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PII: S0308-8146(97)00109-X

Food Chemistry, Vol. 61, No. 1/2, pp. 77–80, 1998

0308-8146/98 $19.00 + 0.00

Effect of milling, soaking, malting, heat-treatment and fermentation on phytate level of four Sudanese sorghum cultivars

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(Received 3 February 1997; accepted 14 May 1997)

Four cultivars of Sudanese sorghum (*Sorghum bicolor*) were analysed for their phytate content, with respect to effects of processing operations, namely milling extraction, water soaking, malting, heat-treatment and fermentation. The conditions of processing used were: decortication to give an 80% extraction meal; 12 and 24 h soaking in tap water; 96 h germination; fermentation for 3,6,9 and 12 h; and cooking at 95°C until starch gelatinized. Total phosphorus, phytate phosphorus and phytic acid were determined. Results showed that phytic acid phosphorus formed > 85% of total phosphorus of the sorghum cultivars studied. All treatments investigated caused phytic acid reduction to various extents. Enzymic methods of phytic acid removal (fermentation and malting) were found to be more effective than physical extraction methods, i.e. milling, soaking and heating.

INTRODUCTION

Phytate (*myo*inositol 1,2,3,5/4,6-hexakis-dihydrogen phosphate) is a naturally occurring organic compound found in plant seeds, roots and tubers. It binds minerals that are necessary as cofactors, thus interfering with several essential metabolic processes, especially the utilization of protein (Harland and Oberleas, 1986), and also minerals that are not cofactors, e.g. calcium and iron. Phytate also complexes with proteins making them less soluble (Smith and Rackis, 1957). The Phytate level in plant material is found to decrease during certain food processing operations, e.g. milling (Nayini and Markakis, 1983), soaking (Chang *et al*., 1977), germination (Eskin and Wiebe, 1983), fermentation (Marfo *et al*., 1990) and heat treatment (Khan *et al*., 1986).

The present study reports on phytic acid level of four sorghum cultivars grown and consumed in Sudan, and the effects of different treatments commonly used in food preparation there.

MATERIALS AND METHODS

Sample preparation

Four cultivars of Sudanese sorghum (*Sorghum bicolor*), locally known as Daber, Fetarita Gadarif (FGD), Fetarita Gazira (FGZ), and Hageen, were purchased from the local market. About 7 kg of each variety was cleaned from damaged seeds and foreign objects, then subjected separately to the following treatments:

(a) 3 kg whole seed was milled to whole grain flour using a falling number laboratory mill: designated fraction 1 (F1),
(b) decortication to 80% extraction rate using a TADD decorticator, then milled: designated fraction 2 (F2),
(c) soaking in a pot filled with tap water. Half of the lot of the grains was removed from the water after 12 h; the rest was removed after 24 h. Both lots were sun-dried, then milled: designated fractions 3 and 4, respectively (F3 and F4),
(d) 1 kg was immersed in water overnight: the grains were spread on trays lined with cloth. It was kept wet by frequent spraying of water. After 96 h, the germinated grains were removed from the trays, sun-dried and milled: designated fraction 5 (F5),
(e) 500 g of F1 were mixed with water at a ratio of 4:5 (w/v); then a previously fermented starter was added (the starter formed 8% of the new dough). Samples from the new dough were taken after 0, 3, 6, 9 and 12 h intervals, designated fractions 6–10, respectively (F6, F7, F8, F9 and F10, respectively). The samples were then oven-dried at 105°C and ground using a mortar.

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(f) 500 g of F1 were mixed with water at a ratio of 4:5 (w/v), then cooked at 95°C until gelatinization of the starch was observed. It was then cooled, sun-dried and ground by the mortar, designated fraction 11 (F11)

Methods

Total phosphorus (TP) and phytate phosphorus (PP) were determined by the method of Chapman and Pratt, (1982). Phytic acid was determined by the method of Wheeler and Ferrel (1971). Data were analysed using the analysis of variance (Snedecor and Cochran, 1976; Duncan, 1955). The Multiple Range Test was applied to determine the statistical probability of observed differences in the mean values.

RESULTS

Table 1 shows the values of TP and PP of the four cultivars studied. FGD contained the highest level of both TP and PP. TP h content of Daber and FGD were not significantly different from one another, whereas FGZ and Hageen were ($p < 0.01$). For the phytic acid content, FGZ and Hageen were not significantly different from each other, while FGD and Daber were. A positive correlation existed between phytic acid and total phosphorus. FGD had the highest PP/TP ratio while Hageen had the lowest.

As shown in Table 2, 80%-extracted flour of Daber and FGZ 3 h contained levels of phytic acid which were not significantly different from one another at $P < 0.01$. Hageen showed the highest reduction by this treatment, while FGZ showed the least reduction. The phytic acid contents of the four varieties, when soaked in water for 12 and 24 h, were significantly different; for the two soaking periods, Daber showed the highest reduction while Hageen showed the least effect for both treatments. Malted flour of the varieties Daber, FGZ and Hageen had similar phytic acid levels and their reduction percentage was higher than that of FGD. FGZ and Hageen showed values of phytic acid which were not significantly different from each other when cooked; the variety FGD showed the least reduction in PP content by cooking. Statistical pairwise comparisons of means of phytic acid by treatment indicate that there were five treatments in which the means were not significantly different ($p < 0.01$) from one another. These treatments were: untreated seeds, 80 extraction, 12 h and 24 h soaking, and cooking. Malting caused a significantly different reduction of phytic acid.

Table 3 shows that in the samples at 0, 9 and 12 h of fermentation, the phytic acid concentrations of FGZ and Hageen were not significantly different from one another. For the 3 h fermentation Daber, FGZ and Hageen were not significantly different from one another. Six hours fermentation resulted in different levels of significance for the four varieties. After 12 h of fermentation, Hageen showed the highest percentage reduction, while Daber showed the least reduction. Statistical pairwise comparisons of means of phytic acid reduction by time of fermentation (3, 6, 9 and 12 h) indicated that the means were not significantly different from one another but were significantly different from the non-fermented flour (0 h).

Table 1. Relationship between total phosphorus (TP) and phytate phosphorus (PP) in sorghum grains

Cultivar	Mean TP (mg per 100 g)	Mean PP (mg per 100 g)	Percentage PP/TP
Daber	319[a] (2.2)	283[b] (1.0)	89
FGD	326[a] (3.2)	300[a] (2.0)	92
FGZ	272[c] (2.1)	248[c] (0.1)	91
Hageen	301[b] (2.1)	255[c] (1.0)	85
LSD values	11.8	5.5	—

Values in parentheses are SDs. All values are means of three replicates. Means with same letters (within columns) are not significantly different at $p < 0.01$.

DISCUSSION

The TP content of FGZ and FGD were similar to those reported by Yossif and Magboul (1972), but the TP content of Daber was slightly higher than that given by them. The phytic acid content of Daber and FGD fall within the range of phytic acid content in sorghum given by Doherty *et al.*, (1982), but that of FGZ and Hageen was lower. The average phytic acid contents of the four varieties of sorghum were lower than both that of millet reported by Khetarpaul and Chauhan (1989) and that of wheat reported by Tangkongchitr *et al.* (1981). The percentage PP/TP of the four varieties falls within the range given by Doherty *et al.* (1982). The difference in TP content among varieties may be due to genetic or environmental factors such as fertilization and available soil phosphorus. Since phytic acid formed 85% of the TP, the same reasons could be true for the difference in phytic acid content of the four varieties.

Eighty per cent extraction rate of sorghum resulted in a reduction percentage lower than that of 80%-extracted wheat reported by Nayini and Markakis (1983). Dehulling of sorghum resulted in a higher reduction percentage (Doherty *et al.*, 1982). In milling extraction, the reduction was due to elimination of phytic acid from the grain by the removal of the outer layers where phytic acid was concentrated.

The reduction due to 12 and 24 h of soaking was lower than that reported for 10 h soaking of beans (Chang *et al.*, 1977). The reduction in phytic acid caused by soaking may be due to water solubilization of some phytic acid salts.

Table 2. Effect of different treatments on phytic acid (PA) content (mg per 100 g) and the percentage (%) of reduction

Cultivar	Phytic acid content of untreated flour (F1)	80% extracted flour (F2)		Flour from seeds soaked for 12 h (F3)		Flour from seeds soaked for 24 h (F4)		Flour from malted seeds (F5)		Cooked F1 flour (F11)	
	PA	PA	%	PA	%	PA	%	PA	%	PA	%
Daber	283^{bx} (1.0)	178^{bx} (1.5)	37.0	242^{bx} (2.1)	14.4	223^{bx} (1.2)	21.3	38.5^{by} (26.2)	86.4	202^{bx} (2.1)	28.5
FGD	300^{ax} (2.0)	196^{ax} (3.8)	34.8	262^{ax} (2.1)	12.6	245^{ax} (1.0)	18.4	95.3^{ay} (3.5)	68.3	247^{ax} (5.8)	17.9
FGZ	248^{cx} (0.1)	175^{bx} (2.1)	29.5	221^{dx} (1.2)	10.7	207^{dx} (1.5)	16.3	43.8^{by} (2.9)	82.3	168^{cx} (0.6)	32.3
Hageen	246^{cx} (1.0)	152^{cx} (9.4)	40.4	234^{cx} (3.2)	8.2	213^{cx} (1.0)	16.3	33.3^{by} (22.4)	86.9	159^{cx} (6.1)	37.5
LSD Values	5.52	11.0		4.86		2.56		28.52		9.04	

Values in parentheses are SDs. All values are means of three replicates. Values with same letters (a, b, c, d within columns) are not significantly different at $p < 0.01$. Values with same letters (x, y within rows) are not significantly different at $p < 0.01$.

Table 3. Effect of fermentation on phytic acid content (mg per 100 g) of sorghum grains

Cultivar	Phytic acid content after different fermentation periods (h) 0(F6)	3(F7)	6(F8)	9(F9)	12(F10)	% of reduction after 12 h fermentation
Daber	283^{bx} (1.0)	203^{by} (2.7)	162^{by} (3.2)	125^{by} (1.7)	121^{by} (1.0)	57
FGD	300^{ax} (2.0)	216^{ay} (3.8)	183^{ay} (4.4)	144^{ay} (1.5)	126^{ay} (0.6)	58
FGZ	248^{cx} (0.1)	197^{by} (5.9)	156^{cy} (3.5)	107^{cy} (1.5)	105^{cy} (3.8)	58
Hageen	255^{cx} (1.0)	195^{by} (4.6)	152^{dy} (2.9)	101^{cy} (1.2)	102^{cy} (1.2)	60
LSD values	5.52	9.49	3.77	6.02	4.46	—

Values in parentheses are SDs. All values are means of three replicates. Means with same letters (a, b, c, d within columns) are not significantly different $p < 0.01$. Means with same letters (x, y within rows) are not significantly different $p < 0.01$.

Malting of sorghum resulted in a substantial reduction of phytic acid content. The reduction range for the four varieties covered the values reported for 10-day-germinated beans given by Eskin and Wiebe (1983). This substantial reduction of phytic acid content may be due to the activity of the enzyme phytase, which hydrolyses phytic acid to inorganic phosphate and inositol (Eskin and Wiebe, 1983).

The reduction due to cooking observed in this study was higher than that reported by Marfo *et al.* (1990) for the reduction of phytic acid in cereals. This reduction may be due to limited activation of phytase enzyme during cooking before it is denatured by heat.

Fermentation for 12 h (the usual practice in Sudan) resulted in a reduction range higher than that reported by Khetarpaul and Chauhan (1989) for the fermentation of millet for 27 h. On the other hand, it falls within the range of phytic acid reduction reported for white maize fermented for 72 h (Marfo *et al.*, 1990). Four per cent yeasted wheat dough fermented for 5 h (Tangkongchiter *et al.*, 1981) had a lower percentage of reduction.

The phytate reduction caused by fermentation was probably due to the activity of the microbial phytase enzyme (Khetarpaul and Chauhan, 1989). As shown in Table 3, higher reduction rates occurred during the first hours of fermentation. This result agrees with the findings of Lopez *et al.* (1983), who reported a reduction of the phytate level of corn by natural lactic acid fermentation, thus increasing the amount of free phosphorus. With an increase of fermentation time, the reduction is limited and is nearly the same as that at 9–12 h. This slow rate of reduction was due to a reduced activity of the enzyme which may be due to, either its denaturation by pH changes, feedback inhibition or inaccessibility of phytic acid salts (Tangkongchiter *et al.*, 1982).

CONCLUSION

PP forms >85% of TP, the range being 85–92%. All the treatments investigated cause reduction of phytate content. This loss occurs to different extents, the ascending order of reduction being: 12 h soaking (8.2–14.4%); 24 h soaking (16.3–21.3%); cooking (17.9–37.5%); 80% extraction (29.5–40.4%); 12 h fermentation (57–60%); malting (68.3–86.9%).

These observations indicate that enzymatic methods (malting and fermentation) for phytic acid reduction are more effective than physical methods (milling, soaking and heating).

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