

Enhancement of Extruded Brown Rice Flour Functionality through Fermentation

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Abstract

To develop high quality extruded functional foods; their content of bioactive compounds should be maintained during and after processing. Therefore, this investigation aimed to study influence of extrusion temperatures on nutritional value of brown rice flour (BRF) and fermented brown rice flour (FBRF). BRF was firstly fermented according to the optimum conditions of moderate acidity (pH 5.5). BRF and FBRF were then extruded at 100, 125, and 150°C. The results indicated that total phenolic content, minerals, riboflavin, nicotinic acid, pyridoxine, tocopherols, crude protein and soluble fiber of extruded brown rice flour (EBRF) and extruded fermented brown rice flour (EFBRF) decreased after extrusion. However, EFBRF had significantly higher values of all the measured components and specifically at the highest extrusion temperature (150 °C). It can be established that applying fermentation prior to extrusion would be promising to obtain extruded brown rice products with enhanced functionality.

Keywords: Extrusion; Fermentation; Brown rice flour; Nutritional value; Moderate acidity

Abbreviations

BRF: Brown Rice Flour; FBRF: Fermented Brown Rice Flour; EBRF: Extruded Brown Rice Flour; EFBRF: Extruded Fermented Brown Rice Flour; TPC: Total Phenolic Content; FRAP: Ferric Reducing Ability Power

Introduction

Extrusion process became a popular technique, which is utilized to produce variety of foodstuffs, such as breakfast cereals, snack foods, and instant foods, due to its greater production, richer functionality and lesser operative expenses in comparison to the other conventional food processing [1,2]. It can be also used to enhance health properties of a food material. For example, extruded foods could enhance loss of body weight by addition of dietary fiber and formation of resistant starch or can influence heart health through antioxidants addition and fat reduction [3].

Moreover, extrusion could improve nutritional and functional properties of a product through destruction of anti-nutritional components, improve digestibility and bioavailability, increment of

soluble dietary fiber and total phenolics [4-6]. On the other hand, in some cases this process might reduce the nutritional components of a product which could affect its functionality. Numerous extrusion process factors such as screw configuration, energy input, barrel temperature, screw speed and moisture content of the raw material could positively or negatively affect the bioactive substances and then the nutritional quality of final extrudates [7].

Production of functional ready to eat foods or instants using extrusion might adversely impact their nutritional value through decreasing phenolics, antioxidants, amino acids and minerals [8-11]. These components are valuable and need to be maintained during processing to achieve functional food requirements. The negative effects of extrusion mainly depend on the harshness of the applied extrusion conditions and the raw material. Consequently, to achieve nutritionally balanced extruded ingredients; moderate extrusion conditions, such as low screw speed, low temperature and high moisture level is essential [11]. Besides, applying a prior process to preserve the mentioned components or increase their quantities or stability such as fermentation could be practically helpful.

Fermentation could be one of the ways to develop functional foods. It is a potential processing method in food technology, where fermented foods represent about one third of human diet worldwide [12]. This process is commonly used to produce high quality products from various food ingredients such as whole cereals [13,14]. Fermentation plays a critical part in enhancing nutritional value and ensuring security of a food material, in addition to improve safety by reducing anti-nutritional components [15]. Additionally, fermented foods could provide health benefits as indicated by some clinical investigations [16]. The biochemical alterations that take place during fermentation can enhance bioactivity, structure stability, digestibility, shortening of cooking time and extending shelf life of the end product [13].

Several studies have reported that nutritional value of millet, sorghum, wheat and rice products was considerably improved through fermentation at different conditions [13,17-19]. Also, fermented foods at moderate acidity (pH 6-5) would be convenient in terms of sensory properties, distinguished nutritional value and extended shelf life [20]. It is was found that nutritional value of fermented brown rice flour at pH (5.5) significantly improved by increasing its protein, phenolics, mineral, antioxidants and B vitamin contents [21]. It is known that fermentation is a primary food processing that needs to be coupled with other food processing techniques such as extrusion to enhance product qualities and to prepare ready to eat cereal based foods. Therefore, combining fermentation with extrusion; a new product could have distinctive nutritional and functional properties.

Starch- and protein-based constituents are the most used raw materials in extrusion process. Thus, Brown rice is a whole cereal and an example of starchy food. Its usage as a direct food is limited due to its dark color and hard texture and it is mainly consumed based on its polished form [22]. However, brown rice was reported to contain higher nutritional compounds than polished rice that should be exploited to prepare functional foods [23,24]. Accordingly, it can be ground to flour and modify its functionality through fermentation to enable its usage in ready to eat functional food preparations.

Certainly, understanding the fate of the bioactive compounds in food products using these processes became important to develop high quality functional foods. Indeed, there is limited knowledge about the effect of extrusion on bioactive components of fermented brown rice flour. Therefore, the objective of this study was to investigate the effect of extrusion temperatures on nutritional components of moderately acidic fermented brown rice flour.

Material and Methods

Participants

Whole rice grains (MR219) and Eagle baker's yeast (QS6540 2801 0001, China) were purchased from local market (Selangor, Malaysia). The used yeast was chosen according to our previous study, which demonstrated that fermented brown rice flour by using Eagle yeast recorded the highest values for the most measured nutrients [25]. The Foss Tecator (Cyclotech™ 1093, Hoganas, Sweden) was used to grind brown rice grains to flour passing through 500 µm sieve. Brown rice flour (BRF) was packaged under vacuum and stored at 4°C in polyethylene bags for further analysis. All the chemicals used were of analytical grade and were obtained from Merck (Darmstadt, Germany) and Sigma (Aldrich-USA).

Fermentation process

Fermentation of BRF was performed according to the optimized conditions of moderate acidity (pH 5.5). The optimum conditions of moderate acidification (6.23h, 32°C, and 1% baker's yeast concentration) were achieved using response surface methodology with central composite design as reported in our previous study [21]. The fermented dough was dried at 50 °C for 3h then ground to pass 500 µm sieve to obtain fermented brown rice flour (FBRF).

Extrusion process

Single screw extruder (KE 19 Extruder from Brabender, Germany) was used to evaluate the effect of extrusion temperatures on the nutritional properties of BRF and FBRF. The extrusion conditions were as follows: screw speed was fixed at 60 rpm, hopper speed was 30 rpm, the first zone temperature was fixed at 70°C, while the second, third and fourth zones temperatures were manipulated at 100, 125, and 150°C. The moisture content of the samples was adjusted to 25% by adding the calculated amount of distilled water. The screw speed was fixed at low speed (60 rpm) and moisture content was adjusted at 25% to minimize the destruction involved with higher screw speed and lower moisture content [11,26]. The extruded products were identified as extruded brown rice flour (EBRF) and extruded fermented brown rice flour (EFBRF).

Determination of proximate and fiber compositions

Moisture, protein, total lipid, soluble fiber and insoluble fiber contents were measured according to AOAC [27], while total ash was measured according to ISO 2171 method [28].

Determination of mineral and phytic acid contents

Calcium, magnesium, iron and zinc contents were determined according to the method of AOAC [29] by flame atomic absorp-

tion spectrophotometry AAS (Perkin Elmer Analyst 400, Shelton, USA). Whereas, phosphorus content was evaluated following the yellow method using spectrophotometer (PerkinElmer, Lambda 25 UV/VIS, Shelton, USA) AOAC [29]. The rapid method of phytic acid evaluation was performed according to Wu., *et al.* [30] with minor modifications in terms of extraction time, which was 3 h.

Determination of total phenolic content and antioxidant activity

Total phenolic content (TPC) was determined using folin-ciocalteu method according to Beta., *et al.* [31] with slight modifications in terms of extraction equipment and solvent composition. Briefly, a 4 mL of 80% methanol solution was mixed with 0.20g of samples, sonicated for 12 minutes at 25°C and then centrifuged at 2000g for 10 minutes. The extraction was repeated twice. Then total phenolic content (TPC) was estimated following the mentioned method. The assay of ferric reducing ability power (FRAP) was applied to determine the antioxidant activity of samples.

Determination of vitamins

Tocopherol, tocotrienol and total γ -oryzanol were measured using high pressure liquid chromatography (HPLC) equipped with fluorescence detector (Agilent Technologies 1200 Series, German) according to the method described by Aguilar-Garcia., *et al* [32]. Stock solutions of tocopherol, tocotrienol were prepared in accordance to Ye., *et al* [33].

Riboflavin, nicotinic acid, and pyridoxine were determined following the method described by AACC [34] with some modifications in terms of sample extraction. A 5 mL of 1N NaOH was used to hydrolyzed 0.5g of samples for 1 h at 50°C. Later the pH was adjusted to 6.8 using 0.1 M HCL [35]; whereas, 0.1N HCL was utilized for thiamine extraction for 30 min at 100 °C. The pH was adjusted to 6.8 using 0.1N NaOH after cooling down according to AOAC method 953.17 [27]. Then, the extracts were filtered through 0.45 μ m membrane filter into vials for HPLC analysis after centrifugation at 2000g for 15 minutes. High performance liquid chromatography equipped with UV detector (Waters 2489 UV/V detector and Empower software, USA) was used to determine vitamin B.

Statistical analysis

Data obtained were subjected to one way analysis of variance (ANOVA) and Tukey's multiple range tests with a confidence interval of 95% were used to report the significant differences between the data obtained.

Results and Discussion

Proximate and fiber compositions

The results of proximate and fiber compositions are shown in table 1. Protein, ash and soluble fiber contents of BRF increased after fermentation at moderate acidity. There was also a significant ($p < 0.05$) increase in protein and ash contents of EBRF and EFBRF with increasing barrel temperature. The increase in both protein and ash contents was more pronounced in EFBRF and the highest values (9.23% and 1.40%, respectively) were detected at the highest temperature (150°C). These results were not in agreement with those reported by Lampart-Szczapa., *et al.* [36], where significant decrease in the extracted protein upon extrusion of fermented and non-fermented lupin samples was observed.

Insoluble fiber content of EBRF significantly increased ($p < 0.05$) with the increase in barrel temperature to 150°C, whereas soluble fiber decreased with increasing temperature from 100 to 125°C, which later significantly increased with the increase in extrusion temperature to 150°C, however its value is still significantly lower than that of the control (BRF). There was a significant reduction in soluble fiber concentration of EFBRF from 2.33% to 0.37% at 100°C, which later increased to 0.46% and 0.66% at 125 and 150°C, respectively. It was observed that EFBRF had higher soluble fiber values than EBRF at 125°C and 150°C (Table 1). The pH of EFBRF is lower than EBRF that could increase fiber solubility, where it is indicated that numerous factors affect solubility of fiber, such as alkaline and acid treatments [11]. Insoluble fiber level of EFBRF significantly increased after extrusion by 75.3%, without a significant difference between the tested temperatures, but its level still lower than its content in EBRF at 150°C. Another study also indicated that total fiber content of barley increased upon extrusion process [6]. Also, an investigation of Wani and Kumar [37] reported an increase in total carbohydrates, fiber and protein contents of snacks after extrusion compared to the raw ingredients.

The increase in insoluble fiber of EBRF and EFBRF compared to their controls might be due to the formation of resistance starch. Starch gelatinization during extrusion followed by retrogradation process was implicated to form resistant starch that could lead to the increment in insoluble fiber [38], mainly in EBRF at 150°C. However, in terms of EFBRF, fermentation could lower or delay retrogradation process because of enzymatic degradation of starch [21], which led to a lesser formation of resistant starch. Lipid content of BRF decreased after fermentation, which further reduced

with the increase in extrusion temperature from 100°C to 150°C with EFBRF having higher lipid content. The reduction in lipid content of samples after extrusion could be due to development of lipid complexes with amylose or protein [39,40].

Mineral and phytic acid contents

It is reported that phytic acid has several biological activities, which include anticancer and antioxidant. However, it plays a major role as anti-nutritional factor, because of its ability to hinder

Sample	Moisture (%)	Protein (%)	Ash (%)	Lipid (%)	Insoluble fiber (%)	Soluble fiber (%)
BRF	9.76 ± 0.01	7.72 ± 0.01 ^f	1.12 ± 0.01 ^e	2.59 ± 0.01 ^a	1.35 ± 0.03 ^c	1.13 ± 0.01 ^b
FBRF	11.38 ± 0.37	8.72 ± 0.01 ^c	1.22 ± 0.01 ^d	2.57 ± 0.02 ^a	1.58 ± 0.04 ^c	2.33 ± 0.02 ^a
EBRF (100 °C)	10.51 ± 0.11	7.71 ± 0.01 ^f	1.31 ± 0.00 ^b	0.84 ± 0.01 ^c	2.52 ± 0.06 ^b	0.45 ± 0.33 ^d
EBRF (125 °C)	10.36 ± 0.07	7.81 ± 0.02 ^e	1.25 ± 0.01 ^{dc}	0.67 ± 0.01 ^d	2.80 ± 0.11 ^b	0.24 ± 0.00 ^e
EBRF (150 °C)	10.30 ± 0.00	8.11 ± 0.02 ^d	1.28 ± 0.00 ^c	0.21 ± 0.04 ^f	4.07 ± 0.06 ^a	0.42 ± 0.03 ^d
EFBRF (100 °C)	11.29 ± 1.45	8.82 ± 0.01 ^b	1.31 ± 0.01 ^b	0.97 ± 0.01 ^b	2.77 ± 0.20 ^b	0.37 ± 0.06 ^d
EFBRF (125 °C)	11.79 ± 0.34	8.77 ± 0.01 ^b	1.31 ± 0.02 ^b	0.84 ± 0.02 ^c	2.70 ± 0.06 ^b	0.46 ± 0.04 ^d
EFBRF (150 °C)	10.36 ± 0.36	9.23 ± 0.02 ^a	1.40 ± 0.01 ^a	0.44 ± 0.01 ^e	2.71 ± 0.30 ^b	0.66 ± 0.05 ^c

Table 1: Effect of extrusion temperatures on proximate and fiber compositions of fermented and non-fermented brown rice flours.

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

Values with the same superscript letter within a column are not significantly different (p > 0.05).

BRF: Brown Rice Flour; FBRF: Fermented Brown Rice Flour; EBRF: Extruded Brown Rice Flour; EFBRF: Extruded Fermented Brown Rice Flour

Sample	Phosphors (%)	Zinc (µg/g)	Iron (µg/g)	Magnesium (µg/g)	Calcium(µg/g)	Phytic acid (µg/g)
BRF	15.85 ± 0.25 ^b	14.24 ± 0.35 ^c	5.09 ± 0.12 ^g	19.73 ± 0.03 ^b	106.30 ± 0.85 ^e	124.37 ± 0.16 ^c
FBRF	19.87 ± 0.05 ^a	19.08 ± 0.11 ^a	5.74 ± 0.14 ^f	22.86 ± 0.05 ^a	121.85 ± 0.64 ^c	75.56 ± 1.85 ^e
EBRF (100 °C)	18.36 ± 0.29 ^a	13.71 ± 0.38 ^c	6.14 ± 0.14 ^e	22.77 ± 0.02 ^a	116.40 ± 0.14 ^d	252.05 ± 1.00 ^a
EBRF (125 °C)	18.36 ± 0.40 ^a	14.23 ± 0.09 ^c	6.88 ± 0.19 ^d	22.66 ± 0.03 ^a	125.35 ± 0.64 ^b	201.41 ± 2.53 ^d
EBRF (150 °C)	19.84 ± 0.05 ^a	14.90 ± 0.14 ^c	13.59 ± 0.01 ^b	22.72 ± 0.06 ^a	124.00 ± 0.71 ^b	239.58 ± 0.42 ^b
EFBRF (100°C)	20.22 ± 0.02 ^a	17.31 ± 0.12 ^b	7.49 ± 0.38 ^c	22.83 ± 0.01 ^a	123.35 ± 0.21 ^b	62.84 ± 2.26 ^f
EFBRF (125°C)	20.09 ± 0.19 ^a	17.12 ± 0.13 ^b	7.57 ± 0.51 ^c	22.87 ± 0.00 ^a	123.70 ± 0.42 ^b	35.31 ± 0.90 ^g
EFBRF (150°C)	20.40 ± 0.14 ^a	17.67 ± 0.02 ^b	16.73 ± 0.96 ^a	22.63 ± 0.04 ^a	138.70 ± 0.99 ^a	35.83 ± 0.37 ^g

Table 2: Effect of extrusion temperatures on mineral and phytic acid contents of fermented and non-fermented brown rice flours.

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

Values with the same superscript letter within a column are not significantly different (p > 0.05).

BRF: Brown Rice Flour; FBRF: Fermented Brown Rice Flour; EBRF: Extruded Brown Rice Flour; EFBRF: Extruded Fermented Brown Rice Flour.

the availability of minerals [8]. From the results (Table 2) extrusion cooking decreased the amount of phytic acid in EFBRF and the decrease was significantly (p < 0.05) greater with increasing the temperature to 150°C (35.38 µg/g). Even though there was a reduction in phytic acid contents of EBRF with increasing barrel temperature up to 150°C, its values at all the examined temperatures were significantly greater than that of its counterparts (BRF).

It was reported that fermentation of wheat and rye bran was effective in breaking down anti-nutritive components such as phytic acid [4], which was observed in this study and the reduction was greater after extrusion. Abd El-Hady and Habiba, [41] reported that soaked legume seeds in acid solution followed by cooking significantly decreased phytic acid content.

The current study showed significant ($p < 0.05$) increase in phosphorus, zinc, iron, magnesium and calcium after fermentation. The same trend was observed for phosphorus, calcium, and iron after extrusion and their highest values were recorded at 150°C with EFBRF having higher values. However, zinc content significantly decreased in EBRF, without a significant difference between the tested temperatures, while its content in EBRF decreased at 100°C, but the decrease was not significant and then increased again with increasing extrusion temperature reaching its same level in the control. There was not any change in magnesium content (Table 2). A study also pointed out that there was an increment in the overall mineral content and a reduction in phytic acid content of extruded snacks [37]. Conversely, another study reported that extrusion did not have any significant impact on the levels of these minerals in pea and kidney bean seeds, except the increment in iron content [42].

The results of this investigation can be explained by the fact that several substances in cereals may decrease the solubility and availability of minerals, such as phytic acid and fiber. It was found that the phytate degradation after extrusion was valued to be up to 90% that causes increment in phosphorus and magnesium solubility [10]. Thus, the increase in mineral contents particularly in EFBRF can be correlated with the reduction in phytic acid content or the occurred alterations in fiber compositions.

Total phenolic content and antioxidant activity

Fermentation significantly ($p < 0.05$) increased TPC in FBRF up to 13%. After extrusion, there was no change in its content in EFBRF at 100 and 125°C, then significantly increased from 1.23 to 1.27 mg GAE/g at 150°C. Also, TPC contents of EBRF significantly ($p < 0.05$) increased compared to their counterparts (BRF) (Table 3). These results are consistent with that of previous study which reported significant increase in TPC of extruded barley flour at 150°C with 20% moisture content [5]. The higher increment of TPC in EFBRF compared to EBRF in the present study might be due to the breakdown of the cell wall matrix and release of insoluble phenolic components by enzymatic action during fermentation followed by shear during extrusion [43]. Recently, it was found that enzymatic extrusion (using α -amylase with different concentrations) considerably improved the retention of free and total phenolics in brown rice in comparison to traditional extrusion [44]. Moreover, the creation or enzymatic transformation of different bioactive components may take place during fermentation [13] that could reflect the higher TPC content of EFBRF compared to EBRF. It was also indicated that

extrusion significantly reduced total phenolic and total flavonoid contents in hulled barley grits and buckwheat seeds [10,45]. The reduction in total phenolic contents was also explained by the fact, that breakdown, polymerization or decarboxylation of some phenolic components could occur during extrusion [7].

Ferric reducing ability power values of BRF significantly increased after fermentation ($p < 0.05$). Nevertheless, heating during extrusion significantly reduced this ability for both EBRF and EFBRF in comparison to their counterparts. Even though, TPC was increased after extrusion for both EBRF and EFBRF, their antioxidant abilities reduced with EFBRF gaining higher values (Table 3). A study also indicated a lower antioxidant capacity of dark-red beans compared to coloured cream and black brown beans, although the dark-red bean extrudates exhibited greater TPC [46]. Thus, antioxidant ability of extruded products is dependent not only on the concentration of bioactive substances but also on their compositions and the raw material. In some cases, antioxidant activity was reported to increase with increasing barrel temperature in extruded products [37,47]. The greater antioxidant ability of EFBRF could be due to its higher content of bioactive components or due to the changes occurred in the composition of BRF as affected by enzymatic actions during fermentation. Brown rice is a significant source of several bioactive components specifically phenolics which play a major role as antioxidants. As a result, they should be taken into consideration, when brown rice or its products processed as functional foods or functional food ingredients.

Vitamin contents

Tocopherols and tocotrienols are naturally found in cereal grains and their biological activities are well recognized. Tocols in EBRF and EFBRF significantly ($p < 0.05$) decreased with increasing extrusion temperature (Table 4). α -Tocopherol and α -tocotrienol were not detected in EBRF, but they were detected in EFBRF. It was demonstrated that both α -tocopherol and α -tocotrienol were the least sensitive to heat among the other forms [8]. It seemed that high temperature short time cooking decreased the stability of tocols. Previous study also reported a 30% decrease in tocopherols and tocotrienols in barley, oat, wheat, buckwheat and rye after extrusion [8]. These components are fat soluble, thus the reduction in their concentrations could be associated with lipid degradation [48]. In this study, the lipid contents significantly decreased with increasing extrusion temperatures and the decrease was more pronounced in EBRF samples as the level of tocols.

Sample	TPC (mg GAE/g)	FRAP (mmol TE/g)
BRF	1.10 ± 0.01 ^e	1.02 ± 0.01 ^b
FBRF	1.24 ± 0.01 ^b	1.19 ± 0.01 ^a
EBRF (100 °C)	1.18 ± 0.00 ^c	0.22 ± 0.01 ^f
EBRF (125 °C)	1.17 ± 0.00 ^c	0.31 ± 0.06 ^e
EBRF (150 °C)	1.14 ± 0.00 ^d	0.27 ± 0.00 ^f
EFBRF (100 °C)	1.23 ± 0.00 ^b	0.85 ± 0.02 ^c
EFBRF (125 °C)	1.23 ± 0.01 ^b	0.64 ± 0.02 ^d
EFBRF (150 °C)	1.27 ± 0.01 ^a	0.61 ± 0.02 ^d

Table 3: Effect of extrusion temperatures on total phenolic content and antioxidant activity of fermented and non-fermented brown rice flours.

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

Values with the same superscript letter within a column are not significantly different (p>0.05).

BRF: Brown Rice Flour; FBRF: Fermented Brown Rice Flour; EBRF: Extruded Brown Rice Flour; EFBRF: Extruded fermented Brown Rice Flour; TPC: Total Phenolic Content; FRAP: Ferric Reducing Ability Power; GAE= Gallic Acid Equivalent; TE: Trolox Equivalent.

Component	BRF	FBRF	EBRF 100 °C	EBRF 125 °C	EBRF 150 °C	EFBRF 100 °C	EFBRF 125 °C	EFBRF 150 °C
Riboflavin (µg/g)	0.24 ± 0.01 ^g	8.92 ± 0.10 ^b	2.99 ± 0.02 ^e	4.80 ± 0.00 ^d	2.15 ± 0.30 ^f	8.45 ± 0.35 ^c	10.57 ± 0.52 ^a	9.94 ± 0.34 ^a
Nicotinic acid (µg/g)	6.87 ± 0.03 ^c	7.52 ± 0.07 ^b	0.27 ± 0.03 ^e	0.50 ± 0.03 ^e	1.49 ± 0.07 ^d	7.55 ± 0.64 ^b	7.80 ± 0.23 ^a	7.83 ± 0.01 ^a
Pyridoxine (µg/g)	0.12 ± 0.01 ^c	0.61 ± 0.01 ^b	0.67 ± 0.06 ^b	0.62 ± 0.17 ^b	0.61 ± 0.04 ^b	0.85 ± 0.07 ^a	0.90 ± 0.03 ^a	0.89 ± 0.01 ^a
γ-oryzanol (µg/g)	262.40 ± 2.82 ^a	206.33 ± 0.46 ^b	60.63 ± 1.38 ^d	43.70 ± 3.1 ^g	24.06 ± 0.00 ^h	73.53 ± 1.75 ^c	57.95 ± 3.86 ^e	45.60 ± 1.10 ^f
α-tocopherol (µg/g)	4.03 ± 0.01 ^a	3.90 ± 0.01 ^b	ND	ND	ND	2.78 ± 0.01 ^c	2.76 ± 0.01 ^c	2.37 ± 0.04 ^d
γ-tocopherol (µg/g)	2.90 ± 0.16 ^a	2.86 ± 0.02 ^a	1.34 ± 0.01 ^d	1.34 ± 0.04 ^d	1.38 ± 0.00 ^d	1.98 ± 0.01 ^b	1.87 ± 0.01 ^b	1.52 ± 0.02 ^c
δ-tocopherol (µg/g)	0.77 ± 0.01 ^a	0.78 ± 0.01 ^a	0.56 ± 0.01 ^c	0.58 ± 0.01 ^c	0.55 ± 0.01 ^c	0.64 ± 0.02 ^b	0.63 ± 0.00 ^b	0.57 ± 0.01 ^c
α-tocotrienol (µg/g)	2.52 ± 0.05 ^a	2.59 ± 0.01 ^a	ND	ND	ND	2.54 ± 0.01 ^a	2.36 ± 0.01 ^b	ND
γ-tocotrienol (µg/g)	10.32 ± 0.16 ^b	11.08 ± 0.13 ^a	2.63 ± 0.07 ^f	2.44 ± 0.00 ^f	2.97 ± 0.00 ^e	6.29 ± 0.22 ^c	5.80 ± 0.06 ^c	3.74 ± 0.18 ^d
δ-tocotrienol (µg/g)	1.23 ± 0.01 ^b	1.37 ± 0.01 ^a	0.64 ± 0.04 ^e	0.61 ± 0.00 ^e	0.53 ± 0.01 ^f	0.87 ± 0.04 ^c	0.80 ± 0.02 ^d	0.64 ± 0.03 ^e

Table 4: Effect of extrusion temperatures on vitamins and γ-oryzanol contents of fermented and non-fermented brown rice flours.

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

Values with the same superscript letter within a row are not significantly different (p > 0.05).

BRF: Brown Rice Flour; FBRF: Fermented Brown Rice Flour; EBRF: Extruded Brown Rice Flour; EFBRF: Extruded Fermented Brown Rice Flour; ND: Not Detected

The initial content of γ -oryzanol of the control (BRF) (266.40 $\mu\text{g/g}$) was significantly ($p < 0.05$) decreased by fermentation and further by extrusion temperature (Table 4). Nevertheless, extruded fermented sample possessed higher values of γ -oryzanol than non-fermented extruded sample. These findings corroborate with that reported by Liu, *et al.* [49], who reported significant decrease in γ -oryzanol with increasing temperature in texturized rice supplemented with 4% rice bran.

The levels of riboflavin, nicotinic acid and pyridoxine were significantly increased after fermentation (Table 4). Riboflavin content of EBRF and EFBRF increased after extrusion barrel temperature elevated from 100°C to 125°C and later decreased with increasing temperature to 150 °C. However, EBRF and EFBRF still had higher values of riboflavin and pyridoxine than the controls (BRF and FBRF) except EFBRF at the extrusion temperature of 100°C. Extrusion also increased the level of nicotinic acid in EFBRF, but its content significantly ($p < 0.05$) reduced in EBRF. It is interesting to note that EFBRF at 125 and 150°C extrusion temperatures had higher values of riboflavin, nicotinic acid and pyridoxine than EBRF. These findings further supported other study by Athar, *et al.* [50] who indicated high retention of B vitamin group (44 - 62%) during extrusion and that such retention depends on the barrel length. These authors also reported that the retention of vitamins during extrusion cooking is not related to the original concentration, but it depends on the stability of the vitamin and the type of raw material. For instance, riboflavin is more stable to heat effect than thiamine and pyridoxine. Anuonye, *et al.* [51] reported a 6% decline in riboflavin and 86.36% in pyridoxine during extrusion of Acha (*Digitaria exilis*)/soy bean mixture. It is possible that fermentation conditions may elevated the stability of these vitamins against heat or increase its extractability during measurements. It was pointed out that the extent of vitamins degradation is subjected to different extrusion parameters that affect their stability such as moisture content, temperature, resident time and pH [7,52]. Since, the conditions of extrusion were the same for both samples, thus the difference in vitamin contents of the extruded samples could be due to the difference in the pH value.

Conclusion

The current study revealed that fermentation process combined with extrusion could be promising to maintain and enhance the functionality of extruded brown rice products. Extruded fermented brown rice flour possesses higher nutritional value in comparison to extruded brown rice flour without fermentation. The results presented that fermentation prior to extrusion might increase the

stability of the macro and micro-components during extrusion or increase their extractability during measurements due to enzyme degradation through fermentation. This positive effect might also increase their availability to the body, which still needs to be investigated.

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Conflict of Interest

No Conflict of interest.

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