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Biological utilization of naturally fermented pearl millet flour *(Pennisetum typhoideum)*

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SUMMARY. Natural fermentation of pearl millet flour at 20,25 and 30º for 72h brought about an improvement in its apparent and true protein digestibility. Utilisable protein, net protein retention and protein retention efficiency values were also enhanced as a result of fermentation. Rats fed on flour fermented at 20 and 25º C had higher food as well as protein efficiency ratios than the flour fermented at 30º C. Feeding of the fermented products did not bring about any histopathological abnormality in rats. Cutlets prepared from the fermented flour were organoleptically acceptable to a panel of judges.

RESUMEN.Utilización biológica de la harina fermentada de mijo perlado *(Pennisetum typhoideum)*. La fermentación natural de la harina de mijo perlado a 20º, 25º y 30º C por 72 horas, mejoró la digestibilidad aparente y verdadera, y los valores de proteina utilizable, retención proteica neta y eficiencia de retención proteinica. Las harinas fermentadas a 20º y 25º C produjeron mejores relaciones de eficiencia proteinica y de alimento en ratas, que las fermentadas a 30º C. Los productos fermentados no provocaron anormalidades histopatológicas en las ratas. Las preparaciones elaboradas con la harina fermentada, se encontró organolépticamente aceptable por un panel de jueces.

INTRODUCTION

Digestibility of carbohydrates as well as proteins bioavailability of minerals from pearl millet *(Pennisetum typhoideum)*, a staple food for a large segment of population in Asian and African countries are low (1,2,3). This may partly be attributed to the presence of antinutritional factors like phytic acid and polyphenol present in considerable amounts in pearl millet (8,9). Phytic acid makes a complex with the divalent cations (6) and reduces their bioavailability and also inhibits the amylolytic and proteolytic enzymes (7) resulting in lower starch and protein digestibility (1,3,8,9).

Natural fermentation of pearl millet flour has been found to be an effective method of improving the **in vitro** starch and protein digestibility and biovailability of minerals (3,10). This also brings down the levels of antinutrients like, phytic acid and polyphenols (3, 10). Before the fermented pearl millet flour having better nutritive value is recommended for human, it is essential to have information its utilization in experimental animals. This paper reports the biological utilization of naturally fermented pearl millet flour 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 the local market in a single lot, were cleaned of dust, broken seeds and other foreign material and ground in an electric grinder (Cemotec, M/s Tecator. Höganäs, Sweden) through a 1.5 mm sieve size.

Weànling Wistar albino rats weighin 28 ± 5 g were procured from the Disease and Germ-Free Small Animal House, Haryan Agricultural University, Hisar, India.

For selection temperature and time period of optimum fermentation, pearl millet flour was fermented at various temperatures or different time periods and subjected to organoleptic evaluation for colour and aroma by a panel of judges using a 9-point hedonic scale. On this basis, the fermentation was confined to 20, 25 and 30º C for 72 h. The pH dropped from 6.40 to 3.98, 3.72 and 3.46 after 72h fermentation of millet flour at 20, 25 and 30º C, respectively.

Pearl millet flour (100 g) was mixed with distilled water (900 ml) in conical flask and autoclaved at 1.05 Kg cm^{-2} pressure for 15 min. After autoclaving and cooling, 10 g freshly ground pearl millet flour was added as inocu-

lum and fermentation was then carried out at 20, 25 and 30º C in an incubator for 72h. In this way, for preparing the diet for rats, 5 Kg fermented flour sample was prepared in respect of each fermentation temperature. The autoclaved unfermented pearl millet flour served as the control. The fermented as well as unfermented control sample were air-dried in an oven for 48h at 65º C to a constant weight. The dried product was finely ground in the cyclone mill (Cyclotec, M/s Teator, Höganäs, Sweden) using 0.5 mm sieve.

Composition of basal diet.

The fermented as well as unfermented pearl millet flour was analysed for protein, fat and ash content by employing standard laboratory methods (11). The basal diets from fermented, unfermented pearl millet flour and egg albumen were prepared (Table 1) so as to provide 8% protein, 10% fat, 4% minerals and 1% vitamin mixture (12). The ingredients were mixed thoroughly and passed through 70 mesh sieve to ensure homogenity and uniform distribution of vitamins and minerals in the diet. The diets made exclusively from the fermented samples were not accepted by the rats, may be due to low pH (sourness) of the sample. In order to overcome this difficulty, the fermented samples were mixed with equal amount of raw pearl millet flour and then incorporated in the diet to provide the required amount of nutrients as mentioned above. The nitrogen free diet was prepared by mixing celulose (5%), fat (10%), minerals (4%) and vitamin mixtures (1%) and starch (80%). This diet did not contain the protein source.

Feeding experiment

Six groups of rats, each consisting of eight rats, were housed individually in cages kept in air conditioned room maintained at 21 ± 1º C with 12h light and dark cycle. After tha adaptation period of 5 days, the rats were fed experimental diets for 28 days with the free access to food and water. Weighed diet was given daily and the unconsumed diet was collected and weighed. Weight of rat was recorded twice a week and final gain in weight on th last day of the experimental period. Food and protein intakes during the period were calculated on dry matter basis for calculating the protein efficiency ratio (PER) and food efficiency ratio (FER)(13).

Apparent protein digestibility (APD), true protein digestibility (TPD) and biological value (BV) were assessed as per formulae given below (14). After 28 days of feeding, the rats were transferred to metabolic cages and after they got 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 endogenous and metabolic nitogen losses.

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

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

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

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

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

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

The value of net protein retention (NPR), protein retention eficiency (PRE) (15) and utilisable protein (16) were also estimated.

Histopathology and haematology

At end of experiment, the rats were lightly anaesthetised with diethyl ether and blood was collected by cardiac puncture. The liver, heart, thymus and adrenal were excised, cleaned of adhering matter, blotted in filter paper, weighed and preseved in 10% formaline solution. Tissues were thoroughly washes in running tap water for 12h, dehydrated in acetone, cleaned in benzene and embedded in paraffin wax (melting point 60-62º C). Sections of 5 microns in thickness were cut stained with routine haematoxylin and eosin method for histopathological studies. Studies were prepared for total leucocyte count (TLC), differential leucocyte count (DLC) and examined microscopically.

Product development from the fermented product:

Various type of cutlets and **chapaties** were prepared from naturally fermented pearl millet flour. For preparing cutlets (A), the ingredients including boiled rice (50 g), boiled, peeled and mashed potato (100 g), fermented

TABLE 1
COMPOSITION OF THE EXPERIMENTAL DIETS[a]

Dietary components (g/Kg diet)	Dietary groups				
	F20	F25	F30	Autoclaved unfermented flour diet (control)	Egg albumen
Egg albumen	-	-	-	-	103
Fermented product	404.0	431.0	435.5	-	-
Raw pearl millet flour	404.0	431.0	435.5	404.0	-
Autoclaved unfermented flour	-	-	-	404.0	-
Mineral mixture	23.9	22.8	22.6	23.8	40.0
Vitamin mixture	10	10	10	10	10
Su crose	50	50	50	50	50
Fat	44.7	40.5	40.0	48.1	100.0
Starch	63.4	14.7	6.4	60.1	647.0
Cellulose	-	-	-	-	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 product.
Refined and deodorised peanut oil was the source of fat.
Protein, fat and ash (g/100 g) content of samples was as follows:
Pearl millet flour: 9.99, 6.42 and 2.0; Autoclaved unfermented flour: 9.90, 6.42 and 2.0; F20: 9.90, 6.85 and 1.99; F25: 9.28, 6.90 and 2.0; F30: 9.18, 6.90 and 2.0, respectively.

pearl millet flour (50 g), onion (10 g),salt (5 g) and spices including red chilli powder, black pepper powder etc. (5 g) were mixed properly. A small portion of the above mixture was shaped into cutlet and deep fried in the hydrogenated vegetable oil (150º C for 3 min.). Another type of cutlet (B) was prepared in the same manner as above by taking fermented pearl millet flour (100 g), boiled potatoes (100 g), bread slices (4 nos.), 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 3:2 (w/w) where as 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. A small amount of dough was taken and shaped into the form of a ball. Then it was rolled and baked on a hot griddle (45º F for 2 min.).

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 q-point hedonic scale and average 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
Neitheir like nor dislike	5		

Statistical analysis

The data were subjected to analysis of variance and correlation coefficients were derived in a completely randomised desing (16).

RESULTS AND DISCUSSION

Protein efficiency ratio and food efficiency ratio:

Natural fermentation at 20 and 25º C did not improved significantly ($P<0.05$) the food intake, gain in body weight, food efficiency ratio and protein efficiency ratio (Table 2). Rats fed with the flour fermented at 30º C had significantly ($P<0.05$) lower food intake, body weight, food efficiency ratio and protein efficiency ratio than

those fed with autoclaved unfermented flour diet. Rats fed egg albumen had much higher gain in body weight, FER and PER values when compared with the fermented (at all the temperatures) flour groups.

Production of lactic acid during fermentation lowers pH of the product and the sourness may account for the lower food intake. Uncontrolled fermentation allowed the growth of diverse types of microflora, some of which are, perhaps, responsible for the production of unpalatable flavour and taste. A temperature of 30º C might be conducive to growth of a relatively wide spectrum of microflora and the growth of undesirable micro-organisms which may account for the product fermented at 30º C being less acceptable. Since protein intake by the rats depends upon their food intake, groups having higher food intake showed the high protein intake too. As the rats fed on fermented flour at 30º C had the lowest food intake, protein intake and gain in body weight; this group had the lowest FER and PER values too.

Biological utilization

Rats fed on fermented flour had significantly ($p < 0.05$) higher apparent and true protein digestibility values than those of the control; there was no significant ($p < 0.05$) difference in the apparent digestibility values among the rats fed on 20 , 25 and 30º C fermented flours (Table 2). Fermentation at 20 and 25º C and not at 30º C significantly ($p<0.05$) improved the utilizable protein. An increase in net protein retention and protein retention efficiency was observed among the rats fed on flour fermented at 20º C; values for the rats fed on 25, 30º C fermented and unfermented flour did not differ significantly ($p<0.05$). The fermentation at any of these temperature did not improved BV and NPU. Among all the groups, rats fed on egg albumen had the highest AD, TD, BV, NPR and PRE values. Rats fed on **dhokla** at 8% protein level had enhanced i.e. 92.5 % true digestibility (17) and rats fed on fermented soybean with ***R. oligosporus*** had greater AD, BV and NPU values (18).

An attempt was made to correlate apparent and true protein digestibility with phytic acid content of the pearl millet as an association of **in vitro** protein digestibility with phytate content of the plant foods had been known to exist (19). Decrease in phytic acid content possibly through hydrolysis by phytase of the fermenting microflora or present in pearl millet grain, may partly be responsible for an improvement in protein digestibility of the fermented flour. In natural fermentation, phytate was found to have a sinificant negative correlation with apparent (-0.9403) and true protein digestibility (-0.8784) of the fermented pro-duct. **In vitro** digestibility of the fermented products could also be used as a yardstick for assessing apparent digestibility of the naturally fermented food as a significant positive correlation between the two was established (3).

Histopathology, haematology and organoleptic acceptability

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. Total leucocyte count (TLC) differential leucocyte count (DCL) and red blood cell counts (RBC) were also within the normal range. It signifies that feeding of the fermented flour in the diets was biologically safe and did not cause any physiological abnormality.

The naturally fermented pearl millet flour was incorporated in the tradicional recipes like **chapaties** and cutlets (Table 4). Cutlets A and B prepared from the fermented flour at different temperatures were found to be "slightly acceptable". **Chapaties** A and B prepared from the flour fermented at 20º C were "neither liked nor disliked" where a those prepared from the flour fermented at 25 at 30º C were "disliked slightly" and "disliked moderately", respectively. According to Khader (20), a fermented food just like **miso** prepared from rice, chickpea and curd or yeast was well accepted by the panel. Taur **et al** (21) did sensory evaluation of chips made from germinated fermented sorghum and they were neitheir liked nor disliked by the panel of judges.

In summary, organoleptically, the recipes incorporating flour fermented at 20º C and 25º C appeared to be better than those incorporating flour fermented at 30º C. Due to sourness of the fermented flour (30º C) food intake was less and there was no improvement in FER and PER. But the rats fed on flour fermented at 20º C and 25º C had higher apparent and true protein digestibility, utilizable protein, net protein retention and protein retention efficiency values. Feeding of the fermented products didn't bring out any physiological, histopathological and haematological changes in rats which needs to be investigated in human beings.

TABLE2
FOOD INTAKE, PROTEIN INTAKE, GAIN IN BODY WEIGHT, FOOD 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)	Feed efficiency ratio	Protein efficiency ratio
Control					
Autoclaved unfermented flour	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
Fermentation (° C)					
20	109.7 ± 6.2	8.77 ± 0.49	9.64 ± 1.40	0.087 ± 0.01	1.09 ± 0.15
25	112.7 ± 9.4	9.01 ± 0.74	9.76 ± 2.50	0.086 ± 0.02	1.08 ± 0.27
30	99.8 ± 6.6	7.98 ± 0.40	6.30 ± 1.47	0.063 ± 0.01	0.79 ± 0.15
CD ($P<0.05$)[b]	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 Critical difference at 5% level. Differences of two means within/between the treatments exceeding this value are significant.

TABLE 3
APPARENT DIGESTIBILITY, TRUE DIGESTIBILITY, BIOLOGICAL VALUE, NET PROTEIN UTILISATION, UTILISABLE PROTEIN, NET PROTEIN RETENTION, 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 retention efficiency
Control							
Autoclaved unfermented flour	76.8 ± 4.6	81.9 ± 4.4	77.6 ± 0.78	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 ± 3.4
Fermentation (° C)							
20	82.5 ± 6.2	88.3 ± 5.3	77.9 ± 2.9	68.7 ± 5.8	5.88 ± 0.51	3.30 ± 0.71	52.8 ± 11.4
25	82.3 ± 2.5	86.9 ± 2.4	79.7 ± 3.7	69.2 ± 3.7	5.92 ± 0.29	2.71 ± 0.23	43.4 ± 3.8
30	81.4 ± 2.6	86.5 ± 2.8	78.1 ± 3.3	67.5 ± 1.4	5.77 ± 0.12	2.71 ± 0.48	43.4 ± 7.8
CD ($P<0.05$)[b]	4.50	4.35	4.35	9.15	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 Critical difference at 5% level. Differences of two means within/between the treatments exceeding this value are significant

TABLE 4
OVERALL ACCEPTABILITY OF FERMENTED PEARL MILLET CUTLES AND CHAPATIES[a]

Fermentation	Cutlets[b]		Chapaties[b]	
	A	B	A	B
20º C	6.66 ± 0.38	6.78 ± 0.29	5.28 ± 0.81	5.46 ± 0.74
25º C	6.92 ± 0.34	6.70 ± 0.36	4.72 ± 1.13	4.82 ± 0.50
30º C	6.16 ± 0.82	6.26 ± 0.44	3.76 ± 0.86	3.84 ± 0.64
CD(P<0.05)	0.54	0.51	0.75	0.54

a Average scores of seven characteristics (odour, appearance, flavour, texture, taste, bitterness and sourness) given by 10 judges on 9-point hedonic scale

b Values are means ± SD of four replicates

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