Journal of Food Technology 3 (4): 523-528, 2005

Antinutritional Factors Content and Availability of Protein, Starch and Mineral of Maize (*Zeamays linnaus*) and Lentil (*Lens culinaris*) As Influenced by Domestic Processing

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Abstract: Domestic processing of maize and lentil were observed to cause a significant reduction in the antinutritional factors (phytic acid, tannins and polyphenols) content and improved the content and availability of protein, starch and minerals. Cooking of maize and lentil greatly reduced phytic acid to 353.0 and 504.0 mg/100g, respectively. However, for maize when cooking was preceded by fermentation, further reduction in phytate was observed (50%). Polyphenols and tannin followed a trend similar to that obtained for phytate. Cooking increased the *in vivo* Protein Digestibility (IVPD) of maize to 34.25% and lentil to 96.1%, while *in vivo* starch digestibility (IVSD) increased to 33.89% and to 83.62%, for the crops respectively. Further improvement in IVPD and IVSD was observed when the grains of maize were fermented and cooked. Domestic processings were observed to decrease major minerals content with increase in their availability. However, trace minerals content and availability were increase with cooking and/or fermentation.

Key words: Maize, lentil, antinutrients, protein, starch, minerals

INTRODUCTION

In most developing countries, the majority of people depend mainly on cereal grains as their staple food due to limited income and higher prices of animal foods. Lentil (*Lens culinaris*) is one of the most important pulse crops in Sudan. As a result of this, there is an increased demand for information on nutritional data for the crop. Nutritional quality and composition are important to consumers, both of food and animal feed, but there is little information available on composition of lentils other than crude protein content. The nutritive value of maize is inadequate due to its deficiency in the essential amino acids (lysine and tryptophan). Corn has a lower nutritive value than that of wheat due to the presence of antinutritional factors such as phytate. Phytate is very efficient as storage form of phosphorus and hydrolyze rapidly in the germinating seed[1]. It has strong binding capacity but phytate levels are reduced during certain food processing such as fermentation and cooking[2], soaking and germination[3] and malt pretreatment[4]. Other antinutritional factors (tannin and polyphenols) also reduce the nutritive value of maize. Legumes seeds are also an important source of dietary minerals with the potential to provide all 15 of the essential minerals required by man with a low concentration of certain minerals (Fe, Zn and Ca) relative to animal food products[5]. Minerals from plant sources, particularly those from plant seeds are less bioaccessible than those from animals' sources due in part to phytic acid, tannins and fibre content[6]. These antinutritional factors chelate dietary mineral in the gastrointestinal tract reducing their bioaccessibility and bioavailability[7]. Processing techniques such as soaking, cooking, germination and fermentation have been found to reduce significantly the levels of phytates and tannins by exogenous and endogenous enzymes formed during processing[8]. Legumes such as lentil contain a high concentration of proteins, carbohydrates and dietary fiber and make an important contribution to human diet in many countries. Legumes have to be processed prior to consumption due to their content of antinutritional compounds, such as trypsin inhibitors, phytic acid, á-galactosides[9]. Maize in Sudan usually consumed after processing such as cooking to prepare thick porridge (Asida) or thin one (Nasha) with or without fermentation. Lentil on the other hand, was only cooked in a thin porridge as soup without fermentation. The objective of this study was to investigate the effect of different domestic processing methods on anti-nutritional factors contents and content and availability of protein, starch and minerals of maize and lentil.

MATERIALS AND METHODS

Materials: Seeds of maize (*Zeamays linnaus*) were obtained from Arab Seeds Company in Khartoum and lentil (*lens culinaris)* seeds were obtained from domestic

market of Khartoum North. Seeds of each were cleaned from dusts, damaged grains and foreign materials by hand. The treated and untreated samples were ground to a fine powder to pass a 0.4 mm screen. All reagents used in this study were reagent grade.

Processing

Cooking: Maize seeds were cooked after soaking while lentil cooked directly without soaking. After soaking in distil water for 12 h, maize seeds were cooked using water to flour ratio of 1:7 until become soft and felt between fingers. Then dried to a constant weight and milled to fine powder to pass a 0.4 mm mesh. Unsoaked lentil seeds were also cooked in the same manner as described above.

Fermentation: The dough was prepared using 5% starter. Fermentation of maize was carried by mixing flour very well in distil water and allowed to ferment for 14 h. Thereafter dried, milled to fine powder. Then the fermented flour was cooked.

Crude protein determination: Crude protein (N% x 6.25) was determined according to AOAC[10] method.

***In vitro* Protein Digestibility (IVPD) determination:** *In vitro* protein digestibility of raw and processed samples was measured according to the method of Saunders *et al.*[11]. About 250 mg sample was suspended in 15 mL of 0.1 N HCl containing 1.5 mg pepsin (1:10,000) in a 100 mL conical flask. The mixture was incubated at 37 °C for 3 h only. The mixture was then neutralized with 0.5 N NaOH and treated with 4 mg pancreatin (Grade VI porcine) in 7.5 mL of 0.2 M phosphate buffer (pH 8.0), containing 0.005 M sodium azide. The mixture solution was incubated at 37 °C for 24 h. Ten milliliters of 10% Trichloro Acetic Acid (TCA) were added to the mixture to stop the reaction. The mixture was then centrifuged at 5000 rpm for 5 min. Five milliliters aliquots from the supernatant were pippetted and analyzed for nitrogen content.

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

Starch determination: Starch was determined using 2.0 gm of each blend by the method of Aman and Hesselman[12].

***In vitro* starch-digestibility determination:** In vitro starch digestibility of samples was determined using pancreatic á-amylase method described by Singh *et al.*[13]. About 50 mg of defatted sample were dispersed in 1.0 mL of 0.2 M phosphate buffer (pH 6.9). About 20 mg of pancreatic α-amylase dissolved in 50 mL of the same buffer and 0.5 mL of it were added to the sample suspension and incubated at 37°C for 2 h. About 2 mL of 3-5 dinitrosalicylic (10% aqueous solution) was added and the mixture was heated for 5 minutes in a water bath. After cooling, the solution was made up to 25 mL with distilled water and filtered prior to measurement of the absorbance at 550 nm. A blank was run simultaneously by adding first 3-5 dinitrosalicylic acid to the sample suspension before the addition of the enzyme solution, and then incubated at 37 °C for 2 h. Maltose was used for the standard curve determination and the *in vitro* starch digestibility values were expressed as percent maltose released from 100% starch present in the sample.

Determination of tannins content: Quantitative estimation of tannin for each sample was carried out using modified vanillin-HCl in methanol method as described by Price *et al.*[14]. A standard curve was prepared expressing the results as catechin equivalents, i.e. amount of catechin (mg mL^{-1}) which gives a colour intensity equivalent to that given by tannins after correcting for blank.

Phytic acid determination: Phytic acid content was determined by the method described by Wheeler and Ferrel[15] using 2.0 gm of a dried sample. A standard curve was prepared expressing the results as Fe $(NO_3)_3$ equivalent. Phytate phosphorus was calculated from the standard curve assuming 4:6 iron to phosphorus molar ratio.

Total polyphenols determination: Total polyphenols were determined according to Purssion Blue spectrophotometric method[16] with a minor modification. Sixty milligrams of ground sample were shaken manually for 1 min in 3.0 mL methanol. The mixture was filtered. The filtrate was mixed with 50 mL of distilled water and analyzed within an hour. About 3.0 mL of 0.1 M $FeCl_3$ in 0.1 M HCl were added to 1 mL of the filtrate followed immediately by timed addition of 3.0 mL of freshly prepared $K_3Fe(CN)_6$. The absorbance was monitor on a spectrophotometer (Pye Unicam SP6-550 UV) at 720 nm after 10 min from the addition of 3.0 mL of 0.1 M $FeCl_3$ and 3.0 mL of 0.008 M $K_3Fe(CN)_6$. A standard curve was prepared expressing the result as tannic acid equivalent i.e. amount of tannic acid (mg $100g^{-1}$) which gives a color intensity equivalent to that given by polyphenols after correction for blank.

Total minerals determination: Minerals were extracted from the samples by dry ashing method that described by Chapman and Pratt[17]. The amount of iron, zinc,

manganese, Cobalt and copper were determined using Atomic Absorption Spectroscopy (Perkin- Elmer 2380). Ammonium Vandate was used to determine phosphorus along with Ammonium Molybdate method of Chapman and Pratt[18]. Calcium and Magnisum were determined by titration method that described by Chapman and Pratt[16]. Sodium and potassium were determined by flame photometer (CORNIG EEL) according to AOAC[10].

Hcl extractable minerals (Availability): Hydrochloric acid extractability of minerals was performed according to Chauhan and Mahjan[19] method. About 1.0 g was extracted using 10 mL of 0.03N HCl with shaking at 37 °C for 3 h. The clear extract obtained was dried at 100 °C and then placed in a muffle furnace at 550°C for 4 h. Thereafter, the samples were cooled and about 5 mL of 5N HCl were added and boiled gently for 10 min and then cooled, diluted to 100 mL with distilled water. Minerals were determined as described above.

Statistical analysis: Each sample was analysed in triplicate and the values were then averaged. Data were assessed by the Analysis Of Variance (ANOVA) described by Snedecor and Cochran[21] and by Duncan'multiple range test[21] with a probability $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of processing on antinutritional factors content and availability and content of protein, starch and minerals of maize and lentil: Phytic acid content of untreated seeds was 473.0 and 817.0 mg/100g for maize and lentil, respectively. Cooking of maize and lentil significantly ($p \leq 0.05$) reduced phytate content to 353 and 504 mg /100g for the crops, respectively, (Table 1).

Further reduction was observed when maize flour was fermented before cooking and more than 50% of the grain phytate was reduced. For both maize and lentil polyphenols and tannin contents follow a trend similar to that obtained for phytate. Results showed that antinutritional factors greatly varied between the crops studied, this variation can be attributed to both genetic and environmental conditions[22] . Moreover, cooking of grains after fermentation was found to be more effective to reduce antinutritional factors levels than fermentation alone. The results comparable with Lopez *et al.*[23] who reported a maximum reduction of phytic acid level in fermented corn meal. The decrease in phytic acid content during fermentation might be due to activity of the enzyme phytase naturally present in cereal, legumes and tubers and microorganisms in the dough. The loss in polyphenols during cooking might be due to the fact that phenols react with protein forming poorly extractable protein/phenolic complexes. The decrease in polyphenols agreed with Dhankhar and Chauhan[24] finding who reported a decrease in polyphenols content of pearl millet with increasing fermentation time. Cooking after fermentation caused more reduction in total polyphenol of maize (kisra) compared to fermentation alone. This effective reduction may be attributed to the presence of polyphenoloxidase that hydrolyzed it during fermentation as well as the heat destruction of polyphenol during cooking. Cooking of maize and lentil seeds reduced tannin as reported by Alonso *et al.*[25] who found a reduction in total tannin content during cooking of legumes due to heat degradation of these molecules as well as changes in their chemical reactivity or the formation of insoluble complexes. The protein content of untreated maize and lentil was found to be 10.90 and 26.10%, respectively. Cooking was found to increase total protein content for both maize and lentil and it was increased to be 13.20 and 28.23%, respectively. The increment in protein content of the cooked samples may be due to quantitative reduction of antinutritional factors (tannin, polyphenols and phytic acid) and other water-soluble constituent as a result of cooking. Fermentation increased protein content for maize dough and it was found to be 14.63%. The increment in protein content during fermentation is also quantitative increment attributed to the utilization of carbohydrates by microorganisms. Cooking of maize after fermentation was found to be more effective in increasing protein content. The results obtained agree with those reported by Bressani[26] who found that cooking of beans in water with or without pressure increase the protein quality and quantity. *In vitro* protein digestibility of untreated maize was found to be 29.03% while those of untreated lentil was found to be 92.27%. Cooking of seeds was found to increase IVPD for both lentil and maize to 34.25% and 96.10%, respectively. Results obtained agree with those reported by Kataria *et al.*[26] who found that soaking of seeds followed by cooking in distilled water improved IVPD of mung bean seeds. The improvement of protein digestibility after cooking could be attributable to the reduction of antinutrients such as phytic acid and condensed tannins and polyphenols, which are known to interact with protein to form complexes. Fermentation was found to increase IVPD of maize dough to 75.23. Results indicated that fermentation was more effective in increasing IVPD of maize which agrees with Hassan and El Tinay[4] who reported that natural fermentation of sorghum dough caused a highly significant improvement in *in vitro* protein and starch digestibilities. Cooking after fermentation slightly increased IVPD of maize to 75.75%

Table 1. Effect of domestic processing on anti-nutritional factors content, protein and starch contents and availability of maize and lentil cultivars

		Antinutritional factors content (mg/ 100g)			Parameter (%)			
Samples	Processing	Phytic acid	Polyphenols	Tannin	Protein	IVPD	Starch	IVSD
Maize	Uncooked	473 (±3.00)^{a}	533 (±2.50)^{a}	673 (±4.00)^{a}	10.90 (±1.10)^{c}	29.03 (±0.42)^{c}	26.99 (±1.70)^{d}	27.05(±1.64)^{d}
	Cooked	353 (±2.62)^{c}	343 (±2.50)^{d}	562 (±2.00)^{d}	13.20 (±0.80)^{b}	34.25 (±1.27)^{b}	33.89 (±1.91)^{c}	33.89(±1.91)^{c}
	Fermented	261 (±2.55)^{b}	243 (±2.10)^{c}	431 (±4.00)^{c}	14.63 (±1.46)^{eb}	75.23 (±0.42)^{a}	51.93 (±2.09)^{b}	65.73(±1.39)^{b}
	Fermented and cooked	211 (±2.55)^{b}	153 (±1.50)^{b}	322 (±3.00)^{b}	16.53 (±2.01)^{a}	75.73 (±0.57)^{a}	64.19 (±1.60)^{a}	72.87(±1.60)^{a}
Lentil	Uncooked	817 (±0.71)^{a}	473 (±2.10)^{a}	531 (±2.00)^{a}	26.10 (±0.26)^{b}	92.27 (±0.45)^{b}	74.75 (±1.18)^{b}	25.42(±1.12)^{b}
	Cooked	504 (±0.71)^{b}	337 (±2.50)^{b}	254 (±2.00)^{b}	28.23 (±0.40)^{a}	96.10 (±0.36)^{a}	85.32 (±1.95)^{a}	83.62(±1.62)^{a}

Values are Means ±SD. Means not sharing a common superscript letter in a column are significantly different at p≤0.05 as assessed by Duncan's Multiple Range tests. IVPD: in *vitro* protein digestibility; IVSD: *in vitro* starch digestibility

Table 2. Effect of domestic processing on some selected major minerals content (mg $100g^{-1}$) and availability (%) of maize and lentil cultivars

		Major minerals							
		Ca		Na		K		P	
Sample	Processing	Total	Available	Total	Available	Total	Available	Total	Available
Maize	Uncooked	133.39 (±0.74)^{d}	12.92 (±1.59)^{c}	0.83 (±0.11)^{b}	25.91 (±1.97)^{b}	64.07 (±2.31)^{c}	37.72 (±1.44)^{c}	18.07 (±1.78)^{a}	22.27 (±1.97)^{a}
	Cooked	322.63 (±6.97)^{a}	44.98 (±1.93)^{a}	1.57 (±0.32)^{a}	34.18 (±1.08)^{a}	57.17 (±1.63)^{d}	53.46 (±0.60)^{b}	17.67 (±1.39)^{a}	21.65 (±1.40)^{a}
	Fermented	310.0 (±1.41)^{b}	26.27 (±1.81)^{b}	1.47 (±0.21)^{a}	14.42 (±2.20)^{c}	72.00 (±3.00)^{b}	81.81 (±1.62)^{a}	18.53 (±0.56)^{a}	15.26 (±1.12)^{b}
	Fermented and cooked	221.00 (±2.65)^{c}	45.19 (±3.15)^{a}	1.67 (±0.33)^{a}	14.21 (±0.44)^{c}	76.80 (±1.31)^{a}	81.37 (±0.84)^{a}	18.93 (±0.56)^{a}	13.16 (±1.12)^{c}
Lentil	Uncooked	285.00 (±5.66)^{b}	32.76 (±2.33)^{b}	3.52 (±0.10)^{a}	24.63 (±1.28)^{b}	86.90 (±1.51)^{a}	63.61 (±2.64)^{b}	16.75 (±1.88)^{a}	15.75 (±2.01)^{a}
	Cooked	343.00 (±5.66)^{a}	43.72 (±1.79)^{a}	3.60 (±0.26)^{a}	32.66 (±2.09)^{a}	77.20 (±1.21)^{b}	84.10 (±1.67)^{a}	13.37 (±0.67)^{b}	14.87 (±1.48)

^{a}Values are Means (±SD). Means not sharing a common superscript letter in a column are significantly different at p≤ 0.05 as assessed by Duncan's multiple range tests

compared to fermentation alone. The increase in IVPD of maize after both fermentation and cooking may be attributed to their efficiency in reducing the levels of antinutrient. As shown in Table 1 the starch content of untreated maize was found to be 26.99% while that of lentil was 74.75%. Cooking increased starch content for both maize and lentil to 33.89 and 85.32%, respectively. The results obtained agree with those obtained by Bishnoi and Khetarpoul[27] and Chau and Cheung[28] using legume seeds. The increment in starch content after cooking could be quantitative and attributed to reduction in antinutritional factors. Fermentation of maize dough increased the starch content to 51.93%. Starch content was improved significantly when maize was cooked after fermentation to 64.19% compared to fermentation alone.

In Vitro Starch Digestibility (IVSD) of untreated maize was found to be 27.05% while that of lentil was 25.42%. Cooking increased IVSD of both maize and lentil to 33.89 and 83.62%, respectively. This improvement may be due rupturing of starch granules or gelatinization of starch which is readily attacked by á-amaylase enzyme. Fermentation increased IVSD of maize dough to 65.73%. Cooking maize flour after fermentation was found to be more effective than fermentation alone and it was found to increase IVSD to 72.87%.

Effect of processing on minerals content and availability of maize and lentil: Calcium content of untreated maize and lentil seeds was found to be 133.39 and 285.00 mg/100 gm, respectively, (Table 2) and out of this amount about 12.92 and 32.76% were found to be available for the grains, respectively. Cooking of both maize and lentil seeds significantly (p≤ 0.05) increased Ca content and availability to 322.63 mg/100gm and 44.98%, respectively for maize while for lentil Ca content and availability were increased to 343.00 mg/100gm and 43.72%, respectively. Results indicated that bioavailability of Ca increased due to heat treatment. Khaterpaul and Chauhan[29] reported similar results showing an increase in bioavailability of calcium and phosphorus after cooking and they attributed the increment in total and available Ca to the decrease in phytic acid content. Moreover, cooking expected to concentrate Ca therefore its amount expected to increase.

Fermentation increased Ca content of maize and it was found to be 310 mg/100g and out of this amount about 26.27% was found to be available (Table 2). Ca content also increased when maize flour was cooked after fermentation and it was found to be 221 mg/100 and out of this amount about 45.14% was found to be available. However, cooking of the fermented maize increased Ca content but to an extend lower than that increment obtained when the flour was fermented. Cooking of the fermented flour significantly (p≤ 0.05) increased the availability of Ca compare to fermentation alone. This was likely due to the fact that cooking of the fermented flour greatly reduced the antinutritional factors, which interfere with the availability of minerals as reported by Antony and Chaudra[30]. Other minerals (Na, K and P)

Table 3. Effect of domestic processing on some selected trace minerals content (mg 100g^{-1}) and availability (%) of maize and lentil cultivars

		Trace minerals					
		Fe		Mn		Zn	
Sample	Processing	Total	Available	Total	Available	Total	Available
Maize	Uncooked	81.10 (±2.14)^{d}	44.83 (±1.15)^{c}	1.43 (±0.15)^{b}	86.59 (±2.44)^{b}	24.03 (±1.99)^{b}	12.79 (±1.71)^{d}
	Cooked	110.33 (±1.53)^{c}	45.53 (±2.28)^{c}	1.73 (±0.33)^{b}	92.65 (±1.33)^{a}	25.00 (±1.73)^{b}	15.41 (±0.56)^{c}
	Fermented	115.44 (±2.28)^{b}	51.13 (±2.61)^{b}	3.20 (±0.26)^{a}	94.08 (±1.79)^{a}	27.50 (±1.28)^{a}	53.67 (±0.60)^{b}
	Fermented and cooked	137.06 (±3.82)^{a}	74.61 (±2.41)^{a}	3.67 (±0.15)^{a}	94.38 (±2.47)^{a}	28.07 (±1.11)^{a}	62.63 (±2.33)^{a}
Lentil	Uncooked	76.90 (±1.73)^{b}	47.08 (±1.69)^{b}	3.37 (±0.21)^{b}	96.40 (±0.76)^{a}	21.83 (±1.38)^{b}	28.55 (±0.66)^{b}
	Cooked	94.12 (±1.22)^{a}	58.31 (±1.51)^{a}	7.80 (±0.36)^{a}	96.61 (±2.22)^{a}	24.07 (±1.31)^{a}	32.99 (±1.66)

[a]Values are Means ±SD. Means not sharing a common superscript letter in a column are significantly different at p≤ 0.05 as assessed by Duncan's Multiple Range tests

follow a trend similar to that obtained for Ca with few exceptions. For lentil, K content was found to be decreased and P content and availability were both decreased as the result of cooking. The reduction may be attributed t heat treatment, which may affect the quantitative determination of such minerals. Iron content of untreated maize and lentil seeds was found to be 81.10 and 76.90 mg/100 gm, respectively (Table 3) and out of this amount about 44.83 and 47.08% were found to be available for the grains, respectively. Cooking of both maize and lentil seeds significantly (p≤ 0.05) increased Fe content and availability to 110.33 mg/100gm and 45.53%, respectively for maize while for lentil Fe content and availability were increased to 94.12 mg/100gm and 58.31%, respectively. Results indicated that bioavailability of Fe increased due to heat treatment. Khaterpaul and Chauhan[29] reported similar results showing an increase in bioavailability of Fe after cooking and they attributed the increment in total and available Fe to the decrease in phytic acid content as well as other antinutritional factors. Moreover, cooking expected to concentrate Fe therefore, its amount expected to increase. Fermentation significantly (p≤ 0.05) increased Fe content of maize and it was found to be 115.44 mg/100g and out of this amount about 51.13% was found to be available (Table 3). Fe content also significantly (p≤ 0.05) increased when maize flour was cooked after fermentation and it was found to be 137.06 mg/100 and out of this amount about 74.61% was found to be available. Results indicated that cooking of the fermented flour significantly (p≤0.05) increased the availability of Fe compare to fermentation alone. This was likely due to the fact that cooking of the fermented flour greatly reduced the antinutritional factors, which interfere with the availability of minerals as reported by Antony and Chaudra[30]. Results obtained agree with the results obtained by Duhan *et al.*[29] who reported that domestic processing of pigeon pea significantly increased the availability of Fe. Increment of Fe after cooking, may be due to reduction in phytate content which is known to chelate the minerals. There was a significant and negative correlation between phytic acid content and HCl-extractability of minerals. Other minerals (Mn and Zn) follow a trend similar to that obtained for Fe.

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