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Effect of Fermentation by Pure Cultures of Yeasts and Lactobacilli on the Available Carbohydrate Content of Pearl Millet

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ABSTRACT

*Single as well as mixed culture fermentations of pearl millet with yeasts (*Saccharomyces cerevisiae *or* Saccharomyces diastaticus*) and lactobacilli (*Lactobacillus brevis *or* Lactobacillus fermentum*) significantly increased the total soluble sugars, reducing and non-reducing sugar content, with a simultaneous decrease in the starch content, of pearl millet flour.* S. diastaticus *and/or its combination with the lactobacilli hydrolysed the starch content to the maximum and, therefore, resulted in higher concentrations of total soluble, reducing and non-reducing sugars when compared to those of unfermented millet flour.*

INTRODUCTION

Pearl millet (*Pennisetum typhoideum*), a staple food for a large segment of population in several Asian and African countries, is a good source of dietary nutrients. Available carbohydrates of pearl millet comprise starch (amylopectin and amylose), reducing sugars and non-reducing sugars. Pure culture fermentation of pearl millet, a novel method for improving its nutritional value, brings about significant changes in its protein, fat, vitamin and available minerals (Khetarpaul & Chauhan, 1989*a*,*b*). Information about the effect of fermentation on the available carbohydrates of pearl

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millet is lacking. This paper reports the effect of fermentation by pure cultures of yeasts and lactobacilli on the level of starch, total soluble sugars, reducing sugars and non-reducing sugars in pearl millet flour.

MATERIALS AND METHODS

Materials

Pearl millet grains purchased from the local market were cleaned of dust, broken seeds and foreign material. The grains were ground coarsely on the day of fermentation using a 1·5 mm sieve.

Four cultures, *Saccharomyces cerevisiae*, *Saccharomyces diastaticus*, *Lactobacillus brevis* and *Lactobacillus fermentum*, were obtained from the Director, National Chemical Laboratory, Poona, India.

Fermentation

The coarsely ground pearl millet (100 g) prepared with an electric grinder (Sumeet, M/s Power Control & Appliances Pvt., Ltd, Bombay, India) and sifted through a 1·5 mm sieve was mixed with water (900 ml), autoclaved at 1·05 kg/cm^2 pressure for 15 min, cooled, inoculated with 10^5 cells/ml of yeast and/or lactobacillus and incubated at 30°C for 72 h. The inocula of yeast and lactobacilli were grown on yeast extract peptone dextrose agar (YEPDA) and MRS medium, respectively, in Roux bottles and transferred to a known volume of sterile saline solution. Different standard dilutions were made and their optical densities and the cell counts by the plate count method were estimated. A particular dilution, providing 10^5 cells/ml in the fermenting mixture of the yeast or lactobacillus, was employed for inoculation. Fresh inocula of the specific optical density providing 10^5 cells/ml of the yeast or lactobacillus were prepared daily from the actively growing cells in the Roux bottles. In case of single culture fermentation, the inoculum of any one of the yeasts or lactobacilli supplied 10^5 cells/ml to the fermenting mixture whereas, in the mixed culture fermentations, the inoculum provided 10^5 cells of the yeast as well as 10^5 cells of the lactobacilli per millilitre of the fermenting 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). Raw pearl millet flour and the autoclaved unfermented millet flour served as the controls. About 20 ml of the fermented as well as unfermented sample was taken out for pH and titratable acidity (TA) determinations (Amerine *et al.*, 1967). The remainder of the fermented as well as unfermented samples

were dried in an air-oven 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 a 0·5 mm sieve.

Chemical analysis

Total soluble sugars were extracted by refluxing in 80% ethanol (Cerning & Guilbot, 1973). Starch, from the sugar free pellet, was extracted in 52% perchloric acid at room temperature (Clegg, 1956). Quantitative determination of total soluble sugars and starch was carried out according to the colorimetric method of Yemm and Willis (1954). Reducing sugars were estimated by Somogyi's modified method (Somogyi, 1945). Non-reducing sugars were determined by calculating the differences between total soluble sugars and reducing sugars.

Statistical analysis

The data were subjected to analysis of variance in a completely randomised design to estimate the significant differences among various treatments (Panse & Sukhatme, 1961).

RESULTS AND DISCUSSION

Titratable acidity and pH

A significant decrease in pH and simultaneous increase in titratable acidity was noticed when the pearl millet flour was fermented by pure cultures of yeasts and lactobacilli at 30°C for 72 h (Table 1). The highest titratable acidity in single culture fermentation was brought about by *Lactobacillus fermentum* followed by *L. brevis*, *S. diastaticus* and *S. cerevisiae*. Among all the single and mixed culture fermentation combinations, SdLf combination brought about the lowest pH and the highest titratable acidity, as reported in various other fermented grains (Nanson & Fields, 1984; Venkatasubbaiah *et al.*, 1984).

Available carbohydrate content

Autoclaving resulted in significant ($P < 0·05$) reduction in the starch content of pearl millet flour, which on fermentation was further reduced significantly ($P < 0·05$) (Table 2). Single culture fermentations by *S. diastaticus*, *S. cerevisiae*, *L. brevis* and *L. fermentum* brought about a marked decrease in

TABLE 1
Changes in pH and Titratable Acidity (g lactic acid per 100 ml) During Pure Culture Fermentation of Pearl Millet Flour[a]

Treatment	*pH*	*Titratable acidity*
Control		
Autoclaved unfermented pearl millet	6·42 ± 0·00	0·55 ± 0·00
Single fermentation		
S. diastaticus (Sd)	4·80 ± 0·01	1·12 ± 0·04
S. cerevisiae (Sc)	5·65 ± 0·04	0·82 ± 0·08
L. brevis (Lb)	4·54 ± 0·01	1·18 ± 0·02
L. fermentum (Lf)	4·41 ± 0·01	1·50 ± 0·05
Mixed fermentation		
Sd + Lb (SdLb)	4·39 ± 0·01	1·31 ± 0·04
Sd + Lf (SdLf)	4·04 ± 0·01	1·95 ± 0·04
Sc + Lb (ScLb)	4·38 ± 0·01	1·35 ± 0·00
Sc + Lf (ScLf)	4·36 ± 0·02	1·48 ± 0·01
SEM	±0·01	±0·02
CD ($P < 0·05$)[b]	0·03	0·06

[a] Values are means ± SD of four replicates.
[b] Critical difference at 5% level. Differences of two means within/between the treatments exceeding this value are significant.

starch content of the millet flour. Yeasts were more effective in reducing the starch content. Among all the microorganisms studied, *S. diastaticus* appeared to be more influential as it is a starch-hydrolysing yeast (Kreger-van Rij, 1984). Mixed culture fermentations also brought about a significant ($P < 0·05$) reduction in the starch content. The combination of *S. diastaticus* with *L. brevis* or *L. fermentum* lowered the starch level more effectively than the combinations of *S. cerevisiae* with the lactobacilli; Sd combinations, with both lactobacilli, did not, however, differ significantly among themselves.

When the raw pearl millet flour was autoclaved, there was a significant ($P < 0·05$) increase in total soluble sugars, reducing and non-reducing sugars (Table 2). Single culture fermentation of this autoclaved flour resulted in a significant ($P < 0·05$) reduction in the total soluble sugars. The extent of decrease in total soluble sugars, reducing and non-reducing sugars was greater in millet flour fermented by lactobacilli than the yeasts. On the other hand, single culture fermentation with yeasts and lactobacilli brought about a significant ($P < 0·05$) increase in the total soluble, reducing and non-reducing sugars when compared to raw pearl millet flour; the resultant total soluble sugar content was highest in the flour fermented by *S. diastaticus* followed by *S. cerevisiae*, *L. brevis* and *L. fermentum*. The reducing sugar content was a maximum in the millet flour fermented by *S. diastaticus* and

TABLE 2
Effect of Pure and Mixed Culture Fermentations on Total Soluble Sugars, Reducing Sugars, Non-reducing Sugars and Starch Content of Pearl Millet Flour[a]

Treatment	*Total soluble sugars*	*Reducing sugars*	*Non-reducing sugars*	*Starch*
Control				
Raw pearl millet	1·76 ± 0·06	0·36 ± 0·02	1·40 ± 0·07	68·5 ± 0·32
Autoclaved unfermented pearl millet	4·52 ± 0·08	1·22 ± 0·02	3·30 ± 0·08	63·3 ± 1·55
Single fermentation				
S. diastaticus (Sd)	3·09 ± 0·10	2·01 ± 0·00	1·08 ± 0·10	50·4 ± 1·63
S. cerevisiae (Sc)	2·82 ± 0·02	0·66 ± 0·03	2·16 ± 0·04	54·2 ± 3·60
L. brevis (Lb)	1·82 ± 0·00	0·24 ± 0·02	1·58 ± 0·02	59·3 ± 1·68
L. fermentum (Lf)	1·77 ± 0·00	0·75 ± 0·03	1·02 ± 0·01	60·2 ± 0·32
Mixed fermentation				
Sd + Lb (SdLb)	4·27 ± 0·27	1·27 ± 0·03	3·00 ± 0·28	50·8 ± 1·82
Sd + Lf (SdLf)	4·11 ± 0·13	0·90 ± 0·01	3·21 ± 0·12	51·0 ± 0·22
Sc + Lb (ScLb)	2·71 ± 0·07	0·90 ± 0·02	1·81 ± 0·06	56·7 ± 1·60
Sc + Lf (ScLf)	2·62 ± 0·01	0·61 ± 0·03	2·01 ± 0·05	57·6 ± 2·20
SEM	±0·06	±0·01	±0·06	±1·03
CD ($P < 0·05$)[b]	0·18	0·03	0·18	3·09

[a] Values are means ± SD of four replicates (g/100 g, on dry matter basis).
[b] Critical difference at 5% level. Differences of two means within/between the treatments exceeding this value are significant.

the lowest in *L. brevis*-fermented flour whereas the *S. cerevisiae*-fermented flour contained the highest amount of non-reducing sugars.

Mixed culture fermentations by the yeasts and lactobacilli combinations also brought down the total soluble sugar content of autoclaved pearl millet flour significantly ($P < 0·05$); maximum reduction was in *S. cerevisiae* combinations with the *lactobacilli* rather than the *S. diastaticus* combinations. During the mixed fermentation, SdLf, ScLb and ScLf combinations had lower amounts of reducing sugars whereas SdLb combination had significantly ($P < 0·05$) higher amount of reducing sugars when compared to autoclaved unfermented pearl millet flour. SdLb combination showed a significant reduction in starch content compared to the uninoculated, autoclaved control even though the total soluble sugars, reducing and non-reducing sugars did not differ significantly. Following starch degradation by amylase content of *S. diastaticus* yeast, the initial concentration of the sugars might have been increased in SdLb-fermented flour. After prolonged fermentation, for 72 h, these sugars might have been utilised by the microflora as a carbon source, finally leading to a non-significant difference

in the total soluble sugar content of the SdLb-fermented and autoclaved unfermented control. As compared to raw pearl millet flour, all the mixed culture fermentation combinations contained significantly higher amounts of reducing sugars. After fermentation, all mixed fermentation groups, except SdLf, had significantly ($P < 0{\cdot}05$) lower concentrations of non-reducing sugars than the autoclaved unfermented control.

Fermentation with the pure culture of *S. diastaticus* resulted in higher levels of reducing sugars than the mixed cultures with lactobacilli. *S. diastaticus* possesses amylase, which is responsible for enhanced amylolysis, and the increased level of reducing sugars. In mixed culture fermentation, both the microorganisms, i.e. yeast and lactobacillus, might have utilised a greater amount of soluble sugars than *S. diastaticus* alone, which may account for the greater amount of soluble sugars in *S. diastaticus* fermentation than mixed culture fermentation.

Reduction of starch in the fermented product may be attributed to amylolytic action of microorganisms in the fermenting mixture. Fermenting microbes have been reported to possess both alpha and beta amylases (Bernfeld, 1962). Dhankher (1985) also found a reduction in starch content of *rabadi*—a pearl millet fermented food—after 9 h fermentation at all the temperature studied. Taur *et al.* (1984) reported a maximum starch degradation by amylases during fermentation of sorghum and found an inverse relationship between starch content and fermentation period. Similar reductions in starch content of food legumes during fermentation have been reported earlier (Zamora & Fields, 1979; Odunfa, 1983).

Moist heating of pearl millet flour might degrade starch and lower the starch content in the autoclaved flour and, thereby, increase the amount of total soluble sugars. In the initial stages of fermentation, higher concentrations of soluble sugars may be seen but during prolonged fermentation these sugars may be utilised and the fermented product may contain lower sugar levels than the initial concentration of sugars in the fermenting mixtures. Odunfa (1983) and Mahajan (1986) also reported an initial increase in the reducing sugar due to hydrolysis of starch and oligosaccharides present in the unfermented sample and a decrease at later stages of fermentation due to utilisation of sugars by the fermenting microflora.

Hence, fermentation brought about a significant change in the total soluble sugars, reducing sugars, non-reducing sugars and starch content of pearl millet flour. Fermented grains had a lower amount of starch and a higher content of soluble and reducing sugars than that of the unprocessed grains. This type of fermented product may have better starch digestibility, too, which needs to be investigated.

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