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Research Article

NERICA-8 brown rice: its pasting properties, nutrients and bioactive compounds as affected by milling, germination temperature and germination time

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Abstract

Effects of milling, germination temperature and time on pasting properties, nutrients and bioactive compounds of NERICA-8 brown rice (UBR) were investigated. Germinated brown rice (GBR) samples were obtained by germinating UBR at 30℃ and 40℃ for 12 and 36 h. Pasting properties, bioactive compounds and nutrients of GBR, UBR and milled rice (UMR) were analyzed and compared. Results showed that setback, trough, final and peak viscosities of UBR reduced significantly ($p<0.05$) in GBR while breakdown viscosity increased. Antioxidant activity (1.20 µg.ml^{-1}), γ-amino butyric acid (GABA) (1.85 mg.100g^{-1}), protein (10.99%), total dietary fiber (8.20%), vitamins and minerals (mg.100g^{-1}) including iron (4.65), zinc (1.70), calcium (106.00), vitamin B_2 (1.66), vitamin E (1.38) contents of UBR increased in GBR up to 355.83%, 329.73%, 28.03%, 9.63%, 113.98%, 17.65%, 74.53%, 110.24%, and 41.30%, respectively at 36 h germination. Milling reduced the levels of these compounds. Total starch, total carbohydrate and amylose contents of GBR decreased as germination time increased. Proteins, amino acids and magnesium were significantly ($p<0.05$) increased at germination temperature of 30℃ than 40℃ while dietary fiber, vitamin E and GABA were higher at 40℃. Germination time of 36 h and temperature of 40℃ were recommended due to optimum contents of the bioactive compounds.

Keywords

antioxidant, dietary fiber, flavonoid, gamma amino butyric acid, mineral, vitamin

Abbreviations

GABA – γ-amino butyric acid; GBR – germinated brown rice; TFC – total flavonoid content; TPC – total phenolic content; UBR – ungerminated brown rice; UMR – ungerminated parboiled milled rice

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Introduction

Amongst the cereals, rice is next to wheat in terms of utilization and consumption worldwide (Sathe et al. 2020). White/milled rice and brown/unmilled rice are the two major products that are obtained from paddy rice. Brown rice is obtained from paddy rice by removing only the inedible cork-like hull/husk. Milled rice is obtained from the brown rice when the bran (aleurone and sub-aleurone layers) and germ/embryo are removed. Amongst these two, milled rice is the form that is mostly consumed but it is predominantly starchy (85-90% starch) and poor in other nutrients and phytochemicals (Bourneow and Toontan 2019). Dietary fiber, minerals, proteins, vitamins, fats, phytochemicals and cellular antioxidants are predominant in the bran and embryo of rice grain which are the layers that are absent in milled rice (Sathe et al. 2020). Thus, brown rice is richer in nutrients and phytochemicals than milled rice but it cannot be used as a stable food because the presence of bran makes its texture to be too hard to cook except with the use of a pressure cooker and too hard to chew (Likittrakulwong et al. 2021). When compared to milled rice, brown rice has a poor aroma and taste and a shorter shelf life (Ravichanthiran et al. 2018).

The poor nutritional quality of milled rice together with the inability to use the nutritionally richer brown rice as stable food resulted in researchers aimed at overcoming these problems. Fortification of polished rice with some micro nutrients such as iron, vitamin B_1 and vitamin B_3 as done in the United States is expensive and cannot fully restore all the lost nutrients (Ukpong and Onyeka 2019). For example, dietary fiber cannot be replaced by fortification. Thus, the solution lies on adopting processing methods that will overcome these structural and organoleptic problems of brown rice to enhance its consumption. Currently, germination process is employed which will apart from overcoming these structural problems, also increases the essential nutrients and enhances the production of beneficial bio-active compounds (Kim et al. 2020; Munarko et al. 2021; Ukpong, Onyeka et al. 2022).

There are two varieties of rice grown all over the world and these are *Oryza sativa* and *Oriza glaberrima*. Of these two, *O. sativa* has its origin from Asia while *O. glaberrima* is indigenous to West Africa (Maji et al. 2017). The different cultivars of rice which are presently grown in different regions are obtained from these two varieties by hybridization, germplasm exchange program or different crosses of genes (Maji et al. 2017). Recently, NERICA-8 cultivar was developed by inclusion of about 13% gene of *O. glaberrima* to *O. sativa* (Maji et al. 2017). The essence of this was to have a rice cultivar that will withstand the harsh environmental factors of the African region while at the same time produce high yield which *O. sativa* is known for. There is parboiled milled rice (UMR) of NERICA-8 cultivar in the market, while its brown rice (UBR) and germinated brown rice (GBR) are lacking. Since it is a newly developed rice cultivar, literature on the nutritional composition and bioactive compounds of its UMR is limited, while literature on its UBR and GBR is lacking. This research was aimed at producing UMR, UBR and GBR from NERICA-8 cultivar after which their pasting properties, nutrients and bioactive compounds were analyzed and compared. Special emphases were paid to the effects of time and temperature of germination on the nutrients, pasting properties and bioactive compounds of GBR.

Materials and Methods

Source of the rice paddy. Rice paddies from NERICA-8 were harvested in rice farm in Ikwo, Ebonyi State, Nigeria, by November, 2022.

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℃) for a period of 40 days to exceed the dormancy period. The method of Ukpong et al. (2021) was used for the 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℃) 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℃ and 40℃ for 12 and 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℃) in oven (England, Gulfex Scientific, model: DHG 9202) to below 13% moisture and storage in plastic tin until they were demanded for analyses.

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℃) after which the paddy rice was steamed at atmospheric pressure for 10 min. When this was completed, the water was decanted and the paddy was dried in oven (England, Gulfex Scietific, model: DHG 9202) first for 10 min at 120℃ and afterward at 78℃ 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 pasting properties. Hammer mill was used to mill the rice grains to flour. 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. (2021).

Determination of bioactive compounds and antioxidant compositions. The total antioxidant activity, total phenol content (TPC), γ-amino butyric acid (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. (2021). Gallic acid was used as standard. The procedure of Jirapa et al. (2016) was adopted for determination of TFC. Catechin was used as standard. DPPH (2,2-diphenyl-1-picrylhydrazyl) procedure described by Munarko et al. (2021) 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. (2013). 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 proximate composition and energy value. Hammer mill was used to mill the rice grains to flour. The flours were sieved through the mesh size of 0.15 mm and proximate composition and gross energy were determined on the rice flour by the method of AOAC (2015). Ash, moisture (drying to constant weight in oven), fat (by extraction using petroleum ether), nitrogen (N) by Kjeldahl method and crudeprotein (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.

Determination of total starch, amylose and reducing sugar compositions. Procedures of AOAC (2015) were employed to determine the total starch and total reducing sugar compositions. ISO (2015) 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 amino acid contents. Methanol was mixed with Chloroform in the ratio of 1:2. The resulting mixture was used to defat 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 (2006).

Determination of mineral composition. The procedure of AOAC (2015) 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 (2015) was used to determine Vitamin E, Vitamin B_1, Vitamin B_2, Vitamin B_3, and Vitamin B_6 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 B_1; 510 nm for Vitamin B_2; 420 nm for Vitamin B_3; and 450 nm for Vitamin B_6.

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

Pasting properties of NERICA-8 brown rice as affected by milling and germination. Table 1 shows the effects of milling, temperature of germination and duration of germination on the pasting properties of UMR, UBR and GBR of NERICA-8 cultivar. The peak viscosity was in the range of 421.0 to 2612.0 cP, trough viscosity ranged 131.0 to 2385.0 cP, final viscosity ranged 342.0 to 4147.0 cP, breakdown viscosity ranged 14.0 to 340.0 cP while setback viscosity ranged 211.0 to 1762 cP. UBR had the highest values of setback, trough, peak and final viscosities. These viscosities were significantly (p<0.05) higher in samples germinated at 40℃ than those germinated at 30℃. Significant (p<0.05) reductions in these viscosities were observed in GBR samples by increasing the duration of germination from 12 h to 36 h. Trough, final, peak and setback viscosities of GBR samples when compared to UMR showed that those of 12 h germination were significantly (p<0.05) higher than their UMR counterparts, while those of 36 h germination were significantly lower. Significant (p<0.05) decrease in breakdown viscosities were observed in the following order: GBR>UBR>UMR. Significantly (p<0.05) higher value of breakdown viscosity was also observed in GBR germinated at 40^{O}C compared to that of 30℃. Also, significant reduction in breakdown viscosity was observed when the duration of germination was raised from 12 to 36 h. This could be attributed to the decrease in the amylose and total starch contents that followed the same order (Table 4). The decrease in these viscosities with an increase in germination time amongst the GBR agrees with previous reports (Chinma et al. 2015; Makinde and Omolori 2020). The ability of starch-based system to swell before breaking down is what peak viscosity indicates (Iwe et al. 2016). The values were, however, lower when compared to the ranges of 1204 to 3336 cp reported by Munarko et al. (2020) for some rice cultivars grown in Indonesia and variation in cultivars could be responsible for the disparities (Makinde and Omolori 2020). The implication of these results is that UBR which 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 in the course of cooling are what the trough viscosity indicates (Ukpong et al. 2021). Low trough viscosity of GBR flour germinated for 36 h indicates that its propensity to collapse during cooking is lower compared to UBR flour. The capability of the starch system to form gel in the course of cooling is what the final viscosity indicates (Ukpong et al. 2023). These results showed that UBR which had the highest final viscosity had higher tendency to form gel compared to GBR and UMR samples. Breakdown viscosity shows how starch molecules will collapse in the course cooking (Iwe et al.2016). The lowest breakdown viscosity of UMR indicates that it would have high resistance to shear stress and heat compared to the other samples. Higher breakdown viscosity of GBR showed that its resistance to shear stress and heat is lower when compared to other samples. The high value of breakdown viscosity of GBR also indicates that it would be palatable when cooked than UBR and UMR samples (Iwe et al. 2016). Retrogradation ability of starch when it cools is what setback viscosity indicates (Iwe et al. 2016). This result indicates that UBR which had the highest setback viscosity, would exhibit higher retrogradation rate than UMR and GBR samples. It also showed that the staling rate of food produced from UBR would be higher than the ones made from either UMR or GBR.

Table 1. Pasting properties of NERICA-8 brown rice as affected by milling and germination

Sample	Pasting properties						
	PV, cP	TV, cP	FV, cP	BV, cP	SV, cP	PT, ℃	PKT, min
UMR	906.00±5.20[d]	892.00±4.21[d]	1256.00±10.11[d]	14.00±1.28[f]	364.00±4.91[d]	87.95±2.11[a]	6.67±0.21[a]
UBR	2490.00±11.01[b]	2385.00±12.00[a]	4147.00±14.00[a]	105.00±3.00[e]	1762.00±11.01[a]	85.30±1.87[b]	6.05±0.18[b]
Germination at 30℃							
$G_{12}T_{30}$	1544.00±9.22[c]	1238.00±9.17[c]	2763.00±11.34[c]	306.00±4.01[c]	1525.00±8.89[c]	84.75±1.90[b]	5.73±0.15[b]
$G_{36}T_{30}$	421.00±4.11[f]	131.00±4.15[f]	342.00±5.70[f]	290.00±4.19[d]	211.00±3.75[f]	84.60±1.76[b]	4.63±0.15[c]
Germination at 40℃							
$G_{12}T_{40}$	2612.00±8.11[a]	2272.00±9.46[b]	3991.00±11.50[b]	340.00±3.72[a]	1719.00±7.48[b]	84.80±1.82[b]	5.73±0.20[b]
$G_{36}T_{40}$	806.00±5.00[e]	487.00±6.11[e]	809.00±7.00[e]	319.00±2.88[b]	322.00±3.21[e]	84.50±1.67[b]	4.63±0.17[c]

Values (mean ± SD) with the same superscripts in each column are not significantly different 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,℃; PV – Peak viscosity; TV – Trough viscosity; BV – Breakdown viscosity; FV – Final viscosity; SV – Setback viscosity; PT – Pasting temperature; PKT – Peak time.

The ranges of pasting temperature and peak time were 84.50 to 87.95℃ and 4.63 to 6.67 min, respectively. The highest value of pasting temperature was found in UMR, while no significant differences existed between UBR and GBR samples. The change in germination time did not produce any significant (p<0.05) effect on the pasting temperature. Significantly (p<0.05) higher value of peak time was observed in UMR compared to UBR and GBR. No significant (p<0.05) difference occurred between the peak time of UBR and GBR that was germinated for 12 h, but GBR germinated for 36 h had a significantly lower peak time than UBR. The minimum temperature that should be used for cooking and the duration of cooking are what peak time indicates (Ukpong et al. 2021). Gelatinization ability and water binding ability of starch are what the pasting temperature stands for (Iwe etal. 2016). The high pasting temperatures of UBR and UMR indicate that their flours could exhibit higher tendency to form gel, lower swelling power, and higher water binding ability (Iwe et al. 2016). 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.

Antioxidant activity and bioactive compounds of NERICA-8 brown rice as affected by milling and germination. Table 2 shows the effect of milling, temperature of germination and duration of germination on total antioxidant activity and bioactive compounds of UMR, UBR and GBR of NERICA-8 cultivar. The TPC (mg GAE.g^{-1}dry weight) of UBR (0.95) reduced significantly (p<0.05) in UMR (0.50) but increased significantly in GBR (1.08). Pro-oxidative and pro-inflammatory effects of certain foods are prevented by foods that have high phenolic contents (Ravichanthiran et al. 2018). Higher quantities of TPC in GBR than UMR and UBR were previously reported (Kaur et al. 2017; Ukpong et al. 2022). The reason for higher TPC in UBR than UMR could be because the phenolics are more in germ and bran; the layers removed in UMR (Ravichanthiran et al. 2018). Liberation of the bound phenolics as a result of modification of the grain's cell wall by the *phenolases* in the course of germination could be responsible for its higher level in GBR (Loan and Thuy 2020). Neither increase in germination time nor temperature resulted in any significant (p<0.05) effect on the TPC.

The TFC (mg CE.g^{-1} dry weight) of UBR (0.05) did not differ significantly from that of UMR (0.02) but significantly (p<0.05) increased in GBR (0.13-0.19). The TFC of GBR of the present work was comparable to the range of 0.08 to 0.15 mg CE.g^{-1} dry weight (Ukpong et al. 2022) but lower than the range of 2.0 to 10.8 mg Rutin.g^{-1} DW (Abubakar et al. 2018) and differences in cultivar could be responsible for the disparity. Higher TFC of GBR

Table 2. Bioactive compounds and antioxidant activity of NERICA-8 brown rice as affected by milling and germination

Sample	Bioactive compounds and antioxidant activity			
	TFC,mg CE. g^{-1}dry weight	**TPC, mg GAE. g^{-1}dry weight**	**DPPH, $\mu g.ml^{-1}$**	**GABA, $mg.100^{-1}.g^{-1}$**
UMR	0.02 ± 0.00^{b}	0.50 ± 0.02^{c}	0.05 ± 0.00^{e}	0.46 ± 0.05^{e}
UBR	0.05 ± 0.00^{b}	0.95 ± 0.03^{b}	1.20 ± 0.02^{d}	1.85 ± 0.09^{d}
		Germination at 30℃		
$G_{12}T_{30}$	0.13 ± 0.00^{a}	1.08 ± 0.01^{a}	1.79 ± 0.04^{c}	3.25 ± 0.16^{c}
$G_{36}T_{30}$	0.14 ± 0.01^{a}	1.08 ± 0.01^{a}	5.47 ± 0.22^{a}	6.95 ± 0.34^{b}
		Germination at 40℃		
$G_{12}T_{40}$	0.15 ± 0.01^{a}	1.08 ± 0.00^{a}	1.30 ± 0.04^{d}	3.89 ± 0.19^{c}
$G_{36}T_{40}$	0.19 ± 0.01^{a}	1.08 ± 0.02^{a}	3.88 ± 0.11^{b}	7.95 ± 0.27^{a}

Values (mean ± SD) with the same superscripts in each column are not significantly different 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,℃; TPC – Total phenolic content; TFC – Total flavonoid content; DPPH – 2,2-diphenyl-1-picrylhydrazyl assay; GABA – γ-amino butyric acid.

than UMR and UBR was previously reported (Jirapa et al. 2016).

Like in the case of TPC, neither changes in germination temperature nor time resulted in any significant (p<0.05) effect on the TFC of GBR. The mean of DPPH ($\mu g.ml^{-1}$) of UBR (1.20) significantly (p<0.05) decreased in UMR (0.05) and but increased in GBR (1.30-5.47). The GBR samples all had higher antioxidant activity than UMR and this is in agreement with previous reports (Jirapa et al. 2015; Bourneow and Toontan 2019). Thus, consumption of GBR instead of UMR may help to preserve some of the vital radicals of the body. Also, the total antioxidant activity increased when the germination time was increased in the GBR samples, this was also previously reported (Kaur et al. 2017; Bourneow and Toontan 2019). Significantly (p<0.05) higher total antioxidant activity was observed in samples that were germinated at 30℃ when compared to that of 40℃.

GABA content ($mg.100g^{-1}$) of UBR (1.85) decreased significantly in UMR (0.46) and increased significantly in GBR (3.25-7.95). Rice milling which detached bran from the endosperm could be responsible for low level of GABA in UMR (Ravichanthiran et al. 2018). 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 $mg.100.g^{-1}$ (Jirapa et al. 2016) but higher than the range of 145.6 to 200.5 $mg.kg^{-1}$ (Kaur et al. 2017) and these disparity could be attributed to the different cultivars used by the different researchers. Significantly (p<0.05) increased GABA content was observed by increasing the time of germination. GABA is reported to be produced primarily by decarboxylation of glutamic acid by the aid of *Glutamate decarboxylase* and report has it that the activity of this enzyme increases when the germination time increases (Munarko et al. 2021). Our results showed that at 36 h germination, GABA was significantly (p<0.05) increased at germination temperature of 40℃ than 30℃.

The physiological functions of GABA include inhibition of creation of cancer cells, reduction of blood pressure, prevention of diabetes, prevention of hypertension, boosting production of high density lipoprotein, prevention of Alzheimer diseases, prevention of high blood cholesterol, impeding production of low density lipoprotein, promotion of neuro-transmission in the brain, and prevention of alcohol-related diseases (Sathe et al. 2020; Ukpong et al. 2023).

Proximate, energy, amylose, total starch and total reducing sugar compositions of NERICA-8 brown rice as affected by milling and germination. Table 3 shows effects of milling, temperature and duration of germination on proximate composition and energy content of UMR, UBR and GBR of NERICA-8 cultivar. The crude protein content of UBR (10.99%) significantly ($p<0.05$) increased in GBR (11.85-14.07%) but reduced in UMR (8.16%). These crude protein contents were higher than the ranges reported for other rice cultivars grown in Nigeria (Chinma et al. 2015; Makinde and Omolori 2020). The possible reasons for the variations could be due to disparities in rice cultivars, types of soil and types of fertilizers (Bourneow and Toontan 2019). Low levels of crude protein in UMR could be due to the loss of aleurone and sub-aleurone layers during milling (Kim et al. 2020), while high levels of protein in GBR could be due to increase in the concentration of nitrogen as a result of loss of organic matter during soaking and germination processes. Observation of the GBR samples showed that increase in germination temperature from 30ºC to 40℃ resulted in a significant ($p<0.05$) reduction in crude protein content. Significant ($p<0.05$) increase in crude protein content was observed by increasing the duration of germination from 12 h to 36 h.

The ash content of UBR (1.32%) also decreased significantly in UMR (1.16%) but increased in GBR (1.35-1.98%). Ash content indicates the mineral composition of food thus, this result suggests higher minerals contents in GBR compared to UMR and UBR. This is in agreement with the results of mineral composition (Table 7). Higher content of ash in UBR than UMR could be due to removal of bran and germ from UMR during milling. Higher content of ash in GBR compared to UBR is in agreement with previous works (Chinma et al. 2015; Kaur et al. 2017; Munarko et al. 2020). The possible explanation for higher ash contents in GBR could be because of loss of total soluble solids during the soaking and germination operations. Significant differences ($p<0.05$) in ash contents did not exist amongst the germination temperatures employed in this work. Also, increase in germination duration from 12 h to 36 h did not result in any significant effect ($p<0.05$) on the ash content.

The total dietary fiber content of UBR (8.20%) also decreased significantly in UMR (5.20%) and increased significantly in GBR (8.30-8.99%).

Table 3. Proximate composition and calorie of NERICA-8 brown rice as affected by milling and germination

Sample	Proximate composition, % and Energy, kCal.100g^{-1} of the rice samples						
	Crude protein	Ash	TDF	Moisture	Fats	Total CHO	Energy
UMR	8.16±0.71^{d}	1.16±0.04^{c}	5.20±0.80^{d}	10.46±0.45^{b}	1.00±0.00^{b}	77.15±4.05^{a}	350.24±8.45^{a}
UBR	10.99±0.84^{c}	1.35±0.03^{b}	8.20±1.10^{c}	12.50±0.67^{a}	2.46±0.09^{a}	72.49±3.43^{b}	356.06±7.59^{a}
			Germination at 30℃				
$G_{12}T_{30}$	12.67±1.01^{ab}	1.37±0.02^{b}	8.30±0.97^{bc}	10.80±0.87^{b}	2.52±0.07^{a}	71.92±3.78^{b}	361.04±8.00^{a}
$G_{36}T_{30}$	14.07±1.01^{a}	1.98±0.03^{a}	8.50±0.87^{b}	10.85±0.56^{b}	2.55±0.09^{a}	61.00±2.97^{c}	323.23±6.90^{b}
			Germination at 40℃				
$G_{12}T_{40}$	11.85±0.83^{b}	1.35±0.03^{b}	8.90±0.87^{a}	10.80±0.81^{b}	2.54±0.11^{a}	71.87±3.00^{b}	357.74±7.89^{a}
$G_{36}T_{40}$	12.56±1.00^{b}	1.91±0.04^{a}	8.99±0.75^{a}	10.59±0.87^{b}	2.50±0.07^{a}	62.98±2.87^{c}	324.66±6.78^{b}

Values (mean ± SD) with the same superscripts in each column are not significantly different 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,℃; CHO – Carbohydrate; TDF – Total dietary fibre

Low total dietary fiber of UMR could be due to loss of aleurone and sub-aleurone layers which contain most of the dietary fiber in rice grain (Chaiyasut et al. 2017). Higher total dietary fiber in GBR agrees with previous report (Chinma et al. 2015) and formation of new cell wall components in the course of germination could be responsible for this (Lee et al. 2019). Both increase in duration and temperature of germination resulted in significant ($p<0.05$) increase in the total dietary fiber content.

The fat contents of UBR (2.46%) did not differ significantly from those of GBR, but it was significantly higher than that of UMR (1.00%). High quantity of fat in UBR and GBR could be due to the presence germ and bran, which contain most of the fats in rice grain (Ukpong and Onyeka 2019). Amongst the GBR, neither increase in duration of germination nor temperature of germination resulted in any significant ($p<0.05$) effect in the fat contents.The total carbohydrate content of UBR (72.49%) increased significantly in UMR (77.15%) but decreased in GBR (61.00-71.92%). UMR is composed of endosperm which is predominantly starchy which could be the reason for higher total carbohydrate of UMR. Increase in germination temperature did not result in any significant effect in total carbohydrate content, while an increase in germination time does. Significant reduction in total carbohydrate was observed by increasing the duration of germination from 12 h to 36 h. Increased catabolism of polysaccharides by *amylases* to oligosaccharides and monosaccharides and increased feeding on these simple sugars by the developing shoots could be responsible for this (Ukpong et al. 2021). The energy value ranged 323.23 to 361.04 kcal. $100g^{-1}$ and the highest significant values ($p<0.05$) were found in UMR, UBR and GBR germinated for 12 h. Amongst the GBR samples, significant ($p<0.05$) reduction in the energy values were observed by increasing the duration of germination from 12 to 36 h. Increased use of this energy by the shoots that also increased in complexities and sizes as the duration of germination increased could be responsible for this.

Table 4 shows effects of milling, temperature and duration of germination on amylose, total starch and total reducing sugar compositions of UMR, UBR and GBR of NERICA-8 cultivar. The total starch ranged 55.07 to 76.06%, while the amylose contents ranged 21.85 to 35.87%. Amylose and total starch decreased significantly ($p<0.05$) in the following order: UMR<UBR<GBR. Hydrolysis of starch by *amylases* to simple sugars and dextrin could be responsible for the lower amylose and total starch contents of GBR (Lee et al. 2019). As the duration of germination was increased, the amylose and total starch contents reduced and could be attributed to increase in activities of *amylases* (Lee et al. 2019). Increase in germination temperature did not result in any significant effects ($p<0.05$) on the total starch and amylose contents.

Table 4. Total starch, amylose and total reducing sugars compositions of NERICA-8 brown rice as affected by milling and germination

Sample	**Total starch, amylose and total reducing sugars compositions, % of rice samples**		
	Total starch	**Amylose**	**Total reducing sugar**
UMR	76.06 ± 4.32^{a}	35.87 ± 3.41^{a}	2.16 ± 0.07^{d}
UBR	67.09 ± 3.90^{b}	35.10 ± 3.21^{a}	1.67 ± 0.05^{e}
	Germination at 30℃		
$G_{12}T_{30}$	62.15 ± 3.00^{bc}	27.66 ± 3.11^{b}	3.11 ± 0.09^{c}
$G_{36}T_{30}$	55.07 ± 3.01^{c}	21.97 ± 2.87^{c}	8.99 ± 0.18^{a}
	Germination at 40℃		
$G_{12}T_{40}$	61.61 ± 3.11^{bc}	29.48 ± 2.77^{b}	8.01 ± 0.09^{b}
$G_{36}T_{40}$	55.87 ± 2.78^{c}	21.85 ± 2.46^{c}	9.44 ± 0.14^{a}

Values (mean ± SD) with the same superscripts in each column are not significantly different 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,℃;

The total reducing sugars content of UBR (1.67%) increased significantly in both UMR (2.16%) and GBR (3.11-9.44%). Reducing sugars are obtained from hydrolysis of starch by *amylases* as germination progresses and this could be the possible explanation for higher value in GBR compared to UMR and UBR. Significant ($p<0.05$) increase in total reducing sugar composition was observed by increasing the duration of germination from 12 h to 36 h. This could also be a result of increase in *amylase* activities as the germination time was increased (Chaijan and Panpipat 2020).

Amino acids composition of NERICA-8 brown rice as affected by milling and germination. Table 5 shows the effect of milling, temperature and duration of germination on the essential amino acid compositions of UMR, UBR and GBR of NERICA-8 cultivar. The essential amino acids contents ($mg.100g^{-1}$) were in the ranges of 5.87 to 6.78 for leucine, 4.01 to 4.59 for lysine, 2.86 to 3.40 for iso-leucine, 3.12 to 4.16 for phenylalanine, 1.80 to 3.00 for tryptophan, 3.71 to 4.50 for valine, 2.00 to 2.41 for methionine, 2.10 to 3.20 for threonine and 1.70 to 2.29 for histidine.

Table 6 shows the effect of milling, temperature and duration of germination on the non-essential amino acid compositions of UMR, UBR and GBR of NERICA-8 cultivar. The non-essential amino acids ($mg.100g^{-1}$) were in the ranges of 2.00-2.98 for proline, 4.00-5.08 for arginine, 9.20-10.16 for glutamic acid, 1.25-2.00 for tyrosine, 0.80-1.21 for cysteine, 2.00-3.04 for alanine, 3.90-4.77 for glycine, 5.84-6.31 for aspartic acid and 2.94-3.30 for serine. Significantly ($p<0.05$) higher essential and non-essential amino acids were observed in UBR compared to UMR. Higher concentration of proteins and amino acids are found in the aleurone and sub-aleurone layers than the endosperm, thus, the removal of these layers at the time of milling could be responsible for the lower amino acid contents of UMR (David et al. 2020; Sathe et al. 2020). Significantly ($p<0.05$) higher essential and non-essential amino acids were also observed in GBR compared to UBR which could be attributed to the enzymatic hydrolysis of storage and complex protein in the course of germination (Chaijan and Panpipat 2020). Significant increase in each of these amino acids was also observed by increasing the duration of germination from 12 h to 36 h. This could be due to increased proteolytic activity as the germination time increased (Chaijan and Panpipat 2020). Increase in the temperature of germination from 30℃ to 40℃ on the other hand resulted in significant ($p<0.05$) reduction in these amino acids. Interestingly, lysine a well known limiting amino acid in cereals was next to leucine in terms of highest essential amino acid content.

Table 5. Essential amino acids composition protein of NERICA-8 brown rice as affected by milling and germination

Sample	**Essential amino acids, mg.100g^{-1} protein**								
	LEU	**LYS**	**ILU**	**PHE**	**TRP**	**VAL**	**MET**	**THR**	**HIS**
UMR	5.87±0.23^{d}	4.01±0.16^{e}	2.86±0.04^{d}	3.12±0.06^{f}	1.80±0.01^{d}	3.71±0.08^{d}	2.00±0.05^{c}	2.10±0.08^{d}	1.70±0.02^{d}
UBR	6.14±0.26^{c}	4.16±0.19^{d}	3.00±0.09^{c}	3.40±0.07^{e}	2.59±0.05^{c}	4.20±0.13c	2.08±0.04^{bc}	2.97±0.10^{b}	1.93±0.04^{c}
					Germination at 30℃				
$G_{12}T_{30}$	6.65± 0.25^{a}	4.30± 0.20^{b}	3.24±0.12^{b}	3.81±0.09^{c}	2.71±0.05^{b}	4.39±0.13^{b}	2.16±0.07^{b}	3.16±0.09^{a}	2.14±0.07^{b}
$G_{36}T_{30}$	6.78± 0.28^{a}	4.59± 0.18^{a}	3.40±0.14^{a}	4.16±0.12^{a}	3.00±0.10^{a}	4.50±0.12^{a}	2.41±0.10^{a}	3.20±0.09^{a}	2.29±0.09^{a}
					Germination at 40℃				
$G_{12}T_{40}$	6.16± 0.23^{c}	4.12± 0.16^{d}	3.00±0.10^{c}	3.59±0.10^{d}	2.60±0.10^{c}	4.21±0.10^{c}	2.10±0.06^{bc}	2.31±0.07^{c}	2.00±0.05^{c}
$G_{36}T_{40}$	6.39± 0.25^{b}	4.37± 0.19^{b}	3.19±0.12^{b}	3.99v0.09^{b}	2.66±0.08^{b}	4.36±0.12^{b}	2.15±0.07^{b}	3.05±0.12^{b}	2.17±0.08^{b}

Values (mean ± SD) with the same superscripts in each column are not significantly different 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,℃. VAL – Valine; PHE – Phenylalanine; MET – Methionine; THR – Threonine; TRP – Tryptophan; HIS – Histidine; ILU – Iso-leucine; LEU – Leucine; LYS – Lysine

Table 6. Non-essential amino acids composition protein of NERICA-8 brown rice as affected by milling and germination

Sample	Non-essential amino acids, mg.100g^{-1} protein								
	PRO	**ARG**	**TYR**	**CYS**	**ALA**	**GLU**	**GLY**	**ASP**	**SER**
UMR	2.00±0.05^{c}	4.00±0.18^{d}	2.00±0.08^{a}	0.80±0.01^{c}	2.00±0.02^{d}	9.20±0.23^{c}	3.90±0.14^{c}	5.84±0.32^{d}	2.94±0.21^{c}
UBR	2.60±0.08^{b}	4.38±0.21^{c}	1.68±0.10^{b}	0.97±0.01^{b}	2.20±0.02^{c}	9.85±0.26^{b}	4.19±0.19^{b}	6.14±0.42^{b}	3.10±0.25^{b}
				Germination at 30℃					
$G_{12}T_{30}$	2.64±0.10^{b}	5.08±0.25^{a}	1.72±0.11^{b}	1.21±0.03^{a}	2.88±0.08^{b}	10.14±0.25^{a}	4.70±0.20^{a}	6.30±0.41^{a}	3.27±0.27^{a}
$G_{36}T_{30}$	2.98±0.12^{a}	5.00±0.21^{a}	1.63±0.12^{b}	1.20±0.04^{a}	3.04±0.15^{a}	10.16±0.20^{a}	4.77±0.23^{a}	6.31±0.39^{a}	3.30±0.25^{a}
				Germination at 40℃					
$G_{12}T_{40}$	2.63±0.09^{b}	4.36±0.21^{c}	1.25±0.09^{c}	0.89±0.07^{bc}	2.21±0.09^{c}	9.65±0.21^{b}	4.17±0.19^{b}	6.00±0.36^{c}	3.00±0.21^{bc}
$G_{36}T_{40}$	2.60±0.11^{b}	4.72±0.19^{b}	1.63±0.10^{b}	1.19±0.10^{a}	2.90±0.09^{b}	10.12±0.26^{a}	4.63±0.21^{a}	6.28±0.40^{a}	3.24±0.25^{a}

Values (mean ± SD) with the same superscripts in each column are not significantly different 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,℃. PRO – Proline; ARG – Arginine; TRY – Tyrosine; CYS – Cystine; ALA – Alanine; GLU – Glutamic acid; GLY – Glycine; ASP – Aspartic acid; SER – Serine.

Minerals and vitamins compositions of NERICA-8 brown rice as affected by milling and germination. Table 7 shows the effects of milling, temperature and duration of germination on mineral contents of UMR, UBR and GBR of NERICA-8 cultivar. The iron content (mg.100g^{-1}) of UBR (4.65) decreased in UMR (1.27) but increased significantly ($p<0.05$) in GBR (3.11-9.95). The zinc content (mg.100g^{-1}) of UBR (1.70) also reduced significantly in UMR (1.03) and increased in GBR (1.74-2.00). The calcium content (mg.100g^{-1}) of UBR (106.00) also reduced in UMR (50.00) andincreased in GBR (94.00-185.00). The phosphorus content (mg.100g^{-1}) of UBR (69.75) was lower in UMR (18.05) and higher in GBR (69.91-189.50). Selenium content (µg.100g^{-1}) of UBR (82.20) also reduced significantly in UMR (43.30) and increased significantly in GBR (89.10-93.20).

Table 7. Mineral compositionsof NERICA-8 brown rice as affected by milling and germination

Sample	Minerals					
	Fe, mg.100g^{-1}	**Zn, mg.100^{-1}.g^{-1}**	**Ca, mg.100g^{-1}**	**Se, µg.100g^{-1}**	**P, mg.100g^{-1}**	**Mg, mg.100g^{-1}**
UMR	1.27±0.04^{e}	1.03±0.01^{c}	50.00±7.10^{d}	43.30±4.52^{c}	18.05±3.12^{c}	39.22±5.97^{c}
UBR	4.65±0.23^{d}	1.70±0.03^{b}	106.00±9.98^{c}	82.20±5.32^{b}	69.75±5.00^{b}	43.58±5.12^{c}
			Germination at 30℃			
$G_{12}T_{30}$	3.11±0.21^{d}	1.74±0.04^{b}	102.00±8.50^{c}	89.10±4.90^{a}	69.91±5.12^{b}	42.18±4.76^{c}
$G_{36}T_{30}$	8.32±0.30^{b}	1.95±0.06^{a}	185.00±7.32^{a}	92.80±6.11^{a}	182.91±7.11^{a}	200.00±9.12^{a}
			Germination at 40℃			
$G_{12}T_{40}$	6.01±0.27^{c}	1.77±0.05^{b}	94.00±6.11^{c}	90.80±7.32^{a}	72.95±6.20^{b}	40.15±4.99^{c}
$G_{36}T_{40}$	9.95±0.29^{a}	2.00±0.05^{a}	145.00±8.41^{b}	93.20±8.00^{a}	189.50±7.11^{a}	150.00±8.77^{b}

Values (mean ± SD) with the same superscripts in each column are not significantly different at $p<0.05$.
MR – ungerminated parboiled milled rice; BR – ungerminated brown rice; GT – germinated brown rice; subscripts 12 and 36 are germination durations, h; subscripts 30 and 40 are the temperatures of germination,℃.

The magnesium content (mg.100g^{-1}) was 39.22 in UMR, 43.58 in UBR and in the range of 40.15-200.00 in GBR. Low mineral composition of UMR than UBR was previously reported on other rice cultivars (Chinma et al.2015; Ukpong et al. 2021) and the reason could be owing to loss of these minerals with the aleurone and sub-aleurone layers through milling (Chaiyasut et al. 2017). High mineral composition in GBR than UBR could be attributed to increase in HCl-extractability of these elements as a result of modifcation of the grains' structure (Benincasa et al. 2019). With the exception of selenium, no significant differences ($p<0.05$) occurred in the mineral contents between UBR and GBR germinated for 12 h while the mineral levels of that of 36 h germination were all significantly higher than their UBR counterparts. Increased in the mineral extraction with increase in time of germination could be responsible for this. Increase in germination temperature from 30 to 40℃ led to significant ($p<0.05$) increase in iron content, decrease in magnesium content while the remaining minerals analyzed in this work were not affected by the change in germination temperatures.

Table 8 shows the effects of milling, temperature and duration of germination on vitamin contents of UMR, UBR and GBR of NERICA-8 cultivar. The vitamin contents ($mg.100g^{-1}$) were vitamin E (0.24-1.95), vitamin B_6 (0.68-1.01), thiamin (0.08-0.35), niacin (0.80-0.92) and riboflavin (0.81-3.49). In each of them, UMR had the lowest levels which could be due to the loss of these vitamins with the bran and embryo which were removed by milling (Bourneow and Toontan 2019). Significant differences ($p<0.05$) did not occur between GBR and UBR in the levels of vitamins B_1, B_2 and B_6 and change in duration of germination did not also result in any significant effect on these vitamins. For vitamins B_3 and E, their levels in GBR were significantly ($p<0.05$) higher than that of UBR and these vitamins increased significantly by increasing the germination time from 12 h to 36 h and this suggest the possibility of biosynthesis of these vitamins during germination. Increase in temperature of germination from 30℃ to 40℃ did not result in any significant effects ($p<0.05$) on these vitamins except vitamin E which increased significantly at germination temperature of 40℃.

Table 8. Vitamin compositions, $mg.100g^{-1}$ of NERICA-8 brown rice as affected by milling and germination

Sample	Vitamins, $mg.100g^{-1}$				
	B_1	B_2	B_3	B_6	E
UMR	0.08±0.00[b]	0.81±0.02[f]	0.80±0.01[a]	0.68±0.02[b]	0.24±0.02[e]
UBR	0.33±0.01[a]	1.66±0.07[e]	0.80±0.01[a]	1.04±0.04[a]	1.38±0.09[c]
Germination at 30℃					
$G_{12}T_{30}$	0.33±0.01[a]	2.28±0.09[c]	0.80±0.01[a]	1.01±0.05[a]	1.19±0.08[d]
$G_{36}T_{30}$	0.35±0.01[a]	3.02±0.11[b]	0.92±0.02[a]	0.88±0.03[a]	1.89±0.14[a]
Germination at 40℃					
$G_{12}T_{40}$	0.34±0.01[a]	3.49±0.10[a]	0.83±0.02[a]	0.98±0.03[a]	1.65±0.10[b]
$G_{36}T_{40}$	0.33±0.02[a]	1.92±0.08[d]	0.81±0.01[a]	0.89±0.02[a]	1.95±0.11[a]

Values (mean ± SD) with the same superscripts in each column are not significantly different at $p<0.05$.
MR – ungerminated parboiled milled rice; BR – ungerminated brown rice; GT – germinated brown rice; subscripts 12 and 36 are germination durations, h; subscripts 30 and 40 are the temperatures of germination,℃.

Conclusions

The work revealed that milling produced adverse effects on nutrients and bioactive compounds of NERICA-8 brown rice while germination improved them. Significantly ($p<0.05$) higher total antioxidant activity, γ-amino butyric acid, aminoacids, protein, total dietary fiber, iron, zinc, calcium, vitamin B_2 and vitamin E contents occurred when the brown rice was germinated for 36 h compared to 12 h. Milling reduced the levels of each of these compounds. The work also showed that germinated brown rice could have a lower resistance to heat and shear sterss and could be highly palatable. Germination for 36 h could also reduce retrogradation of NERICA-8 brown rice flour. For enhanced nutritional contents such as high proteins, amino acids, calcium and magnesium contents, the germination temperature of 30℃ was appropriate, while 40℃ was suitable for vitamin E, total dietary fiber and γ-amino butyric acid contents. The significant increase ($p<0.05$) observed in the level of γ-amino butyric acid level at the germination temperature of 40℃ for 36 h (329.73%) indicates that it could be used as functional food.

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