

Effects of Milling, Germination Temperature and Time on Nutrients, Bioactive Compounds and Pasting Properties of FARO 44 Brown Rice

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Abstract

Effects of milling, germination temperature and time on nutrients, bioactive compounds and pasting properties of FARO 44 brown rice (UBR) were evaluated. Germinated brown rice (GBR) samples were obtained by germinating UBR at 30 and 40 °C for 12 and 36 h. Nutrients, bioactive compounds and pasting properties of the GBR were analyzed and compared to the controls which comprised its counterparts from UBR and non-germinated parboiled milled rice (UMR). Results showed that milling reduced the total dietary fiber (36.59%), fat (59.35%), iron (72.69%), calcium (52.83), phosphorus (74.12%), selenium (47.32%), vitamins B₁ (75.76%), B₂ (51.20%), E (82.61%), total phenolic content (TPC) (50.50%), and γ -amino butyric acid (GABA) (71.43%) contents of UBR. Germination enhanced nutrients, bioactive compounds, and modified the pasting properties of UBR. Ash (1.32%), total dietary fiber (8.20%), protein (10.99%), vitamins and minerals (mg/100 g): B₂ (1.66), B₃ (0.80), B₆ (1.04), E (1.38), calcium (106.00), magnesium (43.58), phosphorus (69.60), iron (4.65), zinc (1.70), and selenium (82.20 μ g/100 g), TPC (1.01 mgGAE/g), DPPH (0.05 μ g/100 g), GABA (2.10 mg/100 g) of UBR decreased significantly ($p < 0.05$) in UMR but increased significantly in GBR. The observed percentage increments at 36 h of germination were 104.55%, 17.07%, 26.84%, 98.715, 31.18%, 19.22%, 173.58%, 432.35%, 138.56%, 44.92%, 43.565, 9460.00%, and 294.76% for ash, total dietary fiber, protein, iron, zinc, selenium, calcium, magnesium, phosphorus, vitamin E, TPC, DPPH, and GABA, respectively. Protein, amino acids, ash, calcium, magnesium, selenium, vitamins B₃ and E and pasting temperature decreased significantly ($p < 0.05$) with increase in germination temperature while GABA, TPC, iron, zinc, amylose, total reducing sugars, pasting viscosities, and peak time increased as the germination temperature increased. Germination temperature-time of 40 °C, 36 h was recommended due to its optimum bioactive compounds' contents.

Keywords

Antioxidant, Gamma amino butyric acid, Milled, Mineral, Parboiled rice, Vitamin

Introduction

Rice is the second most consumed cereal grain after wheat [1]. Rice is harvested as paddy rice and its major layers comprise the husk or hull, bran, germ, and endosperm. The removal of only the husk or hull which is the inedible outer layer results in brown rice. Rice milling detaches the bran and germ from the rice caryopsis (brown rice) results in milled rice/white rice. Milled rice, which is

predominantly rice endosperm, is the type of rice that most people eat.

The main reason for rice milling is to extend its shelf life. When the hull (which is normally inedible) is detached from the rice paddy, the unsaturated fatty acids contained in the bran and germ will be attacked by oxygen resulting in oxidative rancidity [2] or when brown rice is exposed to moisture, the free fatty acids could be attacked by the endogenous *lipases* in the bran resulting in hydrolytic rancidity [3]. The end point of both oxidative and hydrolytic rancidity is a product with off flavor. Milling will prevent these biochemical reactions and thus the shelf life of the grain will be prolonged.

Unfortunately, the bran and the germ which are removed during milling contain the majority of dietary fiber, proteins, fats, minerals, vitamins, bioactive compounds, and cellular antioxidants of rice grain [4, 5]. Starch (85 - 95%) predominates rice endosperm [6]. Thus, rice milling does not only remove the bran and the germ but results in losses of nutrients, bioactive compounds, and beneficial antioxidants which have made people clamor for consumption of brown rice. However, the nutty flavor, hard to cook and chewy texture and poor taste of brown rice have prevented its consumption despite its nutritional and health benefits [7, 8].

The current approach to consume rice with this bran layer without encountering the above-mentioned problems/constraints is by germination to obtain GBR. Germination, which is a bio-modification process employs endogenous enzymes in the bran layer such as *lipase*, *protease*, and *α -amylase* to degrade lipid, protein, and starch, respectively [4]. Germination also increases nutrients such as minerals, proteins, amino acids, and vitamins and helps in the synthesis of bioactive compounds whose examples include vitamin E (tocopherol and tocotrienol), phenolics, flavonoids, dietary fiber, GABA, and antioxidants [3, 5]. According to literature, the quantity of nutrient and bioactive compounds in GBR are dependent on a number of factors such as rice cultivar, the temperature, and duration of germination [9].

FARO 44 rice cultivar is cultivated in Africa and different parts of the world [10]. It is presently the most cultivated rice cultivar in Nigeria owing to its shorter time of maturity (90 days), long slender grain size, high yield even under low farm management, good tolerance to diverse types of soil, and good competitiveness with weed [11]. Currently its UMR and UBR are produced and nutritional data of its UMR and UBR are available while GBR is not produced and data on its nutrients and bioactive compounds are lacking. Previous work on this rice cultivar investigated how germination and milling affect microbial, sensory, functional, and cooking qualities [12]. Researchers who worked on nutrient and bioactive compounds of GBR from other rice cultivars focused on the effect of germination time while literature on the effect of germination temperatures are scarce. Therefore, in this study, the production of UMR, UBR, and GBR from FARO 44 rice cultivars were carried out. Their nutrients, bioactive compounds, and pasting properties were analyzed and compared. Also, the effects of germination temperatures and times on these nutrients, bioactive compounds, and pasting properties were evaluated.

Materials and Methods

Material

FARO 44 paddies were harvested by November 2022 from rice farm in Ikwo, Ebonyi State, Nigeria.

Methods

Production of germinated brown rice

Before commencement of the germination process, the rice paddies were spread on the table at the ambient temperature (29 ± 2 °C) for a period of 40 days to exceed the dormancy period. The method of Ukpong et al. [13] was used for rice germination. The process steps included dehusking the paddies in a rice husker (SATAKE, Australia), soaking in 0.1% NaClO (30 min), rinsing 5 times with distilled water and steeping in distilled water (1 part grain: 10 parts water, w/v) at ambient temperature (29 ± 2 °C) for 24 h. Decanting of the steep water was done at every 6 h interval during steeping to prevent fermentation. The steep water was decanted at the end of the steeping period. This was followed by spreading the dehusked grains thinly on a clean jute bag that was previously dampened with distilled water and covering the grains up with another clean jute bag after which it was positioned in stainless-steel pan, the pan together with its content was introduced into a cabinet incubator (England, Gulfex Scientific. Model: DNP-9082,) for the grains to germinate at 30 °C and 40 °C for 12 to 36 h. A uniform relative humidity was achieved during germination by sprinkling distilled water at every 6 h interval. Germination was followed by drying (50 °C) in oven (England, Gulfex Scientific. Model: DHG 9202) to below 13% moisture and storage in plastic tin until when they were analyzed.

Production of non-germinated parboiled milled rice and brown rice

Laboratory rice husker (SATAKE, Australia) was used to dehusk the paddy rice to obtain the UBR. For UMR, the paddy was soaked in a water bath (40 °C) after which the paddy rice was steamed for 10 min. When this was completed, the water was decanted and the paddy was dried in oven (England, Gulfex Scientific. Model: DHG 9202) first for 10 min at 120 °C and afterward at 78 °C to moisture content below 13%. A rice husker (SATAKE, Australia) was used to dehusk the paddy and was followed by milling in laboratory rice mill (China, LT JIM-2099) to obtain the UMR. UBR and UMR were used as controls.

Determination of proximate composition and energy value

A hammer mill was used to mill the rice grains to flour and proximate composition and gross energy were determined on the rice flour by the method of AOAC [14]. Ash, moisture (drying to constant weight in oven), fat (by extraction using petroleum ether), nitrogen (N) by Kjeldahl method and protein (6.25 multiplied by N), total carbohydrate (by treatment with phenol and tetraoxosulphate (vi) acid), and total dietary fiber (by treatment with enzymes) were all determined. Atwater formula was used to calculate the gross energy value where the fat, carbohydrate and protein contents were multiplied by

9.0 kCal, 4.0 kCal and 4.0 kCal, respectively, and the results were summed up and multiplied by 4184 J.

Determination of mineral composition

The procedure of AOAC [14] was employed to determine the contents of selenium, iron, phosphorus, zinc, calcium, and magnesium in Atomic Absorption Spectrometer (Model 210-VPG).

Determination of composition of vitamins

The procedure of AOAC [14] was used to determine Vitamin E, Vitamin B₁, Vitamin B₂, Vitamin B₃, and Vitamin B₆ contents. The extract solution of each of the vitamins was read in Spectrophotometer (England, Genway 6305) at the following wavelengths: 520 nm for Vitamin E; 360 nm for Vitamin B₁; 510 nm for Vitamin B₂; 420 nm for Vitamin B₃; and 450 nm for Vitamin B₆.

Amino acid analysis of the samples

Methanol was mixed with Chloroform in the ratio of 1:2. The resulting mixture was used to de-fat the samples. Amino Acid Analyzer (Serial no.704520, Model: 120A, Applied Biosystems Inc., USA) was used to determine the amino acids contents by the method of AOAC [15].

Determination of bioactive compounds and antioxidant compositions of the samples

The total antioxidant activity, TPC, GABA composition, and total flavonoid content (TFC) were determined. Extraction for total antioxidant activity, TPC, and TFC determinations was done using 95% ethanol solution. The TPC was determined by the method of Likittrakulwong et al. [16]. Gallic acid was used as standard. The procedure of Jirapa et al. [17] was adopted for determination of TFC. Catechin was used as standard. DPPH (2, 2-diphenyl-1-picrylhydrazyl) procedure described by Munarko et al. [8] was adopted for determination of antioxidant activity. GABA was measured by gradient run using HPLC (Model 363, Varian, Inc. Scientific Instruments, USA) as described by Thitinunsomboon et al. [18]. Two mobile phases, the first was made of a mixture of 0.1 M phosphate buffer and 0.1 M sodium citrate (ratio of 4:1, respectively), and the second ethanol, were used for the gradients run.

Determination of total starch, amylose, and reducing sugar compositions

Procedures of AOAC [14] were employed to determine the total starch and total reducing sugar compositions. The ISO [19] method was used to determine the amylose content. A 0.1 g of the sample, standard or blank was measured and mixed with 95% ethanol (1 ml) and 1 M sodium hydroxide (9 ml) followed by boiling for 20 min in water bath. Furthermore, 0.5 ml of the sample extract, blank or standard, 5% acetic acid (0.1 ml) and iodine (0.2 ml) were mixed in 10 ml test tube and distilled water was added until it reached the 10 ml mark. This was followed by vortex mixing after which the absorbance read using spectrophotometer (England, Genway 6305) at the wavelength of 720 nm against the blank. The composition of amylose was extrapolated from the calibration curve which was prepared with standard graded amylose (Germany, Fluka Chemicals).

Determination of pasting properties of the samples

The flours were sieved through the mesh size of 0.15 mm. Rapid Visco Analyzer (RVA-4 Model, Newport Scientific Warriewood, Australia) was used to analyze and read the pasting temperature, peak time, peak viscosity, final viscosity, trough viscosity, setback viscosity, and breakdown viscosity according to the procedure described by Ukpong et al. [20].

Statistical analysis

Each analysis was done in triplicates. Analysis of Variance was done using R-software (R×64 3.4.2) and Fisher's least significant difference test was used for means separation at $p < 0.05$.

Results and Discussion

Effects of milling, germination temperature, and time on proximate composition and energy content

Table 1 shows the effects of milling, germination temperatures, and times on the proximate composition and energy content of FARO 44 brown rice. The contents were: protein (10.99%, 10.16%, and 11.237 - 13.94%); ash (1.32%, 1.26%, and 1.32 - 2.70%); total dietary fiber (8.20%, 5.20%, and 8.20 - 9.60%); fat (2.46%, 1.00%, and 2.91 - 3.45%); mois-

Table 1: Proximate composition and energy value as affected by germination temperatures and durations.

Sample	Proximate Composition (%) and Energy Value (J)						
	Protein	Ash	Dietary Fiber	Fats	Moisture	Total CHO*	Energy (J)
UMR	10.16 ± 1.01 ^c	1.26 ± 0.04 ^c	5.20 ± 0.91 ^c	1.00 ± 0.00 ^c	10.46 ± 1.21 ^{bc}	77.15 ± 3.00 ^a	85703.35 ± 27.30 ^c
UBR	10.99 ± 1.05 ^{bc}	1.32 ± 0.04 ^b	8.20 ± 0.87 ^b	2.46 ± 0.05 ^b	12.50 ± 1.24 ^a	72.49 ± 2.71 ^b	85281.82 ± 25.50 ^d
Germination at 30 °C							
G ₁₂ T ₃₀	12.03 ± 1.11 ^b	1.33 ± 0.05 ^b	8.90 ± 1.01 ^{ab}	3.40 ± 0.07 ^a	10.63 ± 1.11 ^{bc}	71.53 ± 2.21 ^b	87282.30 ± 30.50 ^a
G ₃₆ T ₃₀	13.94 ± 1.20 ^a	2.70 ± 0.10 ^a	8.98 ± 0.98 ^{ab}	2.98 ± 0.11 ^{ab}	10.98 ± 1.16 ^b	60.65 ± 2.58 ^d	77794.26 ± 14.40 ^f
Germination at 40 °C							
G ₁₂ T ₄₀	11.27 ± 0.99 ^b	1.32 ± 0.08 ^b	8.20 ± 1.11 ^b	2.91 ± 0.12 ^{ab}	10.31 ± 1.08 ^c	71.96 ± 3.01 ^b	85911.48 ± 20.70 ^b
G ₃₆ T ₄₀	11.98 ± 1.00 ^b	2.64 ± 0.14 ^a	9.60 ± 1.20 ^a	3.45 ± 0.34 ^a	10.67 ± 1.01 ^{bc}	64.00 ± 2.46 ^c	80136.36 ± 17.50 ^e

Note: *Carbohydrate. Values are means of triplicates (mean ± SD). Values with the same superscripts in each column are not significant difference at $p < 0.05$. UMR = ungerminated parboiled milled rice; UBR = ungerminated brown rice; GT = germinated brown rice; subscripts 12 and 36 are germination durations (h); subscripts 30 and 40 are the temperatures of germination (°C).

ture (12.50%, 10.46%, and 10.31 - 10.98%); total carbohydrate (72.49%, 77.15%, and 60.65 - 71.96%), and energy (85281.82 J, 85703.35 J, and 77794.26 - 87282.30 J) in UBR, UMR, and GBR, respectively.

Protein was significantly ($p < 0.05$) lower in UMR than UBR which could possibly be caused by the removal of the bran from UMR at the time of milling [21, 22]. Significantly ($p < 0.05$) higher protein content was also observed in GBR than UBR though both contain the bran, and this could be as a result of hydrolysis of proteins or catabolism of protein and bound protein molecules to free amino nitrogen and peptides at the time of germination [23]. Higher quantities of protein in GBR than UBR agree with previous reports [24, 25]. These values were, however, higher than previous reports of 6.54 to 9.99% [25] and 8.56 to 12.52% [24] but less than the range of 10.16 to 16.21% reported for FARO 57 cultivar [26]. This disparity could be due to variation in cultivar, agro-ecological zone, type of fertilizer, or type of soil [21]. Protein was significantly ($p < 0.05$) higher at germination temperature of 30 °C than 40 °C suggesting that proteolytic enzymes perform better at 30 °C. This agrees with the previous report [26]. As the time of germination increased from 12 h to 36 h, protein content also increased which could possibly be ascribed to hydrolytic breakdown of the protein with increase in germination time [27].

Ash content of this study was higher than that of Indonesian rice (1.29 to 1.44%) [28] but lower than that of FARO 57 rice (1.26 to 3.92%) [26]. Again, the disparity could be due to variation in cultivar, type of fertilizer, or type of soil [23]. Significantly ($p < 0.05$) higher ash content in UBR than UMR was observed which could be as a result of the effect of milling [29]. Rice bran which was removed during milling contains most of the ash content of rice grain [3]. GBR had significantly higher ash content than UBR and this could be due to breakdown of the mineral chelator, phytic acid by *phytase* during germination [30]. Ash indicates the composition of dietary minerals in a food [39]. Like protein, the content of ash was significantly ($p < 0.05$) lower at germination temperature of 40 °C than 30 °C suggesting lower *phytase* activity at high temperature of germination. Increase in germination duration from 12 h to 36 h also led to significant ($p < 0.05$) increase in ash content which could be due to increase in the activity of *phytase* as the germination time increased [31]. This agrees with previous reports [24, 26].

Total dietary fiber was significantly ($p < 0.05$) lower in UMR than UBR which could be ascribed to the loss of dietary fiber in UMR along with the bran during the period of milling [29]. GBR also had significantly higher dietary fiber than UBR and this could be as a result of synthesis of new cell wall during the period of germination [32]. The change in germination temperature did not cause any significant effect ($p < 0.05$) in total dietary fiber content but increase in germination time increased its content. The physiological functions of dietary fiber include substrate for beneficial microflora, helps to reduce cholesterol level in the blood, helps to reduce glycemic index, normalizes the absorption of glucose in blood stream, prevention of diabetes mellitus and obesity, helps to reduce

low density lipoprotein, and adds bulk to stool to make egestion easy [13].

Fat was also significantly higher in UBR than UMR and this could be because the embryo and bran which contain most of the fats in rice grain were removed from UMR during milling [22]. GBR also had more fat contents than UBR which could be attributed to breakdown of lipids to lower molecular weight substances which the germinating seedlings utilized for energy during the germination process [27]. This was in agreement with the results of previous works [25, 26]. Increase in temperature and time of germination did not result in any significant ($p < 0.05$) effect in the fat content.

Total carbohydrate decreased significantly as follows: UMR > UBR > GBR. The possible reason for lower total carbohydrate in UBR and GBR compared to UMR could be as a result of the presence of embryo and bran in GBR and UBR. The observed lower total carbohydrate in GBR than UBR could be attributed to breakdown of complex carbohydrate in GBR by *amylases* to simple sugars and lower molecular weight polymers as well as utilization of these lower molecular weight carbohydrates by the developing shoots [33]. Increase in germination temperature did not result in any significant effect ($p < 0.05$) on the total carbohydrate contents. As the time of germination increased from 12 h to 36 h, the total carbohydrate composition decreased and was in agreement with previous report [26]. Increased conversion of the polysaccharides to monosaccharides and oligosaccharides by the *amylases* as the germination duration increased as well as increased utilization of the resulting simple sugars by the shoots which were also increasing in sizes and complexity, could be the possible reasons for this [27].

The moisture content was significantly ($p < 0.05$) lower in UMR than UBR and GBR samples. A possible reason for this could be due to the drying operation that both UMR and GBR had undergone. Amongst the GBR samples, increase in the time of germination did not produce any significant ($p < 0.05$) effect on the moisture content but increase in germination temperature resulted in significant decrease in moisture content. However, the moisture of GBR, UMR, and UBR samples were all lower than 12% which will help to extend the shelf life of the rice grains [34].

Effects of milling, germination temperature, and time on mineral contents

Table 2 shows the effects of milling, germination temperatures, and times on the mineral contents of FARO 44 brown rice. The mineral elements contents (mg/100 g) were: Iron (1.27, 4.65, and 2.02 - 9.45); Zinc (1.03, 1.70, and 1.74 - 2.23); Calcium (50.00, 106.00, and 100.00 - 290.00); Phosphorus (18.05, 69.75, and 69.60 - 166.04); Magnesium (39.22, 43.58, and 40.02 - 232.00), and Selenium (43.30, 82.20, and 58.60 - 98.00 µg/100 g) in UMR, UBR, and GBR, respectively.

These minerals increased significantly in this order: UMR < UBR < GBR. A possible reason why these minerals were higher in UBR than UMR could be because the bran, which contains a greater part of the dietary mineral in rice caryop-

Table 2: Mineral and vitamin composition (mg/100 g) as affected by germination temperatures and durations.

Sample	Minerals and Vitamins Contents (mg/100 g)										
	Fe	Zn	Ca	P	Mg	Se ($\mu\text{g}/100\text{ g}$)	B ₁	B ₂	B ₃	B ₆	E
UMR	1.27 \pm 0.03 ^f	1.03 \pm 0.00 ^d	50.00 \pm 1.60 ^e	18.05 \pm 2.40 ^d	39.22 \pm 4.32 ^c	43.30 \pm 1.12 ^d	0.08 \pm 0.00 ^b	0.81 \pm 0.01 ^f	0.80 \pm 0.01 ^c	0.68 \pm 0.09 ^d	0.24 \pm 0.04 ^d
UBR	4.65 \pm 0.21 ^d	1.70 \pm 0.04 ^c	106.00 \pm 1.20 ^d	69.75 \pm 3.70 ^c	43.58 \pm 3.86 ^c	82.20 \pm 3.88 ^b	0.33 \pm 0.05 ^a	1.66 \pm 0.08 ^e	0.80 \pm 0.00 ^c	1.04 \pm 0.12 ^c	1.38 \pm 0.12 ^c
Germination at 30 °C											
G ₁₂ T ₃₀	2.02 \pm 0.05 ^e	1.74 \pm 0.06 ^c	100.00 \pm 3.67 ^d	69.60 \pm 2.50 ^c	49.60 \pm 3.35 ^c	80.20 \pm 3.05 ^b	0.33 \pm 0.02 ^a	2.40 \pm 0.10 ^c	0.95 \pm 0.02 ^b	1.17 \pm 0.15 ^b	1.38 \pm 0.10 ^c
G ₃₆ T ₃₀	7.36 \pm 0.41 ^b	1.95 \pm 0.06 ^b	290.00 \pm 3.11 ^a	166.04 \pm 3.11 ^a	232.00 \pm 5.00 ^a	98.00 \pm 7.45 ^a	0.34 \pm 0.02 ^a	3.19 \pm 0.15 ^b	1.27 \pm 0.07 ^a	1.07 \pm 0.07 ^c	2.00 \pm 0.14 ^a
Germination at 40 °C											
G ₁₂ T ₄₀	5.04 \pm 0.32 ^c	1.94 \pm 0.05 ^b	129.00 \pm 3.00 ^e	80.59 \pm 3.00 ^b	40.02 \pm 2.99 ^c	58.60 \pm 2.12 ^c	0.34 \pm 0.02 ^a	3.63 \pm 0.16 ^a	0.96 \pm 0.03 ^b	1.24 \pm 0.14 ^a	1.58 \pm 0.13 ^b
G ₃₆ T ₄₀	9.43 \pm 0.71 ^a	2.23 \pm 0.09 ^a	190.00 \pm 2.05 ^b	166.01 \pm 4.14 ^a	159.80 \pm 6.00 ^b	78.20 \pm 3.50 ^b	0.33 \pm 0.03 ^a	1.92 \pm 0.19 ^d	0.80 \pm 0.01 ^c	1.05 \pm 0.11 ^c	1.39 \pm 0.11 ^c

Note: Values are means of triplicates (mean \pm SD). Values with the same superscripts in each column are not significant difference at $p < 0.05$. UMR = ungerminated parboiled milled rice; UBR = ungerminated brown rice; GT = germinated brown rice; subscripts 12 and 36 are germination durations (h); subscripts 30 and 40 are the temperatures of germination (°C).

sis was removed in UMR during the milling operation [35]. Both GBR and UBR contain the bran layer but significantly ($p < 0.05$) lower contents of minerals were observed in UBR compared to GBR, the reason for this could be attributed to liberation of these minerals following the breakdown of phytic acid in GBR by *phytase* [7, 31]. Significant increase ($p < 0.05$) in iron and zinc contents were observed when the germination temperature was increased from 30 to 40 °C. Selenium, magnesium, and calcium contents on the other hand, decreased when the germination temperature was increase from 30 °C to 40 °C while no significant effect was observed in potassium content. These variations could be due to specificity of *phytase* for the different minerals at different temperatures. When the time of germination was increased from 12 h to 36 h, significant increases in the levels of all the minerals were observed and this could be due to increased *phytase* activity [31]. Chinma et al. [24] reported higher contents of magnesium (180.53 - 334.52 mg/100 g) and phosphorus (170.03 - 229.10 mg/100 g) but lower contents of calcium (27.19 - 29.55 mg/100 g), iron (2.85 - 3.97 mg/100 g), and zinc (1.79 - 1.90 mg/100 g) when compared the GBR of this work. Again, by comparing the mineral content to that of FARO 57 rice cultivars [26] showed that they were all lower in the present work. These differences could be due to the different rice cultivars, variation in the types of soil or fertilizers [21].

Effects of milling, germination temperature, and time on vitamin contents

Table 2 shows the effects of milling, temperature, and time of germination on the vitamin contents of FARO 44 brown rice. The vitamin contents (mg/100 g) were: B₁ (0.08, 0.33, and 0.33 - 0.34); B₂ (0.81, 1.66, and 1.92 - 3.63); B₃ (0.80, 0.80, and 0.80 - 1.27); B₆ (0.68, 1.04, and 1.05 - 1.24), and E (0.24, 1.38, and 1.38 - 2.00) in UMR, UBR, and GBR, respectively. Like the minerals, these vitamins increased in the following order: UMR < UBR < GBR. Higher vitamin contents of UBR than UMR could also be because these vitamins were lost in UMR together with the embryo and bran which were removed in the course of the milling operation

[21]. Higher contents of vitamin in GBR than UBR indicates the possibility of biosynthesis of these vitamins during germination. Generally, increase in temperature of germination from 30 °C to 40 °C did not result in any significant ($p < 0.05$) effect in the levels of these vitamins except for vitamins B₃ and E where significant decrease was observed. Furthermore, when the time of germination was increased from 12 h to 36 h in samples that were germinated at 30 °C, a significant increase in the levels of vitamins B₂, B₃, and E occurred while significant decrease was observed on vitamin B₆. On the other hand, when the time of germination was increased from 12 h to 36 h in samples that were germinated at 40 °C, significant decrease in the levels of vitamins B₂, B₃, B₆, and E were observed.

Effects of milling, germination temperature, and time on amino acids contents

Figure 1 shows the effects of milling, germination temperature, and time on the essential amino acid contents of FARO 44 brown rice, while figure 2 shows these effects on the non-essential amino acid contents. The essential amino acid

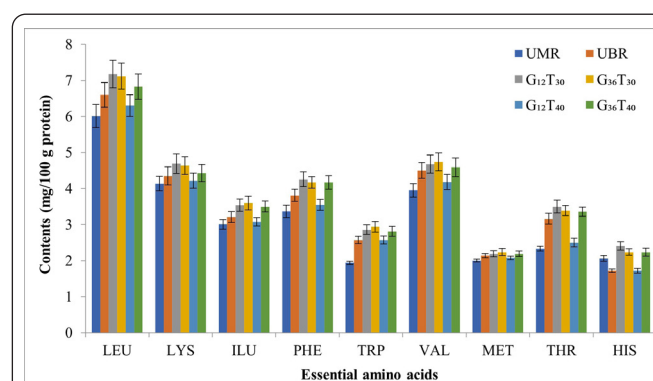


Figure 1: Effects of milling, germination temperature, and time on essential amino acid contents.

Note: UMR = ungerminated parboiled milled rice; UBR = ungerminated brown rice; GT = germinated brown rice; subscripts 12 and 36 are germination durations (h); subscripts 30 and 40 are the temperatures of germination (°C). VAL = Valine; PHE = Phenylalanine; MET = Methionine; THR = Threonine; TRP = Tryptophan; HIS = Histidine; ILU = Isoleucine; LEU = Leucine; and LYS =Lysine.

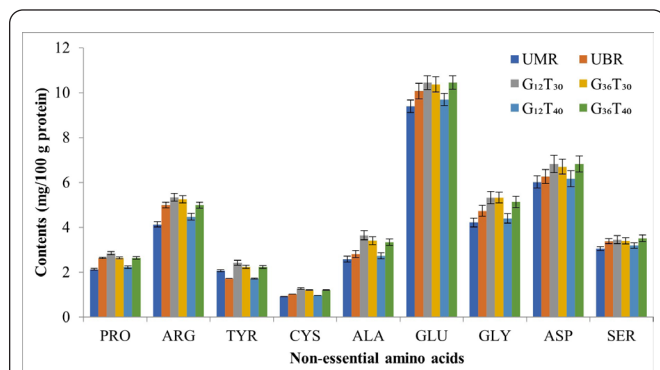


Figure 2: Effects of milling, germination temperature and time on non-essential amino acid contents.

Note: UMR = ungerminated parboiled milled rice; UBR = ungerminated brown rice; GT = germinated brown rice; subscripts 12 and 36 are germination durations (h); subscripts 30 and 40 are the temperatures of germination (°C). PRO = Proline; ARG = Arginine; TYR = Tyrosine; CYS = Cysteine; ALA = Alanine; GLU = Glutamic acid; GLY = Glycine; ASP = Aspartic acid; and SER = Serine.

contents (mg/100 g protein) in UMR, UBR, and GBR were leucine (6.01, 6.60, and 6.30 - 7.18), lysine (4.14, 4.35, and 4.22 - 4.69), iso-leucine (3.01, 3.21, and 3.08 - 3.60), phenyl-alanine (3.37, 3.81, and 3.55 - 4.26), tryptophan (1.94, 2.57, and 2.57 - 2.94), valine (3.95, 4.50, and 4.18 - 4.74), methionine (2.00, 2.14, and 2.08 - 2.24), threonine (2.33, 3.16, and 2.50 - 3.50), and histidine (2.06, 1.72, and 1.72 - 2.41), respectively. The non-essential amino acid contents (mg/100 g protein) in UMR, UBR, and GBR were proline (2.13, 2.64, and 2.23 - 2.84), arginine (4.13, 4.99, and 4.47 - 5.33), tyrosine (2.06, 1.72, and 1.72 - 2.41), cystine (0.91, 1.03, and 0.97 - 1.27), alanine (2.58, 2.81, and 2.73 - 3.64), glutamic acid (9.39, 10.07, and 9.69 - 10.45), glycine (4.21, 4.73, and 4.39 - 5.32), aspartic acid (6.02, 6.26, and 6.17 - 6.82), and serine (3.05, 3.38, and 3.19 - 3.51), respectively.

Variations exist amongst the amino acid contents of GBR, UMR, and UBR. The amino acid contents were lower in UMR compared to UBR which could be attributed to the presence of bran and embryo in UBR which have been reported to contribute greater number of amino acids in rice grain [35]. The only exception to this was found in histidine. Embryo and bran were present in both GBR and UBR, but UBR had

lower amino acids contents when compared to GBR. The explanation for the higher amino acid contents in GBR could be because the proteolytic enzymes degrade complex and storage protein in GBR to these amino acids during the germination process [33]. These findings were in agreement with the previous report [16]. Like the crude proteins (Table 1), increase in germination temperature from 30 °C to 40 °C decreased the levels of each of these amino acids. Similar effect was previously reported on FARO 57 rice cultivar [26]. It seems that the activities of proteolytic enzymes on the rice grains were enhanced more at 30 °C than at 40 °C. Observation made on samples germinated at 30 °C was that, as the germination time increased from 12 h to 36 h, alanine, proline, histidine, threonine, and tyrosine contents decreased significantly. However, samples germinated at 40 °C for the same 12 h to 36 h showed significant increase in all the amino acids. Increase in proteolytic activity could be the possible reason for this [32]. The observed increase in amino acid contents in GBR as the germination time was increased agreed with previous studies [32, 36].

Effects of milling, germination temperature, and time on the bioactive compounds and antioxidant activity

Table 3 shows the effects of milling, germination temperatures, and times on bioactive compounds and total antioxidant activity of FARO 44 brown rice. TFC (mg CE/g dry weight) was 0.02, 0.10, and 0.20 - 0.24 in UMR, UBR, and GBR, respectively. TPC (mg GAE/g dry weight) was 0.50, 1.01, and 1.31 - 1.45 in UMR, UBR, and GBR, respectively. GABA (mg/100 g) was 0.60, 2.10, and 3.75 - 8.29 in UMR, UBR, and GBR, respectively. Total antioxidant activity (µg/ml) was 0.05, 0.05, and 0.15 - 4.78 in UMR, UBR, and GBR, respectively.

Comparing the TFC of GBR of the present work to previous works showed that they were higher than the range of 0.08 to 0.15 mg CE/g dry weight [26] but lower than the range of 2.0 to 10.8 mg Rutin/g DW [37]. By comparing result of TPC of GBR of the present work to previous works, it was also found that they were higher than the range of 0.09 to 0.56 mg GAE/100 g [21] and the range of 1.05 to 1.10 mg GAE/g dry weight [26] but lower than the range of 1288.9 to

Table 3: Bioactive compounds and antioxidant activity as affected by germination temperatures and durations.

Sample	Bioactive compounds and antioxidant activity			
	TFC (mg CE/g dry weight)	TPC (mg GAE/g dry weight)	DPPH (µg/ml)	GABA (mg/100 g)
UMR	0.02 ± 0.00 ^c	0.50 ± 0.01 ^d	0.05 ± 0.00 ^d	0.60 ± 0.06 ^f
UBR	0.10 ± 0.01 ^b	1.01 ± 0.03 ^c	0.05 ± 0.00 ^d	2.10 ± 0.32 ^e
Germination at 30 °C				
G ₁₂ T ₃₀	0.22 ± 0.01 ^a	1.33 ± 0.05 ^b	0.17 ± 0.01 ^c	3.75 ± 0.34 ^d
G ₃₆ T ₃₀	0.20 ± 0.02 ^a	1.31 ± 0.07 ^b	4.78 ± 1.06 ^a	7.62 ± 1.14 ^b
Germination at 40 °C				
G ₁₂ T ₄₀	0.22 ± 0.01 ^a	1.32 ± 0.04 ^b	0.15 ± 0.01 ^c	4.05 ± 0.87 ^c
G ₃₆ T ₄₀	0.24 ± 0.01 ^a	1.45 ± 0.09 ^a	2.94 ± 0.99 ^b	8.29 ± 1.17 ^a

Note: Values are means of triplicates (mean ± SD). Values with the same superscripts in each column are not significant difference at p < 0.05. UMR = ungerminated parboiled milled rice; UBR = ungerminated brown rice; GT = germinated brown rice; subscripts 12 and 36 are germination durations (h); subscripts 30 and 40 are the temperatures of germination (°C). TPC = Total phenolic content; TFC = Total flavonoid content; DPPH = 2,2-diphenyl-1-picrylhydrazyl assay; and GABA = Gamma amino butyric acid.

2361.2 mg/kg [31] and the range of 38.5 - 54.1 mg GAE/g DW [37]. These variations could be attributed to either disparity in cultivars or differences in methodology [21].

TFC and TPC of the present research increased significantly ($p < 0.05$) in this order: UMR < UBR < GBR. Flavonoids and phenolic compounds are abundant in the bran which was removed and discarded in the period of milling, and this could be responsible for their lowest levels in UMR [5, 37]. TPC was however, higher in GBR than UBR though both contain the bran which could be attributed to liberation of bound phenolic compounds due to modification of the grain's cell wall by the *phenolases* [7]. Other researchers who worked on other rice cultivars reported similar results [17, 21, 31]. A careful observation of the GBR samples showed that, neither increase in temperature nor time of germination affected the TFC significantly ($p < 0.05$). The TPC on the other hand was significantly higher at 40 °C than 30 °C. At 40 °C, the increase in time of germination from 12 h to 36 h also increased the TPC significantly.

The total antioxidant activity of UMR and UBR did not differ significantly from each other while those of GBR samples were significantly ($p < 0.05$) higher. The implication of this result is that GBR when consumed could have higher potential to preserve some of the essential radicals of the body than UMR and UBR. Higher total antioxidant activity of GBR than UMR and UBR agree with previous reports [21, 37]. Higher total antioxidant activity was observed at germination temperature of 30 °C than 40 °C which agree with previous report [26]. Increase in time of germination from 12 h to 36 h also resulted in significant increase in total antioxidant activity.

The GABA contents of GBR of this work, when compared to previous works showed that they were lower than the range of 3.22 to 24.14 mg/100 g [17] and the range of 4.92 to 9.45 mg/100 g [26] but higher than the range of 145.6 to 200.5 mg/kg [31] and these disparity could be attributed to the different cultivars used by the different researchers [21]. GABA increased significantly in this order: UMR < UBR < GBR. The GABA content of UBR reduced in UMR by 71.43% and the reason for this could be due to the bran layer that was removed from UMR when milling was done [5, 8]. GABA was higher in GBR than UBR by 78.57 - 294.76% which could be due to conversion of glutamic acid to GABA by *Glutamate decarboxylase* which became active in response to abiotic stress in the course of germination [8, 33]. These findings agree with previous studies [22, 33]. GABA was significantly higher at germination temperature of 40 °C than 30 °C and its contents also increased significantly with increase in time of germination. This could be due to an increase in *Glutamate decarboxylase* activity [33]. The physiological functions of GABA include inhibition of creation of cancer cells, reduction of blood pressure, prevention of diabetes, prevention of hypertension, boost production of high-density lipoprotein, prevention of Alzheimer diseases, prevention of high blood cholesterol, impede production of low-density lipoprotein, promotion of neurotransmission in the brain, and prevention of alcohol-related diseases [5, 36].

Table 4: Total starch, amylose, and total reducing sugar compositions as affected by germination temperatures and durations.

Sample	Total starch (%)	Amylose (%)	Reducing sugar (%)
UMR	76.06 ± 8.50 ^a	35.87 ± 3.25 ^a	2.16 ± 0.22 ^c
UBR	67.09 ± 7.20 ^b	35.10 ± 4.00 ^a	1.67 ± 0.07 ^f
Germination at 30 °C			
G ₁₂ T ₃₀	60.75 ± 6.90 ^c	26.93 ± 2.31 ^c	2.83 ± 0.24 ^d
G ₃₆ T ₃₀	55.83 ± 4.00 ^d	22.15 ± 3.00 ^d	8.33 ± 1.00 ^a
Germination at 40 °C			
G ₁₂ T ₄₀	60.30 ± 5.97 ^c	30.83 ± 3.11 ^b	7.50 ± 1.02 ^b
G ₃₆ T ₄₀	58.15 ± 6.00 ^c	23.68 ± 2.40 ^d	4.17 ± 0.51 ^c

Note: Values are means of triplicates (mean ± SD). Values with the same superscripts in each column are not significant difference at $p < 0.05$. UMR = ungerminated parboiled milled rice; UBR = ungerminated brown rice; GT = germinated brown rice; subscripts 12 and 36 are germination durations (h); subscripts 30 and 40 are the temperatures of germination (°C).

Effects of milling, germination temperature and time on total starch, amylose and total reducing sugars contents

Table 4 shows the effects of milling, germination temperatures and time on the amylose, total starch and total reducing sugars contents of FARO 44 brown rice. The results were: total starch (76.06%, 67.07%, and 55.83 - 60.75%); amylose (35.87%, 35.10%, and 22.12 - 30.83%), and total reducing sugars (2.16%, 1.67%, 2.83 - 8.33%) in UMR, UBR, and GBR, respectively.

Total starch contents decreased significantly in this order: UMR > UBR > GBR. Lower total starch of UBR than UMR could be contributed by the presence of germ and bran in UBR. Lower total starch of GBR than UBR could be attributed to the breakdown of starch by *amylases* to oligosaccharides and monosaccharides in the course of germination process [32]. Significantly ($p < 0.05$) higher amylose contents were observed in GBR than UBR and UMR and the reason for this could be because the germination process resulted in degradation of starch to oligosaccharides and monosaccharides by *amylases* [32]. Total reducing sugars increased significantly in the following order: UBR < UMR < GBR. Bran and germ layers which were present in UBR and absent in UMR could be responsible for lower total reducing sugars in UBR than UMR. The reducing sugars are by-products of degradation of starch by *amylases* and this could have been the possible reason why they were lower in UMR and UBR when compared to GBR. A significant increase in total reducing sugars contents was observed as the temperature of germination was increased from 30 °C to 40 °C suggesting higher *amylase* activity at 40 °C than at 30 °C. It was also observed that when the germination time was increased from 12 h to 36 h, the amylose and total starch contents decreased significantly ($p < 0.05$), whereas the total reducing sugars composition increased significantly. Increase in *amylase* activity when the duration of germination was increased could be responsible for these effects [33].

Effects of milling, germination temperature, and time on pasting properties

Table 5 shows the effects of milling, germination tempera-

Table 5: Pasting properties as affected by germination temperatures and durations.

Sample	Pasting Properties						
	PV (cP)	TV (cP)	FV (cP)	BV (cP)	SV (cP)	PT (°C)	PKT (min)
UMR	906.00 ± 11.70 ^c	892.00 ± 8.50 ^c	1256.00 ± 10.20 ^c	14.00 ± 2.00 ^c	364.00 ± 4.65 ^c	87.95 ± 1.20 ^a	6.67 ± 0.07 ^a
UBR	2487.00 ± 17.00 ^a	2340.00 ± 15.00 ^a	4139.00 ± 21.50 ^a	147.00 ± 11.50 ^c	1799.00 ± 10.67 ^a	83.90 ± 1.21 ^b	6.00 ± 0.05 ^b
Germination at 30 °C							
G ₁₂ T ₃₀	337.00 ± 6.50 ^d	243.00 ± 4.80 ^d	551.00 ± 6.70 ^d	94.00 ± 6.87 ^d	308.00 ± 5.21 ^d	83.90 ± 1.15 ^b	5.13 ± 0.09 ^d
G ₃₆ T ₃₀	137.00 ± 4.00 ^e	53.00 ± 5.40 ^f	110.00 ± 2.20 ^f	84.00 ± 7.50 ^d	57.00 ± 5.70 ^f	83.50 ± 1.00 ^b	4.20 ± 0.05 ^e
Germination at 40 °C							
G ₁₂ T ₄₀	1654.00 ± 10.50 ^b	1226.00 ± 10.60 ^b	2704.00 ± 10.50 ^b	428.00 ± 5.90 ^a	1478.00 ± 16.40 ^b	81.55 ± 1.09 ^c	5.47 ± 0.08 ^c
G ₃₆ T ₄₀	318.00 ± 5.90 ^d	106.00 ± 5.90 ^e	267.00 ± 5.80 ^e	212.00 ± 4.80 ^b	161.00 ± 3.50 ^e	81.50 ± 1.13 ^c	4.47 ± 0.05 ^e

Note: Values are means of triplicates (mean ± SD). Values with the same superscripts in each column are not significant difference at $p < 0.05$. UMR = Ungerminated parboiled milled rice; UBR = Ungerminated brown rice; GT = germinated brown rice; subscripts 12 and 36 are germination durations (h); subscripts 30 and 40 are the temperatures of germination (°C); PV = Peak viscosity; TV = Trough viscosity; BV = Breakdown viscosity; FV = Final viscosity; SV = Setback viscosity; PT = Pasting temperature; and PKT = Peak time.

tures, and times on the pasting properties of FARO 44 brown rice. The pasting properties results reported for UMR, UBR, and GBR were: peak viscosity (cP) (906.00, 2487.00, and 137.00 - 1654.00); trough viscosity (cP) (892.00, 2340.00, and 53.00 - 1226.00); final viscosity (cP) (1256.00, 4139.00, and 110.00 - 2704.00); breakdown viscosity (cP) (14.00, 147.00, and 84.00 - 428.00); setback viscosity (cP) (364.00, 1799.00, and 57.00 - 1478.00); pasting temperature (°C) (87.97, 83.90, and 81.50 - 83.90), and peak time (min) (6.67, 6.00, and 4.20 - 5.47).

Milling decreased the setback, peak, trough, final, and breakdown viscosities of UBR by 79.77%, 63.57%, 63.16%, 69.65%, and 90.48%, respectively. With the exception of breakdown viscosity, which was higher in GBR samples, much significantly ($p < 0.05$) lower values of these viscosities were observed in GBR samples. Amongst GBR, it was observed that the setback, peak, breakdown, final, and trough viscosities increased significantly ($p < 0.05$) with increase in germination temperature but reduced significantly with increase in germination time. The possible reason for this behavior could be attributed to total starch contents which also followed the same trend. The ability of starch-based system to swell before breaking down is what peak viscosity indicates [20]. The implication of these results is that UBR that had the highest peak viscosity could swell more than the rest of the samples. Stability of starch to heat as well as its ability to withstand breakdown while cooling is what the trough viscosity indicates [20]. This shows that the GBR samples can withstand breakdown during cooking than UBR and UMR. The capability of the starch system to form gel in the course of cooling is what the final viscosity indicates [38], thus, UBR could have higher tendency to form gel than GBR and UMR. Breakdown viscosity shows how starch molecules will collapse in the course cooking [20] and this showed that UMR would have higher resistance to shear stress and heat than GBR and UMR. The high values of breakdown viscosity of GBR also indicate that its cooked sample would be more palatable than UBR and UMR [38]. Retrogradation ability of starch when it cools is what setback viscosity indicates [20]. This result indicates that UBR flour would exhibit a higher retrogradation rate than UMR and GBR flours and food products that are produced

from UBR flour would exhibit a higher rate of stalling.

A significantly ($p < 0.05$) higher pasting temperature was observed in UMR than UBR and GBR flours. Amongst the GBR, whereas significantly increase in pasting temperature was observed when the germination temperature was increased from 30 °C to 40 °C, increase in germination time did result in any significant effect. Peak time increased significantly in the following order: GBR < UBR < UMR. In GBR, both increase in germination temperature and time resulted in significant decrease in peak time. The minimum temperature that should be used for cooking as well as the duration of cooking are what peak time indicates [20]. Gelatinization ability and water binding ability of starch are what the pasting temperature stands for [38]. The high pasting temperature of UMR indicates that its flour could exhibit a higher tendency to form gel, lower swelling power, and higher water binding ability [38]. The lower peak time and pasting temperature exhibited by GBR flours compared to UMR and UBR flours could be due to softening of its particles as a result of the soaking and germination processes.

Conclusion

The work showed that milling reduced all the nutrients and bioactive compounds of FARO 44 brown rice investigated apart from amylose, total starch, and total carbohydrate. Germination on the other hand improved these bioactive compounds and nutrients and the effects of germination temperatures and times were evident. The germination temperature of 30 °C resulted in improved calcium, magnesium, selenium, vitamins B₃ and E contents, while 40 °C was optimum for GABA, TPC, iron, and zinc contents. The study therein indicated that germination time of 36 h was suitable for protein, ash, total dietary fiber, iron, zinc, calcium, selenium, phosphorus, magnesium, leucine, lysine, iso-leucine, valine, methionine, total antioxidant activity and GABA. Results of the pasting properties revealed that germinated brown rice could be more palatable, resist retrogradation, and exhibit shorter cooking time than milled rice and brown rice. Thus, for optimum nutraceutical benefits due to the bioactive compounds especially GABA, germination temperature of 40 °C and time of 36 h are recommended.

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Conflict of Interest

The authors declared that they have no conflict of interest with respect to this work.

Credit Author Statement

Ekpeno Sunday Ukpong: Conceptualization, Study design, Writing—original draft preparation, Writing—review and editing, Funding; Emeka Felix Okpalanma: Writing—review and editing, Funding; Roseline Nwabugo Attaugwu: Writing—review and editing, Funding; Babatunde Stephen Oladeji: Writing—review and editing, Funding; Uzoma Charles Onyekwu: Writing—review and editing, Funding. All the authors read and approved the manuscript.

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