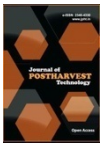

Journal of Postharvest Technology
2022, **10**(2): 35-47
www.jpht.in

RESEARCH ARTICLE

Beneficial effect of diverse fermentation treatments on nutritional composition, bioactive components, and anti-nutritional factors of foxtail millet (*Setaria italica* L.)

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Received: 19.02.2022 Accepted: 16.04.2022

ABSTRACT

The foxtail millet (*Setaria italica* L.) flour was exposed to lactic acid fermentation by using two strains of *Lactobacillus* i.e. with *Lactobacillus brevis* (BF) and *Lactobacillus plantarum* (PF), yeast (*Saccharomyces cerevisiae* L.) (YF), yeast + ammonium sulfate [$(NH_4)_2SO_4$] (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 phenolics enhanced significantly ($p \leq 0.05$) during all fermentation treatments but the highest value was observed during YAF treatment. Similarly, the antioxidant activity improved significantly ($p \leq 0.05$) during all treatments but the highest values were observed during YAF treatment. The fermentation treatments increased significantly ($p \leq 0.05$) the crude protein content during all fermentation treatments. Whereas, there was a significant ($p \leq 0.05$) decrease in crude fiber and fat content. A significant ($p \leq 0.05$) increase in mineral contents such as Cu, Fe, Mn, and Zn was observed after all fermentation treatments. Anti-nutrients such as phytic acid declined significantly ($p \leq 0.05$) during all fermentation treatments but the highest reductions were observed during treatment with *L. brevis* (BF) and with yeast + (NH4)2SO4 (YAF). Similarly, the tannin contents reduced significantly ($p \leq 0.05$) during all fermentation treatments. The results concluded that fermentation could be the most efficient technique of improving the bioactive compounds, nutritional components, and antioxidant activity of foxtail millet flour with a significant reduction in anti-nutritional components.

Keywords: Anti-nutrients, fermentation, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Saccharomyces cerevisiae*

Citation: Jan, S., Kumar, K., Ahmed, N., Thakur, P., Chauhan, D., Rizvi, Q. E. H., and Vyas, P. 2022. Beneficial effect of diverse fermentation treatments on nutritional composition, bioactive components, and anti-nutritional factors of foxtail millet (*Setaria italica* L.). *Journal of Postharvest Technology*, **10**(2): 35-47.

INTRODUCTION

Foxtail millet (*Setaria italica* L.), an individual from the grass family Poaceae, is one of the world's most nutritious millets and is an important food in Asia and Africa. It remained an underutilized crop like different millets but, and its cultivation is easy

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ISSN: 2348-4330

according to the agrarian perspective as it can be grown at places lacking water supply (Sharma and Niranjan, 2018). The dietary profile of foxtail millet is likewise best in class to the staple cereals (rice and wheat), and possess comparable contents of soluble fibers (3-4%), proteins (12-19%), fat (5-9%), and minerals (3.3%) like iron and calcium. It is additionally a more significant source of nutrients and phytochemicals like riboflavin, thiamine, niacin, folic acid, and β-carotene (Sunil and Venkatachalapathy, 2017). The high antioxidant activity of foxtail millet is the other main useful characteristic found in foxtail millet. The phenolic components are one of the essential antioxidant components present in foxtail millet. Phenolics from the foxtail millet in addition to other millets have additionally revealed their capacity as a reducing agent, metal chelating, and singlet oxygen quenching agents (Chandrasekara and Shahidi, 2010). Foxtail millet has gained the attention of food scientists due to its numerous medical advantages like hypoglycemic, and hypolipidemic impacts, and these advantages have principally supported the presence of cancer-preventing agents such as phenolic compounds and carotenoids, which instigates the inactivation of free radicals inside the human body (Sharma and Niranjan, 2018). Foxtail millet is superbly nutritious, non-glutinous, and like buckwheat and quinoa, is simple to digest (Kamara et al., 2009). In day-to-day existence, the foxtail millet is hulled and consumed after removing the hush layer. The de-husking process brings about a significant decline in the anti-nutrients thereby enhancing the bioavailability of dehulled millet (Verma et al., 2015). It is highly suitable for persons suffering from gluten allergy as there has been a remarkable upsurge in the exploration for gluten-free diet formulations (Amadou et al., 2011).

Processing treatments like fermentation have been reported through numerous studies to decrease the contents of anti-nutrients in grains and improve the bioavailability of micronutrients (Chu et al., 2019). The fermentation of millets resulted in the degradation of polysaccharides such as cellulose and hemicellulose in the bran layers, bringing about the development of more permeable and loose structures and improving the edibility and prebiotic properties of foxtail millet (Chu et al., 2019). Moreover, it has been reported to increase the availability of micronutrients like calcium, zinc, manganese, and iron, as well as produced antimicrobial ingredients that helped to inhibit the growth of pathogenic microorganisms (Chaves-López et al., 2020; Xiang et al., 2019). It also enhanced the phenolic contents and antioxidant activity in cereals. The bioavailability of some of these nutrients in foxtail millet is reduced by the presence of anti-nutrients such as phytates, tannins, and oxalates (Popova and Mihaylova, 2019). During fermentation, flour undergoes major biochemical reactions such as starch hydrolysis, sugar transformation, and softening which have been reported to improve the nutritional quality of cereal grain, reduce the anti-nutrients and increase the bioavailability of micronutrients (Gupta et al., 2015). The present study was planned to study the influence of diverse fermentation treatments on the nutritional, anti-nutritional, and bioactive potential of foxtail millet grains to improve the nutritional quality of functional food products to be prepared from this underutilized millet. This study will also help in the promotion of traditional processing techniques in enhancing the utilization of underutilized grains with increased nutritional value and bioavailability of micronutrients due to reduced anti-nutritional components.

MATERIALS AND METHODS

Procurement of raw materials

The study was carried out at the laboratories of Eternal University, Sirmour, Himachal Pradesh, India. Foxtail millet variety (GS-450) used in this study was taken from the experimental farms of Eternal University to ensure the utilization of the same variety throughout the study. 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 starter culture of lactic acid bacteria (*Lactobacillus brevis* and *Lactobacillus plantarum*) in lyophilized form were purchased from National Dairy Research Institute, Karnal. The culture ampules were stored at −20 °C and grown in nutrient agar broth at 37°C.

Physicochemical evaluation

The moisture content (%) of grains was estimated by following the hot air-oven method (AOAC, 1990). The equipment Fibroplus FBS 08P (Pelican Inc.) was used to determine the crude fiber, Soxoplus SPS 06 AS (Pelican Inc.) for crude fat and Kjelodist CAS VA (Pelican Inc.), was used to estimate the crude proteins and ash contents as per the methods defined by Ranganna (1986). The total carbohydrate contents were assessed by deducting the measured moisture, crude protein, ash, crude fat, and crude fiber from 100. The calorific value (kcal/100g) was determined by using the factors of 4.0, 9.10, and 4.2 kcal/g for crude protein (Nx6.25), fats, and carbohydrates, respectively (WHO, 1973). The mineral components such as iron, zinc, manganese, and copper were assessed using Atomic Absorption Spectrometer (AA240FS, Agilent Technology, CA, USA) as per the method of AOAC (1990). The antioxidant activity (%) was evaluated according to the method described by Bouaziz et al. (2008)) and tannins (%) as per the method of Saxena et al. (2013). The extraction and quantification of phytic acid in the biofortified wheat derivatives was evaluated by Gao et al. (2007). Total phenolic contents (TPC) were determined using the Folin-Ciocalteu reagent by following a slightly modified method of Ainsworth and Gillespie (2007).

Fermentation treatments

The lactic acid fermentation of foxtail millet flour (FMF) was conducted by taking the 20 g flour and mixing it with 120 ml distilled water in a 250 ml conical flask. The flask was then autoclaved at 121°C for 30 minutes. The flasks containing the samples were then cooled to 37 °C before adding the starter cultures. The microorganisms such as *L. brevis, L. plantarum,* and *S. cerevisiae* species were grown on agar plates. Fresh cultures were taken out with an inoculation loop and added to the 50 ml nutrient broth. For fermentation with *L. brevis* (BF) the autoclaved media was inoculated with 250µl 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 i.e. 500µl 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 (Gupta et al., 2010).

The fermentation with yeast (YF) was carried out by mixing 20 g of foxtail millet flour (FMF) with 120 ml distilled water in a conical flask of 250 ml capacity and was autoclaved at 121 °C for 30 minutes 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 i.e. 12 (Y12), 24 (Y24), and 36 h (Y36) at 37°C followed by drying in an oven at 50°C. During fermentation with yeast + $(NH_4)_2SO_4$ (used as fermentation activator) (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 (Khetarpaul and Chauhan, 1990). The ammonium sulfate is a rich source of nitrogen and acts as a fermentation activator.

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 foxtail millet and kept in an incubator at different time intervals i.e. 12 (C12), 24 (C24) and 36 h (C36) at 37°C. The foxtail millet was then dried in an oven at 50°C.

Statistical Analysis

Data obtained during the research was analysed using one-way analysis of variance (ANOVA) using SPSS Statistical software. Values in tables are presented as mean ± standard deviation of three replicates and differences at the level of $p \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Physicochemical evaluation of foxtail millet grains was done before and after various fermentation treatments. The results obtained during the study are compiled in tables and are discussed under the following headings.

Nutritional, anti-nutritional, and bioactive components of raw foxtail millet

The nutritional, anti-nutritional, and bioactive components of raw foxtail millet are summarized in Table 1**.** Foxtail millet was found to be a rich source of nutritional components. It contains 7.30% of moisture, 4.20% of crude fat, 7.57% crude fibers, 2.67% of ash content, 12.38% of crude protein, and 65.89% of carbohydrates. It contained about 1.29 mg GAE/g of polyphenolic components. Due to its high polyphenolic content, foxtail millet was found to have high antioxidant activity of 73.57%. The anti-nutrients like tannins and phytic contents were reported as 0.48 mg/g and 591.26 mg/100g respectively. The mineral contents such as Cu, Fe, Zn, and Mn have been reported as 2.30, 2.17, 2.79, and 0.50 mg/100g respectively.

Table 1: Nutritional, anti-nutritional, and bioactive components of raw foxtail millet

Parameters	Raw foxtail millet
Moisture (%)	7.30±0.26
Fat (%)	4.20±0.52
Fiber (%)	7.57±0.40
Ash (%)	2.67±0.29
Protein (%)	12.38±0.59
Carbohydrates (%)	65.89±0.76
Calorific value (Kcal/100g)	364.46±3.81
Phytic acid (mg/100g)	591.26±0.64
TPC (mg GAE/g)	1.29±1.06
Tannin (mg/g)	0.48±0.09
Antioxidant activity (%DPPH inhibition)	73.57±0.92
Cu (mg/100g)	2.30±0.15
Fe (mg/100g)	2.17±0.26
Zn (mg/100g)	2.79±0.22
Mn (mg/100g)	0.50±0.52

Values in the table are presented as mean±SD

Effect of fermentation treatments on nutritional, anti-nutritional, and bioactive components of foxtail millet fermentation with yeast *(Saccharomyces cerevisiae* L.*)*

The effect of different fermentation treatments on foxtail millet is presented in Table 2. It has been observed that the moisture content of raw foxtail millet flour (RFMF) possesses the least moisture content of 7.30% when compared to the YF and YAF treated samples. During YF treatment moisture content got increased from 7.30% (RFMF) to 11.40% (Y36). Similarly during YAF treatment moisture content increased from 7.30% (RFMF) to 11.6% (YA36). Similar results were found by Narayanasamy (2021) who reported that the moisture content of finger millet got increased from 8.96 to 12.82% after fermentation. A significant ($p \leq 0.05$) reduction in fat and fiber content was seen in fermented FMF with fermentation time. Fat content got reduced from 4.20% (RFMF) to 3.13% (Y36) and from 4.20% (RFMF) to 2.65% (YA36) respectively during both YF and YAF treatments. A similar reduction in fat contents was observed in finger millet after the fermentation treatments (Narayanasamy, 2021). Similarly, fiber content got reduced from 7.57% (RFMF) to 5.03% (Y36) and from 7.57% (RFMF) to 3.67% (YA36), respectively during YF and YAF treatment. The decrease in the fiber contents of fermented FMF may probably be caused due to the breakdown of fiber components by the fermenting organisms (Ojokoh and Bello 2014). The protein content increased from 12.38% (RFMF) to 15.24% (Y36) and from 12.38% (RFMF) to 16.45% (YA36) during YF and YAF treatments, respectively. This complies with the results of Cui et al. (2012) who reported about a 35.2% rise in the protein content of fermented maize. The rise in proteins after the fermentation treatment was inferable from a drop-off in carbon proportion in the total mass. Microorganisms employed the carbohydrates as a source of energy and generated CO_2 as a by-product. This resulted in the concentration of nitrogen contents in the fermented slurry, and consequently, the contents of protein in total mass increased.

The ash content of RFMF increased from 2.67% (RFMF) to 3.30% (Y36) during fermentation with yeast. Similarly, during YAF treatment, the ash content gets increased from 2.67% (RFMF) to 4.27% (YA36). From the results, it has been found that the carbohydrate content of raw FMF was 65.89% which is significantly ($p \leq 0.05$) higher than YF and YAF treated samples. During the fermentation treatment, the carbohydrate contents get reduced from 65.89% (RFMF) to 61.90% (Y36). Similarly, during YAF treatment the level of carbohydrate contents get decreased from 65.89% (RFMF) to 61.37% (YA36). The comparable outcomes were found by Chandra et al. (2016) who reported a reduction in carbohydrate content of fermented finger millet. Energy content was reduced from 364.46 Kcal/100g (RFMF) to 349.39 Kcal/100g (Y36) and from 364.46 Kcal/100g (RFMF) to 347.66 Kcal/100g (YA36) during YF and YAF treatments.

The total phenolic contents and antioxidant activity were in the ranges of 1.29 (RFMF) to 1.86 (Y36) mg GAE/g and 73.57 (RFMF) to 78.76 % (Y36) during YF treatment. Similarly, during YAF treatment the total phenols and antioxidant content ranged between 1.29 (RFMF) to 2.06 mg GAE/g (YA36) and 73.57 (RFMF) to 79.68% (YA36), respectively. The highest antioxidant content was seen in the FMF during YAF fermentation treatment. Both phenolic and antioxidant contents increased with an increase in fermentation time. A comparable outcome was found by Cui et al. (2012) who reported a 23.4% increase in phenolic content of fermented maize. The increase in TPC observed in the fermented samples might be because of the liberation of bound phenolic components throughout the fermentation process (Bartolomé and Gómez-Cordovés, 1999).

The effect of fermentation on anti-nutritional components such as phytic acid and tannin content was also studied. The phytic acid got reduced from 591.26 mg/100g (RFMF) to 414.03 mg/100g (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 591.26 mg/100g (RFMF) to 310.80 (YA36). These results conform to the findings of Cui et al. (2012) who reported that the phytic acid content of maize got

reduced from 476 mg/100g to 364 mg/100g after the fermentation. The decrease in phytic acid after fermentation has partly been credited to the activity of phytase present in microflora which hydrolyzed the phytate into inositol and orthophosphate (Ejigui et al., 2005). Similarly the tannin content got reduced from 0.48 mg/g (RFMF) to 0.33 mg/g (Y36) and from 0.48 mg/g (RFMF) to 0.34 mg/g (YA36), respectively during YF and YAF treatments.

There mineral contents such as Cu, Fe, Mn, and Zn contents increased significantly ($p \leq 0.05$) in fermented FMF. The values of Cu content increased significantly ($p \leq 0.05$) by 30% (Y36) and 46% (YA36) and that for Fe content increased by 36.40% (Y36) and 35.02% (YA36), respectively, during YF and YAF treatments. Similarly, the Zn contents increased significantly ($p \leq 0.05$) by 27.95% (Y36) and 41.93% (YA36), and Mn content by 34% (Y36) and 50% (YA36) respectively, during YF and YAF treatments (Figure 1). The increase in minerals during fermentation may be due to the fact that fermentation additionally reduced the phytic acid that binds minerals making them loose and extra available (Lopez et al., 1983).

Table 2: Effect of different fermentations treatments on nutritional, anti-nutritional and bioactive components of foxtail millet

Parameters	Raw Foxtail millet	Fermentation time (h) during fermentation with yeast (*S. cerevisae*)			Fermentation time (h) during fermentation with yeast (*S. cerevisae*) + $(NH_4)2SO4$		
		12hours	24 hours	36 hours	12 hours	24 hours	36 hours
Moisture (%)	7.30±0.26^{d}	9.70±0.20^{c}	10.75±0.22^{b}	11.40±0.36^{a}	10.67±0.32^{b}	11.40±0.36^{a}	11.6±0.26^{a}
Fat (%)	4.20±0.52^{a}	3.87±0.32a^{b}	3.23±0.18^{cd}	3.13±0.11^{d}	3.32±0.02^{bc}	3.20±0.15^{cd}	2.65±0.48^{d}
Fiber (%)	7.57±0.40^{a}	5.72±0.28^{b}	5.37±0.23^{bc}	5.03±0.55^{cd}	5.23±0.20^{bc}	4.57±0.32^{d}	3.67±0.32^{e}
Ash (%)	2.67±0.29^{d}	2.93±0.15^{cd}	3.10±0.10^{c}	3.30±0.35^{bc}	3.33±0.21^{bc}	3.67±0.15^{b}	4.27±0.21^{a}
Protein (%)	12.38±0.59^{d}	14.42±0.49^{c}	14.50±0.18b^{c}	15.24±0.14^{b}	14.52±0.45^{bc}	14.67±0.37^{bc}	16.45±0.33^{a}
Carbohydrates (%)	65.89±0.76^{a}	63.36±0.48^{b}	63.06±0.51b^{c}	61.90±0.53^{cd}	62.93±0.36^{bc}	62.49±0.99^{bcd}	61.37±0.45^{d}
Calorific value (Kcal/100g)	364.46±3.81^{a}	358.99±2.98^{b}	352.20±2.85^{c}	349.39±1.38^{c}	352.63±2.48^{c}	350.30±1.46^{c}	347.66±2.46^{c}
Phytic acid (mg/100g)	591.26±0.64^{a}	431.79±0.46^{b}	415.51±0.56^{bc}	414.03±0.17b^{c}	344.47±0.34^{cd}	338.18±0.27^{d}	310.80±0.18^{d}
TPC (mg GAE/g)	1.29±1.06^{e}	1.58±0.08^{d}	1.67±0.28^{cd}	1.86±0.23^{b}	1.68±0.52^{c}	1.99±0.45^{a}	2.06±0.55^{a}
Tannin (mg/g)	0.48±0.09^{a}	0.42±0.13^{a}	0.40±0.13^{a}	0.33±0.06^{a}	0.36±0.02^{a}	0.34±0.02^{a}	0.34±0.04^{a}
Antioxidant activity (%DPPH inhibition)	73.57±0.92^{c}	77.56±0.47^{b}	77.80±0.77^{b}	78.76±0.68^{b}	77.56±0.47^{ab}	78.38±0.54^{b}	79.68±0.53^{a}
Cu (mg/100g)	2.30±0.15^{b}	2.46±0.28^{b}	2.92±0.32^{a}	2.99±0.23^{a}	3.00±0.30^{a}	3.34±0.31^{a}	3.36±0.22^{a}
Fe (mg/100g)	2.17±0.26^{a}	2.49±0.29^{a}	2.64±0.60^{a}	2.96±0.25^{a}	2.66±0.69^{a}	2.77±0.88^{a}	2.93±0.13^{a}
Zn (mg/100g)	2.79±0.22^{c}	3.12±0.29^{bc}	3.53±0.33^{ab}	3.57±0.38^{ab}	3.15±0.50^{bc}	3.82±0.33^{a}	3.96±0.14^{a}
Mn (mg/100g)	0.50±0.52^{a}	0.61±0.19^{a}	0.62±0.10^{a}	0.67±0.26^{a}	0.67±0.19^{a}	0.70±0.29^{a}	0.75±0.10^{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≤0.05

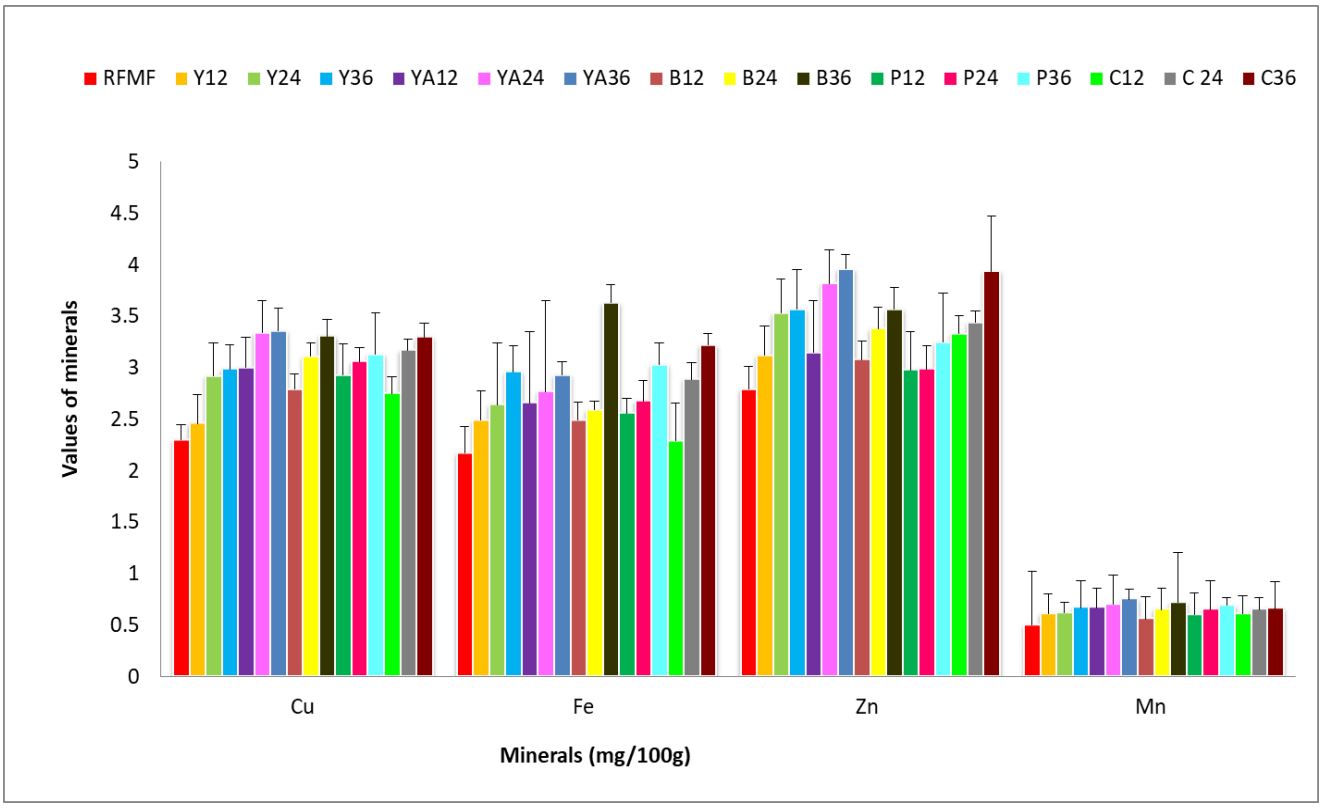

Figure 1. Effect of fermentation treatments on the mineral components of foxtail millet flour (RFMF-Raw foxtail millet flour, Y12-Fermentation with yeast for 12 h, Y24- for 24 h, Y36- for 36 h, YA-12-Fermentation with yeast+$(NH_4)_2SO_4$ for 12 h, YA24- for 24 h, YA36- for 36 h, B12-Fermentation with *L. brevis* for 12 h, B24- for 24 h, B36- for 36 h, P12- Fermentation with *L.Plantarum* for 12 h, P24-for 24, P36- for 36 h, C12-combined treatment for 12 h, C24-for 24 h, C36- for 36 h)

Effect of fermentation with lactic acid bacteria on nutritional, anti-nutritional, and bioactive components of foxtail millet

The influence of lactic acid bacterial fermentation on nutritional, anti-nutritional, and bioactive components of foxtail millet is presented in Table 3. During BF treatment the moisture content increased from 7.30% (RFMF) to 9.45 % (B36). Similarly during PF and CF treatment moisture content increased from 7.30% (RFMF) to 11.33 (P36) and 10.59% (C 36), respectively. Similar results were found by Amadou et al. (2014) who reported that moisture content got increased from 11.38 to 13.31% after fermentation treatment of foxtail millet flour. A significant ($p \leq 0.05$) rise in the ash and protein contents was seen during fermentation. The ash content increased from 2.67% (RFMF) to 3.17% (B36), 3.77% (P36) and 3.43% (C36) and protein contents from 12.38% (RFMF) to 16.85% (B36), 13.77% (P36) and 14.39% (C36), respectively during BF, PF and CF treatments. The increase in protein content is similar to the observations of Amadou et al. (2014) who reported that protein content got increased from 12.02 to 20.51% after fermentation treatment of FMF. This increase in protein content may be because of the degradation of complex protein by the fermenting microorganisms and releasing peptides and amino acids (Pranoto et al., 2013). However, a significant ($p \leq 0.05$) reduction in fat and fiber content of FMF was seen during fermentation, and values for fat content decreased from 4.20 % (RFMF) to 1.27% (B36), 1.23% (P36), and 2.40% (C36), and that for proteins decreased from 7.57% (RFMF) to 5.63% (B36), 4.63% (P36) and 5.47% (C36), respectively, during BF, PF, and CF

treatments. The decline in fiber content can be attributed to the enzymatic breakdown of fiber during fermentation by lactic acid bacteria which utilized the fiber as carbon source (Ojokoh and Bello, 2014). The drift of decrease in crude fiber is reliable with the observations of Sade (2009) in fermented pearl millet flour. Adegbehingbe (2013) observed that the fat content of maize decreased from 2.2 to 1.5% during fermentation and it can be ascribed to biochemical and physiological changes that occurred during fermentation needed energy and part of the lipids contained in the samples was utilized for the production of energy (El-Beltagi et al., 2011).

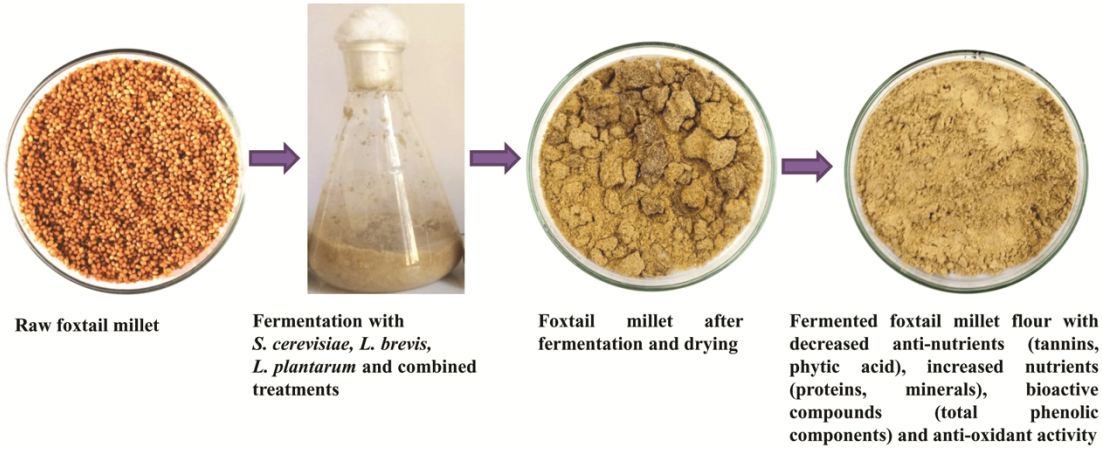

Figure 2. A schematic diagram of fermented foxtail millet flour production

The Carbohydrate Content of the FMF reduced significantly (p≤0.05) from 65.89% (RFMF) to 63.63% (B36), 65.27% (P36), and 63.72% (C36) during BF, PF, and CF treatments respectively. The decrease in carbohydrate content during fermentation of FMF could be due to the fact that the glucose released during fermentation is a favored substrate for microorganisms fermenting the food and could partly give an explanation for the decrease in total carbohydrate after 36 hours of fermentation (Osman, 2011). The calorific value decreased from 364.46 Kcal/100g (RFMF) to 346.17Kcal/100g (B36), 340.39Kcal/100g (P36), and 347.04 Kcal/100g (C36) during BF, PF, and CF treatments, respectively.

The impact of fermentation with lactic acid bacteria and combined treatments on bioactive components of FMF is presented in Table 3. The TPC and antioxidant activity of FMF increased significantly (p≤0.05) from 1.29 mg GAE/g (RFMF) to 1.88 mg GAE/g (B36), 1.79 mg GAE/g (P36) and 1.64 mg GAE/g (C36) and from 73.57% (RFMF) to 79.17% (B36), 77.63% (P36) and 77.87% (C36) during BF, PF and CF treatments, respectively. The increase in total phenolic contents and antioxidant activity may be attributed to the reality that microorganisms wreck down cereal grain matrices causing the release of the bound phytochemicals (Đorđević et al., 2010). Lactobacillus *plantarum* has been formerly pronounced to have β-glucosidase that can cleave glycosidic bonds among phytochemicals and sugars thereby releasing phytochemicals (Kuo et al., 2006).

There was a significant (p≤0.05) decrease in the anti-nutrient components of FMF after different fermentations treatments. The phytic acid content in FMF decreased significantly (p≤0.05) from 591.26mg/100g (RFMF) to 314.50 mg/100g (B36), 416.62 mg/100g (P36), and 460.28 mg/100g (C36) during BF, PF, and CF treatments, respectively and results comply with the findings of Mohamed et al. (2007) in pearl millet grain. This decline in phytic acid can be ascribed to the microbial phytase and endogenous foxtail millet components (Mohamed et al., 2007). Similarly, the tannin content got reduced from 0.48 mg/g

(RFMF) to 0.23 mg/g (B36), 0.29 mg/g (P36) and 0.34 mg/g (C36) during BF, PF and CF treatments, respectively. Similar results were found by El Hag et al. (2002) who reported a decrease in tannin contents during lactic acid fermentation in pearl millet. The decrease in tannin content could be attributed to the binding of tannins with the components present in the endosperm of cotyledon which is usually undetected by a routine method due to their insolubility in the solvent (Emmambux and Taylor, 2003).

The changes in mineral content of FMF subjected to different fermentation treatments are presented in Figure 1. The results indicated a significant ($p \leq 0.05$) increase in mineral contents with an increase in fermentation time. The values for Cu content increased significantly ($p \leq 0.05$) by 43.91% (B36), 36.08% (P36) and 43.47 % (C36) and that for Fe content increased by 67.28% (B36), 39.63% (P36) and 48.38% (C36) during BF, PF and CF treatments, respectively. Similarly, the values for Zn content increased significantly ($p \leq 0.05$) by 27.95% (B36), 16.48% (P36) and 41.21% (C36) and that for Mn content by 44% (B36), 38% (P36) and 32% (C36) during BF, PF and CF treatments respectively. The increase in minerals can be ascribed to the loss of dry matter in the fermentation process due to the microbial degradation of proteins and carbohydrates (Day and Morawicki, 2016).

Table 3: Effect of LAB fermentation and combined treatments on nutritional, anti-nutritional, and bioactive components of foxtail millet

Parameters	Raw Foxtail millet	Fermentation time (h) during fermentation with *L. Brevis*			Fermentation time (h) during fermentation with *L. Plantarum*			Fermentation time (h) during fermentation with *L. brevis* + *S. cerevisiae* (combined effect)		
		12 hours	24 hours	36 hours	12 hours	24 hours	36 hours	12 hours	24 hours	36 hours
Moisture (%)	7.30±0.26^{e}	8.33±0.21^{d}	8.63±0.38^{d}	9.45±0.22^{c}	9.47±0.35^{c}	10.53±0.15^{b}	11.33±0.40^{a}	9.30±0.29^{c}	9.56±0.26^{c}	10.59±0.32^{b}
Fat (%)	4.20±0.52^{a}	1.43±0.02^{c}	1.36±0.33^{c}	1.27±0.21^{c}	2.47±0.25^{b}	1.33±0.54^{c}	1.23±0.33^{c}	2.56±0.31^{b}	2.49±0.29^{b}	2.40±0.47^{b}
Fiber (%)	7.57±0.40^{a}	6.51±0.35^{b}	6.47±0.15^{b}	5.63±0.25^{c}	5.68±0.11^{c}	5.63±0.31^{c}	4.63±0.25^{d}	6.73±0.15^{b}	6.33±0.21^{b}	5.47±0.21^{c}
Ash (%)	2.67±0.29^{c}	2.70±0.20^{c}	3.00±0.44^{bc}	3.17±0.46^{bc}	3.33±0.21^{ab}	3.50±0.10^{ab}	3.77±0.15^{a}	3.17±0.15^{bc}	3.37±0.15^{ab}	3.43±0.40^{ab}
Protein (%)	12.38±0.59^{e}	15.33±0.36^{b}	16.67±0.25^{a}	16.85±0.16^{a}	13.43±0.42^{d}	13.53±0.21^{d}	13.77±0.15^{d}	13.29±0.16^{d}	13.50±0.32^{d}	14.39±0.24^{c}
Carbohydrates (%)	65.89±0.76^{a}	65.69±0.45^{a}	63.87±0.74^{bc}	63.63±0.82^{c}	65.62±0.45^{a}	65.47±0.90^{a}	65.27±0.63^{a}	64.96±0.16^{ab}	64.75±0.47^{abc}	63.72±1.00^{bc}
Calorific value (Kcal/100g)	364.46±3.81^{a}	350.26±3.00^{bc}	347.33±0.96^{c}	346.17±1.50^{c}	351.80±1.26^{b}	341.21±2.20^{d}	340.39±1.83^{d}	349.27±3.21^{bc}	348.58±1.77^{bc}	347.04±0.62^{c}
Phytic acid (mg/100g)	591.26±0.64^{a}	325.23±0.39^{cd}	319.31±0.59^{d}	314.50±0.75^{d}	444.74±0.59^{b}	430.68±0.56^{bc}	416.62±0.10^{bcd}	499.50±0.63^{ab}	489.88±0.87^{ab}	460.28±0.56^{b}
TPC (mg GAE/g)	1.29±1.06^{f}	1.66±0.35^{b}	1.81±0.57^{a}	1.88±0.59^{a}	1.47±0.18^{de}	1.52±0.49^{c}	1.79±0.26^{a}	1.34±0.74^{ef}	1.41±0.65^{cd}	1.64±0.26^{b}
Tannin (mg/g)	0.48±0.09^{a}	0.28±0.14^{a}	0.26±0.16^{a}	0.23±0.17^{a}	0.37±0.24^{a}	0.36±0.18^{a}	0.29±0.17^{a}	0.41±0.13^{a}	0.36±0.15^{a}	0.34±0.15^{a}
Antioxidant activity (% DPPH inhibition)	73.57±0.92^{e}	78.24±0.53^{abc}	78.89±0.37^{ab}	79.17±1.00^{a}	75.24±0.59^{d}	75.58±0.59^{d}	77.63±0.59^{c}	73.63±0.21^{e}	77.73±0.51c	77.87±0.47^{bc}
Cu (mg/100g)	2.30±0.15^{d}	2.79±0.15^{bc}	3.11±0.13^{abc}	3.31±0.16^{a}	2.93±0.30^{abc}	3.06±0.14^{abc}	3.13±0.40^{abc}	2.75±0.16^{c}	3.17±0.11^{ab}	3.30±0.13^{a}
Fe (mg/100g)	2.17±0.26^{f}	2.49±0.18^{def}	2.59±0.09^{cde}	3.63±0.18^{a}	2.56±0.14^{cde}	2.68±0.20^{bcd}	3.03±0.21^{ab}	2.29±0.37^{ef}	2.89±0.16^{abc}	3.22±0.11^{a}
Zn (mg/100g)	2.79±0.22^{c}	3.08±0.18^{bc}	3.38±0.21^{ab}	3.57±0.21^{ab}	2.98±0.37^{bc}	2.99±0.22^{bc}	3.25±0.48^{bc}	3.33±0.18^{bc}	3.44±0.11^{ab}	3.94±0.53^{a}
Mn (mg/100g)	0.50±0.52^{a}	0.56±0.22^{a}	0.65±0.21^{a}	0.72±0.49^{a}	0.60±0.21^{a}	0.65±0.28^{a}	0.69±0.08^{a}	0.61±0.18^{a}	0.65±0.12^{a}	0.66±0.26^{a}

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

CONCLUSIONS

The present study aimed to determine the effect of various fermentation treatments on nutritional composition, anti-nutritional, and bioactive components of foxtail millet. The fermentation treatments were found to decrease the anti-nutritional components like phytic acid and tannin components in foxtail millet flour. A significant increase in the protein content of fermented samples of foxtail millet was observed and it could be of great importance in alleviating protein-energy malnutrition by incorporating foxtail millet in fermented food samples. Total phenolic components and antioxidant activity were found to get increased significantly during fermentation treatments. Mineral contents were also found to get increased significantly ($P \leq 0.05$) after fermentation treatments. Processing by fermentation is found to enhance its nutritive worth. The outcomes suggest that fermented foxtail millet flour can prove to be a valuable food supplement, especially in the eating routine of the metropolitan population consuming milled polished rice. The fermented millet could be consumed after cooking as porridge with salt/sugar and additionally buttermilk. Alternatively, it could be blended with other cereals and steamed items such as 'idli', 'dhokla', or 'appam. Thus, the fermentation treatment can upgrade the dietary benefit and effectiveness of this underutilized grain and it tends to be efficiently utilized for the development of new functional food products.

ACKNOWLEDGMENT

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.

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