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Biological utilisation of pearl millet flour fermented with yeasts and lactobacilli

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Abstract. Mixed culture fermentation of pearl millet flour with *Saccharomyces diastaticus, Saccharomyces cerevisiae, Lactobacillus brevis* and *Lactobacillus fermentum* brought about an improvement in its biological utilisation in rats. Protein efficiency ratio, feed efficiency ratio, apparent protein digestibility, true protein digestibility, net protein utilisation, net protein retention, protein retention efficiency and utilisable protein values in case of pure culture fermented pearl millet four were higher than the control. Feeding of the fermented products did not bring about physiological, histopathological and haematological changes in rats. *Chapaties* and cutlets prepared from the fermented products were organoleptically acceptable.

Introduction

Pearl millet (*Pennisetum typhoideum*) is a staple food for a large section of population in Asian and African countries and contributes a major part of dietary nutrients. Pearl millet is equal or superior to corn, sorghum and rice in protein and oil content. It contains more iron and is similar in phosphorus and calcium contents to cereals [8, 16]. Owing to the presence of antinutritional factors like phytic acid and polyphenols [5, 17], which complex with divalent cations [6], the availability of minerals from the millet for humans is poor [18]. In the presence of phytate, starch as well as protein digestibility is also significantly reduced [24, 21, 15].

Fermentation of pearl millet four with pure cultures of yeasts and lactobacilli has been found to be an effective method of improving its nutritive value; the fermentation improves the starch and protein digestibility (*in vitro*), increases the bioavailability of minerals and brings down the level of antinutrients like phytic acid and polyphenols [14]. Information on the utilisation of this fermented product is lacking. This paper reports the effects of feeding the pearl millet flour fermented by employing pure cultures of yeasts and lactobacilli in rats. Sensory evaluation of the products prepared from fermented flour for human consumption is also reported.

Materials and methods

Materials

Pearl millet grains procured from a local market, were cleaned of foreign matter and ground in an electric grinder using 1.5 mm sieve. Four cultures namely *Saccharomyces cerevisiae, Saccharomyces diastaticus, Lactobacillus brevis* and *Lactobacillus fermentum* were obtained from the Director, National Chemical Laboratory, Poona, India.

Weanling Wistar strain albino rats weighing 28 $\pm$ 5 g were procured from the Disease and Germ Free Small Animal House, Haryana Agricultural University, Hisar, India.

Fermentation

Pearl millet flour (100 g) was mixed with distilled water (900 ml), autoclaved at 1.05 kg/cm^2 pressure for 15 min and inoculated with the above cultures so as to provide an initial count of 10^5 yeast cells/ml and 10^5 lactobacillus cells/ml in the fermentation mixture. 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). The autoclaved unfermented pearl millet flour served as the control. The fermented as well as unfermented control samples were oven dried for 48 h at 65 °C to a constant weight and then finely ground in the cyclone mill (Cyclotec, M/s Tecator, Höganäs, Sweden) using 0.5 mm sieve.

Composition of basal diet

The fermented as well as unfermented pearl millet flour was analysed for protien, fat and ash content by employing standard methods [2]. The basal diets from fermented, unfermented pearl millet flour and egg albumen (EA) were prepared (Table 1) so as to provide 8% protein, 10% fat, 4% minerals and 1% vitamin mixture [19]. The ingredients were mixed thoroughly and passed through 70 mesh sieve to ensure homogeneity and uniform distribution of vitamins and minerals. The diets made exclusively from the feremented samples were not accepted by the rats; this may have been due to low pH of the sample (sourness). In order to overcome this difficulty the fermented samples were mixed with equal amount of raw pearl millet flour and then incorporated in the diets.

Table 1. Composition of the experimental diets.

Dietary groups	Dietary Components (g/Kg Diet)[a]									
	Egg Albumen	Fermented product	Raw pearl millet flour	Zero hour fermented flour	Mineral Mixture	Vitamin Mixture	Sucrose	Fat	Starch	Cellulose
SdLb[b]	–	430.1	430.1	–	22.8	10	50	45.2	11.8	–
SdLf[b]	–	413.5	413.5	–	23.5	10	50	50.0	39.5	–
ScLb[b]	–	430.1	430.1	–	22.8	10	50	46.8	10.2	–
ScLf[b]	–	431.0	431.0	–	23.8	10	50	50.3	3.9	–
Zf[b]	–	–	404.0	404.0	23.3	10	50	48.1	60.1	–
Egg albumen	103	–	–	–	40.0	10	50	100.0	647.0	50

[a] The protein, fat and mineral content of the diets were made to contain 8, 10 and 4%, respectively, after taking into account the level of these constituents in the fermented products. Refined and deodorised peanut oil was the source of the fat. Protein, fat and ash (g/100 g) content of samples was as follows: SdLb: 9.30, 6.37 and 2.0; SdLf: 9.67, 6.04 and 1.90; ScLb: 9.30, 6.19 and 2.0; ScLf: 9.28 5.88 and 2.0; Zf: 9.90, 6.42 and 2.00; and raw pearl millet flour: 9.99, 6.42 and 2.00, respectively.

[b] ScLf, ScLb, SdLb, SdLf represent the mixed fermentations by *S. cerevisiae* and *L. fermentum*. *S. cerevisie* and *L. brevis*, *S. diastaticus* and *L. brevis* and *S. diastaticus* and *L. fermentum*, respectively. Zf is zero hour, unfermented control.

Feeding experiment

Six groups of rats, each consisting of eight rats, were housed individually in cages kept in air conditioned room maintained at 21 + 1 °C with 12 h light and dark cycle. The rats were fed experimental diets for 28 days with free access to food and water. A weighed diet was given daily and the unconsumed diet was collected and weighed. Weight of rats was recorded twice a week and final gain in weight on the expiry date of the experimental period was recorded. Food and protein intake during the period was calculated on dry matter basis for calculating the protein efficiency ratio (PER) and food efficiency ratio (FER) [4].

Apparent protein digestibility (APD), true protein digestibility (TPD) and biological value (BV) were assessed by the method of Chick, Hutchinson and Jackson [7]. After 28 days of feedings, the rats were transferred to metabolic cages and after acclimatised, observations were made for nitrogen intake, gain in body weight, nitrogen excreted in urine and faeces for 5 days. Another group of rats of the same weight and age was fed on a nitrogen free diet to calculate the endogeneous and metabolic nitrogen losses. APD, TPD and BV were determined by using the following formulae:

$$\text{APD} = \frac{\text{N intake} - \text{FN}}{\text{N intake}} \times 100$$

$$\text{TPD} = \frac{\text{N intake} - (\text{FN} - \text{MFN})}{\text{N intake}} \times 100$$

$$\text{BV} = \frac{\text{N intake} - (\text{FN} - \text{MFN}) - (\text{UN} - \text{EUN})}{\text{N intake} - (\text{FN} - \text{MFN})} \times 100$$

where, FN = Faecal nitrogen; MFN = Metabolic faecal nitrogen; UN = Urinary nitrogen; EUN = Endogenous urinary nitrogen.

Net protein utilisation (NPU) was determined by using the following formula:

$$\text{NPU} = \frac{\text{BV} \times \text{TD}}{100}$$

The values of net protein retention (NPR) and protein retention efficiency (PRE) were calculated according to the method of Bender and Doell (1957) [3]. Utilisable proteins (UP) were estimated by the method of Gupta *et al.* [10].

Histopathology and Haematology

Liver, kidney, thymus and adrenals were examined histopathologically. Samples of fixed organ tissues were dehydrated in graded alcohol solutions and embedded in paraffin. Sections of 5 μm thickness were cut and stained with haematoxylin–Cosin. Slides were prepared for total leucocyte count (TLC) and differential leucocyte count (DLC) and examined microscopically.

Development and utilisation of the fermented product

Various types of cutlets and *chapaties* were prepared from the mixed culture fermented pearl millet flour. For preparing cutlets (A), the ingredients including boiled rice (50 g), boiled potato (100 g), fermented pearl millet flour (50 g), onion (10 g) and salt (5 g) and spices (5 g) were mixed properly. A small portion of the above mixture was shaped into cutlet and deep fried in hydrogenated vegetable oil. Another type of cutlet (B) was prepared in the same manner as above by taking fermented pearl millet flour (100 g), boiled potato (100 g), bread slices (4 nos) and salt (5 g) and spices (5 g).

Two types of *chapaties* were prepared. *Chapati* (A) contained fermented pearl millet flour and raw pearl millet flour in the ratio of 3:2 (w/w) whereas in *chapati* (B), fermented pearl millet flour and whole wheat flour were incorporated in 3:1 ratio. Water was added to the ingredients and the dough was kneaded. The rolled *chapaties* were baked on the hot *tawa*.

Organoleptic evaluation

The products developed from the fermented pearl millet flour were evaluated for colour, flavour, taste, texture and appearance by a panel of judges deploying a 9-point hedonic scale and average of scores of all the above characteristics was expressed in terms of overall acceptability. The score sheet of the taste panel was as follows:

Like extremely	9	Dislike slightly	4
Like very much	8	Dislike moderately	3
Like moderately	7	Dislike very much	2
Like slightly	6	Dislike extremely	1
Neither like nor dislike	5		

Statistical analysis

The data were subjected to analysis of variance and correlation coefficients were derived in a completely randomised design [20].

Results and discussion

Protein efficiency ratio and feed efficiency ratio

Food as well as protein intakes of rats fed on pearl millet flour fermented with pure cultures of yeasts and lactobacilli were not significantly different from those fed with zero hour fermented pearl millet flour (Table 2). All the four groups fed on pure culture fermented pearl millet flour except ScLb, had better gain in body weight than the control. Fermentation of pearl millet flour employing all four combinations improved significantly the food efficiency and protein efficiency ratio in rats. Food intake, protein intake, gain in body weight, FER and PER in egg albumen group was of course much higher than in the fermented flour groups.

As fermentation results in low pH of the product, the sourness may account for lower intake of the diets containing the fermented pearl millet flour. Since protein intake by the animals depended upon their food intake, groups having higher food intake showed the high protein intake as well. Fermentation has been known to improve the digestibility of protein (Table 3) and carbohydrates [9]. The better digestibility may explain the higher FER and PER in rats fed with the fermented flour. Fermented foods like *miso, yidli, khaman*, yoghurt and kefir had been found to result in higher PER values in rats [1, 13, 23].

Biological utilisation

Mixed pure culture fermentations improved the apparent protein digestibility, true protein digestibility, net protein utilisation and utilisable protein in all the pure culture combinations except SdLf in which improvement in all these parameters was not statistically significant. An improvement in bioloical value was witnessed only when pearl millet flour was fermented by *S. cerevisiae* and *L. fermentum* combination (Table 3). Improvement in biological utilisation of protein by all the pure culture fermentations of pearl millet flour appeared to be of the same extent. All the combinations of yeasts and lactobacilli increased net protein retention except in SdLb and protein retention efficiency except in ScLb and SdLf groups (Table 3). Khader [11]

Table 2. Food intake, protein intake, gain in body weight, feed efficiency ratio (FER) and protein efficiency ratio (PER) in rats fed fermented pearl millet flour[a]

Dietary groups	Food intake (g)	Protein intake (g)	Gain in body wt. (g)	Food efficiency ratio	Protein efficiency ratio
ScLf[b]	123.0 ± 10.2	9.84 ± 0.80	18.8 ± 3.82	0.152 ± 0.02	1.91 ± 0.24
ScLb[b]	107.6 ± 7.2	8.60 ± 0.57	16.4 ± 2.77	0.152 ± 0.02	1.90 ± 0.28
SdLf[b]	119.3 ± 8.5	9.54 ± 1.90	18.0 ± 4.29	0.150 ± 0.03	1.88 ± 0.32
SdLb[b]	120.8 ± 12.0	9.66 ± 0.96	17.4 ± 3.26	0.144 ± 0.01	1.80 ± 0.24
Zf[b]	114.8 ± 4.8	9.18 ± 0.38	13.0 ± 0.81	0.113 ± 0.01	1.41 ± 0.06
Egg albumen	205.1 ± 12.7	16.4 ± 1.02	42.9 ± 5.11	0.209 ± 0.02	2.62 ± 0.28
CD ($P < 0.05$)	12.5	1.05	3.42	0.02	0.24

[a] Values are means ± SD of eight rats in each group fed for four weeks.

[b] ScLf, ScLb, SdLb, SdLf represent the mixed fermentations by *S. cerevisiae* and *L. fermentum*, *S. cerevisie* and *L. brevis*, *S. diastaticus* and *L. brevis* and *S. diastaticus* and *L. fermentum*, respectively. Zf is zero hour fermented control.

Table 3. Apparent digestibility, true digestibility, biological value, net protein utilisation and utilisable protein, net protein retention and protein retention efficiency of fermented pearl millet flour.[a]

Dietary groups	Apparent digestibility (%)	True digestibility (%)	Biological value (%)	Net protein utilisation (%)	Utilisable protein (%)	Net protein retention	Protein rentention efficiency
ScLf[b]	82.3 ± 2.6	87.7 ± 2.7	82.2 ± 5.21	72.1 ± 4.6	6.17 ± 0.43	3.34 ± 0.61	53.4 ± 9.8
ScLb[b]	82.7 ± 3.0	87.6 ± 3.2	80.3 ± 3.6	70.3 ± 4.5	6.01 ± 0.42	3.13 ± 0.23	50.5 ± 3.8
SdLf[b]	79.1 ± 3.1	84.4 ± 3.3	79.4 ± 3.4	66.7 ± 4.6	5.77 ± 0.39	3.10 ± 0.28	49.6 ± 4.5
SdLb[b]	82.6 ± 1.9	86.8 ± 2.2	80.8 ± 1.9	70.1 ± 0.9	6.00 ± 0.08	2.74 ± 0.52	53.8 ± 8.4
Zf[b]	76.4 ± 2.4	81.9 ± 4.4	77.6 ± 0.7	63.5 ± 4.0	5.43 ± 0.43	2.64 ± 0.21	42.2 ± 3.4
Egg albumen	92.1 ± 1.7	95.8 ± 1.7	93.3 ± 1.9	89.3 ± 3.2	7.64 ± 0.27	5.58 ± 0.20	89.2 ± 3.2
CD ($P < 0.05$)	4.50	4.35	4.35	6.15	0.42	0.45	8.91

[a] Values are means ± SD of six albino rats kept in metabolic cages for five days after acclimatisation.

[b] ScLf, ScLb, SdLb, SdLf represent the mixed fermentations by *S. cerevisiae* and *L. fermentum*, *S. cerevisie* and *L. brevis*, *S. diastaticus* and *L. brevis* and *S. diastaticus* and *L. fermentum*, respectively. Zf is zero hour unfermented control.

Table 4. Overall acceptability of fermented pearl millet cutlets and chapaties.[a]

Fermentation	Cutlet		Chapaties	
	A	B	A	B
SdLb[b]	7.58 ± 0.42	7.19 ± 0.51	6.74 ± 0.47	6.56 ± 0.33
SdLf[b]	7.02 ± 0.48	6.94 ± 0.52	6.37 ± 0.64	6.75 ± 0.39
ScLb[b]	7.14 ± 0.71	6.54 ± 0.81	6.66 ± 0.52	6.45 ± 0.50
ScLf[b]	7.14 ± 0.62	7.12 ± 0.54	6.12 ± 0.70	6.29 ± 0.48
CD ($P < 0.05$)	0.54	0.51	0.75	0.54

[a] Average score of seven characteristics (colour, appearance, flavour, texture, taste, bitterness and sourness) given by 10 judges on 9-point hedonic scale. Values are means ± SD of ten replicates.

[b] ScLf, ScLb, SdLb, SdLf represent the mixed fermentations by *S. cerevisiae* and *L. fermentum, S. cerevisie* and *L. brevis, S. diastaticus* and *L. brevis* and *S. diastaticus* and *L. fermentum*, respectively. Zf is zero hour unfermented control.

fed *dhokla* to rats at 8% protein level and reported 92.5% true digestibility. Zamora and Veum [25] reported an increase in the average daily weight gain for weanling rats fed soybean fermented with *R. Oligosporus*. The fermented soybeans also had greater apparent digestibility, BV and NPU values.

Histopathology and haematology

Histopathological examination of liver, kidney, adrenals and thymus of the rats fed on different dietary groups did not reveal any abnormality. The feeding of the fermented products did not affect the organs adversely. TLC, DLC and RBC counts were also within the normal range. It signifies that the feeding of fermented and control diets was biologically safe and did not cause any physiological abnormality.

Utilisation of fermented products

The pearl millet flour fermented by pure cultures of yeasts and lactobacilli was incorporated in the traditional recipes like *chapaties* and cutlets (Table 4). The cutlets prepared in combination with boiled rice and potato (A) and bread and boiled potatoes (B) were found to be acceptable. Cutlets (A) prepared from mixed fermented pearl millet flour were 'moderately liked'. Cutlets (B) prepared from ScLb combination were 'slightly liked' whereas those prepared from other mixed fermentation combinations were 'moderately liked'. The *chapaties* (A and B) prepared from mixed culture fermented pearl millet flour were 'slightly liked'. Both the mixed fermented pearl millet flour products were acceptable although a slight variation in their overall acceptability score was ocassionally seen. Cutlets were found to

be more acceptable than *chapaties*. Khader [12] prepared a fermented food similar to *miso* from rice, chickpea and curd or yeast. The fermented food when fried was well accepted by a taste panel. Taur, Pawar & Ingle [22] did sensory evaluations of the chips made from germinated-fermented sorghum and they were 'neither liked nor disliked' by the panels.

Fermentation of coarse grains like pearl millet with yeasts and lactobacilli seems to be a potential method of improving the biological utilisation of such staple foods. The fermented products can be beneficially included in the traditional recipes for improving the nutritional status of the masses in the developing countries like India.

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