Journal of Applied Biology & Biotechnology Vol. 10(Suppl 1), pp. 46-52, Mar-Apr, 2022
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2022.10s107

Effect of diverse fermentation treatments on nutritional composition, bioactive components, and anti-nutritional factors of finger millet (*Eleusine coracana* L.)

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ARTICLE INFO

Article history:
Received on: October 30, 2021
Accepted on: January 30, 2022
Available online: March 18, 2022

Key words:
Anti-nutrients,
Antioxidant activity,
Bioactive compounds,
Fermentation,
Lactobacillus brevis,
Lactobacillus plantarum.

ABSTRACT

The finger millet (*Eleusine coracana* L.) flour was subjected to lactic acid fermentation using two strains of *Lactobacillus,* that is, with *Lactobacillus brevis* (BF) and *Lactobacillus plantarum* (PF), with yeast (*Saccharomyces cerevisiae* L.) (YF), and with yeast + ammonium sulfate used as fermentation activator (YAF) and combined treatment of yeast and *L. brevis* (CF) at an interval of 12, 24, and 36 h. The samples after drying were evaluated for their nutritional, anti-nutritional, minerals, and bioactive components. The total phenolic contents enhanced significantly ($P \leq 0.05$) during all fermentation treatments but the highest values were observed after PF treatment. Similarly, there was a significant ($P \leq 0.05$) enhancement in the antioxidant activity during all fermentation treatments, and the highest activity was observed during YAF treatment. Fermentation significantly ($P \leq 0.05$) enhanced the crude proteins content but decreased the crude fiber and fat contents. A significant ($P \leq 0.05$) increase in mineral content such as Cu, Fe, Mn, and Zn was observed after all fermentation treatments. Anti-nutrients such as phytic acid and tannins were reduced significantly ($P \leq 0.05$) and the greatest reductions were observed during treatment with *L. brevis* (BF). Similarly, the tannin contents get reduced significantly ($P \leq 0.05$) during all fermentation treatments. The present study, therefore, shows that fermentation could be the most effective method for improving the nutritional and bioactive components, as well as the antioxidant capacity of finger millet flour with a significant reduction in anti-nutritional components.

1. INTRODUCTION

Finger millet (*Eleusine coracana* L.) also known as ragi is one of the main millets grown in India. It is a rich source of minerals such as calcium, phosphorus, and iron contents [1]. The finger millet contains all essential amino acids such as cysteine, lysine, and methionine. Therefore, it can serve as an important source of vegetable proteins in the diet of vegetarian people. It also contains about 72% carbohydrates, including dietary fiber components and non-starchy polysaccharides which help in preventing constipation and help in decreasing the blood glucose level. It is rich in B-group vitamins such as riboflavin, thiamine, niacin, and folic acid [2]. The bran layers of finger millet comprise phenolic contents, vitamins, and minerals, which provide numerous nutritional and therapeutic benefits [3]. It has nutraceutical properties and is recognized for its antidiabetic, anti-tumorigenic, antidiarrheal, atherosclerogenic, anti-inflammatory, antimicrobial, and antioxidant characteristics [4]. Finger millet is considered as the poor man's food and can be stored for long period without being infested by insects and pests [5]. It is considered a gluten-free grain, with a lower glycemic index, and is generally used as whole grain flour for traditional food formulations, and can be utilized after processing in form of noodles, biscuits, muffins, vermicelli, pasta, and bread [6].

The prominent health benefits of fermented food products make them play a key role in a human diet. Fermentation is generally considered as one of the ancient methods of food preservation generally used in the processing of cereal and millet grains. Studies on fermented food products indicated that it helps to improve the sensory characteristics, for example, taste, flavor, and texture as well as increase the nutritional quality of fermented products. Due to these unique benefits, fermentation has become a main subject of research for the food technologists globally [7]. Fermentation has been found to significantly enhance the nutritional quality of cereal and millet grains by enhancing proteins, improving digestibility, and increasing the lysine content of grains [8]. Moreover, it has been reported to increase the availability of micronutrients such a calcium, zinc, manganese, and iron, as well

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as produce antimicrobial ingredients that help to inhibit the growth of pathogenic microorganisms [9,10]. It also enhances the phenolic contents as well as antioxidant activity in cereals. The bioavailability of some of these nutrients in finger millet is reduced due to the presence of anti-nutrients such as phytates, tannins, and oxalates [11]. Fermentation enhances the biological availability of the micronutrients by reducing the anti-nutrient content of finger millets [12]. During the fermentation process, flour undergoes major biochemical changes such as sugar transformation, softening, and hydrolysis of starch which have been reported to improve the nutritional quality of cereal grain, reduce the anti-nutrients, and increase the bioavailability of micronutrients [13]. This study was undertaken to examine the influence of different fermentation treatments on the biochemical characteristics of soybean and how yeast as well as lactic acid fermentation treatments can be used as a beneficial biotechnological approach to improve the nutritional quality of soybean by decreasing the anti-nutritional factors such as phytic acid and tannin contents as well as increasing the phytochemicals such as phenolic compounds, and antioxidant activity of finger millet.

2. MATERIALS AND METHODS

2.1. Procurement of Raw Materials

The finger millet cultivar, that is, GPHCPB-17 used in this study was procured from the experimental farms of Eternal University. The chemicals and reagents of ultrapure grade were used in the present study. These were obtained from the standard companies of chemicals such as Qualigens, Hi-Media, Merck India, and Sigma-Aldrich. Active dry yeast (commercial baker's yeast) was purchased from the local bakery Baru Sahib and lactic acid bacteria (LAB) starter (*Lactobacillus brevis* and *Lactobacillus plantarum*) in lyophilized form was purchased from National Dairy Research Institute, Karnal. The culture ampules were stored at −20 °C and grown on nutrient broth at 37°C.

2.2. Physicochemical Analysis

The raw grains as well as the fermented ones were subjected to physicochemical analysis at the laboratories of Eternal University, Sirmour, Himachal Pradesh, India. The moisture content (%) of grains was estimated by following the oven-drying method [14]. The equipment Fibroplus FBS 08P (Pelican Inc.) was utilized to estimate the crude fiber, Soxoplus SPS 06 AS (Pelican Inc.) for crude fat, and Kjelodist CAS VA (Pelican Inc.) was used to determine the crude proteins. The ash contents were analyzed as per the methods described by Ranganna [15]. The total carbohydrates were assessed by deducting the measured moisture, crude protein, ash, crude fat, and crude fiber from 100. The calorific value (kcal/100 g) was determined using the factors of 4.0, 9.10, and 4.2 kcal/g for crude protein (Nx6.25), fats, and carbohydrates, respectively [16]. The mineral components such as iron, zinc, manganese, and copper were assessed using Atomic Absorption Spectrometer, Agilent Technology, USA [14]. The antioxidant activity (%) was determined by following the method given by Bouaziz *et al*. [17] and tannins (%) as per the method of Saxena *et al*. [18]. The phytic acid was evaluated by following the methodology described by Gao *et al*. [19]. The phenolic contents (mg GAE/100 g) were analyzed using Folin–Ciocalteu reagent by following the methodology described by Ainsworth and Gillespie [20] with an increase in incubation time to 2.5 h after adding the reagent.

2.3. Fermentation Treatments

2.3.1. Fermentation with yeast (Saccharomyces cerevisiae L.)

The fermentation with yeast (YF) was carried out by mixing 20 g of finger millet flour (FMF) with 120 ml distilled water in a conical flask of 250 ml capacity and was autoclaved at 121°C, for 15 min before adding the starter culture. Then, 125 µL of *S. cerevisiae* was mixed well with autoclaved media and fermentation was carried out in the incubator at different time intervals, that is, 12 (Y12), 24 (Y24), and 36 h (Y36) at 37°C followed by drying in a hot air oven at 50°C. During fermentation with yeast + $(NH_4)_2SO_4$ (YAF), the same procedure was adopted except for the addition of ammonium sulfate at the rate of 2% which was equivalent to 0.4 grams per sample [21]. The ammonium sulfate is a rich source of nitrogen and acts as a fermentation activator.

2.3.2. Fermentation with LAB and combined treatment

The lactic acid fermentation of FMF was performed by taking the 20 g flour in a 250 ml conical flask and mixing it with 120 ml distilled water. The flask was then autoclaved at 121°C for 20 min. The flasks containing the samples were then cooled to 37°C before adding the starter cultures. The lyophilized starter culture of LAB (*L. brevis* and *L. plantarum)* and *S. cerevisiae* was revived on agar plates. The fresh cultures were taken out with an inoculation loop and added to the 50 ml nutrient broth. The fermentation with *L. brevis* (BF) was accomplished by inoculating the autoclaved media with 250 µL of *L. brevis* broth. After inoculation, the sample was put in an incubator at 37°C for 12 (B12), 24 (B24), and 36 h (B36) followed by oven drying at 50°C. In the case of fermentation by *L. plantarum* (PF), higher amount of inoculum, that is, 500 µL was added because it showed slow growth as compared to *L. brevis*. Inoculation was done in a laminar airflow chamber. After inoculation, the samples were kept in an incubator at 37°C for 12 (P12), 24 (P24), and 36 h (P36) followed by oven drying at 50°C [22] [Figure 1].

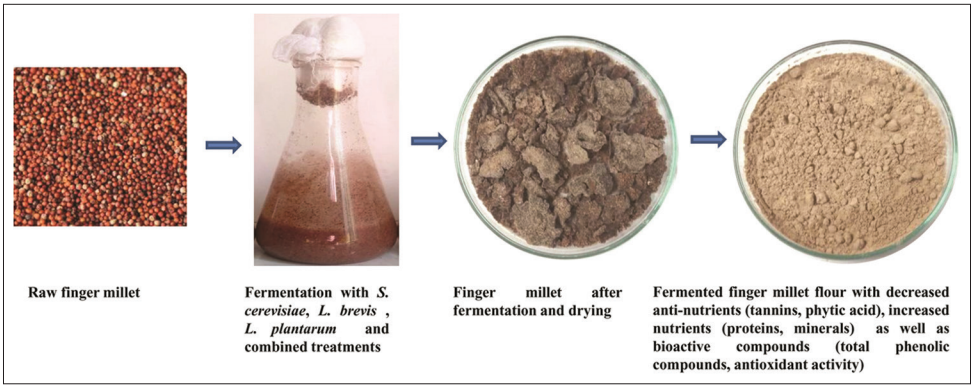

Figure 1: A schematic diagram of fermented finger millet flour production.

The combined effect of fermentation by *L. brevis* and *S. cerevisiae* was studied by adding 125 µL each of *S. cerevisiae* and *L. brevis* into an autoclaved media of finger millet and kept in an incubator for different time intervals, that is, 12 (C12), 24 (C24), and 36 h (C36) at 37°C. The fermented finger millet was then dried in an oven at 50°C.

2.4. Statistical Analysis

The data obtained during the research were evaluated using one-way analysis of variance by SPSS statistics software. Values in tables are represented as mean standard deviation of three replicates and changes were considered as significant at the level of $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Physicochemical Analysis

Finger millet was found to be a rich source of nutritional components. It contains 9.70% of moisture, 9.10% of crude protein, 1.20% of crude fat, 3.10% of ash content, and 71.23% of carbohydrates. It is a rich source of polyphenolic contents and contained 1.07 mg GAE/g of polyphenolic contents. Due to its high polyphenolic contents, finger millet was found to have high antioxidant activity of 74.35% [Table 1]. Anti-nutrients such as tannin and phytic contents were reported as 1.64 mg/g and 629.00 mg/100 g, respectively. The mineral contents such as Cu, Fe, Zn, and Mn have been reported as 1.52, 4.56, 3.31, and 5.04 mg/100 g, respectively. Patil [23] reported a phytic acid of 674.30 mg/100 g in finger millet.

3.2. Fermentation Treatments

3.2.1. Fermentation with yeast (Saccharomyces cerevisiae L.)

The changes in nutritional, anti-nutritional, and bioactive components of finger millet after fermentation with yeast (*S. cerevisiae* L.) are summarized in Table 2. During YF treatment, the moisture content increased significantly ($P \leq 0.05$) from 9.70 (raw flour [RF]) to 10.53% (Y36). Similarly, FMF treated with YAF showed the highest moisture content than unfermented grains and it enhanced significantly ($P \leq 0.05$) from 9.70 (RF) to 10.6% (YA36). There was a significant ($P \leq 0.05$) increase in protein contents of FMF during fermentation treatment and values increased from 9.10% (raw finger millet [RFM]) to 12.70% (Y36) and from 9.10% (RFM) to 12.19 % (YA36) during YF and YAF treatments, respectively. Hamad and Fields [24] reported that the protein content of FMF increased significantly ($P \leq 0.05$) from 11.56% to 12.31% after the fermentation treatment of grains. They stated that an increase in protein content could be credited to the utilization of carbohydrate contents by the action of extracellular enzymes produced by the microorganisms involved in fermentation as well as the degradation of complex proteins resulting in the release of the peptides and amino acids. Whereas, the ash content of FMF increased significantly ($P \leq 0.05$) during YF treatment, and the values increased from 3.10% (RFM) to 3.60% (Y36). Similar results were observed during fermentation with yeast + $(NH_4)_2SO_4$.

Table 1: Nutritional, bioactive, and anti-nutritional components of RFM.

Parameters	RFM
Moisture (%)	9.70±0.26
Fat (%)	1.20±0.52
Fiber (%)	5.67±0.58
Ash (%)	3.10±0.56
Protein (%)	9.10±0.30
Carbohydrates (%)	71.23±0.29
Calorific value (kcal/100 g)	346.50±3.50
Total phenolic component (mg GAE/g)	1.07±0.07
Antioxidant activity (% DPPH inhibition)	74.35±0.97
Phytic acid (mg/100 g)	629.00±0.79
Tannin (mg/g)	1.64±0.05
Cu (mg/100 g)	1.52±0.27
Fe (mg/100 g)	4.56±0.59
Zn (mg/100 g)	3.31±0.23
Mn (mg/100g)	5.04±0.03

Values in the table are presented as mean±SD. RFM: Raw finger millet

There was a significant ($P \leq 0.05$) decline in the fat content of the FMF and values diminished by 32.5 and 89.16%, respectively, during YF and YAF treatments. This is in conformity with the findings of Antony *et al*. [3] who reported about a 42.9% reduction in the total fat content in fermented FMF. The observation in the present work agrees with the results of Adegbehingbe [25] who reported a decrease in fat content in fermented maize samples. El-Beltagi *et al*. [26] stated that this decline in the fat contents during the fermentation process might be due to the fact that physiological as well as biochemical changes during fermentation involve the use of energy resulting in the utilization of lipids for the production of energy. It could also be due to the breakdown of fatty acids as well as glycerol components by the fermenting microorganisms which improve the taste, aroma, and texture of fermented flour products [27]. The low-fat content observed in the fermented samples could help in improving the shelf life of fermented FMF preventing the chances of rancidity and declining the energy value of the fermented samples.

The carbohydrate content of FMF declined from 71.23% (RFM) to 67.96% (Y36) during YF treatment. This is in line with the reports of other researchers who had reported that the carbohydrate content of pearl millet flour decreased significantly ($P \leq 0.05$) from 72.63% to 70.97% after fermentation treatments [28]. Similar results have been observed during YAF treatment where carbohydrate content reduced from 71.23% (RFM) to 69.34% (YA36). It has been observed that there was a gradual decrease in the carbohydrate content during fermentation at different time intervals. These observations are in close conformity with the previous reports that stated that carbohydrates are a major source of carbon for fermenting microbes [29]. It has been observed that the fiber content of fermented FMF reduced with increasing fermentation time in each treatment. The values for fiber content reduced from 5.67% (RFM) to 4.40% (Y36) during YF and 4.13% (YA36) during YAF treatments. There was a 22.39% and 27.16% decline in fiber content of FMF during YF and YAF treatments. The decrease in the fiber contents may be due to the capability of the fermenting microorganisms to metabolize the fiber components [27]. The calorific value (kcal/100 g) varied between 346.50 kcal/100 g (RFM) and 337.45 kcal/100 g (Y36) during YF treatment and 347.34 kcal/100 g (YA36) during YAF treatment.

There was a significant ($P \leq 0.05$) upsurge in minerals such as Cu, Fe, Mn, and Zn contents in the fermented FMF. The values for Cu content augmented significantly ($P \leq 0.05$) by 22.3% (Y36) and 38.8% (YA36), and that for Fe content increased by 22.14% (Y36) and 52.41% (YA36), respectively, during YF and YAF treatments, respectively. Similarly, the values for Zn content increased significantly ($P \leq 0.05$) by 18% (Y36) and 62.53% (YA 36) and that for Mn content by 28.37% (Y36) and 34.92 (YA36), respectively, during YF and YAF treatments. The results were in line with other reports that stated that mineral contents increased significantly ($P \leq 0.05$) with increased fermentation time [8].

Table 2: Effect of fermentation with yeast (*S. cerevisiae* L.) on nutritional, anti-nutritional, and bioactive components of finger millet.

Parameters	Raw finger millet	Fermentation time (h) during fermentation with yeast (*S. cerevisiae*)			Fermentation time (h) during fermentation with yeast (*S. cerevisiae*) +$(NH_4)_2SO_4$		
		12 (Y12)	24 (Y24)	36 (Y36)	12 (YA12)	24 (YA24)	36 (YA36)
Moisture (%)	9.70±0.26^{b}	10.20±0.26^{ab}	10.50±0.36^{a}	10.53±0.38^{a}	10.37±0.38^{a}	10.40±0.30^{a}	10.6±0.44^{a}
Fat (%)	1.20±0.52^{a}	0.90±0.08^{ab}	0.83±0.09^{ab}	0.81±0.06^{b}	0.15±0.01^{c}	0.13±0.06^{c}	0.13±0.06^{c}
Fiber (%)	5.67±0.58^{a}	4.46±0.23^{b}	4.43±0.45^{b}	4.40±0.32^{b}	4.27±0.21^{b}	4.27±0.31^{b}	4.13±0.21^{b}
Ash (%)	3.10±0.56^{a}	3.33±0.55^{a}	3.53±0.55^{a}	3.60±0.35^{a}	3.4±0.36^{a}	3.57±0.31^{a}	3.60±0.26^{a}
Protein (%)	9.10±0.30^{c}	11.66±0.4^{b}	12.62±0.52^{a}	12.70±0.15^{a}	11.78±0.17^{b}	11.92±0.34^{b}	12.19±0.19^{ab}
Carbohydrates (%)	71.23±0.29^{a}	69.44±1.40^{bc}	68.09±1.00^{c}	67.96±1.15^{c}	70.04±0.45^{ab}	69.71±0.41^{ab}	69.34±0.57^{bc}
Calorific value (kcal/100 g)	346.50±3.50^{a}	339.62±4.38^{b}	337.67±1.80^{b}	337.45±4.09^{b}	349.51±1.88^{a}	348.01±1.68^{a}	347.34±1.44^{a}
Phytic acid (mg/100 g)	629.00±0.79^{a}	537.61±0.13^{b}	526.88±0.27^{b}	521.33±0.11^{b}	417.73±0.27^{c}	417.73±0.42^{c}	416.62±0.06^{c}
Total phenolic component (mg GAE/g)	1.07±0.07^{f}	1.37±0.10^{e}	1.57±0.81^{c}	1.80±0.53^{b}	1.48±0.08^{d}	1.86±0.42^{b}	2.10±0.21^{a}
Tannin (mg/g)	1.64±0.05^{a}	0.76±0.07^{b}	0.76±0.12^{b}	0.74±0.15^{b}	0.72±0.04^{b}	0.70±0.12^{b}	0.69±0.30^{b}
Antioxidant activity (%DPPH inhibition)	74.35±0.97^{e}	86.68±0.45^{d}	90.37±0.62^{c}	98.02±0.39^{b}	98.43±0.39^{ab}	98.57±0.53^{ab}	99.35±0.21^{a}
Cu (mg/100 g)	1.52±0.27^{c}	1.61±0.24^{bc}	1.84±0.41^{c}	1.86±0.42^{c}	1.92±0.17^{bc}	2.01±0.15^{b}	2.11±0.02^{b}
Fe (mg/100 g)	4.56±0.59^{c}	5.22±0.58b^{c}	5.32±0.58^{bc}	5.57±0.62^{b}	6.61±0.52^{a}	6.69±0.28^{a}	6.95±0.25^{a}
Zn (mg/100 g)	3.31±0.23^{b}	3.46±0.29^{b}	3.86±0.20^{b}	3.91±0.14^{b}	5.22±0.59^{a}	5.36±0.65^{a}	5.38±0.27^{a}
Mn (mg/100 g)	5.04±0.03^{d}	5.92±0.53b^{c}	6.42±0.31^{abc}	6.47±0.25^{abc}	5.64±0.59^{cd}	6.68±0.51^{ab}	6.80±0.59^{a}

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 $P \leq 0.05$. *S. cerevisiae*: *Saccharomyces cerevisiae*

The effect of fermentation on the anti-nutrients such as phytic acid and tannin contents was also studied. The phytic acid content got reduced from 629.00 mg/100 g (RFM) to 521.33 mg/100 g (Y36) during YF treatment. The highest reduction in phytic acid content was seen in the FMF subjected to YAF treatment as it got reduced from 629.00 mg/100 g (RFM) to 416.62 mg/100 g (YA36) leading to a 33.76% decrease in phytic acid contents. The results are in line with the studies of Makokha *et al*. [30] who reported a reduction in the phytic acid content of finger millet after fermentation treatments. The tannin contents got reduced from 1.64 mg/g (RFM) to 0.74 mg/g (Y36) and from 1.64 mg/g (RFM) to 0.69 mg/g (YA36), respectively, during YF and YAF treatments. The YAF treatment resulted in a higher decline of 57.92% in tannin contents as compared to the YF treatment where it decreased to the extent of 54.87%.

The total phenolic component (TPC) and antioxidant content were in the ranges of 1.07 (RFM) to 1.80 (Y36) mg GAE/g and 74.35% (RFM) to 98.02% (Y36), respectively, during YF treatment. Similarly, during YAF treatment, the total phenols and antioxidant content ranged between 1.07 (RFM) and 2.10 (YA36) mg GAE/g and 74.35% (RFM) and 99.35% (YA36), respectively. The highest antioxidant as well TPC content was seen in the FMF during YAF fermentation treatment. Both phenolic and antioxidant contents increased significantly ($P \leq 0.05$) with an increase in fermentation time. The effects of fermentation on phenolic compounds were reported to be a factor of grain types, microorganism species, as well as fermentation conditions (temp, pH, and time). Antioxidants prevent the damage caused by free radicals by their radical scavenging activity. These have also been reported to act as a reducing agent which can help to remove the free radical intermediates and prevent further oxidation [31].

3.2.2. Fermentation with LAB and combined treatment

The changes in nutritional, anti-nutritional, and bioactive components of finger millet after fermentation with LAB as well as combined treatments are presented in Table 3. The moisture content enhanced significantly ($P \leq 0.05$) from 9.70% (RFM) to 11.85% (B36) during BF treatment. Similarly, FMF treated with PF and combined fermentation treatment by *S. cerevisiae* and *L. brevis* (CF) showed higher moisture content than unfermented grains. The values for moisture content improved significantly ($P \leq 0.05$) from 9.70 (RFM) to 12.03 % (P36) and from 9.70 (RFM) to 10.93% (C36) during PF and CF treatments, respectively. Therefore, the moisture content of fermented FMF was significantly higher than that of raw FMF. Ojokoh *et al*. [32] reported that moisture content values enhanced significantly ($P \leq 0.05$) from 5.94% to 13.23% in fermented pearl millet sprout flour blends. There was a slight increase in ash content during fermentation treatments and values increased from 3.10% (RFM) to 3.63% (B36) and from 3.10% (RFM) to 3.53% (P36), respectively, during BF and PF treatment. No significant ($P \leq 0.05$) difference in the ash content was seen in the fermented FMF during BF and CF treatment.

There was a significant increase in crude protein contents and values increased significantly ($P \leq 0.05$) from 9.10% (RFM) to 13.13% (B36), 9.10% (RFM) to 10.23% (P36), and from 9.10% (RFM) to 10.70% (C36), respectively, during BF, PF, and CF treatments. The net production of protein components during the fermentation process of FMF might have led to the synthesis of some amino acids resulting in increased protein contents during the fermentation process [33]. Inyang and Zakari [8] described that fermentation increased significantly ($P \leq 0.05$) the protein contents of pearl millet flour.

There was a significant ($P \leq 0.05$) decrease in the fiber content of FMF with increased fermentation time. The crude fiber composition of FMF decreased significantly ($P \leq 0.05$) from 5.67% (RFM) to 3.49% (B36), 5.67% (RFM) to 3.67% (P36), and from 5.67% (RFM) to 5.12% (C36), respectively, during BF, PF, and CF treatments. There was a 38.44, 35.27, and 9.70% decline in the crude fiber contents of

Table 3: Effect of fermentation with lactic acid bacteria and combined treatments on nutritional, anti-nutritional, and bioactive components of finger-millet.

Parameters	Raw finger millet	Fermentation time (h) during fermentation with *L. brevis*			Fermentation time (h) during fermentation with *Lactobacillus plantarum*			Fermentation time (h) during fermentation with *Lactobacillus brevis*+*Saccharomyces cerevisiae* (combined effect)		
		12 (B12)	24 (B24)	36 (B36)	12 (P12)	24 (P24)	36 (P36)	12 (C12)	24 (C24)	36 (C36)
Moisture (%)	9.70±0.26^{f}	10.97±0.50^{cd}	11.40±0.32^{bc}	11.85±0.10^{ab}	10.87±0.40^{cde}	11.7±0.21^{ab}	12.03±0.42^{a}	10.32±0.35^{e}	10.45±0.39^{de}	10.93±0.15^{cd}
Fat (%)	1.20±0.52^{a}	1.20±0.10^{a}	1.14±0.10^{a}	1.19±0.12^{a}	1.2±0.1^{a}	1.11±0.06^{a}	1.19±0.20^{a}	1.17±0.15^{a}	1.2±0.15^{a}	1.17±0.15^{a}
Fiber (%)	5.67±0.58^{a}	4.48±0.27^{c}	3.68±0.14^{d}	3.49±0.29^{d}	4.47±0.29^{c}	3.73±0.15^{d}	3.67±0.15^{d}	5.31±0.03^{ab}	5.25±0.17^{ab}	5.12±0.11^{b}
Ash (%)	3.10±0.56^{a}	3.20±0.52^{a}	3.53±0.15^{a}	3.63±0.21^{a}	3.20±0.46^{a}	3.23±0.15^{a}	3.53±0.25^{a}	3.20±0.61^{a}	3.53±0.40^{a}	3.63±0.57^{a}
Protein (%)	9.10±0.30^{d}	10.28±0.22^{b}	10.44±0.37^{b}	13.13±0.12^{a}	9.54±0.31^{c}	9.73±0.21^{c}	10.23±0.15^{b}	10.30±0.29^{b}	10.67±0.25^{b}	10.70±0.20^{b}
Carbohydrates (%)	71.23±0.29^{a}	69.87±0.23b^{c}	69.81±0.45^{bcd}	66.70±0.51^{f}	70.72±0.54^{ab}	70.46±0.18^{abc}	69.35±0.47^{cde}	69.71±0.42^{cde}	68.87±0.32^{de}	68.45±0.47^{e}
Calorific value (kcal/100 g)	346.50±3.50^{a}	345.49±0.72^{a}	345.34±0.60^{a}	343.49±3.84^{a}	346.12±2.43^{a}	344.94±0.42^{a}	342.99±1.58^{a}	344.60±1.99^{a}	343.14±1.42^{a}	340.89±3.08^{a}
Phytic acid (mg/100 g)	629.00±0.79^{a}	250.49±0.25^{c}	230.88±0.57^{c}	207.94±0.62^{c}	538.35±0.62^{ab}	465.09±0.16^{b}	459.91±0.65^{b}	549.82±0.37^{ab}	522.07±0.19^{b}	498.02±0.76^{b}
Total phenolic contents (mg/g)	1.07±0.07^{f}	1.87±0.62^{d}	1.88±0.32^{d}	1.92±0.34^{c}	1.76±0.32^{d}	1.78±0.84^{d}	2.02±0.38^{ab}	1.83±0.19^{d}	1.91±0.38^{abc}	1.95±0.46^{bc}
Tannin (mg/g)	1.64±0.05^{a}	0.97±0.11^{b}	0.97±0.10^{b}	0.72±0.12^{c}	0.90±0.17^{bc}	0.80±0.14^{bc}	0.72±0.12^{c}	0.85±0.15^{bc}	0.83±0.15^{bc}	0.76±0.11^{bc}
Antioxidant activity (% DPPH inhibition)	74.35±0.97^{h}	78.18±0.41^{g}	89.24±0.53^{b}	91.73±0.30^{a}	84.53±0.62^{d}	87.30±0.10^{c}	92.04±0.56^{a}	79.85±0.51^{df}	81.66±0.37^{e}	81.66±0.37^{e}
Cu (mg/100 g)	1.52±0.27^{c}	1.68±0.15^{bc}	1.81±0.52b^{c}	2.54±0.12^{a}	1.90±0.22^{bc}	1.94±0.20^{bc}	2.04±0.21^{b}	1.61±0.19^{bc}	1.92±0.21^{bc}	2.09±0.21^{b}
Fe (mg/100 g)	4.56±0.59^{b}	4.94±0.40^{b}	6.09±0.35^{a}	6.14±0.35^{a}	4.73±0.35^{b}	5.19±0.20^{b}	5.23±0.20^{b}	4.77±0.39^{b}	4.76±0.19^{b}	5.09±0.54^{b}
Zn (mg/100 g)	3.31±0.23^{d}	5.08±0.41^{ab}	5.52±0.39^{a}	5.53±0.34^{a}	4.25±0.41^{c}	4.40±0.54^{bc}	4.61±0.21^{bc}	4.60±0.20^{bc}	4.68±0.41^{bc}	4.71±0.53^{bc}
Mn (mg/100 g)	5.04±0.03^{c}	5.49±0.35^{bc}	5.68±0.19^{b}	5.74±0.20^{b}	5.24±0.20^{bc}	5.30±0.34^{bc}	5.50±0.41^{bc}	6.41±0.36^{a}	6.52±0.34^{a}	6.58±0.19^{a}

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 $P \leq 0.05$

FMF, respectively, during BF, PF, and CF treatments. The general decline in the fiber contents might be attributed to the ability of the microorganisms involved in fermentation to metabolize the fiber contents present in raw grains. It can also be due to the enzymatic degradation of fiber components during the fermentation process by LAB which utilized the fiber as a carbon source [27]. The results are consistent with the findings of Sade [34] who reported that the fiber content of pearl millet flour got reduced from 2.0% to 1.8% in fermented flour of pearl millet. There was a small but non-significant decline in fat content during LAB fermentation as well as the combined fermentation treatments.

The values of carbohydrates got reduced significantly ($P \leq 0.05$) from 71.23% (RFM) to 66.70% (B36), 71.23% (RFM) to 69.35% (P36), and 71.23% (RFM) to 68.45% (C36), respectively, during BF, PF, and CF treatments. Finger millet contains free sugars and starch contents [1] and these compounds worked as a substrate for *Lactobacillus* bacteria and get utilized during fermentation [35]. The enzymes such as α- and β-amylases present in microorganisms could hydrolyze glycosidic bonds resulting in the production of monosaccharides which were utilized for releasing energy required in microbial activities. Moreover, the LAB could have consumed the fermentable sugars for growth as well as other metabolic activities resulting in the lower carbohydrate contents of fermented FMF [36]. Similar results were found by Mutshinyani *et al*. [37] who reported the reduction of carbohydrate from 73.1% to 72.7% and 71.8% to 71.4% in light brown and dark brown varieties of FMF after 96 h of fermentation. The energy value decreased from 346.50 kcal/100 g (RFM) to 343.49 kcal/100 g (B36), 346.50 kcal/100 g (RFM) to 342.99 kcal/100 g (P36), and from 346.50 kcal/100 g (RFM) to 340.89 kcal/100 g (C36), respectively, during BF, PF, and CF treatments. Reduction in carbohydrates and fat components during fermentation resulted in lower energy contents of fermented FMF.

The bioactive components such as TPC) increased significantly ($P \leq 0.05$) from 1.07 mg GAE/g (RFM) to 1.92 mg GAE/g (B36), 1.07 mg GAE/g (RFM) to 2.02 mg GAE/g (P36), and from 1.07 mg GAE/g (RFM) to 1.95 mg GAE/g (C36), respectively, during BF, PF, and CF treatments [Table 3]. It was observed that TPC got increased significantly ($P \leq 0.05$) after fermentation and this change was different at different time intervals. The increase in TPC throughout the fermentation process could be due to the production of different types of enzymes by the microbes used in the fermentation process that contributed to the release of bound phenolic compounds into free phenols [38]. Similar results were found by Mutshinyani *et al*. [37] who reported that the phenolic content got increased from 1.66 mg/g to 3.01 mg/g during the fermentation of FMF.

The DPPH radical scavenging activities of FMF increased significantly ($P \leq 0.05$) with increase in fermentation time. The antioxidant activity increased from 74.35% (RFM) to 91.73% (B36), 74.35% (RFM) to 92.04% (P36), and from 74.35% (RFM) to 81.66% (C36), respectively, during BF, PF, and CF treatments. During the fermentation process, the existence of LAB contributed to the depolymerization of high-molecular-weight phenolic compounds into simple phenolic compounds resulting in increased antioxidant activity [39]. The conversion of

glycosylated isoflavones into their aglycons during fermentation also increased the level of antioxidants in FMF [40]. Similar findings were reported by Đorđević *et al*., Marinković [41], and Moore *et al*. [42], whereby fermentation treatment significantly ($P \leq 0.05$) increased the DPPH radical scavenging activity of fermented cereal flour samples as compared to non-fermented samples.

There was a significant ($P \leq 0.05$) decrease in the anti-nutrient components of FMF after different fermentations treatments. Fermentation decreased significantly ($P \leq 0.05$) the levels of tannin and phytic acid in all treatments. Phytic acid contents reduced from 629.00 mg/100 g (RFM) to 207.94 mg/100 g (B36), 459.91 mg/100 g (P36), and 498.02 mg/100 g (C36) respectively, during BF, PF, and CF treatments. The maximum reduction in phytic acid was seen in the samples treated with *L. brevis*. The values decreased by 67% during BF treatment, 26.9% during PF, and 20.82% during combined treatments. Results also showed that phytic acid got reduced with an increase in fermentation times in all treatments. The reduction in phytic acid could be due to the increased activities of phytase during fermentation [43]. The results are consistent with the findings of Osman [28] who reported that the phytic acid content of pearl millet got reduced from 647.0 to 310.95 mg/100 g resulting in a 51.93% decline after the fermentation process. Therefore, fermentation by *L. brevis* (BF) was the most effective treatment in decreasing the phytic acid in fermented FMF.

Similarly, the level of tannin contents also got reduced with fermentation time in all treatments. The tannin level reduced from 1.64 mg/g (RFM) to 0.72 mg/g (B36), 0.72 mg/g (P36), and 0.76 mg/g (C36), respectively, during BF, PF, and CF treatments. There was a 56.1% decrease in tannin contents during BF and PF treatments and 53.65% during the combined treatment (CF). The reduction in tannin contents can be caused by the hydrolysis of the polyphenolic components to simpler substances by the enzymes such as polyphenol oxidase or due to the breakdown of the tannin complexes such as tannic acid-starch, tannin-protein, and tannin-iron complexes to release the bound nutrients. The tannin components leached into the fermentation medium. The reduced level of anti-nutrients during the fermentation activity resulted in increased availability of micronutrients in the seed [44].

The changes in mineral content of FMF subjected to different fermentation treatments are depicted in Table 3. The results indicated a significant increase ($P \leq 0.05$) in mineral contents with an increase in fermentation time. The values for Cu content increased significantly ($P \leq 0.05$) by 67.10% (B36), 34.21% (P36), and 37.5% (C36), and that for Fe content increased by 34.64% (B36), 14.69% (P36), and 11.62% (C36), respectively, during BF, PF, and CF treatments. Similarly, the values for Zn content increased significantly ($P \leq 0.05$) by 67.06% (B36), 39.27% (P36), and 42.29% (C36) and that for Mn content by 13.88% (B36), 9.12% (P36), and 30.55% (C36), respectively, during BF, PF, and CF treatment. This is in line with other researchers who observed that fermentation increased the bioavailability of minerals in cereal grains [30].

4. CONCLUSION

This study aimed to evaluate the effect of processing techniques on nutritional composition, anti-nutritional compounds, as well as bioactive components of finger millet. Fermentation treatments were found effective in increasing the nutritional value and decreasing the anti-nutritional components in finger millet. There was a significant ($P \leq 0.05$) increase in the protein content of fermented samples. The high-protein content of fermented FMF suggested that it could be of great significance in the alleviation of protein-energy malnutrition. Total phenolic contents and antioxidant activity were found to get increased significantly ($P \leq 0.05$) during fermentation treatments. All fermentation treatments resulted in a significant decline in anti-nutrients such as phytic acid and tannin components which are responsible for binding the micronutrients. The decline in anti-nutrients resulted in a significant ($P \leq 0.05$) increase in mineral contents after all fermentation treatments. Therefore, the use of fermentation techniques can enrich the nutritional and bioactive potential of this underutilized grain. This study will also help in the promotion of traditional processing techniques in enhancing the utilization of underutilized finger millet by incorporating them for the development of functional food products with high nutritional value, lower anti-nutritional components, and better bioavailability of micronutrients.

5. ACKNOWLEDGMENTS

The financial support provided by the Ministry of Food Processing Industries (MoFPI) Govt. of India grant (F. No. 5-11/2010-HRD) for the development of infrastructural capability at the university is duly acknowledged.

6. FUNDING

This research is supported by Ministry of Food Processing Industries (MoFPI) Govt. of India grant (F. No. 5-11/2010-HRD) for the development of infrastructural facilities at the Department of Food Technology, Eternal University, Baru Sahib.

7. CONFLICTS OF INTEREST

The authors state that no conflicts of interest exist in this study.

8. ETHICAL APPROVALS

There is no involvement of experiments on animals or human beings.

9. PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

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How to cite this article:
Jan S, Kumar K, Yadav AN, Ahmed N, Thakur P, Chauhan D, Rizvi QH, Dhaliwal HS. Effect of diverse fermentation treatments on nutritional composition, bioactive components, and anti-nutritional factors of finger millet (Eleusine coracana L.). J App Biol Biotech. 2022;10(Suppl 1):46-52. DOI: 10.7324/JABB.2022.10s107