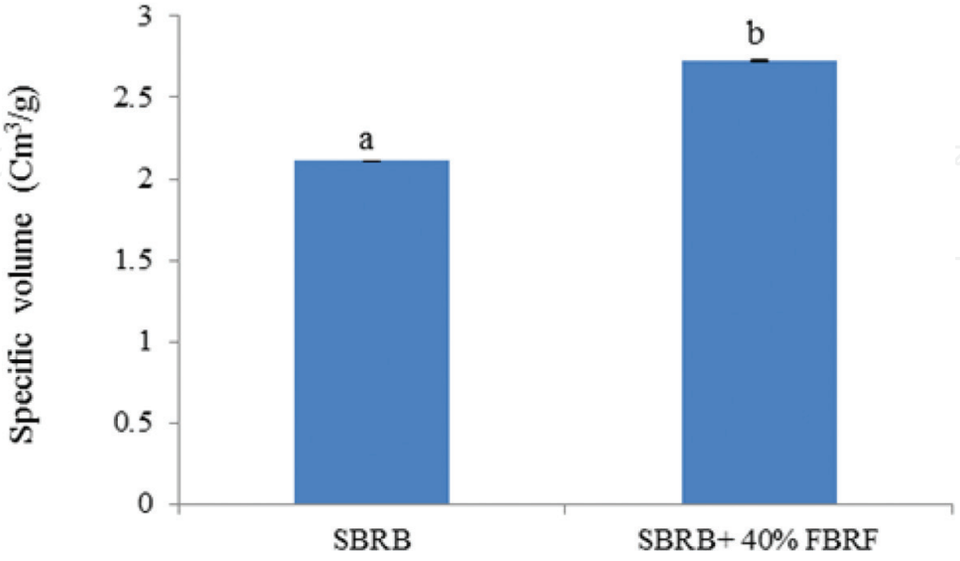


Figure 3. Specific volume of steamed brown rice breads (SBRBs). Error bars: standard deviations of three replicates. Each different small letters above columns means statistically significant ($p > 0.05$) difference.

3.4. Steamed bread texture

The textural characteristics of a food were described as group of physical properties, which are sensed through the feeling of touch [43]. **Figure 4** revealed that FBRF had significant effect on the textural properties of the bread. The springiness, cohesiveness and chewiness were significantly increased due to addition of FBRF, whereas there was no significant impact on the hardness of bread. The increase in springiness indicated higher recovery in the bread height during the first and second bite and also an indication on the increase in the elasticity and softness. Cohesiveness is a measure of fracturability, an increase in its value means higher ability of the bread structure to resists a second deformation in relation to its withstand in the first deformation. Chewiness is an indication of the time required to chew a solid food to be ready for swallowing. Its value was higher in the bread containing FBRF, which reflects higher time needed to masticate it and less breakable in the mouth which is an important attribute of bread. An early study reported that substitution of white rice bread with FBRF significantly decreased the hardness of white rice bread (6398.61 g) compared to the control (6948.13 g). In addition, its chewiness, cohesiveness and resilience were also significantly improved [20]. Moreover, crumb texture properties of wheat bread supplemented with bran were significantly improved due to addition of pre-fermented wheat bran [44]. Meanwhile, it is reported that natural and yeast fermented rice bran protein concentrates, which were used for wheat bread making significantly increased hardness, springiness, chewiness and gumminess of the bread; however, its cohesiveness value was reduced [32]. It was stated that usage of pre-fermented bran with yeast and lactic acid bacteria enhanced retention of CO_2 during dough proofing and as a result increased the bread volume and softness of crumb texture [19, 44].

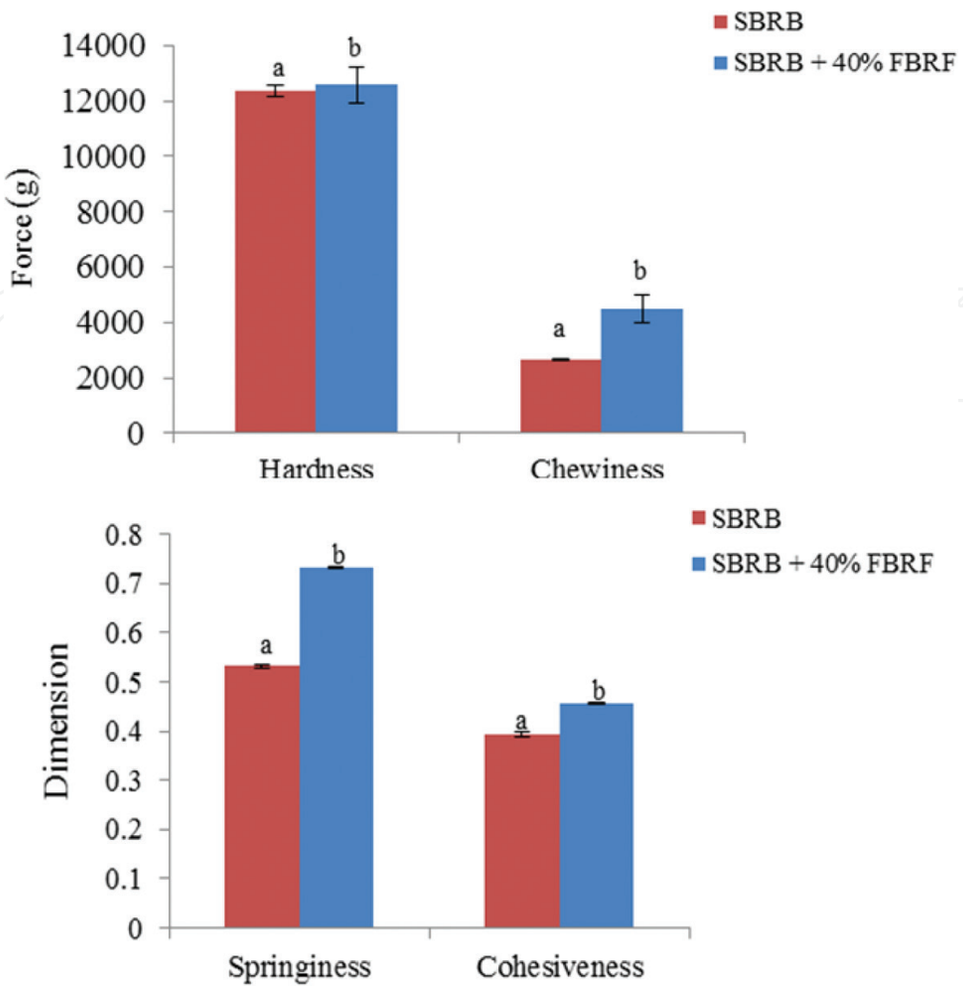


Figure 4. Textural properties of steamed brown rice breads (SBRBs). Error bars: standard deviations of three replicates. Each different small letters above columns means statistically significant ($p > 0.05$) difference.

3.5. Morphological structure of steamed bread

The microstructure organization of SBRBs was investigated using scanning electron microscopy (SEM) at different magnifications (**Figure 5**). As presented in **Figure 5B** and **D**, SBRBs with FBRF demonstrated a smooth structure and the underneath compounds were not simply visualized. The control bread illustrated a compact structure and more continuous surface, coupled with the fact that the underneath substances were not revealed (**Figure 5A** and **C**). Usage of FBRF probably caused a disruption in the protein-starch matrix of the bread that causes these differences in the microstructure between samples.

3.6. Proximate composition and nutritional value

The chemical composition of steamed bread samples is presented in **Table 2**. The protein content of the bread prepared using FBRF (8.67%) was significantly ($p < 0.05$) higher than its content in reference bread (8.29%). The reason could be due to amount of protein added by FBRF, where it possesses greater protein content after fermentation [20]. This is in line with the findings reported by Chinma et al. [36] where wheat bread substituted with natural and yeast fermented

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rice bran protein concentrates had higher values of protein content than the control, whereas lipid content of bread containing 40% FBRF was significantly lower than the control and ash content was not significantly affected. Total and insoluble fibers in both bread samples were greater than their levels in the original flour with bread containing FBRF having higher values. However, the difference was not significant. It can be suggested that the increase in total fiber might be due to formation of resistance starch because of retrograding process after steaming. A similar study indicated that protein content of wheat bread supplemented with sourdough was higher than the control, without significant difference in ash and fiber contents [45]. Another study also demonstrated that sourdough treatment had no significant impact on chemical composition of whole wheat bread, except the increase in water soluble arabinoxylans [46].

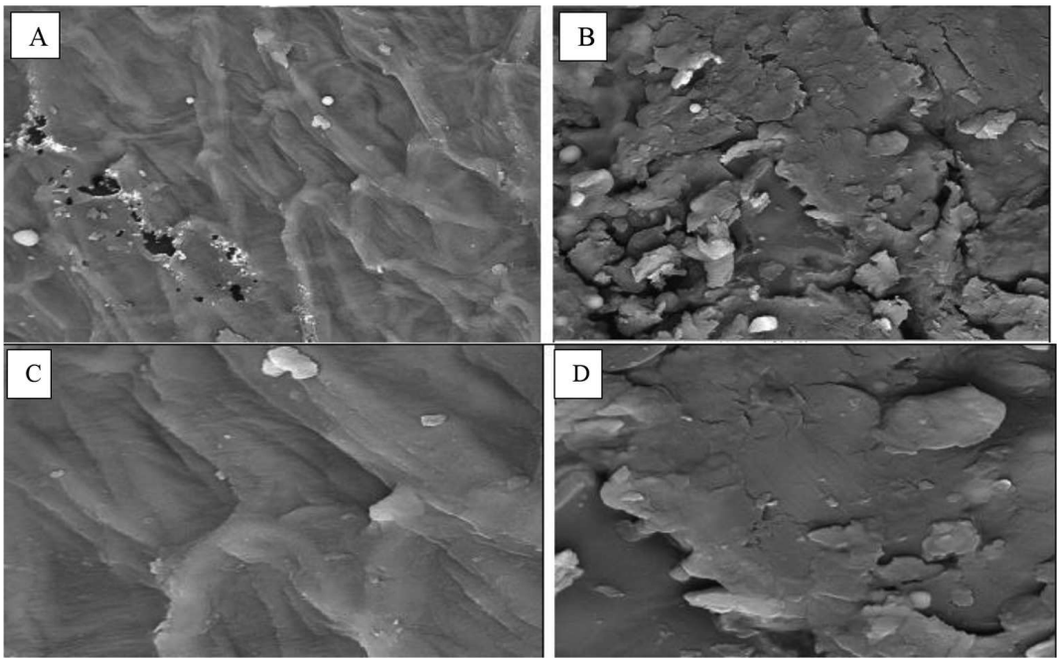


Figure 5. Microstructures of steamed brown rice bread (A and C) and steamed brown rice bread with 40% fermented brown rice flour (B and D).

Phytic acid is the most important anti-nutritive component in cereals, including brown rice due to its ability to bind divalent and trivalent minerals. It is also considered the main storage form of phosphorus and plays a part as an antioxidant factor [47]. Phytic acid content significantly decreased in both SBRBs compared to its content in the flour. Addition of FBRF significantly ($p < 0.05$) reduced phytic acid level in the treated bread (39.34 μg/g) compared to the reference bread (43.23 μg/g) (**Table 2**). Several factors are affecting phytic acid content during bread making such as, fermentation time and the pH that activate phytase enzyme, in addition to baking temperature [48]. Accordingly, the difference in phytic acid content in the SBRBs could be attributed to the supplemented phytase by FBRF. These results agreed with the reports of previous study that indicated application of sourdough with or without yeast significantly decreased phytic acid content compared to conventional yeast fermentation [48]. They also reported that sourdough increased mineral solubility in the whole wheat bread. Fermentation process of the batter may create the optimal pH for endogenous phytase, in addition to that secreted by the yeast, which helps

to breakdown phytic acid and increase the mineral content of the produced bread [7]. This fact might be approved in the current study, since the concentration of calcium, zinc, iron, magnesium and phosphorus significantly increased in both breads. It is noticeable that the increase in their contents was more pronounced in the treated bread, particularly calcium, phosphorus, magnesium and zinc contents. However, the increment was only significant in the zinc content (**Table 2**).

	BRF	SBRB	SBRB with 40% FBRF
Proximate composition			
Moisture (%)	9.77 ± 0.01	44.33 ± 0.11	44.41 ± 0.03
Protein (%)	7.70 ± 0.00[a]	8.29 ± 0.02[b]	8.67 ± 0.01[c]
Ash (%)	1.13 ± 0.01[a]	2.10 ± 0.00[b]	2.03 ± 0.03[b]
Lipid (%)	2.58 ± 0.02[a]	1.57 ± 0.01[b]	1.26 ± 0.03[c]
Nutritional value			
Soluble fibre (%)	1.12 ± 0.01[a]	0.15 ± 0.04[b]	0.13 ± 0.01[b]
Insoluble fibre (%)	1.35 ± 0.04[a]	3.46 ± 0.35[b]	3.85 ± 0.79[b]
Total fibre (%)	2.47 ± 0.01[a]	3.61 ± 0.32[b]	3.98 ± 0.80[b]
Phosphorus (%)	18.90 ± 0.13[a]	20.57 ± 0.04[b]	20.85 ± 0.54[b]
Phytic acid (μg/g)	128.71 ± 0.43[a]	43.23 ± 0.60[b]	39.34 ± 0.58[c]
Magnesium (μg/g)	19.70 ± 0.12[a]	22.38 ± 0.11[b]	22.49 ± 0.13[b]
Zinc (μg/g)	14.21 ± 0.35[a]	18.88 ± 0.16[b]	21.48 ± 2.07[c]
Calcium (μg/g)	105.75 ± 1.48[a]	136.95 ± 0.78[b]	135.30 ± 0.28[b]
Iron (μg/g)	5.09 ± 0.12[a]	6.64 ± 0.23[a]	6.57 ± 0.57[a]
Riboflavin (μg/g)	0.24 ± 0.00[a]	3.28 ± 0.34[b]	2.25 ± 0.35[b]
Nicotinic acid (μg/g)	6.87 ± 0.01[a]	2.76 ± 0.91[b]	4.02 ± 0.74[c]
Pyridoxine (μg/g)	0.12 ± 0.00[a]	0.05 ± 0.01[b]	0.07 ± 0.00[c]
γ-Oryzanol (μg/g)	262.40 ± 2.82[a]	96.10 ± 3.87[b]	76.55 ± 2.18[c]
α-Tocopherol (μg/g)	4.03 ± 0.01[a]	2.45 ± 0.08[b]	2.70 ± 0.05[b]
γ-Tocopherol (μg/g)	2.95 ± 0.02[a]	2.02 ± 0.04[b]	1.99 ± 0.07[b]
δ-Tocopherol (μg/g)	0.76 ± 0.00[a]	0.81 ± 0.04[b]	0.75 ± 0.01[a]
α-Tocotrienol (μg/g)	2.52 ± 0.05	ND	ND
γ-Tocotrienol (μg/g)	10.31 ± 0.16[a]	4.78 ± 0.01[b]	4.75 ± 0.35[b]
δ-Tocotrienol (μg/g)	1.22 ± 0.02[a]	0.81 ± 0.01[b]	0.83 ± 0.01[b]
Antioxidant activity			
TPC (mg GAE/g)	1.10 ± 0.01[a]	1.18 ± 0.00[b]	1.20 ± 0.01[b]
FRAP (mmol TE/g)	1.03 ± 0.01[a]	0.53 ± 0.01[b]	0.43 ± 0.01[c]

[a] Represented values are the means ± standard deviations of three replicates.
[b] Values with the same superscript letter in a row are not significantly different ($p > 0.05$).
[c] ND, not detected; GAE, gallic acid equivalent; TE, trolox equivalent.

Table 2. Proximate compositions, nutritional values and antioxidant activities of steamed brown rice breads (SBRBs).

The effect of FBRF on total phenolic content of SBRB is presented in **Table 2**. The substitution of BRF with 40% FBRF slightly increased TPC. This may be attributed to the difference between control bread and bread containing FBRF in their acidity, where the batter containing FBRF was more acidic than the control. This result is in line with reports of Liukkonen et al. [1] who reported that formation of acidity during sourdough process can increase phenolic substances, or due to the effect of enzymes supplemented by FBRF, which breakdown the cell wall and increased the extractable phenolic compounds. Also, Katina et al. [19] reported an increase in the extractable phenolic compounds with addition of sourdough. Even though, total phenolic content was higher in treated bread, its antioxidant activity (FRAP value) (0.43 mmol TE/g) was significantly ($p < 0.05$) lower than the control bread (0.53 mmol TE/g) (**Table 2**). This is in contrast with another study that reported substitution of natural and yeast fermented rice bran protein concentrates to wheat flour elevated its ferric reducing ability power and radical scavenging activity [36]. In fact, antioxidants in foods play a critical role in the prevention and regulation degenerative maladies in which oxidative destruction has been involved [49].

Brown rice is a good source of vitamin B and E as well as γ-oryzanol. The present study provided new facts on the effects of steaming process and the addition of fermented flour on vitamins and total γ-oryzanol contents in BRF (**Table 2**). Tocopherols and tocotrienols were reduced in SBRBs compared to their levels in the flour that could be due to the heat effect during steaming. Pascual et al. [50] reported that cooking of brown rice caused significant decrease in tocols. It has been reported that sourdough might change the levels of tocopherols and tocotrienols and that depends on sourdough process and the raw material [1, 19]. However, in this study, incorporation of FBRF did not have any significant ($p > 0.05$) change on their concentrations. Total γ-oryzanol content significantly decreased in control bread (96.10 μg/g) and bread containing 40% FBRF (76.55 μg/g) compared to its initial level in the flour (262.40 μg/g) (**Table 2**). This is in contrary to the reports of Pascual et al. [50] that heat treatment of brown rice such as parboiling and cooking did not decrease oryzanol content. Substitution with FBRF caused higher reduction in its concentration. From the obtained results, this compound is sensitive to heat and acidity, where the reduction was higher in the bread containing FBRF, which its batter was more acidic.

Riboflavin content significantly ($p < 0.05$) increased in steamed breads compared to flour. SBRB without FBRF had higher riboflavin content but was not significant. Pyridoxine and nicotinic acid contents significantly decreased in the bread samples and the reduction was greater in the control bread (**Table 2**). Previous study indicated that sourdough fermentation had no influence on riboflavin and pyridoxine contents [51]. It is well known that fermentation increases vitamin B group. Nevertheless, these compounds are sensitive to heat, which may explain their reduction in the bread samples. An early study reported that riboflavin is more stable to heat than the others [51]. Thus, the current investigation supported that where their concentrations in the breads were greater than in the flour (**Table 2**).

4. Conclusions

The results of the present study indicated that pre-fermentation of BRF is an appropriate way to relief the negative effects of the bran fraction and reduce some of the gluten-free bread

drawbacks. As the brown rice batter having 40% pre-fermented brown rice flour had a softer system, as indicated by lower viscoelastic properties and pasting parameters, The softer system could be stronger and more stable during proofing and steaming, which had greater ability to retain more air bubbles and as a result higher specific volume and improved texture of its bread were obtained. Besides, pre-fermented whole flour enhanced the nutritional value of the steamed bread. These improvements might relate to the enzyme actions activated during pre-fermentation, in addition to the acidification rate and microbial metabolism products. The differences in the SBRBs microstructures might explain the whole changes that occurred in the rheological, textural and nutritional properties of the treated bread, which still need to be investigated.

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