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Symbiotic and adverse interplay of hypogeal germination periods on brown rice (*Oryza sativa*): nutrient and non-nutrient characteristics

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Abstract

This study investigated the symbiotic and adverse consequence of hypogeal germination periods on nutrients and non-nutrient characteristics of brown rice (*Oryza sativa*). Brown rice paddy was subjected to hypogeal germination for 0–72 h using one-factor design-response surface methodology (OFD-RSM) and evaluated for nutrients and non-nutrient characteristics. The results showed that hypogeal germination caused a significant ($p < 0.05$) change in the proximate composition: protein (9.42–12.36%), fat (0.88–1.38%), ash (1.87–2.50%); anti-nutrients: saponin (2.03–2.22%), oxalate (2.44–3.45 mg/100 g), phytate (6.99–8.81 mg/100 g); functional properties: water absorption capacity, WAC (121.23–147.78%), oil absorption capacity, OAC (121.39–147.26%); antioxidants properties: 2, 2-diphenyl-1-picrylhydrazyl, DPPH (35.30–43.60%), ferric reducing antioxidant power, FRAP (0.054–0.119 mMolFe^{2+}), metal chelating activity, MCA (44.28–52.99%), total phenolic content, TPC (0.623–0.798 mg gallic acid equipvalent per gram (mgGAE/g)), total flavonoid content, TFC (43.47–50.63 mg rutin equivalent per gram (mgRUTIN/g)); and mineral content: calcium (36.0–41.76 mg/100 g), phosphorus (82.53–94.32 mg/100 g), and magnesium (162.70–168.36 mg/100 g). Germination had significant symbiotic effects (linear and quadratic) on the proximate, DPPH, FRAP, MCA, TPC, WAC, OAC, and anti-nutrients. Whereas, adverse effects (linear and quadratic) of germination were noted in total flavonoids and anti-nutrients. Optimum hypogeal germination period of 72.18 h was established and corresponding protein (12.37 g/100 g), fat (1.37 g/100 g), fibre (2.15 g/100 g), moisture (10.07 g/100 g), DPPH (43.66%), FRAP (0.105mMolFe^{2+}), TPC (0.08mgGAE/g), TFC (50.25MgRUTIN/g), WAC (147.99%), OAC (147.29%), Calcium (41.77 mg/100 g), iron (0.207 mg/100 g), zinc (5.89 mg/100 g), phosphorus (94.77 mg/100 g). Phenolic compounds profile of the optimized germinated brown rice showed the presence of gallic acid (2.84 mg/100 g), 4-hydroxy benzoic acid (3.41 mg/100 g), caffeic acid (4.63 mg/100 g), vanillic acid (6.19 mg/100 g), catechin (3.88 mg/100 g), chlorogenic acid (1.93 mg/100 g), ferulic acid (4.16 mg/100 g), and quercetin (1.27 mg/100 g) whereas, the non-germinated rice showed gallic acid (2.05 mg/100 g), 4-hydroxy benzoic acid (2.53 mg/100 g), caffeic acid (4.11 mg/100 g), vanillic acid (6.08 mg/100 g), catechin (3.35 mg/100 g), chlorogenic acid (1.89 mg/100 g), ferulic acid (4.23 mg/100 g), and quercetin (1.29 mg/100 g). Hypogeal germinated brown rice could find application as a functional ingredient in food formulation.

Keywords: Hypogeal germinated brown rice, Response surface methodology, Proximate, Functional properties, Antioxidant activity, Mineral, Anti-nutrient

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Introduction

Rice is a starchy food and the most important cereal in developing countries. It is a staple food in many parts of the world especially in the East, South, and Southeast Asia, and becomes the second-most consumed cereal grain. Rice has many unique attributes such as ease of digestion; bland taste and hypoallergenic properties. However, rice has relatively low amounts of proteins and most of them are very hydrophobic and therefore resist swelling in the water at neutral pH (Lumduwong & Seib 2000). Due to low protein contents and the absence of gliadin moiety in rice, it is an ideal food material for patients suffering from celiac sprue (gluten-sensitivity disease), other chronic diarrhea diseases, and people that need low protein diets (Hartsook 1984). Rice varieties are classified by grain size and shape into three types; long, medium, and short-grain. Each variety has distinct cooking, eating, and processing qualities. White or polished rice is the popular form of rice consumed; it is produced by milling rough rice to remove the bran and germ. Brown rice is unpolished rice obtained by removing the husk from the rough rice. Rice could be consumed as whole milled grains or as rice flour (tuwo shinkafa) which is obtained by mixing rice flour with boiling water and stirred to produce dough with a smooth consistency. Rice milling which removes the bran and germ reduces the nutrients significantly in white rice. This calls for a global campaign for the consumption of brown rice (Wu et al. 2013).

Germination is one of the most common and effective processes for improving the nutritional quality of cereals and legumes. Besides reducing anti-nutritive compounds, it improves the levels of free amino acids, dietary fiber, and other components, changes the sensory characteristics, and increases the functionality of the legume seeds due to the subsequent increase in the bioactive compounds (Patil & Khan 2011; Onyeka 2019). Germination of cereals and legumes is an economical processing technology. Germinated brown rice (GBR) is among the most prominent cereal products and has gained much attention due to its improved textural properties. The GBR is obtained by steeping brown rice grains in potable water. Consumption of germinated brown rice is associated with improvement in human health because of the wide range of biological properties. Germination of brown rice releases the bound minerals, by reducing phytic acid content making them more absorbable by the body (Kim et al. 2012). Germinated brown rice is rich in protein, dietary fibre, iron, zinc, calcium, and B-vitamins. Germinated brown is also rich in bioactive compounds such as γ-aminobutyric acid, γ-oryzanol, tocopherol, tocotrienol, flavonoids, phenolic compounds, and tannins (Patil & Khan 2011; Kim et al. 2012; Onyeka 2019).

The functional attributes of cereal grains are the fundamental physiochemical properties that reflect the complex interaction between the structure, molecular components, and physicochemical properties of food components. The characteristics and use of flours to produce acceptable sensory characteristic products depend on the functional characteristics of the flours. During the process of germination, some biochemical processes take place and this leads to a change in nutritional parameters, chemical contents, and activities of certain enzymes (Khampang et al. 2009). These changes would affect the functional performance of germinated rice flour, which will directly influence the quality of rice-based products.

Consumers of underdeveloped and developing countries are currently aware of health problems caused by different diets and for this reason, it is a great challenge for food industries to produce foods having sufficient nutrients and bio-active compounds with high quality in terms of specific properties such as antioxidant activity and functional properties. Despite numerous beneficial characteristics of GBR in terms of nutritional and therapeutic properties, it is scarce in different parts of the world. Even in West African countries such as Nigeria, it is scarce if available at all in the market.

There is a current upsurge in the consumption of local cultivars of rice in some developing countries such as Nigeria due to their low starch content and higher bioactive properties. Recent studies reported that some cultivars of rice such as Kwandala, Yardass, Jeep, and Jamila contain a lower rate of starch digestibility with higher nutritional value compared to improved varieties (Odenigbo et al. 2014). There is an increasing trend focusing on the use of germinated cereal flour in the formulation of high-quality healthful products (with sufficient nutrients, bioactive compounds, and good functional properties). Although some studies have been conducted on the germination of cereals, however, there is limited information on the optimization of the germination process and characterization. Thus, this study aimed to optimize the germination process for brown rice from B12 cultivar and evaluate the symbiosis and adversative significances of hypogeal germination on nutritional, antioxidant activity, functional properties, and antinutrients characteristics of brown rice.

Materials and methods

Material and source

Rice paddy (long-grain B12), *Oryza sativa* was purchased from a rice farmer in Amasiri, Afikpo North LGA, Ebonyi State Nigeria. The chemicals were of analytical grades and obtained from Fisher Scientific (Oakville, ON, Canada) and Sigma Chemicals (St. Louis, MO, USA).

Methods

Modeling and optimization of hypogeal germinated brown rice

Rice paddy was winnowed, steeped in potable water for 16 h while the steeping water was changed every 6 h. After the soaking period, it was drained. Hypogeal germination was carried out using One Factor Design of Response Surface Methodology (OFD-RSM). The germination period was varied between 0 and 72 h while proximate, mineral, antioxidants, antinutrients, and functional properties were employed as the dependent variables (Table 1). A sum of 10 experiments was conducted with five replicates ($n = 5$). The germinated rice was parboiled, dried in a hot air oven (Bioevopeak DOF-H-140, Jinan City, China) at 60 °C for 10 h, dehulled, milled into powder, sieved with 100 μm mesh size, packaged in an airtight polythene bag as germinated brown rice flour (GBR) and stored at ambient temperature (28 ± 2 °C) for further analysis. Modeling and optimization were performed using OFD-RSM.

Proximate composition

Moisture, fat, fibre, ash, and protein were determined using AOAC (2005) while carbohydrate was evaluated by a difference.

$$Carbohydrate\ (\%) = 100 - (moisture\ content + ash\ content + fibre + fat + protein) \quad (1)$$

Methanol-aqueous extraction

The extraction was done as described by Alvarez et al. (2016) with slight modification. A 1 g of sample (powder) was dispersed in 20 ml of a methanol-aqueous mixture (80:20, v/v). The resultant was homogenized on a magnetic stirrer at ambient temperature (28 ± 2 °C) for 30 h and centrifuged at 4552 x g (ESCO MCR-88, Singapore, Republic of Singapore) for 25 min after which the supernatant was separated and used for the evaluation of antioxidant activities.

Table 1 Proximate composition and regression analysis of hypogeal germinated brown rice

(a) Expt. No.	Run Order	Hypogeal Germination Period	Protein	Fat	Fibre	Ash	Moisture
		(h)	(%)	(%)	(%)	(%)	(%)
1	10	0(− 1)	9.42 ± 0.06	0.88 ± 0.01	2.01 ± 0.02	1.87 ± 0.02	9.17 ± 0.05
2	2	0(−1)	9.42 ± 0.08	0.89 ± 0.02	2.00 ± 0.04	1.87 ± 0.01	9.17 ± 0.02
3	3	18(−0.5)	9.75 ± 0.15	1.09 ± 0.02	2.09 ± 0.02	2.20 ± 0.02	9.58 ± 0.04
4	9	54(0.5)	11.22 ± 0.05	1.33 ± 0.01	2.16 ± 0.07	2.50 ± 0.02	10.02 ± 0.06
5	7	72(1)	12.36 ± 0.06	1.37 ± 0.01	2.15 ± 0.01	2.47 ± 0.03	10.07 ± 0.04
6	4	72(1)	12.36 ± 0.09	1.38 ± 0.02	2.15 ± 0.02	2.47 ± 0.02	10.07 ± 0.08
7	5	36(0)	10.35 ± 0.04	1.23 ± 0.01	2.13 ± 0.02	2.41 ± 0.01	9.86 ± 0.05
8	6	36(0)	10.35 ± 0.07	1.23 ± 0.01	2.13 ± 0.01	2.41 ± 0.03	9.88 ± 0.02
9	8	36(0)	10.35 ± 0.03	1.23 ± 0.04	2.13 ± 0.01	2.41 ± 0.02	9.87 ± 0.07
10	1	36(0)	10.66 ± 0.10	1.24 ± 0.02	2.14 ± 0.03	2.43 ± 0.01	9.87 ± 0.06
(b) Model Terms	*p*-value						
	Protein	Fat	Fibre	Ash	Moisture		
Model	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
h	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
h^2	0.0003	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
Lack of fit	0.8416	0.5806	0.3970	0.8468	0.5429		
R^2	0.9927	0.9993	0.9968	0.9993	0.9998		
Adj. R^2	0.9907	0.9991	0.9959	0.9991	0.9997		
Pred. R^2	0.9887	0.9984	0.9917	0.9989	0.9996		
Ad. Prec.	51.99	169.469	74.23	159.06	270.05		
CV (%)	0.97	0.44	0.18	0.31	0.06		
Std. Dev.	0.10	0.01	< 0.01	0.01	0.01		

Data are presented as mean ± standard deviation (n = 5). *h* – hypogeal germinated brown rice; R^2 – coefficient of determination; Adj. R^2- adjusted coefficient of determination; Pred. R^2 – predicted coefficient of determination; Ad. Pred. – adequate prediction; CV – coefficient of variation

2, 2-diphenyl-1-picrylhydrazyl determination

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of samples was determined using the method described by Girgih et al. (2011) with slight modifications. Sample (10 mg/ml) was dissolved in 0.1 M sodium phosphate buffer, pH 7.0 containing 1% (v/v) Triton-X. The DPPH was dissolved in 95% methanol to a final concentration of 100 μM. A 100 μL aliquot of each sample was mixed with 100 μL of the DPPH radical solution in a 96-well plate and incubated at ambient temperature (28 ± 2 °C) in the dark for 30 min. The buffer was used in the blank assay while Glutathione (GSH) served as the positive control. Absorbance was measured at 517 nm using a spectrophotometer (DU-8800R Split Beam, Shanghai, China), and the percentage DPPH radical scavenging activity was determined using the following equation:

$$\%\text{DPPH} = x = \frac{\text{absorbance of blank} - \text{absorbance of sample}}{\textit{absorbance of blank}}\, x\, 100 \tag{2}$$

Ferric reducing antioxidant powers

The ferric reducing antioxidant power (FRAP) of the sample was evaluated using the modified method of Benzie and Strain (1999). The FRAP working reagent was prepared by mixing 300 mmol/L acetate buffer of pH 3.6, 10 mmol/L 2,4,6-tri-(2-pyridyl)-1,3,5- triazine, and 20 mmol/L $FeCl_3$ in the ratio of 5: 1: 1, respectively to obtain a straw-colored solution, and the temperature of the mixture raised to 37 °C. Samples were prepared to 10 mg/ml using distilled water. Into a clear and clean test tube, 40 μl of samples and 200 μl of FRAP reagent were added and absorbance was read at 593 nm. Iron II sulphate heptahydrate was used as a standard. This was prepared by making serial dilutions of 0.025 to 0.25 μM from 1 mM of Iron II sulphate. Iron reducing activity of the samples was determined from the standard curve of Iron II sulphate heptahydrate and the results were expressed in Fe^{2+} (mMol).

Metal chelating activity

The metal (iron) chelating activity (MCA) of the samples was determined according to the modified method of Xie et al. (2008). The sample was prepared to final concentrations of 1.0 mg/ml to 5 mg/ml in distilled water. The mixture was hydrated for 1 h and centrifuged (ESCO MCR-88, Singapore, Republic of Singapore). A 1 ml aliquot of the sample solution or blank (distilled water) was mixed with 50 μl of 2 mM$FeCl_2$ and 1.85 ml double distilled water in a reaction tube. This was followed by the addition of 100 μl of 5 mM Ferrozine. The mixture was homogenized thoroughly and incubated at room temperature for 10 min and absorbance values of both the blank (Ab) and samples (As) were measured at 562 nm using a spectrophotometer (DU-8800R Split Beam, Shanghai, China). The metal chelating activity was calculated as follows:

$$\%\text{metal chelating ability} = x = \frac{\text{absorbance of blank} - \text{absorbance of sample}}{\textit{absorbance of blank}}\, x\, 100 \tag{3}$$

Phenolic content

The total phenolic content (TPC) of each extract was determined using the Folin–Ciocalteu method (Hoff & Singleton 1977) with some modifications. A standard calibration curve was prepared using 25–350 mg/ mL gallic acid concentration in 50% (v/v) methanol. The samples were also diluted with 50% methanol to a concentration and centrifuged. A 0.25 mL aliquot of Folin-Ciocalteu reagent was added to 0.25 mL of a gallic acid solution or the sample and then mixed. After standing in the dark at room temperature for 5 min, 0.5 ml 20% sodium carbonate solution was added followed by 4 mL of double-distilled water. The contents were mixed and incubated in the dark for 1 h. The intensity of the green color was then measured at 725 nm using a UV–visible spectrophotometer (DU-8800R Split Beam, Shanghai, China). TPC was expressed as milligrams gallic acid equivalents (GAE) per gram of dry leaf powder (mg GAE/g).

Flavonoids

The concentration of flavonoids in the extract was determined spectrophotometrically according to the procedure of Sun et al. (1999). The sample (0.01 g) was dissolved in 5 ml of extraction solvent and made up to 20 ml to give a final concentration of 0.5 mg/ml and centrifuged (ESCO MCR-88, Singapore, Republic of Singapore). To clean dry test tubes (in triplicate) was pipetted 0.5 ml of working solution of the sample and diluted with 4.5 ml distilled water. To each test tube is then added 0.3 ml of 5% (w/v) Sodium nitrite ($NaNO_2$), 0.3 ml of 10% aluminum chloride ($AlCl_3$) and 4 ml of 4% (w/v) sodium hydroxide (NaOH). The reaction mixtures were incubated at ambient temperature for 15 min. The absorbance was read at 500 nm against the reagent blank containing all reagents except the extract or standard rutin in the case of the standard curve. The standard calibration curve was prepared by pipetting 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 ml of 1 mg/ml rutin (1 mg / ml of rutin standard solution) into clean dry test tubes. The volumes were made up to 5 ml with distilled water. To each of the tubes was added 0.3 ml of 5% (w/v) $NaNO_2$, 0.3 ml of 5% (w/v) AlCl3 and 4 ml of 4% (w/v) NaOH. The reaction mixture is incubated at room temperature (28 ± 2 °C) for 15 min. The absorbance was taken at 500 nm

and was plotted against the concentration to give the standard calibration curve. The concentrations of the flavonoids in the sample were extrapolated from the standard calibration curve and expressed as milligram rutin equivalent per gram of sample.

Bulk density

Bulk density was determined by the method of Okezie and Bello (1988). A 10 ml graduated cylinder, previously tared, was gently filled with the sample. The bottom of the cylinder was gently tapped on a laboratory bench several times until there was no further diminution of the sample level after filling it to the 10 ml mark. Bulk density was calculated as the weight of the sample per unit volume of sample (g/ml).

Least gelation concentration

Least gelation concentration (LGC) was determined according to the method of Adebiyi and Aluko (2011) by suspending the samples in water at different percentages (1 to 27%). The mixture was vortexed, placed in a water bath (JY/OEM HH-S_4, Shanghai, China) at 95 °C for 1 h, cooled rapidly under tap water, and left in the refrigerator (4 °C) for 14 h. The sample concentration at which the gel did not slip when the tube was inverted was taken as the LGC.

Swelling capacity and solubility index

Swelling capacity and solubility index were determined using the method described by Takashi and Sieb (1988). It involved weighing 1 g of the sample into a 50 ml centrifuge tube. 50 ml of distilled water was added and mixed gently. The slurry was heated in a water bath (DU-8800R Split Beam, Shanghai, China) at 80 °C for 15 min. During heating, the slurry was stirred gently to prevent the clumping of the flour. On completion of 15 min, the tube containing the paste was centrifuged at 3000 x g for 10 mins. The supernatant was decanted immediately after centrifuging. The weight of the sediment was taken and recorded. The moisture content of the sediment's gel was, therefore, determined to get the dry matter content of the gel

$$SC = \left(\frac{Weight\ of\ wet\ mass\ sediment}{weight\ of\ dry\ matter\ in\ the\ gel}\right) * 100 \quad (4)$$

$$\mathrm{WSI} = \left(\frac{Weight\ of\ dry\ solid\ after\ drying}{weight\ of\ the\ initial\ powder\ or\ flour}\right) * 100 \quad (5)$$

Tannin content

Tannin content was determined by the AOAC (2005) method. Sample (0.5 g) was dispensed in 50 ml (which was diluted to 5 mg/ml) of distilled water and shaken. The mixture was allowed to stand for 30 min at 28 °C before it was filtered through Whatman no.4 grade of filter paper. The extract (2 ml) was dispensed into a 50 ml volumetric flask. Similarly, 2 ml of standard tannic solution (1 mg/ml tannic acid) and 2 ml of distilled water were put in a separate volumetric flask to serve as a standard. A 2.5 ml of saturated sodium carbonate (Na_2CO_3) solution and 1 ml of Folin-C reagent were added to each flask and volume was made up to 50 ml and thoroughly mixed. After standing for 1½ h, the sample was filtered using Whatman no.4 grade of filter paper, and the absorbance was measured at 760 nm against reagent blank.

Saponin determination

The spectrophotometric method of Brunner (1994) was used for the saponin determination. A 2 g of the finely ground sample was weighed into a 250 ml beaker and 100 ml of Isobutyl alcohol was added. Shaker was used to shake the mixture for 5 h to ensure uniform mixing. The mixture was filtered with No 1 Whatman filter paper into a 100 ml beaker containing 20 ml of 40% saturated solution of magnesium carbonate ($MgCO_3$). The mixture obtains again was filter through No 1 Whatman filter paper to obtain a clear colourless solution. A 1 ml of the colourless solution was put into a 50 ml volumetric flask using a pipette, 2 ml of 5% iron (iii) chloride ($FeCl_3$) solution was added and made up to the mark with distilled water. It was allowed to stand for 30 min for the colour to develop. Saponin standard was prepared in the following concentrations: 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml and treated similarly as the samples. The absorbance was read against the blank at 380 nm. The concentration of saponin was read from the equation of the calibration curve generated from the standard concentrations.

Oxalate

Oxalate was determined using the titrimetric method by Falade et al. (2004). One milliliter of the sample was weighed in triplicate into conical flasks and extracted with 190 ml distilled water and 10 ml 6 M HCl. The suspension was placed in boiling water for 2 h, filtered, and made up to 250 ml with water in a volumetric flask. To 50 ml aliquot was added 10 ml of 6 M HCl and filtered and the precipitate was washed with hot water. The filtrate and the wash water were combined and titrated against concentrated ammonium hydroxide (NH_4OH) until the salmon pink colour of the methyl red indicator changed to faint yellow. The solution was heated to 90 °C and 10 ml of 5% (w/v) calcium chloride ($CaCl_2$) solution was added to precipitate the oxalate overnight.

Table 2 Mineral composition and regression analysis of hypogeal germinated brown rice

(a) Expt. No.	Run Order	Hypogeal Germination Period	Ca	P	Fe	Na	Mn	Cu	K	Zn	Mg
		(h)	(mg/100 g)	(mg/100 g)	(mg/100 g)	(mg/100 g)	(mg/100 g)	(mg/100 g)	(mg/100 g)	(mg/100 g)	(mg/100 g)
1	10	0(−1)	36.00 ± 0.35	82.53 ± 0.78	0.185 ± 0.002	34.41 ± 0.11	0.441 ± 0.005	2.25 ± 0.04	1576.83 ± 1.10	5.01 ± 10	162.70 ± 1.35
2	2	0(−1)	36.00 ± 0.21	82.53 ± 0.34	0.185 ± 0.003	34.41 ± 0.34	0.441 ± 0.010	2.25 ± 0.06	1576.83 ± 0.98	5.01 ± 0.08	162.70 ± 1.72
3	3	18(−0.5)	37.80 ± 0.10	88.36 ± 0.11	0.192 ± 0.001	35.88 ± 0.28	0.470 ± 0.008	2.54 ± 0.08	1674.83 ± 0.49	5.30 ± 0.11	164.21 ± 0.89
4	9	54(0.5)	40.69 ± 0.45	94.41 ± 0.16	0.203 ± 0.004	37.09 ± 0.45	0.506 ± 0.004	2.87 ± 0.10	1762.69 ± 1.38	5.73 ± 0.35	167.04 ± 0.84
5	7	72(1)	41.76 ± 0.29	94.65 ± 0.78	0.207 ± 0.100	36.83 ± 0.12	0.512 ± 0.005	2.90 ± 0.05	1752.56 ± 1.47	5.89 ± 0.01	168.36 ± 0.94
6	4	72(1)	41.76 ± 0.11	94.65 ± 0.49	0.207 ± 0.010	36.83 ± 0.11	0.512 ± 0.011	2.90 ± 0.03	1752.56 ± 1.92	5.89 ± 0.03	168.36 ± 1.36
7	5	36(0)	39.37 ± 0.18	92.32 ± 0.71	0.197 ± 0.007	36.76 ± 0.14	0.493 ± 0.002	2.74 ± 0.07	1737.30 ± 1.38	5.53 ± 0.02	165.69 ± 1.48
8	6	36(0)	39.37 ± 0.21	92.32 ± 0.64	0.197 ± 0.009	36.76 ± 0.21	0.493 ± 0.001	2.74 ± 0.02	1737.30 ± 1.46	5.53 ± 0.10	165.69 ± 1.16
9	8	36(0)	39.37 ± 0.32	92.32 ± 0.56	0.198 ± 0.005	36.77 ± 0.13	0.493 ± 0.004	2.74 ± 0.04	1737.30 ± 0.98	5.54 ± 0.07	165.68 ± 0.97
10	1	36(0)	39.39 ± 0.28	92.38 ± 0.47	0.198 ± 0.004	36.77 ± 0.16	0.4940.010	2.75 ± 0.03	1738.00 ± 0.78	5.53 ± 0.09	165.67 ± 0.76
(b) Model Terms	*p*-value										
	Ca	P	Fe	Na	Mn	Cu	K	Zn	Mg		
Model	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
h	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
h^2	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
Lack of fit	0.8416	0.8417	0.7337	0.8089	0.1290	0.6436	0.1531	0.6954	0.0719		
R^2	0.9999	0.9999	0.9980	0.9999	0.9997	0.9998	0.9999	0.9999	0.9999		
Adj. R^2	0.9999	0.9998	0.9974	0.9998	0.9997	0.9998	0.9999	0.9999	0.9998		
Pred. R^2	0.9998	0.9998	0.9969	0.9998	0.9996	0.9997	0.9997	0.9998	0.9998		
Ad. Prec.	1402.81	1021.42	100.93	1238.45	264.16	330.26	1019.95	455.30	973.58		
CV (%)	0.02	0.02	0.20	0.01	0.10	0.13	0.02	0.06	< 0.01		
Std. Dev.	< 0.01	0.02	< 0.01	< 0.01	< 0.01	< 0.01	0.33	< 0.01	0.01		

Data are presented as mean ± standard deviation ($n = 5$). *h* – hypogeal germinated brown rice; R^2 – coefficient of determination; Adj. R^2- adjusted coefficient of determination; Pred. R^2 – predicted coefficient of determination; Ad. Pred. – adequate prediction; CV – coefficient of variation. Ca – calcium, P – phosphorus, Fe – iron, Na – sodium, Mn – manganese, Cu – copper, K – potassium, Zn – zinc, Mg – magnesium

The precipitate was washed free of calcium with distilled water and then washed into100 ml conical flask with 10 ml of hot 25% (v/v) *sulfuric acid* (H_2SO_4) and then with 15 ml of distilled water. The final solution was heated to 90 °C and titrated against a standard 0.05 M potassium permanganate ($KMnO_4$) until a faint purple solution persisted for the 30s. The oxalate was calculated as the sodium oxalate equivalent.

$$1\text{ml of } 0.05\text{M KMn04} = 2\text{mg sodium oxalate equivalent/g of sample} \quad (6)$$

Phytate

The phytate content of the sample was determined as described in Vaintraub and Lapteva (1988) after extraction of the sample with 2.4% hydrochloric acid (HCl) for 1vh and centrifuged. A 3 ml supernatant was added to 1 ml of wade reagent (0.03% *ferric chloride* hexahydrate and 0.3% sulfosalicylic acid in distilled water). The absorbance of the mixture was measured at 500 nm wavelength using a UV–vis Spectrophotometer (DU-8800R Split Beam, Shanghai, China). The value obtained was subtracted from the blank absorbance value and the phytate content (mg/100 g sample) was estimated from the phytic acid standard calibration curve (5–35 mg/kg) which was prepared similarly as the sample.

Mineral contents

For the mineral contents, 500 mg of the sample was weighed in a conical digesting flask and 10 ml of hydrochloric acid (HCl) and nitric acid (HNO_3) were respectively added to the flask. The mixture was digested for 10 min and allowed to cool. The mixture was filtered using filter paper (Whatman number 1) and made up to 50 ml of distilled water. The digested samples were introduced at 2.0 ml/min into the Atomic Absorption Spectroscopy (Perkin Elmer, model 402, Norwalk, Los Angeles) for calcium, phosphorous, iron, manganese, copper, zinc at 393.37, 766.49, 213.618, 279.55, 259.94, 257.61, 324.75 and 213.856 wavelengths, respectively while sodium and potassium were determined using flame photometer (Martines et al. 2007).

Table 3 Antinutrient compositions and regression analysis of hypogeal germinated brown rice

(a) Expt. No.	Run Order	Hypogeal Germination Period	Tannin	Saponin	Oxalate	Phytate
		(h)	(%)	(%)	(mg/100 g)	(mg/100 g)
1	10	0(−1)	1.08 ± 0.01	2.22 ± 0.10	3.45 ± 0.03	8.81 ± 0.10
2	2	0(−1)	1.08 ± 0.02	2.22 ± 0.09	3.45 ± 0.05	8.81 ± 0.21
3	3	18(−0.5)	1.05 ± 0.02	2.14 ± 0.05	3.37 ± 0.05	8.19 ± 0.15
4	9	54(0.5)	1.01 ± 0.03	2.05 ± 0.08	2.86 ± 0.04	7.28 ± 0.07
5	7	72(1)	1.01 ± 0.01	2.03 ± 0.10	2.44 ± 0.01	6.99 ± 0.04
6	4	72(1)	1.01 ± 0.01	2.03 ± 0.10	2.44 ± 0.05	6.99 ± 0.06
7	5	36(0)	1.03 ± 0.03	2.08 ± 0.04	3.18 ± 0.03	7.68 ± 0.10
8	6	36(0)	1.03 ± 0.01	2.08 ± 0.02	3.18 ± 0.03	7.68 ± 0.14
9	8	36(0)	1.03 ± 0.02	2.08 ± 0.01	3.18 ± 0.02	7.68 ± 0.09
10	1	36(0)	1.02 ± 0.02	2.08 ± 0.04	3.19 ± 0.11	7.66 ± 0.03
(b) Model Terms	*p*-value					
	Tannin	Saponin	Oxalate	Phytate		
Model	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
h	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
h^2	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
Lack of fit	0.5987	0.2028	0.1148	0.8416		
R^2	0.9999	0.9999	0.9998	0.9999		
Adj. R^2	0.9998	0.9999	0.9998	0.9998		
Pred. R^2	0.9998	0.9998	0.9997	0.9998		
Ad. Prec.	362.44	4152.53	327.29	407.32		
CV (%)	0.04	< 0.01	0.18	0.10		
Std. Dev.	< 0.01	< 0.01	< 0.01	< 0.01		

Data are presented as mean ± standard deviation ($n = 5$). *h* – hypogeal germinated brown rice; R^2 – coefficient of determination; Adj. R^2- adjusted coefficient of determination; Pred. R^2 – predicted coefficient of determination; Ad. Pred. – adequate prediction; CV – coefficient of variation

Identification and quantification of phenolic compounds

Extraction was carried out by suspending 0.6 g of sample in 95% ethanol, thoroughly mixed using a vortex mixer for 2 min, and centrifuged at 3664 3 x g using a centrifuge (ESCO MCR-88, Singapore, Republic of Singapore) for 20 min at ambient temperature. The residue was discarded while the supernatant was used for the identification and quantification. The sample analysis was performed using high-performance liquid chromatography (HPLC LI-6200, Haryana, India). The phenolic compounds in the sample were scanned using a Diode Array Detector (DAD) at absorbance wavelengths of 200 to 400 nm. A gradient solvent system was employed with solvent A being water-acetic acid (97:3, v/v) and solvent B being acetonitrile. The column temperature was maintained at 30 °C with a flow rate of 0.8 ml/min. Solutions of the phenolic standards were prepared in the mobile phase. Separate calibration curves were used for phenolic compounds at four different concentrations.

Statistical analysis

A completely randomized experimentation technique was utilized to avoid systematic errors. The model's qualities were evaluated using the coefficient of determination (R^2), adjusted coefficient of determination (Adj. R^2), probability value at 95% confidence interval, predicted R^2, coefficient of variation, lack-of-fit, and analysis of variance (ANOVA). Mathematical modeling and optimization of the hypogeal germination were conducted through One Factor Design-Response Surface Methodology (OFD-RSM) of the Design-Expert software version 10.0 (Stat-ease Inc., MN, USA). Principal component analysis (PCA) and agglomerative hierarchical cluster (AHC) were performed using XLSTAT 2013 version). Pearson correlation was performed using the SPSS version17. Also, evaluation of significant difference among means at 5% significant level by Duncan test was performed using the SPSS version17.

Table 4 Antioxidant properties, antioxidant activity and regression analysis of hypogeal germinated brown rice

(a) Expt. No.	Run Order	Hypogeal Germination Period	DPPH	MCA	FRAP	TPC	TFC
		(h)	(%)	(%)	(mMolFe^{2+})	(mgGAE/g)	(MgRUTIN/g)
1	10	0(−1)	35.30 ± 0.84	52.99 ± 0.29	0.054 ± 0.002	0.770 ± 0.005	50.63 ± 0.92
2	2	0(−1)	36.00 ± 0.21	52.99 ± 0.71	0.054 ± 0.001	0.770 ± 0.003	50.63 ± 0.48
3	3	18(−0.5)	37.01 ± 0.14	46.06 ± 0.32	0.096 ± 0.001	0.657 ± 0.004	45.08 ± 0.32
4	9	54(0.5)	40.85 ± 0.32	47.61 ± 0.47	0.121 ± 0.003	0.671 ± 0.006	45.10 ± 0.14
5	7	72(1)	43.60 ± 0.12	56.10 ± 0.46	0.106 ± 0.002	0.798 ± 0.002	50.17 ± 0.74
6	4	72(1)	43.60 ± 0.11	56.10 ± 0.38	0.106 ± 0.004	0.798 ± 0.009	50.17 ± 0.61
7	5	36(0)	38.80 ± 0.31	44.28 ± 0.14	0.118 ± 0.001	0.624 ± 0.004	43.47 ± 0.18
8	6	36(0)	38.00 ± 0.42	44.28 ± 0.73	0.118 ± 0.003	0.624 ± 0.006	43.47 ± 0.43
9	8	36(0)	38.00 ± 0.15	44.28 ± 0.28	0.119 ± 0.002	0.624 ± 0.003	43.47 ± 0.54
10	1	36(0)	38.00 ± 0.21	44.30 ± 0.51	0.118 ± 0.002	0.623 ± 0.005	43.88 ± 0.31
(b) Model Terms	*p*-value						
	DPPH	MCA	FRAP	TPC	TFC		
Model	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
h	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0421		
h^2	0.0011	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
Lack of fit	0.5135	0.4332	0.7427	0.8416	0.2886		
R^2	0.9874	0.9990	0.9999	0.9999	0.9979		
Adj. R^2	0.9839	0.9998	0.9998	0.9998	0.9973		
Pred. R^2	0.9742	0.9998	0.9998	0.998	0.9967		
Ad. Prec.	39.33	2365.02	353.46	1197.75	74.71		
CV (%)	0.94	0.02	0.34	0.04	0.37		
Std. Dev.	0.37	0.01	< 0.01	< 0.01	0.17		

Data are presented as mean ± standard deviation ($n = 5$). *h* – hypogeal germinated brown rice; R^2 – coefficient of determination; Adj. R^2- adjusted coefficient of determination; Pred. R^2 – predicted coefficient of determination; Ad. Pred. – adequate prediction; CV – coefficient of variation; DPPH - 2, 2-diphenyl-1-picrylhydrazyl; FRAP - ferric reducing powers; MCA - metal chelating activity; TPC - total phenolic content; TFC – total flavonoids content

Results and discussion

Nutrients, ant-nutrients, and non-nutrients attributes of hypogeal germinated brown rice

The proximate composition of the germinated brown rice is presented in Table 1. The study revealed that hypogeal germination caused significant ($p < 0.05$) effects in protein, fat fibre, ash, and moisture contents of the rice samples (Table 1b). The moisture content of the samples was very low and this indicates good keeping or storage quality. Table 1b indicates that both single and quadratic effects of hypogeal germination on the rice samples were significant ($p < 0.05$).

Germination had significant linear and quadratic effects on the mineral (Table 2) and anti-nutrient (Table 3) contents of the brown rice ($p < 0.05$). The result of the antioxidant properties and activity of the hypogeal germinated rice samples (Table 4) showed that hypogeal germination had significant ($p < 0.05$) linear and quadratic effects on the antioxidants and antioxidant activity.

The functional properties of the hypogeal germinated rice (Table 5) showed significant linear and quadratic effects of germination on brown rice ($p < 0.05$). The least gelation concentration is presented in Table 6. Germinated flours had increased the least gelation concentration. The least gelation concentration ranged between 8% w/v in 0GBR and 24GBR to 16%w/v in 48GBR and then reduced to 13% (w/v) in 72GBR. Anuchita et al. (2014) also reported an increased least gelation concentration in germinated brown rice. Gelation is an aggregation of denatured molecules. Germination may break down the rice proteins and, thus caused more denature when heated and aggregation than in the un-germinated rice flour. These results would be useful in food systems that require thin thickening and gelling agents.

The high values of coefficient of determination, R^2 (0.9874–0.9999), adjusted R^2 (0.9839–0.9998) with non-significant lack of fit (0.0719–0.8468) indicated models of a good fit for the proximate, mineral, anti-nutrient, antioxidants, and functional properties of the germinated brown rice. The high adequate precision of range 39.33–2365.02, low coefficient of variation (0.01–0.97%) with very low standard deviation proved the suitability of the generated models. Generally, the values of the R^2 and adj. R^2 are expected to near unity. The closer the value

Table 5 Functional properties and regression analysis of hypogeal germinated brown rice

(a) Expt. No.	Run Order	Hypogeal Germination Period	WAC	OAC	SC	WSI	BD
		(h)	(%)	(%)	(%)	(%)	(g/ml)
1	10	0(−1)	131.91 ± 1.21	121.39 ± 0.83	241.52 ± 1.05	14.98 ± 0.21	0.60 ± 0.02
2	2	0(−1)	131.91 ± 1.42	121.39 ± 1.11	241.52 ± 0.98	14.98 ± 0.31	0.60 ± 0.02
3	3	18(−0.5)	121.92 ± 0.98	129.23 ± 1.25	231.550.75	15.04 ± 0.09	0.59 ± 0.01
4	9	54(0.5)	129.85 ± 0.86	142.17 ± 0.49	226.80 ± 1.32	15.68 ± 0.11	0.57 ± 0.02
5	7	72(1)	147.78 ± 0.78	147.26 ± 0.82	232.02 ± 1.21	16.25 ± 0.16	0.57 ± 0.03
6	4	72(1)	147.78 ± 0.93	147.26 ± 1.35	232.02 ± 0.84	16.25 ± 0.09	0.57 ± 0.02
7	5	36(0)	121.23 ± 0.14	136.43 ± 0.74	226.64 ± 1.28	15.27 ± 0.17	0.58 ± 0.04
8	6	36(0)	121.23 ± 0.21	136.43 ± 1.23	226.64 ± 1.27	15.27 ± 0.32	0.58 ± 0.03
9	8	36(0)	121.23 ± 0.32	136.00 ± 0.49	226.64 ± 0.86	15.27 ± 0.12	0.58 ± 0.04
10	1	36(0)	121.58 ± 0.29	136.43 ± 0.84	227.00 ± 0.99	15.26 ± 0.10	0.58 ± 0.02
(b) Model Terms	*p*-value						
	WAC	OAC	SC	WSI	BD		
Model	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
h	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
h^2	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
Lack of fit	0.8416	0.6712	0.8416	0.6908	0.8510		
R^2	0.9999	0.9998	0.9997	0.9999	0.9997		
Adj. R^2	0.9999	0.9997	0.9996	0.9998	0.9996		
Pred. R^2	0.9998	0.9997	0.9995	0.9998	0.9996		
Ad. Prec.	405.89	309.91	221.19	658.43	267.04		
CV (%)	0.09	0.11	0.05	0.02	0.03		
Std. Dev.	0.12	0.15	0.12	< 0.01	< 0.01		

Data are presented as mean ± standard deviation ($n = 5$). *h* – hypogeal germinated brown rice; R^2 – coefficient of determination; Adj. R^2- adjusted coefficient of determination; Pred. R^2 – predicted coefficient of determination; Ad. Pred. – adequate prediction; CV – coefficient of variation
WAC – water absorption capacity; OAC – Oil absorption capacity; BD – Bulk density; SC – Swelling capacity; WSI – Water solubility index

Table 6 Least gelation concentration of hypogeal germinated brown rice

Flour concentration (% w/v)	0GBR	24GBR	48GBR	72GBR
1	- –	- –	- –	- –
2	- –	- –	- –	- –
3	- +	- +	- –	- –
4	- +	- +	- –	- –
5	- +	- +	- –	- –
6	- +	- +	- –	- –
7	- +	- +	- –	- +
8	+ +	+ +	- –	- +
9	+ +	+ +	- –	- +
10	+ +	+ +	- –	- +
11	+ +	+ +	- –	- +
12	+ +	+ +	- +	- +
13	+ +	+ +	- +	+ +
14	+ +	+ +	- +	+ +
15	+ +	+ +	- +	+ +
16	+ +	+ +	+ +	+ +
17	+ +	+ +	+ +	+ +
18	+ +	+ +	+ +	+ +
19	+ +	+ +	+ +	+ +
20	+ +	+ +	+ +	+ +
21	+ +	+ +	+ +	+ +
22	+ +	+ +	+ +	+ +
23	+ +	+ +	+ +	+ +
24	+ +	+ +	+ +	+ +
25	+ +	+ +	+ +	+ +

-- = not gelled; – + = slightly gelled; ++ = completely gelled; GBR – Germinated brown rice; $n = 5$

to unity, the better the suitability of the model. The value of adequate precision is expected to be > 4; the higher the value, the better the model. Furthermore, the value of the coefficient of variation is expected to be ≤10. The lower the value, the better the model. The low standard deviation indicated high precision among the experimental data. The p-value of < 0.0001 for all models showed the reliability of the models. Thus, the generated models could be used to establish empirical expressions for the effect of hypogeal germination on brown rice.

Optimization of hypogeal germination process for brown rice

The hypogeal germination process was optimized and the results are presented in Table 7. The results showed that 72.18 h was the appropriate optimal germination period. At this optimal period, the proximate composition showed a protein content of 12.37 g/100 g, fat (1.37 g/100 g), fibre (2.15 g/100 g), and moisture (10.07 g/100 g). Antioxidants properties showed DPPH as 43.66%, MCA (56.20%), FRAP (0.105 mMolFe^{2+}), TPC (0.08 mgGAE/g), and TFC (50.25 MgRUTIN/g). Colorimetric methods are usually used for the first-level screening of potential bioactivities of plant foods. However, due to its limitations in evaluating the bioactivity of phenolic compounds adequately (de Camargo et al., 2019), further evaluation of phenolic compounds is needed using the chromatography technique. The functional properties showed a bulk density of 0.567 g/ml, WAC (147.99%), OAC (147.29%), swelling index (232.09%), and WSI (16.26%). The optimal values of the anti-nutrients and minerals were also presented in Table 7.

Phenolic compounds identification and quantification in optimized germinated brown rice

The phenolic compounds in the optimized germinated brown rice (Table 8) were gallic acid (2.84 mg/100 g), 4-hydroxy benzoic acid (3.41 mg/100 g), caffeic acid (4.63 mg/100 g), vanillic acid (6.19 mg/100 g), catechin (3.88 mg/100 g), chlorogenic acid (1.93 mg/100 g), ferulic acid (4.16 mg/100 g), and quercetin (1.27 mg/100 g). Some phenolic compounds such as gallic acid, 4-hydroxy benzoic acid, caffeic acid, vanillic acid, and catechin were significantly improved through germination ($p < 0.05$). Whereas, no significant difference was noted in quercetin, and chlorogenic acid ($p > 0.05$). However, ferulic acid significantly reduced in content as a result of germination ($p < 0.05$). Gallic acid is a secondary metabolite that possesses antioxidant, antimicrobial, anti-inflammatory, and anticancer characteristics (Fernandes & Salgado 2015). Caffeic acid is an antioxidant; it can increase collagen production and prevention of premature aging. Caffeic acid also has antimicrobial activity (Magnani et al. 2014). Vanillic acid is known to be an antioxidant, anti-inflammatory, immune-stimulating, neuroprotective, cardioprotective, and antiapoptotic (Sharma et al. 2020).

Symbiotic and adverse interplay of hypogeal germination periods on brown rice

The illustrations in Fig. 1 show the synergetic and antagonistic effects of hypogeal germination periods on the proximate and functional properties of brown rice. The protein content experienced a steady increase throughout the germination periods whereas, the ash and fibre contents had a sharp increase from 0 h to 54 h and thereafter began to fall after 60 h of germination. Enyinnaya et al. (2015) also reported higher ash content in germinated brown rice. The higher ash content observed in germinated rice samples could be attributed to decreased total soluble solids and probably loss of dry matter. Probably, during the germination process, hydrolytic enzymes did not encourage the accumulation of total soluble solids and consequently increased ash levels

Table 7 Optimal characteristics of hypogeal germinated brown rice

Parameters	Amount								
Independent Variable									
Hypogeal germination period (h)		72.18 ± 0.00							
Dependent variable									
Proximate (g/100 g)		Protein	Fat	Fibre	Ash	moisture			
		12.37 ± 0.10	1.37 ± 0.01	2.15 ± 0.00	2.47 ± 0.01	10.07 ± 0.01			
Antioxidants		DPPH (%)	MCA (%)	FRAP ($mMolFe^{2+}$)	TPC (mgGAE/g)	TFC (MgRUTIN/g)			
		43.66 ± 0.37	56.20 ± 0.01	0.105 ± 0.00	0.08 ± 0.00	50.25 ± 0.17			
Functional properties		WAC (%)	OAC (%)	SC (%)	WSI (%)	BD (g/ml)			
		147.99 ± 0.12	147.29 ± 0.15	232.09 ± 0.12	16.26 ± 0.00	0.567 ± 0.00			
Antinutrients		Tannin (%)	Saponin (%)	Oxalate (mg/100 g)	Phytate (mg/100 g)				
		1.01 ± 0.00	2.03 ± 0.00	2.43 ± 0.01	6.99 ± 0.01				
Mineral (mg/100 g)	Ca	P	Fe	Na	Mn	Cu	K	Zn	Mg
	41.77 ± 0.01	94.64 ± 0.02	0.207 ± 0.00	36.83 ± 0.00	0.512 ± 0.00	2.90 ± 0.00	1752.23 ± 0.33	5.89 ± 0.00	168.37 ± 0.01

WAC – water absorption capacity; OAC – Oil absorption capacity; BD – Bulk density; SC – Swelling capacity; WSI – Water solubility index

(Enyinnaya et al. 2015). The percentage of ash obtained in this study showed that the rice samples were rich in minerals. Ohtsubo et al. (2005) reported an increase in fibre in their separate studies. The increase of fiber may result from the formation of primary cell walls, through an increase in pectic substance in the middle lamella. Increased fibre is of interest because fiber is essential in food as it absorbs water and provides roughage for the bowels, assisting intestinal movement. Enyinnaya et al. (2015) in their studies reported an increase in the protein content of germinated rice. Bau et al. (1997) suggested that the increase in total protein content in germinated grains could be due to the synthesis of enzyme proteins which rapidly transform free amino acids to form new protein compounds.

The WAC of the germinated brown rice (Fig. 1) experienced a decrease from germination periods of 0 h to 27 h and thereafter rose significantly at 36 h, which continued for the rest of the germination time. Similar observations were recorded for the swelling capacity except that the fall continued to 45 h and thereafter recorded an increase. Water absorption capacity is an important processing parameter and has implications for viscosity, consistency of products, as well as in baking applications. Other studies

Table 8 identification and quantification of phenolic compounds in brown rice germinated at optimum condition

Phenolic Compounds	Germinated Brown Rice (mg/100 g)	Non-germinated Brown Rice (mg/100 g)
Gallic acid	2.84 ± 0.02[a]	2.05 ± 0.04[b]
4-Hydroxy benzoic acid	3.41 ± 0.03[a]	2.53 ± 0.03[b]
Caffeic acid	4.63 ± 0.01[a]	4.11 ± 0.03[b]
Vanillic acid	6.19 ± 0.01[a]	6.08 ± 0.05[b]
Catechin	3.88 ± 0.04[a]	3.35 ± 0.02[b]
Chlorogenic acid	1.93 ± 0.02[a]	1.89 ± 0.04[a]
Ferulic acid	4.16 ± 0.05[b]	4.23 ± 0.01[a]
Quercetin	1.27 ± 0.01[a]	1.29 ± 0.03[a]

Data are presented as mean ± standard deviation ($n = 3$)
Values with the same superscript in the same row are not significantly different at 5% significant level using analysis of variance (ANOVA) and Duncan test

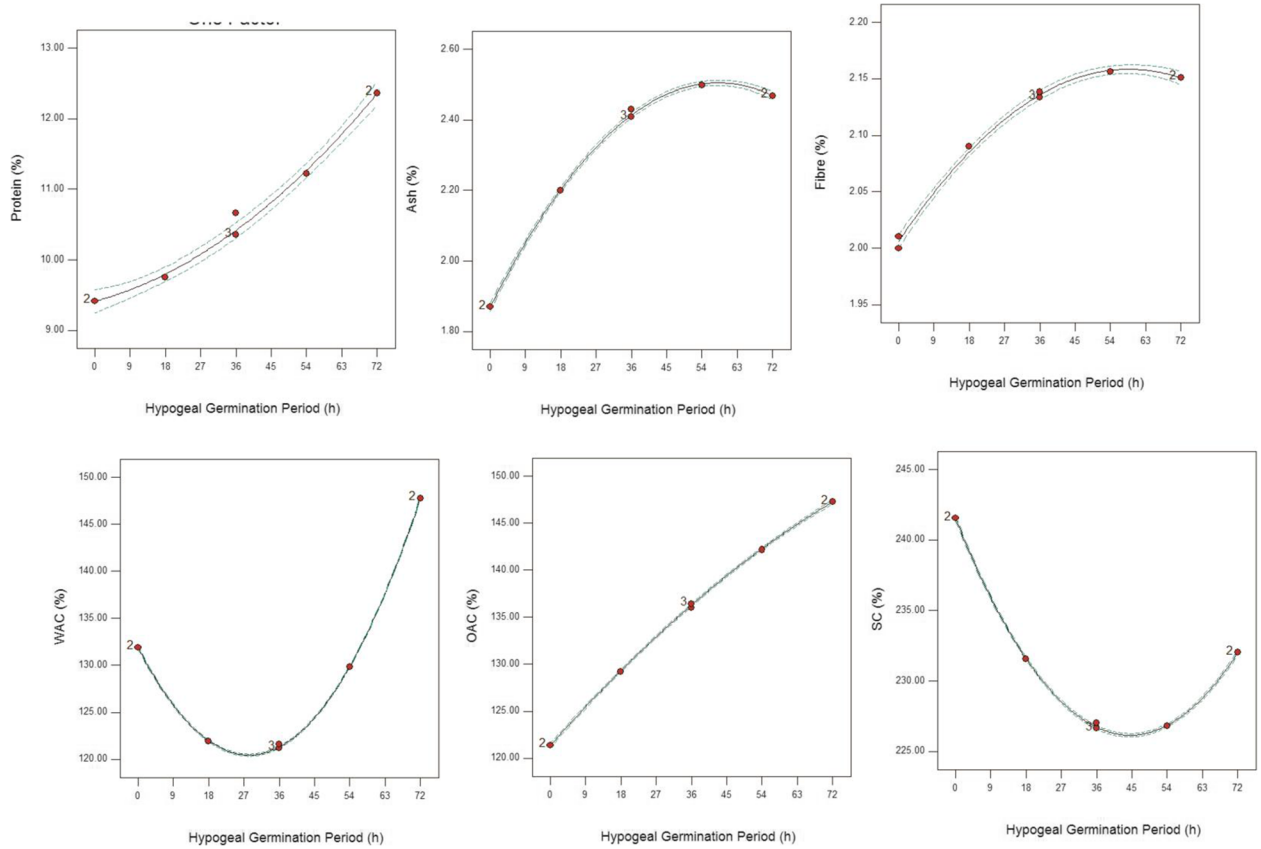

Fig. 1 Response surface plots for proximate and functional properties of hypogeal germinated

reported that the water absorption capacities of cowpea, green gram, lentil, and Bengal gram were improved by germination (Ghavidel & Prakash, 2006). The increase observed in the water absorption of germinated brown rice could be attributed to an increase in sugar content and breakdown of polysaccharide molecules; which, therefore, increased the sites for interaction with water and holding water. Therefore, the germinated rice samples could find application as functional ingredients in soups, sausages, and baked products. The oil absorption capacity depends on the protein content; it has also been attributed to the physical entrapment of oil. This characteristic is important in food formulation since fat acts as flavour retainer and increases the mouth feel of foods. The ability of the rice samples to absorb oil is an important functional property of ingredients used in confectionery industries. The increase in swelling capacity could be attributed to the degradation of fat, fiber, and starch-lipid complex of flour during germination. The presence of amylase and amylopectin due to the degradation of starch could also be responsible for the increase in swelling power of germinated cereals. High swelling capacity is a desirable functional property that makes cereal useful as a thickener in liquid food products.

The graphical illustrations of the effects of hypogeal germination on the antioxidant capacity and activity of brown rice are presented in Fig. 2. The graphs showed that total phenolic content (TPC), total flavonoids content (TFC), and metal chelating activity (MCA) decreased significantly at 0 h and experienced a minimum point at 36 h; and thereafter rose at 36 h through 72 h. Conversely, ferric reducing power had a maximum at 36 h. However, the effect of germination on DPPH was distinct. DPPH is an index for the assessment of antioxidant activity in vitro. Metal chelating measures the ability to chelate metal ions such as iron and copper. The trend observed in the metal chelating could be attributed to free phenol which may have dominated the un-germinated rice 0 h, which could have been broken down to less metal chelating compound at 36 h germination. Release of bound phenolic and flavonoid after 36 h may have enhanced the metal chelating activities of the germinated rice. The FRAP evaluates the reducing properties of food materials. It could be deduced that the

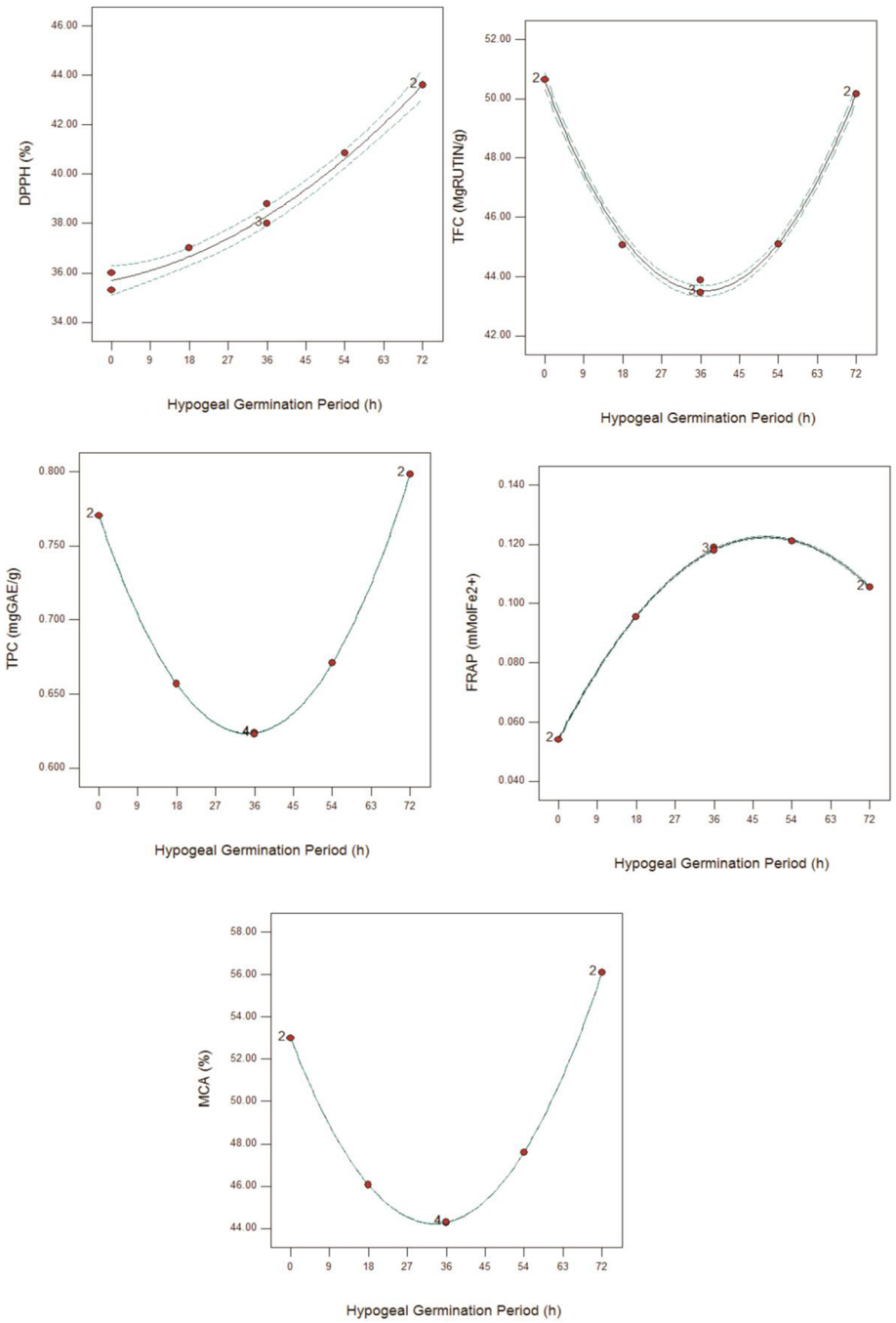

Fig. 2 Response surface plots for antioxidants characteristics of hypogeal germinated rice

FRAP of free phenols dominated up to the period of 36 h germination. After 36 h high FRAP was observed which may suggest the release of bound phenols in addition to free phenols which resulted in better FRAP content. The trend could be attributed to the nature of phenols released. Germination could change the phenolic content, both soluble and insoluble, which may have implications in the content of the antioxidant properties, depending on the germination time and the predominant phenols (Tarasevičienėa et al. 2019).

Figure 3 shows the graphical representations of the effects of hypogeal germination on the mineral contents of brown rice. Calcium, iron, and magnesium had a significant increase from 0 h through 72 h; whereas, phosphorus, sodium, and potassium recorded peaks at 63 h, 54 h, and 54 h, respectively, and then experienced a fall

through 72 h. The results showed that hypogeal germination affects certain elements differently. Calcium contributes to the health of bone and teeth and in the regulation of blood pressure. Calcium is necessary for blood clotting. It regulates the acid-basic balance of blood, thus preventing the latter to be acids. Iron plays an important role in transporting oxygen through the human body and in the prevention of anemia. Potassium regulates the heartbeat and blood pressure, the water content of the body, and neuromuscular excitability. An increase in mineral contents of germinated rice compared to non-germinated samples could be attributed to the reduction in antinutrients thereby making the mineral more available.

The mathematical expressions for hypogeal germination are presented in Table 9. Hypogeal germination had a significant ($p < 0.05$) symbiotic linear effect on proximate composition, antioxidants, and activity except for total flavonoids, functional properties except for swelling capacity and bulk density, and mineral compositions. The adverse linear effect occurred in the total flavonoids, swelling capacity, bulk density, and all the antinutrients parameters. The quadratic effect had significant ($p < 0.05$) symbiotic interplay in protein, antioxidants except for FRAP, functional properties except for OAC, and anti-nutrients except for oxalate. Whereas, the adverse quadratic effect occurred in proximate except protein, FRAP, OAC, oxalate, all the minerals. The protein, total phenolic, DPPH, WAC, and WSI recorded only symbiotic (linear and quadratic) effects.

Evaluation of the correlation analysis among nutrients, anti-nutrients, and non-nutrient properties of hypogeal germinated brown rice

Pearson correlation describes the linear interactions between two parameters (Mukaka 2012; Samuels 2014). The presence of linear interplay is evaluated through the *p*-value whereas, the coefficient of correlation (*R*) depicts the intensity of the linear interplay and this ranged from − 1 to + 1 (Mukaka 2012). The value of *R* equals to + 1 showed a perfect positive association while a value of *R* equals to − 1 represents the inverse perfect association. A value of 0 means there is no interaction. Meanwhile, a value of $R > \pm 0.90$ is rated as very high correlation whereas, a value in the range $\geq \pm 0.70 < \pm 0.90$ is considered high correlation while a value in the range $\geq \pm$

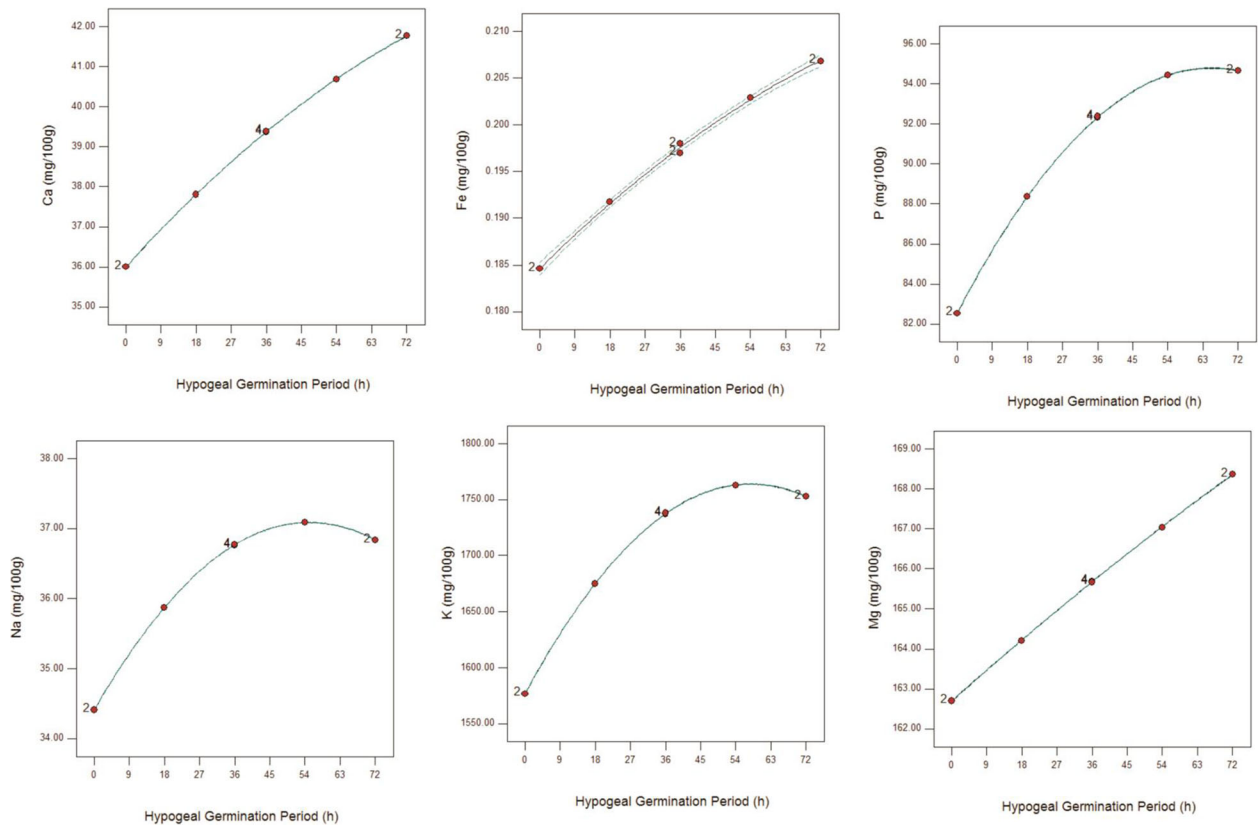

Fig. 3 Response surface plots for mineral contents of hypogeal germinated rice. OAC – water absorption capacity; WAC – water absorption capacity; SC – swelling index; BD – bulk density; WSI – water solubility; TPC – total phenolic content; TFC – total flavonoid content

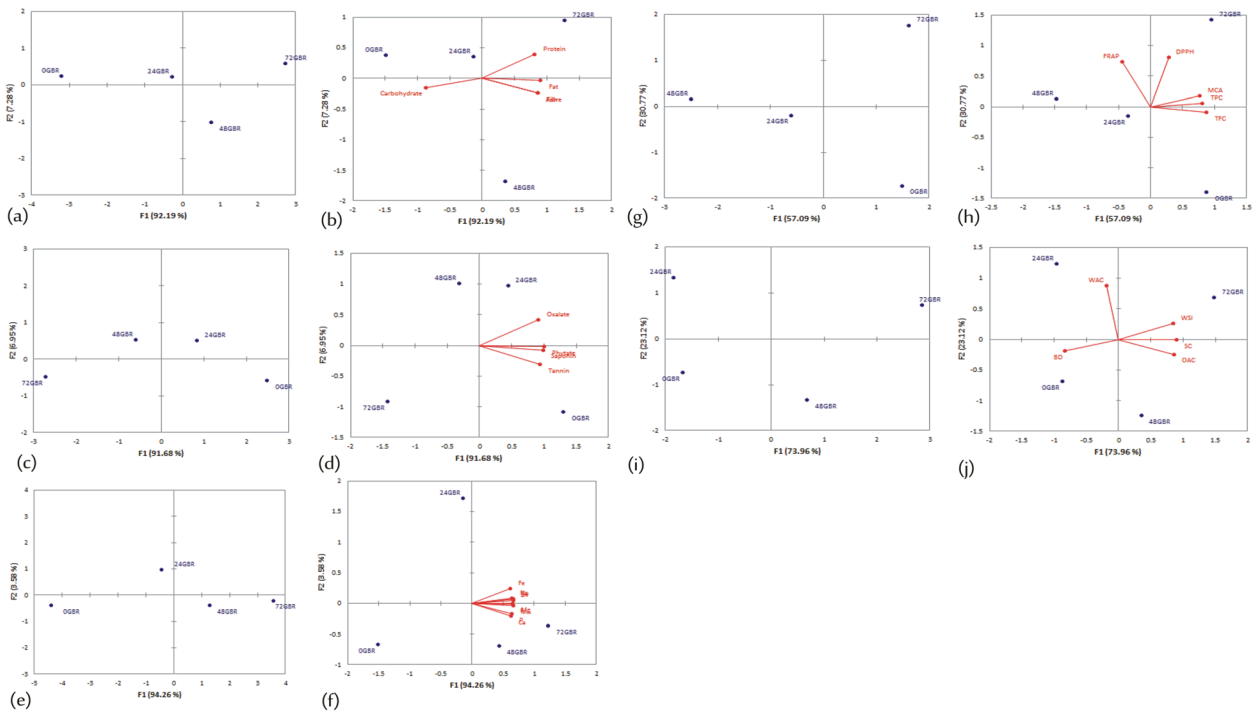

Fig. 4 (**a**) Proximate factor plot (**b**) proximate biplot (**c**) antinutrient factor plot (**d**) antinutrient biplot (**e**) mineral factor plot (**f**) mineral biplot (**g**) antioxidants factor plot (**h**) antioxidants biplot (**i**) functional properties factor plot (**j**) functional properties biplot

$0.50 \leq \pm 0.70$ is termed as moderate correlation. The degree of association and the direction for the functional, mineral, antioxidant, and antinutrient attributes of the hypogeal germinated brown rice are presented in Table 10. The values of the correlation coefficient (R) ranged from 88.00 to 99.90%, and this indicated a high level of association between the proximate and functional attributes. The DPPH had a strong positive interaction with tannin. TPC had a strong positive interaction with some minerals (iron, sodium, and potassium). The TFC had a strong positive association with the functional properties (oil absorption capacity, swelling capacity, and water solubility index). The WAC had a strong positive association with oil absorption capacity and swelling index as well as some minerals (phosphorus, sodium, manganese, potassium, and zinc). Meanwhile, some antioxidants, functional, and antinutrients had an inverse interaction. It could be concluded that there were significant ($p < 0.05$) positive interplay among some antioxidants (DPPH, TPC, and TFC), mineral (P, Fe, Na, K, Mn, and Zn), and functional parameters (oil absorption capacity, water solubility index and swelling capacity).

Principal component and correlation analyses

In this study, principal component analysis (PCA) was employed to express an overview and differences among the hypogeal germinated rice samples. The factor score plot and biplot for F1 against F2 for the proximate, antinutrients, minerals, antioxidants, and functional properties are presented in Fig. 4 (a-j). Generally, hypogeal germination time affected proximate, minerals, and functional properties similarly and an inverse effect was noted for the antinutrients. Whereas, antioxidants experienced a distinct effect. Fig. 4(d) showed that the influence of germination on the antinutrient at 72 h was significantly ($p < 0.05$) more than other germination times. Whereas, a similar observation was noted at 24 h (Fig. 4f).

Agglomerative hierarchical clustering

The dendrogram for the germinated rice samples is shown in Fig. 5. Figure 5(a-b) show similar trends for proximate and antinutrients behaviours at 24 h and 48 h germination time. The mineral contents and antioxidants had similar hierarchical behaviour at 48 and 72 h germination time whereas, functional properties experienced similar hierarchical behaviour at 0 and 72 h germination time.

Conclusion

The study showed that hypogeal germination resulted in a significant ($p < 0.05$) change in proximate, antinutrients, functional properties, antioxidant capacity and

Table 9 Mathematical expressions for symbiotic and adverse effects of hypogeal germination on nutrient and non-nutrient compositions of brown rice

Mathematical Model	Linear Effect		Quadratic Effect	
	Symbiotic	Adverse	Symbiotic	Adverse
Proximate				
$Protein = 10.41 + 1.47h + 0.47h^2$	s	–	s	–
$Fat = 1.23 + 0.24h - 0.10h^2$	s	–	–	s
$Fibre = 2.14 + 0.07h - 0.06h^2$	s	–	–	s
$Ash = 2.41 + 0.30h - 0.24h^2$	s	–	–	s
$Moisture = 9.87 + 0.45h - 0.25h^2$	s	–	–	s
Antioxidant properties and activity				
$TPC = 0.62 + 0.01h + 0.16h^2$	s	–	s	–
$TFC = 43.52 - 0.20h + 6.86h^2$	–	s	s	–
$FRAP = 0.12 + 0.03h - 0.04h^2$	s	–	–	s
$DPPH = 38.31 + 3.96h + 1.36h^2$	s	–	s	–
$MCA = 44.28 + 1.55h + 10.26h^2$	s	–	s	–
Functional properties				
$WAC = 121.30 + 7.93h + 18.54h^2$	s	–	s	–
$OAC = 136.29 + 12.94h - 1.98h^2$	s	–	–	s
$SC = 226.71 - 4.75h + 10.05h^2$	–	s	s	–
$WSI = 15.27 + 0.64h + 0.35h^2$	s	–	s	–
$BD = 0.58 - 0.02h + 4.96x10^{-3}h^2$	–	s	s	–
Anti-nutrients				
$Tannin = 1.02 - 0.04h + 0.02h^2$	–	s	s	–
$Saponin = 2.08 - 0.09h + 0.04h^2$	–	s	s	–
$Oxalate = 3.18 - 0.51h - 0.24h^2$	–	s	–	s
$Phytate = 7.68 - 0.91h + 0.22h^2$	–	s	s	–
Mineral				
$Ca = 39.37 + 2.88h - 0.49h^2$	s	–	–	s
$P = 92.33 + 6.06h - 3.74h^2$	s	–	–	s
$Fe = 0.20 + 0.01h - 1.85x10^{-3}h^2$	s	–	–	s
$Na = 36.77 + 1.21h - 1.14h^2$	s	–	–	s
$Mn = 0.49 + 0.04h - 0.02h^2$	s	–	–	s
$Cu = 2.74 + 0.32h - 0.17h^2$	s	–	–	s
$K = 1737.33 + 87.87h - 72.68h^2$	s	–	–	s
$Zn = 5.53 + 0.44h - 0.08h^2$	s	–	–	s
$Mg = 165.68 + 2.83h - 0.15h^2$	s	–	–	s

S – significant at 5%; DPPH - 1,1-diphenylpicrylhydrazine; FRAP - ferric reducing powers; MCA - metal chelating activity; TPC - total phenolic content; TFC – total flavonoids content; WAC – water absorption capacity; OAC – Oil absorption capacity; BD – Bulk density; SC – Swelling capacity; WSI – Water solubility index

activity, and mineral content of brown rice. The symbiotic linear effect occurred in proximate, DPPH, FRAP, MCA, total phenolic, WAC, OAC; whereas, an adverse linear effect occurred in total flavonoids, tannin, saponin, oxalate, and phytate. The significant symbiotic quadratic effect occurred in protein, DPPH, FRAP, MCA, total phenolic, WAC, saponin, phytate, and tannin; whereas, the adverse quadratic effect occurred in fibre, ash, moisture, fat, FRAP, OAC, oxalate. All the minerals recorded symbiotic for linear and adverse for quadratic. The hypogeal germination period of 72.18 h was established as the optimum period. Hypogeal germinated brown rice produced at the optimum condition could find application as a functional ingredient in food formulation.

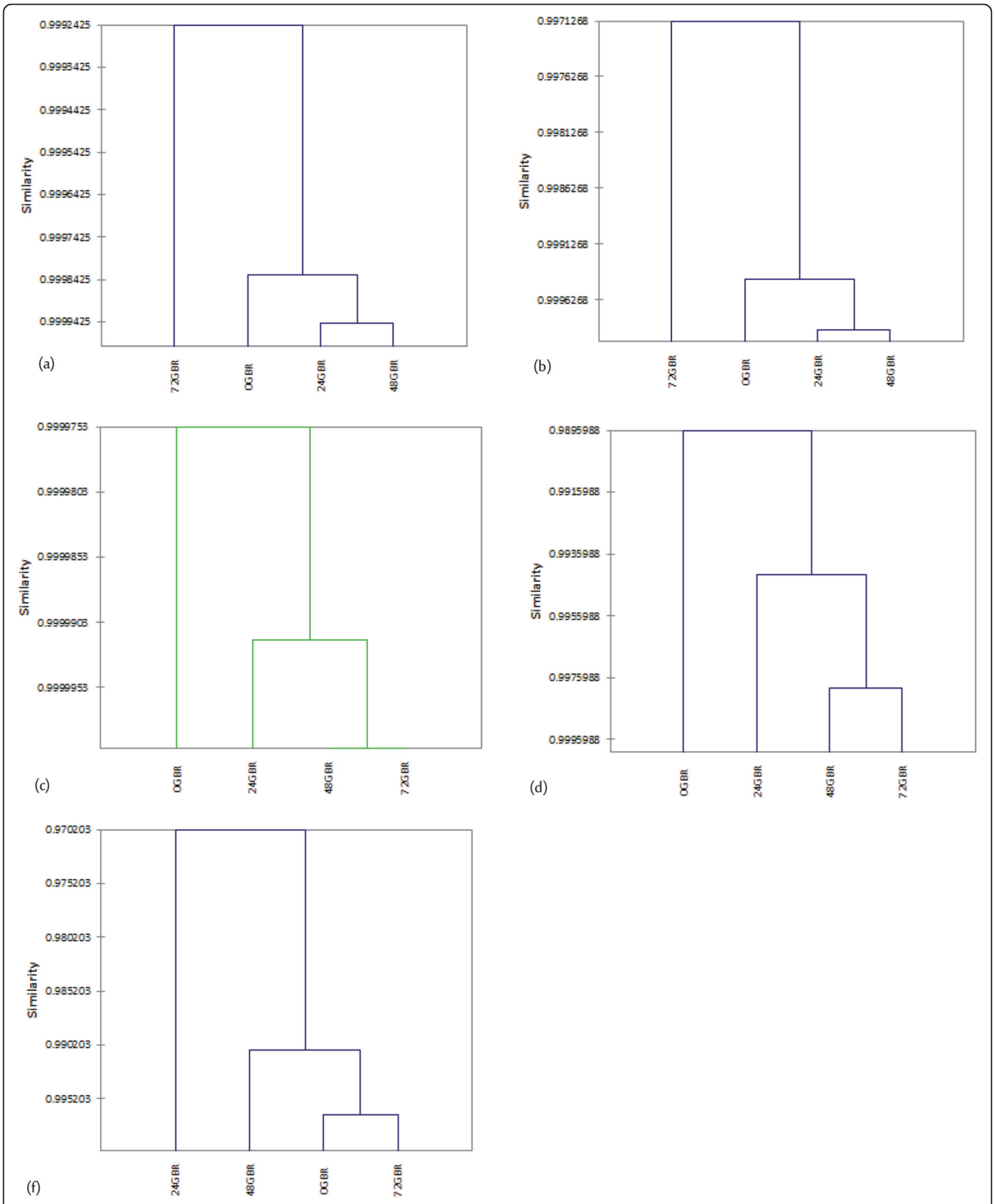

Fig. 5 Dendrogram (**a**) proximate (**b**) antinutrients (**c**) mineral (**d**) antioxidants (**f**) functional properties

Table 10 Significance correlation analysis for the association among functional properties, mineral, antioxidants and antinutrient of hypogeal germinated brown rice

	OAC	SC	BD	WSI	Tannin	Saponin	Oxalate	Phytate	Ca	P	Fe	Na	Mn	K	Zn	Mg
Positive Correlation																
DPPH	–	–	–	–	0.943*	–	–	–	–	–	–	–	–	–	–	–
TPC	–	–	–	–	–	–	–	–	–	–	0.895*	0.918*	–	0.906*	–	–
TFC	0.941*	0.906*	–	0.938*	–	–	–	–	–	–	–	–	–	–	–	–
WAC	0.913*	0.919*	–		–	–	–	–	–	0.929*	–	0.916*	0.910*	0.929*	0.971**	–
OAC	–	0.899*	–	0.905*	–	–	–	–	–	0.894*	–	–	–	–	–	–
SC	–	–	–	–	–	–	–	–	–	–	–	–	0.879*	–	–	–
BD	–	–	–	–	–	–	–	0.902*	–	–	–	–	–	–	–	–
WSI	–	–	–	–	–	–	–	–	–	0.923*	–	–	–	–	–	–
oxalate	–	–	–	–	–	–	–	0.882*	–	–	–	–	–	–	–	–
Ca	–	–	–	–	–	–	–	–	–	–	0.885*	–	0.944*	–	–	0.978**
P	–	–	–	–	–	–	–	–	–	–	–	0.989**	0.936*	0.990**	0.944*	–
Na	–	–	–	–	–	–	–	–	–	–	–	–	0.909*	0.999**	0.958*	–
Mn	–	–	–	–	–	–	–	–	–	–	–	–	–	0.909*	0.924*	0.916*
K	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.964**	–
Negative Correlation																
MCA	–	–	–	–	–	–	–	–	−0.880*	–	−0.898*	–	–	–	–	–
TFC	–	–	− 0.903*	0.938*	–	–	–	− 0.986**	–	–	–	–	–	–	–	–
WAC	–	–	–	–	–	–	−0.938*	–	–	–	–	–	–	–	–	–
OAC	–	–	–	–	–	–	−0.982**	−0.948*	–	–	–	–	–	–	–	–
SC	–	–	−0.900*	–	–	–	–	−0.959**	–	–	–	–	–	–	–	–
BD	–	–	–	–	–	–	–	–	−0.942*	–	–	–	−0.959*	–	–	− 0.970**
WSI	–	–	–	–	–	−0.915*	− 0.885*	−0.879*	–	–	–	–	–	–	–	–
Tannin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Saponin	–	–	–	–	–	–	–	–	–	–	−0.887*	–	–	–	–	–
Oxalate	–	–	–	–	–	–	–	–	–	−0.923*		−0.900*	–	−0.915*	− 0.882*	–

*Correlation is significant at 5% significant level (2-tailed); Correlation is significant at 1% level (2-tailed)

OAC – water absorption capacity; WAC – water absorption capacity; SC – swelling index; BD – bulk density; WSI – water solubility; TPC – total phenolic content; TFC – total flavonoid content

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Both authors designed experiments, collected and analyzed data, prepared the manuscript, and approved the manuscript.

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References

Adebiyi, A. P., & Aluko, R. E. (2011). Functional properties of protein fractions obtained from commercial yellow field pea (Pisum sativum L.) seed protein isolate. *Food Chemistry*, *128*(4), 902–908. https://doi.org/10.1016/j.foodchem.2011.03.116.

Alvarez, R., Araya, H., Navarro-Lisboa, R., & de-Dicastillo, C. L. (2016). Evaluation of polyphenol content and antioxidant capacity of fruits and vegetables using a modified enzymatic extraction. *Food Technology and Biotechnology*, *54*(4), 462–467. https://doi.org/10.17113/ftb.54.04.16.4497.

Anuchita, M., Tanongsak, M., Pisan, D., & Kirati, P. (2014). Functional properties of Brown Rice flour as affected by germination. *APCBEE Procedia*, *8*, 41–46. https://doi.org/10.1016/j.apcbee.2014.01.077.

AOAC (2005). *Official methods of analysis*, (18th ed.,). D.C. USA: Association of Official Analytical Chemists Washington.

Bau, H., Villaume, C., Nicolas, J., & Mejean, L. (1997). Effect of germination of chemical composition, biochemical constituents, and antinutritional factors of soya bean (Glycine max) seeds. *Journal of the Science of Food and Agriculture*, *73*(1), 1–9. https://doi.org/10.1002/(SICI)1097-0010(199701)73:1<1::AID-JSFA694>3.0.CO;2-B.

de Camargo, A. C., Biasoto, A. C. T., Schwember, A. R., Granato, D., Rasera, G. B., Franchin, M., … Shahidi, F. (2019). Should we ban total phenolics and antioxidant screening methods? The link between antioxidant potential and activation of NF-κB using phenolic compounds from grape by-products. *Food Chemistry*, *290*, 229–238. https://doi.org/10.1016/j.foodchem.2019.03.145.

Enyinnaya, C. C., Chukwuemeka, A. J., Comfort, S. O., Ozavize, O. R., & Nahemiah, D. (2015). Effect of germination on the physicochemical and antioxidant characteristics of rice flour from three rice varieties from Nigeria. *Food Chemistry*, 1–23.

Falade O. S, Dare A. F, Bello M. O, Osuntogun B. O & Adewusi S.R.A (2004). Varietal changes in proximate composition and the effect of processing on the ascorbic content of some Nigerian vegetables. Journal of Food Technology 2: 103–108.

Fernandes, F. H. A., & Salgado, H. R. N. (2015). Gallic acid: A review of the methods of determination and quantification. *Critical Reviews in Analytical Chemistry*, 1–40.

Ghavidel, R. A., & Prakash, J. (2006). Effect of germination and dehulling on functional properties of legume flours. *Journal of the Science of Food and Agriculture*, *86*(8), 1189–1195. https://doi.org/10.1002/jsfa.2460.

Girgih, A., Udenigwe, C., Li, H., Adebiyi, A., & Aluko, R. (2011). Kinetics of enzyme inhibition and antihypertensive effects of hemp seed (*Cannabis sativa L.*) protein hydrolysates. *Journal of the American Oil Chemists' Society*, *88*(11), 1767–1774. https://doi.org/10.1007/s11746-011-1841-9.

Hartsook, E. T. (1984). Celiac express: Sensitivity to gliadin cereal foods world. *Advances in Food and Nutrition Research*, *29*(2), 157–158.

Hoff, J., & Singleton, K. (1977). A method for the determination of tannins in foods using immobilized protein. *Journal of Food Science*, *42*(6), 1566–1569. https://doi.org/10.1111/j.1365-2621.1977.tb08427.x.

Khampang, E., Kerdchoechuen, O., & Laohakunjit, N. (2009). Change of chemical composition of Rice and cereals during germination. *Agricultural Science Journal*, *40*, 341–344.

Kim, S. P., Kang, M. Y., Nam, S. H., & Friedman, M. (2012). Dietary rice component -oryzanol inhibits tumor growth in tumor-bearing mice. *Mol Nutrition Food Research*, *56*(6), 935–944. https://doi.org/10.1002/mnfr.201200057.

Lumduwong, N., & Seib, P. A. (2000). Rice starch isolation by alkaline protease digestion of wet-milled rice flour. *Journal of Cereal Science*, *31*(1), 63–74. https://doi.org/10.1006/jcrs.1999.0279.

Magnani, C., Isaac, V. L. B., Correa, M. A., & Salgado, H. R. N. (2014). Caffeic acid: A review of its potentials use in medications and cosmetics. *Analytical Methods*, *6*(10), 3203–3210. https://doi.org/10.1039/C3AY41807C.

Mukaka, M. M. (2012). Statistics corner: A guide to the appropriate use of the correlation coefficient in medical research. *Malawi Medical Journal*, *24*(3), 69–71.

Odenigbo, A. M., Danbaba, N., & Ngadi, M. (2014). Nutrient composition and predicted glycemic index of rice varieties from Nigeria. *British Journal of Applied Science and Technology*, *4*(2), 302–318. https://doi.org/10.9734/BJAST/2014/6203.

Ohtsubo, K., Suzuki, K., Yasui, Y., & Kasumi, T. (2005). Bio-functional components in the processed pregerminated brown rice by a twin-screw extruder. *Journal of Food Composition and Analysis*, *18*(4), 303–316. https://doi.org/10.1016/j.jfca.2004.10.003.

Okezie, B. O., & Bello, A. B. (1988). Physicochemical and functional properties of winged bean flour and isolate compared with soy isolate. *Journal of Food Science*, *53*(2), 450–454. https://doi.org/10.1111/j.1365-2621.1988.tb07728.x.

Onyeka, E. U. (2019). Nutritional and health properties of germinated brown rice – A review. *Nigerian Food Journal*, *37*(1), 92–105.

Patil, S. B., & Khan, M. K. (2011). Germinated brown rice as a value-added rice product: A review. *Journal of Food Science and Technology*, *48*(6), 661–667. https://doi.org/10.1007/s13197-011-0232-4.

Samuels, P. (2014). Pearson correlation. *Technical Report*, 1–5.

Sharma, N., Tiwari, N., Vyas, K. N., & Muthuraman, A. (2020). An overview of therapeutic effects of vanillic acid. *Plant Archives*, *20*, 3053–3059.

Benzie, I. F. F., & Strain, J. J. (1999). Ferric reducing the ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry*, *239*, 70–76.

Sun, J., Chu, Y., Wu, X., & Liu, R. (1999). Antioxidant and antiproliferative activity common fruits. *Journal of Agricultural and Food Chemistry*, *50*, 7449–7454.

Takashi, S., & Seib, R. A. (1988). Paste and gel properties of prime corn and wheat starches with and without native lipids. *Cereal Chemistry*, *65*, 474.

Tarasevičienėa, Z., Akvilė, V., Honorata, D., Pavelas, D., Aurelija, P., & Marek, G. (2019). Effects of germination time on the antioxidant properties of edible seeds. *CYTA – Journal of Food*, *17*(1), 447–454. https://doi.org/10.1080/19476337.2018.1553895.

Wu, F., Yang, N., Touré, A., Jin, Z. Y., & Xu, X. M. (2013). Germinated brown rice and its role in human health. *Critical Reviews in Food Science and Nutrition*, *53*(5), 451–463. https://doi.org/10.1080/10408398.2010.542259.

Xie, Z., Huang, J., Xu, X., & Jin, Z. (2008). Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. *Food Chemistry*, *111*(2), 370–376. https://doi.org/10.1016/j.foodchem.2008.03.078.

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