Food Chemistry **38** (1990) 21–26

Effect of Germination and Fermentation on Available Carhohydrate Content of Pearl Millet

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(Received 30 May 1989; revised version received and accepted 24 October 1989)

ABSTRACT

Germination significantly increased the total soluble sugars, reducing and non-reducing sugar contents of pearl millet with a parallel decrease in its starch content. Fermentation of the sprouts with pure cultures of yeasts (Saccharomyces cerevisiae *or* Saccharomyces diastaticus*) and lactobacilli (* Lactobacillus brevis *or* Lactobacillus fermentum*) further reduced its starch content. Among the various fermentation combinations studied, sprouts fermented with* S. diastaticus *and* L. brevis *combinations had the lowest amounts of starch and the maximum amounts of total soluble and reducing sugars. Other combinations for fermentation of the sprouts also had higher concentrations of total soluble, reducing and non-reducing sugars than those of the unprocessed pearl millet but less when compared with the sprouts alone, perhaps due to utilisation of sugars by the fermenting microflora.*

INTRODUCTION

Pearl millet (*Pennisetum typhoideum*), an important staple dietary constituent of the population in developing countries, is a good and inexpensive source of dietary calories, protein and minerals. Carbohydrates are the major contributors of calories in pearl millet grain where they constitute about 65·4 to 71·2% of the grain (Singh & Popli, 1973). Of the

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Food Chemistry 0308-8146/90/$03·50 © 1990 Elsevier Science Publishers Ltd, England. Printed in Great Britain

available carbohydrates present, starch is the major constituent (56·3 to 63·7%) which is composed of amylose (30·1%) and amylopectin (69·9%). Sugars are present in lower amounts, e.g. 1·2%. Different domestic processing and cooking methods, including soaking, pressure cooking, autoclaving, sprouting etc., have been known to affect the levels of available carbohydrates in food legumes and grains (Jood *et al.*, 1986, 1988; Kumar, 1989).

Germination and fermentation of pearl millet flour has been reported to affect its protein, fat, mineral and vitamin contents (Khetarpaul & Chauhan, 1989). This paper reports the effect of germination and/or fermentation on the level of available carbohydrates including starch, total soluble sugars, reducing sugars and non-reducing sugars.

MATERIALS AND METHODS

Materials

Pearl millet grains were procured from the local market and were cleaned of dust, broken seeds and other foreign material. The cultures of *Saccharomyces cerevisiae*, *Saccharomyces diastaticus*, *Lactobacillus brevis* and *Lactobacillus fermentum* were procured from the Director, National Chemical Laboratory, Poona, India.

Germination

Pearl millet grains were soaked in distilled water overnight (12 h, 30°C). The grains were then sprouted on wet filter paper (24 h, 30°C) in Petri dishes, dried at 65°C to a constant weight, and ground for analysis. Other samples (100 g) were ground to a slurry in distilled water (200 ml), and after removing 20 ml for titratable acidity (as lactic acid) (Amerine *et al.*, 1967) and pH, the rest was autoclaved (20 min, 5 psi), oven-dried (48 h, 65°C) to constant weight and finely ground (0·5 mm sieve).

Fermentation

The cooked autoclaved samples were inoculated with mixtures of actively growing cells of *Saccharomyces cerevisiae* (Sc) or *S. diastaticus* (Sd) and *Lactobacillus brevis* (Lb) or *Lactobacillus fermentum* (Lf) to give 10^5 cells/ml. All were incubated (72 h, 30°C), after which a sample (20 ml) was taken for pH and acidity, and the rest was dried and finely ground as above. Unsprouted cleaned grains were milled as a control.

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 as the differences between total soluble sugars and reducing sugars.

Statistical analysis

The data were statistically analysed for analysis of variance to know the significant differences among various treatments (Panse & Sukhatme, 1961).

RESULTS AND DISCUSSION

Titratable acidity and pH

The initial pH (6·42) dropped slightly on sprouting (5·89), but much further on fermentation (Table 1), as lactic acid produced by the hetero-fermentative organisms increased titratable acidity, as commonly occurs in fermenting grains (Nanson & Fields, 1984; Venkatasubbaiah *et al.*, 1984).

Available carbohydrate content

The concentration of total soluble sugars, reducing and non-reducing sugars increased significantly ($P < 0{\cdot}05$) during germination (Table 2). When the germinated slurry was further processed for homogenisation and autoclaving, a further significant ($P < 0{\cdot}05$) increase in soluble sugars and a decrease in starch content was observed. The increased sugar content of pearl millet during germination and autoclaving may be due to hydrolysis of starch, thereby resulting in release and higher concentration of soluble sugars. Choi (1984) reported that starch in the endosperm was degraded slowly during the course of germination and, with the degradation of starch, the sugar levels were elevated during the period of germination. Rise in reducing sugars may be due to mobilization and hydrolysis of seed polysaccharides, leading to more available reducing sugars. Rapid amylolysis might yield significant amounts of maltose, a reducing sugar. Increased levels of total soluble sugars, reducing sugars and non-reducing sugars during germination have been

TABLE 1
Effect of Germination and Fermentation on pH and Titratable Acidity (g lactic acid/ 100 ml) of Pearl Millet[a]

Treatment	*pH*	*Titratable acidity*
Raw	6·42 ± 0·01	0·55 ± 0·01
Germinated	5·89 ± 0·01	1·27 ± 0·01
Germinated, homogenised and autoclaved	5·89 ± 0·01	1·27 ± 0·01
+ *S. diastaticus* + *L. brevis*	3·67 ± 0·01	2·10 ± 0·0
+ *S. diastaticus* + *L. fermentum*	3·62 ± 0·01	3·31 ± 0·02
+ *S. cerevisiae* + *L. brevis*	3·91 ± 0·0	2·02 ± 0·0
+ *S. cerevisiae* + *L. fermentum*	3·85 ± 0·01	3·23 ± 0·01
CD ($P < 0·05$)[b]	0·015	0·024

[a] Values are means of four replicates.
[b] Critical difference, significant when exceeded.

TABLE 2
Effect of Germination and Fermentation of Sprouted Pearl Millet with Yeasts (Saccharomyces) and Lactobacilli on Available Carbohydrate Content (g/100 g, on dry matter basis)[a]

Treatment	*Total soluble sugars*	*Reducing sugars*	*Non-reducing sugars*	*Starch*
Raw pearl millet flour	1·76 ± 0·06	0·36 ± 0·02	1·40 ± 0·07	68·5 ± 0·32
Germinated	6·13 ± 0·39	3·43 ± 0·29	2·70 ± 0·41	60·3 ± 3·20
Germinated, homogenised and autoclaved	16·3 ± 0·84	6·54 ± 0·12	9·80 ± 0·88	50·2 ± 0·62
+ *S. diastaticus* + *L. brevis*	11·8 ± 0·14	7·25 ± 0·28	4·55 ± 0·42	38·5 ± 0·49
+ *S. diastaticus* + *L. fermentum*	4·2 ± 0·47	0·51 ± 0·04	3·69 ± 0·48	39·6 ± 0·24
+ *S. cerevisiae* + *L. brevis*	5·8 ± 0·28	2·84 ± 0·04	2·96 ± 0·33	42·8 ± 0·98
+ *S. cerevisiae* + *L. fermentum*	4·0 ± 0·71	1·55 ± 0·04	2·45 ± 0·74	41·4 ± 1·31
CD ($P < 0·05$)[b]	0·99	0·24	1·05	1·41

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

reported in chickpea, black gram (Jood *et al.*, 1988), mung beans (Kataria & Chauhan, 1988) and pearl millet (Kumar, 1989).

A further significant ($P < 0{\cdot}05$) reduction in starch content was observed when the sprouts were fermented with different combinations of yeasts and lactobacilli. Starch content of the *S. diastaticus* combinations with both *L. brevis* and *L. fermentum* showed more reduction than the combinations of *Lactobacilli* with *S. cerevisiae*; of all the combinations, the sprouts fermented with *S. diastaticus* and *L. brevis* had the lowest amount of starch. The hydrolysis of starch content, as a result of germination and/or fermentation, resulted in an increase in the total soluble, reducing and non-reducing sugars. The sprouts fermented with the *S. diastaticus* and *L. brevis* combination had the maximum amount of total soluble, reducing and non-reducing sugars. Other combinations also had higher concentrations of total soluble, reducing and non-reducing sugars than the raw pearl millet but less when compared with germinated grain. 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). Amylolysis during germination followed by fermentation has been reported in a number of food grains including pearl millet (Khetarpaul, 1988) and sorghum (Taur *et al.*, 1984).

In the initial stages of fermentation, higher concentrations of the soluble sugars may be seen but with passage of the period of fermentation, these sugars may be utilised and the fermented product may have a sugar level which is lower than the initial concentration of sugars in the germinated and/or fermenting mixture. Hamad and Fields (1979) reported the similar finding that, during the natural lactic acid 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.

Germination, as well as its combination with pure culture fermentation, brought about a significant change in the profile of available carbohydrates in the pearl millet. Fermented sprouts had lower amounts of starch and higher concentrations of soluble sugars as compared with unprocessed pearl millet grain. This type of fermented product may have therapeutic application in the diet of people requiring low levels of available carbohydrates (low glycemic index), particularly where this coarse grain is a staple food.

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