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 combating 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 lysine content and protein digestibility were substantially improved and maintained in a range of African foods. For the genetically engineered sorghums, 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.

KEY WORDS: 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, 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 which can tolerate drought and water-logging (5). From a nutritional point of view, whilst 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 35-90% lower than of other cereals (6). Lysine is essential for growth in infants, for maintenance in adults (7) and is important for bone calcification, for 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), whilst 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, P-721N (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 than P721N (12). This improved digestibility was attributed to increased enzyme susceptibility of the major storage protein, α-kafirin due to 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 in vitro protein digestibility (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 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, Iowa, 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 ex Texas A&M University, Weslaco, Texas 2006; and Macia (developed from SDS 3220, ICRISAT SMIP) cultivated at Makoro Lands, Central District, Botswana, 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. Dialysis tubing used was Spectra/Por 7 (Ø = 20.4 mm) with a molecular mass cut-off (MMCO) of 10 000 Da, obtained from Labretoria, Pretoria.

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. Due to the small amount of transgenic sorghum available, small-scale processing methods were devised.

Preparation of food products. Raw whole grain was analysed 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 an RVA paddle. The porridge was cooked in the RVA using the following profile: heated to 91°C within 20 minutes, held at 91°C for 5 minutes and then cooled to 50°C within 5 minutes and then held at 50°C for a further 3 minutes. 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. Final porridge pH was approximately 9.

Starter culture. Macia flour (25 g) was mixed with 65 ml 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 distilled water in a plastic tube, 2 g inoculum was added and samples incubated at 25°C for 48 h. 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.

Injera. Prepared according to the method of 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 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 diam., were cut out. The dough rounds baked in a pre-heated oven at 180°C for 20 min.

Couscous. Sorghum flour (20 g) was mixed with 12 ml 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 water was added and the particles 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. Tannin content was determined on raw and fermented flour, uji, ugali, tô and couscous. Reactive lysine was determined on raw and fermented flour, uji, ugali and couscous.

Total protein was determined by a Dumas combustion method (19).

Tannin content. The Vanillin HCl assay of Price et al. (20) was used to determine tannin content using 1% conc. HCl in methanol as extractant. Sample extract blanks (extract incubated without vanillin reagent) were used to compensate for colored samples, when colour was not only due to tannins. Results were expressed as catechin equivalents (CE) after blank corrections.

Lysine, reactive lysine and lysine score. Lysine content of the samples was determined after acid hydrolysis and derivatisation 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 the ULPC. Sample volume was 1μl and the solvent system was a gradient of two

solvents AccQ Tag ultra eluent A and AccQTag ultra eluent B. Limit of quantification (LOQ) was 5.3 μM for lysine.

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

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

In vitro protein digestibility (IVPD) and Protein Digestible Corrected Amino Acid Score (PDCAAS). The IVPD method of Mertz, Hassen, Cairns-Whittern, Kirleis, Tu and Axtell (25) was used, as modified (18). Accurately weighed samples (approx. 200 mg) were digested with P7000-100G pepsin, activity 863 units/mg protein for 2 h at 37^{o}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 in vitro protein digestibility as described by Henley et al., 2010 (6).

Statistical analysis. Samples were analysed in duplicate twice (4 values). All data were analysed by one way 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 due to 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 the sorghum types were very fragile and broke into small pieces, due to the use of whole grain flour, as the bran caused discontinuities in the flatbread.

Tannins. These 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 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 1.4-1.9 g CE/100 g flour (**Table 1**). T1 and T2 contained substantially less tannin in the raw grain than C1 and C2. This is probably due only to 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 the traditional processing methods decreased the measurable tannin contents, alkali cooking (tô) decreasing it the most. This is in agreement with Dlamini, Taylor & Rooney (28), who found substantial reductions in assayable tannin contents after cooking sorghum foods. Beta et al., (29), found 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, whilst 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.

Lysine and reactive lysine. Total lysine for raw sorghum ranged from 1.82 to 2.69 g/100 g protein, whilst reactive lysine ranged from 2.38 to 2.97 g/100 g protein (**Table 2**). Values for

reactive 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, T2 2.69 g/100 g protein, respectively) and highest reactive lysine (T1 2.97, T2 2.85 g/100 g protein, respectively). This was probably due to compensatory synthesis of lysine-rich, non-prolamin proteins (32). HD had a total lysine content of 2.42 g/100 g protein and reactive lysine (2.63 g/100 g protein) intermediate between T1, T2, and C1, C2 USC and Macia. C1 and C2 had generally the lowest total lysine (1.86, 1.82 g/100 g protein, respectively) and reactive lysine (2.38, 2.50 g/100 g 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 were 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, and USC, Macia and C1, C2. The overall ranking for all the cultivars for reactive lysine was slightly different. T2 and T1 had the highest overall reactive lysine followed by USC, HD, C2, Macia and C1. Reactive lysine is a measure of lysine availability in foods, which is adversely affected thermal processing (22). The difference in rankings of overall reactive lysine was probably due to differences in the amount of free lysine (more reactive 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 the foods except injera, had lower total lysine than the raw grain. Yeast was added during injera processing and so would be responsible for the higher total lysine, (18). Overall, the cookies had the largest reduction in total lysine for all the sorghum types. The presence of sugar and high temperature during baking resulted in loss of lysine due to the Maillard reaction (34). Serrem et al. (35) found similar reduction in lysine on 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). Reactive lysine was also generally similarly reduced as result of food processing.

Protein. The total protein content of the grains (N x 6.25) ranged from 8.6-13.1%. (**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 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.

In vitro protein digestibility (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 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 similar to that of other cereals, for example maize approx. 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 the T1 and T2 remained higher than C1 and C2 for all the treatments. In spite of the presence of tannins in the transgenic samples, the 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, T2 would presumably reduce the level of cross-linking. It appears that the suppression of the kafirins had a greater effect on IVPD than did the presences of tannins. The IPVD of HD foods was somewhat lower than that of T1, T2 and Macia. This probably due to thermally induced disulfide bonding involving gamma-kafirin, which is still present in HD type sorghums (41).

For all the sorghum types, processing into couscous and cookies resulted in the greatest decrease in IVPD (overall means 50.3% and 41.8% respectively), due to 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 of 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 the sorghum varieties (**Table 3**). Various workers have found decreased IVPD on alkali cooking when compared with raw grain (43, 44**)**. Vivas et al (44) attributed this to increased disulfide bond formation during tô processing.

PDCAAS is a derived unit which 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 and 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, T2 was also generally reflected in the food products, in spite of the presence of tannins. The overall mean PDCAAS over all food products was 0.33 and 0.34 for T1 and T2 compared with 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 since 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 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 due to 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).

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 above

HD, a non-tannin high protein digestibility sorghum

USC, a non-tannin, normal protein digestibility sorghum

CE, catechin equivalents

R Lysine, reactive lysine

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FIGURE CAPTIONS

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

Table 1. The effects of sorghum type and traditional food processing on tannin content (g (CE)/ 100 g db)

Food product	C 1	T 1	C 2	T 2
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)

^{abc}-Values with different superscripts in the same row differ significantly ($p<0.05$)
^{ABC}-Values with different superscripts in the same column differ significantly ($p<0.05$)
Values in parentheses are 1SD of four determinations

Table 2. The effects of sorghum type and traditional food processing on lysine and reactive lysine (g/100 g protein)

Sorghum Type		Sorghum Grain	Wet Cook (Ugali)	Alkali Cook Tô	Fermented	Fermented then Cook Uji	Flatbread	Injera	Couscous	Cookies	Overall Means Sorghum Type
C1	Lysine	1.86aE	1.75aCD	1.62aB	1.68aBCD	1.79aD	1.47aA	2.10bF	1.73aCD	1.66aBC	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	2.38aB	2.57abB	ND	2.15aA	2.27abAB	ND	ND	1.96aA	ND	2.27
T1	Lysine	2.60dC	2.50eB	2.50eB	2.56dB	2.54eB	2.45deB	2.59cdB	2.53dB	2.24bcA	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	2.97dC	2.75bcB	ND	2.80cB	2.35abA	ND	ND	2.25cA	ND	2.62
C2	Lysine	1.82aC	1.73aB	1.64aA	1.64aA	1.73aB	1.62bA	1.94aC	1.68aAB	1.64aA	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	2.50abB	2.50aB	ND	2.51bcB	2.20aA	ND	ND	1.97aA	ND	2.34
T2	Lysine	2.69dC	2.61fB	2.54eAB	2.83eC	2.65fB	2.51eAB	2.50cAB	2.63eB	2.38cA	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	2.85cdB	2.91cdB	ND	2.73cB	2.77cB	ND	ND	2.26cA	ND	2.70
HD	Lysine	2.42cD	2.41dC	2.26dAB	2.26cAB	2.37dBC	2.39eE	2.79eE	2.40cC	2.22bcA	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	2.63bcC	2.77bcdC	ND	2.39abcB	2.36abAB	ND	ND	2.10bA	ND	2.45
USC	Lysine	2.26bD	2.22cC	2.06cA	2.03bA	2.18cC	2.09cAB	2.64dE	2.17bBC	2.09bAB	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	2.76cdB	2.96dB	ND	2.36abcA	2.64bcAB	ND	ND	2.23cA	ND	2.59
Macia	Lysine	2.16bF	1.97bCD	1.87bAB	1.93bBC	2.09bE	2.03cDE	2.50cG	2.12bE	1.79aA	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	2.49abC	2.61abBC	ND	2.00aA	2.31abABC	ND	ND	2.21cAB	ND	2.32
Overall Means 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	

Values in the same column but with different letters (lower case) are significantly different ($p<0.01$)
Values in the same row but with different letters (upper case) are significantly different ($p<0.01$)

Table 3. The effects of sorghum type and traditional food processing on protein digestibility and PDCAAS

Sorghum Type	Total protein (g/100 g dwb)		Sorghum Grain	Wet Cook (Ugali)	Alkali Cook Tô	Fermented	Fermented then Cook Uji	Flatbread	Injera	Couscous	Cookies	Overall Mean sorghum Types
C1	12.3	IVPD	72.5aD	42.9aB	56.4aC	81.2aE	56.2aC	47.8aB	56.3bC	33.2aA	36.3aA	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	88.4dG	62.0dC	73.4dEF	90.7dG	74.1bF	65.0bcCD	69.2deDE	45.5bcA	54.3bB	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	73.2abE	45.2abB	58.7aD	82.1aF	59.2aD	51.5aC	52.2aC	31.2aA	34.3aA	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	88.0dF	61.3cdC	73.0cdE	91.4dF	73.1bE	63.9bcCD	68.2cdeD	45.9bcA	53.0bB	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	83.4cE	55.4cB	68.9bcD	88.1cF	71.7bD	61.0bC	64.8cC	45.4bcA	63.5cC	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	75.0bF	49.7bB	67.8bDE	85.5bG	70.7bE	62.0bC	66.2cdD	41.9bA	59.2bcC	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	86.4dF	64.9dC	76.7dE	90.4dF	80.2cE	69.3cD	72.3eD	49.6cA	51.8bB	71.3
		PDCAAS	0.35	0.25	0.28	0.33	0.32	0.27	0.35	0.20	0.18	0.28
Overall Means 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	

Values in the same column but with different letters (lower case) are significantly different ($p<0.01$)
Values in the same row but with different letters (upper case) are significantly different ($p<0.01$)

Figure 1