Influence of micronization (infrared treatment) on the protein and functional quality of a ready-to-eat sorghum-cowpea African porridge for young child-feeding

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Abbreviations: CE - catechin equivalent, CP – Cowpeas, CPL – Cowpea leaves, DFS - defatted soy flour, HTST - high temperature-short time, IVPD - in vitro protein digestibility, LMW - low molecular weight, MCP 1 – Micronized cowpea, MCP 2 – Micronized cowpea 2, MRP - Maillard reaction products, PDCAAS - protein digestibility corrected amino acid score, TIA - trypsin inhibitor activity, TIU - trypsin inhibitor units, TPC - total phenolic content, WAI - water absorption index, WSI - water solubility index,

Abstract

Indigenous plant foods play a major nutritional and cultural role in the diets of rural people in Africa. However, they can contain high levels of antinutrients, which may exacerbate nutritional and health problems in young children consuming nutrient deficient diets. Also, the rapid increase in urbanization in Africa has led to the need for convenience type meals. This study investigated the potential of micronization (infrared treatment) in combination with extrusion cooking in developing a ready-to-eat sorghum and cowpea based porridge supplemented with cooked cowpea leaves for young child-feeding. Micronization not only inactivated the trypsin inhibitors in cowpea, it also produced an instantized product with excellent hydration properties. When served as a stiff porridge with cooked cowpea leaves in the recommended portion sizes for children aged 2-5 years, one daily serving would meet 40% of the children’s protein and lysine requirements. Further, the calculated Protein Digestibility Corrected Amino Acid Score would be comparable to commercial maize-soy instant products. This is notwithstanding that the cowpea leaves had a negative effect on protein digestibility due to their high tannin content. This nutritious ready-to-eat meal from locally available plant foods could contribute substantially to food security in both urban and rural communities in Africa.

1. Introduction

Traditionally, in many resource poor African communities, meals are prepared mainly using indigenous cereals and legumes (Young & Pellett, 1994). The use of legumes and cereals to make composite foods suitable for infant and young child feeding is well founded (Food and Agriculture Organization/World Health Organization, 1994). Sorghum and cowpea are indigenous African pulse-type legume and cereal grains of considerable nutritional and cultural importance in Africa (Anyango, De Kock, & Taylor, 2011). Indigenous green leafy vegetables also make an important contribution to rural African diets, adding high levels of vitamins and minerals (Uusiku, Oelofse, Duodu, Bester, & Faber, 2010). African green leafy vegetables are widely consumed in farming communities in Africa. Traditional dishes made from indigenous African green leafy plants were found to be well-accepted and are consumed by children 7-10 years (Van der Hoeven, Osei, Greeff, Kruger, Faber, & Smuts, 2013).

However, these African plant foods have substantial amounts of antinutrients such as enzyme inhibitors, polyphenolic compounds and anti-metals (phytates and oxalates) (Soetan & Oyewole, 2009). Consumption of foods that contain antinutrients by infants and young children can predispose them to malnutrition (Gibson, Ferguson, & Lehrfeld, 1998). This in turn affects the children's immune systems leading to diseases such as pneumonia, diarrhoea, malaria and acute malnutrition (Black, Allen, Bhutta, Caulfield, De Onis, Ezzati, Mathers, & Rivera, 2008).

In addition to these nutritional problems, the rapid increase in urbanization in Africa has led to the need for convenience type meals prepared from easily available foods (De Pee & Bloem, 2009) such as sorghum and cowpea. High Temperature-Short Time (HTST) extrusion cooking has been used successfully to produce nutritious ready-to-eat meals such as a protein-rich instant porridge (Pelembe, Erasmus, & Taylor, 2002; Singh, Gamlath, & Wakeling, 2007). Another thermal technology, micronization (infrared heating) (Sharma, 2009), has been found to be particularly effective at reducing the cooking time of legumes such as cowpea (Mwangwela, Waniska, &

Minnaar, 2006) and eliminating antinutrients in cowpea (Khattab & Arntfield, 2009). Micronization has also been found to improve the starch pasting properties of kidney beans, green beans, black beans, lentils and pinto beans (Fasina, Tyler, Pickard, Zheng, & Wang, 2001).

Thus, this study investigated the potential of micronization in combination with extrusion cooking in developing a ready-to-eat sorghum and cowpea based porridge supplemented with cooked cowpea leaves for young child feeding (2-5 years) and the effects of these technologies on the protein quality and functional quality of the meal. The products were compared with a commercial maize-soy composite instant porridge flour.

2. Materials and methods

2.1. Raw materials

Red non-tannin sorghum (cultivar MR Buster) was produced by the Agricultural Research Council, Potchefstroom, North-West Province, South Africa and cowpeas (cultivar Bechuana white) were from Delareyville, North West Province. The grains were cleaned and stored at 4°C. Young cowpea leaves were handpicked at the Ukulima Research Farm, Limpopo Province. Super (highly refined) white maize meal (Premier, Johannesburg), defatted soy flour (DSF) (Nedan Oil Mills, Potgietersrus) and a commercial instant maize-soy composite porridge flour suitable for children from one year to four years (FUTURELIFE®, Durban) were obtained.

2.2. Sorghum processing

Sorghum grains were decorticated to an extraction rate of 70–80% using an abrasive dehuller (Rural Industries Innovation Center, Kanye, Botswana). The decorticated grains were milled using a hammer mill with a 1.5 mm opening screen size. The milled grains were extruded in a TX 32 twin-screw, co-rotating extruder (CFAM Technologies, Potchefstroom). The feed rate was 30 kg/h and moisture content of the feed was adjusted to 20%. The screw rotation speed was 200 rpm and barrel temperature was maintained between 130°C and 159°C with a residence time of 30–90 s. The die diameter was 3 mm and the cutter speed 310 rpm. The extrudate was cooled at ambient temperature for 8 h before being packaged into plastic buckets with tight fitting lids.

2.3. Cowpea processing

Micronized cowpea flour type 1 (pre-conditioned, micronized, cooled, dehulled and then milled).

The cowpeas were pre-conditioned to 41% moisture by steeping in de-ionized water for 6 h and then allowed to equilibrate for 12 h. The grains were micronized using a table top micronizer (Technilamp, Johannesburg). After micronization, the grains were cooled to ambient temperature and then manually dehulled. The dehulled grains were milled using a hammer mill fitted with a 500 μm opening screen and then packed into zip lock-type polyethylene bags and kept at 10°C.

Micronized cowpea flour type 2 (pre-conditioned, dehulled, micronized, cooled and then milled).

This was prepared by first pre-conditioning as described, after which the grains were manually dehulled. Micronization, milling and packaging of the dehulled grains was as described.

2.4. Processing of cowpea leaves

Cowpea leaves were prepared essentially as described by Faber, Van Jaarsveld, Wenhold, & Van Rensburg (2010) for boiled amaranth. The leaves were washed using running tap water and then cooked in the water remaining trapped between them. The leaves were then frozen at -20°C. These steps were completed on the day the leaves were picked. They were then freeze dried and stored at 4°C. Freeze-drying was used simply as a matter of convenience for research purposes as it is the best method of preserving the nutritional quality of fresh produce. In real practice, fresh or air-dried leaves would be used.

2.5. Formulation of meals

Composite flours were prepared from the raw and extruded sorghum flour and the raw and micronized cowpea flours at a 70:30 (w/w) ratio. Composites of sorghum, cowpea flour and freeze dried cooked cowpea leaves were prepared at a 7:3:5 (w/w/w) ratio. Cowpea leaves were included in accordance with recommendations by the Nutrition Information Centre of the University of Stellenbosch (NICUS) (2003) and Vorster, Badham & Venter (2013) that children's diets should include a high intake of fruit and vegetables in order to provide better micronutrient nutrition.

2.6 Analyses

Protein content (N x 6.25) was determined by Dumas combustion, Method 46-30 (AACC International, 2000).

In vitro pepsin protein digestibility was determined using the procedure of Hamaker, Kirleis, Mertz, & Axtell (1986) as modified by Taylor & Taylor (2002) and using pepsin ≥250 units/mg solid (P7000) (Sigma–Aldrich, St. Louis, MO).

In vitro multi-enzyme protein digestibility was determined according to Hsu, Vavak, Satterlee, & Miller (1977). Samples were digested with trypsin, 13,000-20,000 BAEE units/mg protein (T03030, Sigma-Aldrich), bovine Chymotrypsin type II, 60 units/mg protein (C 4129, Sigma-Aldrich) and Protease XIV, 3.5 units/mg solid (P5747, Sigma-Aldrich).

Lysine was determined by the Pico-Tag method after acid hydrolysis of the protein (Bidlingmeyer, Cohen, & Tarvin, 1984).

Trypsin inhibitor activity was determined according to Method 22-40 (AACC International, 2000).

Total phenolic content (TPC) was determined by a Folin–Ciocalteu method (Waterman & Mole, 1994) with catechin as standard.

Tannin content was determined using the modified Vanillin-HCl method of Price, Van Scoyoc, & Butler (1978) with catechin as standard. Extract blanks were used to correct for highly coloured samples.

Flour water absorption (WAI) and water solubility (WSI) indexes were determined according to Anderson, Conway, Pfeifer, & Griffin (1969).

2.7. Statistical analysis

Data were analyzed using one way analysis of variance. Means were compared using Fisher's least significant difference test at a 95% level. All the experiments were conducted three times.

3. Results and discussion

3.1. Protein and lysine contents

As expected, the protein contents of the raw and micronized cowpea flours were some three times higher than the sorghum and raw maize flours (Table 1). The protein content of the cowpea flours (28-29%) was within the range reported for cowpea flour processed using traditional preparation methods (Akinyele, 1989). The protein content of the freeze-dried cooked cowpea leaves (27%) was similar to that of the cowpea flours (29%) and to that of other dried leafy vegetables such as spinach and amaranth (Singh, Kawatra, & Sehgal, 2001).

The two micronized cowpea flours had a slightly higher protein content compared to raw cowpea flour. This was presumably as a result of leaching out of minerals, simple sugars and amino acids (Barampama & Simard, 1995) during pre-conditioning prior to micronization.

Compositing sorghum flour with cowpea flour in the ratio 70:30 resulted in a 36-38% higher protein content compared to sorghum flour alone. This increase was similar to where the same ratio

Table 1: Effects of cooking sorghum by extrusion and cowpea by micronization in combination with compositing sorghum with cowpea and the inclusion of cooked cowpea leaves on the protein, lysine contents and lysine scores of the flours, in comparison with the maize-soy composites

Treatment	Percentage contribution (dry basis) of sorghum and cowpea flours and cowpea leaves in the formulations					Protein (g/100 g dry basis)[1]	Lysine (g/100 g dry basis)[1]	Lysine (g/100 g protein)[1]	Lysine score (amino acid score)[4]	
	Sorghum	Cowpea	Micronize cowpea1	Micronize cowpea2	Cowpea leaves				2 years	3-5 years
Raw										
Decorticated sorghum flour	100	0	0	0	0	9.2^{a}	0.20[6]	2.17	0.42	0.45
Dehulled cowpea flour	0	100	0	0	0	28.3^{g}	1.60[6]	5.65	1.09	1.18
Super maize meal flour	0	0	0	0	0	8.6^{a}	0.25[6]	2.91	0.56	0.61
Defatted soy flour	0	0	0	0	0	49.3^{i}	2.65[6]	5.38	1.03	1.12
Cooked										
Extruded sorghum flour	100	0	0	0	0	9.4^{ab}	0.16^{a}	1.70^{a}	0.33	0.35

Micronized cowpea 1 flour[2]	0	0	100	0	0	29.3^{h}	1.60[6]	5.46	1.05	1.14
Micronized cowpea 2 flour[3]	0	0	0	100	0	29.4^{h}	1.60[6]	5.44	1.05	1.13
Wet cooked cowpea leaves	0	0	0	0	100	27.0^{f}	1.16^{c}	4.30^{b}	0.83	0.90
Composites[5]										
Raw decorticated sorghum and dehulled cowpea flour	70	30	0	0	0	14.9^{c}	0.62[7]	4.16	0.80	0.87
Raw maize and defatted soy flour	0	0	0	0	0	23.8^{e}	0.97[8]	4.08	0.78	0.85
Extruded sorghum and micronized cowpea 1 flour	70	0	30	0	0	14.7^{c}	0.61^{b}	4.15^{b}	0.80	0.86
Extruded sorghum and micronized cowpea 2 flour	70	0	0	30	0	15.0^{c}	0.61^{b}	4.07^{b}	0.78	0.85
Extruded sorghum and micronized cowpea 1 flour+ cooked cowpea leaves	46.7	0	20	0	33.3	18.9^{d}	0.80^{b}	4.23^{b}	0.81	0.88
Extruded sorghum and micronized cowpea 2 flour+ cooked cowpea leaves	46.7	0	0	20	33.3	19.1^{d}	0.80^{b}	4.19^{b}	0.81	0.87
Commercial maize-soy instant flour	0	0	0	0	0	26.9^{f}	0.97[8]	3.61	0.69	0.75

[1]Values are means (n=3 for protein and n=2 for lysine). Means followed by different superscripts in a column are significantly different ($p < 0.05$)

[2]Pre-conditioned, micronized, cooled, dehulled and then milled into flour

[3]Pre-conditioned, dehulled, micronized, cooled and then milled into flour

[4]WHO/FAO/UNU Expert Consultation (2007)

[5]7 parts sorghum flour: 3 parts cowpea flour: 5 parts cowpea leaves (dry basis)

[6]Lysine values are from FAO (1981), except for extruded sorghum, wet cooked cowpea leaves, extruded sorghum and micronized cowpea flours 1 &2 and extruded sorghum and micronized cowpea flours + cooked cowpea leaves 1 & 2.

[7]Calculated using the raw sorghum and cowpea values based on a 70%sorghum and 30% cowpea formulation.

[8]Calculated using the raw maize and soy values based on 70% maize and 30% soy formulation

for sorghum and dehulled cowpea flours were used to formulate composite traditional African food products (Anyango et al., 2011). The protein contents of the sorghum and cowpea composites were, however, considerably lower than the laboratory prepared and commercial maize-soy composite flours. This is due to the notably high protein content of DFS, 47% (USDA, 2009). As a result of their high dry basis protein content, inclusion of cowpea leaves further increased protein content of the extruded sorghum and micronized cowpea flour composite, by approximately 30%.

Lysine is well-known as being the first limiting indispensable amino acid in cereals (WHO/FAO/UNU Expert Consultation, 2007). As expected, the sorghum and maize had by far the lowest total lysine and the protein lysine contents (Table 1). Compositing sorghum with cowpea flour more than tripled the lysine content. Inclusion of cowpea leaves further increased the lysine content of the meal because although lysine content of the cowpea leaves was somewhat lower than that of the cowpea flour, because the portion of the sorghum in the meal was reduced, the lysine content of the meal was increased.

The Lysine Scores of the extruded sorghum flours of 0.33 and 0.35 for 2 and 3-5 year olds fell far below the WHO/FAO/UNU Expert Consultation (2007). The sorghum Lysine Score was, however, within the range reported in a study of seven Algerian sorghum varieties (Mokrane, Amoura, Belhaneche-Bensemra, Courtin, Delcour, & Nadjemi, 2010). Micronized cowpea, however, met the lysine requirements of 2 to 5 year old children. The cowpea leaves were also much higher in lysine than sorghum but had a slightly lower Lysine Score than the cowpea flour. Hence, inclusion of cowpea leaves with the extruded sorghum and micronized cowpea composite flour did not affect the Lysine Score.

3.2. In vitro protein digestibility (IVPD) and Protein Digestibility Corrected Amino Acid Score (PDCAAS)

The multi-enzyme and pepsin *in vitro* methods were used as they are simple, rapid and reproducible and have given comparable results to *in vivo* methods in rat models (Boisen & Eggum, 1991) and in young children (Mertz, Hassen, Cairns-Whittern, Kirleis, Tu, & Axtell, 1984). There was generally good agreement in terms of ranking of the treatments between the multi-enzyme and pepsin IVPD methods (Table 2). Overall, the raw sorghum flour and the cowpea leaves had the lowest IVPD, while the extruded sorghum and micronized cowpea composite and the commercial maize-soy instant flour composite had the highest. Compared to raw sorghum flour, the extruded sorghum flour had a seven and 10 percentage points higher IVPD with the pepsin and the multi-enzyme methods, respectively. The high IVPD of the extruded sorghum and micronized cowpea composite flour was presumably a result of structural changes to the sorghum protein during the extrusion cooking process. Changes include improvement in the solubility of sorghum protein kafirin due to its disruption by friction during the extrusion cooking process (Hamaker, Metz, & Axtell, 1994).

Micronizing cowpea resulted in little or no change in IVPD using the multi-enzyme method, while the pepsin method gave an 11 percentage point decrease in the IVPD. This difference is probably due to reactions between amino groups and reducing sugars during micronization, which could result in the formation of low molecular weight Maillard reaction products (Oste, Dahlqvist, Sjostrom, Noren & Miller, 1986). The Maillard reaction products formed have some antioxidant activity (Vhangani and Van Wyk, 2013), which can inhibit protein digestion (Oste & Sjodin, 1986). It is likely that the pepsin method was more sensitive to inhibition by Maillard reaction products as only one enzyme is used, unlike the multi-enzyme method. The multi-enzyme method estimates hydrolysis in the stomach, small intestine and hind-gut of simple-stomach animals (Hsu et al., 1977). In contrast, the pepsin IVPD method, only estimates hydrolysis in the stomach (Boisen & Eggum, 1991).

Table 2: Effects of cooking sorghum by extrusion and cowpea by micronization in combination with compositing sorghum with cowpea and the inclusion of cooked cowpea leaves on the *in vitro* protein digestibility (IVPD) and Protein Digestibility Corrected Amino Acid Score (PDCAAS) of the flours, in comparison with maize-soy composites

Treatment	IVPD-Multi-enzyme (%)[1]	IVPD-Pepsin (%)[1]	PDCAAS[4]			
			IVPD-Multi-enzyme (%)		IVPD-Pepsin (%)	
			2 years	3-5 years	2 years	3-5 years
Raw						
Decorticated sorghum	78.8^{b} ±0.1	80.1^{ab} ±6.9	0.34	0.35	0.34	0.36
Dehulled cowpea flour	83.7^{c} ±0.4	98.6^{g} ±0.1	0.91	0.99	1.07	1.16
Super maize meal flour	87.9^{efg} ±0.3	86.5^{d} ±6.6	0.49	0.54	0.48	0.53
Defatted soy flour	87.6^{ef} ±0.3	98.1^{g} ±2.6	0.90	0.98	1.01	1.10
Cooked						
Extruded sorghum flour	88.8^{fg} ±0.6	87.0^{de} ±1.0	0.29	0.31	0.29	0.30
Micronized cowpea 1 flour[2]	86.4^{de} ±0.1	88.7^{e} ±2.8	0.91	0.98	0.93	1.01

Micronized cowpea 2 flour[3]	84.8 ^{cd} ±0.3	87.3 ^{de} ±1.3	0.89	0.96	0.92	0.99
Wet cooked cowpea leaves	76.5 ^{a} ±0.6	79.3 ^{a} ±2.3	0.63	0.69	0.66	0.71
Composites[5]						
Raw decorticated sorghum and dehulled cowpea flour	83.2^{c} ±0.3	92.3 ^{f} ± 3.9	0.67	0.72	0.74	0.80
Raw maize and soy composite flour	86.7^{e} ±0.1	93.4 ^{f} ± 4.1	0.68	0.74	0.79	0.79
Extruded sorghum and micronized cowpea 1 composite flour	90.5^{h} ±0	91.9 ^{f} ± 1.4	0.72	0.78	0.74	0.79
Extruded sorghum and micronized cowpea 2 composite flour	89.5^{gh} ±0.6	93.0 ^{f} ± 0.1	0.70	0.76	0.73	0.79
Extruded sorghum and micronized cowpea 1 flour+ cooked cowpea leaves	78.8^{b} ±0	81.7^{bc} ± 0.4	0.64	0.69	0.66	0.72
Extruded sorghum and micronized cowpea 2 flour+ cooked cowpea leaves	79.0^{b} ± 1.4	82.9 ^{c} ± 0.4	0.64	0.69	0.67	0.72
Commercial maize-soy instant flour	88.5^{fg} ±.5	92.0 ^{f} ± 0.2	0.61	0.66	0.63	0.69

[1]Values are means ± standard deviation (n=3). Means followed by different superscripts in a column are significantly different ($p< 0.05$)

[2]Pre-conditioned, micronized, cooled, dehulled and then milled into flour

[3]Pre-conditioned, dehulled, micronized, cooled and then milled into flour

[4]PDCAAS = Lysine score x IVPD

[5]7 parts sorghum flour: 3 parts cowpea flour: 5 parts cowpea leaves (dry basis)

Compositing cowpea flour with sorghum flour generally increased IVPD, due to the higher digestibility of the cowpea. Similar increases in the digestibility of several traditional sorghum African foods when composited with cowpea have been reported (Anyango et al., 2011).

The cooked cowpea leaves had the lowest IVPD. Adding the leaves to the composites reduced IVPD by 10-11 percentage points. The low IVPD of the cooked cowpea leaves and the sorghum-cowpea flour composite with cowpea leaves was undoubtedly due to the high tannin content of the leaves (2.19 g CE/100 g) (Table 3). The tannins interacted with the grain proteins forming complexes, which reduce enzyme action (De Bruyne, Pieters, Deelstra, & Vlietinck, 1999).

PDCAAS is a standard measure of how well the protein in a food can be used by the body (WHO/FAO/UNU Expert Consultation, 2007). PDCAAS of the products ranged from 0.29 for extruded sorghum flour to 1.16 in raw cowpea flour (Table 2). Raw and extruded sorghum and raw maize flours had the lowest PDCAAS because of their low lysine contents (Table 1). Due to the addition of cowpea flours with high lysine contents, the PDCAAS of the extruded sorghum and micronized cowpea composite flours were 2-3 times higher than the extruded sorghum flour (Table 2). In fact, the PDCAAS for extruded sorghum and micronized cowpea composites were all >0.70, the level recommended by the WHO as a reference for children 2–5 years (Codex Alimentarius Commission, 1991). A similar increase in PDCAAS after compositing traditional African sorghum foods with cowpea has been reported (Anyango et al., 2011). Considerably increased PDCAAS of other cereal and legume composites have been reported for a rice and beans weaning product (Kannan, Nielsen, & Mason, 2001) and a sorghum-soy composite biscuit (Serrem, De Kock, & Taylor, 2011).

Adding the cowpea leaves somewhat reduced the PDCAAS compared to the extruded sorghum and micronized cowpea composite flour alone (Table 2). Nevertheless, the PDCAAS of the composites with cowpea leaves (0.64-0.72) were the same as the calculated PDCAAS for the commercial maize-soy instant flour (0.61-0.69).

Table 3: Effects of cooking sorghum by extrusion and cowpea by micronization in combination with compositing sorghum with cowpea and the inclusion of cooked cowpea leaves on the trypsin inhibitor activity, total phenolic content and the tannin content of the flours, in comparison with maize and soy composite flour

	Trypsin inhibitory units (TIU/ g)[1]	Total phenolic content (g CE/100 g)[1]	Tannin content (g CE/100 g)[1]
Raw			
Decorticated sorghum flour	ND[a4]	$0.09^{a} \pm 0.00$	$0.24^{abc} \pm 0.1$
Dehulled cowpea flour	$29292^{f} \pm 727$	$0.19^{c} \pm 0.01$	$0.25^{abc} \pm 0.2$
Super maize meal flour	$3340^{cd} \pm 295$	$0.26^{e} \pm 0.01$	ND[a]
Defatted soy flour	$2146^{cd} \pm 746$	$0.43^{g} \pm 0.03$	$0.09^{ab} \pm 0.2$
Cooked			
Extruded sorghum flour	ND[a4]	$0.18^{c} \pm 0.01$	$0.18^{abc} \pm 0$
Micronized cowpea 1 flour[2]	$3421^{cd} \pm 72$	$0.19^{c} \pm 0.00$	ND[a]
Micronized cowpea 2 flour[3]	$2983^{cd} \pm 284$	$0.18^{c} \pm 0.01$	ND[a]
Wet cooked cowpea leaves	$4476^{d} \pm 432$	$3.35^{k} \pm 0.01$	$2.19^{d} \pm 1.7$
Composites[5]			
Raw decorticated sorghum and dehulled cowpea flour	$10413^{e} \pm 442$	$0.16^{b} \pm 0.01$	$0.05^{a} \pm 0.1$
Raw maize and defatted soy flour	$1925^{bcd} \pm 74$	$0.35^{f} \pm 0.00$	ND[a]
Extruded sorghum and micronized cowpea 1 flour	$3590^{cd} \pm 70$	$0.23^{d} \pm 0.01$	$0.14^{abc} \pm 0$
Extruded sorghum and micronized cowpea 2 flour	$4343^{d} \pm 72$	$0.23^{d} \pm 0.01$	$0.13^{abc} \pm 0$
Extruded sorghum and micronized cowpea 1 flour+ cooked cowpea leaves	$768^{bc} \pm 72$	$0.83^{i} \pm 0.03$	$0.71^{c} \pm 0.5$

Extruded sorghum and micronized cowpea 2 flour+ cooked cowpea leaves	$1378^{bcd} \pm 358$	$0.86^{j} \pm 0.01$	$0.65^{bc} \pm 0.5$
Commercial maize-soy instant flour	$1436^{bcd} \pm 145$	$0.53^{h} \pm 0.06$	ND^{a}

[1]Values are means ± standard deviation (n=3). Means followed by different superscripts in a column are significantly different ($p < 0.05$)

[2]Pre-conditioned, micronized, cooled, dehulled and then milled into flour

[3]Pre-conditioned, dehulled, micronized, cooled and then milled into flour

[4] ND not detected

[5]7 parts sorghum flour: 3 parts cowpea flour: 5 parts cowpea leaves (dry basis)

3.3. Antinutrients

The highest TIA was in the raw dehulled cowpea flour (Table 3). Micronizing cowpea, however, significantly reduced the TIA by 88-90%. The micronization procedures (micronized cowpea 1 and 2) were equally effective in reducing TIA. The percentage reduction was similar to that reported for micronization of cowpeas, kidney beans and peas (Khattab & Arntfield, 2009). Exposure of the trypsin inhibitors to heat causes denaturation and subsequent inactivation of the inhibitors (Lajolo & Genovese, 2002). Because of micronization of the cowpea, the level of TIA in the extruded sorghum and micronized cowpea composite flours and cowpea leaves was reduced to the same level as the commercial maize-soy instant flour.

It appeared that TIA inactivation by micronization of the cowpea resulted in only small changes in IVPD, a 0-3% increase with the multi-enzyme method and a 10-11% decrease with the pepsin method (Table 2). Roasting and micronization treatment of cowpeas, peas and kidney beans was reported to reduce their IVPD (Khattab, Arntfield, & Nyachoti, 2009). It is likely that micronization, a thermal treatment led to some non-enzymatic browning and cross-linking of the protein (Fagbemi, Oshodi, & Ipinmoroti, 2005; Hsu et al., 1977), resulting in reduced protein digestibility, as reported for sorghum (Duodu, Taylor, Belton, & Hamaker, 2003). Notwithstanding this, the level of protein digestibility of the extruded sorghum and micronized cowpea composites were comparable to (pepsin method) or higher than (multi-enzyme method) the commercial maize-soy instant flour.

Generally the TPC and tannin contents of the products followed the same trend. Levels were by far the highest in the cowpea leaves (Table 3). High levels of TPC in several leafy vegetables, ranging from 1.11 g/100 g (db) to 7.17 g/100 g in spinach have been reported (Ismail, Marjan, & Foong, 2004). Polyphenols in higher plants serve as protection against oxidative damage (Larson, 1988). Among the raw flours, DFS had the highest TPC (0.43 g/100 g). Soy is well known for its high level of isoflavone phytochemicals (King & Young, 1999). The TPC of the sorghum flours were within the range previously reported for non-tannin sorghum varieties (Chiremba, Taylor, & Duodu, 2009). TPC levels for the cowpea flours were slightly lower than reported elsewhere (Preet & Punia, 2000).

The tannin levels in the sorghum flours were low and within the acceptable recommended range for sorghum flour of < 0.3% (Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission, 1996). Importantly, adding the cowpea leaves to the extruded sorghum and micronized cowpea greatly increased the tannin content of the meals to 0.65-0.71 g/100 g db. This tannin level is more than twice the recommended Codex maximum level for sorghum flour (Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission, 1996). Further, as described, the tannins in the cowpea leaves substantially reduced the IVPD of the meals (Table 2).

3.4 Hydration properties of the flours

The instant porridge making properties of the flours were determined using the water absorption index (WAI) and water solubility index (WSI) parameters (Anderson *et al.*, 1969). All the raw flours had low WSI and had low WAI, with exception of DFS (Table 4). The high DFS WAI was due to the high globulin type protein content of soy (Koshiyama, 1983). As previously reported, (Pelembe et al., 2002), extrusion cooking greatly increased the WAI and WSI of the sorghum flour.

Both micronization treatments increased the WAI and WSI of cowpea flours substantially, 1.7-2.4 and 2.7 times, respectively. Micronization causes changes in cellular structure and gelatinization of starch (Cenkowski, Ames, & Muir, 2006). Gelatinization of starch by micronization has been shown in other pulse-type legumes, navy and black beans (Bellido, Arntfield, Cenkowski, & Scanlon, 2006). Although micronization of cowpeas has been investigated in-depth as a technology to reduce cooking time (Mwangwela, Waniska, & Minnaar, 2007) and inactivate TIA (Khattab & Arntfield, 2009), this is the first report of micronization instantizing of cowpea flour. Importantly,

Table 4: Effects of cooking sorghum by extrusion and cowpea by micronization in combination with compositing sorghum with cowpea and cooked cowpea leaves on the Water Absorption Index (WAI) and Water Solubility Index (WSI) of the flour in comparison with the maize-soy composites

Treatment	WAI (%)[1]	WSI (%)[1]
Raw		
Decorticated sorghum flour	2.47[b]	12.7[bcd]
Dehulled cowpea flour	1.94[a]	9.4[a]
Maize flour	2.85[bc]	15.6[de]
Defatted soy flour	4.23[fg]	10.8[abc]
Cooked		
Extruded sorghum flour	4.26[fg]	33.1[i]
Micronized cowpea 1 flour[2]	4.70[g]	25.7[g]
Micronized cowpea 2 flour[3]	3.22[cd]	25.6[g]
Composites[4]		
Raw decorticated sorghum and dehulled cowpea flour	2.34[ab]	9.95[ab]
Raw maize and defatted soy flour	3.17[c]	13.1[cd]
Extruded sorghum and micronized cowpea 1 flour	3.33[cde]	26.1[gh]
Extruded sorghum and micronized cowpea 2 flour	3.28[cde]	25.6[g]
Commercial maize-soy instant flour	2.83[bc]	22.2[f]

[1]Values are means (n=3). Means followed by different superscripts in a column are significantly different ($p < 0.05$)

[2]Pre-conditioned, micronized, cooled, dehulled and then milled into flour

[3]Pre-conditioned, dehulled, micronized, cooled and then milled into flour

[4]7 parts sorghum flour: 3 parts cowpea flour (dry basis)

both extruded sorghum and micronized cowpea composite flour products had the same WAI and a higher WSI than the commercial maize-soy instant composite flour.

Of the two micronization treatments, treatment 1 (pre-conditioning and micronization before dehulling) showed a significantly greater increase in WAI than treatment 2 (pre-conditioning and dehulling before micronization). This was probably because in treatment 1 the cowpea hull retained more moisture within the grain during micronization. A similar effect was observed during micronization of hulled barley where the hull provided some resistance to the moisture removal leading to expansion of the kernel (Fasina, Tyler, Pickard, & Zheng, 1999).

3.5. Estimated contributions of the composite porridge meals to the protein and lysine requirements of young children

When the lysine and protein contents in a 100 g serving of a composite porridge meal for children aged 2-3 years and 125 g serving for those aged 4-5 years were calculated, there was a 36-50% increase in protein and a 74% increase in lysine in the extruded sorghum and micronized cowpea composite porridge meal compared to extruded sorghum porridge (Table 5). Inclusion of cowpea leaves with the extruded sorghum and micronized cowpea composite porridge meal resulted in further increases of 21-22% and 3-11% in protein and lysine, respectively.

However, compared to commercial maize-soy instant composite porridge meal, extruded sorghum and micronized cowpea composite porridge meal with cooked cowpea leaves contribution was 30-35% lower in terms of both their protein and lysine requirements (WHO/FAO/UNU Expert Consultation, 2007). This is because of the higher protein content and quality of the soy. Notwithstanding this, if the sorghum-cowpea porridge and cowpea leaf meal was consumed by the children once a day, it would meet 40% of their protein and lysine requirements.

Table 5: Effects of cooking sorghum by extrusion and cowpea by micronization in combination with compositing sorghum with cowpea and cooked cowpea leaves on the protein and lysine contents of the flours and the calculated percentage contribution of stiff porridges made from these flours to the WHO/FAO/UNU Expert Consultation (2007) protein and lysine requirements of young children (2-5 years), in comparison with maize-soy composites

Treatment	Protein per serving of porridge (g) [Lysine per serving (mg)]		% per serving of porridge of safe level of protein intake[5]		% per serving of porridge of lysine requirement[5]			
	2-3 years (portion size 100 g)[4]	**4-5 years** (portion size 125 g)[4]	**2-3 years** (safe level of protein intake 0.9-0.97g protein/kg body wt/ day)[5,9]	**4-5 years** (safe level of protein intake 0.85-0.86 g protein/kg body wt/day)[5,9]	**2-3 years** (lysine requirement 45 mg/kg body wt/day)[5]		**4-5 years** (lysine requirement 35 mg/kg body wt/day)[5]	
					Girls	Boys	Girls	Boys
Raw								
Decorticated sorghum flour	3.04 [66]	3.77 [82]	23.8-26.7	24.3-27	12.8-13.5	12.2-13.0	13.2-13.8	12.8-13.4
Dehulled cowpea flour								

Maize flour	2.84 [83][6]	3.53 [103][6]	22.1-24.9	22.8-25.3	16.0-16.9	15.3-16.4	16.6-17.3	16.1-16.8
Defatted soy flour								
Cooked								
Extruded sorghum flour	3.10 [53]	3.85 [65]	21.8-26.4	21.1-23.7	10.2-10.8	9.8-10.4	10.6-10.9	10.2-10.6
Micronized cowpea 1 flour[1]								
Micronized cowpea 2 flour[2]								
Wet cooked cowpea leaves								
Composites[3]								
Raw decorticated sorghum and dehulled cowpea flour	4.92 [205][7]	6.11 [254][7]	34.5-41.9	33.5-37.6	39.6-41.5	38.0-40.4	41.1-42.7	39.8-41.5
Raw maize and defatted soy flour	7.85 [320][8]	9.76 [398][8]	55.1-66.8	53.5-60.1	61.8-65.3	59.3-63.1	64.2-66.9	62.3-65.0
Extruded sorghum and micronized cowpea 1 flour	4.85 [201]	6.03 [250]	34-41.3	33-37.1	38.8-41.0	37.2-39.2	40.4-42.0	39.1-40.8

Extruded sorghum and micronized cowpea 2 flour	4.95 [201]	6.15 [250]	34.7-42.1	33.7-37.8	38.8-41.0	37.2-39.2	40.4-42.0	39.1-40.8
Extruded sorghum and micronized cowpea 1 flour+ cooked cowpea leaves	6.24 [208]	7.75 [258]	43.8-53.1	42.5-47.7	40.2-42.4	38.5-41.0	41.7-44.3	40.4-45.7
Extruded sorghum and micronized cowpea 2 flour+ cooked cowpea leaves	6.30 [225]	7.83 [280]	44.2-53.6	42.9-48.2	43.5-45.9	41.7-44.3	45.2-47.1	43.8-45.7
Commercial maize-soy instant flour	8.88 [320][8]	11.0 [397][8]	62.3-75.6	60.3-67.7	61.8-65.3	59.3-63.1	64.2-66.7	62.2-64.8

[1]Pre-conditioned, micronized, cooled, dehulled and then milled into flour,

[2]Pre-conditioned, dehulled, micronized, cooled and then milled into flour

[3] 7 parts sorghum flour: 3 parts cowpea flour: 5 parts cooked cowpea leaves (dry basis)

[4]Portion size data from the Nutrition Information Centre of the University of Stellenbosch (NICUS) (2003)

[5]Child weight data is from WHO (2014)

[6]Lysine values from FAO (1981)

[7]Calculated using the raw sorghum and cowpea lysine values based on the 70% maize and 30% soy formulation

[8]Calculated using the raw maize and soy lysine values based on the 70% maize and 30% soy formulation

[9] protein/kg body wt/ day – protein per kilogram body weight per day

4. Conclusions

Micronization can not only be used to reduce cooking time and inactivate TIA in cowpea, it can produce instant flours for making porridges with excellent water absorption and solubility. Composite flours of micronized cowpea with HTST extrusion cooked sorghum have equivalent, if not better, hydration properties than a commercial instant maize-soy porridge product. When served as a stiff porridge in the recommended portion sizes for children aged 2-5 years once a day, the micronized cowpea and extruded sorghum composite porridge with cooked cowpea leaves would meet 40% of the children's protein and lysine requirements and the calculated PDCAAS would be comparable to commercial maize-soy instant products. This is notwithstanding the negative effects of the cooked cowpea leaves on protein digestibility.

Furthermore, using sorghum and cowpeas to produce such nutritious ready-to-eat foods can contribute substantially to food security in both urban and rural communities in Africa. This is a much better option than relying on international food aid, as it develops local food processing enterprises, which in turn create a commercial market for small-holder farmers (Mwaniki, 2006). Further, elsewhere in the world where other grains are traditional staples, for example amaranth and common beans in South America, these could be similarly processed to produce nutritious ready-to-eat foods. Also, as the product is in the form of a dry powder it has the advantage that it will remain microbiologically stable if kept dry. However, the powder would be subject to oxidation. The use of ascorbic acid as an antioxidant which is a permitted additive in cereal-based infant foods (European Food Safety Authority, 2010) is suggested.

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5. References

AACC International. (2000). *Approved methods of the American Association of Cereal Chemists*, (10th Ed.). St. Paul, MN: AACC International.

Akinyele., I. O. (1989). Effects of traditional methods of processing on the nutrient content and some antinutritional factors in cowpeas (*Vigna unguiculata*). *Food Chemistry, 33*, 291-299.

Anderson, R. A., Conway, H. F., Pfeifer, V. F., & Griffin, E. L., Jr. (1969). Roll and extrusion-cooking of grain sorghum grits. *Cereal Science Today*, *14,* 372-385, 381.

Anyango, J. O., De Kock, H. L., & Taylor, J. R. N. (2011). 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 Chemistry, 124*, 775-780.

Barampama, Z., & Simard, R. E. (1995). Effects of soaking, cooking and fermentation on composition, in-vitro starch digestibility and nutritive value of common beans. *Plant Foods for Human Nutrition, 48*, 349-365.

Bellido, G., Arntfield, S. D., Cenkowski, S., & Scanlon, M. (2006). Effects of micronization pretreatments on the physicochemical properties of navy and black beans (*Phaseolus vulgaris* L.). *LWT - Food Science and Technology, 39*, 779-787.

Bidlingmeyer, B. A., Cohen, S. A., & Tarvin, T. L. (1984). Rapid analysis of amino acids using pre-column derivatization. *Journal of Chromatography, 336*, 93-104.

Black, R. E., Allen, L. H., Bhutta, Z. A., Caulfield, L. E., De Onis, M., Ezzati, M., Mathers, C., & Rivera, J. (2008). Maternal and child undernutrition: global and regional exposures and health consequences. *The Lancet*, *371*, 243-260.

Boisen, S., & Eggum, B. (1991). Critical evaluation of in vitro methods for estimating digestibility in simple-stomach animals. *Nutrition Research Reviews, 4*, 141-162.

Cenkowski, S., Ames, N., & Muir, W. (2006). Infrared processing of oat groats in a laboratory-scale electric micronizer. *Canadian Biosystems Engineering, 48*, 3.17-3.25

Chiremba, C., Taylor, J. R. N., & Duodu, K. G. (2009). Phenolic content, antioxidant activity, and consumer acceptability of sorghum cookies. *Cereal Chemistry 86*, 590-594.

Codex Alimentarius Commission. (1991). *Guidelines on formulated complementary foods for older infants and young children* CAC/GL 8-1991. Rome: FAO.

De Bruyne, T., Pieters, L., Deelstra, H., & Vlietinck, A. (1999). Condensed vegetable tannins: Biodiversity in structure and biological activities. *Biochemical Systematics and Ecology, 27*, 445-459.

De Pee, S., & Bloem, M. W. (2009). Current and potential role of specially formulated foods and food supplements for preventing malnutrition among 6-to 23-month-old children and for treating moderate malnutrition among 6-to 59-month-old children. *Food & Nutrition Bulletin, 30*, S434-S462.

Duodu, K. G., Taylor, J. R. N., Belton, P. S., & Hamaker, B. R. (2003). Factors affecting sorghum protein digestibility. *Journal of Cereal Science*, *38,* 117-131.

European Food Safety Authority (2010). Scientific opinion on the use of sodium ascorbate as a food additive in vitamin D preparations intended to be used in formulae and weaning food for infants and young children. *European Food Safety Authority Journal, 8*(12), 1942.

Faber, M., Van Jaarsveld, P., Wenhold, F., & Van Rensburg, J. (2010). African leafy vegetables consumed by households in the Limpopo and KwaZulu-Natal provinces in South Africa. *South African Journal of Clinical Nutrition, 23*, 30-38.

Fagbemi, T., Oshodi, A., & Ipinmoroti, K. (2005). Processing effects on some antinutritional factors and in vitro multienzyme protein digestibility (IVPD) of three tropical seeds: breadnut (*Artocarpus altilis*), cashewnut (*Anacardium occidentale*) and fluted pumpkin (*Telfairia occidentalis*). *Pakistan Journal of Nutrition, 4*, 250-256.

FAO (1981). *Amino acid content of foods and biological data on protein*. Rome: FAO. FAO/WHO (1994). *Codex Alimentarius: Foods for special dietary uses (including foods for infants and children).* (2nd ed.) Rome: FAO.

Food and Agriculture Organization/ World Health Organization. (1994). Codex Alimentarius: Foods for special dietary uses (including foods for infants and children) *2nd edition. Rome: Food and Agriculture Organization.*

Fasina, O. O., Tyler, R. T., Pickard, M. D., & Zheng, G. H. (1999). Infrared heating of hulless and pearled barley. *Journal of Food Processing and Preservation, 23*, 135-151.

Fasina., O. O., Tyler, B., Pickard, M., Zheng, G.-H., & Wang, N. (2001). Effect of infrared heating on the properties of legume seeds. *International Journal of Food Science & Technology, 36*, 79-90.

Gibson, R., Ferguson, E., & Lehrfeld, J. (1998). Complementary foods for infant feeding in developing countries: their nutrient adequacy and improvement. *European Journal of Clinical Nutrition, 52*, 764-770.

Hamaker., B., R., Mertz., E., T., & Axtell., J., D. (1994). Effect of extrusion on sorghum kafirin solubility. *Cereal Chemistry, 71*, 515-515.

Hamaker, B. R., Kirleis, A. W., Mertz, E. T., & Axtell, J. D. (1986). Effect of cooking on the protein profiles and in vitro digestibility of sorghum and maize. *Journal of Agricultural and Food Chemistry, 34*, 647-649.

Hsu, H. W., Vavak, D. L., Satterlee, L. D., & Miller, G. A. (1977). A multienzyme technique for estimating protein digestibility. *Journal of Food Science, 42*, 1269-1273.

Ismail, A., Marjan, Z. M., & Foong, C. W. (2004). Total antioxidant activity and phenolic content in selected vegetables. *Food Chemistry, 87*, 581-586.

Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission. (1996). Codex Alimentarius Cereals, pulses, legumes and derived products and vegetable proteins. Volume 7. Food and Agriculture Organization of the United Nation, World Health Organization, Rome.

Kannan, S., Nielsen, S. S., & Mason, A. C. (2001). Protein Digestibility-Corrected Amino Acid Scores for bean and bean–rice infant weaning food products. *Journal of Agricultural and Food Chemistry, 49*, 5070-5074.

Khattab, R. Y., & Arntfield, S. D. (2009). Nutritional quality of legume seeds as affected by some physical treatments. 2. Antinutritional factors. *LWT - Food Science and Technology, 42*, 1113-1118.

Khattab, R. Y., Arntfield, S. D., & Nyachoti, C. M. (2009). Nutritional quality of legume seeds as affected by some physical treatments. Part 1: Protein quality evaluation. *LWT - Food Science and Technology, 42*, 1107-1112.

King, A. M. Y., & Young, G. (1999). Characteristics and occurrence of phenolic phytochemicals. *Journal of the American Dietetic Association, 99*, 213-218.

Koshiyama, I. (1983). Storage proteins of soybean. In W. Gottschalk & H. Müller (Eds.), *Seed proteins, vol. 2* (pp. 427-450). Berlin: Springer.

Lajolo, F. M., & Genovese, M. I. (2002). Nutritional significance of lectins and enzyme inhibitors from legumes. *Journal of Agricultural and Food Chemistry, 50*, 6592-6598.

Larson, R. A. (1988). The antioxidants of higher plants. *Phytochemistry, 27*, 969-978.

Mertz, E. T., Hassen, M. M., Cairns-Whittern, C., Kirleis, A. W., Tu, L., & Axtell, J. D. (1984). Pepsin digestibility of proteins in sorghum and other major cereals. *Proceedings of the National Academy of Sciences, USA. 81*, 1-2.

Mokrane, H., Amoura, H., Belhaneche-Bensemra, N., Courtin, C. M., Delcour, J. A., & Nadjemi, B. (2010). Assessment of Algerian sorghum protein quality [*Sorghum bicolor* (L.) Moench] using amino acid analysis and in vitro pepsin digestibility. *Food Chemistry, 121*, 719-723.

Mwangwela, A.M, Waniska, R. D., & Minnaar, A. (2006). Hydrothermal treatments of two cowpea (*Vigna unguiculata* L. Walp) varieties: effect of micronisation on physicochemical and structural characteristics. *Journal of the Science of Food and Agriculture, 86*, 35–45.

Mwangwela, A. M., Waniska, R. D., & Minnaar, A. (2007). Effect of micronisation temperature (130 and 170 ^{o}C) on functional properties of cowpea flour. *Food Chemistry, 104*, 650-657.

Mwaniki, A. (2006). Achieving food security in Africa: Challenges and issues. UN Office of the Special Advisor on Africa (OSAA) http://www.un.org/africa/osaa/reports/Achieving% 20Food% 20Security% 20in% 20Africa-Challenges% 20and% 20Issues.pdf (last accessed 10 October 2010).

Nutrition Information Centre of the University of Stellenbosch (NICUS) (2003). Dietary Reference Intakes (DRI's). *Compiled by the Nutrition Information Centre of the University of Stellenbosch (NICUS)* (Last accessed 15 July 2014).

Oste, R. E., Dahlqvist, A., Sjostrom, H., Noren, O., & Miller, R. (1986). Effect of Maillard reaction on Protein digestion . In vitro studies. *Journal of Agricultural and Food Chemistry 34*, 355-358.

Oste, R. E., & Sjodin, P. (1984). Effect of Maillard reaction products on protein digestion. In vivo studies on rats. *Journal of Nutrition, 114*, 2228–2234

Pelembe, L. A. M., Erasmus, C., & Taylor, J. R. N. (2002). Development of a protein-rich composite sorghum–cowpea instant porridge by extrusion cooking process. *LWT-Food Science and Technology, 35*, 120-127.

Preet, K., & Punia, D. (2000). Antinutrients and digestibility (in vitro) of soaked, dehulled and germinated cowpeas. *Nutrition and Health, 14*, 109-117.

Price, M. L., Van Scoyoc, S., & Butler, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry 26*, 1214-1218.

Sharma, G. K. (2009). Micronization. *DRDO Science Spectrum*, (3) 169-171.

Serrem, C. A., De Kock, H .L., & Taylor, J. R. N. (2011). Nutritional quality, sensory quality and consumer acceptability of sorghum and bread wheat biscuits fortified with defatted soy flour. *International Journal of Food Science and Technology, 124*, 74-83

Singh, G., Kawatra, A., & Sehgal, S. (2001). Nutritional composition of selected green leafy vegetables, herbs and carrots. *Plant Foods for Human Nutrition, 56*, 359-364.

Singh, S., Gamlath, S., & Wakeling, L. (2007). Nutritional aspects of food extrusion: a review. *International Journal of Food Science and Technology, 42*, 916-929.

Soetan, K. O., & Oyewole, O. E. (2009). The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: A review. *African Journal of Food Science, 3*, 223-232.

Taylor, J., & Taylor J. R .N. (2002). Alleviation of the adverse effects of cooking on protein digestibility in sorghum through fermentation in traditional African porridges. *International Journal of Food Science and Technology, 37*, 129-138.USDA. (2009). National Nutrient Database for Standard Reference. Release 22. http://0-www.nal.usda.gov. (last accessed 4 August 2014).

Uusiku, N. P., Oelofse, A., Duodu, K. G., Bester, M. J., & Faber, M. (2010). Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A

Van der Hoeven, M., Osei, J., Greeff, M., Kruger, A., Faber, M., & Smuts, C. M. (2013). Indigenous and traditional plants: South African parents' knowledge, perceptions and uses and their children's sensory acceptance. *Journal of Ethnobiology and Ethnomedicine, 9,* 1-12

Vhangani, L.N., & Van Wyk, J. (2013). Antioxidant activity of Maillard reaction products (MRPs) derived from fructose–lysine and ribose–lysine model systems. *Food Chemistry, 137,* 92–98

Vorster, H.H., Badham, J.B., & Venter, C.S., (2013) An introduction to the revised food-based dietary guidelines for South Africa. *South African Journal of Clinical Nutrition, 6),* S1-S164

Waterman, P. G., & Mole, S. (1994). *Analysis of phenolic plant metabolites*. Oxford: Alden Press, pp. 66-103.

WHO/FAO/UNU Expert Consultation. (2007). Protein and Amino Acid Requirements in Human Nutrition, Report of a Joint WHO/FAO/UNU Expert Consultation, World Health Organization Technical Report No. 935. Geneva: World Health Organization

WHO. (2014). *Child growth standards: The WHO Child Growth Standards.* http://www.who.int/childgrowth/standards/en/ (Last accessed 10 October 2014).

Young, V. R., & Pellett, P. L. (1994). Plant proteins in relation to human protein and amino acid nutrition. *The American Journal of Clinical Nutrition, 59,* 1203S-1212S.

KEY REFERENCES AND WHY THEY ARE KEY

Anyango, J. O., De Kock, H. L., & Taylor, J. R. N. (2011). 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 Chemistry, 124*, 775-780.

This article was used to support the success of using a sorghum and cowpea composite to formulate an acceptable food. What the researchers found with the sorghum:cowpea (70:30) composite formulation is that it has improved protein quality compared to use of sorghum alone

Bellido, G., Arntfield, S. D., Cenkowski, S., & Scanlon, M. (2006). Effects of micronization pretreatments on the physicochemical properties of navy and black beans (*Phaseolus vulgaris* L.). *LWT - Food Science and Technology, 39,* 779-787.

This article displays the effects of micronization on important physichochemical properties such as the colour and hardness. These are important properties in the present study as it shows that micronization can reduce the cooking time and also improve the products appearance.

De Pee, S., & Bloem, M. W. (2009). Current and potential role of specially formulated foods and food supplements for preventing malnutrition among 6-to 23-month-old children and for treating moderate malnutrition among 6-to 59-month-old children. *Food & Nutrition Bulletin, 30,* S434-S462.

This article focuses on the important role that foods formulated for infants and young children play in the prevention of childhood malnutrition. The article reviews the locally prepared diets that can be used for the nourishment of moderately malnourished children which the present study is doing.

Khattab, R. Y., Arntfield, S. D., & Nyachoti, C. M. (2009). Nutritional quality of legume seeds as affected by some physical treatments. Part 1: Protein quality evaluation. *LWT - Food Science and Technology, 42,* 1107-1112.

Some food processing methods may be detrimental to the nutritional value of legumes and cereals. Thise article investigated the effects of a some methods on on the nutritional quality of cowpea, kidney bean and pea, which was an important aspect of the present study

Uusiku, N. P., Oelofse, A., Duodu, K. G., Bester, M. J., & Faber, M. (2010). Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. *Journal of Food Composition and Analysis, 23,* 499-509.

The present study investigated the potential contributions of indigenous foods to children's diets as an instant product. This article provided evidence that African leafy vegetables can help to reduce malnutrition in developing countries.