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Effect of fermentation on protein, fat, minerals and thiamine content of pearl millet

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Abstract. Natural as well as single, mixed and sequential pure culture (*S. diastaticus, S. cerevisiae, L. brevis* and *L. fermentum*) fermentations of pearl millet flour for 72 h lowered pH and raised titratable acidity. The fermentation either decreased or did not change the protein content of pearl millet flour. Natural fermentation increased whereas pure culture fermentation decreased the fat content. Ash content did not change. Natural fermentation at 20 °C and 25 °C increased whereas at 30 °C it decreased the thiamine content of the pearl millet flour. Yeast fermentation raised the level of thiamine two- to three-fold, while lactobacilli fermentation lowered it significantly.

Introduction

Pearl millet (*Pennisetum typhoideum*) is widely grown and consumed in Asian and African countries. Like other cereals, the quality as well as digestibility of pearl millet protein is low [7, 18, 27]. Carbohydrates of pearl millet consist of starch and smaller amounts of soluble sugars and its starch digestibility is relatively low [28]. Pearl millet contains only marginal amounts of essential vitamins.

Pearl millet has a relatively better mineral profile but the availability of these minerals to human system is low [19]. Phytic acid present in considerable amounts in the pearl millet grains [7, 8] may be partly responsible for the low digestibility of starch [10, 32], protein [10, 16, 27] and low bioavailability of minerals [21, 29].

Fermentation of food grains is known to be an effective method of improving the starch and protein digestibility [6, 10] and bioavailability of minerals [9, 19]. Fermentation also brings down the level of antinutrients like phytic acid and polyphenols [11, 15, 29]. Fermented foods having acidic pH are microbiologically safe and can be stored for a long period.

Besides improving the digestibility, bioavailability of minerals and reducing the level of antinutrients, fermentation may also change the level of

nutrients in the food grains [17, 30]. This paper reports the effect of various types of fermentations on the contents of protein, fat, mineral matter and thiamine of pearl millet.

Materials and methods

Pearl millet grains, used for fermentation, were procured from the local market in a single lot. They were cleaned of broken seeds, dust and other foreign material. The grains were coarsely ground using 1.5 mm sieve size on the day of fermentation by an electric grinder.

Fermentation

The coarsely ground pearl millet flour (100 g) was mixed with distilled water (900 ml) and autoclaved at 15 psi for 15 min. Ten g freshly ground pearl millet was added as inoculum and fermented at 20, 25 and 30 °C for 72 h.

Saccharomyces diastaticus (Sd), *Saccharomyces cerevisiae* (Sc), *Lactobacillus brevis* (Lb) and *Lactobacillus fermentum* (Lf) obtained from National Chemical Laboratory, Poona (India) were employed for carrying out single, mixed and sequential pure culture fermentations of the above-mentioned autoclaved pearl millet flour. For single culture fermentation, the culture of any one of the above microorganisms which supplied 10^5 cells/ml to the fermenting mixture was added and incubated at 30 °C for 72 h. For mixed fermentation, a combination of yeast (10^5 cells/ml) and lactobacillus (10^5 cells/ml) was deployed. Four different types of mixed fermentations included *S. diastaticus* and *L. brevis* (SdLb), *S. diastaticus* and *L. fermentum* (SdLf), *S. cerevisiae* and *L. brevis* (ScLb) and *S. cerevisiae* and *L. fermentum* (ScLf). In the case of sequential culture fermentation, after fermenting with yeast (providing 10^5 cells/ml of the fermenting mixture) at 30 °C for 72 h, the same sample was inoculated with *Lactobacillus spp.* (10^5 cells/ml) and again fermented at 30 °C for 72 h. Four types of sequential fermentations included *S. diastaticus* followed by *L. brevis* (Sd + Lb), *S. diastaticus* followed by *L. fermentum* (Sd + Lf), *S. cerevisiae* followed by *L. brevis* (Sc + Lb), and *S. cerevisiae* followed by *L. fermentum* (Sc + Lf).

The autoclaved unfermented pearl millet flour (Zf) served as control. About 20 ml slurry was taken out for determining the pH and titratable acidity (TA) [2]. The remaining fermented sample was oven dried for 48 h at 65 °C to a constant weight. It was finely ground in the cyclone mill using a 0.5 mm sieve size and analysed for crude protein, crude fat, ash and thiamine

content according to standard methods [3]. A value of 6.25 was employed to convert N to crude protein.

Statistical analysis

The data were subjected to analysis of variance and correlation coefficients in a completely randomised design [23].

Results and discussion

Titratable acidity and pH

After 72 h natural fermentation at 20, 25 and 30 °C, the pH of pearl millet flour dropped from 6.42 to 4.35, 4.01 and 3.55 with corresponding increase in TA from 0.55 to 0.90, 2.85 and 4.35 g lactic acid per 100 ml, respectively. The pH was the lowest and titratable acidity the highest when fermentation was carried out at 30 °C. Similarly, a significant ($P < 0.05$) decrease in pH and simultaneous increase in TA was recorded when the pearl millet flour was fermented by pure cultures of yeast and lactobacilli at 30 °C for 72 h (Table 1). In case of single culture fermentation, *L. fermentum* exhibited the greatest pH lowering effect followed by *L. brevis, S. diastaticus* and *S. cerevisiae*. The pH of the flours after 72 h mixed culture fermentations ranged from 4.04 to 4.39 and the corresponding TA from 1.95 to 1.31 g lactic acid per 100 ml. The lowest pH and the highest TA were observed in the SdLf fermented group. Other mixed fermentations were significantly different from SdLf in respect of the pH and TA values. There were no significant differences between the pH of the products fermented by SdLb and ScLb. The values of TA in the mixed culture fermentation groups were significantly different from one another; the highest in SdLf and the lowest in SdLb group. In sequential culture fermentation, the final pH and TA varied from 3.73 to 4.26 and 1.58 to 2.74 g lactic acid per 100 ml, respectively. The lowest pH's and the highest TA's were in Sd + Lf groups, followed by Sd + Lb, Sc + Lf and Sc + Lb groups. The TA's in Sc + Lf and Sc + Lb groups were not significantly different from one another (Table 1).

From the data on single, mixed and sequential culture fermentations, it can be visualized that *S. diastaticus, L. fermentum* and their combinations were responsible for relatively low pH and corresponding high TA of the fermented product. On the whole, sequential culture fermentation yielded the lowest pH followed by mixed and single culture fermentations. A signifi-

Table 1. Changes in pH and titratable acidity (g lactic acid per 100 ml) during pure culture fermentation of autoclaved pearl millet flour

Fermentation	pH	Titratable acidity
Single fermentation		
Sd	4.80	1.12
Sc	5.65	0.82
Lb	4.54	1.18
Lf	4.41	1.50
Mixed fermentation		
SdLb	4.39	1.31
SdLf	4.04	1.95
ScLb	4.38	1.35
ScLf	4.36	1.48
Sequential fermentation		
Sd + Lb	3.99	1.95
Sd + Lf	3.73	2.74
Sc + Lb	4.26	1.58
Sc + Lf	4.02	1.97
Zf (Control)	6.42	0.55
SE(m)	±0.01	±0.02
CD ($P < 0.05$)	0.03	0.06

Values are means of four replicates. Sd, Sc, Lb and Lf represent the fermenting microorganisms namely *S. diastaticus, S. cerevisiae, L. brevis* and *L. fermentum,* respectively. Mixed fermentation represents the combination of two microorganisms whereas sequential fermentation was carried out by two different microorganisms in two stages. Zf is zero hour fermented control.

cant negative correlation was found between pH and TA in natural ($P < 0.05$) and pure culture ($P < 0.01$) fermentations.

The heterofermenters like *L. fermentum* and *L. brevis* convert glucose to equimolar mixtures of lactic acid, ethanol and CO_2. Due to the production of organic acids by the microflora, the pH of the fermented product is lowered. Rapid drops in pH with corresponding increase in TA have been reported in lactic acid fermentation of corn [5,20], sorghum [24, 25], pearl millet [18], food legumes [33] and the fermented products like *idli* [31], *ogi* [4] and *rabadi* [12].

Crude protein

Natural fermentation brought about a marginal non-significant change in the protein content of the pearl millet flour (Table 2). The temperature did not seem to affect the protein content of the naturally fermented product.

Single, mixed and sequential pure culture fermentations either decreased

or did not change the protein content of the pearl millet flour (Table 3). Protein content of *S. cerevisiae* fermented pearl millet flour was significantly higher than that of the flour fermented by *S. diastaticus, L. brevis* and *L. fermentum*. The protein contents of the mixed culture fermented pearl millet

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

Fermentation	Protein	Fat	Ash	Thiamine
F20	9.90	6.85	1.99	336
F25	9.28	6.90	2.00	330
F30	9.18	6.90	2.00	154
Zf	9.90	6.42	2.00	197
SE (m)	±0.26	±0.05	±0.08	±1.70
CD ($P < 0.05$)	NS	0.15	NS	5.10

Values are means of four replicates.
NS: Non-significant at 5% level.
F20, F25 and F30 represent the samples naturally fermented at 20, 25 and 30 °C, respectively.
Zf is zero hour fermented control.

Table 3. Effect of pure culture fermentation on total protein, fat, ash (g/100 g) and thiamine content (μg/100 g) of pearl millet flour (on dry matter basis)

Fermentation	Protein	Fat	Ash	Thiamine
Single fermentation				
Sd	9.42	5.96	2.00	5.76
Sc	9.99	5.41	2.01	840
Lb	9.30	5.17	2.01	54
Lf	9.26	5.79	2.00	148
Mixed fermentation				
SdLb	9.30	6.37	2.00	70
SdLf	9.67	6.04	1.99	142
ScLb	9.30	6.19	2.00	118
ScLf	9.28	5.88	2.00	201
Sequential fermentation				
Sd + Lb	9.89	5.63	2.01	149
Sd + Lf	9.26	5.35	1.99	170
Sc + Lb	9.90	5.62	2.00	202
Sc + Lf	9.38	5.65	1.99	257
Zf (Control)	9.90	6.42	2.00	1.97
SE (m)	±0.18	±0.20	±0.08	±1.56
CD ($P < 0.05$)	0.54	0.60	NS	4.68

Values are means of four replicates. Sd, Sc, Lb and Lf represent the fermenting microorganisms namely *S. diastaticus, S. cerevisiae, L. brevis* and *L. fermentum*, respectively. Mixed fermentation represents the combination of two microorganisms whereas sequential fermentation was carried out by two different microorganisms in two stages. Zf is zero hour fermented control.

flours were not significantly ($P < 0.05$) different from each other. All the mixed culture fermentation combinations except SdLf had significantly lower protein values than the zero hour control. In case of sequential culture fermentation, only Sd + Lf and Sc + Lf fermented pearl millet flours had significantly ($P < 0.05$) lower protein content than that of the control. Protein content of Sd + Lf fermented pearl millet flour was significantly less as compared to that of Sd + Lb or Sc + Lb fermented flour ($P < 0.05$).

Thus, it appears that the fermentation of pearl millet either decreased or did not alter the protein content of this grain. Increased protein catabolism by fermenting microorganisms may account for loss of protein by escaping ammonia, a by-product of metabolic deamination. Some strains of bacteria are known to possess deaminases. According to Aliya and Geervani [1], the fermentation decreased the crude protein contents of bengalgram and greengram products by 6 to 8% and those of pearl millet and ragi by 4 to 6% whereas sorghum products were unaffected. Fermenting microorganisms including bacteria and yeast resulted in decreased cassava protein when no external source of nitrogen was added [14].

Crude fat

Irrespective of temperature, natural lactic fermentation improved the fat content of pearl millet significantly (Table 2).

Pure culture fermentation either decreased or did not alter the fat content (Table 3). Pearl millet flour fermented with *S. cerevisiae, L. brevis* and *L. fermentum* had significantly less fat as compared to the control whereas the fat content of *S. diastaticus* fermented flour was not affected significantly ($P < 0.05$). The Lb group had the lowest fat content. The mixed culture fermentations did not change the fat content irrespective of the types of culture combinations used. Sequential culture fermentation reduced the fat content.

Some yeast strains are known to be fat producing [22] and their likely participation in the uncontrolled fermentation may account for the increased amount of fat in the naturally fermented pearl millet. According to Eka [13], natural fermentation increased the fat content of locust beans.

Ash

Ash content of pearl millet flour remained unaffected during natural as well as pure culture fermentations (Tables 2 and 3).

Thiamine

Natural fermentation at 20 and 25 °C raised the thiamine content of autoclaved pearl millet flour significantly ($P < 0.05$); values at 20 °C were significantly ($P < 0.05$) higher than those at 25 °C (Table 2). But the fermentation at 30 °C lowered the thiamine content significantly ($P < 0.05$); the amount was less than 50% of that present at 20 and 25 °C.

Pure culture fermentations by lactobacilli, alone or in combination with yeasts, lowered the thiamine content of the pearl millet flour significantly (Table 3); whereas, the concentrations improved almost two to three fold when yeasts alone carried out the fermentation. According to Rao and Basu [26] thiamine in curd increased when yeasts were the fermenting microorganisms; whereas, it decreased during fermentation with lactobacilli.

Summary and conclusion

The pH dropped and TA increased as a result of natural as well as pure culture fermentation of pearl millet flour. Natural fermentation at 30 °C resulted in the lowest pH and the highest TA.

S. diastaticus and *L. fermentum* and their combinations in mixed or sequential culture fermentations were more effective in lowering pH and raising TA of the fermented product.

Protein content of the flour did not improve during fermentation. Natural fermentation did not change whereas pure culture fermentations either decreased or did not affect the protein content of pearl millet flour. At all temperatures, natural lactic fermentation improved the fat content of pearl millet. Mixed culture fermentation combinations did not alter; whereas, the sequential culture fermentation reduced the fat content. Fermentation had no effect on the total ash content of pearl millet flour. Only natural fermentation at 20 and 25 °C raised the thiamine content of autoclaved pearl millet flour significantly ($P < 0.05$). Yeasts increased the thaimine content almost two and three folds whereas *Lactobacilli* decreased it.

Fermentation does not seem to be a viable method of raising the level of protein in the pearl millet; whereas, its fat contents can be improved by selective fermentation. Use of yeasts and not lactobacilli in fermentation may be exploited for raising the thiamine contents of plant foods for human nutrition.

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