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Protein Biofortified Sorghum: Effect of Processing into Traditional African Foods on Their Protein Quality

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ABSTRACT: Protein biofortification into crops is a means to combat childhood protein-energy malnutrition (PEM) in developing countries, by increasing the bioavailability of protein in staple plant foods and ensuring sustainability of the crop. Protein biofortification of sorghum has been achieved by both chemically induced mutation and genetic engineering. For this biofortification to be effective, the improved protein quality in the grain must be retained when it is processed into staple African foods. Suppression of kafirin synthesis by genetic engineering appeared to be superior to improved protein digestibility by chemical mutagenesis, because both the lysine content and protein digestibility were substantially improved and maintained in a range of African foods. For the genetically engineered sorghums, the protein digestibility corrected amino acid score was almost twice that of their null controls and considerably higher than the high protein digestibility sorghum type. Such protein biofortified sorghum has considerable potential to alleviate PEM.

KEYWORDS: Sorghum, biofortification, kafirin, protein digestibility, lysine

INTRODUCTION

One of the most common forms of child malnutrition in developing countries is protein-energy malnutrition (PEM).[1] Direct causes are insufficient food and the lack of dietary diversity coupled with the outbreak of diseases.[2] Plant foods are the most important part of the diet in most developing countries.[3] Sorghum is the staple food of some 300 million people in Africa, who live in the desert margins and semi-arid tropics.[4] Sorghum is well-adapted for growth in these areas, being a hardy crop that can tolerate drought and water-logging.[5] From a nutritional point of view, while sorghum has the same amount of protein as other major cereals, the quality of the protein is inferior. Lysine, the first limiting essential (indispensable) amino acid is between 35 and 90% lower than other cereals.[6] Lysine is essential for growth in infants and maintenance in adults,[7] is important for bone calcification and gastric secretions, and also plays a vital role in the immune system.[7,8] Additionally, the digestibility of sorghum protein is lower than, for example, maize, especially when wet cooked into food, despite the proteins of these two cereals being very similar.[9]

Biofortification aims to increase the bioavailability of nutrients in plant foods through the genetic selection of specific traits and putting them into the crop,[10] while at the same time ensuring sustainability of the crop. Two different approaches to protein biofortification of sorghum have been used, chemically induced mutation and genetic engineering.

In the 1970s, high lysine sorghum was obtained by chemical mutagenesis of a normal, non-tannin line, P721N.[11] This mutant line, P721Q, has more albumins and globulin proteins and less kafirins and cross-linked kafirins than normal sorghum types, resulting in 60% higher lysine content than normal sorghum types. More recently, sorghum lines derived (P851171 and P850029) from P721Q have been shown to have some 10−15% higher uncooked and approximately 25% higher cooked *in vitro* protein digestibility (IVPD) than P721N.[12] This improved digestibility was attributed to increased enzyme susceptibility of the major storage protein, α-kafirin, because of changes in protein body morphology.[13]

The Africa Biofortified Sorghum project led by Africa Harvest Biotechnology Foundation International has used recombinant DNA technology to develop a nutritionally enhanced sorghum with improved lysine and wet-cooked protein digestibility.[14] This has been achieved by suppression of the synthesis of kafirin species using RNA interference technology,[15] as demonstrated with zein, the maize prolamin.[16] Henley et al.[6] reported that early transgenic biofortified sorghums had irregular protein bodies, which looked similar to those of the high digestible lines and was thought to be due to the suppression of kafirin synthesis. These sorghum types had 52−115% more lysine, 23−102% higher IVPD, and double the protein digestible corrected amino acid score (PDCAAS) for 1−2-year-old children than normal sorghum types.

The aim of this research was to establish whether protein quality improvements in these different types of protein biofortified sorghum, high protein digestibility and suppressed kafirin synthesis, would be retained when they are processed into the types of sorghum foods consumed in Africa.

MATERIALS AND METHODS

Materials. The following sorghum types were used for the preparation of food products: two transgenic samples with suppression of kafirin synthesis (T1 and T2) and their null controls (C1 and C2) (parent P898012, type-II tannin sorghum), supplied by Pioneer HiBred, Johnston, IA, 2008; a non-tannin high protein digestibility line, 07HW PRGE 103 (BTx635*P850029)-CS9-CS1-CS1 (HD); a non-tannin, normal protein digestibility line, 06CS7302/7301 ATx2928/RTX436, (USC), both from Texas A&M University, Weslaco, TX, 2006; and

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Macia (developed from SDS 3220, ICRISAT SMIP) cultivated at Makoro Lands, Central District, Botswana, Africa, 2004, an improved non-tannin variety grown widely in sub-Saharan Africa. Macia and USC were included as controls.

All chemicals were obtained from Merck, Darmstadt, Germany or Sigma, St. Louis, MO, unless otherwise stated.

Methods. All samples were milled using either a laboratory hammer mill (Falling Number, Huddinge, Sweden) fitted with a 500 μm opening screen or, for the transgenic samples, a coffee mill (IKA A11 Basic, Staufen, Germany) and then passed through a 500 μm opening sieve to give whole grain flour, which was stored at 10 °C prior to food product preparation. The seven sorghum types were used to prepare six different types of traditional African sorghum-based foods, an unfermented porridge (ugali), a fermented porridge (uji), an alkali cooked porridge (tô), an unfermented flatbread, a fermented flatbread (injera), and a steamed product (couscous). Cookies were also prepared, a product baked at high temperature and often used in relief feeding schemes.[17] Raw and raw, fermented flours were included for comparison. Because of the small amount of transgenic sorghum available, small-scale processing methods were devised.

Preparation of Food Products. Raw whole grain was analyzed as is.

Cooked Unfermented Porridge (Ugali). Distilled water (25.1 g at 25 °C) was weighed into a Rapid Visco Analyser (RVA) (Newport Scientific, Warriewood, Australia) canister. Flour (2.9 g, 10% moisture) was added to the canister containing the water and mixed thoroughly using a RVA paddle. The porridge was cooked in the RVA using the following profile: heated to 91 °C within 20 min, held at 91 °C for 5 min, cooled to 50 °C within 5 min, and then held at 50 °C for a further 3 min. Samples were prepared in duplicate.

Alkali Cooked Porridge (Tô). Samples were cooked as described above, except 0.025 M KOH (1.37 g/L) was used instead of distilled water. The final porridge pH was approximately 9.

Starter Culture. Macia flour (25 g) was mixed with 65 mL of tap water and incubated for 48 h at 25 °C. This was used as inoculum for fermentation.

Fermented Uncooked Flour. Raw grain, 3 g, was mixed with 8 g of distilled water in a plastic tube. A total of 2 g of inoculum was added, and samples were incubated at 25 °C for 48 h. The sample pH was approximately 3.4.

Fermented Cooked Porridge (Uji). Fermented flour samples, prepared as described above, were mixed thoroughly and transferred to a RVA canister with distilled water to a total weight of 28 g. Samples were cooked in the RVA using the profile described above.

Fermented Flatbread (Injera). Fermented flatbread samples were prepared according to the method by Anyango et al.,[18] using 15 g of flour and reducing the amounts of all other ingredients in proportion to this.

Unfermented Flatbread. Margarine (4 g) was rubbed into flour (15 g) and then mixed with 8 mL of warm water to form a dough. The dough was divided into two, chilled (10 °C), placed between two pieces of foil, and pressed into flat circles using a rolling pin. The dough circles were then dry cooked on a griddle.

Cookies. Sorghum flour (25 g), sugar (6 g), and baking powder (0.75 g) was mixed together. Sunflower oil (7.5 g) and water (8−10 mL) was added to the dry ingredients to form a stiff dough. The dough was rolled to a thickness of 5 mm, and cookie rounds, 4.8 cm in diameter, were cut out. The dough rounds were baked in a preheated oven at 180 °C for 20 min.

Couscous. Sorghum flour (20 g) was mixed with 12 mL of water and agglomerated by hand. The agglomerated mixture was rubbed through a 1.4 mm sieve and then steam cooked for 10 min. The mixture was broken into particles and steam cooked for another 10 min. A further 5 mL of water was added, and the particles were further agglomerated before passing through a 2.36 mm sieve. The resulting couscous was then steamed for 20 min.

All products were freeze-dried and milled to pass through a 500 μm opening sieve before analysis. Total protein, protein digestibility, and total lysine was determined on all samples. The tannin content was determined on raw and fermented flour, uji, ugali, tô, and couscous. Reactive lysine (R lysine) was determined on raw and fermented flour, uji, ugali, and couscous.

The total protein was determined by a Dumas combustion method.[19]

Tannin Content. The vanillin−HCl assay by Price et al.[20] was used to determine the tannin content using 1% concentrated HCl in methanol as an extractant. Sample extract blanks (extract incubated without vanillin reagent) were used to compensate for colored samples, when color was not only due to tannins. Results were expressed as catechin equivalents (CEs) after blank corrections.

Lysine, R Lysine, and Lysine Score. The lysine content of the samples was determined after acid hydrolysis and derivatization by ultra-performance liquid chromatography (UPLC) using the AccQ Tag method.[21] An Acquity system (Waters, Milford, MA) equipped with a 2996 photodiode array detector set at 260 nm and a BEH C 18 column at 55 °C (Waters) was used for ULPC. The sample volume was 1 μL, and the solvent system was a gradient of two solvents, AccQ Tag ultra eluent A and AccQTag ultra eluent B. The limit of quantification (LOQ) was 5.3 μm for lysine.

R lysine (chemically available lysine) was determined by the rapid dye-binding (RDB) lysine method,[22] as modified by Kim et al.[23] using Crocein Orange G dye (70% dye content). Two RDB measurements are required, an untreated sample (A), measuring histidine, arginine, and R lysine, and a propionic-anhydride-treated sample (B), which measures histidine and arginine. The difference between A and B gives a measure of R lysine. A solution of dye (0.0389 mM) in oxalic acid−acetic acid phosphate buffer (pH 1.25) was used to prepare a standard curve from 0 to 0.0389 mM at an absorbency of 482 nm. The milled samples (approximately 0.5 g of sample A and 0.7 g of sample B) were accurately weighed into plastic centrifuge tubes, and 5 mL of 16% sodium acetate solution was added. Propionic anhydride (0.2 mL) was added to sample B. All samples were shaken at 300 rpm on an orbital shaker (25 °C) for 15 min, and then 12 mL of 3.89 mM dye solution was added, before shaking for a further 2 h. After centrifugation at 3880*g* for 10 min, the supernatant was diluted 1:100 with oxalic acid−acetic acid phosphate buffer and the absorbance was read at 482 nm. The dye concentration remaining in the supernatant was determined using the dye standard curve. The millimolar basic amino acids per gram of sample was calculated by the difference between the original dye concentration and final dye concentration divided by the weight of the sample. R lysine was the difference between millimolar basic amino acids per gram of samples A and B. Results were expressed as milligrams of R lysine per gram of sample.

Lysine score was calculated by dividing the mg lysine/g protein in the food product by 52 mg/g protein, the protein requirement for a 1−2 year old child.[24] This value was used to determine PDCAAS as described below.

IVPD and PDCAAS. The IVPD method by Mertz et al.[25] was used, as modified.[18] Accurately weighed samples (approximately 200 mg) were digested with P7000-100G pepsin, with an activity of 863 units/mg of protein for 2 h at 37 °C. Residual protein was determined by the Dumas combustion method.[19] Protein digestibility was calculated by the difference between the total protein and the residual protein after pepsin digestion, divided by the total protein, and expressed as a percentage. PDCAAS was calculated by multiplying the lysine score by the IVPD as described by Henley et al.[6]

Statistical Analysis. Samples were analyzed in duplicate twice (four values). All data were analyzed by one-way analysis of variation (ANOVA) at a confidence level of $p < 0.05$ or $p < 0.01$, as stated below each table.

RESULTS AND DISCUSSION

Food Products. All sorghum grain types could be satisfactorily processed into all of the food products (Figure 1). Except for the reddish color because of the presence of tannins, the food products: flatbread, injera, couscous, and cookies made from C1, C2, T1, and T2 were essentially identical to those made from the other sorghums types. The flatbreads from all of the sorghum types were very fragile and broke into small pieces, as a result of the use of whole grain flour, because the bran caused discontinuities in the flatbread.

Tannins have a detrimental effect on the nutritional quality of sorghum foods as they bind proteins.[26,27] It should be noted that type-II tannin sorghums as used in this study are widely used in north and west Africa for the preparation of food products, for example, feterita in Sudan and farafara in Nigeria.

Macia, HD, and USC did not contain tannins (results not shown). The tannin content of T1 and T2 and their null controls C1 and C2 varied between 1.4 and 1.9 g of CE/100 g of flour (Table 1). T1 and T2 contained substantially less tannin in the raw grain than C1 and C2. This is probably due to only natural variation and unrelated to the fact that T1 and T2 were transgenic. The tannin content of the raw grains of these sorghum types was low. All of the traditional processing methods decreased the measurable tannin contents, with alkali cooking (tô) decreasing it the most. This is in agreement with Dlamini et al.,[28] who found substantial reductions in assayable tannin contents after cooking sorghum foods. Beta et al.[29] found an 83−100% decrease in tannin content on alkaline treatment. This was attributed to oxidation of the phenolic groups forming highly polymeric and probably nutritionally inactive compounds. Other workers have suggested decreased levels of measurable phenols, on cooking of sorghum, may be due to the reaction of phenolic hydroxyl groups with food components, such as protein, forming insoluble complexes.[30] Beta et al.[29] also suggested that fermentation or just the addition of water may result in decreased extractability of the phenolic compounds, while Towo et al.[31] proposed that polyphenol oxidase activity caused the reduction in tannins with natural lactic acid fermentation of sorghum, with enzyme activity coming from either the cereal itself or the microorganisms of fermentation.

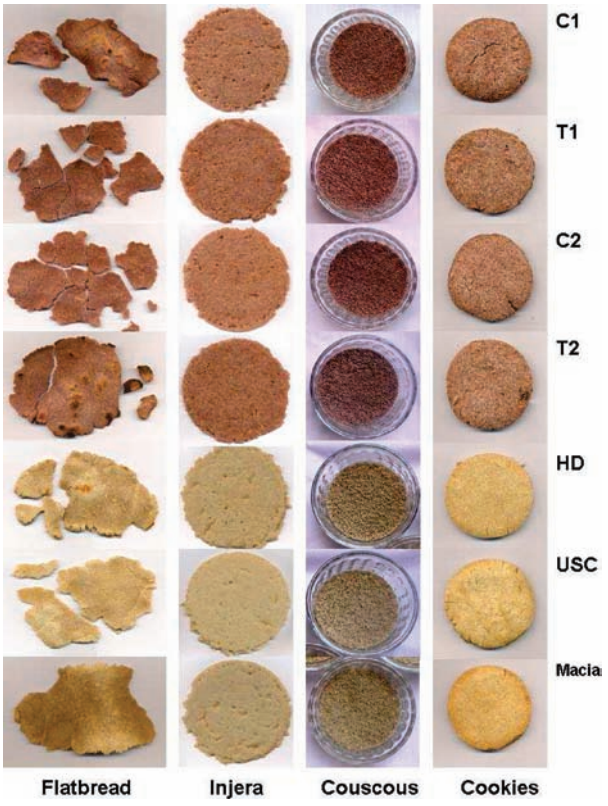

Figure 1. Food products made from biofortified and control sorghum types.

Lysine and R Lysine. Total lysine for raw sorghum ranged from 1.82 to 2.69 g/100 g of protein, while R lysine ranged from 2.38 to 2.97 g/100 g of protein (Table 2). Values for R lysine were generally higher than the corresponding total lysine contents. This was also found by Anyango et al.[18] working with traditional sorghum food products. They suggested that higher values may be due to excess dye.

For raw sorghum, the transgenic types had the highest total lysine (T1, 2.60 g/100 g of protein; T2, 2.69 g/100 g of protein) and highest R lysine (T1, 2.97 g/100 g of protein; T2, 2.85 g/100 g of protein). This was probably because of compensatory synthesis of lysine-rich, nonprolamin proteins.[32] HD had a total lysine content of 2.42 g/100 g of protein and R lysine (2.63 g/100 g of protein) intermediate between T1, T2, C1, C2, USC, and Macia. C1 and C2 had generally the lowest total lysine (1.86 and 1.82 g/100 g of protein, respectively) and R lysine (2.38 and 2.50 g/100 g of protein, respectively). Although the actual lysine values obtained in this study were lower than those reported by Henley et al.,[6] the ranking of the samples was the same.

With regard to the foods, the overall mean total lysine and lysine scores for the different types of sorghums ranked in essentially the same order as for the raw grains (Table 2). T2 and T1 had the highest overall total lysine and lysine score, followed by HD, USC, Macia, C1, and C2. The overall ranking for all of the cultivars for R lysine was slightly different. T2 and T1 had the highest overall R lysine, followed by USC, HD, C2,

Table 1. Effects of Sorghum Type and Traditional Food Processing on Tannin Content (g of CE/100 g of db)[a]

food product	C1	T1	C2	T2
raw flour	1.90 bD (0.14)	1.40 aF (0.11)	1.80 bD (0.12)	1.50 aD (0.06)
fermented flour	0.41 bC (0.03)	0.33 abE (0.02)	0.66 cC (0.03)	0.28 aC (0.12)
ugali (unfermented)	0.26 cB (0.02)	0.22 dC (0.01)	0.41 dB (0.01)	0.14 aB (0.01)
uji (fermented)	0.39 bC (0.01)	0.32 aD (0.01)	0.51 cC (0.01)	0.33 bC (0.02)
tô (alkali cook)	0.06 aA (0.02)	0.04 aA (0.01)	0.06 aA (0.03)	0.06 aA (0.03)
couscous	0.30 cB (0.05)	0.14 bB (0.01)	0.23 aB (0.01)	0.22 bBC (0.03)

[a] Values with different lowercase letters in the same row differ significantly ($p < 0.05$). Values with different capital letters in the same column differ significantly ($p < 0.05$). Values in parentheses are 1 standard deviation (SD) of four determinations.

Table 2. Effects of Sorghum Type and Traditional Food Processing on Lysine and R Lysine (g/100 g of Protein)[a]

sorghum type		sorghum grain	wet cook (ugali)	alkali cook (tô)	fermented	fermented then cook (uji)	flatbread	injera	couscous	cookies	overall mean sorghum type
C1	lysine	1.86 aE	1.75 aCD	1.62 aB	1.68 aBCD	1.79 aD	1.47 aA	2.10 bF	1.73 aCD	1.66 aBC	1.74
	lysine score	0.36	0.34	0.31	0.32	0.34	0.28	0.40	0.33	0.32	0.33
	R lysine	2.38 aB	2.57 abB	ND	2.15 aA	2.27 abAB	ND	ND	1.96 aA	ND	2.27
T1	lysine	2.60 dC	2.50 eB	2.50 eB	2.56 dB	2.54 eB	2.45 deB	2.59 cdB	2.53 dB	2.24 bcA	2.50
	lysine score	0.49	0.48	0.48	0.50	0.49	0.47	0.50	0.49	0.43	0.48
	R lysine	2.97 dC	2.75 bcB	ND	2.80 cB	2.35 abA	ND	ND	2.25 cA	ND	2.62
C2	lysine	1.82 aC	1.73 aB	1.64 aA	1.64 aA	1.73 aB	1.62 bA	1.94 aC	1.68 aAB	1.64 aA	1.71
	lysine score	0.35	0.33	0.32	0.31	0.33	0.31	0.37	0.32	0.32	0.36
	R lysine	2.50 abB	2.50 aB	ND	2.51 bcB	2.20 aA	ND	ND	1.97 aA	ND	2.34
T2	lysine	2.69 dC	2.61 fB	2.54 eAB	2.83 eC	2.65 fB	2.51 eAB	2.50 cAB	2.63 eB	2.38 cA	2.59
	lysine score	0.52	0.50	0.49	0.54	0.51	0.48	0.48	0.51	0.46	0.50
	R lysine	2.85 cdB	2.91 cdB	ND	2.73 cB	2.77 cB	ND	ND	2.26 cA	ND	2.70
HD	lysine	2.42 cD	2.41 dC	2.26 dAB	2.26 cAB	2.37 dBC	2.39 eE	2.79 eE	2.40 cC	2.22 bcA	2.39
	lysine score	0.46	0.46	0.44	0.43	0.46	0.46	0.54	0.46	0.43	0.46
	R lysine	2.63 bcC	2.77 bcdC	ND	2.39 abcB	2.36 abAB	ND	ND	2.10 bA	ND	2.45
USC	lysine	2.26 bD	2.22 cC	2.06 cA	2.03 bA	2.18 cC	2.09 cAB	2.64 dE	2.17 bBC	2.09 bAB	2.19
	lysine score	0.43	0.43	0.40	0.39	0.42	0.40	0.51	0.42	0.40	0.42
	R lysine	2.76 cdB	2.96 dB	ND	2.36 abcA	2.64 bcAB	ND	ND	2.23 cA	ND	2.59
Macia	lysine	2.16 bF	1.97 bCD	1.87 bAB	1.93 bBC	2.09 bE	2.03 cDE	2.50 cG	2.12 bE	1.79 aA	2.05
	lysine score	0.41	0.38	0.36	0.37	0.40	0.39	0.48	0.41	0.34	0.39
	R lysine	2.49 abC	2.61 abBC	ND	2.00 aA	2.31 abABC	ND	ND	2.21 cAB	ND	2.32
overall mean food processing treatment	lysine	2.26	2.17	2.07	2.13	2.19	2.08	2.44	2.18	2.00	
	lysine score	0.43	0.42	0.40	0.41	0.42	0.40	0.47	0.42	0.39	
	R lysine	2.65	2.72	ND	2.42	2.41	ND	2.18	2.14	ND	

[a] Values in the same column but with different lowercase letters are significantly different ($p < 0.01$). Values in the same row but with different capital letters are significantly different ($p < 0.01$). ND, not determined.

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Macia, and C1. R lysine is a measure of lysine availability in foods, which is adversely affected by thermal processing.[22] The difference in rankings of overall R lysine was probably due to differences in the amount of free lysine (more R lysine), in each sorghum type. High-lysine opaque-2 maize and mutant barley cultivars have higher contents of free amino acids than normal varieties.[33]

Overall, all of the foods, except injera, had lower total lysine than the raw grain. Yeast was added during injera processing and, therefore, would be responsible for the higher total lysine.[18] Overall, the cookies had the largest reduction in total lysine for all of the sorghum types. The presence of sugar and high temperature during baking resulted in the loss of lysine because of the Maillard reaction.[34] Serrem et al.[35] found similar reduction in lysine upon baking of sorghum cookies and attributed this loss to the Maillard reaction. Alkali-cooked porridge and flatbread had the next greatest loss of total lysine overall. In the case of the former, this was probably due to formation of lysinoalanine under alkaline conditions.[36] R lysine was also generally similarly reduced as a result of food processing.

Protein. The total protein content of the grains ($N \times 6.25$) ranged from 8.6 to 13.1%. (Table 3). HD had the highest protein content (13.1%). The grain protein contents fell within the normal range for sorghum.[37] Suppression of kafirin synthesis in T1 and T2 did not result in substantial reduction in the protein content. This shows that there was complementary synthesis of other proteins as described above, with reference to Table 2. For reasons unknown, USC had a much lower protein content than any of the other sorghum types.

IVPD of the raw samples ranged from 72.5 to 88.4% (Table 3). These values are within the highly variable range of IVPD for raw sorghum quoted in the literature, for example, from 55.8−59.1%[38] to 88.6−93%.[39] The raw IVPD of T1 and T2 was approximately 15% higher than C1 and C2 and was the same as Macia. This was despite the fact that T1, T2, and their controls contained tannins (Table 1), which are known to reduce sorghum protein digestibility by binding to the proteins.[26,27] Probably with the tannin component removed, the protein digestibility of the suppressed kafirin synthesis transgenic sorghum would be similar to that of other cereals, for example, maize at approximately 81.5% IVPD.[40] As expected, the IVPD of raw HD was high and similar but statistically lower ($p < 0.01$) than T1, T2, and Macia.

All food processing treatments using heat decreased IVPD (Table 3). However, the IVPD of T1 and T2 remained higher than C1 and C2 for all of the treatments. Despite the presence of tannins in the transgenic samples, IVPD was generally the same or higher than the other sorghums, except for Macia. This was probably due to the broad kafirin synthesis suppression, which T1 and T2 had undergone, and the concurrent expression of other more digestible proteins. This would be consistent with the proposal that disulfide bonding protein cross-linking at the protein body periphery, involving γ- and β-kafirin, is the major factor influencing sorghum protein digestibility.[41,9] The reduction in kafirin synthesis in T1 and T2 would presumably reduce the level of cross-linking. It appears that the suppression of the kafirins had a greater effect on IVPD than the presence of tannins. The IPVD of HD foods was somewhat lower than that of T1, T2, and Macia. This was probably due to thermally induced disulfide bonding involving γ-kafirin, which is still present in HD-type sorghums.[41]

For all of the sorghum types, processing into couscous and cookies resulted in the greatest decrease in IVPD (overall means

Table 3. Effects of Sorghum Type and Traditional Food Processing on Protein Digestibility and PDCAAS[a]

sorghum type	total protein (g/100 g of dwb)		sorghum grain	wet cook (ugali)	alkali cook (tô)	fermented	fermented then cook (uji)	flatbread	injera	couscous	cookies	overall mean sorghum type
C1	12.3	IVPD	72.5 aD	42.9 aB	56.4 aC	81.2 aE	56.2 aC	47.8 aB	56.3 bC	33.2 aA	36.3 aA	53.6
		PDCAAS	0.26	0.15	0.17	0.26	0.19	0.13	0.22	0.11	0.12	0.18
T1	12.1	IVPD	88.4 dG	62.0 dC	73.4 dEF	90.7 dG	74.1 bF	65.0 bcCD	69.2 deDE	45.5 bcA	54.3 bB	69.2
		PDCAAS	0.43	0.30	0.35	0.45	0.36	0.31	0.35	0.22	0.23	0.33
C2	12.1	IVPD	73.2 abE	45.2 abB	58.7 aD	82.1 aF	59.2 aD	51.5 aC	52.2 aC	31.2 aA	34.3 aA	54.2
		PDCAAS	0.25	0.15	0.19	0.25	0.20	0.16	0.19	0.10	0.11	0.18
T2	11.6	IVPD	88.0 dF	61.3 cdC	73.0 cdE	91.4 dF	73.1 bE	63.9 bcCD	68.2 cdeD	45.9 bcA	53.0 bB	68.6
		PDCAAS	0.46	0.31	0.36	0.49	0.37	0.31	0.33	0.23	0.24	0.34
HD	13.1	IVPD	83.4 cE	55.4 cB	68.9 bcD	88.1 cF	71.7 bD	61.0 bC	64.8 cC	45.4 bcA	63.5 cC	66.9
		PDCAAS	0.38	0.25	0.30	0.38	0.33	0.28	0.35	0.21	0.27	0.31
USC	8.6	IVPD	75.0 bF	49.7 bB	67.8 bDE	85.5 bG	70.7 bE	62.0 bC	66.2 cdD	41.9 bA	59.2 bcC	64.2
		PDCAAS	0.32	0.21	0.27	0.33	0.30	0.25	0.34	0.18	0.24	0.27
Macia	10.6	IVPD	86.4 dF	64.9 dC	76.7 dE	90.4 dF	80.2 cE	69.3 cD	72.3 eD	49.6 cA	51.8 bB	71.3
		PDCAAS	0.35	0.25	0.28	0.33	0.32	0.27	0.35	0.20	0.18	0.28
overall mean food processing treatment			81.0	54.5	67.8	87.1	69.3	60.1	64.2	41.8	50.3	
			0.35	0.23	0.27	0.36	0.30	0.24	0.30	0.18	0.20	

[a] Values in the same column but with different lowercase letters are significantly different ($p < 0.01$). Values in the same row but with different capital letters are significantly different ($p < 0.01$).

of 50.3 and 41.8%, respectively), because of the fact that they had undergone the most severe heat treatment (Table 3). Fermented sorghum had the highest overall IVPD (87.1%). Cooking fermented sorghum into uji and injera reduced the IVPD of all sorghum types but not to the level of ugali (wet cooked). This is in agreement with the work by Taylor and Taylor[42] and Anyango et al.[18] The former workers suggested that the low pH, resulting from the lactic acid produced during fermentation, could modify the structure of the sorghum proteins, rendering them more accessible to pepsin enzyme. Tô (alkali cooking) resulted in IVPD lower than raw grain but higher than wet cooking alone (ugali) and similar to that of uji (ferment and cook) for all of the sorghum varieties (Table 3). Various workers have found decreased IVPD on alkali cooking when compared to raw grain.[43,44] Vivas et al.[44] attributed this to increased disulfide bond formation during tô processing.

PDCAAS is a derived unit that can be used to predict the biological value of protein in a food.[24] T1 and T2 had much higher PDCAAS (0.43 and 0.46, respectively) in the raw grain than their null controls (C1, 0.26; C2, 0.25) and all other raw sorghum types, which ranged from 0.32 for UCS to 0.38 for HD (Table 3).

The higher PDCAAS of T1 and T2 was also generally reflected in the food products, despite the presence of tannins. The overall mean PDCAAS over all food products was 0.33 and 0.34 for T1 and T2 compared to 0.18 for both C1 and C2 (Table 3). HD had slightly lower mean PDCAAS (0.31) than T1 and T2. This would be expected because HD had a slightly lower IVPD and lower lysine than T1 and T2.

Processing into couscous and cookies resulted in the lowest PDCAAS for all of the sorghum types (0.18 and 0.2, respectively) when compared to the other food processing methods (Table 3). This is probably due to the severity of the heat treatment, reducing the IVPD considerably, and also the reduction in lysine as a result of Maillard reactions, especially for the cookies.[34]

Traditional African sorghum foods made from biofortified sorghum have maintained improved protein quality. Of the two methods of protein biofortification investigated, suppression of kafirin synthesis appears to be superior because both lysine content and protein digestibility are substantially improved. This results in an almost doubling of PDCAAS compared to their null controls and considerably higher PDCAAS than the high protein digestibility sorghum type. Development of tannin-free protein biofortified transgenic sorghum with these traits is needed. Such protein biofortified sorghum has considerable potential to alleviate PEM in children, as indicated by recent findings with quality protein maize in Ethiopia.[45]

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ABBREVIATIONS USED

PEM, protein-energy malnutrition; UPLC, ultra-performance liquid chromatography; LOQ, limit of quantification; RDB, rapid dye binding; IVPD, *in vitro* protein digestibility; PDCAAS, protein digestible corrected amino acid score; T1 and T2, transgenic sorghum with suppression of kafirin synthesis; C1 and C2, null controls of transgenic sorghum with suppression of kafirin synthesis; HD, non-tannin, high protein digestibility sorghum; USC, non-tannin, normal protein digestibility sorghum; CE, catechin equivalent; R lysine, reactive lysine

REFERENCES

(1) Muller, O.; Krawinkle, M. Malnutrition and health in developing countries. *Can. Med. Assoc. J.* **2005**, *173*, 279–286.

(2) Ruel, M. J.; Levine, C. E. *Assessing the Potential of Food Based Strategies To Reduce Vitamin Aand Iron Deficiencies: A Review of Current Evidence*; International Food Policy Research Institute (IFPRI): Washington, D.C., 2000; Food Consumption and Nutrition Division (FCND) Discussion Paper 92, www.ifpri.org/sites/default/files/publications/fcndp92.pdf (accessed Oct 2010).

(3) Tucker, G. Nutritional enhancement of plants. *Curr. Opin. Biotechnol.* **2003**, *14*, 221–225.

(4) International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). *Sorghum*; ICRISAT: Andhra Pradesh, India, 2009; www.icrisat.org/crop-sorghum.htm (accessed Oct 2010).

(5) Doggett, H. *Sorghum*, 2nd ed.; Longman Scientific and Technical: London, U.K., 1988; pp 1−3.

(6) Henley, E. C.; Taylor, J. R. N.; Obukosia, S. D. The importance of dietary protein in human health: Combating protein deficiency in sub-Saharan Africa though transgenic biofortified sorghum. *Adv. Food Nutr. Res.* **2010**, *60*, 21–52.

(7) Jansen, G. R. Lysine in human nutrition. *J. Nutr.* **1962**, *76*, 1.

(8) Klasing, K. C.; Calvert, C. C. Amino acid availability. In *Protein Metabolism and Nutrition*; Loberly, G. E., White, A., MacRae, J. C., Eds.; Wageningen Pers: Wageningen, The Netherlands, 1999.

(9) Duodu, K. G.; Taylor, J. R. N.; Belton, P. S.; Hamaker, B.R. Factors affecting sorghum protein digestibility. *J. Cereal Sci.* **2003**, *38*, 117–131.

(10) Welch, R. M.; Graham, R. D. Agriculture: The real nexus for enhancing bioavailable micronutrients in food crops. *J. Trace Elem. Med. Biol.* **2005**, *18*, 299–307.

(11) Guiragossian, V.; Chibber, B. A. K.; Van Scoyoc, S.; Jambunathan, R.; Mertz, E. T.; Axtell, J. D. Characteristics of proteins from normal, high lysine, and high tannin sorghums. *J. Agric. Food Chem.* **1978**, *26*, 219–223.

(12) Weaver, C. A.; Hamaker, B. R.; Axtell, J. D. Discovery of grain sorghum germ plasm with high uncooked and cooked in vitro protein digestibilities. *Cereal Chem.* **1998**, *75*, 665–670.

(13) Oria, M. P.; Hamaker, B. R.; Axtell, J. D.; Huang, C. P. A highly digestible sorghum mutant cultivar exhibits a unique folded structure of endosperm protein bodies. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, 5065–5070.

(14) Wambugu, F. Developing sustainable food security through agricultural biotechnology: The ABS project model. *Science Based Improvements of Rural/Subsistence Agriculture*; Academy of Science of South Africa (ASSAf): Pretoria, South Africa, 2007.

(15) Jung, R. Grain quality improvement through altered expression of seed proteins. U.S. Patent Application 20080134361, 2008.

(16) Shewry, P. R. Improving protein content and compostion of cerel grain. *J. Cereal Sci.* **2007**, *46*, 239–250.

(17) Miller Del Rosso, J. School feeding programs: Improving effectiveness and increasing the benefit to education. *A Guide for Program Managers*; Partnership for Child Development, University of Oxford: Oxford, U.K., 1999.

(18) Anyango, J. O.; De Kock, H. L.; Taylor, J. R. N. Impact of cowpea addition on the protein digestibility corrected amino acid score

and other protein quality parameters of traditional African foods made from non-tannin and tannin sorghum. *Food Chem.* **2011**, *124*, 775–780.

(19) American Association of Cereal Chemists (AACC). Crude protein-combustion, standard method 46-30; *Approved Methods of the AACC*, 10th ed.; AACC: St. Paul, MN, 2000.

(20) Price, M. L.; Van Scoyoc, S.; Butler, L. G. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *J. Agric. Food Chem.* **1978**, *26*, 1214–1218.

(21) Boogers, I.; Plugge, W.; Stokkermans, Y. Q.; Duchateau, A. L. L. Ultra-performance liquid chromatographic analysis of amino acids in protein hydrolysates using an automated pre-column derivatisation method. *J. Chromatogr.* **2008**, *1189*, 4.06–409.

(22) Hurrell, R. F.; Carpenter, K. J. The estimation of available lysine in foodstuffs after Maillard reactions. *Prog. Food Nutr. Sci.* **1981**, *5*, 159–176.

(23) Kim, J. S.; Kim, K. J.; Ma, W. C. J.; Chung, H. Y. Development of a method to quantify lysine in small amount of rice grain. *Korean J. Sanit.* **2007**, *22*, 75–84.

(24) World Health Organization (WHO)/Food and Agriculture Organization (FAO)/United Nations University (UNU) Expert Consultation. *Protein and Amino Acid Requirements in Human Nutrition*, Report of a Joint WHO/FAO/UNU Expert Consultation; WHO: Geneva, Switzerland, 2007; World Health Organization Technical Report 935.

(25) Mertz, E. T.; Hassen, M. M.; Cairns-Whittern, C.; Kirleis, A. W.; Tu, L.; Axtell, J. D. Pepsin digestibility of proteins in sorghum and other major cereals. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1–2.

(26) Butler, L. G.; Riedl, D. J.; Lebryk, D. G.; Blyit, H. J. Interaction of proteins with sorghum tannin: Mechanism, specificity and significance. *J. Am. Oil Chem. Soc.* **1984**, *61*, 916–920.

(27) Emmambux, N. M.; Taylor, J. R. N. Sorghum kafirin interaction with various phenolic compounds. *J. Sci. Food Agric.* **2003**, *83*, 402–407.

(28) Dlamini, N. R.; Taylor, J. R. N.; Rooney, L. W. The effect of sorghum type and processing on the antioxidant properties of African sorghum-based foods. *Food Chem.* **2007**, *105*, 1412–1419.

(29) Beta, T.; Rooney, L. W.; Marovatsanga, L. T.; Taylor, J. R. N. Effect of chemical treatments on polyphenols and malt quality in sorghum. *J. Cereal Sci.* **2000**, *31*, 295–302.

(30) Matuschek, E.; Towo, E.; Svanberg, U. Oxidation of polyphenols in phytate-reduced high-tannin cereals: Effect on different phenolic groups and on in vitro accessible iron. *J. Agric. Food Chem.* **2001**, *49*, 5630–5638.

(31) Towo, E.; Matuschek, E.; Svanberg, U. Fermentation and enzyme treatment of tannin sorghum gruels: Effects on phenolic compounds, phytate and in vitro accessible iron. *Food Chem.* **2006**, *94*, 369–376.

(32) Ufaz, S.; Galili, G. Improving the content of essential amino acids in crop plants: Goals and opportunities. *Plant Physiol.* **2008**, *147*, 954–961.

(33) Talberg, A. Protein and lysine content in high-lysine double-recessives of barley, I. Combinations between mutant 1508 and Hiproly back-cross. *Hereditas* **1981**, 253–260.

(34) Horvatic, M.; Eres, M. Protein nutrititive quality during production and storage of dietetic biscuits. *J. Sci. Food Agric.* **2002**, *82*, 1617–1620.

(35) Serrem, C. A.; De Kock, H. L.; Taylor, J. R. N. Nutritional quality, sensory quality and consumer acceptability of sorghum and bread wheat biscuits fortified with defatted soy flour. *Int. J. Food Sci. Technol.* **2011**, *46*, 74–83.

(36) Damodaran, S. Amino acids, peptides and proteins. In *Food Chemistry*, 3rd ed.; Fennema, O. R., Ed.; Marcel Dekker: New York, 1996; pp 321−429.

(37) Serna-Saldivar, S.; Rooney, L. W. Structure and chemistry of sorghum and millets. In *Sorghum and Millets: Chemistry and Technology*; Dendy, D. A. V., Ed.; American Association of Cereal Chemists (AACC): St. Paul, MN, 1995; pp 69−124.

(38) Duodu, K. G.; Nunes, A.; Delgadillo, I.; Parker, M. L.; Mills, E. N. C.; Belton, P. S.; Taylor, J. R. N. The effect of grain structure and cooking on sorghum and maize *in vitro* protein digestibility. *J. Cereal Sci.* **2002**, *35*, 161–175.

(39) Axtell, J. D.; Kirleis, A.; Hassen, M. M.; D'Crox-Mason, N.; Mertz, E. T.; Munck, L. Digestibility of sorghum proteins. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 1333–1335.

(40) Hamaker, B. R.; Kirleis, A. W.; Mertz, E. T.; Axtell, J. D. Effect of cooking on the protein profiles and *in vitro* digestibility of sorghum and maize. *J. Agric. Food Chem.* **1986**, *34*, 647–649.

(41) Oria, M. P.; Hamaker, B. R.; Shull, J. M. Resistance of sorghum α-, β-, and γ-kafirins to pepsin digestion. *J. Agric. Food Chem.* **1995**, *43*, 2148–2153.

(42) Taylor, J.; Taylor, J. R. N. Alleviation of the adverse effect of cooking on sorghum protein digestibility through fermentation in traditional African porridges. *Int. J. Food Sci. Technol.* **2002**, *37*, 129–137.

(43) Bach-Knudsen, K. E.; Kirleis, A. W.; Eggum, B. O.; Munck, L. Carbohydrate composition and nutritional quality for rats of sorghum tô prepared from decorticated white and whole grain red flour. *J. Nutr.* **1988**, *188*, 588.

(44) Vivas, N. E.; Waniska, R. D.; Rooney, L. W. Effects on proteins in sorghum and maize, and pearl millet when processed into acidic and basic tô. *Cereal Chem.* **1992**, *69*, 673–676.

(45) Akalu, G.; Taffesse, S.; Gunaratna, N. S.; De Groote, H. The effectiveness of quality protein maize in improving the nutritional status of young children in the Ethiopian highlands. *Food Nutr. Bull.* **2010**, *31*, 418–430.