ELSEVIER

Food Chemistry 84 (2004) 613–619

Food Chemistry

www.elsevier.com/locate/foodchem

Effect of genotype, malt pretreatment and cooking on in vitro protein digestibility and protein fractions of corn

Aisha S.M. Fageer*, A.H. El Tinay

Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, Shambat, Sudan

Received 5 February 2003; received in revised form 20 February 2003; accepted 26 May 2003

Abstract

Twelve corn genotypes, designated as: 12, 14, 22, 35, 36, 37, 41, 42, 43, 91, 113 and Mugtama 45 were used in this study. Investigation showed that corn had 4.3–6.7% moisture, 1.0–2.0% ash, 1.3–2.2% crude fibre, 4.9–6.2% fat, 11.3–16.9% crude protein, 74.7–81.1% carbohydrate, 256–436 mg/100 g phytate, 12.6–16.9% (2 h) in vitro protein digestibility (IVPD) before cooking and 10.4–13.7% IVPD after cooking. The percentages of the protein fractions: albumin + globulin, prolamin (zein), G_1-glutelins, G_2-glutelins, G_3-glutelins and insoluble protein were in the ranges of 16.8–22.7, 31.9–50.3, 4.9–11.3, 3.0–6.9, 10.8–21.9 and 6.0–16.2%, respectively. The genotypes 91, 22 and 43 were rich in the globulin + albumin fraction and consequently could be considered as having higher nutritional quality than other genotypes. Corn seeds of cultivar Giza 2 were germinated for 6 days to obtained 2-, 4- and 6-day old malts. Corn malt was added in concentrations of 4, 8 and 12% to corn flour. The mixtures were incubated with shaking for 2 h. The protein solubility fractions for cooked and uncooked flour indicated that the albumin + globulin fractions increased significantly ($P \leqslant 0.05$) by increasing concentration and age of the malt, accompanied by a decrease in the prolamin zein fraction. Cooking significantly ($P \leqslant 0.05$) decreased the albumin + globulin fraction but the rate of reduction was comparatively lower with increasing age and concentration of the malt. For all treatments, the G_1-glutelin and residual protein increased significantly ($P \leqslant 0.05$) while G_2-glutelin and G_3-glutelin decreased.

Keywords: Corn; Cooking; In vitro protein digestibility; Protein fractions

1. Introduction

It is well established that the majority of the people in the developing countries depend mainly on cereal grains as their staple food due to limited income and the high prices of animal foods. Like other cereals, the nutritive value of corn is nutritionally inadequate due to its deficiency in the essential amino acids lysine and tryptophan.

A comparison of available data for wheat, corn and rice puts corn as the second most important cereal grain after wheat and before rice in terms of yield per hectare (FAO, 1992). However, corn production increased significantly from 1984 to 1991 (AOAD, 1992). Although part of the increase resulted from additional land area planted, significant increases of production resulted from genetic improvement and more efficient technological field practices as well as from the introduction of new more highly productive varieties. While most of the production in developing countries is for human consumption, in the developed world it is mainly for industrial use and animal feed (FAO, 1992). Because of its economic importance, genetic improvement of corn has played a key role in the development of genotypes with high protein quantity and quality. This could be achieved through either a reduction in the zein storage protein fraction or an increased proportion of other protein fractions or a combination of the two (Or, Boyer, & Larkin, 1993). Although improvement of protein quality of corn and other cereals with protein supplements from other sources and with synthetic amino acids is possible, in the long run, improvement by genetic manipulation appears to be the most economical and least complicated approach.

Corn grain protein can be separated into six solubility fractions according to Landry and Moureaux (1970), namely albumin, globulin, zein, G_1-glutelin, G_2-glutelin and G_3-glutelin. The alcohol-soluble protein (zein) accounts for about 50% of the total endosperm protein which is characterized by high contents of glutamine,

* Corresponding author.

0308-8146/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0308-8146(03)00286-3

leucine, proline and is partically devoid of lysine and tryptophan. The overall lysine and tryptophan contents of normal maize are only 1.8 and 0.35%, respectively (Paiva, Kriz, Peixoto, Wallace, & Larkins, 1991).

Germination is an inexpensive and simple method of improving protein quality of corn and can be achieved in a short period of time. The relative nutritive value (RNV) of corn meal, made from germinated corn, is higher than the meal from non-germinated corn (Hashim & Field, 1979; May-Gilay, 1980).

Abdelmoneim, El Tinay, and Abdalla (1996) found that, during germination, the albumin and globulin fractions increased, whereas the prolamin zein decreased. Tsai, Dalby, and Jones (1975) reported improvement in lysine and tryptophan as a result of germination.

The present study was designed to evaluate the chemical composition and to determine variations of in vitro protein digestibility, as well as protein quality through its protein solubility fractions, for twelve recently introduced corn genotypes in Sudan and to study the effect of malt pretreatment and cooking on protein solubility fractions of corn using the Landry and Moureaux (1970) technique.

2. Material and methods

2.1. Materials

Twelve corn genotypes 12, 14, 22, 35, 36, 37, 41, 42, 43, 91, 113 and Muglama 45 were obtained from the Department of Agronomy, University of Khartoum. These samples were carefully cleaned and freed from foreign materials and the grains were ground to pass a 0.4-mm screen.

Commercial corn cultivar Giza 2 was obtained from Sudanese Agricultural Enterprises, Khartoum, and was used for the malt pretreatment experiment. The grains were carefully cleaned and freed of broken seeds. 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 in polyethylene bags at 4 °C.

2.2. Addition and incubation of the malt to corn flour

Two-, 4- and 6- day old malt were added to corn flour at the following concentrations: 4, 8 and 12%, in triplicate. Samples were mixed by shaking for 30 min and then mixed with water 1:2 (w/v) and incubated at 30 °C±2 °C in a shaker for 2 h. Samples were dried at 65 °C and finely ground.

For cooking, the flour was suspended in water 1:10 (w/v) and boiled in a boiling water bath for 20 min, producing gelatinous gruel. The cooked gruel was then dried at 65 °C and reground to pass through 0.4 mm screen.

2.3. Methods

2.3.1. Chemical analysis

Moisture was determined according to the AACC (1980). Ash, fibre and fat were determined according to the AOAC (1984). Protein (N×6.25) was determined according to the AOAC (1975). The phytate was estimated on a dry weight basis by the method of Wheeler and Ferrel (1971).

2.3.2. Determination of in vitro protein digestibility

The in vitro protein digestibility was carried out according to the method of Maliwal (1983) in the manner described by Monjula and John (1991) with 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 of 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:

$$\text{Protein digestibility} = \frac{\text{N in supernatant} - \text{N in blank}}{\text{N in sample}}$$

2.3.3. Protein factions

2.3.3.1. General. The nitrogen from the defatted meal was extracted stepwise by a series of solvents according to the Landry and Moureaux (1970) technique. Thus, triplicate 3.5 g samples were kept in suspension with 35 ml of extractant by magnetic stirring in 50-ml centrifuge tubes.

2.3.3.2. Step 1. To obtain the first fraction, 0.5 M NaCl was added to the sample powder and the mixture was stirred three times, for 60, 30 and 30 min at 4 °C.

2.3.3.3. Step 2. The residue was extracted with the same volume of distilled water, twice for 15 min at 4 °C.

2.3.3.4. Step 3. To obtain the third fraction, the residual material was stirred with 60% ethanol, twice, for 30 min at 20 °C, and then at 60 °C for 30 min, followed by extraction with 55% isopropanol (Pro.OH) at 20 °C three times for 60, 30 and 15 min with stirring.

2.3.3.5. Step 4. To obtain the fourth fraction the residue was extracted with 60% ethanol plus 0.6% 2-mercaptoethanol (2-ME) and stirred, twice, for 30 min (20 °C),

then extracted with 55% Pro.OH containing 2-ME (0.6%) at 20 °C, twice for 30 min.

2.3.3.6. Step 5. To obtain the fifth fraction, borate buffer, pH10 (0.0125 M $Na_2B_4O_7.12H_2O$ and 0.02 M NaOH) with 0.6% 2-ME and 0.5 M NaCl, was used with stirring for 60, 30 and 30 min (20 °C).

2.3.3.7. Step 6. To obtain the sixth fraction, borate buffer, pH10 with 0.6% 2-ME and 0.5% sodium dodecyl sulphate (SDS) was used with stirring for 60, 30 and 15 min (20 °C).

Fractions I and II contained globulin and albumin, the free amino acids and small peptide fragments. Fraction III contained the prolamin zein. Fraction IV contained the zein like protein (G_1-glutelins). Fraction V contained the glutelin like protein (G_2-glutelins). Fraction VI contained the true glutelin (G_3-glutelins). The solid material was isolated from extractants by centrifugation at 30 000*g* for 15 min. For each solvent, supernatants were combined to give the total extract. The nitrogen content of each of these six fractions was determined by the micro-Kjeldahl method. The residue left after extraction was also analysed for nitrogen content.

2.3.4. Statistical analysis

Each determination was carried out on three separate samples and analysed in triplicate and results were then averaged. Data were assessed by analysis of variance (ANOVA) (Snedecor & Cochran, 1987) and by Duncan's multiple range test with a probability $P \leqslant 0.05$ (Duncan, 1955).

3. Results and discussion

3.1. Chemical composition

The chemical composition of twelve corn genotypes is shown in Table 1. Moisture content ranged from 4.3 to 6.7%, being lower than the range reported by Abdelmoneim (1995). Protein content ranged from 11.3 to 16.9g. Values obtained are higher than the range of 8.9–11.2% reported by Amer, Khalil, Zoueil, and Mesallam (1986) for Egyptian varieties and were near to the range reported by Manoharkumar, Gerstenkorn, Zwingelberg, and Bolling (1978) for German varieties. The fat content ranged from 4.9 to 6.2%. This range is higher than the range reported by Peplinski, Paulsen, and Bouzaher (1992) for American varieties. Crude fibre ranged from 1.3 to 2.2%. Values obtained in this study were lower than the range 1.7 to 3.5% reported by Abdelmoneim (1995) for Sudanese varieties, but were close to the range 1.4–1.9% reported by Amer et al. (1986). Ash content ranged from 1.0 to 2.0%. These were lower than the values reported by Uprety and Austin (1973) and Sanderson, Paulis, Porcuna, and Wall (1979). Carbohydrate ranged from 74.7 to 81.1%. The values obtained were higher than those given by Abdelmoneim (1995) and Peplinski et al. (1992). Phytic acid level ranged from 256 to 436 mg/100 g. These values were much lower than the range of 686–734 mg/100 g for white and yellow corn, respectively reported by Marfo, Simpson, Idowu, and Oke (1990). Khan, Zaman, and Elahi (1991) reported very high levels of 715–760 mg/100g, while Deshmukh, Adsule, and Kachare (1995) reported phytate phosphorus content ranging from 132 to 234 mg/100 g. Variation in phytic acid content among different genotypes can be attributed to both genetic and environmental factors, as reported by Simwemba, Hoseney, Varrianomarston, and Zelezank (1984).

3.2. In vitro protein digestibility

The in vitro protein digestibility is shown in Table 2. It ranged from 12.6 to 16.9% for uncooked samples. Differences in protein digestibility were related to enzyme susceptibility of the major storage protein, prolamin (Weaver, Hamaker, & Axtell, 1998). After cooking, pepsin digestibility significantly ($P \leqslant 0.05$) decreased and ranged from 10.4 to 13.6%. Higher percentage reduction in protein digestibility after cooking is evident in lines 12, 113 and Mugtama 45, in comparison to lines 42, 43 and 91, respectively. Reduction in protein digestibility was reported by Yousif (2000) and was attributed to formation of disulphide bonding resulting in the folding of the protein molecule and hence decreasing its susceptibility to digestive enzymes.

3.3. Protein fractions

As shown in Table 2 the water- and salt-soluble proteins, albumins and globulins make up 16.8% of total protein for genotype 37 and 22.7% for genotype 91. Values among cultivars are significantly ($P \leqslant 0.05$) different except for the genotypes 12 and 22, while genotypes 37, 41 were similar. The prolamin zein fraction was 31.9% for genotype 22 and 50.3% for genotype 35. Zein was the predominant fraction for all corn lines. The zein-like fraction (G_1-glutelins) varied from 4.9 to 11.3%, the glutelin like fraction (G_2-glutelins) ranged from 3.0 to 6.7% for genotypes 14 and 37, respectively. The true glutelin (G_3-glutelins) varied from 10.8 to 21.9%. Residual protein for the corn lines studied ranged from 6.0 to 16.2%. The genotypes can arbitrarily be grouped into low, medium and high protein entities with protein contents of 12.5 and 14.7% as dividing lines. The five low-protein genotypes were rich in fraction I (albumin + globulin) and fraction V (true glutelin G_3), except for the two lines 12 and 14, which were characterized by a low level of glutelin and a high level

Table 1
Chemical composition of twelve corn genotypes

Genotype	Moisture%	Ash%	Fibre%	Oil%	Protein%	Carbohydrate%	Phytate mg/100 g
12	6.3±(0.18)d	1.5±(0.15)c	2.2±(0.11)a	5.1±(0.43)fg	11.9±(0.17)i	79.2±(0.55)c	256±(38)k
14	6.3±(0.08)d	2.0±(0.08)a	1.6±(0.09)d	5.7±(0.69)cd	11.6±(0.06)j	79.2±(0.62)c	279±(19)j
22	6.7±(0.05)a	1.0±(0.00)g	1.3±(0.07)f	5.2±(0.15)f	11.5±(0.22)k	81.1±(0.35)a	403±(00)c
35	4.8±(0.21)h	1.7±(0.15)b	1.8±(0.00)b	5.0±(0.21)gh	16.9±(0.22)a	74.7±(0.14)I	428±(00)b
36	4.3±(0.02)j	1.4±(0.04)d	1.3±(0.14)f	4.9±(0.13)h	14.4 (+0.11)e	78.0±(0.31)d	360±(00)e
37	4.5±(0.36)i	1.3±(0.00)e	1.3±(0.13)f	5.8±(0.39)bc	14.1±(0.00)g	77.5±(0.44)e	339±(37)g
41	6.1±(0.22)f	1.4±(0.08)d	1.5±(0.03)e	5.4±(0.36)e	14.2±(0.16)f	77.3±(0.16)ef	389±(19)d
42	6.2±(0.05)e	1.2±(0.10)f	1.8±(0.08)b	6.2±(0.21)a	11.3±(0.00)l	79.5±(0.4)b	322.9±(19)I
43	6.00±(0.03)g	1.2±(0.16)f	1.5±(0.16)e	5.9±(0.28)b	14.7±(0.16)c	76.7±(0.16)g	333±(33)h
91	6.5±(0.02)c	1.2±(0.13)f	1.6±(0.28)d	5.7±(0.24)cd	12.5±(0.17)h	79.1±(0.61)c	414±(51)c
113	6.3±(0.24)d	1.4±(0.11)d	1.5±(0.12)e	5.6±(0.38)d	14.5±(0.11)d	77.0±(45)fg	357±(38)f
Mugtama 45	6.6±(0.13)b	1.7±(0.05)b	1.7±(0.12)c	5.4±(0.16)e	15.0±(0.17)b	76.2±(0.25)h	437±(33)a

Values are means±standard deviation. Means not sharing a common superscript letter(s) in a column are significantly different at $P \leqslant 0.05$ as assessed by Duncan's multiple range test.

Table 2
Protein fractions and in vitro protein digestibility of 12 corn genotypes

Samples	I + II Globulin + albumin (%)	III (Zein) (%)	IV G_1-glutelin (%)	V G_2-glutelin (%)	VI G_3-glutelin (%)	Insoluble protein (%)	Total protein recovered (%)	IVPD before cooking (%)	IVPD after cooking (%)
12	21.9±(1.01)b	38.4±(0.92)cde	11.3±(0.77)a	3.62±(0.12)g	11.8±(0.00)c	16.2±(0.4)a	103.2	12.6±(0.61)g	10.4±(0.33)gh
14	21.2±(0.31)c	39.2±(0.31)cd	9.00±(0.26)b	2.96±(0.11)h	10.8±(0.34)c	15.9±(0.18)a	99.0	15.9±(0.76)c	11.1±(0.6)f
22	21.9±(0.17)b	31.9±(0.45)e	8.34±(0.33)cd	6.85±(0.13)a	21.8±(0.34)a	7.34±(0.17)f	98.1	16.4±(0.28)b	12.0±(0.17)d
35	17.4±(0.33)g	50.3±(0.39)a	4.92±(0.71)f	5.54±(0.09)d	16.0±(0.19)b	7.99±(0.53)ef	102	14.5±(0.28)f	10.4±(0.35)gh
36	19.8±(1.09)d	43.3±(1.03)bc	5.55±(0.26)e	6.06±(0.10)c	19.1±(0.31)ab	5.97±(0.62)g	99.8	15.9±(0.25)c	12.4±(0.33)c
37	16.8±(0.3)g	46.2±(0.72)ab	4.90±(0.71)f	6.69±(0.2)ab	18.7±(0.11)ab	7.77±(0.47)ef	101	16.9±(0.00)a	12.4±(0.83)c
41	17.4±(0.74)g	39.6±(0.00)cd	8.09±(0.13)d	5.77±(0.4)cd	18.7±(0.69)ab	9.12±(0.62)c	98.7	14.8±(0.39)ef	11.5±(0.19)e
42	18.9±(0.05)ef	35.0±(0.01)de	8.48±(0.38)cd	6.52±(0.75)b	21.9±(0.98)a	8.82±(0.58)cd	99.7	15.2±(0.52)d	13.6±(0.1)a
43	20.6±(0.48)c	32.4±(1.15)e	9.12±(0.14)b	5.25±(0.58)de	18.1±(0.58)b	12.0±(1.47)b	97.4	15.0±(0.28)de	13.3±(0.42)b
91	22.7±(0.61)a	34.9±(0.13)de	8.56±(0.45)c	5.89±(0.39)c	18.7±(0.34)ab	8.33±(0.54)de	99.1	15.0±(0.13)de	13.3±(0/34)b
113	18.5±(0.46)f	39.1±(0.22)cd	9.11±(0.77)b	4.76±(0.10)f	18.3±(0.34)b	7.53±(0.46)f	97.3	15.7±(0.29)c	10.7±(0.33)g
Mugtama 45	19.4±(0.69)de	41.6±(0.57)bc	9.06±(0.43)b	5.16±(0.75)e	17.4±(0.43)b	7.85±(0.68)ef	101	15.9±(0.08)c	11.2±(023)ef

Values are means±standard deviation. Means not sharing a common superscript letter(s) in a column are significantly different at $P \leqslant 0.05$ as assessed by Duncan's multiple range test.

of residual protein. The five medium protein lines have relatively higher prolamin contents and relatively poor albumin + globulin contents except for the line 43 which showed a high value of albumin + globulin and a low value of zein fraction. The two abundantly high protein genotypes, 35 and Mugtama 45, had the highest prolamin contents, indicating that the increase in protein content in corn varieties is attributable mainly to higher levels of the prolamin fraction (Virupaksha & Sastry, 1968).

Of especial interest to the nutritionist is the lysine level in these fractions. The lysine level of fraction I was reported to be in the range 4.3–6.3 g/100g protein. Similarly, the lysine levels in fractions II, III and IV fall in or near the ranges of 0.1–0.5, 0.4–0.8, 1.4–2.9 g/100 g protein, respectively. Fraction V (true glutelin) had a high levels of lysine, 5.7–7.0 g/100 g (Nwasike, Mertz, Pickett, Glover, Chibber, & VanScoyoc, 1979). The existence of high negative correlation between zein and lysine contents is well documented in corn (Esen, 1980; Paulis, Wall, & Kwolek, 1974). The same holds true for zein and tryptophan because lysine and tryptophan levels are positively correlated (Hernandez & Bates, 1969).

The effects of concentration and age of the malt and cooking on protein solubility fractions are shown in Tables 3–5. Albumin + globulin fractions increased significantly ($P \leqslant 0.05$) as a result of increasing age and concentration of the malt, while cooking significantly ($P \leqslant 0.05$) reduced them, but the extent of the reduction was lower in treated samples. The albumin + globulin fractions are characterized by higher levels of the amino acid lysine, as reported by Wu and Wall (1980) and Patterson, Brown, Linkswiter, and Harper (1980). The nutritional value of corn is expected to improve, due to malt pre-treatment, as a result of increased levels of the albumin + globulin fractions.

Table 3
Effect of 2 day-old malt pretreatment and cooking on protein fractions

Malt concentration%	I + II Globulin + albumin (%)	III Zein (%)	IV G_1-glutelin (%)	V G_2-glutelin %)	VI G_3-glutelin (%)	Insoluble protein (%)	Total protein recovered (%)
0 Uncooked	16.4±(0.76)ef	45.2±(0.13)a	5.28±(0.19)l	6.63±(0.41)a	18.5±(0.4)d	8.99±(0.56)h	101
0 Cooked	9.32±(0.83)n	24.0±(0.80)jk	12.3±(0.84)e	6.18±(0.27)b	35.8±(1.28)a	12.9±(0.91)fg	101
4 Uncooked	16.1±(0.38)f	40.7±(0.03)b	7.59±(0.39)k	5.11±(0.86)d	19.4±(0.47)cd	12.3±(0.97)fg	101
4 Cooked	9.97±(0.17)m	25.5±(0.96)ij	13.5±(2.9)d	3.33±(0.31)i	14.5±(0.35)f	33.2±(0.94)bc	99.9
8 Uncooked	16.5±(0.36)ef	39.2±(0.2)bcd	8.42±(0.72)j	6.24±(0.15)b	20.2±(0.36)bc	11.7±(0.68)g	102
8 Cooked	10.53±(0.32)l	31.0±(0.2)g	12.4±(1.38)e	3.70±(0.03)g	13.0±(0.99)g	31.5±(0.82)c	102
12 Uncooked	16.6±(0.85)de	39.8±(0.14)bc	8.50±(0.34)j	5.56±(0.38)c	20.9±(0.45)b	11.6±(1.71)g	103
12 Cooked	11.1±(0.42)k	35.0±(1.89)f	11.4±(0.36)fg	3.72±(0.28)g	10.6±(0.2)h	27.3±(0.19)d	99.7

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

Table 4
Effect of 4-days old malt pretreatment and cooking on protein fractions

Malt concentration %	I + II Globulin + albumin (%)	III Zein (%)	IV G_1-glutelin (%)	V G_2-glutelin (%)	VI G_3-glutelin (%)	Insoluble protein (%)	Total protein recovered (%)
0 Uncooked	16.4±(0.76)ef	45.2±(0.13)a	5.28±(0.19)l	6.63±(0.41)a	18.5±(0.4)d	8.99±(0.56)h	101
0 Cooked	9.32±(0.83)n	24.0±(0.8)jk	12.3±(0.84)e	6.18±(0.27)b	35.8±(1.28)a	12.9±(0.91)fg	101
4 Uncooked	16.52±(0.51)def	38.2±(1.15)cde	9.30±(0.14)i	5.41±(0.09)cd	19.9±(0.76)c	11.1±(0.19)g	100
4 Cooked	11.4±(0.29)jk	26.0±(1.65)hi	15.3±(0.34)c	3.66±(0.19)gh	8.93±(0.25)i	35.7±(1.08)a	101
8 Uncooked	17.1±(0.3)bc	37.9±(0.27)de	9.96±(0.3)h	5.68±(0.23)c	19.4±(0.69)cd	11.7±(0.77)g	102
8 Cooked	10.9±(0.28)kl	27.5±(1.00)h	15.7±(0.07)c	3.38±(0.06)hi	9.46±(0.63)i	36.3±(0.24)a	103
12 Uncooked	16.8±(0.27)cde	37.3±(0.6)e	12.6±(0.35)e	4.70±(0.11)e	16.9±(0.57)e	12.9±(2.36)fg	101
12 Cooked	11.6±(0.19)j	25.4±(0.07)ij	16.8±(0.17)b	3.28±(0.15)I	8.83±(0.2)i	37.1±(0.06)a	103

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

Table 5
Effect of 6 day-old malt pretreatment and cooking on protein fractions

Malt concentration %	I + II Globulin + albumin (%)	III Zein (%)	IV G_1-glutelin (%)	V G_2-glutelin (%)	VI G_3-glutelin (%)	Insoluble protein (%)	Total protein recovered (%)
0 Uncooked	16.4±(0.76)ef	45.2±(0.13)a	5.28±(0.19)l	6.63±(0.41)a	18.5±(0.4)d	8.99±(0.56)h	101
0 Cooked	9.32±(0.83)n	24.0±(0.8)jk	12.3±(0.84)e	6.18±(0.27)b	35.8±(1.28)a	12.9±(0.9)fg	101
4 Uncooked	17.0±(0.27)bcd	35.1±(0.09)f	12.0±(0.6)ef	4.30±(0.17)f	16.4±(0.25)e	13.9±(0.21)f	98.6
4 Cooked	12.2±(0.28)i	23.9±(0.4)jk	17.0±(0.14)b	3.22±(0.10)ij	9.79±(0.26)hi	36.5±(0.47)a	103
8 Uncooked	17.4±(0.37)b	35.6±(0.43)f	11.01±(0.56)g	4.30±(0.19)f	15.2±(0.55)f	17.3±(0.98)e	102
8 Cooked	13.6±(0.57)h	22.5±(0.38)k	18.1±(0.68)a	2.79±(0.15)k	9.60±(0.34)i	36.4±(0.99)a	101
12 Uncooked	19.2±(0.31)a	34.9±(1.89)f	11.1±(0.14)g	4.19±(0.37)f	14.9±(1.23)f	17.2±(0.38)e	103
12 Cooked	14.4±(0.47)g	23.2±(2.14)k	18.6±(0.15)a	2.96±(0.04)jk	9.42±(1.13)i	33.5±(1.9)b	102

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

Prolamin or zein fractions decreased in treated samples and the reduction was maximum (35.1%) in the 4% malt of 6 days; thereafter, it remained unchanged. Cooking resulted in a further reduction of the zein fraction.

G_1-gutelins (zein-like) increased significantly as a result of treatment with malt, showing higher levels with increasing age and concentration of the malt, reaching a maximum value of 12.0%, and then started to decrease. Unlike the prolamin fraction, cooking brought about a further increase in G_1-glutelin fraction; the overall increment was 251%.

G_2-glutelins decreased as a result of malt treatment but the rate of reduction fluctuated as a function of age and concentration of the malt. However, cooking significantly ($P \leqslant 0.05$) lowered the G_2-glutelins.

Cooking of untreated samples caused a significant ($P \leqslant 0.05$) increase in the G_3-glutelins, from 18.5 to 35.8% whereas, after treatment and cooking of samples, it was significantly reduced. Heat-treatment of cereals has been reported to significantly increase the G_3-glutelins (Arbab & El Tinay, 1997; El Khalifa, Chandrashekar, & El Tinay, 1999; El Khalifa, Chanderashekar, Mohamed, & El Tinay, 1999; Yousif, 2000). It appears that malt pretreatment prevented polymerization of peptide chains forming the high molecular weight G_3-polypeptide. Lowering the level of G_3-glutelins suggests better digestibility of cooked corn proteins and hence better bioavailability.

4. Conclusion

The genotypes studied represent new breeding lines which are characterized by wide variability in chemical composition, in vitro protein digestibility and distribution of protein fractions. Three genotypes, 22, 43 and 91, were characterized by a higher protein quality as they have high levels of fractions I + II and V (albumin + globulin and G_3-glutelin) which have markedly higher lysine contents. However. genotype 43 is superior to the other two because it is also rich in protein and, moreover, it has relatively high in vitro protein digestibility after cooking.

The results show that malt pretreatment greatly improved protein quality of uncooked and cooked corn flour by increasing the levels of albumin and globulin fractions. Also, the deleterious effect of cooking on cereal protein availability was significantly diminished through the lowering of the G_3-glutelin fraction. This constitutes a hitherto unreported observation.

Acknowledgements

Thanks and gratitude are extended to the German Academic Exchange Service (DAAD) for financing and supporting this work.

References

AACC. (1980). *Approved methods of American Association of Cereal Chemists*. Saint Paul, Minnesota, USA: American Association of Cereal Chemists.

Abdelmoneim, Z. (1995). *Influence of genotype on corn protein fractions and the effect of germination on corn proteins*. MSc thesis, Faculty of Agriculture. University of Khartoum, Sudan.

Abdelmoneim, Z. S., El Tinay, A. H., & Abdalla, A. H. (1996). Effect of germination on protein fractions of corn cultivars. *Food Chemistry*, *57*, 381–384.

Amer, M., Khalil, M., Zoueil, M., & Mesallam, A. (1986). Chemical composition of some new cultivated maize varieties and hybrids in Egypt. *Pakistan Journal of Agricultural Research*, *7*, 32–36.

AOAC. (1975). *Official methods of analysis* (12th ed.). Washington, DC: Association of Official Agricultural Chemists.

AOAC. (1984). *Official methods of analysis* (14th ed.). Washington, DC: Association of Official Agricultural Chemists.

AOAD. (1992). *Year book of agricultural statistics*. Khartoum, Sudan: Arab Organization for Agricultural Development.

Arbab, M. E., & El Tinay, A. H. (1997). Effect of cooking and treatment with sodium bisulphite or ascorbic acid on the in vitro protein digestibility of two sorghum cultivars. *Food Chemistry*, *59*, 339–343.

Bhise, V. J., Chavan, J. K., & Kadam, S. S. (1988). Effect of malting on proximate composition and in vitro protein and starch digestibility of grain sorghum. *Journal of Food Science and Technology*, *23*, 327–329.

Deshmukh, K. S., Adsule, R. N., & Kachare, D. P. (1995). Evaluation of protein quality of improved maize cultivars. *Journal of Maharashtra Agricultural Universities*, *20*(1), 155–156.

Duncan, D. B. (1955). Multiple range and multiple F-test. *Biometrics*, *11*, 1–42.

El Khalifa, A. O., Chandrashekar, A., & El Tinay, A. H. (1999). Effect of pre-incubation of sorghum flour with enzymes on the digestibility of sorghum gruel. *Food Chemistry*, *66*, 339–343.

El Khalifa, A. O., Chandrashekar, A., Mohamed, B. E., & El Tinay, A. H. (1999). Effect of reducing agents on the in vitro protein and strach digestibility of cooked sorghum. *Food Chemistry*, *66*, 323–326.

Esen, A. (1980). A simple colorimetric method for zein determination in corn and its potential in screening for protein quality. *Journal of Cereal Chemistry*, *57*(2), 129–132.

FAO. (1992). *Maize in human nutrition; report series 25*. Rome: Food and Agricultural Organization.

Hashim, N. B., & Fields, M. L. (1979). Germination and relative nutritive value of corn meal and corn chips. *Journal of Food Science*, *44*, 936–937.

Hernandez, H., & Bates, L. S. (1969). Cited by Esen, A (1980). A simple colorimetric method for zein determination in corn and its potential in screening for protein quality. *Journal of Cereal Chemistry*, *57*(2), 129–132.

Khan, N., Zaman, R., & Elahi, M. (1991). Effect of heat treatments on phytic acid content of maize products. *Journal of Science of Food and Agriculture*, *54*, 153–156.

Landry, J., & Moureaux, T. (1970). Heterogeneity of the glutelins of the grain corn. Selective extraction and composition in amino acids of the three isolated fractions. *Bull. Soc. Chem. Bio.*, *52*, 1021–1037.

Maliwal, B. P. (1983). In vitro method to assess the nutritive value of leaf concentrate. *Journal of Agricultural and Food Chemistry*, *31*, 315–319.

Manoharkumar, B., Gerstenkorn, P., Zwingelberg, H., & Bolling, H. (1978). On some correlation between grain composition and physical characteristics to the dry milling performance in maize. *Journal Food Science and Technology, India*, *15*(1), 1–6.

Marfo, E. K., Simpson, B. K., Idowu, J. S., & Oke, O. L. (1990). Effect of local food processing on phytate levels in cassava, cocoyam, yam, maize, sorghum, rice, cowpea and soybean. *Journal Agricultural and Food Chemistry*, *38*, 1580–1585.

May-Gilay, M., & Field, M. L. (1981). Nutritive value of germinated corn and corn fermented after germination. *Journal of Food Science*, *46*, 1069–1073.

Monjula, S., & John, E. (1991). Biochemical changes and in vitro protein digestibility of the endosperm of germinating Dolichos lablab. *Journal of the Science of Food and Agriculture*, *55*, 229–538.

Nwasike, C. C., Mertz, E. T., Pickett, R. C., Glover, D. V., Chibber, B. A. K., & VanScoyoc, W. (1979). Lysine level in solvent fractions of pear millet. *Journal of Agricultural and Food Chemistry*, *27*(6), 132–1331.

Or, E., Boyer, S. K., & Larkins, B. A. (1993). Opaque-2 modifiers act post-transcriptionally and in a polar manner on y-zein gene expression in maize endosperm. *Plant Cell*, *5*, 1599–1609.

Paiva, E., Kriz, A. L., Peixoto, M. J. V. D., Wallace, J. C., & Larkins, B. A. (1991). Quantitation and distribution of y-zein in the endosperm of maize kernel. *Journal of Cereal Chemistry*, *68*, 276–279.

Patterson, J. I., Brown, R. R., Linkswiter, H., & Harper, A. E. (1980). Extraction of tryptophan-niacin metabolites by young men: effect of tryptophan, leucine and vitamin B_6 intakes. *American Journal of Clinical Nutrition*, *33*, 2157–2167.

Paulis, J. W., Wall, J. S., & Kwolek, W. F. (1974). A ripid turbidimetric analysis for zein in corn and its correlation with lysine content. *Journal of Agricultural and Food Chemistry*, *22*, 313–317.

Peplinski, A. J., Paulsen, M. R., & Bouzaher, A. (1992). Physical, chemical and dry milling properties of corn of varying density and breakage susceptibility. *Journal of Cereal Chemistry*, *69*(4), 397–400.

Sanderson, J. E., Paulis, J. W., Porcuna, F. N., & Wall, J. S. (1979). Sweet corn: varietal and developmental, differences in amino acid content and composition of grain. *Journal Food Science*, *44*, 636–638.

Simwemba, C. G., Hoseney, R. C., Varrianomarston, E., & Zelezank,

K. (1984). Certain vitamin and phytic acid contents of pearl millet (P. americanum L.). *Journal of Agriculture and Food Chemistry*, *32*, 31–34.

Snedecor, G. W., & Cochran, W. G. (1987). *Statistical methods* (17th ed.). IA, USA: The Iowa State Univeristy Press.

Tsai, C. Y., Dalby, A., & Jones, R. A. (1975). Lysine and tryptophan increase during germination of maize seeds. *Journal of Cereal Chemistry*, *52*, 356–358.

Uprety, D. C., & Austin, A. (1973). Chemical composition of some improved maize hybrids and composities. *Bulletin of Grain Technology*, *11*(2), 124–128.

Virupaksha, T. K., & Sastry, L. V. (1968). Studies on the protein content and amino acids composition of some varieties of grain sorghum. *Journal of Agricultural and Food Chemistry*, *16*, 199–203.

Weaver, A. C., Hamaker, B. R., & Axtell, J. D. (1998). Discovery of grain sorghum germplasm with high uncooked and cooked in vitro protein digestibilities. *Journal of Cereal Chemistry*, *75*(5), 665–670.

Wheeler, E., & Ferrel, R. E. (1971). A method for phytic acid determination in wheat and wheat fractions. *Journal of Cereal Chemistry*, *48*, 312–320.

Wu, V., & Wall, J. S. (1980). Lysine content of protein increased by germination of normal and high lysine sorghum. *Journal of Agricultural and Food Chemistry*, *28*, 455–458.

Yousif, N. E. (2000). *Effect of fermentation and dry cooking following fermentation on protein fractions and in vitro protein digestibility of sorghum, corn and rice*. PhD thesis, Faculty of Agriculture, University of Khartoum, Sudan.