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Analysis of the effects of natural and pure culture fermentation for the qualitative enhancement of pearl millet flour

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ABSTRACT

Its high nutritive quality and ability to survive in harsh conditions makes pearl millet a suitable crop for arid and semi-arid regions, but anti-nutrient factors reduce the bioavailability and digestibility of its nutrients. Fermentation reduces anti-nutrients and hence increases protein digestibility and mineral bioavailability. Thus, the present work examines the effects of fermentation on the nutritive, physio-chemical and functional properties and anti-nutrient composition of pearl millet flour. Natural fermentation at 20°C, 25°C and 30°C and pure culture fermentations with *Saccharomyces cerevisiae, Saccharomyces diastaticus, Lactobacillus fermentum* and *Lactobacillus brevis* were performed. Analysis revealed an increase in moisture and fat content and a decrease in protein content following all types of fermentation. Although the variation in protein levels in different fermentations was not significant, ranging from 10.29% to 9.83%, the effect on thiamine content was significant as it decreased with an increase in temperature in the range 20–30°C in natural fermentation but was increased in pure culture yeast fermentations as compared with bacterial fermentations at their favourable temperatures, respectively. Total soluble sugar content decreased in pure culture fermentation but increased in natural fermentation. Fermentation decreased pH, thereby increasing titratable acidity. Oil and water absorption capacities were increased, while least gelation concentration was decreased in all types of fermentations. Also, anti-nutritional factors like tannins and phenolic compounds were significantly decreased in all fermentations. We conclude that fermentation is an efficient process to improve the quality of pearl millet flour.

Keywords
Pearl millet
Natural fermentation
Pure culture fermentation
Anti-nutritional factors
Phenolic compounds

Introduction

Pearl millet (*Pennisetum typhoideum*) is grown in tropical and subtropical regions of the world and is a staple crop of Asian and African countries [1]. It can withstand harsh conditions such as low rainfall, high temperatures and poor soil and can thus survive in areas where other cereals such as maize, sorghum and wheat will not. It is drought resistant and is commonly eaten by those living below the poverty line in arid countries [2]. It has a high nutritional value and is mainly consumed in the form of unleavened pancakes made from flour. Its carbohydrate content of 67.5% is less than that of rice, sorghum and wheat but higher than that of maize [2, 3]. The carbohydrates consist of starch and some soluble sugars. Pearl millet also has a crude fibre content of 2.6–4%, ash content of 1.6–2.4%, fat content of 2.7–7.1%, crude protein content of 8.5–15.1% and phytate content of 354–796 mg/g. In addition, it has a high mineral content consisting of calcium (10–80 mg/g), magnesium (180–270 mg/g), phosphorous (450–990 mg/g), potassium (70–110 μg/g), zinc (53–70 μg/g), sodium (4–13 μg/g), copper (10–18 μg/g), manganese (18–23 μg/g) and iron (70–80 μg/g) [2–4]. Pearl millet also has health benefits as it increases haemoglobin to counter anaemia, its high fibre content helps treat constipation, the low glycaemic index (55) protects against diabetes, its anticancer property inhibits tumour progression, and it also has anti-allergic activity and is beneficial for autoimmune disorders like coeliac disease [5]. Although pearl millet has a high nutritional composition and contains most of the essential nutrients, the presence of some anti-nutritional factors reduces the bioavailability of these nutrients. For instance, phytate reduces the bioavailability of divalent ions, and hence inhibits several amylolytic and proteolytic

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enzymes [6]. The polyphenol content is quite high and negatively effects carbohydrate and protein digestibility and the bioavailability of minerals [7]. And the presence of diversely distributed proteinaceous α-amylase inhibitors reduces the utilization of the starch present in pearl millet [8]. Many processes such as soaking, decortication, germination and fermentation have been used to combat these drawbacks and to enhance the nutritional bioavailability of pearl millet, with fermentation known to be the most effective.

The fermentation of pearl millet grains is an efficient method to enhance the digestibility of proteins and starch, and to increase mineral bioavailability [8]. Fermentation reduces antinutrients such as polyphenols and phytic acid [6, 8], and as the pH of the food decreases with fermentation, the growth of undesirable microbes is inhibited and the shelf-life of food is increased [9]. Fermentation also affects nutrient levels [7]. Studies have reported that natural fermentation with a mixed culture for 72 hours at different temperatures reduced phytic acid levels in pearl millet flour, with phytic acid content completely eliminated at 30°C [10, 11]. Noticeable starch and protein digestibility improvements were also reported after natural fermentation at temperatures up to 30°C [8]. Arora *et al* reported a reduction in pH following both natural and pure culture fermentation for 72 hours, thus increasing titratable acidity [12]. The protein content of pearl millet flour was found to either decrease or remain unchanged after fermentation. Another study reported that fat content was increased with natural fermentation but decreased with pure culture fermentation. In the same study, thiamine content was reported to increase at temperatures below 30°C but to decrease at 30°C with natural fermentation [13]. Fermentation by yeast increased the thiamine content two to threefold, while *Lactobacillus* fermentation decreased thymine content [14]. Therefore, the present work examined the effect of natural and pure culture fermentation on the functional and nutritional properties of pearl millet flour in order to determine the best conditions and culture to improve the low bioavailability of nutrients and the digestibility of starch and proteins.

Materials and methods

Raw materials

Pearl millet was bought from a local market in Kanpur in a single lot. Broken grain, dust and foreign material were removed. Microorganisms (*Saccharomyces cerevisiae*, *Saccharomyces diastaticus*, *Lactobacillus fermentum* and *Lactobacillus brevis*) for pure culture fermentation were bought from the National Chemical Laboratory in Pune (India).

Flour preparation

The flour was prepared using three methods.

First method

Foreign material, dust and broken grains were removed from 500 g of millet grains which were then washed with tap water followed by thorough washing in distilled water. The grains were then dried in a hot air oven at 60°C, ground in a Polar MG600 electric grinder and sieved using a sieve size of 40 mm (Fig. 1). This flour was used as control.

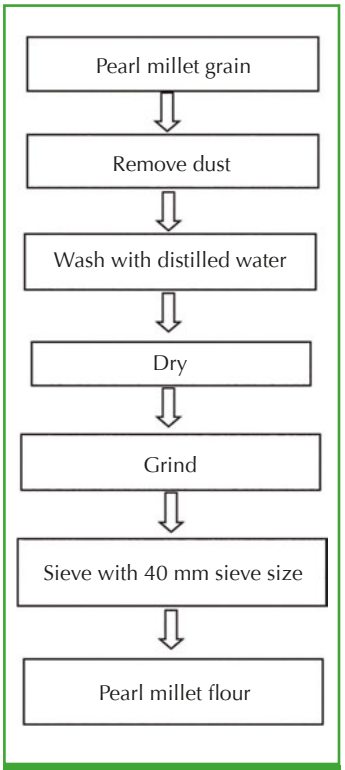

Figure 1 - Method for producing raw millet flour

Second method

Three 200 g samples of millet grains were thoroughly cleaned and then steeped for 72 hours in distilled water at 20°C, 25°C and 30°C. After soaking, the grains were rinsed with water and dried in a hot air oven at 45–50°C for 10 hours. The grains were then ground in a grinder (Fig. 2). This flour samples were naturally fermented millet flour samples.

Third method

Millet grains (100 g) were weighed, cleaned to remove unwanted particles and ground to produce flour. The flour was next mixed with 900 ml of distilled water and autoclaved for 15 min at 15 psig. The flour was then divided into four portions which were fermented with *S. cerevisiae*, *S. diastaticus*, *L. fermentum* or *L. brevis* for 72 hours (Fig. 3).

Proximate analysis of pearl millet flour

The nutritional, functional and physiochemical properties of pearl millet flour samples obtained using the three methods described above were analysed according to AOAC methods [15, 16].

Analysis of moisture content

A 5 g sample was placed in a pre-weighed petri dish which was placed in an oven at 105°C for 2 hours. The dish was then put in a desiccator to reduce it to room temperature and

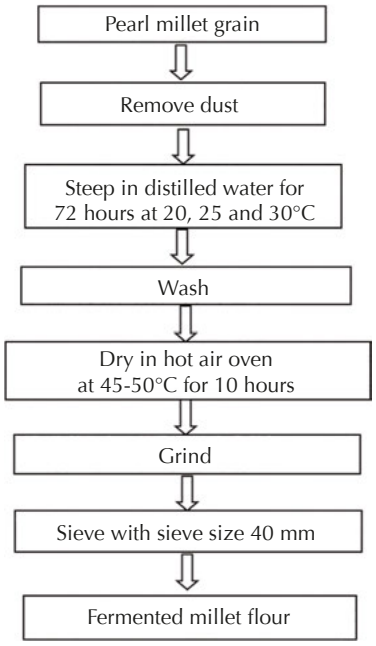

Figure 2 - Method for producing naturally fermented pearl millet flour

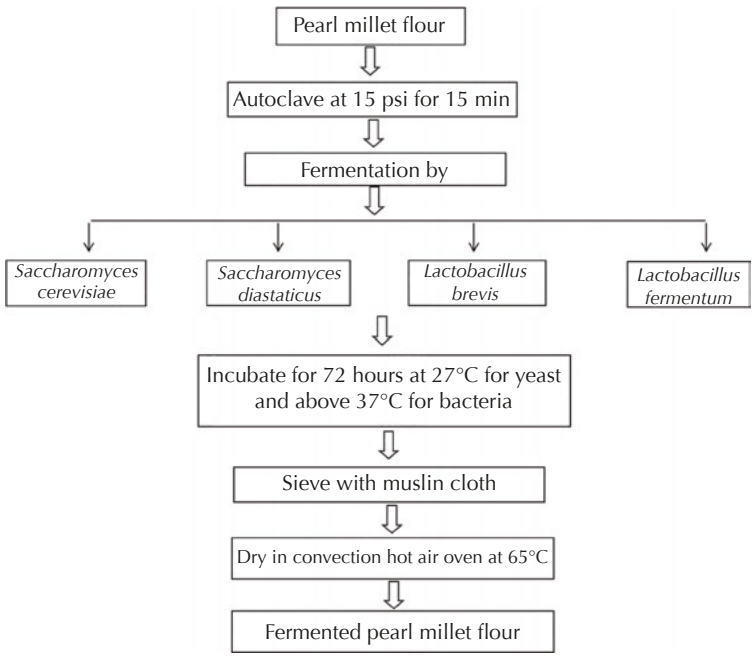

Figure 3 - Method for pure culture fermentations

weighed until a constant weight was reached. The percentage of moisture was estimated using the following formula [15]:

$$\% \text{ Moisture} = \frac{(\text{Weight of sample taken - weight of dried sample}) \times 100}{(\text{Weight of sample})}$$

Analysis of crude fat

Fat content was determined using the Soxhlet extraction method. A 20 g sample was placed in a thimble which was placed in the reflux flask of the Soxhlet extractor system which had an attached condenser and oil extraction flask containing 200 ml petroleum ether. The ether was heated to boiling point. The ether vapour condensed in the condenser and was collected in the reflux flask so that the sample was completely immersed in the ether, allowing extraction to occur. When the reflux flask was full, the overflowing ether containing the oil was siphoned back into the extraction flask containing boiling solvent. The sample was removed at the end of the process and the fat or oil extract present in the flux was dried for 30 min at 60°C in an oven and weighed [15]. The percentage of fat was calculated using the following formula:

$$\% \text{ Fat} = \frac{(W1\text{-}W2) \times 100}{S1}$$

where $W1$ is the weight of the round bottom extraction flask, $W2$ is the weight of the round bottom extraction flask containing fat, and $S1$ is the weight of the sample.

Ash content

A sample of dried flour was placed in a pre-weighed silica crucible which was heated for 3 hours at 600°C in a muffle furnace. The crucible was next placed in a desiccator to allow the temperature to return to normal and weighed until a constant weight was achieved. The ash content was then estimated using the following formula [16]:

$$\% \text{ Ash content} = \frac{\text{Weight of ash} \times 100}{\text{Weight of sample}}$$

Crude protein

The percentage of crude protein was estimated using the micro Kjeldahl method; a 6.25 conversion factor was used to convert nitrogen to crude protein. A 1 g sample was digested in a Kjeldahl flask with 20 ml of concentrated H_2SO_4, which was then cooled and transferred to a 250 ml volumetric flask. The volume was made up to 250 ml with distilled water and thoroughly mixed. Then 5 ml of this mixture was poured into a distillation flask which was connected to a receiving flask containing 10 ml of 4% boric acid. NaOH (40%) was then added to the distillation flask, and ammonia liberated from the reaction of the NaOH with the digested mixture in the digestion flask was collected in the receiving flask containing the boric acid to form ammonium borate. The nitrogen content of the sample was estimated by titrating the ammonium borate with 0.1N H_2SO_4 [15, 16]. A blank sample used for comparison was also subjected to the same

process. The following formula was used for estimating the percentage of nitrogen:

$$\% N = \frac{x \text{ mol}}{1000 \text{ cm3}} \times \frac{Vs - Vb \text{ cm3}}{\text{mg}} \times \frac{14 \text{ g}}{\text{mol}} \times 100$$

where Vs and Vb are the titration volume of the sample and the blank, respectively.

The percentage of protein was calculated as:

$$\% \text{ Protein} = \% N \times 6.25$$

Carbohydrate content

The total carbohydrate content was extracted by refluxing with 80% ethanol. Colorimetry was used to determine total soluble sugars [17], reducing sugar content was estimated using the modified method of Somogyi [18], and non-reducing sugar content was estimated by taking the difference between the total and reducing sugars [18].

Physiochemical properties of pearl millet flour

Colour

The colour of the sample was measured using a HunterLab D25 optical sensor colorimeter (Hunter Associates, Reston, VA, USA). A glass cell containing a flour sample was held over the light source which was covered with white paper, and the L*, a* and b* values were noted [19]. The heu angle ($\tan^{-1}(a^*/b^*)$) and colour difference Δ were also calculated.

Bulk density

A 10 ml graduated measuring cylinder was filled with flour and tapped several times until the sample level touched the 10 ml mark. Bulk density was calculated as the weight of sample per unit volume of sample [20].

pH and titratable acidity

The pH of the sample was measured using the AOAC method. Titratable acidity was estimated by performing titration with 0.1N NaOH and phenolphthalein indicator. The acidity of the flour was estimated as lactic acid in g/100 g [20].

Functional properties of pearl millet flour

Water absorption capacity

The centrifugation method was used to estimate the water absorption capacity (WAC) of the flour. A 3 g sample of flour was dispersed in 25 ml distilled water and transferred to a pre-weighed centrifuge tube. The dispersion was stirred, held for 30 min and then centrifuged at 3,000 rpm for 25 min. The supernatant was decanted, excess moisture from the centrifuge tube was removed, and the sample was weighed again. The WAC was expressed on a dry matter basis in grams of sample by the following formula [15]:

$$\text{WAC (g/g)} = \frac{\text{Weight of centrifuge tube after drying - (centrifuge tube weight+weight of sample)}}{\text{Sample weight}}$$

Oil absorption capacity

A 0.5 g sample was mixed with 6 ml corn oil in a pre-weighed centrifuge tube. The dispersed sample was stirred occasionally, held for 30 min and then subjected to centrifugation at 3,000 rpm for 25 min. The oil as supernatant was pipetted out and the tube was kept inverted for 25 min to drain out any remaining oil, and then reweighed [21]. The oil absorption capacity (OAC) was expressed in grams of sample on a dry matter basis and calculated using the following formula:

$$\text{OAC(g/g)} = \frac{\text{Weight of tube after draining oil - (weight of tube + sample weight)}}{\text{Sample weight}}$$

Least gelation concentration

Suspensions were made up of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 g of flour in 100 ml of distilled water. Equal amounts of these suspensions were added to 5 ml of distilled water in different test tubes which were heated in a boiling water bath for 1 hour, followed by rapid cooling under running water. The test tubes were then cooled for 2 hours at 4°C. The least gelation concentration was determined as the concentration at which there was no slipping or falling of the sample when the test tube was kept in the inverted position [20].

Estimation of the anti-nutrient factors in pearl millet flour

Estimation of tannin content

Tannin content was measured using a Jenway model 6305 spectrophotometer and applying the vanillin–HCl method at 500 nm. A standard curve was plotted for the tannin content expressed as catechin equivalent [22].

Estimation of polyphenols

Polyphenol content was estimated using the Prussian blue assay. About 60 mg of flour and 3 ml of methanol for extraction were placed in a 50 ml conical flask. The mixture was then poured on to a filter paper. The flask was quickly rinsed with 3 ml of additional methanol which was also poured on to the filter paper. The filtrate was then diluted with distilled water to 50 ml and mixed with 0.1 M 3 ml $FeCl_3$ in 0.1N HCl for 3 min followed by timed addition of 3 ml 0.008 M KFe(CN). This mixture was left to stand for

10 min and absorption was read at 720 nm with a spectrophotometer [23].

Results

In the present study we examined the effects of fermentation on pearl millet flour. Analysis of the total moisture, protein, ash, fat and thiamine content of pearl millet flour was conducted for raw flour samples, samples naturally fermented for 72 hours at 20°C, 25°C and 30°C, and samples fermented with pure cultures of *S. cerevisiae, S. diastaticus, L. fermentum* and *L. brevis* (Tables 1 and 2). Fermentation increased moisture content but slightly reduced protein content. The different temperatures for natural fermentation did not have a significant effect on protein content. However, with pure culture fermentation, protein content decreased or remained unchanged in all except the *S. cerevisiae* culture which showed an increase. With natural fermentation, crude fat increased but the effect of temperature was insignificant. Pure culture fermentations showed a slight decrease in fat content compared with natural fermentation. *L. brevis* fermentation had the lowest and *S. diastaticus* the highest fat content of the pure culture fermentations. Thiamine content at 20°C and 25°C was higher than that at 30°C. In pure culture fermentations, *S. cerevisiae* gave the highest and *Lactobacillus* strains the lowest thiamine content.

The carbohydrate content of raw pearl millet flour, naturally fermented flour and pure culture fermented flour was measured (Table 3). Raw flour contained the least total soluble sugars, followed by pure culture fermentations and then natural fermentation which had the highest levels. Of the pure culture fermentations, *S. diastaticus* gave the highest soluble sugar concentration, while *L. fermentum* gave the lowest. The amount of non-reducing sugar was higher in pure culture fermentations than in natural fermentation and was highest in *S. cerevisiae*. The lowest increase was observed in *L. brevis*.

Raw, naturally fermented and pure culture fermented pearl millet flours were subject to physiochemical analysis. The variation in pH was significant, with pH decreasing as fermentation time increased, thereby increasing titratable acidity in all types of fermentation (Tables 4 and 5). The greatest decrease in pH was seen with *L. fermentum* and the lowest with *S. cerevisiae* (Table 5). The effect of fermentation on colour and bulk density was also examined. Fermentation enhanced colour lightness (Table 6) but decreased bulk density, with pure culture fermentation showing a greater decrease than natural fermentation (Table 6).

Fermentation	Moisture	Protein	Fat	Ash	Thiamine
Raw millet flour	13.39	12.03	5.01	0.91	425
Fermentation at 20°C	13.46	10.01	6.89	2.01	352
Fermentation at 25°C	13.49	9.83	7.05	2.13	347
Fermentation at 30°C	13.53	9.72	6.77	2.14	167

Table 1 - Effect of natural fermentation on total protein, fat, ash (g/100 g) and thiamine content (µg/100 g) of pearl millet flour (on a dry matter basis)

Fermentation	Moisture	Protein	Fat	Ash	Thiamine
Saccharomyces diastaticus	14.06	10.02	6.13	2.22	600
Saccharomyces cerevisiae	13.82	10.39	5.39	2.24	872
Lactobacillus brevis	14.01	9.81	5.18	2.23	68
Lactobacillus fermentum	13.76	9.73	5.72	2.22	176

Table 2 - Effect of pure culture fermentation on total protein, fat, ash (g/100 g) and thiamine content (µg/100 g) of pearl millet flour (on a dry matter basis)

Treatment	Total soluble sugar	Reducing sugar	Non-reducing sugar
Raw millet flour	2.74±0.65	1.35±0.04	1.41±0.06
Naturally fermented flour	5.53±0.07	2.23±0.03	3.31±0.07
Saccharomyces diastaticus	4.01±0.12	3.03±0.02	1.06±0.12
Saccharomyces cerevisiae	3.04±0.03	1.65±0.02	2.04±0.05
Lactobacillus brevis	2.11±0.03	1.21±0.01	0.97±0.04
Lactobacillus fermentum	2.58±0.02	1.78±0.03	0.91±0.02

Table 3 - Effect of natural and pure culture fermentations on content of total soluble sugars, reducing sugars and non-reducing sugars of pearl millet flour (g/100 g) (on a dry matter basis)

Treatment	pH	Titratable acidity
Natural fermentation		
20°C	3.39±0.01	1.91±0.03
25°C	3.07±0.01	3.85±0.09
30°C	2.61±0.01	5.29±0.11
Control		
Raw millet flour	5.91±0.01	1.51±0.03

Table 4 - Effect of natural fermentation for 72 hours on pH and titratable acidity (g lactic acid per 100 ml) of autoclaved pearl millet flour

Fermentation	pH	Titratable acidity
Saccharomyces diastaticus	5.03	1.12
Saccharomyces cerevisiae	6.12	0.93
Lactobacillus brevis	4.73	1.51
Lactobacillus fermentum	4.05	2.05

Table 5 - Change in pH and titratable acidity (g lactic acid per 100 ml) during pure culture fermentation at 30°C

Treatment	L*	a*	b*	ΔE	BD	Heu angle tan-1(a*/b*)
Raw millet flour	82.06±0.01	1.06±0.01	16.03±0.02	14.25±0.00	0.61	3.783
Naturally fermented flour	81.94±0.01	1.06±0.02	16.06±0.01	14.27±0.01	0.56	3.776
Saccharomyces diastaticus	83.21±0.021	1.08±0.01	16.08±0.01	15.21±0.01	0.47	3.842
Saccharomyces cerevisiae	83.05±0.002	1.07±0.02	17.00±0.01	16.06±0.01	0.46	3.601
Lactobacillus brevis	83.01±0.21	1.08±0.01	17.02±0.02	16.07±0.02	0.46	3.630
Lactobacillus fermentum	83.03±0.03	1.08±0.01	17.03±0.01	16.06±0.01	0.47	3.628

Table 6 - Effect of natural fermentation and pure culture fermentation on colour and bulk density (BD)

The different fermentations had different effects on WAC, OAC and least gelation concentration (Table 7). There was an increase in WAC and OAC, but the least gelation concentration was decreased compared with raw flour. *S. diastaticus* gave highest increase in WAC and OAC, with *Lactobacillus* strains giving the lowest. In pure culture fermentations, the least gelation concentration decreased most with *S. cerevisiae* and least with *L. fermentum*.

Changes in anti-nutrient factor levels due to the different fermentation processes are summarized in Table 8. Fermentation reduced tannin and polyphenol content. The reduction in tannins was highest with *S. diastaticus*, while the reduction in polyphenol content was highest with *S. cerevisiae*.

Natural fermentation showed the same decrease in polyphenol content as pure culture fermentation. These findings indicate that fermentation is effective for improving the nutritive and storage quality of pearl millet flour.

Sample	Oil absorbing capacity (OAC %)	Water absorbing capacity (WAC %)	Least gelation concentration (LGC %)
Raw millet flour	161±0.06	235±0.06	12
Naturally fermented flour	175.9±0.06	277±0.06	10
Saccharomyces diastaticus	177.1±0.07	273.9±0.07	10
Saccharomyces cerevisiae	177±0.07	273.6±0.07	6
Lactobacillus brevis	176.9±0.06	273.4±0.07	6
Lactobacillus fermentum	176.7±0.07	273.5±0.06	8

Table 7 - Effect of natural and pure culture fermentation on the functional properties of pearl millet flour

Sample	Tannin[a]	Total phenol[b]
Raw millet flour	0.49	0.33
Naturally fermented flour	0.29	0.28
Saccharomyces diastaticus	0.23	0.27
Saccharomyces cerevisiae	0.27	0.24
Lactobacillus brevis	0.25	0.29
Lactobacillus fermentum	0.24	0.30

[a]Tannin acid equivalents per 100 g
[b]Total phenol equivalents per 100 g

Table 8 - Anti-nutritional factors in raw and fermented millet flour

Discussion

The high nutritional value of pearl millet and its ability to withstand adverse growing conditions makes it an important crop for populations living below the poverty line in semi-arid and arid regions of the world. The many health benefits associated with pearl millet also make it valuable in developing and developed countries [1, 2, 5]. Pearl millet is most commonly eaten as flour, but the presence of some anti-nutrients reduces the bioavailability of minerals and the digestibility of proteins and starch [4]. However, fermentation can enhance nutrient bioavailability and digestibility, and decrease the pH of the flour, thereby increasing its shelf-life by reducing microbial growth [24, 25]. This study examined the effects of fermentation on various properties of pearl millet flour. The analysis was conducted on raw flour samples, samples naturally fermented for 72 hours at 20°C, 25°C and 30°C, and samples fermented with pure cultures of *S. cerevisiae, S. diastaticus, L. fermentum* and *L. brevis*.

We found significant changes in the nutritional composition of pearl millet flour following fermentation. The moisture content increased due to fermentation but the protein content was decreased compared with raw millet flour. There was no or very little effect of fermentation temperature on protein content. However, although the protein content decreased or remained unchanged in most pure culture fermentations, a significant increase was noted with *S. cerevisiae*. Similar observations were reported by Abdalla *et al* who observed no or insignificant changes in the protein content of pearl millet after fermentation for 14 hours [11]. Minimum changes in protein content were also reported by other researchers [24, 26]. Studies on green gram and Bengal gram products showed a 6–8% decrease in protein after fermentation, with a 4–6% decrease in protein in pearl millet and ragi [24, 27]. In contrast, some authors reported a significant increase in pearl millet protein content after fermentation [28], while El Hag *et al* reported a decrease in pearl millet protein content after fermentation [4].

The effects of fermentation on crude fat content were also studied. The results showed that fat content increased following natural fermentation but that temperature did not have a significant effect. However, there was either a reduction or no change in the fat content of pearl millet flour following pure culture fermentation. The *L. brevis* culture showed the lowest and *S. diastaticus* the highest fat content. Previous reports have demonstrated that some fat-producing yeast strains participate in natural fermentation, thus increasing the amount of fat [29].

The ash content did not change significantly under the different fermentation conditions, as previously shown for sorghum and green gram blends [30, 31]. However, the effect of fermentation on thiamine content was highly significant: natural fermentation at 20°C and 25°C increased the thiamine content but natural fermentation at 30°C halved it. In pure cultures, the thiamine content was highest with *S. cerevisiae* and lowest with the *Lactobacillus* strains. Some researchers reported that thiamine content in curd is increased due to presence of yeast as *Lactobacillus* actually decreases the thiamine content [32].

Total soluble sugars increased with natural fermentation, but less so with pure culture fermentation, with *S. diastaticus* showing the greatest increase in soluble sugar content and *L. fermentum* the least. Compared with natural and the other pure culture fermentations, reducing sugar content was significantly increased with *S. diastaticus*, while non-reducing sugar content increased most with *S. cerevisiae*. The smallest increase in sugar content was found with *L. brevis*. A similar finding of increased sugar content was reported by Osman when pearl millet was fermented during lohoh preparation [6] and by Chavan and Kadam for wheat, rice, oats and sorghum [3].

The effect of fermentation on the chemical properties of pearl millet flour was investigated by studying changes in pH and titratable acidity. As the natural fermentation time increased, the pH decreased significantly due to the formation of organic acids in the fermentation mixture, thus increasing titratable acidity. There was also a significant decrease in pH in pure culture fermentations, resulting in increased titratable acidity. The greatest decrease in pH was seen with *L. fermentum* followed by *L. brevis, S. diastaticus* and *S. cerevisiae*. Similar findings of decreased pH and increased titratable acidity were reported by Nanson and Fields and by Venkatasubbaiah *et al* [10, 33]. Fermentation enhanced the colour lightness and decreased the bulk density of pearl millet flour. A greater decrease in bulk density was seen in pure culture fermentations than in natural fermentations. Akinola *et al* reported a similar decrease in bulk density and increase in the lightness of pearl millet flour [34].

OAC, WAC and least gelation concentration were also examined to determine the effects of fermentation on the functional properties of pearl millet flour. We found a significant increase in the OAC and WAC and a moderate decrease in least gelation concentration compared with raw millet flour. The highest increase in WAC and OAC was observed with *S. diastaticus* and the lowest with the *Lactobacillus* strains. Least gelation concentration decreased due to the effect of fermentation; the greatest decrease was observed with *S. cerevisiae* and the least with *L. fermentum*. Kaushal *et al* reported similar findings for rice, taro and pigeon pea flours [20].

The effect of fermentation on anti-nutritional factors was significant. We analysed the effect of fermentation on the tannin and total phenol content of pearl millet flour. The significant reductions in tannins and polyphenol content observed increase the digestibility of proteins and the bioavailability of minerals in pearl millet flour. The greatest decrease in tannins was found with *S. diastaticus* and the least with *S. cerevisiae* and *L. fermentum*. The tannin content of natural fermentations was higher than that of the pure culture fermentations. The greatest reduction in polyphenol concentration was seen with *S. cerevisiae* and the least with *L. fermentum*. The polyphenol content decrease was similar for natural and pure culture fermentations. Similar findings in pearl millet flour were reported by Sharma and Kapoor using different processing techniques and types of fermentation [35]. A decrease in anti-nutrients due to fermentation was also reported by Osman [6]. Sokrab *et al* reported a reduction in anti-nutrients in two corn varieties following fermentation [36].

The above findings demonstrate that fermentation can help to improve the nutritional composition, digestibility and storage of pearl millet flour. This study is an effort to better understand the effect of fermentation on various parameters of pearl millet flour quality.

Conclusion

Pearl millet is a staple crop of Asian and African countries: it is very nutritious and cheap to cultivate and can tolerate adverse growing conditions. The presence of anti-nutrients in pearl millet reduces the bioavailability and digestibility of its nutrients, but this problem can be reduced by fermentation. In the present work, we studied the effect of fermentation on various properties of pearl millet flour and found that fer-

mentation enhances its nutritional quality, the bioavailability of minerals, the digestibility of proteins, and storage duration. Natural and pure culture fermentations affect the various properties differently. This study was an effort to study the effects of different culture fermentations which might be used for large-scale application in the future.

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