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Effect of malt pretreatment and/or cooking on phytate and essential amino acids contents and in vitro protein digestibility of corn flour

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Abstract

Corn grains of cultivar Mugtama 45 (phytate: 526 mg/100 g and 2 h pepsin digestion: 13.8%) were germinated for 6 days to obtain 2, 4 and 6 day-old corn malts. Corn malt was added at concentrations of 4%, 8% or 12% to corn flour. The mixtures were incubated with shaking for 2 h. Phytic acid and in vitro protein digestibility (IVPD) were assayed for all treatments, while amino acid composition was assayed for the 12% mixture. The results revealed that phytate content was significantly reduced in both cooked and uncooked mixtures. The 12%, 6 day-old malt reduced the phytate content by 71% and 42% for uncooked and cooked mixture, respectively. The IVPD was significantly ($P \leqslant 0.05$) improved as a result of malt pretreatment, while cooking significantly reduced IVPD. Amino acid content of cooked and uncooked mixture depended on the malt age. The rate of reduction of phytate content and the rate of increment in IVPD increased with age and concentration of the malt.

Keywords: Malt; Phytate; Amino acids; Protein; Digestibility

1. Introduction

Corn nutritional quality is dictated mainly by its chemical composition and the presence of anti-nutritional factors, such as phytic acid. Phytic acid and/or phytate is the 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 with several minerals, which thereby restricts their bioavailability. Many attempts to reduce phytate have been tried. 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 Fretzdorff and Weiper (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 a 72 h fermentation significantly decreased phytate content in foodstuffs. Other attempts to reduce the phytate content were fertilization (Elsheikh, Fadul, & El Tinay, 2000) and activation of the indigenous enzyme phytase and/or addition of microbial phytase (Barrier, Casado, Jondreville, Gatel, & Larbier, 1996). It was found that protein digestibility of sorghum flour significantly improved as a result of malt pretreatment (Elkhalil, El Tinay, Mohamed, & Elsheikh, 2001). Taylor (1983) reported that essential amino acids, protein and starch digestibility were significantly increased when sorghum seeds were germinated for only 2 days. Germination of cereal grains significantly increased both lysine and tryptophan (Dalby & Tsai, 1976). The objective of this study was to develop a simple and rapid method to eliminate the anti-nutritional factor (phytate) associated with corn grain and improve protein digestibility.

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2. Materials and methods

2.1. Source and germination of seeds

Seeds of corn cultivar Mugtama 45 were obtained from Sudanese Agricultural Enterprises, Khartoum. The seeds were carefully cleaned and freed from broken 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 corn flour

Two, 4 or 6 day-old corn malt was added to corn flour at differential concentrations (4%, 8% or 12%) in triplicate. Samples were shaken for 30 min and then mixed with water 1:2 (w/v) and incubated at 30 °C in a shaker for 2 h, thereafter dried at 65 °C and finely ground. Cooking was performed by suspending flour or its mixture in a boiling water bath at 100 °C for 30 min.

2.3. Determination of phytic acid and in vitro protein digestibility (IVPD)

The phytate content of all samples was estimated on a dry weight basis by the method of Wheeler and Ferrel (1971). IVPD 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 N 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 (AOAC, 1980). Digestibility was estimated by using the following equation:

$$\text{IVPD}\% = \frac{\text{N in supernatant} - \text{N in pepsin}}{\text{N in sample}}.$$

2.4. Determination of essential amino acid content

Essential amino acid content was determined by the method described by Cohen, Meys, and Travin (1989). Tryptophan was determined colorimetrically in the alkaline hydrolysate according to the method of Blauth, Chatezinski, and Brber (1963).

2.5. 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 analysis of variance (ANOVA) (Snedecor & Cochran, 1987). Duncan's multiple range test was used to separate means. Significance was accepted at $P \leqslant 0.05$ (Duncan, 1955).

3. Results and discussion

3.1. Effect of malt pretreatment and/or cooking of corn flour on phytate content

Table 1 shows the effect of malt pretreatment on phytate content during incubation of corn flour with corn malt at different concentration (4%, 8% or 12%) before and after cooking. Phytic acid content of untreated corn flour was 526 mg/100 g. Cooking of untreated corn flour was observed to reduce the amount of phytic acid by 16%. Treatment of corn flour with 4% corn malt resulted in lowering of phytate content by 45% for 2 day malt after incubation for 2 h while for 4 and 6 day-old malt reduction amounted to 59% and 65%, respectively. A similar trend was observed for the 8% and 12% malt treatment; however, the reductions were significant ($P \leqslant 0.05$) for 8% malt treatment, amounting to 65%, 68% and 68% for 2, 4 and 6 day-old malt, respectively. When the concentration of the malt was increased up to 12%, the reduction in phytate was significant ($P \leqslant 0.05$) and reached 71% compared to that observed for all other treatments. Similar results were reported when sorghum flour was treated with malt and incubated for different time intervals (Elkhalil et al., 2001). Malt pretreatment, followed by cooking (Table 1), was observed to reduce phytate content but the rate of reduction was lower than to that of malt pretreatment alone. Pretreatment of corn flour with 4% corn malt, followed by cooking, resulted in lowering of phytate content by 27%, 27% and 29% for 2, 4 and 6 day-old malt, respectively. The rate of phytate reduction increased gradually with the concentration of malt and reached 42% for 12%, 6 day-old malt when mixed and cooked with corn flour. The rate of phytate reduction was observed to be lower for malt pretreatment, followed by cooking, compared to uncooked treatments. This is likely due to the fact that cooking of malt-treated corn flour at 100 °C resulted in inactivation of indigenous phytase in sprouted seeds; this finding agreed with the Fretzdorff and Weiper (1986) finding in which they observed that, there was no reduction of phytate content when the whole rye or its flour were cooked at 100 °C. Valverde et al. (1994) reported that germination of lentils greatly reduced phytate content compared to soaking or cooking. The results indicate that phytic acid reduction is significantly affected by addition of corn malt. The rate of reduction depends upon the age as well as the amount of corn malt.

Table 1
Effect of malt pretreatment and/or cooking on phytate content (mg/100 g) of corn flour

Malt age (days)	Malt concentration (%)					
	4		8		12	
	Content	Reduction (%)	Content	Reduction (%)	Content	Reduction (%)
Uncooked						
0	526 (±40.0)[a]	–	526 (±40.0)[a]	–	526 (±40.0)[a]	–
2	290 (±42.0)[d]	45	186 (±37.0)[d]	65	201 (±22.0)[f]	62
4	214 (±12.0)[e]	59	166 (±06.0)[e]	68	151 (±14.0)[g]	71
6	184 (±19.0)[f]	65	168 (±01.0)[e]	68	153 (±02.0)[g]	71
Cooked[a]						
0	441 (±24.0)[b]	16	441 (±24.0)[b]	16	441 (±24.0)[b]	16
2	384 (±14.0)[c]	27	376 (±13.0)[c]	29	361 (±21.0)[c]	31
4	384 (±12.0)[c]	27	360 (±15.0)[c]	32	348 (±14.0)[d]	34
6	373 (±19.0)[c]	29	368 (±01.0)[c]	30	304 (±02.0)[e]	42

Values are means (±SD).
Means not sharing a common superscript letter in a column are significantly different at $P \leqslant 0.05$.
[a] Phytate reduction (%) of cooked samples was calculated as a percent of total (526).

3.2. Effect of malt pretreatment and/or cooking of corn flour on in vitro protein digestibility (IVPD)

Table 2 shows the effect of malt pretreatment and/or cooking on IVPD during incubation of corn flour with corn malt of different concentration (4%, 8% or 12%). IVPD of untreated corn flour was found to be 13.8%. Corn flour incubated with 4% of 2, 4 and 6 day-old malt for 2 h gradually increased IVPD of uncooked mixture. A similar trend to that of 4% malt was also observed for 8% and 12% malt.

For 12% malt, IVPD of uncooked mixture was significantly ($P \leqslant 0.05$) increased and reached 17.5% for 6 day-old malt. Similar results were obtained for IVPD when sorghum flour was treated with sorghum malt (Elkhalil et al., 2001). Cooking of untreated corn flour (Table 2) significantly ($P \leqslant 0.05$) reduced the IVPD of the flour to 10.0%. However, addition of malt at different concentrations, followed by cooking, gradually increased the IVPD of the mixture. Corn flour incubated with 4% of 2, 4 and 6 day-old malt, and cooked at 100 °C for 30 min, gradually increased the IVPD of the mixture but the rate of increment was lower and depended on the malt age as compared to that of the uncooked one. 6 day-old malt significantly increased the IVPD of the mixture to 15.0%. A similar trend was observed when 8% and 12% malt were added and cooked with corn flour. A maximum value of IVPD (18.0%) was observed for the 12%, 6 day-old malt, followed by cooking. This is likely to be attributed to the fact that addition of malt reduced the formation of disulphide bonds after heating, which resulted in protein folding and hence decreased its susceptibility to digestive enzymes. Also, cooking was observed to reduce the amount of phytic acid, which is expected to improve IVPD of the protein (Hamaker, Kirleis, Mertz, & Axtell,

Table 2
Effect of malt pretreatment and/or cooking of corn flour on in vitro protein digestibility (IVPD)[a]

Malt age (days)	Malt Concentration (%)		
	4	8	12
	IVPD (%)		
Uncooked			
0	13.8 (±1.00)[b]	13.8 (±1.00)[b]	13.8 (±1.00)[d]
2	14.5 (±0.10)[a]	14.6 (±0.30)[b]	15 (±0.10)[b]
4	15 (±0.10)[a]	15.5 (±0.00)[a]	16.2 (±0.50)[b]
6	15.9 (±0.20)[a]	16.8 (±0.30)[a]	17.5 (±0.30)[a]
Cooked			
0	10 (±1.00)[c]	10 (±1.00)[c]	10 (±1.00)[e]
2	12.8 (±0.70)[b]	13.1 (±13.0)[b]	14 (±0.20)[c]
4	13.9 (±0.30)[b]	14 (±0.30)[b]	15 (±0.20)[c]
6	15 (±0.70)[a]	16.5 (±0.30)[a]	18 (±0.50)[a]

Values are means (±SD).
Means not sharing a common superscript letter in a column are significantly different at $P \leqslant 0.05$.
[a] IVPD was done using pepsin for 2 h.

Table 3
Effect of malt pretreatment (12%) and/or cooking on essential amino acids content (mg/100 g) of corn floor

Amino acid	Age of malt (days)							
	0		2		4		6	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
Threonine	1.44 (±0.03)^{c}	0.15 (±0.04)^{e}	1.42 (±0.21)^{c}	1.61 (±0.31)^{b}	1.90 (±0.17)^{a}	1.41 (±0.08)^{c}	1.00 (±0.14)^{d}	1.31 (±0.23)^{c}
Methionine	0.35 (±0.01)^{b}	0.14 (±0.03)^{d}	0.27 (±0.02)^{c}	0.12 (±0.00)^{d}	0.12 (±0.03)^{d}	0.09 (±0.00)^{e}	1.00 (±0.08)^{a}	0.43 (±0.04)^{b}
Lysine	0.04 (±0.00)^{e}	0.06 (±0.01)^{e}	0.08 (±0.00)^{e}	1.68 (±0.24)^{a}	0.16 (±0.01)^{d}	0.22 (±0.01)^{d}	1.06 (±0.06)^{c}	1.20 (±0.07)^{b}
Valine	0.38 (±0.04)^{b}	0.69 (±0.02)^{a}	0.31 (±0.00)^{b}	0.70 (±0.00)^{a}	0.21 (±0.03)^{c}	0.68 (±0.02)^{a}	0.25 (±0.03)^{c}	0.18 (±0.01)^{d}
Leucine	0.01 (±0.00)^{e}	0.50 (±0.01)^{d}	0.90 (±0.07)^{c}	1.11 (±0.09)^{b}	1.82 (±0.11)^{a}	0.03 (±0.00)^{e}	0.90 (±0.09)^{c}	0.83 (±0.02)^{c}
Isoleucine	0.90 (±0.02)^{b}	0.03 (±0.00)^{f}	2.52 (±0.13)^{a}	0.34 (±0.01)^{d}	0.76 (±0.04)^{c}	0.12 (±0.01)^{e}	0.74 (±0.05)^{c}	0.15 (±0.03)^{e}
Histidine	1.36 (±0.16)^{c}	0.01 (±0.00)^{g}	0.21 (±0.05)^{e}	2.17 (±0.40)^{a}	0.13 (±0.02)^{f}	0.98 (±0.06)^{d}	1.48 (±0.13)^{b}	0.14 (±0.02)^{f}
Phenylalanine	0.01 (±0.00)^{f}	2.40 (±0.13)^{a}	1.19 (±0.28)^{d}	1.63 (±0.06)^{c}	1.82 (±0.21)^{b}	0.03 (±0.00)^{f}	0.90 (±0.08)^{e}	0.83 (±0.09)^{e}
Tryptophan	0.62 (±0.02)^{c}	0.43 (±0.04)^{d}	0.39 (±0.03)^{d}	0.71 (±0.04)^{b}	0.52 (±0.04)^{d}	0.81 (±0.07)^{a}	0.35 (±0.01)^{e}	0.49 (±0.05)^{d}

Values are means (±SD).
Means not sharing a common letter in a column are significantly different at $P \leqslant 0.05$.

1986). The results indicate that cooking and addition of corn malt significantly affected the IVPD increment. The IVPD increment depended on the age and the amount of corn malt.

3.3. Effect of 12% malt pretreatment and/or cooking of corn flour on essential amino acid content

Table 3 shows the effect of malt pretreatment (12% mixture) and/or cooking on essential amino acid content of corn flour. Addition of malt to corn flour, up to 12%, significantly affected both phytate content and IVPD of the flour. Therefore, the effect of this concentration on essential amino acid content was studied. Cooking of untreated corn flour significantly ($P \leqslant 0.05$) reduced threonine and histidine contents and significantly increased phenylalanine content while other amino acids were only slightly affected by cooking (Table 3). Addition of 12%, 2 day-old malt to corn flour greatly increased essential amino acid content, except isoleucine and methionine. For 4, and 6 day-old malt, amino acid content was fluctuated and either slightly increased or decreased. It was observed that lysine content for 2 and 6 day-old malt was higher for uncooked samples and increased even after cooking. This finding agreed with Dalby and Tsai (1976) who observed that, germination of cereal grains significantly increased both lysine and tryptophan. Also similar results were observed by Taylor (1983) who reported that essential amino acids were significantly increased when sorghum grains were germinated for only 2 days.

4. Conclusion

Utilization of corn malt to lower phytic acid content and to improve the IVPD as well as amino acid content is a promising and simple method. The rate of reduction of phytate and increment of IVPD depend on the age, incubation period and concentration of the malt. The addition of malt to corn flour, followed by cooking, could be a complete process for preparing fermented corn food products.

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