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Effect of malt pretreatment on phytic acid and in vitro protein digestibility of sorghum flour

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Abstract

Sorghum seeds of cultivar Wad Ahemed (phytate: 2.7 mg/g, tannin: 0.96% and 2 h pepsin digestion: 18%) were germinated for three days to obtain 1-, 2- and 3- days old sorghum malts. Sorghum malt was added in concentrations of 1, 2.5, 5, 7.5 or 10% to sorghum flour. The mixtures were incubated with shaking for 0, 30, 60, 90 or 120 min. Phytic acid and in vitro protein digestibility were assayed for all treatments. The results revealed that phytate content was significantly reduced. The 10% 3-day-old malt after 120 min incubation, reduced the phytate content by 83%. The in vitro protein digestibility (IVPD) was significantly improved as a result of malt pretreatment. The rate of reduction of phytate content and the rate of increment in IVPD increased with time of incubation, age and concentration of the malt. © 2000 Published by Elsevier Science Ltd.

1. Introduction

Sorghum nutritional quality is dictated mainly by its chemical composition and the presence of anti-nutritional factors, such phytic acid. Phytic acid and/or phytate is a principal storage form of phosphate, ubiquitously distributed in plants, particularly in cereal grains and in legumes. The effects of phytic acid in human and animal nutrition are related to the interaction of phytic acid with proteins, vitamins and it several minerals, and thereby restricts their bio-availability.

In view of the anti-nutritional effects of phytic acid, many attempts to reduce phytate have been made. It is reported that phytate is reduced in malted oats by 99% (Larrson & Sandberg, 1995) and malted pea by 75% (Beal & Mehta, 1985). Fredlunk, Larrson, Marklinder and Sandberg (1997) found that hydrothermal treatment decreased phytate content in wheat, rye, barley and oats by 46–77% and 84–99%, in water and acetate incubation, respectively. According to Fretzdroff and Weipert (1986) there was no reduction of phytate content when whole rye or its flour were cooked at 100°C but, at 170°C, phytic acid was reduced by 23%. Moreover, Marfo, Simpson, Idowu and Oke (1990) found that 72 h fermentation significantly decreased phytate content in foodstuffs, (80–98% for rice, cassava, and cocoyam and 52–65% for sorghum, maize, soybean, cowpea, and yam). Other attempts to reduce the phytate content such as fertilisation (Elsheikh, El Tinay & Fadul, 1999; Elsheikh, Fadul & El Tinay, 2000) and activation of the indigenous enzyme phytase and/or addition of microbial phytase (Barrier, Casado, Maupetit, Jondreville, Gatel & Larbier, 1996) have been tried. The objective of this study was to develop a simple and rapid method to eliminate the anti-nutritional factors associated with sorghum grain and improve protein digestibility.

2. Materials and methods

2.1. Source and germination of seeds

Seeds of sorghum cultivar Wad Ahemed were procured from Senar Research Station, Sudan. The grains were carefully cleaned and freed from broken seeds and extraneous matter. Seeds were germinated according to the method described by Bhise, Chavan and Kadam (1988). The germinated seeds were sun-dried and the root portions were manually removed. The seeds were milled into fine flour to pass a 0.4 mm sieve and kept at 4°C.

2.2. Addition and incubation of malt to sorghum flour

One-, 2- or 3- day-old sorghum malt was added to sorghum flour at the following concentrations: 1, 2.5, 5, 7.5 or 10% in triplicate. Samples were shaken for 30 min and then mixed with water 1:2 (w/v) and incubated

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at 28°C±2 in a shaker for 0, 30, 60, 90 or 120 min. Samples were dried at 70°C and finely ground.

2.3. Determination of phytic acid and in vitro protein digestibility

The phytate content of all samples was estimated on dry weight basis by the method of Wheeler and Ferrel (1971). In vitro protein digestibility was carried out according to the method of Maliwal (1983) in the manner described by Monjula and John (1991) with a minor modification. A known weight of the sample containing 16 mg nitrogen was taken in triplicate and digested with 1 mg pepsin in 15 ml of 0.1 M HCl at 37°C for 2 h. The reaction was stopped by the addition of 15 ml 10% trichloroacetic acid (TCA). The mixture was then filtered quantitatively, through Whatman No. 1 filter paper. The TCA-soluble fraction was assayed for nitrogen using the micro-Kjeldahl method. Digestibility was obtained by using the following equation:

$$\text{Protein digestibility \%} = \frac{N \text{ in supernatant} - N \text{ in pepsin}}{N \text{ in sample}} \times 100$$

2.4. Statistical analysis

Each determination was carried out on three separate samples and analyzed in triplicate and figures were then averaged. Data was assessed by the correlation, regression and analysis of variance (ANOVA). The Duncan multiple range test was used to separate means. Significance was accepted at $P \leqslant 0.05$.

3. Results and discussion

3.1. Effect of malt pretreatment of sorghum on phytate contents (Tables 1 and 2)

Treatment of sorghum flour with 1% sorghum malt resulted in lowering phytate content by 16.5% for 1-day malt after incubation for 120 min while, for 2- and 3-day-old malt reduction amounted to 27.7 and 33.0%, respectively.

Tables 1 and 2 show the reduction of phytate content during incubation of sorghum flour with 2.5% sorghum malt. The reductions in phytate caused by 1-, 2- and 3-day-malt, for 60 min incubation, were 11.0, 16.0 and 23.0%, respectively. A similar trend was observed for the 120 min treatment; however, the reductions were significant ($P \leqslant 0.05$), amounting to 22.1, 33.0 and 43.3%, respectively.

With 5% malt, the reduction in phytate was significant ($P \leqslant 0.05$) compared to that observed for the 2.5%. Incubation of one day malt with sorghum flour for 90 and 120 min resulted in phytate losses of 21.0 and 28.1%, respectively. For the same treatment with 2-day malt, the reductions were 32.8 and 45.0%, respectively, while, for the three-day-old malt, the reductions were highly significant, amounting to 50.1 and 66.1%, respectively.

Percent phytic acid reduction in relation to incubation time with 7.5% malt is shown in Table 1. For 1-day malt, phytate content was reduced after incubation for

Table 2
The regression equations and their level of significance for the phytic acid reductions

	Sorghum malt concentration				
	1%	2.5%	5%	7.5%	10%
First day malt					
a_0	−0.14	−0.06	−0.02	−0.03	−0.10
a_1	0.14	0.18	0.25	0.32	0.37
r	0.999	0.999	0.999	0.999	0.999
Second day malt					
a_0	−0.64	0.20	−0.50	0.30	0.30
a_1	0.24	0.27	0.38	0.45	0.62
r	0.996	0.999	0.999	0.998	0.999
Third day malt					
a_0	0.38	0.35	−0.23	0.52	0.35
a_1	0.27	0.37	0.55	0.58	0.67
r	0.999	0.998	0.999	0.999	0.999

Table 1
Effect of malt pretreatment of sorghum flour on phytic acid reduction (%)[a]

Time	Sorghum malt concentration														
	First day malt					Second day malt					Third day malt				
	1%	2.5%	5%	7.5%	10%	1%	2.5%	5%	7.5%	10%	1%	2.5%	5%	7.5%	10%
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	3.8	5.4	6.9	9.0	11.5	5.0	9.0	10.0	13.5	18.3	9.2	11.0	16.5	18.9	21.0
60	8.2	11.0	14.2	19.0	20.0	14.5	16.0	22.5	27.0	37.0	16.5	23.0	32.0	34.8	42.8
90	11.8	16.5	21.0	29.0	32.8	20.0	24.3	32.8	42.0	55.5	24.0	35.0	50.1	53.3	61.1
120	16.5	22.1	28.1	38.5	44.5	27.7	33.0	45.0	52.5	75.0	33.0	43.3	66.1	70.0	83.0

[a] Phytic acid content of sorghum flour at zero time = 2.7 mg/g.

30, 60, 90 and 120 min by 9.0, 19.0, 29.0 and 38.5%, respectively. For 2-day-malt, for the same incubation periods, the reductions increased to 13.5, 27.0, 42.0 and 52.5%, respectively, while, for the 3-day malt, the reductions were highly significant ($P \leqslant 0.005$) amounting to 18.9, 34.8, 53.3 and 70.0%, respectively.

When the concentration of malt was increased up to 10%, the reduction in phytate was significant ($P \leqslant 0.05$) compared to that for all other treatments. The 1-day malt, incubated for 90 and 120 min, reduced phytate by 32.8 and 44.5%, respectively. For 2-day malt, the reduction was more pronounced; it increased to 55.5 and 75.0%, respectively, while for the 3-day malt, the loss in phytate was highly significant ($P \leqslant 0.05$), amounting to 61.1 and 83.0%, respectively.

The results indicate that phytic acid reduction is significantly affected by addition of sorghum malt. The rate of phytic acid reduction depends upon the age and the amount of sorghum malt, as well as the incubation period. The reduction in phytic acid content is likely due to indigenous phytase activity in sprouted seeds.

3.2. Effect of malt pretreatment of sorghum on in vitro protein digestibility (IVPD) (Tables 3 and 4)

Sorghum flour incubated with 1% of 1-, 2- and 3-day-old malt for 120 min underwent significant ($P \leqslant 0.05$) increase in IVPD, to 19.5, 20.8 and 22.5%, respectively.

A similar trend to that of 1% malt was also observed for 2.5% of malt. For 1-day-old malt incubated for 120 min, the IVPD increased significantly ($P \leqslant 0.05$) to 20.8% while, for the 2-day malt, the IVPD was significantly ($P \leqslant 0.05$) increased to 22.7%. The 3-day-old malt gave significant ($P \leqslant 0.05$) increase in the IVPD (25.0%).

Incubation of sorghum flour for 60 and 120 min with 5% of the 1-, 2- and 3-day-old malt showed that the 1-day malt caused significant ($P \leqslant 0.05$) increases in the IVPD amounting to 19.8 and 21.4%, respectively. For the two days malt incubated for 60 and 120 min, the IVPD significantly ($P \leqslant 0.05$) increased to 21.6 and 24.0%, respectively; while for three day malt it showed significant ($P \leqslant 0.05$) increase in the IVPD amounting to 22.8 and 27.5%, respectively.

Tables 3 and 4 show significant ($P \leqslant 0.05$) increases in the IVPD when sorghum flour was incubated with 7.5% malt for all malt ages. For 60 and 120 min incubation periods, with 1-day malt, the increases in the IVPD were 20.3 and 22.0%, respectively. For 2-day malts the increases in the IVPD were significant ($P \leqslant 0.05$) reaching up to 21.7 and 25.4%, respectively; while, the 3-day-old malt gave significant ($P \leqslant 0.05$) increases of 22.4 and 29.2%, respectively.

Percent IVPD, in relation to incubation time with 10% malt, is shown in Table 2. For 1-day malt, the increases in the IVPD were significant ($P \leqslant 0.05$) 20.0, 20.9, 22.5 and 24.0% for the four incubation periods, respectively. For 2-day 10% malt, the IVPD were significantly ($P \leqslant 0.05$) higher than that of the 7.5% malt, reaching 20.0, 23.0, 25.5 and 27.7%, respectively. The 3-day-old malt caused highly significant ($P \leqslant 0.05$) increases of 21.6, 24.6, 28.0 and 31.3%, respectively.

The results indicate that the IVPD increment was significantly affected by addition of sorghum malt. The

Table 4
The regression equations and their level of significance for the in vitro protein digestibility

	Sorghum malt concentration				
	1%	2.5%	5%	7.5%	10%
First day malt					
a_0	17.89	18.06	17.94	18.41	18.14
a_1	0.01	0.22	0.03	0.03	0.05
r	0.990	0.984	0.999	0.960	0.995
Second day malt					
a_0	17.77	17.86	18.31	18.00	17.81
a_1	0.02	0.04	0.06	0.06	0.08
r	0.986	0.999	0.953	0.996	0.998
Third day malt					
a_0	17.99	17.91	18.02	17.71	17.71
a_1	0.04	0.06	0.08	0.10	0.11
r	0.999	0.999	0.999	0.992	0.999

Table 3
Effect of malt pretreatment of sorghum flour on the in vitro protein digestibility (%)

	Sorghum malt concentration														
	First day malt					Second day malt					Third day malt				
Time	1%	2.5%	5%	7.5%	10%	1%	2.5%	5%	7.5%	10%	1%	2.5%	5%	7.5%	10%
0	17.9	17.9	17.9	17.9	17.9	17.9	17.9	17.9	17.9	17.9	17.9	17.9	17.9	17.9	17.9
30	18.3	19.0	18.8	20.0	20.0	18.5	19.0	19.5	20.0	20.0	19.2	19.8	20.5	20.8	21.6
60	18.5	19.3	19.8	20.3	20.9	18.8	20.3	21.6	21.7	23.0	20.3	21.3	22.8	22.4	24.6
90	19.0	19.9	20.5	21.7	22.5	20.0	21.5	23.8	23.0	25.5	21.3	23.3	25.0	26.6	28.0
120	19.5	20.8	21.4	22.0	24.0	20.8	22.7	24.0	25.4	27.7	22.5	25.0	27.5	29.2	31.3

IVPD increment depends on the age and the amount of sorghum malt, as well as the incubation period. The increment in IVPD is likely due to the activity of indigenous protease in sprouted seeds.

Alternative methods available in the literature, such as germination, which also results in loss of dry matter, hydrothermal treatment, cooking and fermentation, have the disadvantage of prolonged periods of incubation and are expensive. The method described in this work overcomes all these shortcomings in addition to being easy, rapid and efficient. It could be concluded that utilization of sorghum malt to lower phytic acid and to improve IVPD is a promising and simple method for reducing phytate in sorghum. The rate of reduction of phytate and increment of IVPD depend on the age of the malt, incubation time and concentration of the malt. The addition of malt to sorghum flour could be part of the process of preparing fermented sorghum food products.

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