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Technical Note

Sequential Fermentation of Pearl Millet by Yeasts and Lactobacilli: Changes in Available Carbohydrates Content

ABSTRACT

*Sequential culture fermentation with yeasts (*Saccharomyces cerevisiae *or* Saccharomyces diastaticus*) followed by lactobacilli (*Lactobacillus brevis *or* Lactobacillus fermentum*) (at 30°C for 72 h each) significantly reduced the starch content of pearl millet flour. Starch content was the lowest when fermentation was carried out by* S. diastaticus *followed by* L. brevis. *All the sequential fementations significantly reduced the amount of total soluble, reducing and non-reducing sugars which may be ascribed to utilisation of sugars by the fermenting microflora.*

INTRODUCTION

Available carbohydrates of pearl millet (*Pennisetum typhoideum*), a staple food for a large segment of population in Asian and African countries, consist of starch and smaller amounts of soluble reducing and non-reducing sugars. Amylopectin (67·9%) is the major constituent of pearl millet starch. Fermentation by yeasts and lactobacilli has been reported to affect the protein, fat, mineral and vitamin contents of pearl millet flour (Khetarpaul & Chauhan, 1989). Information regarding the effect of sequential culture fermentation of yeasts and lactobacilli on the level of available carbohydrates of pearl millet is still lacking. This paper reports the effect of sequential culture fermentation by yeasts and lactobacilli on the level of starch, total soluble sugars, reducing sugars and non-reducing sugars in pearl millet flour.

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MATERIALS AND METHODS

Materials

For fermentation, pearl millet grains were purchased from the local market in a single lot and were cleaned of dust, broken seeds and other foreign matter. The cultures of *Saccharomyces cerevisiae*, *Saccharomyces diastaticus*, *Lactobacillus brevis* and *Lactobacillus fermentum* were procured from the Director, National Chemical Laboratory, Poona, India.

Fermentation

Pearl millet grains were coarsely ground in an electric grinder (Sumeet, M/s Power Control & Appliances Pvt. Ltd, Bombay, India) on the day of fermentation. Coarsely ground pearl millet flour (100 g) was mixed with distilled water (900 ml), autoclaved (15 psi for 15 min) and cooled. The inocula of yeasts 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 optimal 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 was employed for inoculation in the cooled autoclaved slurry and incubated at 30°C for 72 h. After the yeast fermentation was over, the yeast fermented slurry was again inoculated with lactobacillus spp. providing 10^5 lactobacilli cells/ml to the fermenting slurry and inoculated at 30°C for 72 h. Thus, the total fermentation period was 144 h. 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 bottle. Four different combinations of sequential culture fermentations included *S. diastaticus* followed by *L. brevis* (Sd + Lb), *S. diastaticus* followed by *L. fermentum* (Sd + Lf), *S. cerevisiae* followed by *L. brevis* (Sc + Lb) and *S. cerevisiae* followed by *L. fermentum* (Sc + Lf). 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 the determination of pH and titratable acidity (TA) determinations (Amerine *et al.*, 1967). The rest of the sample was oven-dried in an air-oven for 48 h at 65°C to a constant weight and 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 & 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 & Sukhatma, 1961).

RESULTS AND DISCUSSION

Titratable acidity and pH

The sequential culture fermentations by yeasts and lactobacilli resulted in a significant drop in pH with a simultaneous rise in titratable acidity (Table 1). Among all the sequential culture combinations, the Sd + Lf combination brought about the lowest pH and the highest titratable acidity followed by the Sd + Lb, Sc + Lf and Sc + Lb combinations. Fermentation has been

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

Treatment	*pH*	*Titratable acidity*
Sequential fermentation		
S. diastaticus + *L. brevis* (Sd + Lb)	3·99 ± 0·01	1·95 ± 0·00
S. diastaticus + *L. fermentum* (Sd + Lf)	3·73 ± 0·02	2·74 ± 0·04
S. cerevisiae + *L. brevis* (Sc + Lb)	4·26 ± 0·00	1·58 ± 0·03
S. cerevisiae + *L. fermentum* (Sc + Lf)	4·02 ± 0·00	1·97 ± 0·00
Control		
Autoclaved unfermented flour	6·42 ± 0·00	0·35 ± 0·00
SE(m)[b]	± 0·01	± 0·02
CD ($P < 0·05$)[c]	0·03	0·06

[a] Values are means ± SD of four replicates.
[b] Standard error of means.
[c] Critical difference. Differences of two means within/between the treatments exceeding this value are significant.

known to decrease pH and increase titratable acidity in several foods (Nanson & Fields, 1984; Venkatasubbaiah *et al.*, 1984; Gupta, 1989).

Available carbohydrate content

Autoclaving significantly ($P < 0.05$) increased the concentration of total soluble sugars, reducing and non-reducing sugars (Table 2). When the autoclaved slurry was further fermented sequentially with pure cultures of yeasts and lactobacilli, a significant ($P < 0.05$) reduction in total soluble sugars occurred; the Sd + Lf combination produced the maximum reduction followed by the Sc + Lb, Sd + Lb and Sc + Lf combinations. During sequential fermentation, reducing and non-reducing sugars also decreased and the extent of reduction varied significantly among the various fermentation combinations. During the sequential culture fermentation, the Sd + Lb, Sd + Lf and Sc + Lb combinations lowered the non-reducing sugars.

Autoclaving resulted in a significant ($P < 0.05$) reduction in the starch content of pearl millet (Table 2) which on fermentation was further

TABLE 2

Effect of Sequential Culture Fermentation by Yeasts and Lactobacilli on Total Soluble Sugars, Reducing Sugars, Non-Reducing Sugars and Starch Content of Pearl Millet Flour (g/100 g, on dry matter basis)[a]

Treatment	*Total soluble sugars*	*Reducing sugars*	*Non-reducing sugars*	*Starch*
Sequential fermentation				
S. diastaticus + *L. brevis* (Sd + Lb)	0·94 ± 0·02	0·09 ± 0·00	0·85 ± 0·02	27·1 ± 0·67
S. diastaticus + *L. fermentum* (Sd + Lf)	0·66 ± 0·01	0·13 ± 0·01	0·53 ± 0·02	37·1 ± 0·52
S. cerevisiae + *L. brevis* (Sc + Lb)	0·85 ± 0·05	0·04 ± 0·00	0·81 ± 0·05	36·1 ± 2·13
S. cerevisiae + *L. fermentum* (Sc + Lf)	1·79 ± 0·14	0·22 ± 0·01	1·57 ± 0·13	37·6 ± 2·65
Control				
Raw pearl millet flour	1·76 ± 0·06	0·36 ± 0·02	1·40 ± 0·07	68·5 ± 0·32
Autoclaved unfermented flour	4·52 ± 0·08	1·22 ± 0·02	3·30 ± 0·08	63·3 ± 1·55
SE(m)	± 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.

[b] Critical difference. Differences of two means within/between the treatments exceeding this value are significant.

significantly reduced ($P < 0{\cdot}05$). The flour fermented by *S. diastaticus* followed by *L. brevis* (Sd + Lb) combination had maximum reduction in starch content followed in descending order by Sc + Lb, Sd + Lf and Sc + Lf combinations. The fermentation by *S. diastaticus* or *S. cerevisieae* with both the lactobacilli resulted in a significant reduction in starch content.

Fermenting microbes have been reported to possess both alpha and beta amylases (Bernfeld, 1962). Amylolytic action of the fermenting microbes may account for reduced starch content of the fermented product. Amylolysis during fermentation has been reported in a number of food grains including pearl millet (Khetarpaul, 1988). sorghum (Taur *et al.*, 1984) and food legumes (Zamora & Fields, 1979; Odunfa, 1983). In addition, moist heating of pearl millet flour during autoclaving might degrade the starch, and thereby lower the starch content and increase the total soluble sugars. The soluble sugars in the fermenting mixture may be utilised by the microflora as a carbon source and the fermented product may ultimately contain a level of sugars lower than that of the autoclaved pearl millet flour.

Hamad and Fields (1979) reported that during the natural lactic fermentation of cereals, the level of reducing sugars increased 4·38 times on the first day but decreased on the second and third days and this increase and decrease was attributed to the action of microflora during fermentation. Odunfa (1983) also reported an initial increase in the reducing sugar due to hydrolysis of starch and oligosaccharides present in the unfermented sample and decrease in later stages of fermentation due to utilisation of sugars by the fermenting microflora.

Conclusively, sequential culture fermentation by yeasts and lactobacilli for 72 h each at 30°C brought about a significant change in the profile of available carbohydrates, i.e. total soluble sugars, reducing and non-reducing sugars and starch content of pearl millet flour. The fermented product had lower amounts of starch and a higher concentration of non-reducing sugars as compared to that of unprocessed grain. This type of fermented product may have a better starch digestibility too, which needs to be investigated. Since carbohydrate-rich diets have been known to result in hyperlipidemia and hyperglycemia, the therapeutic value of a diet containing such a fermented food containing low carbohydrates should not be underestimated.

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