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Effects of Milling, Germination Durations and Germination Temperatures on Bioactive Compounds and Nutritional Composition of FARO 57 Brown Rice Cultivar

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Abstract

Effects of milling, temperature and duration of germination on bioactive compounds and nutrients of FARO 57 brown rice were evaluated. Germinated brown rice (GBR) was produced by germinating FARO 57 brown rice at 30 and 40 °C for 12, 24 and 36 h. Bioactive compounds and nutrients analyzed were compared to that of ungerminated parboiled milled rice (MR) and ungerminated brown rice (BR) of the same cultivar which served as controls. Gamma amino butyric acid (GABA) (2.57 mg/100 g), total phenolics (1.00 mgGAE/g), total antioxidant activity (DPPH) (2.09 µg/ml), protein (11.05%), lysine (4.19 mg/100g), ash (1.38%), total dietary fibre (8.25%), minerals (mg/100 g) such as calcium (110.00), phosphorus (68.18), magnesium (44.05), iron (3.91), zinc (1.79), selenium (80,10 µg/100 g), vitamins (mg/100 g) examples B_2 (1.56) and E (1.39) of BR increased in GBR by 91.44-267.70%, 5.00-10,00%, 43.54-252.63%, 31.31-46.70%, 8.59-14.32%, 139.13-184.06%, 8.73-16.51%, 354.55-422.73%, 82.55-197.79%, 130.58-492.92%, 28.13-168.54%, 40.22-56.42%, 13.48-34.71%, 17.31-129.49%, and 11.51-113.67% respectively but decreased significantly in MR. These variables also increased significantly in GBR as the duration of germination was increased. Total carbohydrate (61.25-72.00%), total starch (54.93-60.15%) and amylose (22.91-30.40) contents of GBR were significantly lower ($p<0.05$) than the controls and they decreased as the duration of germination was increased. Increase in germination temperature from 30 °C to 40 °C decreased the DPPH, protein, amino acids, ash, phosphorus, magnesium and vitamin E contents significantly but increased the total reducing sugars, iron, calcium and GABA contents. Germination temperature of 40 °C and time of 36 h were recommended due to improved bioactive compounds contents.

Keywords

Antioxidant activity, Brown rice, Dietary fibre, Gamma amino butyric acid, Milled, Minerals, Parboiled, Vitamins

Abbreviations

BR: Ungerminated brown rice; **GABA:** Gamma amino butyric acid; **GBR:** Germinated brown rice; **MR:** Ungerminated milled rice; **TFC:** Total flavonoid content; **TPC:** Total phenolic content

Introduction

Rice is a staple food in several regions of the world and it the second most consumed cereal grain after wheat [1, 2]. Most calories of people in the

developing countries come from rice [2]. The rice grain has many layers such as the hull or husk, bran, aleurone layer and endosperm. The removal of only the husk or hull which is the inedible outer layer results in brown rice. A further milling which removes the bran and aleurone layers of the endosperm including the germ (embedded in the endosperm) results in milled rice/white rice.

Rice grain contains a small amount of fats which are mostly unsaturated fatty acids which exist in the bran layer [3]. The removal of the inedible husk from the rice paddy exposes these unsaturated fatty acids to oxygen resulting in oxidative rancidity [4]. Furthermore, endogenous lipases in the bran do breakdown the oil in the bran and germ to free fatty acid and this result in hydrolytic rancidity [5]. As a result of these, the shelf-life of the rice is adversely affected. For this reason, rice is milled a little further by removing the bran and the germ.

Unfortunately, the bran and the outer layers contain majority of dietary fiber, proteins, fats, minerals and vitamins of rice grain as against the endosperm which is predominantly starch [3, 6]. Again, bioactive compounds and cellular antioxidants are plenty in the bran and aleurone layers of the endosperm [1, 7]. Thus, rice milling does not only remove the bran and the aleurone layers, but also removes these nutrients, bioactive compounds, and beneficial antioxidants. This resulted in clamour for consumption of brown rice owing to its high nutrients, phytochemicals and antioxidant activity compared to milled rice. However, the numerous disadvantages of brown rice such as its short shelf life, nutty flavor, longer cooking time, hard to chew texture irrespective of the extent of cooking, and poor taste have prevented its use as a staple food [8].

The current approach to consuming rice with these bran layer and aleurone layer without encountering the above-mentioned problems is by germination to obtain germinated brown rice (GBR). Germination which is a bio-modification process employs enzymes such as α-amylase, protease and lipase in the bran layer, to degrade starch, protein and lipid, respectively [9]. It has been reported that GBR contains more nutrients, antioxidants, phytochemicals and bioactive compounds than brown rice and milled rice [1, 7, 9-11]. According to literature, these beneficial properties of GBR among other factors depend on the rice cultivar, the temperature and duration of germination [6, 12].

FARO 57 rice cultivar has its origin from China but has become a popular cultivar of rice in many countries including Nigeria due to its high yield and long slender grains [13]. Currently parboiled milled rice (MR) and ungerminated brown rice (BR) from FARO 57 are available while its GBR is not found in the market. Also, data on nutritional composition and bioactive compounds of GBR of FARO 57 cultivar are scarce. Again, works on GBR are mostly based on the duration of germination while effects of the germination temperature are often neglected. Thus, the aim of the present study was to produce GBR from FARO 57 rice cultivar, analyze its bioactive compounds and nutritional composition and compare them with MR and BR from the same cultivar. The study specifically focused on the effects of temperature and duration of germination on these bioactive compounds and nutrients.

Materials

Source of the rice paddy

FARO 57 paddy rice was harvested in November 2021 in Ikwo, Ebonyi State, Nigeria.

Methods

Production of germinated brown rice

Germination was done according to the method of Abbas et al. [14] with some modifications. A laboratory rice husker (SATAKE, Australia) was used to de-husk the paddy. It was followed by soaking the dehulled grains in 0.1% NaClO solution for 30 min for sterilization, and then rinsing with distilled water for 5 times. The sterilized grains were steeped in distilled water for 24 h at ambient temperature (29 ± 2 ^{0}C) such that the ratio of the grains to water was 1:10 (w/v), the steep water was changed every 6 h. At the end of steeping, the water was drained off. The rice grains were spread on a layer of damp sterile jute bag and placed in a stainless-steel tray. The other layer of the damp jute bag was used to cover the rice kernels. Germination was carried out in a laboratory cabinet incubator (Gulfex Scietific DNP-9082, England) at 30 and 40 ^{o}C for 12, 24, and 36 h. During germination, distilled water was sprinkled every 6 h to maintain a uniform relative humidity. After the germination, the samples were dried in a hot air oven (Gulfex Scietific DHG 9202, England) at 50 ^{o}C to moisture content of less than 13% and stored in plastic cans until needed for analyses.

Production of ungerminated parboiled milled rice and brown rice

Ungerminated brown rice (BR) was obtained by dehusking the paddy rice using laboratory rice husker (SATAKE, Australia). For parboiled milled rice (MR), the paddy rice was soaked in potable water (40 ^{0}C) for 15 h in a water bath followed by steaming in a stainless-steel pot for 10 min. It was followed by drying using hot air oven (Gulfex Scietific DHG 9202, England) first at 120 ^{0}C for 10 min and then at 78 ^{0}C to less than 13% moisture content. This was followed by dehusking in laboratory rice husker (SATAKE, Australia) and milling using laboratory rice mill (LT JIM – 2099, China). BR and MR were used as controls.

Determination of bioactive compounds and antioxidant compositions of the samples

Parameters determined here included of total phenol content (TPC), total flavonoid content (TFC), total antioxidant activity determined as 2, 2- diphenyl-1-picrylhydrazyl (DPPH) - assay and gamma amino butyric acid (GABA) contents. The samples for determination of TPC, TFC and DPPH were extracted using 95% ethanol as described by Song et al. [15]. The flour sample (10 g) was measured and mixed with 50 mL of 95% ethanol for 15 min using vortex mixer. This was followed by centrifuging for 10 min at 4000 rpm and extraction for 24 h at 4 ^{0}C. Whatman No. 1 filter paper was used to filter the supernatant formed. The entire process was repeated thrice on the residue. The solvent from the extracts

was then evaporated under reduced pressure using a rotary vacuum evaporator (Buchi Rotary Evaporator, Model R-124).

Determination of total phenol content

Folin-Ciocalteau reagent (FC reagent) was used to determine the TPC of the ethanol extract as described by Likittrakulwong et al. [16]. The absorbance of the extract was read at 20 °C using a spectrophotometer (Genway 6305, England) at 765 nm wavelength. The TPC was extrapolated from the calibration curve using gallic acid as standard. The result was expressed as mg GAE/g dry weight.

Determination of total flavonoid content

The TFC were measured using the method of Jirapa et al. [17] using catechin as standard. The absorbance was read in spectrophotometer (Genway 6305, England) at 510 nm wavelength. The TFC was extrapolated from the calibration curve and expressed as mg catechin equivalents (CE) per g of dry weight.

Determination of antioxidant activity - DPPH method

DPPH method of Munarko et al. [18] was employed. A solution of 1.5 mL of 0.1 nM DPPH in ethanol was used as negative control. The absorbance of the extract and control were read using spectrophotometer (Genway 6305, England) at 517 nm wavelength. The DPPH free scavenging activity was then calculated using Equation 1.

$$\%\ Radical\ Scavenging = \frac{[ACxAS]}{AC} X100 \qquad \text{equation 1}$$

Where: AC =Absorbance of negative control; AS = Absorbance of the sample

The percentage of the radical scavenging activity was converted to IC_{50} concentration (µg/mL) where 50% inhibition of the DPPH radical is obtained.

Determination of gamma-amino butyric acid content

The method of Thitinunsomboon et al. [19] was used. The rice samples were ground to pass through 0.425 mm standard mesh sieve after which 5 g was measured for extraction. The extraction was done by mixing the flour with 20 mL of 80% ethanol, vortexed for 2 min and centrifuged for 15 min at 10000 rpm. The supernatant was collected, and the residue was further extracted but this time with 25 mL of the 80% ethanol solution. After extraction, the supernatant collected was poured into a 50 mL volumetric flask, 80% ethanol solution was added to make it up to the 50 mL mark after which the solution was shaken to mix and was followed by filtering through 0.45 µm aperture syringe. Furthermore, 40 µL of 2-mercaptoethanol was mixed with 1 mL of 0.054% o-phthaldialdehyde (OPT) solution and poured into a measuring cylinder and the volume was made up to 10 mL by addition of 0.4 M borate buffer. This was followed by mixing of 100 µL of the mixture of 2-mercaptoethanol and OPT with 20 µL of the supernatant and the solution was allowed to stand for 2 min to react. After that, 20 µL was measured and dispensed into a high-performance liquid chromatography (Model 363, Varian, Inc. Scientific Instruments, USA) using Prevail C_{-18} column (250×4.6 mm, 5 µm, Alltech, Deerfield, Illinois, USA). Two mobile phases (X and Y) were used to run the gradients. Mobile phase X was made up of a mixture of 0.1 M sodium citrate and 0.1 M phosphate buffer mixed at the ratio of 1:4. Mobile phase Y was made up of ethanol. GABA was eluted in a pre-set gradient run using the column temperature of 40^0C and flow rate of the mobile phase was 1 $mLmin^{-1}$. The ratio of mobile phase X – Y which was 80:20 at 0 min changed to the ratio of 20:80 at 10-15 min. A standard GABA (Fluka Analytical, Jiangsu, China) was compared with the area under the curve to obtain the GABA content by extrapolation in mg/100 g of rice (dry basis).

Determination of proximate composition and energy value

The germinated brown rice grains were first milled to flour by the use of hammer mill. The nitrogen value (N) by Kjeldahl, the crude protein (N x 6.25), total dietary fiber (enzymatic-gravimetric method), ash, total carbohydrate (phenol and sulphuric acid method), fat (solvent extraction with petroleum ether) and moisture contents were determined by AOAC [20] methods. The caloric value was calculated by Atwater formula using the factors of 9.0 kCal for fat and 4.0 kCal for carbohydrate and protein. These energies were summed up to give the calorie value in kCal and were then converted to their S.I. units (J) by multiplying with 4184 J.

Amino acid analysis of the samples

The samples were defatted using a solution of chloroform and methanol mixed in the ratio of 2:1 respectively and the AOAC [21] method was used to determine the amino acid profile. The extract was hydrolysed and dispensed into the cartridge of the Applied Biosystems PTH Amino Acid Analyzer (Applied Biosystems Inc., USA. Model: 120A; Serial no.704520). An integrator attached to the Analyzer calculates the peak area proportional to the concentration of each of the amino acids. For determination of tryptophan, the defatted sample was modified by adding 10 mL of 4.2 M sodium hydroxide.

Determination of mineral composition

Atomic Absorption Spectrometer (AAS) (model 210 VPG) was used to determine the calcium, phosphorus, magnesium, iron, zinc and selenium as described by AOAC [22]. Calibration of the AAS was done using working standards prepared from commercially available standard solutions. Results were expressed as mg/100 g sample.

Determination of composition of vitamins

Thiamin (B_1), riboflavin (B_2), niacin (B_3), vitamin B_6, B_9 and vitamin E were determined by AOAC [20] method. The sample (5 g) was finely homogenized, extracted and digested. For thiamin, the sample was homogenized in 50 mL ethanolic sodium hydroxide and then filtered through Whatman No. 1 filter paper. Furthermore, 10 mL potassium dichromate was mixed with 10 mL filtrate for color development. For riboflavin, the sample was extracted for 1 h with 100 mL ethyl alcohol after which the extract was filtered through Whatman No. 1 filter paper. This was followed by measuring 10 mL of the filtrate and mixing with 10 mL 5% potassium permanganate and 10 mL 3% hydrogen peroxide. The mixture was allowed to stand on hot water bath for 30 min after which 2 mL of 40%

Na_2SO_4 was added, and the volume was made up to 50 mL by addition of distilled water. It was followed by centrifuging at 1500 rpm. For niacin, sample was treated with 50 mL of 1 N H_2SO_4 for 30 min. This was followed by addition of 0.5 mL ammonia solution and filtering through Whatman No. 1 filter paper. Furthermore, 10 mL of the filtrate was mixed with 5 mL of 0.5% potassium cyanide and was followed by acidification with 5 mL of 0.02 N H_2SO_4. For vitamin B_6, the sample was extracted with 10 mL 0.1 M HCl accompanied with vigorous shaking for 10 min. The sample was filtered through Whatman No. 1 filter paper and the filtrate was made to 10 mL by addition of distilled water. Furthermore, 5 mL of the slightly acidic filtrate was treated with 1 mL 0.40% ferric chloride. For vitamin E, the sample was extracted with 50 mL petroleum ether after which it was concentrated to dryness. The residue was saponified with 5 mL of 0.1 M potassium hydroxide under reflux. Furthermore, 20 mL of petroleum ether was used to extract the unsaponified matter and the filtrate concentrated to dryness. Furthermore, 20 mL of ethanol was added to dissolve the concentrate and 1 mL was transferred to a test tube after which 1 mL 0.2% ferric chloride in ethanol was added. Furthermore, 1 mL 0.5% dipyridyl in ethanol was also added. Ethanol was then added to the resulting product to make it up to the 5 mL mark level. Spectrophotometer (Genway 6305, England) was used to read the absorbance of the resultant digest at 360, 510, 420, 450 and 520 nm wavelengths for B_1, B_2, B_3, B_6 and E respectively.

Determination of total starch, amylose and reducing sugar compositions

AOAC [20] method was used to determine the percentage of total starch in the samples. The sample was treated with α-amylase and amyloglucosidase before diluting with acetate buffer and addition glucose oxidase/peroxidase reagent and the absorbance was read against the blank at 510 nm wavelength. The amylose content was determined by the ISO [23] method. In this method, the 0.1 g of each sample, blank or standard was measured into volumetric flask (100 ml) and 95% ethanol (1 ml) and 1 M NaOH (9 ml) were added. The water bath was made to boil and the flask together with the mixture was introduced into it and allowed to heat for 20 min after which it was removed and placed on the laboratory table to cool to ambient temperature. Distilled water was then added to make it up to the 100 ml mark. This was followed by pouring 5 ml of distilled water into 10 ml test tube and pipetting 0.5 ml of test sample, standard or blank, 0.1 ml of 5% acetic acid, 0.2 ml of iodine and adding distilled water to make it up to the 10 ml mark. This was followed by mixing with the aid of vortex mixer and the absorbance read at 720 nm wavelength against the blank. A calibration curve was obtained using standard graded amylose (Fluka chemicals, Germany) and the percentage amylose was extrapolated from the curve.

The total reducing sugar was determined by AOAC [24] method by titrating the sample extract with 20 ml Soxhlet reagent and D-glucose standard. The quantity of reducing sugar was calculated using Equation 2.

$$RS = \frac{[100\ (AxB)]}{W} \qquad \text{Equation 2}$$

Where, RS = Reducing sugar (in %); A= D-glucose used to reduce 20 ml soxhlet reagent (in ml) B= Concentration of D-glucose standard used to reduce 20 ml soxhlet reagent W= weight of the sample titrant used (in g)

Statistical analysis

Each experiment will be performed in triplicate. R-software (R×64 3.4.2) was used for the Analysis of Variance (ANOVA). Significantly different means were separated at $p<0.05$ using Fisher's least significant difference.

Results and Discussion

Effects of milling and germination on bioactive compounds and antioxidant activity

Table 1 shows the results of bioactive compounds and antioxidant activity. The TPC (mg GAE/g dry weight) was significantly lower ($p<0.05$) in MR (0.05), followed by that of BR (1.00) but higher in GBR samples (1.05-1.10). Phenolic compounds are found predominantly in the embryo and in the bran and they exist in 3 forms namely free phenolics, soluble and conjugated phenolics, and bound phenolics [7]. Thus, absence of embryo and bran in MR which were removed during milling could be the reason for lower TPC in MR. However, both BR and GBR contain embryo and bran, but TPC was significantly higher in GBR – they were 5-10% higher in GBR than BR and 110-120% higher in GBR than MR. The possible reason could be because of liberation of bound phenolic compounds due to modification of the grain's cell wall by phenolases [8]. Amongst the GBR, changes in duration and temperature of germination did not result in any significant effect on the TPC. Results of TPC of GBR of the present study is lower than the range of 38.5-54.1 mg GAE/g DW reported by Abubakar et al. [25] as well as the range of 1288.9-2361.2 mg/kg reported by Kaur et al. [12] but higher than the range of 0.09-0.56 mg GAE/100 g reported by Bourneow & Toontan [6] for ND and HN GBR cultivars. These variations could be due to the different cultivars of rice used by the different researchers.

The TFC (mg CE/g dry weight) was significantly lower ($p<0.05$) in MR (0.02) than BR (0.08) and GBR (0.08-0.15) which are all lower than the range of 2.0-10.8 mg Rutin/g DW previously reported [25] and again, the reason for the variation could be due to the different cultivars used by both researchers. Flavonoids are also abundant in the bran layers [25] thus; the absence of bran layer in MR could also be responsible for its lowest level. There was no significant difference ($p<0.05$) in TFC between BR and GBR and changes in temperatures and durations of germination also did not have any significant effect on the TFC.

The DPPH assay is one of the ways of evaluating the total antioxidant capacity of foods. The lowest level of DPPH (μg/ml) was found in MR (0.07) followed by that of BR (2.09) and they were significantly higher ($p<0.05$) in GBR (3.00-7.37). High DPPH value of GBR implies that when consumed, they could have higher capability to preserve some of the essential radicals of the body. Like TPC and TFC, the antioxidants in rice grains are plenty in the bran, thus, the removal of the bran could be the reason for its lower level in MR [12]. Higher

Table 1: 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)
MR	0.02_{B}^{b}	0.50_{C}^{c}	0.07_{D}^{g}	0.65_{D}^{h}
BR	0.08_{AB}^{ab}	1.00_{B}^{b}	2.09_{C}^{f}	2.57_{C}^{g}
Germination at 30 ^{0}C				
$G_{12}T_{30}$	0.11^{a}	1.08^{a}	3.09^{e}	4.92^{f}
$G_{24}T_{30}$	0.08^{ab}	1.08^{a}	5.10^{c}	6.70^{d}
$G_{36}T_{30}$	0.11^{a}	1.05^{ab}	7.37^{a}	8.15^{b}
Mean	$\mathbf{0.10_{A}}$	$\mathbf{1.07_{A}}$	$\mathbf{5.19_{A}}$	$\mathbf{6.59_{B}}$
Germination at 40 ^{0}C				
$G_{12}T_{40}$	0.13^{a}	1.09^{a}	3.00^{e}	6.10^{e}
$G_{24}T_{40}$	0.13^{a}	1.10^{a}	4.95^{d}	7.18^{c}
$G_{36}T_{40}$	0.15^{a}	1.08^{a}	6.70^{b}	9.45^{a}
Mean	$\mathbf{0.14_{A}}$	$\mathbf{1.09_{A}}$	$\mathbf{4.88_{B}}$	$\mathbf{7.58_{A}}$

Values with the same superscripts or subscripts in each column are not significant difference at p>0.05. Upper case subscript letters compare means at different germination temperatures with the controls.

MR= ungerminated parboiled milled rice; BR= ungerminated brown rice; GT= germinated brown rice; subscripts 12, 24 and 36 are germination durations (h); subscripts 30 and 40 are the temperatures of germination (^{0}C).

TPC = Total phenolic content; TFC = Total flavonoid content; DPPH = 2,2-diphenyl-1-picrylhydrazyl assay; GABA = Gamma amino butyric acid.

level of DPPH in GBR than BR was previously reported for other cultivars of rice [6, 25] and amongst the GBR samples it increased significantly as the duration of germination was increased which is also in line with previous reports [12, 17]. The value of DPPH decreased significantly as the germination temperature was increased from 30 °C to 40 °C which implies lower antioxidant activity at high temperature of germination

GABA is another bioactive compound that plays very important roles in human body such as neuro-transmitter of the nervous system, prevention of intelligence degeneration due to old age, prevention of hypertension, prevention of cancer and inhibition of cancer cells proliferation, prevention of diabetes, reduction of blood pressure, enhancing of high density lipoprotein, reduction of low density lipoprotein, prevention of hypercholesterolemia, prevention of alcohol-related diseases as well as functioning as antioxidant [1, 11]. GABA (mg/100 g) was significantly higher (p<0.05) in GBR (4.92-9.45) than BR (2.57) and least in MR (0.65). The low level of GABA in MR could be due to the removal of bran layer by rice milling [7]. It was also higher in GBR than BR which could be due to actions of enzymes during germination [18, 26]. Amongst the GBR, GABA increased significantly as the duration of germination was increased. GABA is reported to be produced primarily by decarboxylation of glutamic acid by the aid of glutamate decarboxylase and the activity of this enzyme is reported to increase as the germination time increases [18] which could be the reason for significant increase in the level of GABA with increase in germination time observed in this work. GABA increased significantly when the temperature of germination was increased from 30 °C to 40 °C. It is also worth pointing out that the GABA content of GBR of this study was higher than the ranges of 145.6-200.5 mg/kg reported by Kaur et al. [12] but comparable to the range of 3.22-24.14 mg/100 g reported by Jirapa et al. [17] and differences in cultivars could account for this variation.

Effects of milling and germination on proximate composition and calorie value

Table 2 shows the proximate composition and energy value of the samples as affected by milling, temperatures and durations of germination. The protein content was significantly higher (p<0.05) in GBR (14.51-16.21%) than BR (11.05%) and least in MR (10.16%). The lower quantity of protein in MR could be due to the loss of protein along with the bran during milling [6, 10]. Higher quantity of protein in GBR than BR was previously reported [27, 28] and the reason could either be due to synthesis of new proteins or the breakdown of bound proteinous substances and storage proteins into peptides and free amino nitrogen during germination [29, 30]. The protein contents increased significantly as the germination duration was increased and this could be due to the synthesis of new enzymes and proteins as the time of germination increased [29]. The protein contents of the present study were higher than the range of 8.56-12.52% reported by Chinma et al. [27] as well as the range of 6.54-9.99% by Makinde & Omolori [28] for some local cultivars of germinated brown rice in Nigeria. The possible reasons for the variations could be due to differences in rice cultivars, types of soil and types of fertilizers [6].

The ash content was significantly higher (p<0.05) in GBR (3.30-3.92%) than BR (1.38%) and least in MR (1.26%). Lower quantity of ash in MR than BR could be due to the loss of ash along with the bran during milling [31]. Higher content of ash in GBR than BR could be due to reduction in phytic acid content, a mineral chelator by phytase produced during germination [12, 32]. Ash content indicates the mineral composition of food and higher ash content as found in GBR indicates that GBR could have higher mineral contents which agree with the result of mineral composition of this work (Table 5). Amongst the GBR samples, the ash contents

Table 2: Proximate composition and Calorie Value of FARO 57 rice cultivar as affected by temperatures and durations of germination.

Sample		Proximate composition (%) and energy value (J) of rice samples						
	Nitrogen	Protein	Ash	Dietary fibre	Moisture	Fats	Total CHO	Energy
MR	1.63_{D}^{f}	10.16_{D}^{h}	1.26_{D}^{f}	5.20_{C}^{f}	10.46_{D}^{e}	1.00_{C}^{c}	77.15_{A}^{a}	1498876.16_{A}^{d}
BR	1.77_{C}^{e}	11.05_{C}^{g}	1.38_{C}^{e}	8.25_{B}^{e}	12.48_{A}^{a}	2.35_{B}^{b}	72.45_{B}^{b}	1485947.60_{D}^{f}
Germination at 30 ^{0}C								
$G_{12}T_{30}$	2.51^{b}	15.66^{c}	3.33^{d}	9.25^{c}	10.97^{b}	2.99^{a}	72.00^{b}	1579669.20^{a}
$G_{24}T_{30}$	2.56^{ab}	15.97^{b}	3.70^{b}	9.26^{c}	10.85^{c}	3.00^{a}	66.97^{c}	1501051.84^{c}
$G_{36}T_{30}$	2.59^{a}	16.21^{a}	3.92^{a}	9.35^{b}	10.85^{c}	3.01^{a}	61.25^{e}	1409715.12^{h}
Mean	$\mathbf{2.55_{A}}$	$\mathbf{15.95_{A}}$	$\mathbf{3.65_{A}}$	$\mathbf{9.29_{A}}$	$\mathbf{10.89_{B}}$	$\mathbf{3.00_{A}}$	$\mathbf{66.74_{D}}$	$\mathbf{1496812.05_{B}}$
Germination at 40 ^{0}C								
$G_{12}T_{40}$	2.32^{d}	14.51^{f}	3.30^{d}	8.97^{d}	10.98^{b}	2.92^{a}	71.89^{b}	1555945.92^{b}
$G_{24}T_{40}$	2.37^{c}	14.79^{e}	3.60^{c}	9.38^{b}	10.67^{d}	2.95^{a}	67.96^{c}	1495989.20^{e}
$G_{36}T_{40}$	2.40^{c}	14.98^{d}	3.69^{b}	9.62^{a}	10.69^{d}	2.99^{a}	64.19^{d}	1437580.56^{g}
Mean	$\mathbf{2.36_{B}}$	$\mathbf{14.76_{B}}$	$\mathbf{3.53_{B}}$	$\mathbf{9.32_{A}}$	$\mathbf{10.78_{C}}$	$\mathbf{2.95_{A}}$	$\mathbf{68.01_{C}}$	$\mathbf{1496505.23_{C}}$

Values with the same superscripts or subscripts in each column are not significant difference at p>0.05.

Upper case subscript letters compare means at different germination temperatures with the controls.

CHO = carbohydrate; MR= ungerminated parboiled milled rice; BR= ungerminated brown rice; GT= germinated brown rice; subscripts 12, 24 and 36 are germination durations (h); subscripts 30 and 40 are the temperatures of germination (^{0}C).

increased as the duration of germination was increased and this is in agreement with previous works [27, 28]. This could be because increase in time of germination resulted in increase in phytase activity with a corresponding decrease in phytic acid content [12]. Increase in germination temperature from 30 °C to 40 °C significantly reduced (p<0.05) the ash content. The ash content of GBR of this research is higher than the range of 1.29-1.44% reported for some cultivars of Indonesian brown rice [33] and this variation could be due to the differences in cultivars and agroecological zone [6].

The total dietary fiber was significantly higher in GBR (8.97-9.62%) than BR (8.25%) while MR had the lowest (5.20%). Rice milling is reported to result in significant loss of dietary fiber, and this could be the reason for its lower content in MR [9, 31]. The total dietary fiber content was also significantly higher (p<0.05) in GBR than BR and amongst the GBR samples, it increased as the duration of germination was increased which could be due to the formation of new cell wall components during germination [34]. There was no significant difference in the total dietary fiber content amongst the germination temperatures employed in this work. The fat content was lower in MR (1.00%) than BR (2.35%) and the reason could be because fat is contained in the bran and embryo which were removed in MR during milling [6, 10]. The fat contents however were significantly higher (p<0.05) in GBR (2.92-3.01%) than BR though both contain bran layer and embryo, and this could be due to the action of lipolytic enzymes in GBR which break down fat containing molecules to simpler substances to be used as energy by the seedlings [29]. The higher values of fats in GBR than in BR was also in agreement with the report of Makinde & Omolori [28] but however, contrasted with that of Chinma et al. [27] who reported lower quantities of fats in GBR than BR. It is most likely that there was a conversion of fat to glucose in the work of Chinma et al. [27] since they used longer period of germination (48 h). Increase in germination temperature and duration did not result in any significant effect (p<0.05) in the fat content.

The total carbohydrate composition was significantly higher (p<0.05) in MR (77.15%) than BR (72.45%) and lowest amongst the GBR samples (61.25-72.00%). The bran and embryo were not removed in BR and GBR samples which might have contributed to the low total carbohydrate contents. Amongst the GBR samples, the total carbohydrate contents were found to decrease significantly with increased time of germination. This could be due to increased metabolism whereby amylases break down complex carbohydrates to simpler and more absorbable sugars as well as increased feeding on these carbohydrates by the shoots which also increased in size and complexity with increase in time of germination [29]. Increase in temperature of germination from 30 °C to 40 °C increased the total carbohydrate contents significantly. The moisture contents in MR, BR and GBR were generally low which is good for control of microbial spoilage. These moisture contents range fall below the 14% reported to prolong the storage life of GBR [30].

The energy value was 1498876.16 J in MR which was reduced to 1485947.60 J in BR and they were in the range of 1409715.12-1579669.20 J in GBR samples. The energy value in the samples germinated for 12 h were all significantly higher (p<0.05) than those of the controls (MR and BR) while those of samples germinated for 36 h were significantly lower than those of the controls. Generally, significant decrease in the energy value as the duration of germination increased was observed amongst the GBR samples. The possible reason for this could be due to increased utilization of the energy by the sprouts which also increased in sizes and complexity as the germination duration was increased. Samples germinated at 30 °C had significantly higher energy value than those germinated at 40 °C and this effect could be attributed to significantly higher protein contents of the samples germinated at 30 °C.

Effects of milling and germination on amino acids compositions

Essential amino acids are the ones that the body cannot produce and as such must be provided through food. The essential amino acids compositions are shown in table 3. In most cereals, the limiting amino acid is lysine but lysine content (mg/100 g protein) of this study was 4.11 in MR, 4.19 in BR and in the range 4.55-4.79 in GBR which are quite high. The lysine content was higher than the range of 0.48-0.60 reported for other rice cultivars grown in Nigeria [35] but was however lower than 7.84-11.04 reported by Likittrakulwong et al. [16]. Differences in cultivars, types of soil and types of fertilizers used could be the reason for the variation [6]. The levels of leucine, phenylalanine, tryptophan and valine were also high especially amongst the GBR samples. Generally, each of these essential amino acids increased significantly ($p<0.05$) in the following order: MR>BR>GBR. The essential amino acids were higher in GBR than MR by 12.46-20.98, 10.71-16.55, 2.31-21.78, 21.99-45.45, 83.08-111.44, 21.32-26.96, 10.84-37.93, 12.33-57.71 and 28.77-68.95% for leucine, lysine, iso-leucine, phenylalanine, tryptophan, valine, methionine, threonine and histidine respectively. Rice milling could be the reason for the lower level of the essential amino acids in MR since proteins and amino acids are abundant in the bran layers that are removed during milling [1, 36]. The essential amino acids were also significantly higher in GBR than BR and increase in the duration of germination from 12 h to 36 h also resulted in increase in the levels of the essential amino acids which agree with previous reports [1, 33]. Higher level of essential amino acids in GBR than BR could be due to breakdown of complex protein by proteolytic enzymes during germination [26]. The observed increase in the levels of the essential amino acids as the germination duration increased could be due to increased proteolytic activity [26]. Like the proteins (Table 2), the essential amino acids compositions reduced significantly when the germination temperature was increased from 30 to 40 °C. It seems that the activities of proteolytic enzymes on the rice grains are enhanced at 30 °C than at 40 °C.

The non-essential amino acids are shown in table 4. Apart from thyrosine, the remaining eight non-essential amino acids were significantly higher in BR than MR which could be due to the removal of bran during milling [34]. They were also significantly higher in GBR than both BR and MR which could be caused by breakdown of complex proteins by the proteolytic enzymes or the synthesis of new amino acids during germination [26]. These amino acids were higher in GBR than MR by 29.03-61.75, 12.17-31.98, 11.65-41.75, 12.50-53.75, 18.18-48.33, 3.50-13.27, 2.86-28.10, 17.87-22.62 and 5.32-15.61% for proline, arginine, tyrosine, cystine, alanine, glutamic acid, glycine, aspartic acid and serine respectively. Like the essential amino acids (Table 3) and protein (Table 2), the non-essential amino acids also decreased significantly ($p<0.05$) when the germination temperature was increased from 30 °C to 40 °C. Again, this could suggest that the proteolytic enzymes do not function optimally at higher temperatures of germination. The decrease in the level of glutamic acid as the temperature of germination was increased can also be explained by the fact that glutamate decarboxylase converts it to GABA [31].

Effects of milling and germination on the mineral compositions

Table 5 shows the effects of milling, temperature and duration of germination on the mineral composition of the samples. The minerals decreased significantly ($p<0.05$) in this order: GBR>BR>MR. These minerals were higher in BR than MR by 207.87, 73.79, 120.0, 277.73, 12.32 and 84.99% for iron, zinc, calcium, phosphorus, magnesium and selenium respectively. The reason for this could be due to loss of these

Table 3: Essential amino acid composition (mg/100 g protein) as affected by germination temperatures and durations.

Sample	Essential amino acids (mg/100 g protein)								
	LEU	LYS	ILU	PHE	TRP	VAL	MET	THR	HIS
MR	6.10_{D}^{e}	4.11_{D}^{e}	3.03_{C}^{f}	3.41_{D}^{g}	2.01_{D}^{f}	4.08_{D}^{e}	2.03_{D}^{e}	2.27_{D}^{g}	2.19_{D}^{f}
BR	6.68_{C}^{d}	4.19_{C}^{d}	3.14_{B}^{e}	3.89_{C}^{f}	2.65_{C}^{e}	4.48_{C}^{d}	2.11_{C}^{d}	3.00_{B}^{d}	2.78_{C}^{e}
Germination at 30 °C									
$G_{12}T_{30}$	7.35^{a}	4.77^{a}	3.60^{ab}	4.43^{b}	3.89^{b}	5.09^{b}	2.30^{b}	3.58^{a}	3.20^{b}
$G_{24}T_{30}$	7.33^{a}	4.75^{a}	3.58^{b}	4.43^{b}	3.87^{b}	5.12^{a}	2.33^{b}	3.48^{b}	3.22^{b}
$G_{36}T_{30}$	7.38^{a}	4.79^{a}	3.69^{a}	4.96^{a}	4.25^{a}	5.18^{a}	2.80^{a}	3.52^{ab}	3.70^{a}
Mean	$\mathbf{7.35_{A}}$	$\mathbf{4.77_{A}}$	$\mathbf{3.62_{A}}$	$\mathbf{4.61_{A}}$	$\mathbf{4.00_{A}}$	$\mathbf{5.13_{A}}$	$\mathbf{2.48_{A}}$	$\mathbf{3.53_{A}}$	$\mathbf{3.37_{A}}$
Germination at 40 °C									
$G_{12}T_{40}$	6.86^{c}	4.55^{c}	3.10^{e}	4.16^{e}	3.68^{d}	4.95^{c}	2.25^{c}	2.55^{f}	2.82^{d}
$G_{24}T_{40}$	6.88^{c}	4.58^{c}	3.19^{d}	4.21^{d}	3.75^{c}	4.96^{c}	2.27^{c}	2.90^{e}	2.96^{c}
$G_{36}T_{40}$	6.98^{b}	4.68^{b}	3.28^{c}	4.30^{c}	3.94^{b}	5.11^{ab}	2.32^{b}	3.27^{c}	3.24^{b}
Mean	$\mathbf{6.91_{B}}$	$\mathbf{4.60_{B}}$	$\mathbf{3.19_{B}}$	$\mathbf{4.22_{B}}$	$\mathbf{3.79_{B}}$	$\mathbf{5.01_{B}}$	$\mathbf{2.28_{B}}$	$\mathbf{2.91_{C}}$	$\mathbf{3.01_{B}}$

Values with the same superscript or subscript in each column are not significant different ($p>0.05$).

Upper case subscript letters compare means at different germination temperatures with the controls.

MR= ungerminated parboiled milled rice; BR= ungerminated brown rice; GT= germinated brown rice; subscripts 12, 24 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 = Iso-leucine; LEU = Leucine; LYS =Lysine.

Table 4: Non-essential amino acid composition (mg/100 g protein) as affected by germination temperatures and durations.

Sample	Non-essential amino acids (mg/100 g protein)								
	PRO	**ARG**	**TYR**	**CYS**	**ALA**	**GLU**	**GLY**	**ASP**	**SER**
MR	2.17_{D}^{e}	4.19_{D}^{f}	2.06_{C}^{d}	0.80_{C}^{f}	2.09_{D}^{e}	9.42_{C}^{e}	4.20_{D}^{f}	6.10_{D}^{e}	3.01_{C}^{e}
BR	2.80_{C}^{d}	4.71_{C}^{e}	1.80_{D}^{e}	1.00_{B}^{e}	2.26_{C}^{d}	10.10_{B}^{c}	4.36_{C}^{e}	7.00_{C}^{d}	3.33_{B}^{b}
Germination at 30 ^{0}C									
$G_{12}T_{30}$	2.94^{bc}	5.51^{a}	2.92^{a}	1.21^{a}	3.03^{a}	10.60^{a}	5.30^{a}	7.44^{a}	3.46^{a}
$G_{24}T_{30}$	2.99^{b}	5.53^{a}	2.41^{b}	1.22^{a}	3.04^{a}	10.50^{b}	4.89^{c}	7.40^{a}	3.40^{a}
$G_{36}T_{30}$	3.51^{a}	5.40^{b}	2.41^{b}	1.23^{a}	3.10^{a}	10.67^{a}	5.38^{a}	7.48^{a}	3.48^{a}
Mean	$\mathbf{3.15_{A}}$	$\mathbf{5.48_{A}}$	$\mathbf{2.58_{A}}$	$\mathbf{1.22_{A}}$	$\mathbf{3.06_{A}}$	$\mathbf{10.59_{A}}$	$\mathbf{5.19_{A}}$	$\mathbf{7.44_{A}}$	$\mathbf{3.45_{A}}$
Germination at 40 ^{0}C									
$G_{12}T_{40}$	2.80^{d}	4.70^{e}	2.84^{a}	0.90^{d}	2.47^{c}	9.75^{d}	4.32^{e}	7.19^{c}	3.17^{d}
$G_{24}T_{40}$	2.90^{c}	4.92^{d}	2.30^{c}	0.98^{c}	2.70^{b}	9.98^{c}	4.57^{d}	7.28^{b}	3.23^{c}
$G_{36}T_{40}$	2.97^{b}	5.21^{c}	2.30^{c}	1.17^{b}	3.05^{a}	10.67^{a}	5.25^{b}	7.43^{a}	3.47^{a}
Mean	$\mathbf{2.89_{B}}$	$\mathbf{4.94_{B}}$	$\mathbf{2.48_{B}}$	$\mathbf{1.02_{B}}$	$\mathbf{2.77_{B}}$	$\mathbf{10.13_{B}}$	$\mathbf{4.71_{B}}$	$\mathbf{7.30_{B}}$	$\mathbf{3.29_{B}}$

Values with the same superscript or subscript in each column are not significant different ($p>0.05$).

Upper case subscript letters compare means at different germination temperatures with the controls.

MR= ungerminated parboiled milled rice; BR= ungerminated brown rice; GT= germinated brown rice; subscripts 12, 24 and 36 are germination durations (h); subscripts 30 and 40 are the temperatures of germination (^{0}C).

PRO = Proline; ARG = Arginine; TRY = Tryrosine; CYS = Cystine; ALA = Alanine; GLU = Glutamic acid; GLY = Glycine; ASP = Aspartic acid; SER = Serine.

Table 5: Mineral composition (mg/100 g) as affected by germination temperatures and durations**.**

Sample	Minerals					
	Fe	**Zn**	**Ca**	**P**	**Mg**	**Se (µg/100 g)**
UMR	1.27_{D}^{g}	1.03_{C}^{f}	50.00_{D}^{f}	18.05_{D}^{f}	39.22_{D}^{h}	43.30_{C}^{f}
UBR	3.91_{C}^{f}	1.79_{B}^{e}	110.00_{C}^{e}	68.18_{C}^{e}	44.05_{C}^{g}	80.10_{B}^{e}
Germination at 30 ^{0}C						
$G_{12}T_{30}$	5.01^{e}	2.51^{d}	450.00^{d}	170.83^{c}	101.57^{e}	100.10^{c}
$G_{24}T_{30}$	9.42^{c}	2.71^{b}	500.00^{c}	190.14^{ab}	177.63^{b}	103.20^{b}
$G_{36}T_{30}$	9.50^{c}	2.78^{a}	540.00^{b}	203.03^{a}	261.18^{a}	103.20^{b}
Mean	$\mathbf{7.98_{B}}$	$\mathbf{2.67_{A}}$	$\mathbf{496.67_{B}}$	$\mathbf{188.00_{A}}$	$\mathbf{180.13_{A}}$	$\mathbf{102.17_{A}}$
Germination at 40 ^{0}C						
$G_{12}T_{40}$	7.93^{d}	2.63^{c}	450.00^{d}	124.46^{d}	79.05^{f}	90.90^{d}
$G_{24}T_{40}$	10.32^{b}	2.74^{a}	540.00^{b}	179.48^{bc}	120.60^{d}	104.00^{b}
$G_{36}T_{40}$	10.50^{a}	2.80^{a}	575.00^{a}	183.19^{b}	147.97^{c}	107.90^{a}
Mean	$\mathbf{9.58_{A}}$	$\mathbf{2.72_{A}}$	$\mathbf{521.67_{A}}$	$\mathbf{162.38_{B}}$	$\mathbf{115.87_{B}}$	$\mathbf{100.93_{A}}$

Values with the same superscripts in each column are not significant difference at $p>0.05$.

Upper case subscript letters compare means at different germination temperatures with the controls.

MR= ungerminated parboiled milled rice; BR= ungerminated brown rice; GT= germinated brown rice; subscripts 12, 24 and 36 are germination durations (h); subscripts 30 and 40 are the temperatures of germination (^{0}C).

minerals with the bran through milling [9, 34] 36. The minerals were also higher in GBR than BR by 28.13-168.54, 40.22-56.42, 309.09-422.73, 82.55-197.79, 79.46-492.92 and 13.48-34.71% for iron, zinc, calcium, phosphorus, magnesium and selenium respectively. One possible reason for higher mineral compositions in GBR than BR could be due to the action of phytase on phytic acid to free these minerals and increase their quantity as a result [8, 12]. It is also worth pointing out that amongst the GBR samples, each of these minerals increased as the time of germination was increased which could be due to increased phytase activity [12]. Phosphorus and magnesium were significantly higher at germination temperature of 30 °C than 40 °C, iron and calcium were higher at 40 °C than 30 °C while there were no significant differences in the levels of zinc and selenium between the two germination temperatures. This effect could be caused by temperature and substrate specificity of the phytase. It is also worthy to point out that the minerals contents of the GBR of this work were higher than the ranges reported by Chinma et al. [27] for iron (2.85-3.97 mg/100 g), calcium (27.19-29.55 mg/100 g) and zinc (1.79-1.90 mg/100 g) but lower than the ranges for phosphorus (170.03-229.10 mg/100 g) and magnesium (180.53-334.52 mg/100 g). The variation could be due to the differences in rice cultivars, ecological factors and types of fertilizers used [6].

Effects of milling and germination on the vitamin's compositions

Table 6 shows the effects of milling, temperature and duration of germination on the vitamin compositions of the samples. Vitamin B_1 (mg/100 g) in BR (0.33) did not differ significantly ($p<0.05$) from that of GBR samples (0.33-0.34) but it was significantly higher than that of MR (0.08). Vitamin B_2 (mg/100 g) was significantly higher in GBR (1.83-3.58) than BR (1.56) and lowest in MR (0.81). Vitamin B_6 (mg/100 g) was significantly higher in BR and GBR (1.00-1.18) than MR (0.68), B_9 was not detected in MR and almost negligible in BR and GBR samples while vitamin E (mg/100 g) was significantly higher in GBR (1.55-2.97) than BR (1.39) and lowest in MR (0.24). The low levels of these vitamins in MR could be due to the effect of milling which they were removed and discarded along with the bran and embryo [6]. Generally, levels of vitamins B_2 and E were lower at 40 °C than 30 °C and temperature-time interaction played a major role in the levels of vitamins B_2 and B_3. Significant increase in the level of vitamins B_2 and B_3 with increase in germination time was observed for samples germinated at 30 °C while the opposite was observed when they were germinated at 40 °C. Vitamin B_6 content of the samples germinated at 40 °C decreased significantly as the germination duration was increased and this suggests the possibility of utilization of this vitamin by the developing radicles and plumules for their metabolic activities. For vitamin E, increase in germination time from 12 to 24 h resulted in its significant increase but a further increase in germination time to 36 h resulted in its significant decrease. It is possible that vitamin E started playing its antioxidant role on the developing radicles and plumules at 36 h of germination and this could be the reason for the decrease in its level.

Effects of milling and germination on total starch, amylose and total reducing sugars compositions

Table 7 shows the effects of milling, temperature, and duration of germination on the total starch, amylose and total reducing sugar compositions of the samples. Total starch was significantly higher ($p<0.05$) in MR (76.06%) than BR (67.03%) and lowest in GBR (54.93-60.15%). These values were higher than the range of 33.41-49.86% reported for GBR from some local rice cultivars in Nigeria [27] and the variation could be attributed to differences in cultivar. The low values of total starch in GBR could be due to amylase activity on the starch duration germination [33]. Amylose was significantly lower in GBR (22.91-30.40) while there was no significant difference ($p<0.05$) between MR (35.87%) and BR

Table 7: Total starch, amylose and total reducing sugar composition of FARO 57 rice as affected by germination temperatures and durations.

Sample	Total starch, amylose and total reducing sugar		
	Total Starch (%)	Amylose (%)	Total reducing sugar (%)
MR	76.06_A^a	35.87_A^a	2.16_C^f
BR	67.03_B^b	35.05_A^a	1.79_D^g
Germination at 30 °C			
$G_{12}T_{30}$	60.15c	30.40^b	4.33^e
$G_{24}T_{30}$	57.99^d	28.13^c	9.56^b
$G_{36}T_{30}$	55.25^e	23.52^d	8.40^c
Mean	**57.80_C**	**27.35_B**	**7.43_B**
Germination at 40 °C			
$G_{12}T_{40}$	60.00^c	29.95^b	7.21^d
$G_{24}T_{40}$	58.14^d	27.03^c	14.81^a
$G_{36}T_{40}$	54.93^e	22.91^d	9.25^b
Mean	**57.69_C**	**26.63_B**	**10.42_A**

Values with the same superscripts or subscripts in each column are not significant difference at $p>0.05$.

Upper case subscript letters compare means at different germination temperatures with the controls.

MR= ungerminated parboiled milled rice; BR= ungerminated brown rice; GT= germinated brown rice; subscripts 12, 24 and 36 are germination durations (h); subscripts 30 and 40 are the temperatures of germination (°C).

Table 6: Vitamin composition (mg/100 g) of FARO 57 rice cultivar as affected by germination temperatures and durations.

Sample	Vitamins					
	Vitamin B_1	Vitamin B_2	Vitamin B_3	Vitamin B_6	Vitamin B_9	Vitamin E
MR	0.08_B^b	0.81_D^h	0.80_A^b	0.68_B^c	Nil	0.24_D^f
BR	0.33_A^a	1.56_C^g	0.81_A^b	1.03_A^b	0.01_A^a	1.39_C^e
Germination at 30 °C						
$G_{12}T_{30}$	0.33^a	2.16^d	0.80^b	1.04^b	0.01^a	1.64^d
$G_{24}T_{30}$	0.34^a	2.52^c	0.92^a	1.03^b	0.01^a	2.97^a
$G_{36}T_{30}$	0.34^a	2.94^b	0.92^a	1.00^b	0.01^a	2.04^c
Mean	**0.34_A**	**2.54_A**	**0.88_A**	**1.02_A**	**0.01_A**	**2.22_A**
Germination at 40 °C						
$G_{12}T_{40}$	0.33^a	3.58^a	0.83^b	1.18^a	0.01^a	1.55^d
$G_{24}T_{40}$	0.33^a	2.01^e	0.82^b	1.07^b	0.01^a	2.60^b
$G_{36}T_{40}$	0.33^a	1.83^f	0.80^b	1.02^b	0.01^a	2.00^c
Mean	**0.33_A**	**2.47_B**	**0.82_A**	**1.09_A**	**0.01_A**	**2.05_B**

Values with the same superscripts in each column are not significant difference at $p>0.05$.

Upper case subscript letters compare means at different germination temperatures with the controls.

MR= ungerminated parboiled milled rice; BR= ungerminated brown rice; GT= germinated brown rice; subscripts 12, 24 and 36 are germination durations (h); subscripts 30 and 40 are the temperatures of germination (°C).

(35.05%). Both total starch and amylose decreased significant as the duration of germination was increased and similar effect was also previously reported for other rice cultivars [12, 27]. The possible reason for this could be due to increase in amylase activity with increase in duration of germination [26]. The total reducing sugars compositions were significantly higher in GBR (4.33-14.81%) than MR (2.16%) and lowest in BR (1.79%). The reducing sugars are products obtained from the action of amylases on starch during germination and this could be the reason why they were higher in GBR than MR and BR. Amongst the GBR samples, increase in germination time from 12 h to 24 h also resulted in significant increase in total reducing sugar composition. This could also be as result of increase in amylase activity as the germination time was increased [26]. However, a further increase in time of germination from 24 to 36 h resulted in significant decrease in total reducing sugars compositions. It is most likely that the developing radicles and plumules started utilizing these sugars for energy at 36 h of germination. Significantly higher ($p<0.05$) total reducing sugars contents were observed on samples germinated at 40 °C than 30 °C.

Conclusions

This study revealed that germination improved the bioactive compounds and nutritional contents of FARO 57 brown rice while milling adversely affected these attributes. Milling reduced these bioactive compounds and nutrients except total carbohydrate while germination increased them. Germination duration of 36 h was optimum for most of the bioactive compounds and nutrients. Germination of FARO 57 brown rice for 36 h decreased the total carbohydrate, total starch and amylose contents but increased the gamma amino butyric acid contents (267.70%) and other bioactive compounds and nutrients. Protein, amino acids, ash, phosphorus, magnesium, vitamins B_2 and E were much higher at germination temperature of 30 ^{0}C while gamma amino butyric acid, iron, calcium, total carbohydrate, energy and total reducing sugars were much higher at germination temperature of 40 ^{0}C. Thus, to obtain maximum nutraceutical benefits due to gamma amino butyric acid, FARO 57 brown rice should be germinated at 40 ^{0}C for 36 h.

Acknowledgements

None.

Conflict of Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Authors' Contribution

The work was designed by authors ESU and EFO. Statistical analyses were done by authors ESU and CCE. The first draft was written by ESU after which authors EFO and CCE read through and made their inputs, and the final drafts was written by author ESU. The work was funded by all the authors.

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