Food Chemistry **39** (1991) 347–355

Effect of Pure Sequential Culture Fermentation by Yeasts and Lactobacilli on HCl-Extractability of Minerals from Pearl Millet

Neelam Khetarpaul & B. M. Chauhan*

Department of Foods and Nutrition, Haryana Agricultural University, Hisar-125 004, India

(Received 8 January 1990; revised version received and accepted 29 March 1990)

ABSTRACT

*Sequential culture fermentation of pearl millet flour first by yeasts (*Saccharomyces diastaticus; Saccharomyces cerevisiae*) at 30°C for 72 h and secondly by* lactobacilli *(*Lactobacillus brevis; Lactobacillus fermentum*) at 30°C for 72 h brought about a significant increase in inorganic, HCl-extractable and non-phytate phosphorus with a corresponding decline in phytate phosphorus. The HCl-extractabilities of calcium, iron, zinc, copper and manganese from the fermented millet flour were also improved. Higher HCl-extractability of the minerals may be partly ascribed to the decreased content of phytic acid as a significant negative correlation between the phytic acid and HCl-extractability of dietary essential minerals was obtained.*

INTRODUCTION

The mineral profile of pearl millet (*Pennisetum typhoideum*), a staple food for a large segment of the population in many developing countries, is relatively superior to other cereals (Dhillon *et al.*, 1982; Kumar & Kapoor, 1984; Chauhan *et al.*, 1986). But the availability of these dietary essential minerals from pearl millet to the human system may be poor (Nolan & Duffin, 1987; Mahajan & Chauhan, 1987) due to the presence of considerable amounts of antinutrients including phytic acid and polyphenols (Chauhan *et al.*, 1986;

*To whom correspondence should be addressed.

Food Chemistry 0308-8146/90/$03·50

Dhankher & Chauhan, 1987; Khetarpaul, 1988) as they complex with the divalent cations. Solubility of minerals in foodstuffs subjected to in-vitro gastric simulated conditions is indicative of their bioavailability from the foodstuffs (Lock & Bender, 1980; Wien & Schwartz, 1985; Kim & Zemel, 1986).

Previous work from our laboratory (Mahajan & Chauhan, 1987; Khetarpaul, 1988) has shown that natural fermentation significantly reduced the levels of phytic acid and polyphenols and improved the HCl-extractability of minerals. As yeasts and lactobacilli were found to be dominating microorganisms in the naturally fermented pearl millet flour (Mahajan, 1986; Khetarpaul & Chauhan, 1989*a*), an attempt has been made to study the effect of pure sequential culture fermentation by yeasts and lactobacilli on the HCl-extractability of minerals in 0·03N HCl, the concentration of the acid found in gastric contents of the human stomach.

MATERIALS AND METHODS

Materials

Pearl millet grains (50 kg), procured from the local market in a single lot were cleaned of broken seeds, dust and other foreign materials and were coarsely ground (1·5 mm sieve size) in an electric grinder on the day of fermentation.

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

Pearl millet flour (100 g) was mixed with distilled water (900 ml), autoclaved (15 psi for 15 min), cooled and inoculated with the above cultures to carry out sequential culture fermentation in triplicate.

In sequential culture fermentation, first, yeast inoculum providing 10^5 cells/ml of the fermenting mixture was used and the samples were fermented at 30°C for 72 h. After the expiry of the first fermentation, the same sample was inoculated with *Lactobacillus* spp. (10^5 cells/ml) and again fermented at 30°C for 72 h more. Thus, the total fermentation period was 144 h. Four different types 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). The autoclaved unfermented pearl millet served as the control. The fermented, as well as unfermented, control samples were oven-dried at 65°C for 48 h to a constant weight. They were finely ground in the cyclone mill (Cyclotec, M/s Tecator, Höganäs, Sweden) using a 0·5 mm sieve.

Phytate P, non-phytate P and inorganic P

The samples were extracted in 0·2M HCl with continuous shaking for 3 h in a mechanical shaker at room temperature and phytic acid in the extract was estimated colorimetrically (Haug & Lantzsch, 1983).

Phytate phosphorus was derived by using the following formula (Reddy *et al.*, 1982):

$$\text{Phytate phosphorus (mg)} = \frac{A \times 28{\cdot}18}{100}$$

where A = Phytate content (mg)

Non-phytate phosphorus was calculated as a difference between the total phosphorus and phytate phosphorus.

For determining inorganic phosphorus, a 1 g sample was extracted in 20 ml distilled water by shaking on a rotary shaker for 3 h at room temperature. It was filtered through Whatman No. 42 filter paper and inorganic P in the filtrate was determined colorimetrically (Chen *et al.*, 1956).

Total minerals

The samples were wet acid-digested using a nitric acid and perchloric acid mixture (HNO_3:$HClO_4$, 5:1 v/v). The amounts of iron, copper, zinc and manganese in the digested sample were determined by atomic absorption spectrophotometry (Lindsey & Norwell, 1969). Calcium in the digested samples was estimated by the titration method (Vogel, 1962) whereas phosphorus was determined colorimetrically (Chen *et al.*, 1956).

HCl-extractable minerals

The minerals in the fermented samples were extracted with 0·03N HCl by shaking the contents at 37°C for 3 h. The clear extract obtained after filtration with Whatman No. 42 filter paper was oven-dried at 100°C and wet acid-digested. The amounts of the extractable phosphorus, calcium, iron, zinc, copper and manganese in the digested samples were determined by the methods described above for estimation of total amounts of the minerals.

$$\text{Mineral extractability (\%)} = \frac{\text{Mineral extractable in 0·03N HCl}}{\text{Total mineral}} \times 100$$

Statistical analysis

The data were subjected to analysis of variance and correlation coefficients were derived in a completely randomised design (Panse & Sukhatme, 1961).

RESULTS AND DISCUSSION

Phytate, non-phytate, HCl-extractable and inorganic P

The unfermented pearl millet flour contained phytate P, non-phytate P and inorganic P, constituting 57, 43 and 9% of total phosphorus, respectively. All the sequential culture fermentation combinations of yeasts and lactobacilli resulted in a significant ($P < 0.05$) decline in phytate P with a corresponding significant ($P < 0.05$) increase in non-phytate, inorganic and HCl-extractable P (Table 1). Sequential culture fermentation combinations of *S. diastaticus* with *L. brevis* or *L. fermentum* produced a greater reduction in phytate P than *S. cerevisiae* combination with either of these lactobacilli. Of all the four fermentation combinations, *S. diastaticus* followed by *L. brevis* (Sd + Lb) appeared to be most effective for lowering phytate P and raising the non-phytate P; phytate P was completely eliminated in the millet flour fermented by Sd + Lb combination. The increase in inorganic P was the highest when sequential fermentation was carried out by Sd + Lb combination followed by that of Sc + Lb, Sc + Lf and Sd + Lf fermentation combinations; the inorganic P content was increased more than threefold in flour fermented by Sd + Lb combination than the control. HCl extracta-

TABLE 1

Effect of Pure Sequential Culture Fermentation on Phytate P, Non-Phytate P, Inorganic P (% of total P) and HCl-Extractability of P (%) of Pearl Millet Flour[a]

Treatments	*Phytate P*	*Non-phytate P*	*Inorganic P*	*P Extractability*
Sequential fermentation				
S. diastaticus + *L. brevis* (Sd + Lb)	00·0 ± 0·00	100·0 ± 0·00	30·8 ± 0·10	60·6 ± 0·47
S. diastaticus + *L. fermentum* (Sd + Lf)	19·4 ± 0·08	80·6 ± 0·08	17·7 ± 0·10	47·6 ± 0·23
S. cerevisiae + *L. brevis* (Sc + Lb)	38·9 ± 0·28	61·1 ± 0·28	21·1 ± 0·10	45·0 ± 0·34
S. cerevisiae + *L. fermentum* (Sc + Lf)	43·6 ± 0·25	56·4 ± 0·23	19·5 ± 0·31	43·1 ± 0·21
Control				
Raw pearl millet flour	80·4 ± 0·27	19·6 ± 0·27	6·20 ± 0·15	32·5 ± 0·26
Autoclaved unfermented flour	57·1 ± 0·63	42·9 ± 0·63	9·30 ± 0·10	34·8 ± 0·29
CD($P < 0.05$)[b]	0·57	0·57	0·27	0·69

[a] Values are means ± SD of four replicates (on dry matter basis).

[b] Critical difference at 5% level. Differences of two means within/between the different treatments exceeding this value are significant.

bility of P improved as a result of all the types of sequential culture fermentation combinations; the Sd + Lb combination had the highest P extractability followed by Sd + Lf, Sc + Lb and Sc + Lf combinations.

The reduction in phytate phosphorus during sequential culture fermentation may be due to hydrolysis of phytic acid by phytase elaborated by fermenting microflora (Lopez *et al.*, 1983; Dhankher & Chauhan, 1987). Cleavage of phosphorus from the phytic acid may explain the increased level of inorganic P and higher HCl-extractability of phosphorus in the fermented pearl millet flour. A significant negative correlation between the phytic acid and Pi and extractable P (Table 3) further confirms this. Natural fermentation has been reported earlier to increase the HCl-extractability of phosphorus with corresponding decrease in phytic acid content of pearl millet flour (Mahajan & Chauhan, 1987; Khetarpaul & Chauhan, 1989*b*).

HCl-extractable minerals

A significant ($P < 0{\cdot}05$) improvement occurred in the HCl-extractability of calcium by all the four combinations of sequential culture fermentation using yeasts and lactobacilli (Table 2). The HCl-extractability of calcium did not vary significantly among the four sequential culture combinations.

All the sequential culture fermentation combinations raised the iron extractability of pearl millet flour significantly (Table 2). Fermentation by both the yeasts with *L. brevis* had a better improving effect than their combination with *L. fermentum*; Sc + Lb had the highest iron extractability but did not differ significantly from the Sd + Lb combination. The combination of *S. diastaticus* and *S. cerevisiae* with *L. fermentum* resulted in an improvement in the iron extractability to a similar extent. The extractability increased more than twofold by fermentation with Sc + Lb and Sd + Lb combinations.

HCl-extractability of zinc increased significantly ($P < 0{\cdot}05$) in all the four fermentation combinations (Table 2). Zinc extractability was the highest in flour fermented with Sd + Lb combination followed by Sd + Lf, Sc + Lb and Sc + Lf fermentation combinations; Sd + Lf- and Sc + Lb-fermented products did not differ significantly.

Copper extractability during sequential culture fermentation was more than doubled in all the fermentation combinations except Sc + Lf. Fermentation by Sd + Lb and Sc + Lf combinations had the highest and the lowest copper extractability, respectively; all the combinations except Sc + Lf had copper extractabilities which were not significantly different from each other.

A significant improvement in manganese extractability was noticed as a

TABLE 2
Effect of Pure Sequential Culture Fermentation on HCl-Extractability of Ca, Fe, Zn, Cu and Mn of Pearl Millet Flour[a]

Treatments	*Ca*	*Fe*	*Zn*	*Cu*	*Mn*
Sequential fermentation:					
S. diastaticus + *L. brevis* (Sd + Lb)	72·0 ± 9·93[a]	47·5 ± 8·16	62·5 ± 3·24	88·7 ± 6·85	77·9 ± 7·88
S. diastaticus + *L. fermentum* (Sd + Lf)	76·0 ± 3·12	36·0 ± 5·19	55·3 ± 3·05	74·9 ± 5·86	72·4 ± 3·92
S. cerevisie + *L. brevis* (Sc + Lb)	73·4 ± 1·84	54·9 ± 6·64	52·3 ± 2·15	74·9 ± 5·86	66·8 ± 1·90
S. cerevisiae + *L. fermentum* (Sc + Lf)	76·2 ± 4·55	32·9 ± 1·83	50·7 ± 1·55	66·6 ± 0·00	66·8 ± 1·90
Control:					
Raw pearl millet flour	36·0 ± 5·22	19·4 ± 1·66	42·2 ± 3·21	35·2 ± 8·34	52·8 ± 2·85
Autoclaved unfermented flour	44·8 ± 3·49	20·5 ± 2·95	44·5 ± 1·81	37·4 ± 0·46	55·7 ± 0·00
CD($P < 0·05$)[b]	8·16	7·50	4·08	8·97	6·93

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

TABLE 3
Correlation Coefficients of Group Means of Inorganic Phosphorus, Extractable Zinc, Copper, Iron, Manganese, Phosphorus and Calcium with Phytic Acid Content of Fermented Pearl Millet Flour

Minerals	*Correlation coefficient*
Inorganic P	−0·8609**
Extractable Zn	−0·8800**
Extractable Cu	−0·8644**
Extractable Fe	−0·5843*
Extractable Mn	−0·7000**
Extractable P	−0·8280**
Extractable Ca	−0·9751**

* Values are significant at 5% level.
** Values are significant at 1% level.

result of fermentation of pearl millet flour first by yeasts and then followed by that of lactobacilli (Table 2). The millet flour fermented by *S. diastaticus* and *L. brevis* combination had the highest manganese extractability followed by that of Sd + Lf, Sc + Lb and Sc + Lf combinations; the difference between Sd + Lb and Sd + Lf fermented products was not significant. The flours fermented by *S. cerevisiae*, with both the lactobacilli, had similar extractable manganese contents and they did not differ significantly from the Sd + Lf fermentation combination.

All the sequential culture fermentation combinations significantly improved the HCl-extractability of minerals, an index of their bioavailability to the human system. Higher HCl-extractability of calcium, iron, zinc, copper and manganese from the fermented pearl millet flour may be partly ascribed to the decreased content of phytic acid which had a significant negative correlation with the minerals studied (Table 3). Decrease in phytic acid content, possibly through hydrolysis by phytase of the fermenting microflora (Dhankher & Chauhan, 1987), may indicate that the divalent cations are freed from the phytate mineral complex which may account for their increased HCl-extractability in the fermented millet flour. Improvement in HCl-extractability of minerals through natural fermentation of pearl millet flour has been reported earlier (Mahajan & Chauhan, 1988; Khetarpaul & Chauhan, 1989*b*). Fermentation has also been shown to enhance the HCl-extractability of minerals in *corn* and *soybean* (Chompreeda & Fields, 1984) and *rabadi* (Dhankher, 1985)—a fermented pearl millet food.

Pure sequential culture fermentation by yeasts and lactobacilli is, thus, a potential method for improving the HCl-extractability of minerals including

calcium, phosphorus, copper, iron, zinc and manganese. Consumption of such a fermented food may help to ameliorate the prevalent mineral deficiencies due to their limited bioavailability from such coarse grains and may lead to a better mineral status of the population.

REFERENCES

Chauhan, B. M., Suneja, N. & Bhat, C. M. (1986). Nutritional value and fatty acid composition of some high yielding varieties of bajra. *Bull. Grain Technol.* **21**, 41–2.

Chen, P. S., Tosibara, T. Y. & Warner, H. (1956). Microdeterminations of phosphorus. *Anal. Chem.*, **28**, 1756–9.

Chompreeda, P. T. & Fields, M. L. (1984). Effect of heat and fermentation on the extractability of minerals from soybean meal and corn meal blends. *J. Food Sci.*, **49**, 566–8.

Dhankher, N. (1985). Studies on nutritional value of *rabadi*—A fermented bajra product. MSc Thesis, Haryana Agricultural University, Hisar, India.

Dhankher, N. & Chauhan, B. M. (1987). Effect of temperature and fermentation time on phytic acid and polyphenol content of *rabadi*—a fermented pearl millet food. *J. Food Sci.*, **52**, 828–9.

Dhillon, S., Popli, S. & Dhindsa, K. S. (1982). Chemical composition and protein fractions of some high yielding varieties of bajra. *Bull. Grain. Technol.*, **20**, 155–60.

Haug, W. & Lantzsch, H. J. (1983). A sensitive method for rapid determination of phytate in cereals and cereal products. *J. Sci. Food Agric.*, **34**, 1423–6.

Khetarpaul, N. (1988). Improvement of nutritional value of pearl millet by fermentation and utilisation of the fermented product. PhD Thesis. Haryana Agricultural University, Hisar, India.

Khetarpaul, N. & Chauhan, B. M. (1989*a*). Improvement in HCl-extractability of minerals from pearl millet by natural fermentation. *Food Chem.* (in press).

Khetarpaul, N. & Chauhan, B. M. (1989*b*). Microorganisms of naturally fermented pearl millet. *Indian J. Microbiol.*, **29**, 143–5.

Kim, H. & Zemel, M. B. (1986). *In vitro* estimation of the potential bioavailability of calcium from sea mustard (*Undaria pinnatifida*), milk and spinach under stimulated normal and reduced gastric acid conditions. *J. Food Sci.*, **51**, 957–9.

Kumar, V. & Kapoor, A. C. (1984). Trace mineral composition of different varieties of cereals and legumes. *Ind. J. Nutr. Dietet.*, **21**, 137–43.

Lindsey, W. L. & Norwell, M. A. (1969). A new DPTA-TEA Soil test for zinc and iron. *Agron. Absts.*, **61**, 84.

Lock, S. & Bender, A. F. (1980). Measurement of chemically available iron in foods by incubation with human gastric juice *in vitro*. *Brit. J. Nutr.*, **43**, 413–15.

Lopez, Y., Gordon, D. T. & Fields, M. L. (1983). Release of phosphorus from phytate by natural lactic acid fermentation. *J. Food Sci.*, **48**, 953–4.

Mahajan, S. (1986). Studies on improvement of nutritional value of bajra through fermentation. MSc Thesis, Haryana Agricultural University, Hisar, India.

Mahajan, S. & Chauhan, B. M. (1987). Phytic acid and extractable phosphorus of pearl millet as affected by natural lactic acid fermentation. *J. Sci. Food Agric.*, **41**, 381–6.

Mahajan, S. & Chauhan, B. M. (1988). Effect of natural fermentation on the extractability of minerals from pearl millet flour. *J. Food Sci.*, **53**, 1576–8.

Nolan, K. B. & Duffin, P. A. (1987). Effect of phytate on mineral bioavailability. *In vitro* studies on Mg^{2+}, Ca^{2+}, Fe^{3+}, Cu^{2+} and Zn^{2+} solubilities in the presence of phytate. *J. Sci. Food Agric.*, **40**, 79–83.

Panse, Y. G. & Sukhatme, P. V. (1961). *Statistical Methods of Agricultural Workers* (2nd edn). Indian Council of Agricultural Research, New Delhi.

Reddy, N. R., Sathe, S. K. & Salunkhe, D. K. (1982). Phytates in legumes and cereals. *Adv. Food Res.*, **28**, 1–92.

Vogel, A. (1962). *Textbook of Quantative Inorganic Analysis* (3rd edn). Longmans.

Wien, E. M. & Schwartz, R. (1985). Dietary calcium exchangeability and bioavailability. Evaluation and potential use of an *in vitro* gastric digestion procedure. In *Nutritional Bioavailability of Calcium*, ed. C. Kies. American Chemical Society, Washington, DC.