Effect of Various Processing Methods on Nutritional and Anti-Nutritional Compositions of Brown Rice

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ABSTRACT

This study was conducted to evaluate the qualitative changes in brown rice (IC-577410) during different processing treatments such as soaking, germination and fermentation. Soaking was carried out for 12, 24 h and germination for 24, 48, 72 h. Natural fermentation was carried out by microorganisms already present in the grain and with baker's yeast (*Saccharomyces cerevisiae*) for 12, 24, and 36 h interval. Dehulled rice that contains the embryo, endosperm and bran is called brown rice. Brown rice can be utilized as a functional meal and is an excellent source of nutrients as compared to the fantastic so white or polished rice. By the use of various processing treatments significant effect ($p \leq 0.05$) observed on the chemical composition and antioxidant activity of brown rice. Germination and fermentation help to enhance the nutritional profile of cereals and legumes by causing significant changes in chemical composition. Crude protein and crude fibre content increased by 11.94 and 17.04%, respectively but the crude fat content decreased by 8.24% after germination treatment. Total polyphenolic content (TPC) and antioxidant activity increased significantly ($p \leq 0.05$) by 58.08 and 77.62%, respectively during germination treatment. After the fermentation treatment, crude protein content increased significantly ($p \leq 0.05$) by 11% but the crude fat and crude fibre decreased by 4.12 and 3.41%, respectively. Bioactive components mainly total phenolic components (TPC) and anti-oxidant activity increased significantly ($p \leq 0.05$) by 33.52 and 48.02%, respectively during the fermentation process. Therefore, it was observed that throughout the germination and fermentation treatments, there was a significant ($P \leq 0.05$) increment observed in protein, total phenolic components, and antioxidant activity of brown rice.

Keywords: Antioxidant, Fermentation, Germination, Physicochemical Properties, Rice, Soaking.

INTRODUCTION

Rice botanically named as *Oryza sativa* has been a staple food in Asian nations for quite a long time. During the milling process, the external bran and germ part of intact rice grains (*i.e.* brown rice) are eliminated to produce white rice that fundamentally comprises starchy endosperm. Higher dietary glycemic index (GI) has been reliably connected with an eminent risk of type 2 diabetes (T2D). Brown rice consumption may confer beneficial impacts on T2D hazards due to the presence of nutrients like fiber, and minerals, most of which are lost during refining and processing treatments (Sun *et al,* 2010). Cereals are major sources of carbohydrates and provide the majority of the energy in the human diet, particularly in underdeveloped countries. Both whole brown rice (BR) and polished white rice are made up of carbohydrates mainly starch. In rice kernel, the endosperm contains the highest amount of starch (Zhou *et al,* 2002).

Brown rice (BR) is the grain that remains after the husk has been removed and has higher nutrients than polished rice (Pal *et al*, 2016). During milling, bran and germ are removed for the consumption of polished grains. Bran and germ fractions of rice are a rich source of micro-nutrients but these decrease during the milling process. The bran fraction of rice has approximately 65% of fibre content, phenolics, vitamins, and minerals out of the total nutrient in the grain (Lerma-García *et al,* 2009).

Brown rice contains higher lipid contents than milled rice because outer bran layers contain more concentrated lipids (up to 20%) as described by Thomas *et al* (2015) and Zhou *et al* (2002). (Bryant et al., 2013) evaluated 202 genotypes of brown rice, where protein content ranged from 5.40 to 11.90%. Protein content may vary significantly depending on the geographical area and genetic diversity. The mineral contents are found abundant in brown rice, where the concentration of calcium, manganese, zinc,

and iron ranges from 72-128, 21.90-43.80, 22.50-32.70, and 5.10- 9.75 mg/kg, respectively (Srinuttrakul & Busamongkol, 2014).

Conventional processing methods such as soaking and germination used to enhance the nutritional value, bioactive components such as anti-oxidant activity, total polyphenolic, and flavonoid content, and decrease the anti-nutrients such as trypsin inhibitor, tannin, and phytic content in food grains (Embaby, 2010). In the food processing industry, fermentation is a widely used method to preserve and enhance the shelf-life of food products. It helps to improve the food texture, flavour, and digestibility (Khalil, 2006). During the roasting treatment, the breakdown of cell walls and membranes occurs, resulting in a release of insoluble ester bonds with enhanced soluble phenolic components and an increase in antioxidant properties. During roasting treatment, the physical qualities, flavour, and chemical composition of food get altered and antioxidant activity gets increased due to the Maillard reaction as reported by (Dewanto *et al*, 2002).

Germination has been reported to decrease the fat content (21.16%) after the germination in legumes and rice (Megat Rusydi *et al,* 2011). (Hahm *et al,* 2009) reported a decline in crude fat content with an increase in germination time in sesame seeds. A significant increment in antioxidant activity during germination has been reported in several studies. (Jan *et al,* 2017) reported an increased trend in antioxidant activity and values increased from 26.51 to 29.08% after 48 h of quinoa germination in various cultivars. According to (Acosta-Estrada*et al,* 2014), the interaction between the seed's protein and starch molecules during germination or the release of phenolic compounds from cell walls are both associated with an increment in antioxidant activity.

MATERIALS AND METHODS

Materials

The IP-577410 cultivars of paddy were procured from ICAR- Pusa New Delhi and multiply in the Eternal University field. The chemicals utilized in the current studies were purchased from Qualigens, BDH chemicals, Hi-Media, and Sigma.

Physico-chemical evaluation

Raw paddy and paddy after the processing treatments were subjected to physico-chemical

analysis in the laboratories, of Eternal University in the Department of Food Technology located at Sirmour, Himachal Pradesh, India.

Physical and functional parameters

` Physical parameters such as 1000 kernel weight were estimated as per the method of (Committee, 2000). Vernier calliper was used to measure the length, breadth and thickness. The procedure described by Huang *et al,* 2005 was used to evaluate the bulk density (BD), Jones *et al,* 2000 for tap density (TD), Sosulski (1962) for water absorption capacity (WAC), Kaur *et al* (2015) for oil absorption capacity (OAC) and Stojceska *et al* (2008) for water solubility index (WSI).

Chemical parameters

The moisture content was measured by using the hot air oven to dry the sample as per (AOAC, 1990) Various instruments such as Fibroplus FBS 08P (Pelican Inc.), Soxoplus SPS 06 AS (Pelican Inc.), and Kjeloplus Kjelodist CAS VA (Pelican Inc.) were used to determine the crude fibre, crude fat, and crude proteins, respectively. Ash content was measured as per the method described by (Ranganna, 1986). The total carbohydrate content was determined by difference, and calculated by subtracting the measured crude fat, crude protein, crude fibre, ash, and moisture content from 100. The Atwater factor was used to determine the calorific value. The calorific value was determined by multiplying crude protein, crude fats, and carbohydrates by the Atwater factor of 4.0, 9.10, and 4.2 KCal/g, respectively (WHO, 1973). The method described by (AOAC, 1990) was used to analyse the mineral content such as Cu, Fe, Mn, and Ca by using AAS (Atomic Absorption Spectrometer).

The antioxidant activity was determined as free radical scavenging activity according to the method described by (Bouaziz *et al,* 2008). The tannins (%) were estimated as per the method described by (Saxena *et al,* 2013) with minor modifications. The phytic content (mg/g) was evaluated as per the method described by (Gao *et al,* 2007). The total phenolic content (mg GAE/100g) was estimated by using the Folin-Ciocalteu reagent following a slightly modified method of (Ainsworth and Gillespie, 2007).

Figure 1. Soaking and germination treatment of paddy

PROCESSING TREATMENTS

Soaking and germination processing treatments were carried out in accordance with the methodology employed by (Egli *et al,* 2002). As depicted by Figure 1, the cleaned seeds of brown rice were soaked in distilled water in 1:5. The seeds were soaked for 12 and 24 h at room temperature and subjected to dried in hot air oven for 24 h at 40°C. For germination treatment, seeds being soaked for 16 h and divided into three group (approximately 20 g soaked seeds for each group) and allowed to germinate for varying time interval. For 24, 48, and 72 h, the germination process was conducted in an incubator set at 25°C with periodically sprinkling the water to keep the muslin cloth wet during germination. Following each germination interval, germinated samples were dried over 24 h at 40°C in hot air oven. Samples were then sealed and kept in airtight packages at 4°C until they could be examined further.

With a few modest modifications, natural fermentation process using the methodology developed by (Park *et al,* 2020). Sample of brown rice flour (20 g) was gently combined with distilled water (60 ml). An incubator set at 37°C was used to conduct natural fermentation for 12, 24, and 36 h. naturally occurring microbes in seeds were the cause of fermentation. The samples taken at each time point were oven-dried at 50°C to equilibrate the moisture content. After being dried in a blender, the samples were filtered through a 60-mesh stainless steel sieve, packed in polyethylene bags, and kept at 4°C until they were used.

As per the method describe by (Day and Morawicki, 2016) with minor modification grains were subjected to ferment with baker's yeast (*Saccharomyces cerevisiae*). The 20g brown rice flour and 120 ml distilled water was added in 500 ml conical flask and mixed properly. The samples were autoclaved after adjusting the pH to 4.5 and

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Table1. Physical, functional and optical characteristics of raw grains.

Parameter	Brown Rice
Length (mm)	5.37±0.06
Width (mm)	1.67±0.06
Thickness (mm)	1.73±0.06
1000 kernel Wt. (g)	21.50±0.10
Bulk density (g/cm$^{3)}$)	0.79±0.02
Tap density (g/cm$^{3)}$)	0.83±0.02
Swelling Capacity (%)	5.00±1.0
Water absorption capacity (ml/g)	1.23±0.06
Water solubility index (%)	5.20±0.10
Oil absorption capacity (ml/g)	2.57±0.12
L*	81.00
a*	2.39±0.01
b*	10.21±0.01

The table's values are presented as mean±SD

Table 2. Nutritional profile of raw grains of brown rice.

Parameter	Values
Moisture (%)	9.29± 0.01
Fat (%)	1.94±0.04
Fibre (%)	1.76±0.04
Ash (%)	1.47±0.01
Protein (%)	7.54±0.01
Carbohydrate (%)	78.01±0.07
Calorific Value (Kcal/100g)	375.42±0.08
Antioxidant activity (% inhibition)	40.61±0.12
Tannin (%)	0.557±0.006
Phytic acid (%)	1.30±0.03
Total Phenolic content (mg GAE/100g)	184.60±0.87
Flavonoids (mgRE/100g)	48.97±0.64
Trypsin inhibitor (mgTIA/100g)	50.25±
Cu (mg/kg)	0.96±0.02
Fe (mg/kg)	0.66
Zn (mg/kg)	0.24
Mn (mg/kg)	0.42
Ca (mg/kg)	6.07

The table's values were presented as mean±SD

Saccharomyces cerevisiae (0.4g) was then added for conducting fermentation process. Conical flasks were properly mixed and then placed in incubator at 37°C for 12, 24, and 36 h. Following each fermentation interval, the fermented samples were hot-dried at 50°C and converted to fine powder. Until further examination, flour was kept in sealed airtight packages at 4°C.

RESULTS AND DISCUSSION

Physical properties

The data pertaining to physical and optical parameters of raw grains is presented in Table1. The brown rice length, breadth, and thickness were found to be 5.37, 1.67, and 1.73 mm, respectively. The 1000 kernel weight was observed as 21.50g.

The optical parameters such as L* value for brown rice grains were determined as 81.10, a* value as 2.39, and b* value as 10.21, respectively. The greater the value of L*, the brighter will be the flour as L* represents brightness in the sample. The values for a* represent the red to the green color quality of the samples. Similarly, the value of b* represents the blue to yellow quality of samples. The functional parameters such as bulk and tap density of brown rice were 0.79 and 0.83 g/cm^3 respectively. Water absorption capacity, water solubility index, oil

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Table 3. Nutritional profile of brown rice affected by soaking and germination at different time intervals.

Parameter	Time interval (h)					
			Soaking		Germination	
	0	12	24	24	48	72
Moisture (%)	9.29 ±0.01[c]	9.40 ±0.05 [b]	9.47 ±0. 04 [ab]	9.50 ±0.13 [ab]	9.55 ±0.06 [a]	9.23 ±0.02[c]
Fat (%)	1.94 ±0.04[a]	1.85 ±0.01[b]	1.83 ±0.01[bc]	1.82 ±0.01[bc]	1.81 ±0.01[cd]	1.78 ±0.01[d]
Fibre (%)	1.76 ±0.04[e]	1.81 ±0.01 [d]	1.83 ±0.0[d]	1.97 ±0.02[c]	2.02 ±0.01[b]	2.06 ±0.02[a]
Ash (%)	1.47 ±0.01[a]	1.46 ±0.01 [ab]	1.45 ±0.01[bc]	1.44 ±0.01 [c]	1.43 ±0.01[d]	1.41 ±0.01[e]
Protein (%)	7.54 ±0.01[f]	7.71 ±0.01[e]	7.83 ±0.02[d]	8.27 ±0.02[c]	8.33 ±0.03[b]	8.44 ±0.02[a]
Carbohydrates(%)	78.01 ±0.07[a]	77.76 ±0.04[b]	77.59 ±0.03[c]	77.00 ±0.15[d]	76.87 ±0.04[e]	77.07 ±0.03[f]
Calorific value (Kcal/100g)	375.42 ±0.08[a]	374.31 ±0.26[b]	373.81 ±0.20[bc]	373.05 ±0.61[c]	372.62 ±0.20[d]	373.06 ±0.16[d]
Antioxidant activity (% inhibition)	40.61 ±0.12[f]	42.23 ±0.29[e]	43.17 ±0.16[d]	60.18 ±0.16[c]	67.14 ±0.06[b]	72.13 ±0.10[a]
TPC (mg GAE/100g)	184.60 ±0.87[f]	218.38 ±1.28[e]	227.01 ±0.43[d]	247.69 ±1.35[c]	258.98 ±0.85[b]	291.81 ±3.86[a]
Flavonoids (mgRE/100g)	48.97 ±0.64[f]	50.49 ±0.39 [e]	53.28 ±0.81[d]	59.19 ±1.40[c]	61.47 ±0.39[b]	64.76 ±0.39[a]
Trypsin inhibitor (mgTIA/100g)	0.86 ±0.03[a]	0.84 ±0.02[a]	0.81 ±0.01[b]	0.79 ±0.01[b]	0.58 ±0.01[c]	0.41 ±0.01[d]
Tannin (%)	0.557 ±0.006[a]	0.420 ±0.006[b]	0.410 ±0.008[b]	0.312 ±0.010[c]	0.275 ±0.005[e]	0.241 ±0.009[f]
Phytic acid(%)	1.30 ±0.03[a]	1.28 ±0.01[ab]	1.26 ±0.01[b]	1.21 ±0.02[c]	1.19 ±0.01[d]	1.14 ±0.01 [d]
Cu (mg/kg)	0.96 ±0.01[e]	0.98 ±0.01[d]	0.99 ±0.01 [d]	1.03 ±0.01[c]	1.05 ±0.01[b]	1.10 ±0.01[a]
Fe(mg/kg)	0.56 ±0.03[d]	0.59 ±0.01[c]	0.61 ±0.01[c]	0.98 ±0.01[b]	1.02 ±0.01[a]	1.04 ±0.01[a]
Zn (mg/kg)	0.24 ±0.01[e]	0.25 ±0.01[e]	0.28 ±0.01[d]	0.35 ±0.01[c]	0.38 ±0.01[b]	0.41 ±0.01[a]
Mn (mg/kg)	0.42 ±0.01[e]	0.43 ±0.01[e]	0.49 ±0.01[d]	0.63 ±0.02[c]	0.67 ±0.01[b]	0.72 ±0.01[a]
Ca (mg/kg)	5.17 ±0.06 [e]	5.26 ±0.04[d]	5.33 ±0.01[d]	5.76 ±0.03[c]	5.90 ±0.01[b]	6.03 ±0.01[a]

The table's values were presented as mean±SD; Values with in rows sharing the same letters were not significantly different according to Duncan's LSD post hoc analysis at P≤0.05

absorption capacity, and swelling capacity of brown rice were 1.23ml/g, 5.20%, 2.57 ml/g and 5% respectively.

The data (Table 2) described the nutritional profile of raw grains of brown rice and found to contain 9.29% of moisture, 1.94% of crude fat, 1.76% of crude fiber, 1.47% of ash content, 7.54% of crude protein, 78.01% of carbohydrates, and 375.42 Kcal/100g of calorific value. It also contained about 40.61% of antioxidant activity, 184.60 mg GAE/100g of TPC, and 48.97 mg RE/100g of flavonoid contents. Anti-nutritional components such as tannins, phytic acid, and trypsin inhibitor activities in brown rice were reported as 0.21%, 1.36%, and 0.86 mg TIA/100g, respectively. The mineral contents such as Zn, Cu, Fe, Mn, and Ca have been reported as 0.24, 0.96, 0.56, 0.42, and 5.17 mg/kg, respectively.

Effect of soaking and germination on the nutritional profile of brown rice

The data (Table 3) illustrated the nutritional profile of brown rice affected by soaking and germination at different time intervals i.e. 24 h (G24), 48 h (G48) and 72 h (G72) and revealed that, in brown rice, the moisture and fat content ranged between 9.29 in raw grain (RG) to 9.23 % in (G72) and 1.94 (RG) to

1.78% (G72), respectively. Following germination treatment, the fat content was decreased by 8.25%. when compared to the fiber content of raw and germinated, the fiber content of brown rice increased significantly (P≤0.05) from 1.76% (RG) to 2.06% (G72) resulting in a 17.05% increased. Ash content decreased by 4.14% which is non-significant and values varied from 1.47(RG) to 1.41% (G72).

Crude protein

After 72 h of germination, the crude protein content increased from 7.54% (RG) to 8.44% (G72) resulting in a significant (P≤0.05) and an increment of 11.94%. In brown rice, the carbohydrate contents and calorific value decreased significantly (P≤0.05) from 78.01 (RG) to 77.07% (G72) to and from 375.42 (RG) to 373.06 (G72) Kcal/100g, respectively. The antioxidant activity increased by 40.61 (RG) to 72.13% (G72) with a significant increase (P≤0.05). The antioxidant activity increased by 77.62%. The phenolic components increased from 184.60 (RG) to 291.81 mg GAE/100g (G72) with a significant increment of (P≤0.05). After 72 h of germination, the phenolic content increased by 58.08%.

Flavonoid content

A significant (P≤0.05) increment in flavonoid content also observed during germination process increased from 48.97 (RG) to 64.76 mg RE/100g (G72) resulting in a 32.24% increase in flavonoid contents. Anti-nutrients such as tannin, phytic, and trypsin inhibitor activity declined from 0.557 (RG) to 0.241% (G72); 1.30 (RG) to 1.14% (G72); and 0.86 (RG) to 0.41 mg TIA/100g (G72), respectively, with a significant (P≤0.05) decrease. There was reduction in tannins (56.73%), phytic acid (12.31%), and trypsin inhibitor activity (52.33%) during the germination treatment.

Cu content

Although Cu content increased by 14.58% after germination and the value ranges from 0.96 (RG) to 1.10 mg/kg (G72), germination of the grains for 72 h is the best method to enhance the mineral content. After soaking and germination treatment, the Fe content increased from 0.56 (RG) to 1.04 mg/kg (G72) and Zn from 0.24 (RG) to 0.41mg/kg (G72). Following these treatments, there were 85.71 and 70.84% increase in Fe and Zn content, respectively observed. After germination, the manganese content increased by 71.43 and 16.63%, respectively as the values for Mn and Ca content increased from 0.42 (RG) to 0.72 mg/kg (G72) and 5.17 (RG) to 6.03 mg/kg (G72).

Effect of fermentation

The brown rice was subjected to fermentation treatments i.e. natural fermentation carried out for 12, 24 and 36 h named as (NF12), (NF24), and (NF36) respectively and fermentation with *S. cerevisiae* for 12, 24, and 36 h named as (SCF12), (SCG24), and (SCF36), respectively. The fat content decreased significantly (P≤0.05) from 1.94 (RG) to 1.86% (SCF36), resulting in a 4.12% reduction in fat content observed after the fermentation treatment as per table 4. Similarly, in comparison to the fiber content of raw and fermented brown rice sample significant (P≤0.05) reduction observed from 1.76% (RG) to 1.70% (NF36) or 3.41%. After 36 h of fermentation, the crude protein content was increased significant (P≤0.05) from 7.54 (RG) to 8.34% (SCF36) showing a 10.61% increase in protein content. The carbohydrate and calorific values declined significantly (P≤0.05) from 78.01 (RG) to 77.37% (SCF36) and 375.42 (RG) to 375.28 Kcal/100g (SCF12), respectively.

In brown rice, bio-active components such as antioxidant activity, total phenolic and flavonoid content increased significantly (P≤0.05) from 40.61 (RG) to 60.11% (SCF36), 184.60 (RG) to 246.47 mgGAE/100g (SCF36), and 48.97 (RG) to 57.67 mgRE/100g (NF36), respectively.

The results obtained during the study reported the increase in antioxidant activity (48.02%), TPC (33.52%), and flavonoid (17.77%) content of treated grains. Trypsin inhibitor, tannin and phytic content decreased significantly (P≤0.05) from 0.86 (RG) to 0.45 mgTIA/100g (SCF36), 0.557 (RG) to 0.272% (NF36) and 1.30 (RG) to 0.74% (SCF36), respectively. Reduction in anti-nutritional components i.e. trypsin inhibitor activity (47.67%), tannin (51.17%) and phytic (43.08%) content after the fermentation for 36 h was observed.

Mineral content in brown rice increased significantly (P≤0.05) by 16.67% in Cu content after fermentation. The values increased from 0.96% (RG) to 1.12 mg/kg (NF36) after the fermentation treatments. Similarly, Fe content increased from 0.56 (RG) to 0.71 mg/kg (NF36), Zn content from 0.24 (RG) to 0.42 mg/kg (NF36), Mn content from 0.42 (RG) to 0.62 mg/kg (NF36), and Ca content from 5.17 (RG) to 5.54 mg/kg (NF36). There were 26.79, 75, 47.62, and 7.16% increase in Fe, Zn, Mn, and Ca content, respectively observed after the fermentation process.

Table 4. Effect of fermentation at different time intervals on the nutritive value of Brown rice.

Parameter	Raw (0 h)	Natural Fermentation			Fermentation with *S. cerevisiae*		
		12 h	24h	36 h	12 h	24h	36 h
Moisture (%)	9.29 ±0.01[b]	9.32 ±0.01[a]	9.33 ±0.02[a]	9.33 ±0.01[a]	9.24 ±0.02[d]	9.26 ±0.01[c]	9.28 ±0.01[bc]
Fat (%)	1.94 ±0.04[a]	1.92 ±0.04[a]	1.92 ±0.03[a]	1.91 ±0.01[ab]	1.92 ±0.01[a]	1.89 ±0.01[ab]	1.86 ±0.01[c]
Fibre (%)	1.76 ±0.04[a]	1.74 ±0.03[ab]	1.73 ±0.01[ab]	1.70 ±0.01[b]	1.75 ±0.01[a]	1.73 ±0.01[ab]	1.70 ±0.02[b]
Ash (%)	1.47 ±0.01[a]	1.45 ±0.01[bc]	1.44 ±0.01[cd]	1.42 ±0.01[f]	1.46 ±0.01[ab]	1.44 ±0.01[cd]	1.43 ±0.01[de]
Protein (%)	7.54 ±0.01[f]	7.66 ±0.01[e]	7.70 ±0.01[d]	8.13 ±0.02[c]	8.30 ±0.02[b]	8.33 ±0.01[a]	8.34 ±0.02[a]
Carbohydrate (%)	78.01 ±0.07[a]	77.91 ±0.05[b]	77.88 ±0.02[b]	77.51 ±0.03[c]	77.29 ±0.01[d]	77.34 ±0.01[d]	77.37 ±0.03[d]
Calorific Value (Kcal/100g)	375.42 ±0.08[a]	375.36 ±0.19[a]	375.34 ±.18[a]	375.43 ±0.07[a]	375.28 ±0.09[a]	375.31 ±0.05[a]	375.40 ±0.09[a]
Anti-oxidant activity (%inhibition)	40.61 ±0.12[g]	51.07 ±0.56[f]	52.74 ±0.21[e]	54.64 ±0.06[c]	53.72 ±0.12[d]	58.54 ±0.26[b]	60.11 ±0.21[a]
Total Phenolic content (mg GAE/100g)	184.60 ±0.87[f]	187.60 ±0.22[e]	189.60 ±0.22[d]	190.15 ±0.05[d]	198.39 ±0.56[c]	232.52 ±1.54[b]	246.47 ±1.09[a]
Flavonoids (mgRE/100g)	48.97 ±0.64[e]	53.28 ±0.81[c]	55.39 ±0.39[b]	58.43 ±0.39[a]	51.42 ±0.25[d]	53.95 ±0.25[c]	57.67 ±1.48[a]
Trypsin inhibitor (mgTIA/100g)	0.86 ±0.03[a]	0.83 ±0.01[b]	0.76 ±0.01[c]	0.55 ±0.02[d]	0.75 ±0.01[c]	0.54 ±0.01[d]	0.45 ±0.03[e]
Tannin (%)	0.557 ±0.006[a]	0.519 ±0.006[b]	0.511 ±0.005[b]	0.486 ±0.007[c]	0.298 ±0.006[d]	0.282 ±0.006[e]	0.272 ±0.01[e]
Phytic acid (%)	1.30 ±0.03[e]	1.28 ±0.01[ab]	1.27 ± 0.01[bc]	1.25 ±0.01[c]	0.97 ±0.01[d]	0.80 ±0.01[f]	0.74 ±0.01[g]
Cu (mg/kg)	0.96 ±0.02[e]	1.05 ±0.03[c]	1.09 ±0.01[b]	1.12 ±0.01[a]	0.98 ±0.01[e]	1.01 ±0.01[d]	1.05 ±0.03[c]
Fe (mg/kg)	0.56 ±0.03[e]	0.63 ±0.01[c]	0.67 ±0.01[b]	0.71 ±0.01[a]	0.60 ±0.02[d]	0.62 ±0.01[c]	0.64 ±0.01[c]
Zn (mg/kg)	0.24 ±0.01[f]	0.27 ±0.02[e]	0.34 ±0.03[b]	0.42 ±0.02[a]	0.29 ±0.09[de]	0.30 ±0.01[cd]	0.32 ±0.01[bc]
Mn (mg/kg)	0.42 ±0.01[e]	0.53 ±0.02[c]	0.58 ±0.02[b]	0.62 ±0.01[a]	0.43 ±0.01[e]	0.45 ±0.01[d]	0.52 ±0.01[c]
Ca (mg/kg)	5.17 ±0.06[d]	5.24 ±0.01[c]	5.39 ±0.02[b]	5.54 ±0.05[a]	5.40 ±0.01[b]	5.44 ±0.01[b]	5.45 ±0.01[b]

Values in the table were presented as mean±SD; Values with in rows sharing the same letters were not significantly different according to Duncan's LSD post hoc analysis at P≤0.05

Effect of processing treatments

The effect of processing treatment (soaking and germination) on brown rice revealed that there was 8.25% reduction observed in fat content after germination processing treatment. During the germination of brown rice, Megat Rusydi *et al* (2011) also reported similar decreased trend in fat content With an increase in germination time, Hahm *et al* (2009) reported that crude fat content decreased because fatty acids are converted to water and carbon dioxide through oxidation, which provides energy for germination. This was due to the fact that fat served as the primary carbon source for seed growth (Bau *et al,* 1997). The crude fiber and crude protein content significantly (P≤0.05) increased in present study during germination of brown rice by 17.05 and 11.94%. (Moongngarm and Saetung, 2010), reported 7.96% increment in crude fibre and 28.65% in crude protein content. According to Bau *et al* (1997), the increment in protein content was either caused by the process of enzymatic production of protein or a change

in composition brought out by the breakdown of other components. Nonogaki *et al (*2010) reported that during the germination treatment hormonal changes were considered responsible for increasing the protein content. The antioxidant activity, TPC, and flavonoid content increased significantly (P≤0.05) and there was a 77.62% increment observed in antioxidant activity, 58.08% increase in phenolic, and 32.24% increase in flavonoid content after 72 h of germination. Similar increasing trend after germination of grain was also reported by (Kaur *et al,* 2017). Similarly, Chauhan (2018) reported that the trypsin inhibitor activity decreased in germinated finger millet to the extent of 52.22%. According to (Kumar *et al,* 2019) the apparent decrease in trypsin inhibitor activity during germination might be due to the reason that trypsin inhibitors are used as a source of energy and undergo breakdown by pancreatic and peptic hydrolytic enzymes. Germination of the grain for 72h is the suitable way to enhance the mineral content and there was significant (P≤0.05) increases in Cu, Fe, Zn, Mn and Ca content by 14.58, 85.71, 70.84, 71.43, and 16.63%, respectively. (Chinma *et al,* 2015) also reported similar increase in Ca, Fe, and Zn by 8, 33.80, and 59.82%, respectively after the germination process.

The moisture and fat content of brown rice during fermentation process varied non-significantly (P≤0.05) and there was 4.12% reduction in fat content after fermentation treatment. The fiber content also gets decreased significantly (P≤0.05) during fermentation resulting in a 3.41% reduction when compared to the fiber content of raw grains. In brown rice, there was a significant (P≤0.05) increase in crude protein content, resulting in a 4.75% increase in protein content after 36 h of fermentation. Ilowefah *et al* (2015) also reported 7.75% decrease in fat content and 12.99% increase in crude protein content after fermentation of brown rice. This result was comparable with other studies that reported increase in protein content of cereals and legumes after natural fermentation for 4 days. The values for carbohydrate decreased significantly (P≤0.05) and calorific value increased significantly (P≤0.05).

In brown rice, bio-active components such as antioxidant activity, total phenolic and flavonoid content increased significantly (P≤0.05) during fermentation treatments. There was significant increase in antioxidant activity (31.59%), TPC (52.65%), and flavonoids (6.62%) content. Ilowefah *et al* (2015) reported 11.71% increment in TPC after fermentation treatment of rice. There was significant

reduction in anti-nutritional components i.e. trypsin inhibitor activity (47.67%), tannin (31.25%), and phytic (33.98%) content after the fermentation for 36 h. During the fermentation of brown rice, (Nnam and Obiakor, 2003) also reported similar decrease trends in anti-nutrional components after 48 h. Liang *et al* (2008) reported similar decreasing trend (48.8%) during fermentation of brown rice. Mineral content in brown rice grains, were increased significantly (P≤0.05) and there was an increase in Cu (45.50%), Fe (26.04%), Zn (31.93%), Mn (49.44%), and Ca (9%) after the fermentation process. (Nnam and Obiakor, 2003) reported increase in Ca (55.81%), Fe (12.77%), and Zn (33.99%) after the fermentation of brown rice. (Katina *et al,* 2005) reported that during fermentation, the pH of phytase enzyme was get declined which reduces the phytic acid complex and enhance the mineral content.

CONCLUSION

The study revealed that during processing treatments such as soaking, germination, and fermentation treatments, different physico-chemical parameters such as crude fiber, mineral content, antioxidant activity, TPC, and flavonoid content increased significantly (P≤0.05). The processing treatments resulted in a significant decreased (P≤0.05) in anti-nutritional components such as tannin and phytic components which are responsible for binding the micro-nutrients. Therefore, the use of different processing treatments helps to enhance the nutrional and bioactive components of brown rice. The flour obtained after milling of germinated and fermented brown rice can be utilized with other flour for the preparation of innovative bakery products. Thus, it can be stated that the application of different processing techniques in enhancing the utilization of brown rice by incorporating them and can be used for value addition of functional food products with high nutrional value, decreased anti-nutrients, and increased bioavailability of micro-nutrients.

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