

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

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ABSTRACT

Protein malnutrition is a problem in Africa where sorghum is a staple foodstuff. Improvement in the protein quality of traditional African sorghum (*Sorghum bicolor* L. Moench) foods through addition of cowpea (*Vigna unguiculata* L. Walp), an indigenous African legume, was investigated. Two sorghum cultivars, a red, tannin-type (NS 5511) and a white tan-plant, non-tannin type (Orbit) were complemented with cowpea (70:30 ratio). Ugali (thick porridge), uji (fermented thin porridge) and injera (fermented flatbread) were prepared. Protein lysine scores of cowpea-complemented foods were about double the levels of sorghum-only foods. *In vitro* protein digestibility of the foods increased by 13% to 62%. Increase in lysine and protein digestibility improvement resulted in three- and two-fold improvement in the Protein Digestibility Corrected Amino Acid Score (PDCAAS) of NS 5511 and Orbit foods, respectively. Addition of cowpea to tannin- as well as non-tannin sorghum is a viable option for improving the protein quality of a wide range of traditional African foods.

Keywords: Sorghum, Cowpea, Protein quality, Lysine score, Protein Digestibility Corrected Amino Acid Score (PDCAAS), Tannins, Traditional African foods

1. Introduction

Protein malnutrition is a serious problem in Africa where sorghum is a staple food. The nutrient content of sorghum grain is generally similar to other cereals (Food and Agriculture Organization (FAO), 1995). However, the lysine content is particularly low because the kafirin storage proteins are very low in lysine. For example, Taylor and Schüssler (1986) studying protein composition of different parts of sorghum grain reported an average of about 2% lysine in kafirin, while the recommended value for a 1 to 2 year-old child (WHO/FAO/UNU Expert Consultation, 2007) is 5.2%. In addition, the digestibility of sorghum proteins decreases especially on wet cooking (reviewed by Duodu, Taylor, Belton & Hamaker, 2003), and work by Maclean, De Romana, Placko & Graham (1981) has shown that poorly digestible, high lysine sorghum proteins might not provide substantial protein nutritional benefits to children who consume sorghum. Further, the tannins in tannin-containing sorghum cultivars bind proteins (Butler, Riedl, Lebryk & Blyit, 1984), which can further reduce protein digestibility. It has been shown that higher sorghum tannin levels result in a greater reduction in *in vivo* protein digestibility of cooked high tannin sorghum compared to low tannin sorghum (Bach Knudsen, Munck & Eggum, 1988). Therefore, sorghum-based foods require improvement to enhance their protein nutritional value. Cowpea is an important legume in the tropics, particularly in Africa. With an average of 24 g protein per 100 g and about 7 g lysine per 100 g protein (USDA, 2009), cowpea is protein-rich. Therefore cowpea could be used to complement traditional sorghum-based foods (Pelembé, Erasmus & Taylor, 2002).

To assess the protein nutritional adequacy achieved through cowpea addition, it is important to measure protein quality of the foods in terms of the digestibility and biological value of the protein. This is particularly crucial because, as plant protein sources their protein quality is a

major concern in meeting protein nutritional sufficiency of their consumers (Millward, 1999). The biological value of protein in a food can be predicted by Protein Digestibility Corrected Amino Acid Score (PDCAAS) (WHO/FAO/UNU Expert Consultation, 2007). PDCAAS, which is the amino acid score (in this case lysine score as the first limiting indispensable amino acid) of the food protein \times digestibility, was introduced as a means of assessing the protein quality of both individual food sources and food mixtures as in this work. The reasoning here is that utilization of a protein will be limited by its digestibility, which determines the overall available amino acid nitrogen from food (WHO/FAO/UNU Expert Consultation, 2007).

The total lysine values from amino acid analysis do not always reflect lysine availability to the body for metabolism (reviewed by Moughan & Rutherfurd, 2008). This is because the ϵ -amino group of lysine can react with many other food components including reducing sugars, fats and their oxidation products, polyphenols, vitamins, food additives and other amino acids, rendering the lysine nutritionally unavailable (Hurrell & Carpenter, 1981). Therefore, apart from increasing protein lysine content of sorghum foods, it is necessary to increase the protein digestibility as well, in order to realize protein nutritional adequacy derived from a plant food such as sorghum, where it presents a problem (Millward, 1999). As already mentioned the approach used must also take into account the presence or absence of tannins in sorghum. Accordingly, this study examined the effects of adding cowpea to both tannin- and non-tannin sorghums as a means of enhancing the protein quality of traditional African sorghum-based foods.

2. Materials and methods

2.1. Grain samples and preparation of whole grain flour

Grains of two sorghum cultivars and one cowpea variety were used in this study. NS 5511 (red, tannin sorghum, 11.0% protein) was a year 2007 harvest grown in the Free State Province, South Africa; Orbit (white tan plant, non-tannin sorghum, 8.4% protein) was a year 2005 harvest from the Agricultural Research Council, Potchefstroom, South Africa, and cowpea (Bechuana white variety, 23.5% protein) was a year 2007 harvest, grown in Delareyville, North West Province, South Africa. The grains were separately milled using a laboratory hammer mill (Falling Number 3100, Huddinge, Sweden) fitted with a 500 µm opening screen to give whole grain flour, which was then stored at 10°C prior to food preparation and other treatments. Composite flours were prepared by thoroughly mixing sorghum and cowpea flours at a ratio of 70:30 (w/w).

Four flour samples, NS 5511 and Orbit sorghum, and sorghum-plus-cowpea flours were used to prepare three different types of traditional African sorghum-based foods: unfermented thick porridge (ugali), fermented thin porridge (uji) and fermented flatbread (injera).

2.2. Preparation of sorghum-based foods

Ugali was prepared with tap water (40 mL) which was brought to boil in a 400 mL beaker. Flour (30 g) was made into slurry with 20 mL water. The slurry was added to the boiling water, then cooked with constant heating and vigorous mixing until a uniform and well-cooked product was formed in 1 min.

For uji and injera preparation, a natural inoculum was previously obtained. Orbit sorghum flour was chosen for use in the preparation of the natural inoculum because preliminary tests showed that it fermented relatively rapid and also to ensure that all the samples received the same bacteria in the starter culture. A natural inoculum was prepared according to the procedure used by Taylor & Taylor (2002), with some modifications. Whole Orbit sorghum

flour (40 g) was made into a slurry with 80 mL tap water. The slurry was incubated at 25°C for 7 days. A portion of the fermented slurry (40 mL), pH 3.7, was then taken and added to a freshly prepared slurry containing the same ratio of flour to water as before. This was mixed and incubated at 25°C for 3 days. The procedure was repeated thus maintaining a natural inoculum. To prepare the starter culture, a slurry containing 40 g Orbit sorghum flour and 80 mL tap water was inoculated with 40 mL natural inoculum prepared previously and incubated at 25°C for 3 days by which time the pH dropped to 3.7.

For preparation of uji, a slurry obtained with 30 g flour and 60 mL tap water was inoculated with 10 mL starter culture in a closed plastic bucket and incubated at 25°C for 24 h. The fermented slurry was added to 160 mL boiling water and cooked while stirring (for 2 min) until a smooth product formed.

Injera was prepared according to Yetneberk, de Kock, Rooney & Taylor (2004) with modification. To initiate the second fermentation, 0.5 g commercial instant dried baker's yeast (protein content 38 g/100 g and lysine content 8 g/100g protein) and 1.5 g sugar was added to the rest of the fermented batter and stirred thoroughly to get a uniform mix. The cooked-and-cooled portion was added to this. Then, 30 mL water was added and stirred in to obtain uniform mixture. The plastic bucket containing the batter was covered with a lid and then incubated for 1 h in a water bath at 35°C. The actively fermenting dough was stirred to obtain uniform consistency. The yeast fermented batter (20 g) was weighed into a 90 mm plastic Petri dish and baked in a 900 Watt microwave oven (for 45 s) until it formed a honeycombed structured surface ('eyes'). All the food samples were freeze-dried and then pulverized using a Waring Commercial[®] laboratory blender (New Hartford, CT), set at high (Hi) for 60 s.

2.3. Tannin content

Tannin content was determined using a modified Vanillin-HCl method (Price, Van Scoyoc & Butler, 1978). Extract blanks were prepared to compensate for highly coloured samples, where colour was not only due to tannins. Results were calculated with blank correction, then tannin concentration expressed as catechin equivalents (CE).

2.4. Protein content

Protein content (N x 6.25) was determined by the Dumas combustion method (American Association of Cereal Chemists (AACC) International, 2000) Method 46-30.

2.5. In vitro protein digestibility (IVPD)

A pepsin digestion method based on that of Hamaker, Kirleis, Butler, Axtell & Mertz (1987) was used. Accurately weighed samples (200 mg) were digested with P7000-100G pepsin, activity 863 units/mg protein (Sigma-Aldrich, St. Louis, MO) for 2 h at 37°C. Residual protein was determined by the Dumas combustion method.

2.6. Lysine and reactive lysine content

After acid hydrolysis, the lysine contents of the samples were determined by the Pico-Tag method, which is a reversed phase HPLC procedure (Bidlingmeyer, Cohen & Tarvin, 1984).

Reactive lysine content was determined by a rapid dye-binding lysine (DBL) method as modified by Kim, Kim, Ma & Chung (2007) using Crocein Orange G dye (70% dye content) (Fluka grade 27965: Sigma-Aldrich, Buchs, Switzerland).

2.7. Statistical analysis

The data were analysed by one-way analysis of variance (ANOVA) using sample as the independent variable and the measured parameters as the dependent variables. The means

were compared by Fisher's least significant differences (LSD). The calculations were performed using Statgraphics Centurion XV (Stat Point, Herndon, VA).

3. Results and discussion

Non-tannin and tannin sorghum cultivars were used because tannins in sorghum are known to bind proteins (Butler et al., 1984) and thus reduce the protein nutritional quality of sorghum foods (Bach Knudsen et al., 1988). In addition, consumers in Africa frequently use tannin sorghums to prepare a wide variety of foods by different processing methods (personal observation). Three different types of traditional sorghum foods were selected based on their importance in the diets of consumers across sub-Saharan Africa and the differences in cooking methods.

3.1. Tannin content

As expected, much higher tannin contents were measured for foods prepared from tannin sorghum (NS 5511) than those from non-tannin sorghum (Orbit) (Table 1). Cooking resulted in decreases in assayable tannin contents by between 18% and 69%. These reductions are in agreement with Dlamini, Taylor & Rooney (2007), who found substantial reductions in assayable tannin contents after cooking sorghum foods. The reductions in assayable tannin after cooking were probably due to interaction with other grain components such as proteins, forming insoluble complexes (Butler et al., 1984; Emmambux & Taylor, 2003), thus lowering tannin extractability.

There was a greater reduction (determined by the difference between the tannin content of the raw flour and that of the corresponding food, expressed as a proportion of raw flour tannin content) in the assayable tannin content of foods prepared from NS 5511 sorghum-only than

the corresponding cowpea composited foods. The higher tannin content of NS 5511 may have enhanced tannin-protein interactions, thereby lowering extractability of the tannins in composite foods. These results are in agreement with findings of Emmambux & Taylor (2003) who studied the affinity of tannins for sorghum kafirin proteins. These authors reported a linear increase of tannins bound with increase in tannin content. As cowpea had a higher tannin content than Orbit sorghum (Table 1), cowpea addition increased the tannin content proportionately. However, tannin levels were still equivalent to the group I category sorghum (less than 1% CE) according to the classification of Price et al. (1978), and therefore would not be expected to have significant effect on protein quality.

3.2. Protein content

Cowpea addition at a sorghum to cowpea ratio of 70:30 (w/w) increased the protein contents of raw flour and foods by approximately 32% to 35% and 35% to 57%, for NS 5511 and Orbit sorghums, respectively (data not shown). Sorghum-cowpea composite injera had the highest protein content, some 4-7% higher than that of the other composite foods. This may be partly because the yeast added during preparation of the food is protein-rich, containing about 38 g protein/100 g (USDA, 2009). Additionally, it can be attributed to the decrease in carbohydrate to protein ratio during fermentation. Onyango, Noetzold, Bley & Henle (2004) also reported an increase in protein content of maize-finger millet blend when it was fermented. Similar results were reported for fermented sorghum porridge (Taylor & Taylor, 2002). In the present study, it was noted that uji (fermented porridge) prepared from NS 5511 sorghum flour had the same protein content as raw flour and ugali. This may be attributed to inhibited activity of the fermentation bacteria by tannins in sorghum. Tannins have been reported to be bacteriostatic and/or bactericidal for many bacteria species (Scalbert, 1991). These authors outlined various mechanisms of tannin antimicrobial activity including

inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation.

3.3. Lysine and reactive lysine content

Addition of cowpea increased the lysine contents of the proteins in all the foods by between 67% and 139% (Table 2). An increase was expected because the cowpea flour had a high lysine content of around 1.1%. However, contrary to expectation, there were inconsistencies in the increase in lysine contents of the food proteins. With respect to the corresponding sorghum-only foods, cowpea addition increased the lysine content of proteins in NS 5511 foods by between 81 and 139%, while the percent increases in lysine content of protein in Orbit foods were relatively lower (67-87%). These inconsistencies are probably due to the effects of different cooking methods on the interactions among different food components such as that between tannins and proteins, which have been shown to be quite specific. Such specificity of protein-tannin interactions was demonstrated by Asquith & Butler (1986) when they studied the reactions of the condensed tannins (procyanidins) from sorghum, with different proteins. These authors found that protein-tannin interactions are both protein and tannin dependent.

Cowpea addition resulted in a 10-75% increase in the reactive (chemically available) lysine content of the sorghum foods (Table 2). However, the increase in reactive lysine was in general rather lower than the increase in total lysine. In fact, ugali and uji proteins had slightly lower reactive lysine contents, compared to the corresponding raw flour. On the other hand, injera proteins had generally the same reactive lysine content with respect to the

corresponding raw flour. A reduction in reactive lysine content was expected due to the effect of heat on lysine (reviewed by Moughan & Rutherfurd, 2008).

Injera had higher reactive lysine than the other foods, probably due to the lysine-rich protein from the yeast added. There was generally little effect of cooking on reactive lysine content of proteins in some of the foods. Short time, moist heat used during preparation of the foods, especially ugali and uji, apparently had minimal effect on the reactive lysine.

There were inconsistencies, as some of reactive lysine content values were higher than the corresponding lysine contents particularly for proteins in sorghum-only foods. As proteins from sorghum-only foods contained lower contents of the basic amino acids compared to their composite food counterparts (data not shown), it is possible that there was excess dye in reactions involving sorghum-only foods as an equal amount of dye was used for all the foods. This may have caused an overestimation of the reactive lysine content of the proteins in the food samples, which concurs with observations from similar studies (Hurrell & Carpenter, 1981).

The protein lysine contents of ugali were the same as the corresponding raw flours. Proteins in uji had the lowest lysine content of all the foods studied with about 13% to 23% less lysine than the corresponding raw flour. An increase in lysine content of up to 20% occurred in proteins in injera. Onyango et al. (2004) suggested that there is preferential utilization of lysine by lactic acid bacteria during fermentation. This is because lactic acid bacteria, the principal bacteria in uji, are fastidious in their amino acid requirements for growth and metabolism. These workers also suggested that yeasts have minimal nutrient requirements and hydrolyze storage proteins into peptides and amino acids, thereby increasing the lysine

content of the proteins. These microbial activities might explain the reductions in lysine contents of proteins in uji as well as the increase in lysine content of proteins in injera.

Addition of cowpea had little effect on contents of other essential amino acids of the food proteins (data not shown). This was expected because apart from lysine and leucine, there are generally little differences in the contents of other essential amino acids between sorghum and cowpea proteins (USDA, 2009).

3.4. In vitro protein digestibility

Cowpea addition increased the IVPD of the NS 5511 and Orbit sorghum foods by about 54% to 74% and 4% to 13%, respectively (Table 3). The reason for the improvement in IVPD is probably that addition of cowpea increased the content of more digestible cowpea globulin proteins, with a concomitant decrease in less digestible sorghum kafirin proteins. Cowpea is rich in globulins (reviewed by Chavan, Kadam & Salunkhe, 1989), which become more digestible after cooking because of reduction of antinutrients such as trypsin inhibitors (Akinyele, 1989), as opposed to the poorly digestible sorghum proteins (kafirins) (Hamaker et al., 1987). Furthermore, by reducing tannin content of the foods, in the case of NS 5511 (Table 1), foods made from NS 5511-plus-cowpea had relatively less tannins complexing with protein, which probably improved their IVPD. This observation concurs with findings by Emmambux & Taylor (2003) on the affinity of tannins to bind with kafirin proteins. Similarly, Nguz & Huyghebaert (1998) working on eight sorghum cultivars with different tannin contents found that high levels of tannins in sorghum grain reduces their protein digestibility. NS 5511 sorghum foods had lower IVPD than Orbit sorghum foods attributed to the presence of high level of tannins in NS 5511 (Table 1).

Cooking decreased the IVPD of NS 5511, NS 5511-plus-cowpea, Orbit and Orbit-plus-cowpea foods, by approximately 34% to 47%, 18% to 25%, 5% to 20%, and 7% to 16%, respectively (Table 3). Duodu et al. (2003) reviewed the effects of cooking on IVPD of sorghum foods. They also proposed that protein cross-linking (probably between γ - and β -kafirin proteins at the protein body periphery) might be the main factor that influences sorghum protein digestibility. The fermented foods, uji and injera, had higher IVPD than the unfermented thick porridge (ugali). This agrees with work by Taylor & Taylor (2002) who suggested that the lactic acid produced during fermentation, by lowering the pH, could modify the structure of the sorghum proteins rendering them more accessible to pepsin enzyme. Injera had the highest IVPD compared to the other foods. This was possibly due to its yeast protein.

Cowpea addition increased the PDCAAS by approximately two-fold and three-fold for the cowpea-complemented Orbit and NS 5511 sorghum foods, respectively (Table 3). The great increase in the PDCAAS of the foods as a result of cowpea addition was due to the combined effects of the improvement in lysine content (Table 2) and protein digestibility (Table 3). The cowpea-complemented sorghum foods have similar protein quality in terms of PDCAAS compared to maize and wheat but rather lower (50-80%) than Quality Protein Maize (QPM). Complemented foods provide about the same protein quality as high lysine, high protein digestibility transgenic sorghum (Biosorghum) and a 50-80% biological value of Quality Protein Maize (QPM) predicted by PDCAAS (Table 3). However, notwithstanding the greater proportional increase in PDCAAS in the NS 5511-cowpea foods, the levels were still lower than in the Orbit-cowpea foods. This is probably because NS 5511 protein had a lower protein digestibility as it contained tannins.

4. Conclusions

Cowpea addition substantially increases the protein digestibility and biological value predicted by PDCAAS, of traditional African sorghum foods to levels comparable with maize and wheat because cowpea proteins are richer in lysine and are more digestible than sorghum proteins. With both tannin- and non-tannin sorghum cultivars, addition of cowpea is a viable option for improving the protein quality of a wide range of different traditional African sorghum foods prepared by various processing methods.

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Table 1

Effects of cowpea addition on the tannin content (catechin equivalents, g/100 g, db) of raw flour and traditional African sorghum foods

Grain	Raw flour	Ugali	Uji	Injera
Cowpea	2.30 ^j ±0.01 ¹			
NS 5511 Sorghum	5.62 ^l ±0.04	1.79 ^h ±0.01	1.78 ^{gh} ±0.01	1.75 ^g ±0.02
NS 5511 Sorghum +Cowpea	4.71 ^k ±0.03	2.11 ⁱ ±0.01	2.30 ^j ±0.02	2.07 ⁱ ±0.01
Orbit Sorghum	0.23 ^b ±0.01	0.08 ^a ±0.01	0.11 ^a ±0.02	0.10 ^a ±0.02
Orbit Sorghum + Cowpea	0.76 ^f ±0.02	0.45 ^c ±0.01	0.62 ^e ±0.02	0.56 ^d ±0.01

¹Values are means ±standard deviations (n=3). Values within the table followed by different superscript letters were significantly different (p≤ 0.05).

Table 2

Effects of cowpea addition on the lysine and reactive lysine contents (g/100 g protein, db) of raw flour and traditional African sorghum foods

Grain	Lysine content				Reactive lysine content			
	Raw flour	Ugali	Uji	Injera	Raw flour	Ugali	Uji	Injera
Cowpea	4.56 ^g ±0.18 ¹ (0.88) ²				4.89 ^h ±0.24			
NS 5511 Sorghum	1.62 ^b ±0.14 (0.31)	1.58 ^b ±0.00 (0.30)	1.25 ^a ±0.20 (0.24)	1.88 ^c ±0.00 (0.36)	1.77 ^b ±0.16	1.75 ^{ab} ±0.29	1.51 ^a ±0.22	1.88 ^b ±0.17
NS 5511 Sorghum + Cowpea	3.43 ^f ±0.11 (0.66)	3.37 ^f ±0.00 (0.65)	2.99 ^e ±0.05 (0.58)	3.40 ^f ±0.19 (0.65)	2.65 ^{def} ±0.27	2.63 ^{def} ±0.06	2.64 ^{def} ±0.15	2.82 ^g ±0.07
Orbit Sorghum	1.90 ^c ±0.00 (0.37)	2.05 ^{cd} ±0.00 (0.39)	1.59 ^b ±0.08 (0.31)	2.28 ^d ±0.14 (0.44)	2.40 ^{cd} ±0.13	2.35 ^c ±0.26	2.52 ^{cde} ±0.20	2.33 ^c ±0.26
Orbit Sorghum + Cowpea	3.45 ^f ±0.18 (0.66)	3.43 ^f ±0.17 (0.66)	2.97 ^e ±0.11 (0.57)	3.92 ^g ±0.05 (0.65)	2.82 ^g ±0.18	2.71 ^{ef} ±0.28	2.76 ^{ef} ±0.07	2.82 ^g ±0.16

¹Values are means ±standard deviations (n=3). Values of a parameter within the table, followed by different superscript letters were significantly different (p≤0.05)

²Lysine score based on 52 mg/g protein requirement for a 1 to 2 year old child (WHO/FAO/UNU Expert Consultation, 2007).

Table 3

Effects of cowpea addition on the *in vitro* protein digestibility (PD) and Protein Digestibility Corrected Amino Acid Score (PDCAAS)¹ of raw flour and traditional African sorghum foods, compared to data obtained for other cereals.

	PD (%)	PDCAAS
Cowpea, raw	91.3 ^m ±2.3 ²	0.80
NS 5511 Sorghum		
Raw	61.8 ^{ef} ±0.2	0.19
Ugali	32.6 ^a ±0.8	0.10
Uji	38.8 ^b ±0.1	0.09
Injera	40.7 ^b ±0.2	0.15
NS 5511 Sorghum + Cowpea		
Raw	76.0 ⁱ ±0.2	0.50
Ugali	56.7 ^d ±0.6	0.37
Uji	61.0 ^e ±0.1	0.35
Injera	62.5 ^{ef} ±0.3	0.41
Orbit Sorghum		
Raw	80.6 ^k ±0.9	0.29
Ugali	64.6 ^f ±0.3	0.26
Uji	68.3 ^g ±1.9	0.21
Injera	76.4 ^{ij} ±1.2	0.34
Orbit Sorghum + Cowpea		
Raw	85.8 ^l ±0.2	0.57
Ugali	72.2 ^h ±0.4	0.48
Uji	77.0 ^{ij} ±0.7	0.44
Injera	79.8 ^{ik} ±1.1	0.60
High lysine sorghum (cooked)	45.3 ³	0.25 ⁴
Maize, normal (cooked)	85.3 ⁵	0.46 ⁶
Wheat flour, whole grain (cooked)	85.5 ⁵	0.45 ⁶
Quality Protein Maize (QPM) (raw)	96.2 ⁷	0.74 ⁷

¹Calculated as Lysine Score × Protein Digestibility

²Values of PD for cowpea, NS 5511, Orbit and complemented flours and foods are means ± standard deviations (n=3). Values followed by different superscript letters were significantly different (p≤0.05)

³Axtell et al. (1981)

⁴Based on lysine content values from Maclean et al. (1981)

⁵Mertz et al. (1984)

⁶Based on lysine content value from USDA (2009)

⁷National Research Council (1988)