ISSN: 2347-467X, Vol. 13, No. (12) 2025, Pg. 749-763

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Impact of various Fermentation Treatments on Nutritional, Anti-Nutritional and Bioactive Compounds of Black Rice

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Abstract

The black rice flour was subjected to a comprehensive fermentation process, incorporating a diverse range of microorganisms and treatment methodologies. Specifically, the fermentation process utilized two distinct strains of *Lactobacillus*, namely *Lactobacillus brevis* (BF) and *Lactobacillus plantarum* (PF), and yeast *Saccharomyces Cerevisiae* L (YF). Additionally, the fermentation process also involved the use of yeast in conjunction with ammonium sulphate, a fermentation enhancer (YAF), and a combined treatment (CF) of yeast and *Lactobacillus brevis.* The fermentation process was conducted over a range of multiple time points (12, 24 and 36 hr), to assess the impact of varying fermentation durations on the various parameters of the black rice flour. The fermented samples were then dried and analyzed for their nutritional content, anti-nutritional properties, mineral contents and bioactive compounds. Crude protein and ash content were elevated significantly ($p \leq 0.05$) i.e. from 10.29 to 16.59% and 1.49 to 2.67% , respectively following fermentation, however, resulted in a significant reduction of crude fat (77.35%) and crude fibre (61.11%) content. A significant elevation in total phenolic (8.16 to 10.73 mg GAE/g) and flavonoid content (125.51 to 187.21 mg RE/100g) was observed throughout fermentation treatments, resulting in enhanced antioxidant activity (28.91%). The fermentation process also enhanced the nutritional value of black rice flour, reducing anti-nutrients like phytic acid (65.65%) and tannins (50.47%) and boosting mineral levels, particularly copper, iron, zinc and manganese. Although all fermentation treatments enhanced the nutritional and bioactive potential of black rice, but the effect of fermentation with *Lactobacillus brevis* was highest in improving the

Article History
Received: 02 March 2025
Accepted: 25 April 2025

Keywords
Anti-Nutrients;
Bioactive Compounds;
Black Rice;
Fermentation;
Minerals;
Nutrients.

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Doi: https://dx.doi.org/10.12944/CRNFSJ.13.2.14

nutritional and bioactive components and decreasing the anti-nutritional compounds of black rice. This study suggests that fermentation is a highly valuable technique to elevate the nutritional value, bioactive components and antioxidant activity of black rice flour while reducing the anti-nutrients.

Abbreviations

BRF	Black rice flour
FBRF	Fermented black rice flour
YF	Yeast Fermentation
YAF	Fermentation with Yeast and Ammonium Sulphate
BF	Fermentation by *L. brevis*
PF	Fermentation by *L. plantarum*
CF	Combined fermentation by *S. Cerevesiae* and *L.Brevis*
TPC	Total Phenolic Content
TFC	Total Flavonoid content
TAC	Total anthocyanin content

Introduction

Black rice is a specialized strain of *Oryza sativa* L., recognizable by its unique black bran coating on the endosperm. The rice endosperm is translucent and ranges in colour from grey to nearly black, turning a deep purple when cooked. As a whole grain, black rice is regarded as a highly nutritious nutraceutical food owing to its rich content of phenolic compounds, particularly anthocyanins, found in the pericarp.[1] Black rice has been traditionally consumed in Japan and China and is valued for its health benefits, particularly its rich antioxidant content, which helps combat oxidative stress. Chinese black rice is known for its high iron content, often regarded as a natural blood tonic. It also contains more protein, vitamins, and minerals compared to regular white rice.[2] Black rice is generally richer in minerals such as iron, zinc, manganese, and phosphorus compared to white rice. However, the exact mineral content can vary significantly depending on the specific variety of black rice and the soil conditions where it is cultivated.[3] The black glutinous rice is notable for its accumulation of bioactive compounds, particularly anthocyanins and γ-oryzanol.[4] These bioactive compounds found in black glutinous rice, have been identified to exhibit potent antioxidant and free radical neutralizing properties.[5] These properties may contribute to lowering blood fat levels and lower the risk of developing heart diseases and certain types of cancers.[6] Due to its distinctive colour, flavour, and numerous health benefits, black rice has seen a significant surge in demand in recent years. This increased popularity has led to a notable expansion in its production. The surge in black rice production sparked an innovation, leading to the creation of exciting new products like black rice bread, cakes and noodles.[7] Researchers are constantly improving high-quality rice varieties, like black rice, to make processed rice products more competitive.[8] Rice starch comes in many different forms, which is important because it allows food makers to create a wide range of products that meet the different needs and preferences of consumers. Therefore, it is essential to clarify the nutritional and functional characteristics of black rice to better understand its potential applications and uses.

Preserving food through fermentation is a timeless technique that is been used for generations. This process not only extends shelf life but also enhances flavour complexity and increases nutrient availability. Additionally, fermentation can reduce anti-nutrient levels, thereby improving the nutritional profile of foods.[9] Researchers are using fermentation to upgrade the nutritional quality and safety of traditional staple foods, making them more nutritious and healthy.[10] Fermentation of black rice could enhance its nutritional profile and expands its culinary applications. This research aimed to investigate the impact of fermentation on the nutritional content, anti-nutritional factors, and bioactive compounds present in black rice.

Materials and Methods

Raw Material Procurement and Sample Preparation

The research was conducted at the Department of Food Technology, Dr Khem Singh Akal College of Agriculture, Eternal University Baru Sahib, H.P, India. Black rice was procured from SKUAST (Kashmir), J&K, to maintain uniformity and consistency during the entire study. The required chemicals employed in this study were of the highest purity grade, sourced from recognized distributers including BDH chemicals, Merck India, Qualigens, Sigma and Hi-Media. Commercial baker's yeast was obtained from the local bakery in Baru Sahib. Lyophilized starter cultures of *Lactobacillus brevis* and *Lactobacillus plantarum* were obtained from the National Dairy Research Institute, Karnal. The lyophilized cultures were stored at −20 °C and reactivated by growth in nutrient agar broth at 37°C.

Physico-chemical Evaluation

The physicochemical analysis of raw and fermented black rice flour was conducted at the Department of Food Technology, Eternal University, Baru Sahib, Himachal Pradesh, India. Moisture content was determined using the Air-oven drying method of AOAC.[11] Crude fibre, fat and protein were analyzed using Fibroplus FBS 08P (Pelican Inc.), Soxoplus SPS 06 AS (Pelican Inc.), and Kjeloplus Kjelodist CAS VA (Pelican Inc.), respectively. Ash content was determined according to Ranganna.[12] The calorific value was calculated using the Atwater factors, considering the contents of crude protein (N × 6.25), fats, and carbohydrates, with energy values of 4.0, 9.10, and 4.2 KCal/g, respectively.[13] The minerals such as copper, manganese, iron and zinc were analyzed using an Atomic Absorption Spectrometer (AAS), (AA240FS Agilent Technology, CA, USA) according to AOAC[11] procedures. Antioxidant activity (%), tannin content (% and phytic acid content were evaluated according to the methods of Bouaziz *et al*.,[14] Saxena *et al*.[15] and Gao *et al*.[16] Total Phenolic contents (mg GAE/g) were evaluated using the Folin-Ciocalteu assay, following the method of Ainsworth and Gillespie[17] and the flavonoid contents (mg RE/100g) were assayed using the aluminium chloride calorimetric method of Chang *et al*.[18] The total anthocyanin content (mg cyanidin-3-glucoside equivalent/100g) was evaluated by using the method described by Trinovani *et al*.[19] with slight modifications.

Fermentation Treatments

Fermentation with Lactic Acid Bacteria (LAB)

Lactic acid fermentation of black rice was performed by mixing 20 g of black rice flour with 120 mL distilled water in a 250 mL conical flask. After autoclaving for 30 minutes at 121°C, the flasks were subsequently cooled to a temperature of 37 °C. Once the flasks had reached this temperature, the starter cultures were carefully added. Lactic acid bacteria (LAB) including *L. brevis*, *L. plantarum*, and yeast i.e. *S. cerevisiae* were reactivated on agar plates. Using an inoculation loop, fresh cultures were obtained and transferred to 50 mL of nutrient broth.

L. brevis fermentation (BF) was initiated by inoculating autoclaved media with 250 µL of *L. brevis* broth. The inoculated samples were incubated at 37 °C for 12h (B12), 24h (B24), and 36 h (B36). Upon the completion of the incubation period, the samples were subjected to oven drying at a temperature of 50 °C.

For *L. plantarum* fermentation (PF), a larger inoculum volume of 500 µL was used due to its slower growth rate compared to *L. brevis*. Inoculation was performed in a laminar airflow chamber to ensure sterility. The inoculated samples were incubated at 37 °C for 12h (P12), 24h (P24), and 36h (P36) and dried at 50 °C.[20]

Fermentation with Yeast (YF)

For yeast fermentation, 20g of black rice flour was mixed with 120 mL distilled water in a 250 mL conical flask and then autoclaved at 121 °C for 30 minutes before the addition of the starter culture. The autoclaved media was then inoculated with 125 µL of *S. cerevisiae*, thoroughly mixed and subjected to fermentation in an incubator at 37°C for 12h (Y12), 24h (Y24), and 36 h (Y36), and then dried in a hot air oven at 50 °C.[21]

For yeast fermentation with ammonium sulphate $(NH_4)_2SO_4$ (YAF), the same protocol was used, with the addition of 0.4 g of ammonium sulphate (2%) to each sample to boost nitrogen levels activation of the fermentation process, as described by Khetarpaul and Chauhan.[22]

Combined Fermentation (CF)

The combined fermentation effects of *L. brevis* and *S. cerevisiae* on black rice flour were examined. Autoclaved black rice media was inoculated with 125 µL of each microorganism, *L. brevis* and *S. cerevisiae*, and then incubated at 37 °C for varying durations 12h (C12), 24h (C24) and 36 h (C36) and subsequently fermented samples were dried at 50 °C in a hot air oven.[23]

Statistical Analysis

Data analysis was performed using one-way analysis of variance (ANOVA) via IBM SPSS Statistics 26 software. Samples were analyzed in triplicates to ensure consistency. Results are presented in tables as Mean ± Standard Deviation (SD) and significant differences were determined at a probability level of $p \leq 0.05$.

Results

Physico-chemical evaluation of black rice was conducted both prior to and following various fermentation treatments. Research outcomes are tabulated and elaborated upon under the following headings.

Nutritional, Anti-nutritional and Bioactive Components of Raw Black Rice

Black rice was found to be a rich source of nutritional components. It contains 11.23% moisture, 10.29% crude protein, 2.87% crude fat, 1.49% ash content and 70.52% carbohydrates. It is a rich source of polyphenolic contents and contains 8.16 mg GAE/g of polyphenolic contents, 125.51 mg RE/100g of flavonoid contents and 169.2 mg CGE/100g of anthocyanin contents, respectively. Due to the high polyphenolic content, black rice was found to have a high antioxidant activity of 82.10% (Table 1). Anti-nutritional components such as tannin and phytic acid content were reported as 1.05 mg/g and 328.56 mg/100g, respectively. The mineral contents such as Cu, Fe, Zn and Mn have been reported as 3.26, 5.02, 3.18 and 2.04 mg/100g respectively.

Effect of Fermentation Treatments on Nutritional, Antinutritional and Bioactive Components of Black Rice

Effect of Yeast Fermentation (*Saccharomyces Cerevisiae* L.)

The impact of diverse fermentation methods on black rice is presented in Table 2. A significant ($p \leq 0.05$) increase in moisture content was observed following yeast fermentation (YF) treatment, rising from 11.23% in raw black rice flour (RBRF) to 12.97% after 36 hr (Y36) of fermentation. Similarly, YAF-treated black rice flour had the highest moisture content, which increased significantly ($p \leq 0.05$) from 11.23% (RBRF) to 13.23 % (YA36).

Table 1: Nutritional, antinutritional and bioactive components of raw black rice

Parameters	Raw black rice
Moisture (%)	11.23±0.25
Fat (%)	2.87±0.55
Fiber (%)	3.60±0.17
Ash (%)	1.49±0.49
Protein (%)	10.29±0.19
Carbohydrates (%)	70.52±0.69
Calorific value (Kcal/100g)	363.42±3.27
Phytic acid (mg/100g)	328.56±0.28
TPC (mg GAE/g)	8.16±0.53
Tannin (mg/g)	1.05±0.15
Antioxidant activity (%DPPH inhibition)	82.10±0.48
Cu (mg/100g)	3.26±0.29
Fe (mg/100g)	5.02±0.20
Zn (mg/100g)	3.18±0.51
Mn (mg/100g)	2.04±0.29
Flavonoid content (mg RE/100g)	125.51±0.63
Anthocyanin content (mg cyanidin-3-glucoside equivalent/100g)	169.2±0.7

Values in the table are presented as mean±SD

Fermentation treatments led to an increase in protein content. Specifically, protein levels increased from 10.29% (RBRF) to 13.85% (Y36) during YF treatment and from 10.29% in (RBRF) to 14.51% (YA36) during YAF treatment. A significant increase ($p \leq 0.05$) in ash content was also observed, rising from 1.49% (RBRF) to 2.17% (Y36) during YF treatment and from 1.49% (RBRF) to 2.53% (YA36) during YAF treatment. Statistically significant reduction (p≤0.05) in fat content was observed in fermented flour, with YF and YAF treatments yielding a decrease of 55.05% (Y36) and 66.20% (YA36), respectively.

Similarly, fibre content declined significantly (p≤0.05) from 3.60% (RBRF) to 2.50% (Y36) and 1.83% (YA36) respectively during YF and YAF treatments. Fiber content was found to decrease as fermentation time increased, with the greatest reduction occurring during YAF treatment, resulting in a 49.16% decline. In comparison, YF treatment led to a 30.55% reduction. During YF treatment, the carbohydrate content of BRF reduced from 70.52% (RBRF) to 67.23% (Y36)."

The carbohydrate content reduced from 70.52% (RBRF) to 66.93% (YA36) during YAF treatment. The calorific value (kcal/100g) varied from 363.42 kcal/100g (RBRF) to 349.48 kcal/100g (Y36) during YF treatment and to 347.94 kcal/100g (YA36) during YAF treatment. The total phenolic content (TPC) and total flavonoid content (TFC) during YF treatment ranged from 8.16 (RBRF) to 10.60 mg GAE/g (Y36) and 125.51 (RBRF) to 188.91 mg RE/100g (Y36), respectively. Likewise, under YAF treatment the TPC and TFC values spanned from 8.16 (RBRF) to 10.70 mg GAE/g (YA36) and 125.51 (RBRF) to 195.24 mg RE/100g (YA36), respectively.

Both TPC and TFC values were increased during YAF treatment. Due to the enhancement of phytonutrients components such as polyphenols and flavonoids, the antioxidant activity was enhanced by 18.30% (Y36) and 26.09% (YA36), respectively during YF and YAF treatments. The anthocyanin contents during fermentation treatments vary significantly (p≤0.05) and the values reduced from 169.2 mg CGE/100g (RBRF) to 158.0 mg CGE/100g (Y36) and from 169.2 mg CGE/100g (RBRF) to 145.8 (YA36), respectively during YF and YAF treatments. Additionally, the impact of fermentation on the levels of anti-nutritional factors, including phytic acid and tannins, was analyzed. Phytic acid content decreased significantly during YF treatment from 328.56 mg/100g (RBRF) to 204.24 mg/100g (Y36). However, the most substantial reduction (64.41%) was observed in fermented flour subjected to YAF treatment, where phytic acid levels declined substantially from 328.56 mg/100g (RBRF) to 116.92 mg/100g (YA36). Tannin content was markedly reduced in both YF and YAF treatments. YF treatment decreased tannin content from 1.05 mg/g (RBRF) to 0.77 mg/g (Y36), with a decline of 26.66%. In contrast, YAF treatment resulted in a more pronounced decrease, from 1.05 mg/g (RBRF) to 0.50 mg/g (YA36), representing a 52.38% reduction in tannin content.

Significant (p≤0.05) enhancement was identified in the mineral content of fermented flour, particularly for copper, iron, zinc and manganese. A significant (p≤0.05) increase was observed in Cu and Fe contents after YF and YAF treatments. Specifically, Cu content increased by 54.60% (Y36) and 61.65% (YA36), whereas Fe content increased by 34.26% (Y36) and 37.05% (YA36). Similarly, significant (p≤0.05) increases were observed in Zn and Mn contents following YF and YAF treatments. The Zn content increased by 60.69% (Y36) and 68.55% (YA36), whereas Mn content increased by 56.86% (Y36) and 67.15% (YA36).

Effect of LAB and Combined Fermentation Treatments

The effect of fermentation with lactic acid bacteria (LAB) on the nutritional, anti-nutritional and bioactive components of black rice is presented in Table 3. The fermentation was carried out with *L. brevis* (BF), *L. plantarum* (PF) and combined treatment with *L. brevis* + *S. cerevisiae* (CF). During BF treatment, the moisture content increased significantly (p≤0.05) from 11.23% (RBRF) to 11.63% (B36). Similarly, moisture content increased from 11.23% (RBRF) to 12.80% (P36) and from 11.23% (RBRF) to 11.67% (C36), correspondingly under PF and CF treatments.

Moreover, protein content increased during fermentation, with values rising from 10.29% (RBRF) to 16.59% (B36); from 10.29% (RBRF) to 13.00% (P36) and from 10.29% (RBRF) to 13.49% (C36), respectively during BF, PF and CF treatments. A statistically significant (p≤0.05) enhancement in ash content was noted during BF treatment and the values increased from 1.49% (RBRF) to 2.67% (B36). Similarly, ash content increased significantly (p≤0.05) from 1.49% (RBRF) to 2.37% (P36) and from 1.49% (RBRF) to 2.33% (C36), respectively during PF and CF treatments.

Table 2: Effect of yeast fermentation on nutritional, anti-nutritional and bioactive components of black rice

Parameters	Raw Black rice flour (RBRF)	Fermentation time during fermentation with yeast (*S. cerevisiae*)			Fermentation time during fermentation with yeast (*S. cerevisiae*) + $(NH_4)_2SO_4$		
		12 hr (Y12)	24 hr (Y24)	36 hr (Y36)	12 hr (YA12)	24 hr (YA24)	36 hr (YA36)
Moisture (%)	11.23±0.25^{d}	12.60±0.10^{abc}	12.73±0.06^{ab}	12.97±0.50^{a}	11.77±0.21^{cd}	11.94±1.02^{bcd}	13.23±0.60^{a}
Fat (%)	2.87±0.55^{a}	1.46±0.39^{b}	1.31±0.46^{b}	1.29±0.31^{b}	2.30±0.35^{a}	1.43±0.50^{b}	0.97±0.21^{b}
Fiber (%)	3.60±0.17^{a}	3.47±0.40^{a}	3.23±0.15^{ab}	2.50±0.35^{c}	3.20±0.30^{ab}	2.73±0.21^{bc}	1.83±0.35^{d}
Ash (%)	1.49±0.49^{b}	1.57±0.49^{b}	1.80±0.61^{ab}	2.17±0.31^{ab}	1.83±0.12^{ab}	2.07±0.15^{ab}	2.53±0.32^{a}
Protein (%)	10.29±0.19^{d}	10.70±0.57^{d}	11.59±0.35^{c}	13.85±0.13^{b}	10.53±0.15^{d}	11.47±0.37^{c}	14.51±0.39^{a}
Carbohydrates (%)	70.52±0.69^{a}	70.21±1.02^{a}	69.33±1.09^{a}	67.23±0.83^{b}	70.37±0.31^{a}	70.36±1.67^{a}	66.93±0.95^{b}
Calorific value (Kcal/100g)	363.42±3.27^{a}	350.94±2.05^{c}	349.50±4.38^{c}	349.48±0.71^{c}	358.60±2.86^{ab}	354.43±3.38^{bc}	347.94±5.91^{c}
Phytic acid (mg/100g)	328.56±0.28^{a}	284.53±0.94^{ab}	253.08±0.71^{abc}	204.24±0.69^{bcd}	191.66±0.10^{bcd}	156.14±0.56^{cd}	116.92±0.67^{d}
TPC (mg GAE/g)	8.16±0.53^{f}	9.57±0.26^{d}	10.40±0.35^{c}	10.60±0.35^{b}	8.52±0.98^{e}	9.65±0.35^{d}	10.70±0.20^{a}
Tannin (mg/g)	1.05±0.15^{a}	0.82±0.19^{ab}	0.80±0.36^{ab}	0.77±0.19^{ab}	0.67±0.17^{ab}	0.66±0.15^{ab}	0.50±0.11^{b}
Antioxidant activity (% DPPH inhibition)	82.10±0.48^{e}	94.02±0.85^{d}	95.22±0.87^{cd}	97.13±0.10^{bc}	97.95±0.10^{b}	98.29±0.51^{b}	103.52±2.55^{a}
Cu (mg/100g)	3.26±0.29^{c}	4.23±0.28^{b}	4.54±0.10^{ab}	5.04±0.29^{a}	3.96±0.29^{bc}	4.19±0.56^{b}	5.27±0.78^{a}
Fe (mg/100g)	5.02±0.20^{a}	5.79±0.25^{b}	6.48±0.57^{a}	6.74±0.49^{a}	4.78±0.28^{a}	6.71±0.32^{a}	6.88±0.14^{a}
Zn (mg/100g)	3.18±0.51^{c}	4.43±0.24^{b}	5.07±0.29^{ab}	5.11±0.53^{ab}	4.77±0.52^{ab}	4.92±0.69^{ab}	5.36±0.31^{a}
Mn (mg/100g)	2.04±0.29^{c}	2.68±0.15^{b}	3.02±0.34^{ab}	3.20±0.34^{ab}	3.21±0.28^{ab}	3.24±0.24^{ab}	3.41±0.45^{a}
Flavonoid content (mg RE/100g)	125.51±0.63^{e}	174.75±0.37^{d}	179.40±0.63^{c}	188.91±0.63^{b}	179.61±0.37^{c}	179.82±0.37^{c}	195.24±0.63^{a}
Anthocyanin content (mg CGE/100g)	169.2±0.7^{a}	159.0±1.0^{b}	158.1±0.4^{b}	158.0±1.0^{b}	148.5±0.6^{c}	147.3±0.6^{c}	145.8±0.4^{d}

Values in the table are presented as mean±SD; Values within rows sharing the same letters are not significantly different according to Duncan's LSD post hoc analysis at $P \leq 0.05$

Table 3: Effect of LAB fermentation treatments on nutritional, anti-nutritional and bioactive components of black rice

Parameters	Raw Black rice flour (RBRF)	Fermentation time during fermentation with *L. Brevis* (BF)			Fermentation time during fermentation with with *L. Plantarum* (PF)		
		12 hr (B12)	24 hr (B24)	36 hr (B36)	12 hr (P12)	24 hr (P24)	36 hr (P36)
Moisture (%)	11.23 ± 0.25^{c}	11.47 ± 0.37^{c}	11.60 ± 0.27^{bc}	11.63 ± 0.38^{bc}	11.4 ± 0.26^{c}	12.07 ± 0.38^{b}	12.80 ± 0.10^{a}
Fat (%)	2.87 ± 0.55^{a}	1.34 ± 0.53^{cd}	1.33 ± 0.72^{cd}	0.65 ± 0.23^{d}	2.23 ± 0.32^{ab}	1.57 ± 0.12^{bc}	1.10 ± 0.26^{cd}
Fiber (%)	3.60 ± 0.17^{a}	3.47 ± 0.45^{a}	3.23 ± 0.31^{ab}	1.40 ± 0.44^{c}	3.07 ± 0.51^{ab}	2.6 ± 0.17^{b}	1.5 ± 0.40^{c}
Ash (%)	1.49 ± 0.49^{c}	1.63 ± 0.15^{c}	1.80 ± 0.17^{bc}	2.67 ± 0.32^{a}	1.6 ± 0.26^{c}	2.30 ± 0.53^{ab}	2.37 ± 0.15^{ab}
Protein (%)	10.29 ± 0.19^{f}	12.73 ± 0.21^{cd}	13.72 ± 0.51^{b}	16.59 ± 0.47^{a}	11.63 ± 0.59^{e}	12.00 ± 0.46^{de}	13.00 ± 0.44^{bc}
Carbohydrates (%)	70.52 ± 0.69^{a}	69.36 ± 0.93^{ab}	68.32 ± 0.91^{bc}	67.06 ± 0.31^{c}	70.07 ± 0.80^{a}	69.47 ± 1.21^{ab}	69.23 ± 0.42^{a}
Calorific value (Kcal/100g)	363.42 ± 3.27^{a}	354.41 ± 1.56^{b}	353.94 ± 5.78^{b}	353.94 ± 1.62^{b}	361.14 ± 0.72^{a}	354.02 ± 3.84^{b}	352.79 ± 2.34^{b}
Phytic acid (mg/100g)	328.56 ± 0.28^{a}	156.51 ± 0.70^{b}	153.18 ± 0.74^{b}	112.85 ± 0.76^{b}	200.17 ± 0.63^{b}	197.95 ± 0.69^{b}	161.69 ± 0.12^{b}
TPC (mg GAE/g)	8.16 ± 0.53^{e}	9.76 ± 0.42^{b}	9.97 ± 0.23^{b}	10.73 ± 0.21^{a}	9.00 ± 0.61^{b}	9.92 ± 0.64^{b}	9.96 ± 0.60^{b}
Tannin (mg/g)	1.05 ± 0.15^{a}	0.80 ± 0.18^{ab}	0.75 ± 0.16^{ab}	0.52 ± 0.21^{b}	0.73 ± 0.17^{ab}	0.69 ± 0.16^{b}	0.60 ± 0.14^{b}
Antioxidant activity (%DPPH inhibition)	82.10 ± 0.48^{d}	88.97 ± 1.76^{c}	92.28 ± 2.34^{b}	105.84 ± 0.20^{a}	87.70 ± 0.20^{c}	87.74 ± 0.26^{c}	88.01 ± 0.47^{c}
Cu (mg/100g)	3.26 ± 0.29^{c}	3.42 ± 0.40^{c}	4.12 ± 0.15^{b}	5.48 ± 0.53^{a}	4.14 ± 0.40^{b}	4.61 ± 0.20^{b}	4.69 ± 0.30^{b}
Fe (mg/100g)	5.02 ± 0.20^{c}	5.20 ± 0.17^{c}	5.52 ± 0.61^{c}	8.24 ± 0.16^{a}	5.13 ± 0.34^{c}	6.40 ± 0.41^{b}	6.85 ± 0.40^{b}
Zn (mg/100g)	3.18 ± 0.51^{d}	3.92 ± 0.16^{c}	4.59 ± 0.15^{b}	5.37 ± 0.19^{a}	3.64 ± 0.21^{cd}	3.99 ± 0.21^{cd}	4.14 ± 0.53^{b}
Mn (mg/100g)	2.04 ± 0.29^{c}	2.20 ± 0.21^{bc}	2.34 ± 0.20^{bc}	3.26 ± 0.18^{a}	2.27 ± 0.20^{bc}	2.61 ± 0.42^{b}	2.66 ± 0.39^{b}
Flavonoid content (mg RE/100g)	125.51 ± 0.63^{g}	135.45 ± 0.37^{e}	168.20 ± 0.37^{b}	187.21 ± 0.37^{a}	128.05 ± 0.63^{f}	141.36 ± 0.63^{d}	147.07 ± 0.63^{c}
Anthocyanin content (mg CGE/100g)	169.2 ± 0.7^{a}	146.6 ± 0.3^{c}	144.3 ± 0.6^{d}	141.5 ± 0.5^{e}	151.4 ± 0.4^{b}	151.3 ± 0.2^{b}	145.9 ± 0.5^{c}

Values in the table are presented as mean±SD; Values within rows sharing the same letters are not significantly different according to Duncan's LSD post hoc analysis at $P\leq0.05$

Table 4: Effect of combined fermentation treatments on nutritional, anti-nutritional and bioactive components of black rice

Parameters	Raw Black rice flour (RBRF)	Fermentation time during fermentation with *L. brevis* + *S. cerevisiae* (combined effect) (CF)		
		12 hr (C12)	24 hr (C24)	36 hr (C36)
Moisture (%)	11.23±0.25^{c}	11.33±0.53^{c}	11.53±0.35^{bc}	11.67±0.25^{bc}
Fat (%)	2.87±0.55^{a}	1.57±0.29^{bc}	1.23±0.21^{cd}	1.20±0.26^{cd}
Fiber (%)	3.60±0.17^{a}	2.73±0.21^{b}	2.50±0.40^{b}	2.27±0.15^{b}
Ash (%)	1.49±0.49^{c}	1.6±0.15^{c}	1.77±0.15^{c}	2.33±0.15^{ab}
Protein (%)	10.29±0.19^{f}	12.33±1.00^{cd}	13.48±0.21^{bc}	13.49±0.42^{bc}
Carbohydrates (%)	70.52±0.69^{a}	70.40±1.65^{a}	69.49±0.53^{ab}	69.04±0.41^{ab}
Calorific value (Kcal/100g)	363.42±3.27^{a}	359.27±3.45^{b}	356.99±3.71^{b}	354.88±2.51^{b}
Phytic acid (mg/100g)	328.56±0.28^{a}	302.66±0.64^{a}	205.72±0.65^{b}	201.65±0.62^{b}
TPC (mg GAE/g)	8.16±0.53^{e}	8.99±0.15^{d}	9.61±0.16^{b}	9.67±0.42^{b}
Tannin (mg/g)	1.05±0.15^{a}	0.76±0.23^{ab}	0.70±0.10^{ab}	0.61±0.20^{b}
Antioxidant activity(%DPPH inhibition)	82.10±0.48^{d}	99.66±0.56^{a}	99.76±0.43^{a}	100.82±0.62^{a}
Cu (mg/100g)	3.26±0.29^{c}	4.49±0.20^{b}	4.53±0.20^{b}	4.96±0.12^{b}
Fe (mg/100g)	5.02±0.20^{c}	6.28±0.34^{b}	6.33±0.20^{b}	6.81±0.42^{b}
Zn (mg/100g)	3.18±0.51^{d}	4.03±0.20^{b}	4.19±0.37^{b}	4.41±0.20^{b}
Mn (mg/100g)	2.04±0.29^{c}	2.47±0.40^{bc}	2.52±0.21^{b}	3.12±0.40^{a}
Flavonoid content (mg RE/100g)	125.51±0.63^{g}	153.41±0.63^{b}	155.10±0.37^{b}	162.28±0.63^{b}
Anthocyanin content (mg CGE/100g)	169.2±0.7^{a}	152.8±0.5^{b}	151.3±0.6^{b}	144.3±0.6^{d}

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

A significant (p≤0.05) reduction in the fat content of fermented flour was observed, with values decreasing by 77.35% (B36), 61.67% (P36), and 58.18% (C36) during BF, PF, and CF treatments, respectively." Likewise, fiber content in the flour declined across all fermentation treatments, with the most significant reduction occurring during the BF treatment as it was reduced by 61.11% (B36) as compared to PF and CF-treated samples where it reduced to the extent of 58.33% (P36) and 36.94% (C36), respectively. The carbohydrate content of the BRF declined from 70.52% (RBRF) to 67.06% (B36); 70.52% (RBRF) to 69.23% (P36) and from 70.52% (RBRF) to 69.04% (C36), respectively during BF, PF and CF treatments.

The calorific value (kcal/100g) ranged from 363.42 kcal/100g (RBRF) to 353.94 kcal/100g (B36), 352.79 kcal/100g (P36) and 354.88 kcal/100g (C36) during BF, PF and CF treatments respectively.

The TPC content ranged from 8.16 mg GAE/g (RBRF) to 10.73 mg GAE/g (B36), 9.96 mg GAE/g (P36) and from 8.16 mg GAE/g (RBRF) to 9.67 mg GAE/g (C36), respectively during BF, PF and CF treatments respectively. The highest value was observed during BF treatment leading to a 31.49% rise in TPC content. Similarly, the total flavonoid content (TFC) ranged from 125.51 mg RE/100g (RBRF) to 187.21 mg RE/100g (B36) during BF, 147.07 mg RE/100g (P36) during PF and 162.28 mg RE/100g (C36) during CF treatments. Due to the enhancement of phytonutrients components such as polyphenols and flavonoids, the antioxidant activity was enhanced by 28.91% (B36), 7.19% (P36) and 22.80% (C36), following BF, PF and CF treatments respectively. The anthocyanin contents varied significantly (p≤0.05) during fermentation treatments and the values reduced from 169.2 mg CGE/100g (RBRF) to 141.5 mg CGE/100g (B36), 145.9 mg CGE/100g (P36) and 144.3 mg CGE/100g (C36) during BF, PF and CF treatments, respectively.

Furthermore, this study examined the impact of fermentation on anti-nutritional compounds, specifically phytic acid and tannins. The results showed a decline in both phytic acid and tannin levels as fermentation time progressed in each treatment. The phytic acid content was reduced from 328.56 mg/100g (RBRF) to 161.69 mg/100g (P36), and 201.65 mg/100g (C36), during PF and CF treatments, respectively. The most significant decline in phytic acid content was observed in fermented samples subjected to BF treatment, where values decreased from 328.56 mg/100g (RBRF) to 112.85 mg/100g (B36), representing a 65.65% decline in phytic acid. The BF treatment led to a greater reduction in tannin content, decreasing by 50.47% (B36), compared to PF and CF treatments, which showed reductions of 42.85% (P36) and 41.90% (C36), respectively."

A significant ($p \leq 0.05$) rise was observed in the mineral content of fermented flour, specifically in the levels of copper, iron, zinc and manganese. The copper (Cu) content showed a significant rise ($p \leq 0.05$) of 68.09% (B36), 43.86% (P36) and 52.14% (C36) while iron (Fe) content increased significantly ($p \leq 0.05$) by 64.14% (B36), 36.45% (P36) and 35.65% (C36), following BF, PF and CF treatments, respectively. Zinc (Zn) content increased significantly ($p \leq 0.05$) by 68.86% (B36), 30.18% (P36) and 38.67% (C36) and manganese (Mn) content also increased significantly ($p \leq 0.05$) by 59.80% (B36), 30.39% (P36) and 52.94% (C36), respectively during BF, PF and CF treatments.

Discussion

Nutritional, Anti-nutritional and Bioactive Components of Raw Black Rice

The nutritional composition of black rice highlights its high levels of polyphenols, flavonoids, and anthocyanins. The results of this study are consistent with those of Sethi *et al.*,[s] who reported an anthocyanin content of 167.12 mg CGE/100g in black rice. Additionally, black rice contains essential minerals such as copper, iron, zinc, and manganese, which contribute to its nutritional value. However, the presence of anti-nutritional factors like tannins and phytic acid may affect mineral bioavailability. These findings suggest that black rice is a valuable functional food, and further research should explore processing techniques to enhance nutrient absorption while maintaining its bioactive properties.

Effect of yeast fermentation (*Saccharomyces Cerevisiae* L.)

The effects of different Yeast fermentation (YF) methods on black rice led to an improvement in protein content. Both YF and YAF treatments significantly increased protein levels compared to raw black rice flour, with YAF showing the highest enhancement. Comparable findings were reported by Nnam and Obiakor,[9] who observed an increase in protein content from 6.61% to 9.03% in rice grains following 24 hr of fermentation. The enhancement in the protein content of fermented black rice flour can be attributed to the yeast's enzymatic activity, which breaks down starch and polysaccharides into monosaccharides. These simpler sugars can then be readily converted into protein by the yeast.[25] The yeast fermentation (YF) resulted in higher ash levels, while YAF treatment showed the most pronounced enhancement. Similarly, Sukma *et al.*[26] reported 18.6% rise in the ash content of fermented rice bran. The elevated ash content in fermented products is likely due to the proliferation of fungal populations within the substrates. Fungal cell walls which are dense in mineral deposits, contribute to the increased mineral residue, thereby raising the ash content.[27] A significant decrease in fat content was observed after the fermentation treatments (YF and YAF) of black rice flour and similar results were found by Nnam and Obiakor [9] who reported that the fat content of rice grains was reduced by 81.14% after 24 hr of fermentation. The decline in fat content during fermentation may result from increased lipase activity in the fermenting medium. Similarly, there was a significant decrease in the fiber content of black rice flour during YF and YAF treatments. Fiber content was found to decrease as fermentation time increased, with the greatest reduction occurring during YAF treatment.

Both YF and YAF treatments resulted in a decrease in carbohydrate and calorific value of black rice flour and results are in conformity to the findings of Nnam and Obiakor[9] who reported that carbohydrate content was reduced from 90.63% to 90.36 % in rice grains after 24 hr of fermentation. According to Yafetto,[28] yeast exhibits versatility in utilizing diverse carbon sources, including monosaccharides, disaccharides and polysaccharides, to facilitate mycelial growth. Consequently, a reduction in the carbohydrate content of fermented flour may be attributable to the use of carbohydrates by yeast in the course of the fermentation treatment.

During yeast fermentation treatments, the bioactive compounds in black rice flour, including total phenols, total flavonoid content (TFC), and antioxidant activity, showed a significant increase ($P \leq 0.05$). The rise in TPC and TFC was more pronounced during YAF treatment as compared to YF treatment. This enhancement in phytonutrients, such as polyphenols and flavonoids, contributed to a corresponding increase in antioxidant activity in both YF and YAF treatments. Noviasari *et al*.[29] reported that TPC, TFC and antioxidant activity of fermented rice bran increased during fermentation treatment and the values increased by 57.39%, 168.08 to 184.54 mg/100g and 26.28 to 76.91% respectively. The rise in total phenolic content (TPC) can be linked to the organic acids produced during fermentation, which disrupt cell membrane permeability and facilitate the release of phenolic compounds from black rice. Additionally, phenolics may also be generated as metabolic byproducts of microorganisms.[30] An increase in flavonoid content during fermentation may additionally contribute to antioxidant activity.[31] As a result of increase in TPC and TFC, the hydroxyl group of phenolic compounds becomes a hydrogen donor for DPPH free radicals, thereby increasing the antioxidant activity of fermented flour.[32] Significant ($p \leq 0.05$) reduction in anthocyanin content was observed during both YF and YAF treatments of black rice. Trinovani *et al*.[19] documented a decrease in the anthocyanin content of black rice subsequent to fermentation. It has also been reported that increasing fermentation time causes a reduction in anthocyanin content. This is caused by the degradation of anthocyanin caused by the hydrolysis process. The breakdown of anthocyanin involves the action of various enzymes, including glucosidase and polyphenol oxidase. Specifically, glucosidase catalyzes the hydrolysis of sugar bonds linking the aglycone and glycone moieties, resulting in the synthesis of chalcone compounds through the rearrangement of aromatic rings.[33]

Antinutrients such as phytic acid and tannins were declined in both treatments, with a more pronounced decrease in YAF treatment. Nnam and Obiakor [9] reported a 61.53% and 50% reduction in phytic acid and tannins content of fermented rice grains post 72 hr fermentation. The decrease in phytic acid content in fermented flour may be due to the enhanced activity of phytase during fermentation. The enzyme facilitates a stepwise dephosphorylates of phytate, culminating in the production of inositol phosphoric acid. This process liberates certain metals, enhancing their bioavailability while concurrently reducing phytate levels.[9] Significant ($p \leq 0.05$) enhancement was identified in the mineral content of fermented flour, particularly for copper, iron, zinc and manganese. A significant ($p \leq 0.05$) increase was observed in Cu, Fe, Zn and Mn contents after YF and YAF treatments. Nnam and Obiakor 9 reported a 33.33% and 19.24% increase in Cu and Zn content of rice grains after 48 hr of fermentation. The elevated mineral contents of fermented black rice flour were likely a result of the fermenting microorganism's metabolic activities, which break down metal-phytate complexes and release minerals.[34] Furthermore, the decrease in dry matter during fermentation also contributed to the apparent increase in mineral content.[35]

Effect of LAB and Combined Fermentation Treatments

The effect of fermentation with lactic acid bacteria (LAB) on the nutritional, anti-nutritional and bioactive components of black rice is discussed in Table 3 and 4. The fermentation was carried out with *L. brevis* (BF), *L. plantarum* (PF) and combined treatment with *L. brevis* + *S. cerevisiae* (CF).

The moisture content of black rice flour was found to increase significantly ($p \leq 0.05$) during fermentation treatments. Additionally, protein and ash content showed a notable rise across all fermentation treatments, with the highest increase observed in BF treatment. These findings align with those of Steve[36] who reported an increase in the protein content of wheat flour from 10.77% to 13.70% following fermentation. Fermentation treatment resulted in a rise in protein content, mainly attributed to a decrease in the carbohydrate-to-total-mass ratio. During fermentation, microorganisms break down carbohydrates to produce energy, releasing carbon dioxide as a byproduct. As a result, the nitrogen content in the fermented slurry becomes more concentrated, thereby increasing the proportion of protein in the total mass.[37]

A significant (p≤0.05) reduction in the fat and fiber content of fermented black rice flour was observed across all fermentation treatments with the most significant reduction occurring during the BF treatment as compared to PF and CF-treatment. Steve [36] also reported a reduction in fat and fibre content of fermented wheat flour and the values decreased by 41.45% and 33.52%, respectively. The decrease in fibre content may be due to lactic acid bacteria breaking down fibre during fermentation. Similarly, the decline in fat content could be due to the breakdown of fatty acids and glycerol by fermenting microorganisms, resulting in improved aroma, taste and texture of fermented products.[38]

Significant (p≤0.05) reduction in carbohydrate content and calorific value of black rice flour was observed during BF, PF and CF treatments. Steve 36 observed a similar reduction in the carbohydrate content of wheat flour, and values declined from 84.63 to 83.27% following fermentation. Studies have consistently shown that an increase in glucose levels in the early phases of fermentation is linked to the starch-hydrolyzing activities of activated maltase and α-amylase. The released glucose serves as a preferred energy source for fermenting microorganisms, contributing to the observed decrease in carbohydrate content following fermentation.[39]

Bioactive components, including TPC, TFC, and antioxidants, significantly (p≤0.05) increased during LAB fermentation, with the highest enhancement observed in BF treatment compared to PF and CF treatments. Additionally, all components showed a progressive increase with extended fermentation time, indicating a positive correlation between fermentation duration and bioactive compound enrichment. Xu *et al*.[40] reported a 70.61% rise in TPC of brown rice after fermentation. Studies have demonstrated that fermentation significantly improves both free and bound phenolic content. The action of microorganisms and enzymes during fermentation facilitates the easier extraction of phenols from the food matrix.[41] Comparable findings were reported by Saharan *et al*.,[42] who observed an increase in TFC and antioxidant activity of fermented *T. aestivum* extracts. Additionally, Singh *et al*.[43] found that the enhanced antioxidant activity of soybean products fermented with *Trichoderma harzianum* using solid-state fermentation (SSF) was attributed to the increased levels of phenolic and flavonoid compounds compared to unfermented products. The anthocyanin contents varied significantly (p≤0.05) during fermentation treatments and the values were reduced.

The results indicated a significant (p≤0.05) reduction in antinutrients, including phytic acid and tannins, as fermentation time increased across all treatments. BF treatment showed the most substantial decline in both components over time compared to PF and CF treatments, highlighting its effectiveness in reducing antinutritional factors. Steve [36] found that the phytic acid and tannin contents of fermented wheat flour decreased by 20.60% and 60.71% respectively. The lowering in phytic acid levels following fermentation can be partially attributed to the phytase activity of microflora, which breaks down phytate into inositol and orthophosphate.[44] Additionally, the decline in tannin content is likely attributed to the degradation of tannin complexes, including tannic acid-starch, tannin-protein, and tannin iron complexes, releasing bound nutrients. Furthermore, tannin compounds may leach into the fermentation medium, contributing to their decreased levels.[45]

A significant (p≤0.05) rise was observed in the mineral content of fermented black rice flour, specifically in the levels of copper, iron, zinc and manganese. These results conform to the findings of Steve [36] who reported an increase in the mineral contents of wheat flour after fermentation. The enhanced mineral content of fermented flour can be attributed to the degradation of carbohydrates and proteins by microbes during fermentation, resulting in dry matter loss. Additionally, Fermentation increased Fe bioavailability, likely due to the breakdown of oxalates and phytates, which complexes with minerals and reduces their bioavailability.[46]

Conclusion

This study aimed to assess the impact of diverse fermentation treatments on nutritional composition, anti-nutritients and bioactive components of black rice. The black rice, a type of pigmented rice, was found to be a rich source of minerals, phenolic compounds with strong antioxidant activity, dietary fiber, and anthocyanins, among other nutritional and bioactive components. The fermentation treatments substantially enhanced the nutritional profile and bioactive potential of black rice flour. Significant enhancements in protein content, total phenolic compounds and antioxidant activity was observed during fermentation treatments. Further, significant reductions in anti-nutritional factors like phytic acid and tannins, emphasize the benefits of fermentation in upgrading the nutritional quality of this pigmented rice. Furthermore, the increased accessibility of minerals highlights the potential of fermentation in creating functional food products with elevated nutritional value. Therefore, this study will support the use of fermentation processing methods to increase the utilization of underutilized black rice by incorporating them into the creation of functional food products that have better micronutrient bioavailability, lower anti-nutritional components, and high nutritional and bioactive potential.

To fully explore the potential health benefits of fermented black rice, future research should investigate its effects on human health through well-designed clinical trials. These trials should examine the impact of fermented black rice consumption on various health outcomes, including cardiovascular health, glucose metabolism, and weight management. Additionally, the anti-inflammatory and antioxidant effects of fermented black rice should be assessed. A randomized controlled trial design would be suitable, with participants receiving either fermented black rice or a control diet. Outcome measures could include biomarker analysis, anthropometric assessments, and self-reported surveys. The results of these trials would provide valuable insights into the potential therapeutic applications of fermented black rice.

Acknowledgement

The authors gratefully acknowledge the Eternal University, Baru Sahib, Sirmour, HP for facilitating this study and the and The Vice Chancellor, Eternal University for providing the motivation and research infrastructure.

Funding Sources

The authors are grateful for the funding provided by the Ministry of Food Processing Industries (MoFPI) through the Government of India grant (F. No. 5-11/2010-HRD) for the development of infrastructure facilities at Eternal University, Baru Sahib.

Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to Reproduce Material from Other Sources

This study did not involve any figures and tables materials that have been previously published in other sources.

Author Contributions

- **Sumaira Jan:** Data Collection, Analysis, Writing – Original Draft
- **Krishan Kumar:** Conceptualization, Methodology, Statistical analysis, Review, Editing.

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