Effect of Fermentation on the Primary Nutrients in Finger Millet *(Eleusine coracana)*

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The effect of fermentation using endogenous grain microflora at 30 °C on the primary nutrients in finger millet *(Eleusine coracana)* is reported. The fermentation decreases the starch and long-chain fatty acid content. The pH drops by 2.1 units, leading to an increase of about 6.5 and 3.7 times in lactic and acetic acid contents, respectively. These are the major organic acids produced during fermentation. The total fat content decreased by about 42.9%, which favorably agrees with total loss in long-chain fatty acid content. The total microbial flora increased rapidly during the first 24 h of fermentation. Controlled fermentation using a mixed-culture inoculum taken from 18 and 48 h fermented millet decreased the fermentation time markedly as measured in terms of pH and titratable acidity.

Keywords: *Finger millet; Eleucine coracana; natural fermentation; primary nutrients; biochemical changes; carbohydrates; fatty acids*

INTRODUCTION

Finger millet is the most important among the five small millet species and is cultivated in many parts of India by dryland farming. Although the 8−11% total protein content of finger millet is comparable to fine cereals, it is limiting in lysine, threonine, and tryptophan but has sulfur-containing amino acids equal to that of milk protein. The carbohydrates (72%) include starch as the main constituent, and the non-starch polysaccharides (dietary fiber 19%) are higher than in other cereals and pulses. Fat content (1.5−2.0%), although low, is high in polyunsaturated fatty acids (PUFA). No other millet or finer cereal is as rich as finger millet in total minerals (2.7%), calcium (334 mg/100 g), and iron (2.4−6.4 mg/100 g). Among vitamins, thiamine content (0.42 mg/100 g) and β-carotene content (42 μg/100 g) is more than in rice. The phytate content is lower than that of maize and more than in pearl millet. Substances interfering with the absorption of nutrients are the phytates, tannins, and amylase and trypsin inhibitors, which could probably be overcome by suitable processing (Malleshi and Hadimani, 1993).

Finger millet is consumed whole, thereby retaining the fiber, minerals, and vitamins present in the outer layer of the grain, which is nutritionally advantageous.

Traditionally it is consumed in the form of thick porridge (mudde or dumpling), thin fermented porridge (ambali), fried or baked pancake (roti, dosa), and beverages (chang/jnard) (Malleshi and Hadimani, 1993; Gomez, 1993; Madhavi and Vaidehi, 1990). Most of these involve a fermentation step. There are so far no reports of toxicity in any of these products.

Although finger millet is traditionally fermented for consumption, there are no systematic studies on the beneficial changes of this processing, although it is claimed to be a health food. Aliya and Geervani (1981) have assessed the protein quality and B vitamin content of ambali made from finger millet. However, the changes in other primary nutrients, namely, carbohydrates, fats, long-chain fatty acids (LCFA), and organic acids, have not been reported. We report here the biochemical changes in these primary nutrients during mild fermentation by the endogenous grain microflora. In addition, an attempt has been made to follow the changes in the microbial population during fermentation and then to control the fermentation process by use of a mixed-culture inoculum from these various stages.

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MATERIALS AND METHODS

The millet purchased from the local market was cleaned, washed, dried, and powdered (100 mesh). Millet flour (50 g) was mixed with 100 mL of distilled water in a 250 mL Erlenmeyer flask. The flask was covered with aluminium foil and fermented at 30 °C in a BOD incubator for 24 or 48 h. In order to simulate home conditions, the containers were not sterilized. Fermentation was carried out in triplicate. The control consisted of unfermented slurry prepared in a similar manner. After the fermentation, the slurries were frozen, lyophilized, and stored in air-tight containers at 5 °C for further analyses. The samples were analyzed as follows:

1. Biochemical Changes. (a) The pH of the slurry was determined using a pH meter, and the titratable acidity was determined by titration against 0.05 M NaOH using phenolphthalein as an indicator. Titratable acidity was calculated in terms of grams of lactic acid per 100 g of millet flour (Egan et al., 1981).

(b) A 500 mg aliquot of the sample was extracted four times with 20 mL of 80% hot ethanol. The extract was evaporated and made to 10 mL. An aliquot was assayed for total soluble sugars by the phenol−sulfuric acid method (Dubois et al., 1956) and reducing sugars by the Nelson-Somogyi method (Nelson, 1957). The individual free sugars were separated and quantified by HPLC (Zygmunt, 1982) using a Shimadzu Model 6A system, Shimpack CLC-NH_2, 4.6 mm × 25 cm column, with pressure at 165 mmHg, and a flow rate of 1.5 mL/min with refractive index detector.

(c) The alcohol-insoluble residue was analyzed for the starch content (McCready et al., 1950).

(d) The defatted millet was sequentially extracted for albumin/globulin, prolamine, and glutelin fractions on the basis of solubility in organic and aqueous solvents (Monteiro et al., 1982). The nitrogen content of the millet and protein fractions was estimated using the Tecator Kjeltec system 1028 and the protein value calculated using the factor 6.25 (AOAC, 1990).

(e) The total free amino acids and soluble peptides in the alcohol extract were estimated by reaction with ninhydrin and absorbance measurement using a Spectronic 20 spectrophotometer (Magne and Larher, 1992).

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Table 1. Changes in the Primary Nutrients of Unfermented and Fermented Finger Millet[a]

		fermented	
biochemical parameters	unfermented	24 h	48 h
pH	6.4 ± 0.1	5.2 ± 0.1	4.3 ± 0.1
titratable acidity (%)	0.4 ± 0.0	0.8 ± 0.1	1.9 ± 0.2
lactic acid (%)	0.15 ± 0.01	0.41 ± 0.01	0.98 ± 0.04
acetic acid (%)	0.14 ± 0.00	0.22 ± 0.01	0.50 ± 0.01
starch (%)	59.4 ± 0.8	56.0 ± 1.7	52.0 ± 2.0
total soluble sugars (%)	1.4 ± 0.1	0.5 ± 0.1	1.4 ± 0.3
reducing sugars (%)	0.43 ± 0.0	0.13 ± 0.0	0.95 ± 0.1
total protein (%)	8.1 ± 0.3	8.3 ± 0.3	8.3 ± 0.2
protein extractability (%)	59.9 ± 3.8	64.4 ± 4.3	76.2 ± 4.5
total free amino acids (%)	0.5 ± 0.0	1.4 ± 0.0	1.8 ± 0.2
fat (%)	2.1 ± 0.2	1.9 ± 0.0	1.2 ± 0.1

[a] Percent dry matter.

Table 2. Free Sugar Composition of Unfermented and Fermented Finger Millet[a]

		fermented	
sugars	unfermented	24 h	48 h
D-xylose	1.12	0.35	1.30
D-fructose	0.09	0.04	nd[b]
D-glucose	0.09	0.09	0.12
sucrose	0.12	nd	nd

[a] Values in milligrams per gram of dry matter. [b] nd, not detectable.

(f) The fat content of the samples was estimated gravimetrically after extraction with petroleum ether (AOAC, 1990). The LCFA were saponified and extracted into ether and estimated by gas chromatography in Pye Unicam 4550 gas chromatograph (Cohen et al., 1969). The column packed with 5% EGSSX was operated at 200 °C with the same ignition temperature and the FID temperature at 250 °C.

(g) The organic acids present were estimated by HPLC (Shimadzu Model 7A system Shimpack SCR 101H 9.2 mm × 30 cm column, flow rate 1.2 mL/min, column pressure 140 mmHg, 40 °C) (Anderson and Hedlund, 1983).

2. Microbial Population Counts. Fresh fermenting millet samples were diluted using the serial dilution technique and, the microbial population was enumerated by colony counts using the pour plate method on standard plate count agar (M091, HiMedia, Bombay, India) containing (in g/L) tryptone 5.0, yeast extract 2.5, dextrose 1.0, and agar 15.0 of pH 7.0 ± 0.2.

3. Controlled Fermentation. Controlled fermentation differed from the natural fermentation in that a slurry from 18 and 48 h naturally fermented millet was added at the 5% level. The millet samples were not sterilized.

All values expressed are the means of two or more reproducible estimations.

RESULTS AND DISCUSSION

pH, Titratable Acidity, and Organic Acids. A considerable drop in pH with a simultaneous rise in titratable acidity was observed (Table 1). The predominant organic acids include lactic and acetic acids. Only trace amounts of butyric, isobutyric, and valeric acids were detected. The total organic acids produced in finger millet fermentation was higher compared to that produced in foxtail millet fermented under similar conditions (Usha Antony et al., 1996) suggesting greater fermentability of finger millet. The increased acidity and low pH can enhance the keeping quality of the millet by inhibiting microbial growth and also contribute to the flavor of the processed millet (Giese, 1994). Umeta and Faulks (1989) have reported lactic and acetic acids as the predominant organic acids detected during the fermentation of 'tef' (*Eragrostis tef*), which is in close agreement with the present study.

Free Sugars and Starch. Fermentation of finger millet resulted in a considerable change in the profile of available carbohydrates (Table 1). The starch content decreased by 7.4% during fermentation for 48 h, indicating utilization of the carbohydrates by the fermenting microbes. A similar decrease in starch content during fermentation has been reported in 'tef' (Umeta and Faulks, 1988), pearl millet (Khetarpaul and Chauhan, 1990, 1991), and foxtail millet (Usha Antony et al., 1996).

The total soluble sugars and reducing sugars decreased after 24 h of fermentation followed by a marked increase at the end of 48 h (Table 1). This suggests hydrolysis of starch, cellulose, and hemicellulose as both free xylose and glucose increased on fermentation (Table 2). A decrease in sugars at 24 h could be due to the high consumption of sugars by the microbes, which are highly active at this stage. This consumption is probably higher than the rate of production at the first stage of 24 h. In the second stage after 24 h, the consumption is probably slower due to the inhibition of the microbes by accumulated acidity (pH 5.2−4.3) thereby resulting in a high free sugar content at 48 h. The free sugar content of finger millet before and after fermentation consisted largely of xylose (Table 2). Small amounts of sucrose, glucose, and fructose were also detected in the unfermented millet.

Protein and Free Amino Acids. The total protein content of the millet was found to be unaltered by fermentation (Table 1). This is in close agreement with the report of Chavan and Kadam (1989) on other cereals and foxtail millet (Usha Antony et al., 1996). However, the extractability of the proteins and the free amino acid content showed a definite increase, suggesting solubilization of the protein. This may be the result of microbial protease activity and a breakdown of tannins and phytates which are known to bind proteins (Chavan and Kadam, 1989).

Fats and Long-Chain Fatty Acids (LCFA). The total fat content of the millet was found to decrease by 42.9% during a period of 48 h (Table 1). Table 3 presents the LCFA content of unfermented and fermented finger millet. The results show that 87.6 and 56.2% of the total LCFA are left after fermentation for 24 and 48 h, respectively. These changes in the LCFA content of the finger millet probably suggest their utilization by the fermenting microbes. The present observation contradicts our earlier report on foxtail millet (Usha Antony et al., 1996). It was found that the fermentation process had no significant effect on the LCFA profile of foxtail millet. The possible explanation

Table 3. Long-Chain Fatty Acid Content[a] of Unfermented and Fermented Finger Millet

	LCFA content (mg/g of dry matter)						
sample	lauric (12:0)	myristic (14:0)	palmitic (16:0)	stearic + oleic (18:0, 18:1)	linoleic (18:2)	linolenic (18:3)	total
unfermented	0.20 ± 0.05	nd[b]	5.29 ± 1.34	8.99 ± 2.28	5.98 ± 1.52	0.89 ± 0.23	21.35 ± 5.05
fermented							
24 h	0.34 ± 0.00	nd	4.44 ± 0.08	7.69 ± 0.03	5.44 ± 0.02	0.80 ± 0.00	18.71 ± 0.20
48 h	0.29 ± 0.03	0.03 ± 0.00	3.00 ± 0.27	5.03 ± 0.46	3.18 ± 0.29	0.52 ± 0.05	12.03 ± 1.09

[a] Mean ± SD, $n = 3$. [b] nd, not detectable.

for this contradiction may be the type of microbes present during the fermentation of different species of millet.

The results also show that the total percentage of saturated LCFA is nearly equal to the total percentage of unsaturated fatty acids (Table 3). The percentage of PUFA is nearly constant (32.2, 33.4, and 30.8% in the unfermented and the 24 and 48 h fermented samples, respectively). This suggests that the nutritionally beneficial fatty acid component (PUFA) is unaffected by fermentation.

Microbial Counts and Controlled Fermentation. The total microbial count increased rapidly during the first 24 h from log cfu/g of 4.4 to 9.9. At 48 h the microbial count was log cfu/g of 9.2, indicating no substantial increase in fermentation in the second 24 h.

An observation of the colony characteristics and microscopic morphology indicated five major groups at 24 h and only one predominant type at 48 h. All appeared to be Gram-positive rods and cocci. Yeasts counts were low, and fungi were not detectable. This indicated the predominance of lactic acid bacteria in this naturally fermented millet. The identification of the microbial isolates is under study and is not reported here. It is of interest to note that this profile of microbial groups and the total colony counts were remarkably consistent with repeated fermentation (using endogenous grain flora) over six times.

When the 18 h slurry and 48 h slurry (5% v/v) were added to the naturally fermenting millet at 0 h, the pH fell to 4.9 in 12 h and 4.7 in 6 h, respectively. This suggests that the fermentation time can be considerably reduced by controlled fermentation, which could be advantageous for further technological development. The changes in the nutrient profile and toxicological effects, however, need to be further studied.

In conclusion, the biochemical changes in the primary nutrients during the fermentation of finger millet by natural grain microflora have been characterized. Changes in starch, reducing sugars, fat, LCFA, free amino acids, and organic acids were observed. The percentage of essential fatty acids remains unchanged as does the total protein content. The 24 and 48 h fermentation time could be reduced to 6−12 h by use of a starter microbial consortium obtained from 18 or 48 h naturally fermented millet. Further investigations are required to assess the changes in tannins, phytates, phenols, vitamins, dietary fiber, amino acid profile, and enzyme inhibitor activity. Our preliminary work on controlled fermentation encourages efforts for new methods of processing to exploit the nutrient potential of this millet.

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