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The effect of fermentation on the primary nutrients in foxtail millet (*Setaria italica*)

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Foxtail millet (*Setaria italica*), consumed by the rural and tribal populations in South India, is a rich source of nutrients. Fermentation by endogenous microflora increased the total soluble sugars and reducing sugars with a simultaneous decrease in the starch content. The protein extractability and albumin/globulin fractions were improved. The beneficial long-chain fatty acid profile of raw flour was retained. Acetic and butyric acid were the major short-chain fatty acids produced. Most of these changes occurred in the first 24 h of fermentation.

INTRODUCTION

Millets play an important role in the traditional diet of people in the semi-arid and arid tropics. Fermentation, a traditional method of food processing, is used extensively in Asia and Africa to process cereals and millets. Table 1 lists some of the popularly consumed fermented foods in these countries. These foods constitute part of the daily food intake of the population. Fermented steamed foods (e.g. 'idli') are considered to be easily digestible and are particularly recommended for growing children and during convalescence. Nutritional enhancement of millets by fermentation is significant in view of the fact they constitute the staple food in these regions.

Millets are consumed whole or hand-pounded, unlike rice and wheat which are milled and polished. Thus, they contribute significantly not only to carbohydrate and protein intake, but also to the vitamin and fibre intake. Fermented foods have a special significance in the diet of a predominantly vegetarian population as in India. In such diets the major source of vitamin B_{12} is from lactic acid fermented foods. Other benefits include the antipathogenic effect during fermentative processing (Hesseltine, 1979, 1983).

In recent years there has been renewed interest in millets for human consumption, as is evident from the release of improved millet cultivars in South Asia. Among the five common millets, foxtail millet is consumed by some rural and tribal populations of South India. The hand-pounded millet is largely consumed after dry or moist heat processing without fermentation, in the form of porridge or bread (like pancake) or steamed (like rice). The possibility of nutritional enhancement by fermentative processing does not appear to be documented.

In the present study, the biochemical changes in the primary nutrients (i.e. carbohydrates, proteins and fats) accompanying fermentation of the millet with endogenous microflora have been analysed. Such a premilinary study can throw light on its potential for the development of a suitable fermented product that can add variety to the predominantly rice-based diet of South India.

MATERIALS AND METHODS

The millet was purchased from the local market. It was cleaned, washed, dried and powdered (100 mesh). Fermentations were carried out with a slurry prepared by mixing 50 g of flour with 100 ml of distilled water in a 500 ml Erlenmeyer flask. The flasks were covered with aluminium foil and left undisturbed in an incubator at 30°C for 48 h. To simulate home conditions of fermentation, the flasks and water were not sterilized. Samples of triplicate fermentations and control were prepared. At the end of fermentation the slurries were frozen, lyophilized and stored at 4°C until analyses of the following:

1. pH and titratable acidity were determined with a 5% aqueous slurry.
2. A 500 mg portion of sample was extracted four times with 20 ml of hot 80% ethanol and the extract evaporated and made up to 10 ml. An aliquot was assayed for total soluble sugars by the phenol–sulphuric acid method (Dubois *et al.*,

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Table 1. Cereal and millet fermented products of India and Africa

Fermented food	Cereal/millet	Type	Country
'Idli'	Rice with black gram dhal	Bread-like, steamed	South India
'Dosai'	Rice with black gram dhal	Pancake-like	South India
'Appam'	Rice	Pancake-like	South India
'Dhokla'	Bengal gram dhal	Bread-like, steamed	West India
'Rabadi'	Wheat/sorghum/pearl millet	Porridge	North India
'Ambali'	Sorghum/pearl millet/finger millet with rice	Porridge	South India
'Injera'	*Tef*	Bread-like	Ethiopia
'Ogi'	Maize/sorghum	Porridge	West Africa
'Kenkey'	Maize	Dumpling	Ghana

1956) and for reducing sugars by the Nelson–Somogyi method (Nelson, 1957). The non-reducing sugar content was obtained by calculation, by subtracting the reducing sugar from the total soluble sugar. The individual free sugars were separated and quantified by high-performance liquid chromatography (Zygmunt, 1982) using a Shimadzu Model 6A system: Shimpack CLC-NH_2 4.6 mm×25 cm column, pressure 165 mm, flow rate 1.5 ml min^{-1}, with a refractive index detector.

3. The alcohol-insoluble residue was analysed for starch content (McCready *et al.*, 1950).
4. The defatted millet was sequentially extracted for the albumin/globulin, prolamin and glutelin fractions based on solubility in organic and aqueous solvents (Monteiro *et al.*, 1982). The nitrogen content was estimated using a Tecator Kjeltec System 1028, and the protein value was calculated using the factor 6.25 (Association of Official Analytical Chemists, 1990).
5. The fat content of the millet samples was estimated gravimetrically after extraction with ether (Association of Official Analytical Chemists, 1990).
6. The short-chain fatty acids (SCFA) were extracted into ether, separated and quantified using a Pye Unicam 4550 gas chromatograph (Cochrane, 1975; Whitehead *et al.*, 1976).
7. The long-chain fatty acids (LCFA) were saponified and extracted into ether and estimated by gas chromatography (Cohen *et al.*, 1969).

RESULTS AND DISCUSSION

Acidity, pH and SCFA

A considerable drop in pH with a simultaneous rise in titratable acidity was noted (Table 2). Production of organic acids during fermentation inhibits the growth of microbes, including pathogens, and enhances the keeping quality (Hesseltine, 1983). Although increased acidity in fermented cereals and millets has been reported, there is little information on the type of organic acids produced. Here, we have attempted to characterize the organic acids responsible for the change.

The predominant SCFA in the fermented millet was acetic acid followed by butyric acid (Fig. 1). Isobutyric and valeric acids were detected in negligible amounts. The difference between the total titratable acidity and SCFA content is presumably due to lactic acid; this is yet to be confirmed. Acetic acid can enhance keeping quality as the acid and its salts are used as antimicrobials in foods (Giese, 1994). The lower fatty acids may contribute to flavour. The only other study which reports on the type of organic acids during millet fermentation is on *tef* (*Eragrostis tef*) (Umeta & Faulks, 1989).

Free sugars and starch

Fermentation of the millet resulted in a marked change in the profile of available carbohydrates (Table 2). The fact that the reducing sugar content increases during

Table 2. Changes in pH, titratable acidity, available carbohydrate protein, fat and short-chain fatty acids (SCFA) of fermented foxtail millet

Biochemical parameters	Unfermented	Fermented	
		24 h	48 h
pH	6.7±0.1	4.7±0.1	4.3±0.2
Titratable acidity (%)	0.8±0.08	1.6±0.08	2.0±0.15
SCFA (%)	0.03±0.003	0.18±0.01	0.22±0.01
Starch (%)	47.3±4.1	43.5±3.9	37.8±3.5
Total soluble sugars (%)	0.81±0.03	1.18±0.05	1.55±0.08
Reducing sugars (%)	0.52±0.04	0.83±0.08	1.11±0.10
Non-reducing sugars (%)	0.29±0.02	0.35±0.03	0.44±0.04
Total protein (%)	10.2±0.4	10.5±0.5	10.5±0.4
Protein extractability (%)	68.5±3.5	75.2±4.1	76.9±4.3
Fat (%)	5.41±0.21	5.36±0.04	5.50±0.14

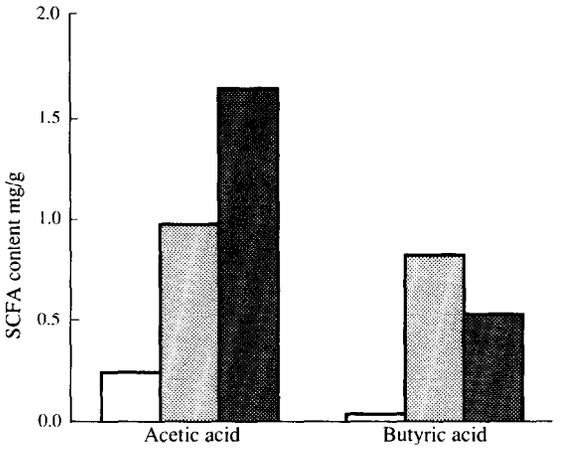

Fig. 1. SCFA content of fermented foxtail millet (No shading: unfermented; light shading: fermented 24 h; dark shading: fermented 48 h.)

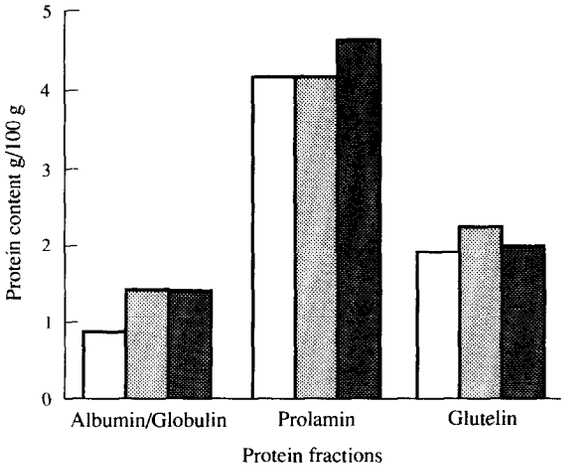

Fig. 2. Protein fraction extracted from fermented foxtail millet (No shading: unfermented; light shading: fermented 24 h; dark shading: fermented 48 h.)

fermentation suggests that starch was utilized as the main energy source by the fermenting flora, resulting in a 10% decrease of starch. Thus, fermentation enhanced the availability of carbohydrate in this millet. Utilization of starch during fermentation of *tef* (Umeta & Faulks, 1988) and pearl millet (Khetarpaul & Chauhan, 1990, 1991*a*) has been reported.

The free sugar contents of foxtail millet before and after fermentation are shown in Table 3. The predominant sugar in both raw and fermented millet was xylose. This is nutritionally beneficial as xylose utilization is insulin-independent (Demetrakopoulos & Amos, 1978). Umeta & Faulks (1988) report the presence of sucrose as the predominant sugar in unfermented *tef*, and fructose on fermentation. Glucose increased markedly after 48 h of fermentation, possibly due to starch hydrolysis. Fructose and sucrose were detected in small amounts.

Protein

The total protein contents of cereals and millets are generally known to be unaffected by fermentation (Chavan & Kadam, 1989). Changes in the quality of proteins based on animal studies (PER, BV, TD and NPU) have been reported in fermented foods (Aliya & Geervani, 1981; Khetarpaul & Chauhan, 1991*b*).

Table 3. Free sugar composition of fermented foxtail millet (mg g^{-1} DM)

Sugars	Unfermented	Fermented	
		24 h	48 h
D-Xylose	4.73	7.74	6.58
D-Fructose	0.16	ND	0.06
D-Glucose	0.31	0.56	3.92
Sucrose	ND	0.36	ND

Values are the mean of two independent estimations.
DM, dry matter; ND, not detectable.

However, biochemical characterization of the types of protein has not been undertaken. The changes in the protein fractions of raw and fermented foxtail millet were determined (Fig. 2). The increased extractability of proteins, particularly the albumin/globulin fraction, indicates increased protein availability. This can make a significant contribution in millet-based diets which are usually high in protein-binding components. These changes may be the result of breakdown of tannins and phytates and increased microbial protease activity.

Fat

The total fat content of the millet was not affected by fermentation (Table 2), as reported in other cereals (Chavan & Kadam, 1989).

Long-chain fatty acids (LCFA)

There are no data in the literature on the LCFA changes during fermentation of foxtail millet. Therefore, the changes in LCFA were monitored (Table 4). The raw

Table 4. Long-chain fatty acid (LCFA) content of fermented foxtail millet (mg g^{-1} DM)

LCFA	Unfermented	Fermented	
		24 h	48 h
Lauric acid ($C_{12:0}$)	0.27	0.07	0.18
Myristic acid ($C_{14:0}$)	ND	0.02	ND
Palmitic acid ($C_{16:0}$)	5.53	5.40	6.23
Stearic acid ($C_{18:0}$) + oleic acid ($C_{18:1}$)	9.98	8.59	9.32
Linoleic acid ($C_{18:2}$)	35.1	34.3	38.5
Linolenic acid ($C_{18:3}$)	0.86	0.87	0.94
Total	50.2	49.2	55.2

Values are the mean of two independent estimations.
DM, dry matter; ND, not detectable.

millet was rich in polyunsaturated fatty acids (PUFA: linoleic acid) and C_{18} fatty acids (oleic and stearic acid). The C_{12}–C_{16} fatty acid levels were low. Among the saturated fatty acids, only C_{14} and C_{16} fatty acids increase the blood cholesterol level, while PUFA are known to lower it. The fatty acid composition of the millet seems nutritionally beneficial. The fact that fermentation did not alter this profile is encouraging (Table 4).

CONCLUSIONS

We report here, for the first time, some of the biochemical changes that accompany the fermentation of foxtail millet by endogenous microflora. The types of organic acids formed during the processing have been characterized. Starch is the major source of energy utilized by the microflora and results in increased total and reducing sugars. The non-reducing sugars remained almost the same. Increased albumin/globulin fraction and total protein extractability suggests improved protein quality which needs confirmation by *in vivo* biological tests. The LCFA profile of the raw millet, which is nutritionally favourable, remains unchanged by fermentation. All these changes occurred within the first 24 h. The biochemical profile of the major nutrients of foxtail millet is suggestive of its nutrient potential. Processing by fermentation is shown to enhance its nutritive value.

The results suggest that millet fermented as above could form a useful food supplement, particularly in the diet of the urban population consuming milled polished rice. The fermented millet could be consumed after boiling in the form of porridge with salt/sugar and/or buttermilk. Alternatively, it could be mixed with other cereals and steamed products such as 'idli', 'dhokla' or 'appam'. The microbiological profile, mineral and vitamin availability and antipathogenic effects seen in other fermented foods need further investigation in this fermented foxtail millet.

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