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Improvement in the protein quality of African sorghum foods through compositing with cowpea

By

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DECLARATION

I hereby declare that the dissertation herewith submitted at the University of Pretoria for the award of MSc (Food Science) degree is my work and has not been submitted by me for a degree at any other university or institution of higher education.

Joseph Ochieng' Anyango

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ABSTRACT

Improvement in the protein quality of African sorghum foods through compositing with cowpea

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Supervisor: Prof J.R.N. Taylor

Co-supervisor: Dr H.L. de Kock

Lysine deficiency is a major nutritional problem faced by poor people living in the arid and semi-arid tropics who depend on sorghum as their staple food. This is because of poor lysine content and digestibility of sorghum proteins, which aggravates when sorghum is cooked in food. To address this nutritional problem, compositing with locally available lysine-rich legumes has been proposed. Therefore, this study investigated the effects of compositing with the African grain legume, cowpea, on the protein and functional quality of important traditional African sorghum foods.

Two sorghum cultivars, a red, tannin (NS 5511) and a white tan plant, non-tannin (Orbit) composited with cowpea at 70:30 ratio, were used to prepare three traditional sorghum foods, ugali (unfermented thick porridge), uji (fermented thin porridge) and injera (fermented flatbread). The protein quality of the traditional sorghum foods was determined by measuring their protein contents, lysine and reactive lysine contents, and in vitro protein digestibility. The functional properties of the foods were studied using instrumental texture analysis. Other sensory properties of ugali were determined using a trained sensory panel.

Compositing with cowpea increased the protein contents of the foods by up to 35% and 57% for NS 5511 and Orbit foods, respectively. Lysine contents of the food proteins increased by 67% to 139%. Reactive lysine content increased by 10% to 75%. Protein digestibility of the foods increased by 13% to 62%. There was approximately three- and two-fold increase in protein digestibility corrected amino score (PDCAAS) of NS 5511 and Orbit foods, respectively, due to addition of cowpea. However, Orbit-plus-cowpea foods still had better

protein quality than NS 5511-plus-cowpea foods, primarily because of the tannins in the latter which bind the proteins thereby lowering their digestibility.

Compositing reduced paste peak viscosity (PV) and cool paste viscosity (CPV) of uji porridge by 6% to 23%, and 6% to 12%, respectively, probably as a result of decreasing porridge starch content. Principal component analysis (PCA) showed that compositing contributed 38% of the variation in 17 sensory attributes of ugali. Compositing imparted cowpea flavour to ugali. Most of the variation in sensory properties (59%) of ugali was due to the quality characteristics of the sorghum cultivars. Compositing increased the stiffness of NS 5511 injera by up to 25%, while it reduced the stiffness of Orbit injera by up to 12%. These differences in stiffness suggested a weakening effect of weaker H-bonding between tannins and other food polymers such as proteins instead of stronger covalent bonds like those involved in proteins-protein interactions.

Compositing important traditional sorghum foods with cowpea has potential for helping to solve lysine deficiency faced by sorghum consumers in the semi-arid tropics. However, it introduces cowpea flavour which may need to be eliminated, in foods intended for consumers not accustomed to cowpea flavour.

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1 INTRODUCTION

Sorghum (*Sorghum bicolor* [L.] Moench) is one of the most important staple crops in Africa and is uniquely adapted to the semi-arid and sub-tropical climatic conditions of the continent (Doggett 1988). Many of the world's most food insecure people depend on it as their primary food grain (Food and Agriculture Organisation (FAO) and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) 1996).

Sorghum grain is eaten in a variety of forms that vary from region to region (Murty and Kumar 1995). As stated by these authors, the most common traditional foods made from sorghum are thin and thick porridges and flatbreads. The nutrient content of sorghum grain is generally similar to other cereals (FAO 1995). However, studies have revealed poor protein quality of sorghum foods because sorghum protein is deficient in lysine (Taylor and Schüssler 1986) and becomes less digestible after cooking into foods (Hamaker et al 1986).

Effort to improve the lysine content of sorghum foods, such as that of Daniel et al (1966) has shown that supplementation with synthetic essential amino acids may improve protein quality of sorghum diets. However, this practice is regarded as inefficient and impractical among populations where sorghum is the dietary staple as it may be too expensive for the poor to afford such amino acid supplements (Allen 2003). Furthermore, such an approach requires infrastructure, access to markets and healthcare systems for their success, often not available to people living in remote rural areas (reviewed by Mayer et al 2008). Compositing with lysine-rich legumes has been used to enhance the protein quality of sorghum food products. For example, Bookwalter et al (1987) reported an improved protein quality of sorghum-soy blend used in the United States' Food for Peace Program. As the semi-arid tropics are characterised by unpredictable weather, limited and erratic rainfall and nutrient poor soils among other agricultural constraints (reviewed by Maqbool et al 2001), efforts to improve nutritional insufficiency should focus on indigenous crops which are accessible to the poor consumers in such environments (reviewed by Sharma et al 2002).

Cowpea (*Vigna unguiculata* [L.] Walp) is the most important food legume in the dry savannas of tropical Africa, where it accounts for more than 12.5 million hectares and is consumed by nearly 200 million people (African Agricultural Technology Foundation (AATF) 2005). As a legume, it is richer in high-quality protein and it contains almost as

much energy by weight as cereal grains (United States Department of Agriculture (USDA) 2008). Therefore, it provides a suitable way to complement protein-deficient sorghum diets. The techniques commonly employed in traditional weaning food formulations include the use of composite foods made from cereals and legumes such as cowpeas (reviewed by Sefa-Dedeh et al 2001).

Studies indicate that functional properties of sorghum foods depend on the nutrient composition of the sorghum grain type. For example, when Lorri and Svanberg (1993a) studied viscosity in relation to flour concentration, of lactic acid-fermented sorghum gruel used as a weaning food in Tanzania, they noted less reduction in viscosity of tannin-sorghum gruels. These authors attributed the limited reductions in viscosity of tannin-sorghum gruels to inhibition of bacterial growth by tannins. A study such as this on thin porridge viscosity provides useful information on the flour concentration that can be used without making the food unpalatable (Lorri and Svanberg 1993a). High dietary bulk makes it hard for infants fed on these gruels to meet their nutrient requirement, as a small child's immature digestive system may not be able to process enough food to meet the nutritional needs (Ljungqvist et al 1981). Similarly, studies on fermented flatbread such as injera have shown effects of sorghum grain quality on its textural attributes such as softness, rollability and fluffiness (Yetneberk et al 2004).

Determination of the effects of compositing with cowpea on the protein and functional quality of traditional sorghum foods is part of the ongoing research aimed at finding a solution to lysine deficiency. The aim of this project is to establish whether compositing traditional sorghum foods with cowpea, an indigenous tropical legume, can deliver the essential amino acid lysine to African populations in the arid and semi-arid tropics dependent on sorghum as their major diet component. This knowledge will be useful in helping to improve the health of the protein-malnourished people living in these regions.

2 LITERATURE REVIEW

This review examines the research on protein quality of sorghum and cowpea grains as applicable in their use in traditional food systems. It highlights studies on the grain structure, protein composition and quality. An overview of the different kinds of traditional sorghum foods and the effects of their preparation methods on protein quality is given. Studies on the factors that influence the protein and functional quality of traditional sorghum foods are reviewed. Various approaches in improving the protein quality of traditional sorghum foods are also examined and a review of analytical methods for determining their protein and functional quality is presented.

2.1 SORGHUM

Sorghum is a tropical cereal able to survive both arid and semi-arid climates, and waterlogged soils (Doggett 1988). It belongs to the grass family Poaceae, producing dry indehiscent fruits referred to as caryopses (reviewed by Rooney and Serna-Saldivar 2000). The average dimensions of a sorghum caryopsis (grain) are length 4 mm, width 2 mm and weigh about 25 to 35 mg (reviewed by Rooney and Serna-Saldivar 2000).

2.1.1 Structure of the sorghum caryopsis

The sorghum caryopsis is composed of three distinct parts: outer layer (pericarp), storage tissue (endosperm) and embryo (germ) (Figure 1).

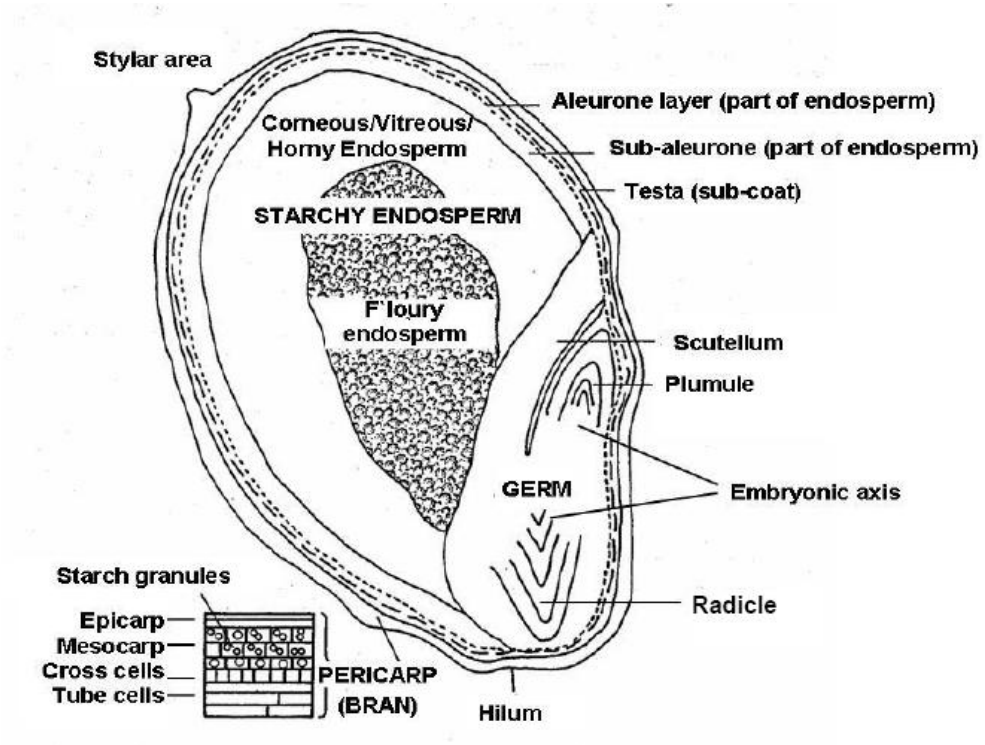

Figure 1: Longitudinal section of sorghum grain (adapted from: Taylor and Belton 2002).

2.1.1.1 Pericarp

The pericarp constitutes 4.3% to 8.7% of the sorghum caryopsis (reviewed by Waniska and Rooney 2000). It has a thickness of 8 to 160 µm (reviewed by Earp and Rooney 1982) varying within individual mature caryopses (Earp et al 2004a). It is subdivided into three tissues: epicarp, mesocarp and endocarp. The epicarp is covered with a thin layer of wax and is usually pigmented. The sorghum mesocarp contains starch granules, a characteristic unique to sorghum and pearl millet (reviewed by Serna-Saldivar and Rooney 1995). The tube cells, which are part of the pericarp, conduct water during germination while, the cross cells form a layer that impedes moisture loss (reviewed by Waniska and Rooney 2000). The pericarp contains approximately 5% to 8% of the grain protein.

Some sorghum cultivars have pigmented sub-coat (testa) (Earp et al 2004b) located between the pericarp and the endosperm (Figure 1). The pigmented testa contains tannins (proanthocyanidins) (reviewed by Waniska and Rooney 2000). Tannins protect the grain against insects, birds and fungal attack but condensed tannins are associated with nutritional disadvantages and reduced food quality (reviewed by Serna-Saldivar and Rooney 1995). The nutritional disadvantages of sorghum tannins lie primarily in their ability to form poorly digestible complexes with dietary protein (Butler et al 1984).

2.1.1.2 Endosperm

The endosperm constitutes 82% to 87% of the sorghum grain (reviewed by Waniska and Rooney 2000). It is composed of aleurone layer, peripheral, and floury and corneous (horny, vitreous, glassy) areas (Figure 1). The aleurone layer cells possess large amounts of proteins (protein bodies and enzymes), ash (phytin bodies), and oil (spherosomes). The peripheral region has several layers of dense cells containing more protein bodies and smaller starch granules than the corneous area. The peripheral and corneous areas affect processing and nutrient digestibility. In a review of the composition of the sorghum endosperm cells, Taylor et al (2006) noted that both the floury and corneous endosperm cells are composed of starch granules, protein matrix, protein bodies and the cell walls are predominated by water-insoluble glucuronoarabinoxylans (GAX). The endosperm contains approximately 81% of sorghum protein (reviewed by Waniska and Rooney 2000). In normal sorghum cultivars, most of the proteins in the endosperm are prolamins (soluble in alcohol-water mixtures) as well as some limited amounts of glutelins (soluble in dilute acid and dilute alkali) (Taylor and Schüssler 1986).

To understand the phenomenon of sorghum endosperm hardness and factors responsible for it, Shull et al (1990) observed the differences in developing sorghums of varying hardness. They found that corneous sorghum endosperm had more and evenly distributed proteins. Similarly, in a review of biochemical basis and implications of hardness and grain strength in sorghum and maize, Chandrashekar and Mazhar (1999) noted that the protein bodies in the corneous endosperm contained more γ-prolamins, which seemed to be cross-linked by disulphide bonds, than in soft grains. These authors suggested that the amounts of α- and γ-prolamins relative to the total prolamin content may be essential for corneous texture, in which these prolamins are usually higher in hard grains than in soft grains. Furthermore, Ioerger et al (2007) investigated the role of cross-linking of sorghum storage proteins (kafirins) into larger polymeric groups in influencing grain hardness. They used a number of protein analytical techniques to study the protein composition of isolated corneous and floury endosperm. These authors found that corneous endosperm had a greater level of kafirin cross-linking than did floury endosperm and that the cross-linking produced a larger molecular weight distribution than in the floury endosperm. These workers also reiterated that the γ-kafirins in the corneous endosperm may have the most obvious relationships to indicators of kafirin cross-linking in the corneous endosperm.

Reviewing the traditional food applications of sorghum, Murty and Kumar (1995) reported that sorghum endosperm texture determines the food making properties of sorghum. However, there are differing reports on the preferences for traditional sorghum foods based on the endosperm hardness. For example, Bello et al (1990) and Da et al (1982) found that tô (a West African thick porridge) prepared using corneous endosperm sorghum produced desirable firmer texture than floury endosperm sorghum. On the other hand, Fliedel (1995), working on tô and Aboubacar et al (1999) using tuwo (tô) (a sorghum porridge consumed in Niger) did not find any correlation between thick porridge firmness and endosperm texture. However, there is a consensus that corneous endosperm sorghum is not suitable for preparation of fermented (Yetneberk et al 2004) and unfermented (Rooney et al 1988) flatbreads as it produces undesirable stiffer bread.

2.1.1.3 Germ

The germ is the living part of the sorghum grain. It consists of two main parts: embryonic axis and scutellum (Figure 1). The embryonic axis contains the new plant (reviewed by Evers and Millar 2002). As explained by these authors, during germination and development the radicle forms the primary roots while the plumule forms the shoot. The scutellum is the cotyledon and has reserve nutrients: moderate quantity of oil, protein, enzymes, and minerals, doubling up as a link between endosperm and germ (reviewed by Waniska and Rooney 2000). The germ contains approximately 15% of the protein in sorghum. It is rich in albumins (water-soluble) and globulins (soluble in dilute salt solution) which are rich in lysine and other essential amino acids (Taylor and Schüssler 1986).

2.1.2 Sorghum protein composition and quality

Sorghum protein content is quite variable, ranging from approximately 7% to 15% (National Research Council 1996). In a review of the structure and chemistry of sorghum grain, Serna-Saldivar and Rooney (1995) stated that sorghum grain quality is affected by both environmental and genetic factors, which affect the chemical composition and nutrient value. Working on the protein composition of the different anatomical parts of sorghum grain, Taylor and Schüssler (1986) identified four fractions of protein in sorghum grain (Table I). These are the aqueous alcohol-soluble prolamins (deposited mainly in endosperm protein

bodies), the alkali-soluble glutelins (in endosperm protein matrix), water-soluble albumins and globulins (in the germ) (FAO 1995).

To investigate the qualitative and quantitative differences in protein compositions of floury and corneous endosperm sorghums, Watterson et al (1993) used SDS-PAGE and enzyme-linked immunosorbent assay (ELISA). These workers found that kafirins were the most abundant storage proteins in sorghum grain. Kafirins are deposited primarily in the endosperm during grain development (Shewry et al 1995) forming protein bodies that surround starch granules (Chandrashekar and Kirleis 1988).

Kafirins are of low nutritional quality, very heterogeneous (Sastry et al 1986), very deficient in lysine but rich in leucine, proline and glutamine (reviewed by Duodu et al 2003; Shewry et al 1995). Table II shows the amino acid composition of sorghum proteins. Glutelins, albumins and globulins are richer in lysine than prolamins (Taylor and Schüssler 1986). These authors also indicated that the most abundant nitrogenous fractions in the germ are the low molecular weight nitrogenous compounds, which are amino acids, peptides and nucleotides important for the physiological function of the embryo.

Table I: Protein composition of the sorghum grain and its anatomical tissues

Nutrient	**Composition**			
	Whole grain	Pericarp	Endosperm	Germ
Protein[a]	7.3-15.6	5.2-7.6	8.7-13.0	17.8-19.2
Protein fraction[b]				
Low molecular weight nitrogen	3.9-4.9	4.8-22.6	1.5-2.1	40.1-48.2
Albumins + Globulins	17.5-19.1	9.3-11.4	5.2-6.4	32.0-34.6
Prolamins	42.9-45.1	11.2-12.0	67.3-69.3	5.0-12.3
Glutelins	18.0-18.9	18.3-19.5	13.3-14.8	6.6-7.1
Residue	14.6-15.1	36.5-54.4	9.4-10.7	5.5-8.5

[a] Values expressed as % of whole grain or anatomical tissue (Waniska and Rooney 2000);

[b] Values expressed as g/100 g protein (Taylor and Schüssler 1986).

Table II: Approximate amino acid composition of sorghum protein (g/100 g protein)

Amino acid	Composition[a]	WHO standard[b]
Essential amino acids		
Histidine	2.2	1.8
Isoleucine	3.8	3.1
Leucine	13.2	6.3
Lysine	2.0	5.2
Methionine	1.5	2.6[c]
Phenylalanine	4.8	4.6[d]
Threonine	3.1	2.7
Tryptophan	1.1	0.7
Valine	5.0	4.2
Non-essential amino acids		
Alanine	9.1	
Arginine	3.1	
Aspartic acid	6.6	
Cysteine	1.1	
Glutamine	21.6	
Glycine	3.1	
Proline	7.5	
Serine	4.1	
Tyrosine	2.8	

[a] Adapted from: USDA (2008);

[b] This pattern is based on the essential amino acid requirement of a 1 to 2 year-old child, World Health Organization (WHO) (2007);

[c] Methionine + cysteine;

[d] Phenylalanine + tyrosine. Cysteine and tyrosine are not essential amino acids but they can spare the requirement for methionine and phenylalanine, respectively.

2.1.2.1 Types of kafirins

Four types of kafirins have been identified in grain sorghum. These are the α-, β-, γ- (characterized by Shull et al 1991) and δ- kafirins (reviewed by Belton et al 2006). Their classification is based on molecular weight, solubility, and structure. Watterson et al (1993) working on three sorghum varieties with varying hardness and percent vitreousness, found that the α-kafirins constituted the highest proportion of the kafirin proteins in sorghum endosperm accounting for 66% to 84% of the total kafirin fraction in the sorghum endosperm. This fraction contained 1 mol % cysteine. The β-kafirins accounted for 7% to 13% of the total kafirins in the sorghum endosperm tissue and contained about 5.8 mol % cysteine. They occurred as monomers with intra-chain disulphide bonds or as oligomers and polymers with intra- and inter-chain disulphide bonds. The γ-kafirins accounted for 9% to 21% of the total kafirin fraction of the sorghum endosperm. Shull et al (1992) working on purified kafirins, found that γ-kafirins contained about 7 mol % cysteine. The γ-kafirins were readily soluble in water as reduced subunits but were insoluble in native state due to their presence in polymers stabilized by inter-chain disulphide bonds. The δ-kafirins have not yet been identified at the protein level though it has been suggested that they have lower methionine contents than δ-zeins in maize (reviewed by Belton et al 2006).

2.2 COWPEA

Cowpea is a leguminous crop widely grown in the marginal lands of sub-Saharan Africa (AATF 2005). It is a relatively small dicotyledonous seed, which is either kidney, globular (Taiwo 1998; Henshaw et al 1996) or oval (Giami 2005) shaped. It is widely used to fortify cereal-based weaning foods in West African countries (Uwaegbute 1991). Cowpea flour and paste are utilised in preparation of a number of traditional West African dishes such as akara (a fried cowpea paste), moin moin (a steamed cowpea paste) (Phillips et al 2003) and kpejigaou (a griddled cowpea-paste) (Amonsou et al 2008).

2.2.1 Cowpea seed chemical composition related to protein quality

The chemical composition and nutritional properties of cowpeas vary considerably according to varietal differences (Longe 1980). According to data from the USDA (2008), cowpea contains an average of 24% crude protein and 7 g lysine per 100 g protein (Table III). Studies

indicate that globulins are the major cowpea seed proteins, ranging from about 48% to 90% of the total protein content. For example, Freitas et al (2004) studying the protein composition of cowpea cotyledons, found that 51% and 45% of cowpea proteins were globulins and albumins, respectively. Similarly, Chan and Phillips (1994) reported 67% globulins followed by 25% albumins. Reviewing distribution of proteins in cowpea Chavan et al (1989) reported ranges of about 48% to 90% globulins, 3% to 15% albumin, 5% to 13% prolamins and 7% to 23% glutelins. These findings, however contradict those of Ragab et al (2004) who found 71% albumin and 11% globulin proteins.

The cowpea globulin, vignin, is a 7S (Svedberg) vicillin type glycoprotein composed of three main units designated α-, β-, and γ-vignin (Freitas et al 2004). As a legume, cowpea is far richer in the essential amino acid, lysine, which is limiting in sorghum.

Table III: Approximate amino acid composition of cowpea protein (g/ 100 g protein)

Amino acid	Composition[a]	WHO standard[b]
Essential amino acids		
Histidine	3.1	1.8
Isoleucine	4.0	3.1
Leucine	7.8	6.3
Lysine	6.8	5.2
Methionine	1.4	2.6[c]
Phenylalanine	5.8	4.6[d]
Threonine	3.8	2.7
Tryptophan	1.2	0.74
Valine	4.7	4.2
Non-essential amino acids		
Alanine	4.6	
Arginine	6.9	
Aspartic acid	12.1	
Cysteine	1.1	
Glutamic acid	18.9	
Glycine	4.1	
Proline	4.5	
Serine	5.1	
Tyrosine	3.2	

[a] Adapted from: USDA (2008);

[b] This pattern is based on the essential amino acid requirement of a 1 to 2 year old child, WHO (2007);

[c] Methionine + cysteine;

[d] Phenylalanine + tyrosine.

2.2.2 Antinutrients in cowpea grain

Incorporation of cowpea seed flour, as with other grain legumes, into foods to increase their protein quality has certain limitations because of its antinutrients. Ologhobo and Fetuga (1983) studied the antinutritional factors in 10 varieties of cowpea. They found approximately 24 trypsin inhibitor units (TIU) per mg protein, 0.3 g phytic acid per 100 g db (dry basis) and 0.6 g tannic acid per 100 g db. In similar work, Giami (2005) found somewhat lower values of polyphenolic compounds (0.1 to 0.2 g per 100 g db) and phytic acid (0.1 to 0.2 g per 100 g db).

Various studies have shown that thermal treatment generally destroys most of the antinutrients in cowpea. For example, Giami (2005) found a 2% to 25% and a 31% to 51% reduction in phytic acid content through steaming and boiling, respectively. In the same research, polyphenol contents were decreased by 29% to 51% and 47% to 61% after steaming and boiling, respectively. However, Egounlety and Aworh (2003) working with dehulled-cooked cowpea, found a 35% increase in phytic acid content. These authors suggested that the increase in phytic acid may have been due to concentration. This was probably because other grain chemical components such as polyphenols located in seed coat (Phirke et al 1982) may have been removed by dehulling while retaining phytates, which are located in the cowpea cotyledons. Plahar et al (1997) reported a 3% to 8% increase in protein digestibility by roasting four West African cowpea cultivars. Akinyele (1989) working with four cowpea cultivars, found an average of 82% reduction in trypsin inhibitor activity after cooking them into boiled whole grains, fried dehulled paste, steamed dehulled paste and cowpea soup.

However, in general processing can also lead to Maillard reactions, cross-linking and the oxidative destruction of lysine, resulting in an overall decrease in reactive lysine content of food proteins as explained by Nursten (1981). Studies on various methods of processing cowpeas (section 2.3.1), such as soaking, dehulling (Phillips et al 1988), germination, cooking (Giami et al 2001) and fermentation (Zamora and Fields 1979; Kiers et al 2000) have shown improvements in nutritional value and digestibility of cowpea proteins.

2.3 TRADITIONAL SORGHUM FOODS

Sorghum is eaten in different forms in different places. Detailed descriptions of the traditional sorghum foods are found elsewhere in the literature (Murty and Kumar 1995; Rooney and Waniska 2000; Taylor and Belton 2002; Taylor and Dewar 2000; Taylor and Emmambux 2008). Traditional sorghum foods are broadly grouped into four categories (FAO and ICRISAT 1996). These are flatbreads, porridges, boiled products, and snacks and special foods. The simplest and the most common traditional foods made from sorghum are thin porridge (gruel); thick porridge (fermented and unfermented); flat, unleavened fermented bread; and unfermented bread such as chapatti. Table IV provides a summary of the different categories of traditional sorghum foods in terms of food category, examples, countries or region of origin and a brief description of the products. It is worth noting though, that the summary table is not complete because of the variations of sorghum foods dictated by preferences of the source community.

Table IV: Traditional sorghum foods

Food category	Examples	Country or Region	Description and variations
Flatbreads	injera	Ethiopia	Fermented batter baked into large thin pancakes
	kisra, dosa, dosai	Sudan, Southern India, Sri Lanka	Fermented batter baked into large thin pancakes. Dosa contains black grams or sometimes rice flour is used.
	roti, chapatti, rotte	India	Unfermented dry pancakes.
	tortilla	Mexico, Central America	Unfermented bread, lime-cooked grains ground and pressed into cakes, which are, baked dry on a flat plate.
Porridges	uji, amabali, edi, eko, kamu, nasha, obungi, bwa, kal, obushera, atole	Africa, India, Mexico, Central America	Thin porridge. Acid or alkali may be added to sorghum flour (made from sprouted grain, pearled grain or whole grain with varied particle sizes). At times part of the flour may be fermented before cooking. The variations depend on local preference.
	ogi, oko, akamu kafa, koko, akasa	Nigeria, Ghana	Thin porridge. Sorghum grains steeped in water to ferment. Fermented grain milled, the bran removed, and the sediment cooked in a pot with water to produce porridge consumed warm or cooled to form gel or pudding.
	ugali, tuwo (tô), saino, sadza, dalaki, aceda, atap, bogobe, ting, tutu, kalo, karo, nshimba, nuchu, zaafi, mato	Africa, India, Mexico, Central America.	Stiff porridge, made from either dehulled or whole grain flour, which may be course or finely ground. pH can be neutral, acidic (fermented or direct acid addition) or alkaline. The variations depend on the local preferences.

Table IV continued

Food category	Examples	Country or Region	Description and variations
Boiled or steamed products	couscous, acha, sankati, mudde, kali, piti, soru, kaoliang mi fan, lehata wagen, balila, nufro, wesla, gumba, kande	Africa, India, Haiti, China	Sorghum ground finely into flour and kneaded with water to agglomerate, forced through coarse screen and steamed (couscous) or the grains pearled to remove the pericarp and cooked like rice. Variations exist depending on origin.
Snacks and special foods		Worldwide	Sorghums are popped, puffed, and parched. They may be consumed directly or ground and mixed with other ingredients. Fried snacks are common with numerous variations in snacks.

Adapted from: Murty and Kumar (1995); Rooney and Waniska (2000); Taylor and Belton (2002); Taylor and Dewar (2000); Taylor and Emmambux (2008)

2.3.1 Traditional food preparation processes and their effects on protein quality

2.3.1.1 Soaking

Soaking is a procedure used in the preparation of some traditional sorghum foods, especially in West Africa, for example in the early stages in the production of kenkey, a traditional fermented Ghanaian cereal-based porridge (Nche et al 1994), and ogi, a thin fermented porridge which can be made from sorghum (Egounlety 2002). Akinyele and Akinlosotu (1991) working on two cowpea varieties (brown and white), reported about a 6% increase in protein content of cowpea after 4 hr soaking in distilled water. Similarly, Guerra and Bressani (2008) working on a mixture of cowpea and common beans for development of new food products, found a slight increase in protein content after soaking. The workers suggested that the slight increase on protein content might have been a result of concentration effect due to leaching of some of the soluble grain components especially polyphenols into the soaking water discarded during the research.

2.3.1.2 Decortication

Decortication is the abrasive removal of the outer layers of the grain including hull, pericarp, seed coat and germ. By removing 17.5% of original weight of raw and parboiled sorghum, Serna-Saldivar et al (1994) found about 6% and 8% reductions in protein and lysine contents, respectively. In addition, *in vivo* tests revealed a 5% less weight gain by the rats fed on the decorticated normal sorghum, which the workers suggested to be due to the losses of lysine content. Much work has been done on the effects of decortication of cowpea seeds on the protein quality. For example, Eusebio (1991), studying the effects of dehulling on the nutritive value of cowpea and rice bean as protein sources, reported a 2% increase in protein content and digestibility of cowpea after decortication. Similarly, Ghavidel and Prakash (2007), studying the impact of dehulling of cowpea on its nutritional quality, found about 11% and 21% increases in protein content and protein digestibility, respectively. Vasagam et al (2007) studied the effect of decortication on antinutrient content in cowpea. They reported 10%, 11% and 77% reductions in trypsin inhibitors, phytic acid and tannins, respectively.

2.3.1.3 Milling

This is a process aimed at reducing the size of decorticated grain or whole grain into flour. It can also involve separating grains in their anatomical parts. Sorghum can be milled using various methods such as stone milling, hand pounding, hammer milling, plate milling and roller milling (Kebakile et al 2007; Murty and Kumar 1995). Different milling processes have different effects on sorghum protein quality. Milling processes that involve the removal of germ and pericarp of sorghum grain may reduce the sorghum protein quality (reviewed by Serna-Saldivar and Rooney 1995). In addition, Kebakile et al (2007), studying the effects of sorghum type and milling process on the protein content of sorghum meal, found a 10% to 15% increase in sorghum protein content compared to the whole grain depending on the extraction rate of the milling process used, because of pericarp removal.

2.3.1.4 Wet cooking

This is a food preparation method that involves boiling of the food material in variable amounts of water. Studies show a reduction in sorghum protein digestibility after wet cooking. For example, Axtell et al (1981) working on condensed tannin-free sorghum, found 45% and 41% reductions in protein digestibility for whole and decorticated sorghum flours, respectively. Similarly, Hamaker et al (1986) working with whole condensed tannin-free sorghum, reported about a 20% decrease in protein digestibility, using in vitro pepsin, trypsin-chymotrypsin and pepsin–trypsin–chymotrypsin assays. Arbab and El Tinay (1997) also worked on wet cooked tannin and condensed tannin-free sorghum varieties. They found 50% and 48% reductions in protein digestibility for tannin and condensed tannin-free sorghum, respectively. Duodu et al (2002) working with whole condensed tannin-free sorghum varieties, found 48% and 34% reductions in protein digestibility for red and white sorghum varieties, respectively.

To explain why the kafirins are less digestible after cooking, Hamaker et al (1987) proposed two theories. The first theory was that, kafirin proteins might form polymeric units bound by intermolecular disulphide bonds. The polymers may thereby be less susceptible to digestion compared to the lower molecular weight protein units that are presumably present in the raw flour. The second theory focused on the inaccessibility of the protein bodies to attack by proteolytic enzymes. This could be due to formation of either a disulphide-bound protein coat

produced by proteins surrounding the protein body or an interior "toughening" of the periphery of the protein body because of formation of disulphide bonds. El Nour et al (1998) working with low tannin soft-endosperm sorghum, proposed that β-kafirin (which is rich in cysteine) could act as a chain extender by linking together oligomers of γ- and α-kafirin by disulphide bridges to form high molecular weight polymers. Rom et al (1992) working with whole low-tannin sorghum, used in vitro pepsin assay and scanning electron microscopy (SEM) to examine the effects of cooking and treatment with sodium bisulphite (reducing agent) on protein digestibility and protein microstructure. They found about a 26% increase in protein digestibility by treating samples with the reducing agent. SEM revealed that in all the treatments, the endosperm protein matrix was digested before the protein bodies. However, cooking changed the protein bodies so that they could not be digested. Narayan et al (2007) working on sorghum fortified with defatted soy flour, found a 10% loss in available lysine as result of extrusion cooking. Oria et al (1995) working with low tannin sorghum harvested at selected days after half-bloom (DAHB) and at maturity, found in vitro protein digestibility of the uncooked sorghum flour reduced by 19% at maturity. There was a marked decrease due to cooking, starting at 35 DAHB and continuing through maturity (13%, 25% and 18% decrease) at 35 and 40 DAHB and mature grain, respectively.

Cooking increases cowpea protein quality. This is attributed to a decrease in antinutrients in cowpea, discussed previously. For example, by boiling or steaming cowpea seeds, Giami (2005) found a reduction in polyphenols and phytates with a concomitant increase in protein digestibility of up to 39%. Similarly, Onigbinde and Akinyele (1989) reported an increase in cowpea protein digestibility in the early stages of heating. These authors noted that, prolonged heating caused a sharp decrease in protein digestibility, which depended on the water activity of the sample and heating temperature. In a review of developments in studies of the Maillard reaction, Nursten (1981) noted that food processing conditions, especially high heat treatment could lead to Maillard reactions, cross-linking and the oxidative destruction of lysine, resulting in overall decrease in lysine availability.

2.3.1.5 Alkali cooking

This involves cooking of sorghum with alkali (i.e. ashes or lime) for the production of alkaline cooked products such as tô and tortillas. In an in vivo study, Serna-Saldivar et al (1987) reported that lime treatment and cooking did not affect the total protein content or

amino acid content of sorghum but reduced the protein digestibility. Vivas et al (1987), investigating in vitro, the solubility and molecular distribution of proteins in sorghum tortilla, found a 60% reduction in protein digestibility and changes in the structure of the proteins. They also noted a reduction in the lysine-rich protein fractions. In a review of nutritional value of proteins from different food sources, Friedman (1996) discussed the theory about alkali-induced racemisation of amino acids and consequent formation of lysinoalanine in proteins. The author noted that the process may impair the nutritional quality and safety of foods by decreasing the amount of essential amino acids, decreasing digestibility and bioavailability of proteins, and possibly form toxic products.

Babiker and El Tinay (1992) working with high tannin and low tannin sorghum cultivars, found extractable tannin content substantially reduced by alkali treatment depending on time of incubation, temperature and alkali concentration. They reported about 9% to 10% increase in in vitro protein digestibility (IVPD), after 24 hr and 20 hr treatment with alkali at 30^{o}C and 100^{o}C, respectively. Similarly, when Laurena et al (1986) soaked dark red seeds of cowpea in ash or lime, they reported a 6% to 14% increase in IVPD depending on the soaking treatment. These authors attributed the apparent increases in IVPD to reduction of tannin content by the alkali. It has been suggested that the apparent reduction in assayable sorghum tannin content after alkali treatment is probably due to formation of corresponding phenates (salts of phenols) which possess properties different from polyphenols (Babiker and El Tinay 1992; Price et al 1979). Alternatively, some authors have shown that under basic conditions, epimerization of (+)-catechin and its rearrangement to catechinic acid may occur (Kiatgrajai et al 1982; Jorgensen et al 2004). As catechin residues are an important component of tannin, formation of unstable flavanoids by these competitive reactions could potentially interfere with tannin extraction.

From these reports, it seems that the overall effect of alkali treatment on the protein quality of the traditional sorghum and/or cowpea foods may not be certain and probably requires further investigation.

2.3.1.6 Fermentation

Traditional fermented sorghum foods largely rely on spontaneous fermentation (reviewed by Taylor and Dewar 2000). Additionally, some lactic acid fermented foods may also undergo unintentional alcoholic fermentation. Much work has been done to determine how

fermentation affects sorghum protein quality. For instance, Osman (2004) worked on the effects of traditional fermentation on trypsin inhibitor activity, phytic acid, tannin content and IVPD of three sorghum varieties. This author found a 31% to 58% reduction in trypsin inhibitor activity, a 42% to 58% decrease in phytic acid content, a 15% to 35% reduction in tannin content and a 5% to 7% increase in IVPD after 24 hr fermentation. Moneim et al (1995) working with low and high tannin sorghum cultivars, found a general increase in protein digestibility in the first 6 hr fermentation. Hassan and El Tinay (1995) working with both intermediate and high tannin sorghum, found a 15% and a 13% increase in protein digestibility of the former and latter, respectively. Working with five sorghum cultivars, Taylor and Taylor (2002) found, up to 85% increase in protein digestibility depending on the protein content of the raw sample. They postulated that higher total protein content would allow relatively more proteins to be exposed to pepsin resulting in a higher IVPD. There was, however, a marked reduction in soluble protein content after 8 hr fermentation, which was attributed to rapid growth of microorganisms at that stage. In addition, these authors showed that cooking after fermentation reduced gains in protein digestibility but not to the level of wet cooking alone. Working with sorghum, green gram and sorghum-green gram blends, Chavan et al (1988) found about 50%, 34% and 24% increases in soluble proteins, respectively. The free amino acids in the fermented samples were 3 to 5 times more than the raw samples. Working on fermented sorghum weaning food, Asiedu et al (1993) found a 25% increase in lysine content. However, the amount (2 g/100 g protein) in fermented sorghum food was far below the 5.2 g/100 g protein recommended standard for infants (WHO 2007).

It is apparent from these reviews that fermentation generally improves the protein quality of sorghum foods and two theories have been suggested to explain this phenomenon. First, lactic acid fermentations may alter the sorghum protein structure rendering them more accessible for digestion as was proposed by Taylor and Taylor (2002) from their pepsin digestion experiments. Elkhalifa et al (2006) also discussed this hypothesis in their study on the molecular and structural modifications occurring in protein and starch components of sorghum flour when used to prepare kisra (a Sudanese fermented flatbread). From this study, SEM images revealed a release of individual starch granules from the kafirin-rich protein matrix after fermentation. A second theory may be that the resultant low pH from lactic acid fermentation could inhibit a possible complex formation (“protective effect”) between sorghum proteins and other substances such as polyphenols, which is optimal at the

isoelectric point of the protein. This hypothesis was proposed by Eggum et al (1983), in the context of their nutritional studies with rats, in which the pH of one ugali (an East African unfermented stiff porridge) was adjusted to 3.9 before cooking. They found that cooking had no substantial effects on protein digestibility, biological value and amino acid composition of the ugali proteins at such acidic pH. However, the authors did not find evidence of this "protective effect" of acidification on protein digestibility in a similar experiment using aceda (a Sudanese unfermented stiff porridge). Therefore, this theory may require further investigation.

2.3.1.7 Malting

The process of malting comprises three unit operations, namely: steeping, germination and drying (Taylor and Dewar 2000). A major objective of malting is to promote the development of hydrolytic enzymes, which are not present in the non-germinated grain. Studies have shown that malting improves the quality of sorghum proteins attributed to metabolism of lysine deficient kafirins and increase in lysine rich proteins in the shoots and roots during germination (reviewed by Taylor and Belton 2002). For example, Taylor (1983) reported a reduction in the levels of kafirin protein fractions, with concomitant increases in albumins and globulins after malting sorghum. He also reported seven-fold increase in all the nine essential amino acids. In addition, Taylor et al (1985), using transmission electron microscopy (TEM) to examine the endosperm of germinating sorghum, found that the protein bodies were degraded predominantly by progressive hydrolysis of the prolamins from their surface accompanied by internal hydrolysis. Chemical analyses of protein bodies isolated at different stages during germination showed that their amino acid composition and electrophoretic pattern remained relatively unchanged during hydrolysis. These findings seem to support the suggestion that the simple technology of malting could offer a means by which to improve the quality and digestibility of sorghum protein. A study by Asiedu et al (1993) on germinated weaning foods, however, indicated that germination did not significantly improve the protein quality of sorghum as the lysine content was slightly increased to a level that could not meet the nutritional needs of infants.

Rivas-Vega et al (2006), studying the nutritional value of cowpea meals prepared using different processing methods, reported a 13% and 16% increase in protein content and IVPD, respectively, after germination. Similarly, Vasagam et al (2007) working with cowpea and

mung bean, found that germination improved the protein content by 18% and 16% in cowpea and mung bean, respectively. These workers also noted reductions of tannin and phytic acid contents ranging between 55% to 60% and 70% to 81%, respectively. However, the trypsin inhibitor activity increased substantially on germination. Some studies have shown that germination does not have a significant effect on the IVPD of cowpea. For example, Herken et al (2006) working on germinated, fermented, cooked, ground cowpea flour fortified with standard durum wheat semolina at 20% level to prepare macaroni, found no significant effect on protein digestibility. Nnanna and Phillips (1989) working on cowpea seeds germinated at 25°C or 30°C for 24 hr, reported similar results.

2.3.2 Lysine availability

As defined by Bender (1998), lysine availability refers to protein-bound lysine in which the ε-amino group is free, so that after digestion, the lysine is available for absorption. Through balance studies in 13 children 6 to 30 months of age, Maclean et al (1981) assessed the protein quality of two high lysine (2.9 to 3.0 g/100 g protein) and two conventional (lysine content 2.1 to 2.2 g/100 g protein) sorghum varieties. The molar ratio of lysine/total concentration of essential amino acids (Lys/TEAA) was lowest in all the varieties indicating that lysine was the first-limiting amino acid in sorghum. Similarly, Tuan et al (1999) working on extruded sorghum, cowpea and sorghum-cowpea blend studied the relationship between in vivo availability of amino acids and overall sorghum protein quality as reflected by nitrogen balance. They reported that Protein Digestibility Corrected Amino Acid Score (PDCAAS) (discussed in section 2.5) for lysine was the lowest. Consequently, information on the amount of available lysine in traditional sorghum foods is required for appropriate dietary formulation.

As heat is one of the most common and effective methods of food processing that may be used alone or in combination with other techniques, this literature review will focus briefly on the general principles of heat damage to lysine. In a review of the consequences of a thermal treatment on the nutritive value of proteins, Papadopoulos (1989) discussed the changes that occur in proteins involving lysine during processing. According to this author, different types of damage can occur on lysine during processing depending on the prevailing conditions. As explained in a review by Moughan and Rutherfurd (2008), the reducing sugar/lysine Maillard reaction (Figure 2) initially involves a reversible condensation reaction

that results in formation of a Schiff's base. This undergoes an irreversible rearrangement to produce ε-N-deoxyketosyllysine (the Amadori compound), also known as the early Maillard product. With more severe heat, the Amadori product can, in less well defined reactions, further produce brown pigments or melanoidins (late Maillard products) leading to an additional reduction in available lysine. Nutritionally, mammals can utilize the Schiff's base (at least of the aliphatic aldehydes and reducing sugars) almost entirely. However, the metabolic utilization of the Amadori compound seems negligible.

To model storage, autoclaving, baking/broiling and charring, Smith and Friedman (1984) heated casein and mixtures of casein with starch, sucrose, and glucose at temperatures between 37^{o}C and 300°C. The lysine contents decreased by up to 99%. The effects of the three carbohydrates in affecting these changes were different at the four temperatures studied. Similarly, Horvatić and Ereš (2002) did a comparative investigation of the changes in available lysine content during industrial production of two dietetic hard biscuits based on whole grain wheat flour/grits and a standard hard biscuit based on white wheat flour. They found that dough preparation (at 19^{o}C to 39^{o}C, for 20 to 60 min) did not significantly affect the available lysine content. However, after baking (at 245^{o}C to 285^{o}C, for 7 to 10 min), a loss of between 27% and 47% of available lysine was found in all three types of biscuit studied. In the absence of reducing sugars, much higher temperatures, above 100°C for several hours, are required to bring about loss of available lysine (Carpenter and Booth 1973). Under these conditions cross-links form between the ε-amino group of lysine and of the carboxyl group of aspartic acid and glutamic acid (or their amides) to form new peptide-like cross-links (Hurrell et al 1976). In addition, cystine may lose hydrogen sulphide to form a dehydroalanine residue and a cysteine residue. The dehydroalanine and cysteine then recombine to form lanthionine creating a new C-S-C cross-link between peptide chains.

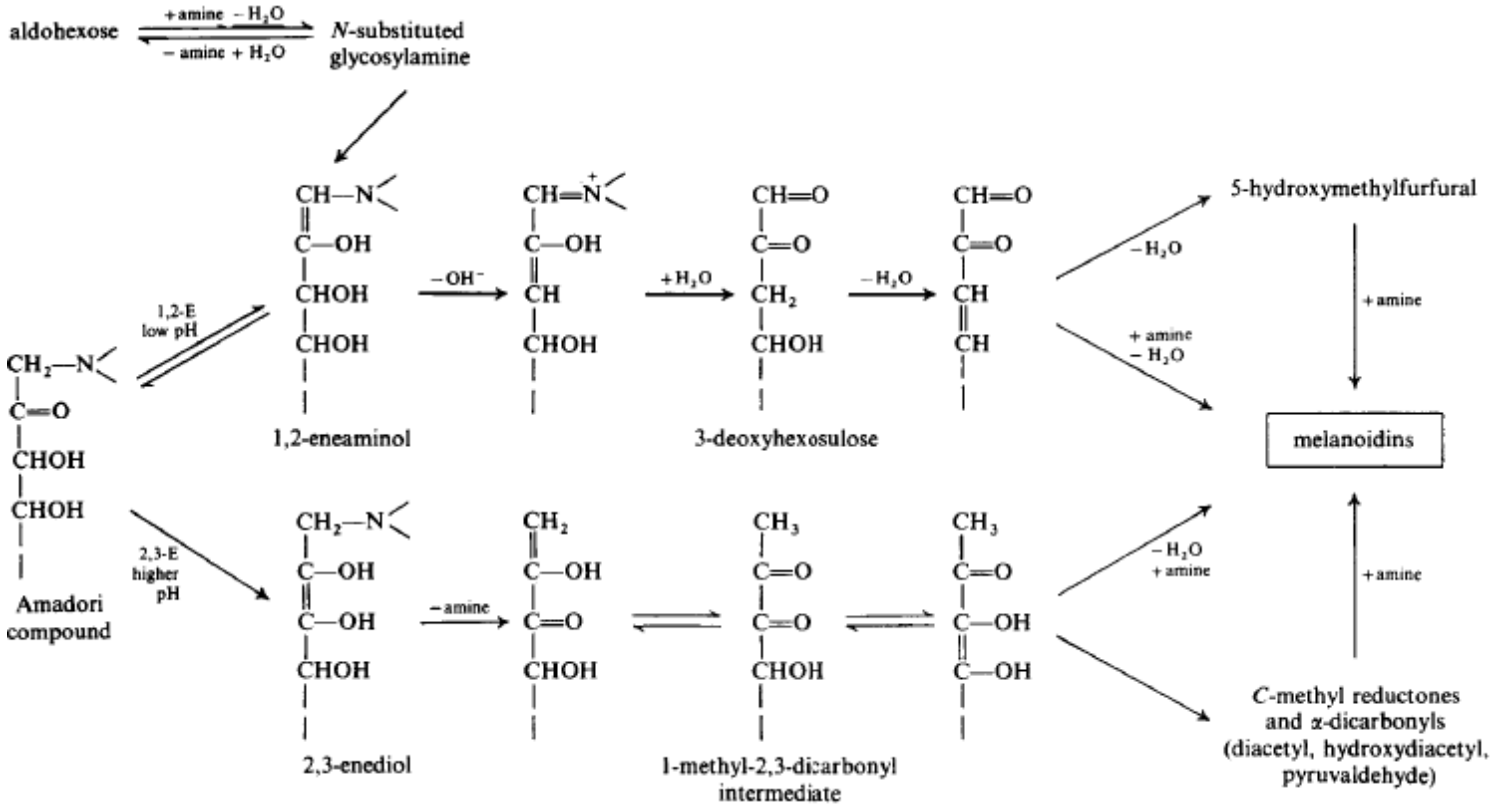

Figure 2: The Maillard reaction: two major pathways from Amadori compounds to melanoidins (source: Nursten 1981)

Under certain conditions, especially in alkaline pH, the ε-amino group of lysine reacts with dehydroalanine to form a lysinoalanine cross-link (Friedman and Pearce 1989). These new cross-links reduce the digestibility of the protein and hence the availability of all amino acids not just those directly involved. However, these are extreme conditions. Hence, it is unlikely that such reactions occur in a sorghum food when cooked normally.

Heating protein in the absence of reducing sugars under much milder conditions (70^{o}C to 120°C for 20 min) brings about a loss of sulphydryl groups (cysteine residues) and an increase in disulphide bonds (cystine residues) with little loss of the total cysteine plus cystine (Opstvedt et al 1984). These authors postulated that heating causes the formation of new S-S cross-links from -SH oxidation and the rearrangement of existing disulphide bonds during denaturation of the protein. These changes are associated with reduction in protein digestibility.

2.3.3 Protein digestibility

For a protein to serve as nutritional source of amino acids, it has to be digested. Protein digestibility is a measure of the proportion of food nitrogen that would be absorbed after ingestion (Damodaran 1996a). Protein digestibility studies indicate that sorghum proteins are generally less digestible than proteins of other cereals (reviewed by Klopfenstein and Hoseney 1995). For example, when Ejeta et al (1987) compared the IVPD of sorghum, pearl millet and maize, they found sorghum least digestible of the three cereals after wet cooking. Axtell et al (1981) working on sorghum, found decline in protein digestibility from highs of 78% to 100% (in uncooked sorghum proteins) to lows of 45% to 55% (in wet cooked sorghum proteins). Similarly, Duodu et al (2002) working with a white and a red condensed-tannin-free sorghum varieties, found reductions in IVPD of 34% and 48%, respectively after wet cooking.

2.3.3.1 Factors that influence protein digestibility of sorghum-based foods

In a review of factors that affect sorghum protein digestibility Duodu et al (2003) concluded that depending on the nature of the sorghum grain used, different factors may contribute with some being more important than others. In this review, the factors will be addressed briefly under four categories.

Protein conformation

Conformational properties of proteins are involved in functional characteristics such as hydrophilicity and hydrophobicity (Pour-El 1981), which are known to influence the hydrolysis by proteases (Damodaran 1996a). It has been reported that sorghum proteins are more hydrophobic than other cereal proteins, such as the maize storage proteins, zeins (reviewed by Duodu et al 2003; Wall and Paulis 1978). This could be a reason for the poor digestibility of the sorghum foods. To understand the effect of structure on the digestibility of sorghum proteins, Duodu et al (2001) studied wet cooked and popped sorghum proteins (known to be relatively more digestible) using Fourier transform infrared (FTIR) and solid state ^{13}C nuclear magnetic resonance (NMR) spectroscopic methods. The workers reported more extensive changes in the protein secondary structure, from α-helical to antiparallel, intermolecular β-sheet conformation after wet cooking than on popping probably due to the heat energy applied during the wet cooking process breaking hydrogen bonds which stabilize the α-helical conformation. The polypeptides would then become unravelled and aligned next to each other in a β-sheet conformation stabilised by disulphide and possibly non-disulphide cross-links between polypeptides causing reduction in kafirin protein digestibility.

Grain antinutritional factors

As stated, sorghum, like other grains, has some protein nutritional limitations, due to the presence of antinutritional factors, such as tannins (in some varieties), phytic acid and trypsin inhibitors (Osman 2004). These compounds are known to affect protein digestion. Many workers have reported effects of tannins on protein digestibility. For example, Baxter et al (1997) demonstrated that tannins bind and form complexes with a mouse salivary proline-rich peptide. These workers showed that proline residues may act as binding sites and help to keep the peptide extended and thus maximizing the available binding surface area. Similarly, reviewing the interactions of sorghum tannins with proteins, Butler et al (1984) explained that proline residues disrupt the α-helix, forming open structures with carbonyl and amide groups extending into the solvent. These authors also stated that protein-tannin interactions are hydrophobic. In addition, when Emmambux and Taylor (2003), compared ferulic acid, catechin, tannic acid and condensed tannins, they noted that only the condensed tannins exerted significant negative effects on the digestibility of sorghum proteins. Condensed tannins (Figure 3), also known as proanthocyanidins or procyanidins, are high-molecular

weight polyphenols that consist of polymerized flavan-3-ol and/or flavan-3,4-diol units linked mainly by C4→C8 interflavan bonds (Dykes and Rooney 2006).

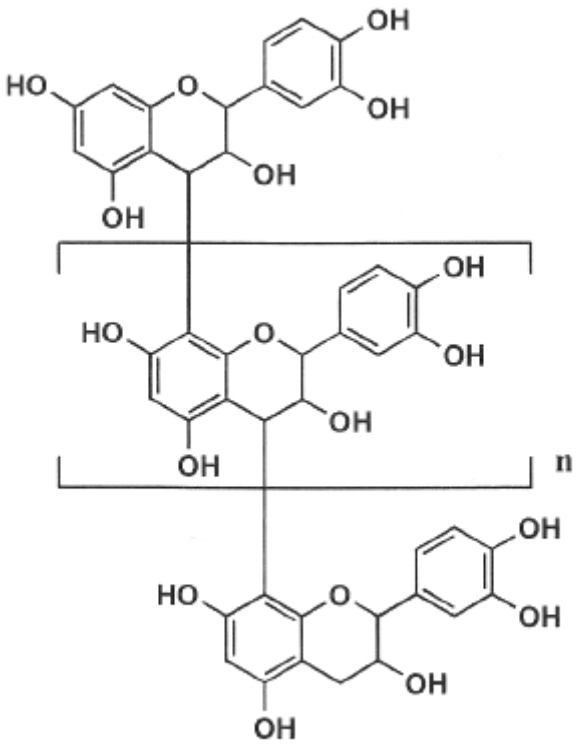

Figure 3: Structure of condensed tannin (source: Shahidi and Naczk 2005)

It appears that no research has established a direct effect of sorghum phytates on the digestibility of sorghum proteins. However, a study by Elkhalil et al (2001) on malt-pre-treated sorghum flour showed an inverse relationship between phytate content and IVPD. Likewise, Knuckles et al (1985) working on casein and bovine serum albumin, found a reduction in IVPD after treatment with phytates. These, negative effects of phytate on protein digestibility are thought to be due to formation of phytate-protein complexes, which are less susceptible to enzymatic attack (reviewed by Duodu et al 2003). To understand the effects of traditional fermentation of sorghum on trypsin inhibitors, Osman (2004) related trypsin inhibitor activity to IVPD. Results suggested an inverse relationship between trypsin inhibitor activity and IVPD.

Associations between cell wall components and proteins

A number of cell wall components that may influence sorghum protein digestibility have been identified. For example, Glennie (1984) studying the changes on the sorghum

endosperm cell wall during germination, isolated deferulic and ferulic acids which were associated with alkali-soluble protein fraction of the endosperm cell walls. Other cell wall components associated with sorghum proteins are dietary fibre, mainly composed of water-insoluble glucuronoarabinoxylans (GAX), which were isolated by Verbruggen et al (1993). Duodu et al (2003) suggested that associations between sorghum proteins and cell wall components may lower protein digestibility by either reducing accessibility to enzymes or the formation of indigestible complexes. These authors proposed two mechanisms of the associations. Firstly, there can be a direct attachment of the proteins to the carbohydrate moieties forming a glycoprotein such as the hydroxyproline-rich glycoproteins (HRGPs) or extensins reported in sorghum by Raz et al (1991). As explained by Duodu et al (2003), these structural glycoproteins are highly resistant to most proteases, especially when the oligoarabinose side chains are still attached probably because GAX have been shown to hinder enzyme activity (Verbruggen et al 1998). Secondly, the association of proteins to cell walls may be by ferulic acid-mediated cross-linking. Ferulic acid has the ability to couple oxidatively to another ferulic acid-bearing arabinoxylan to form a diferulate cross-link (reviewed by Fry 1988). As explained by this author, the formation of such phenolic cross-links is dependent on the synthesis of the phenol-bearing arabinoxylan, the presence of peroxidase enzyme and a supply of hydrogen peroxide or an equivalent oxidising agent. Therefore, as suggested by Duodu et al (2003), during cooking the oxidizing conditions could promote the formation of phenolic or specifically, ferulic cross-links.

Food processing methods

The effects of food processing methods on sorghum protein digestibility are discussed in section 2.3.1. As suggested by Duodu et al (2003), it appears that food processing methods that enhance cross-linking of sorghum proteins may be the greatest factor that influences sorghum protein digestibility.

2.4 APPROACHES TO IMPROVING THE PROTEIN QUALITY OF SORGHUM FOODS

2.4.1 Food compositing

Sorghum can be combined with suitable protein sources such as the lysine-rich legumes to provide the level of protein required in formulation for optimum nutrition (reviewed by Klopfenstein and Hoseney 1995). This is referred to as food compositing. One such legume is

cowpea, which is an important local legume in sub-Saharan Africa and is a relatively inexpensive source of legume protein (Nnanna and Phillips 1988). When Pelembe et al (2002) composited sorghum with cowpea, they produced a protein-rich extruded instant porridge. Composite food products for children containing sorghum and quality protein sources like soya meal served as thin porridge or gruel are common as formulations of high protein complete foods. For example, Akinyele and Fasaye (1988) demonstrated that mixing sorghum with cowpea at ratio of 70:30 (w/w) improved nutritional quality of fermented gruel (ogi) substantially.

2.4.2 Biofortification

Biofortification is the process used to increase the amounts of nutrients in food crops through conventional breeding (White and Broadley 2005) or targeted genetic engineering (use of recombinant DNA technology) (Zimmermann and Hurrell 2002). Polleti et al (2004) reviewed the progress made in the nutritional fortification of cereals. According to these authors, effective biofortification of a cereal, such as sorghum, can reach the poor in rural areas, has low recurrent costs, is sustainable in the long term, and in the case of genetic improvement, it only requires an up-front investment. Similarly, O'Kennedy et al (2006), reviewing the use of sorghum and millet biotechnology for food and health, indicated that the ability to improve the nutritional quality of sorghum grain protein by classical plant breeding is limited to level of variation in the gene pool available for crossing. They identified genetic engineering as a potential means to overcome this limitation by introducing wild type or mutant genes from other organisms.

Early attempts to improve sorghum protein quality focused on the identification of high-lysine mutants. For example, two mutants were identified in sorghum, *hl* gene in an Ethiopian line (Singh and Axtell 1973). In another work, *P721 opaque* gene was induced by chemical mutagen diethyl sulphate (Axtell et al 1979), following identification of similar lines in maize (Mertz et al 1964). Both sorghum lines are low in prolamins. The proportion of kafirin is reduced by about 50% with compensatory increases in other more lysine-rich proteins and free amino acids, thus increasing lysine by 40% to 60% (reviewed by O'Kennedy et al 2006). However, as noted by these authors, such lines are associated with deleterious effects on seed weight and yield. Sorghums with easier-to-digest proteins have been identified (Weaver et al 1998). It is believed that the improved protein digestibility is caused by more invaginations in

the protein bodies (Oria et al 2000). As explained by these authors, it appears that the change in structure of protein bodies locates the highly cross-linked γ-kafirins to the base of the folds, improves accessibility to the major storage protein α-kafirin by proteolytic enzymes, and increases surface area for enzymic activity. All these modifications probably increase their protein digestibility.

Another approach in biofortification of sorghum may be to genetically engineer sorghum to express additional nutritionally enhanced proteins following a model for increasing lysine content in maize using genetic engineering proposed by (Rao et al 1994). As mentioned by O'Kennedy et al (2006) little work of this kind has been done to produce lysine-rich proteins, possibly because lysine is positively charged at cellular pHs, and high proportions are less readily accommodated in proteins. According to these authors, current genetic modification of sorghum involves the use of DNA particle gun technology and *Agrobacterium*-mediated transformation protocols for selected lines to provide the technological basis for improvement of the nutritional quality and tolerance to, biotic and abiotic stress of grain sorghum.

2.4.3 Supplementation

Supplementation involves the addition of synthetic essential amino acids to improve the protein quality of sorghum diets (Daniel et al 1966; Polleti et al 2004). The practice is however, considered inefficient and impractical among populations where sorghum is the staple food because economically disadvantaged households that cannot afford to buy fortified food products (Allen 2003).

2.4.4 Processing

Indigenous sorghum consumers of Africa and Asia have developed traditional processes that make the sorghum palatable, digestible, and a good source of ingredients for food (Rooney and Waniska 2000). The traditional sorghum processing methods that improve protein quality were discussed earlier in section 2.3.1. They include fermentation (Obizoba 1988; Taylor and Taylor 2002), malting of sorghum (Palmer 1989; Taylor 1983; Taylor and Dewar 2000), and alkali (lime) cooking (Babiker and El Tinay 1992; reviewed by Taylor and Belton 2002).

2.5 ANALYTICAL METHODS FOR DETERMINING SORGHUM PROTEIN QUALITY

As has been discussed earlier in this review, the protein quality of traditional sorghum food depends on the quantity and the availability of the essential amino acids, particularly lysine and the digestibility of the proteins. Various ways may be used to estimate of the protein quality of traditional sorghum foods. The more important of these are briefly discussed next.

2.5.1 Analytical methods for determining lysine content

A common method of determining lysine content is by the use of High-Performance Liquid Chromatography (HPLC) (reviewed by Aristoy and Toldrá 2004). The method initially involves hydrolysis of the protein and peptides into their constituent amino acids usually by acid digestion. This is typically achieved by constant boiling in a 6 M HCl at around 110^{o}C for 20 to 96 hr. Then the amino acids are derivatized to improve separation and/or to enhance their detection. The derivatized amino acids are then introduced into a mobile phase and separated based on their differences in affinity for the stationary phase in the HPLC column. Two basic separation principles are discussed.

2.5.1.1 Ion Exchange-High Performance Liquid Chromatography (IE-HPLC)

IE-HPLC separation is based on the ionic interactions between charged molecules on the stationary phase and in the mobile phase, and the ionic sample species. The quantity of each amino acid is determined by spiking the sample with a known quantity of internal standard (reviewed by Aristoy and Toldrá 2004). Its major drawbacks are the laborious nature of its optimization, the corrosive effects of salts formed, the interference by hydrophobic interactions with column packing and the longer time it takes compared to Reverse Phase (RP)-HPLC (reviewed by Van Camp and Dierckx 2004) discussed next.

2.5.1.2 Reverse Phase-High Performance Liquid Chromatography (RP-HPLC)

RP-HPLC separation is based on differences in surface hydrophobicity among the amino acid molecules (reviewed by Van Camp and Dierckx 2004). As explained by these authors, this is achieved on an inert column packing, usually covalently bonded with a high density of hydrophobic functional groups such as linear hydrocarbons typically C4, C8 or C18 or the relatively more polar phenyl group. A differential desorption is achieved by applying a gradient of increasing concentration of an organic solvent. The main advantages of RP-HPLC

are its high resolution, suitability for analysis at low ionic strength and quicker elution (less than 30 min) compared to IE-HPLC (Bidlingmeyer et al 1984). Its main drawbacks are the interference by hydrophobic contaminants and the use of toxic solvents (reviewed by Van Camp and Dierckx 2004).

2.5.2 Analytical methods for determining lysine availability

The most accurate measure of how suitable a food is as a source of nutritionally available lysine is the response of the human or animal in a situation where lysine is limited (Hurrell and Carpenter 1981). However, reviewing the history of the development of science addressing lysine availability in foods, Moughan and Rutherfurd (2008) considered an in vivo method such as the Slope-Ratio Assay (Batterham et al 1984) to be technically an estimate of utilized rather than available lysine. In addition, Baker (1986) reiterated that in vivo techniques are labour-intensive and costly, have a high degree of imprecision and are prone to inaccuracy. In respect of these considerations and the ethical concerns about use of animals (European Commission (EC) 1986), in vitro assays offer an alternative approach and have the advantage of being relatively rapid and less expensive.

As stated by Moughan and Rutherfurd (2008), structural changes to lysine residues are not detected by conventional amino acid analysis. Furthermore, total lysine values appear to overestimate the amount of nutritionally available lysine (Kivi 2000). Procedures have been developed for determining “reactive lysine,” which describes the lysine molecules that have not undergone any form of structural change and thus have a chemically reactive (unblocked) ε-amino group considered potentially available nutritionally (Bender 1998). Among the chemical methods used to measure reactive lysine content of food proteins, the fluorodinitrobenzene (FDNB) (Carpenter 1960) and the dye-binding lysine (DBL) methods (Hurrell et al 1979) are commonly used. The FDNB method is considered to detect accurately, the amount of lysine in food, which has not undergone any structural alterations, which may occur during storage or processing (Hurrell and Carpenter 1981; Moughan 1991). The two methods are discussed next.

2.5.2.1 Fluorodinitrobenzene (FDNB) method

This is a method for a direct determination of available lysine, which depends on the reaction of the ε-amine with a chromophore reagent and then spectrophotometric measurement (Kivi

2000). The method using 1- fluoro-2, 4-dinitrobenzene (FDNB) chromophore was established by Carpenter (1960). Its current usage is detailed in the Association of Official Analytical Chemists (AOAC) International (1990) Method 975.44 and can be used to detect whether food preparations have reduced the availability of lysine in the food proteins. This method may suffer from the problem that the reaction product of the FDNB-derivatized lysine (dinitrophenyllysine) is not completely stable during acid hydrolysis especially in the presence of carbohydrates (Hurrell and Carpenter 1981) therefore, correction factors are recommended. In addition, Hendriks et al (1994) noted that FDNB-reactive lysine values may underestimate the extent of availability because it does not detect structurally unaltered lysine units, which may be resistant to digestion and absorption. Other disadvantages of the FDNB method are the long period required for assay, the small number of samples dealt with simultaneously and the considerable technical skill required. The DBL method offers advantages in respect of these latter considerations.

2.5.2.2 Rapid dye-binding lysine (DBL) method

The DBL method using dyes such as Acid Orange 12 (Crocein Orange G) for determining reactive lysine involves blocking the ε-amino group by propionylation (Hurrell et al 1979). The nutritional damage of food proteins due to heat treatments can be estimated since the dye-binding capacity of the protein with Acid Orange 12 is reduced by the loss of reactive ε-amino group of lysine. The rapidity of this method contrasts with other chemical methods for determining reactive lysine in foodstuffs, which are laborious (Carpenter and Booth 1973). A major drawback of the DBL is that it may over estimate the reactive lysine of samples (Hendriks et al 1994; Molnár-Perl et al 1986). According to Hurrell et al (1979), the ratio of added dye to the basic amino acids influences the amount of dye bound. Therefore, the method is unsuitable for determination of reactive lysine content when the amount of dye added to achieve a binding ratio of dye and basic amino acids of 1:1, is unknown (Hendriks et al 1994). However, Hurrell et al (1979) concluded that for purposes of assessment of the first 15% of nutritional damage to foods, considered the region of practical importance, the DBL method may still be suitable.

2.5.3 Analytical methods for determining protein digestibility

2.5.3.1 Biological methods

Damodaran (1996a) reviewed the procedures for evaluating protein nutritive value. The author explained that biological methods are based on gain in weight or nitrogen retention in test animals when fed on test protein. A protein free diet is used as control. Data obtained are then used to evaluate protein digestibility as apparent protein digestibility or coefficient of protein digestibility. A correction can then be made for the true digestibility.

A basic protein quality criterion that also utilizes animal assays is protein efficiency ratio (PER). As defined in the AOAC International (1990) Method 960.48, PER estimates the protein nutritional quality in an in vivo assay by measuring rat growth as weight gain per gram of protein fed and is calculated using the equation below.

$$\text{PER} = \frac{\text{Total weight gain of test group (g)}}{\text{Total protein consumed (g)}}$$

According to Smith (2003), use of PER method is time consuming and does not provide an allowance for the protein requirement for maintenance the body. This implies that, a protein that produces no weight gain in this assay has PER of zero. In addition, as stated previously, there are ethical concerns about the use of animals assays (EC 1986). Furthermore, there are high costs involved in the biological assays. For example the number of test animals must be enough to ensure results that are statistically reliable with test period of 9 days commonly used (reviewed by Damodaran 1996a).

2.5.3.2 Enzymatic methods

Pepsin digestion

Based on the method of Hamaker et al (1986), this involves first determining the protein content of the food and digesting the protein in the food by pepsin under a specified set of conditions. This is then followed by determination of the residual protein content of the digested food sample and expressing that as a percentage of the original protein content.

Studies have shown that pepsin digestion values parallel those found in humans and may be a better sorghum protein digestibility indicator than some animal digestibility tests. For example, Axtell et al (1981) working on sorghum protein digestibility found that rat digestibility test values were higher and poorly correlated with the values reported in literature for children. However, these authors noted that pepsin digestion values paralleled those reported for children. They suggested that the differences may be because a young rat might be more efficient than a child in digesting sorghum proteins. Mertz et al (1984) did similar work with a modified version of pepsin digestion used by Axtell et al (1981) on sorghum and other cereals. They reported protein digestibility values that were similar to those found in children for sorghum and the other three cereals analysed.

Three enzyme protein digestibility assay

In this method, which simulates human digestion, the protein is digested with three enzymes: pancreatic trypsin, chymotrypsin, and porcine intestinal peptidases (reviewed by Damodaran 1996a). This method is usually a pH-shift method and so it has the disadvantage that it may not offer the optimum pH level for the digestive enzymes in the assay (reviewed by Smith 2003).

2.5.4 Protein quality assessment method for specialized applications

In a review of protein quality assessment methods, Smith (2003) noted concerns about protein nutritional quality including meeting the requirements for nutritional labelling, formulating products of high protein quality and testing the effects of food processing on protein digestibility. This section covers the calculation required for nutritional labelling and mentions briefly, the more important assessment method for specialized applications.

2.5.4.1 Protein Digestibility-Corrected Amino Acid Score (PDCAAS)

PDCAAS combines the amount of first limiting essential amino acid in a protein to the amount of that amino acid in a reference pattern for the target consumer group, and the digestibility of the protein (FAO/WHO 1991; WHO 2007). The following equation is used for estimating PDCAAS:

$$\text{PDCAAS (\%)} = \frac{\text{mg of limiting amino acid in 1 g of test protein}}{\text{mg of same amino acid in 1 g of reference protein X protein digestibility (\%)}} \text{ X } 100$$

PDCAAS was adopted by FAO/WHO as the preferred method for the measurement of the protein value in human nutrition since it takes into account the protein digestibility (FAO/WHO 1991).

2.6 TEXTURAL PROPERTIES OF TRADITIONAL SORGHUM FOODS

Rosenthal (1999) defined food texture as the mouth feel of a food, which relates to the sensory assessment of its physical properties. Textural properties have been identified as a very important determinant of food acceptability. For example, Aboubacar et al (1999) when evaluating the sorghum porridge quality parameters that affect consumer acceptance found that textural characteristics were the most important sensory attributes. Food texture also influences palatability of the food. This is important particularly in sorghum foods meant for children such as gruel (Lorri and Svanberg 1993a).

2.6.1 Factors that influence the textural properties of sorghum foods

Sorghum food texture appears to be influenced by an interplay of various factors that may be grouped as follows.

2.6.1.1 Sorghum grain physico-chemical properties

Cagampang and Kirleis (1984) studied the relationship between physico-chemical properties and textural quality of sorghum rice using 15 sorghum cultivars. They found sorghum rice prepared from sorghum with high grain hardness (measured as percent vitreousness) had high cooked sorghum grain texture (determined by the amount of energy required to back extrude the cooked sorghum rice). Kobue-Lekalake et al (2007) also worked on the textural properties of sorghum rice made from six sorghum cultivars with various grain textures and total phenol contents. They reported that sorghum rice that had the softest texture was from sorghum with relatively corneous endosperm texture and lowest total phenol content. They suggested that

this soft texture was probably due to splitting of kernels. These authors also found that the hardest sorghum rice was from sorghum with relatively soft endosperm texture and highest total phenol content. It seemed therefore, that the perceived hardness of the (cooked) sorghum rice was inversely related to grain endosperm hardness. These observations by Kobue-Lekalake et al (2007) appear to contradict the findings of Cagampang and Kirleis (1984) on the relationship between grain endosperm hardness and the texture of the sorghum product. This suggests that grain hardness may not be the only factor that influences the texture of sorghum foods.

2.6.1.2 Food preparation techniques

Food preparation methods have been found to influence sorghum food texture and may be manipulated to produce foods with preferred texture. One such food preparation technique is fermentation. Lorri and Svanberg (1993a) evaluated the dietary bulk properties, i.e. viscosity in relation to flour concentration, of lactic acid-fermented cereal (including sorghum, maize and millet) gruels used as weaning foods in Tanzania. They found that lactic acid fermentation had a substantial viscosity-lowering effect attributed to the hydrolysis of starch by bacterial enzymes. These authors also noted that presence of relatively higher tannin content in some sorghum varieties affected the reduction in viscosity of the gruel. This was attributed to tannins, which are known to inhibit bacterial growth (reviewed by Chung et al 1998; Scalbert 1991).

Another traditional food processing technique, identified as a means of reducing volume/viscosity ratio (dietary bulk) of traditional sorghum weaning foods, is malting (germination). As observed by Mosha and Svanberg (1983), germination activates amylolytic enzymes that degrade the starch components in the gruels. This reduces the water-holding capacity of the gruels and the inherent problem of dietary bulk associated with the traditional starchy weaning foods (reviewed by Nnam 2001).

In addition, alkali cooking has been shown to improve functional quality of some sorghum products such as tortilla, an important flatbread in Latin American countries (reviewed by Taylor and Belton 2002). As explained by these authors, lime may modify the cellular structure of the grain forming a cohesive, pliable, semi-plastic food material similar to a gluten-containing product after baking.

2.6.1.3 Compositing

Compositing with other cereals and/or legumes has been shown to influence the texture of sorghum foods. For example, to evaluate the effect of compositing on the rheological properties of traditional complementary foods, Nnam (2001) examined the viscosities of thin porridges made from blends of sprouted sorghum, bambara groundnuts and fermented sweet potatoes. Composited porridges were up to 11 times less viscous than pure sorghum porridge, depending on the level of compositing applied. However, in a different study Kulamarva (2005) determined the effects of compositing with wheat, soya and black gram flours, on the rheological properties of sorghum dough, used for preparation of sorghum roti (an Indian unfermented dry pancake). This author noted that compositing substantially increased the apparent biaxial extensional viscosity of the doughs as determined using an Instron-Universal Testing Machine, particularly when boiling water was used in dough preparation. Dynamic rheological measurements of the doughs revealed that the complex modulus (G*) values were higher for dough samples made with composite flours, meaning that composite doughs were more viscous but less elastic. As explained by the author, the rheological properties of doughs are largely governed by the contribution of starch, protein and water. The viscoelastic behaviour of doughs is influenced by the capability of starch and proteins to form a continuous network. Addition of wheat or soya alters the composition of the starches and proteins in the dough samples as compared to sorghum flour doughs. Supplementation with wheat or soya flours lead to a higher protein content and lesser carbohydrate content than pure sorghum flour dough. The higher protein content in case of composite flours may have increased starch-protein interactions.

These observations by Nnam (2001) and Kulamarwa (2005) show the complexities of interactions that determine the texture of sorghum products. These interactions appear to be as diverse as the food compositions.

2.6.2 Measurement of sorghum food texture

Both sensory (utilizing human sense receptors as analytical tools) and instrumental textural evaluations (mechanical tests) are important in the development of alternative food ingredients or processing methods. For instance, in order to find an alternative grain to millet for fura (a West African semi-solid dumpling cereal meal) production, Jideani and Danladi

(2005) used both instrumental and sensory textural assessments of the different cereal grains studied. The same techniques were used to determine the influence of sorghum cultivar on injera making and keeping quality (Yetneberk et al 2004), and to study sorghum grain decortication and compositing with tef as methods for improving the quality of injera made from tannin and tannin-free sorghums (Yetneberk et al 2005).

To determine the effects hydrothermal treatment in modifying the physicochemical properties of sorghum grains, Bolade and Buriamoh (2006) also used instrumental and sensory textural quality assessments. Similarly, Kebakile et al (2007) used instrumental and sensory textural parameters to assess the effects of different milling methods on sorghum porridge quality. Sensory texture evaluation is generally a clearer predictor of expected consumer perception of the food than instrumental measurements. However, this technique suffers the disadvantage of being generally time consuming, expensive in training and maintaining qualified panellists particularly in the case of descriptive sensory evaluation (Einstein 1991). Workers in this area of research appear to agree that sensory evaluation results are largely affected by natural differences in the physiological and psychological behaviours of the human panellists (Fischer et al 1994; Wolters and Allchurch 1994; Kobue-Lekalake 2008). On the other hand, instrumental texture analyses are considered to be fast, reproducible and relatively cheaper. However, some instrumental definitions of attributes may be an oversimplification and may not correspond to the human perception of the attributes (Meullenet et al 1998). These authors explained that some textural perceptions involve additional attributes such as saliva flow, in-mouth mechanical breakdown and manipulation, which may not contribute equally to the perception of food. To improve accuracy some workers such as Meullenet et al (1997) proposed the use of multiple instrumental parameters to predict a single sensory attribute.

Having highlighted how important texture is, to the acceptability of traditional sorghum foods, it is deemed appropriate to discuss briefly, the relationship between the two texture analysis techniques identified thus far. Correlations between sensory panel and instrumental measurements of food texture are generally used to predict consumer perception of food (Szczesniak 1987). It has been noted in most cases that instrumental textural assessments provide results that positively correlate to corresponding sensory attributes (Aboubacar et al 1999; Kebakile 2008). Meullenet et al (1998) studied texture relationships using both sensory and instrumental texture profile analysis (TPA) techniques to evaluate 21 foods from a wide

variety of sources. They reported high correlations between sensory and instrumental TPA parameters for hardness (force required to bite completely through the food sample when placed between molars) (r = 0.76) and springiness (degree or rate at which the food returns to its original size/shape after partial compression between the tongue and palate) (r = 0.83). However, they found poor correlations for cohesiveness (the amount of deformation undergone by a food before rupture when biting completely through it using molars) and chewiness (total amount of work necessary to chew a food to a state ready for swallowing).

These observations underscore the challenges encountered in relating some textural quality parameter values obtained from instrumental measurements to sensory assessment results. Where there are strong correlations, instrumental tests may be used to replace humans in sensory evaluation. However, as was noted by Aboubacar et al (1999), some tests may show the effects of different treatments on food textural properties but cannot be used as reliable predictors of consumer response to sorghum texture.

2.7 CONCLUSIONS

Various factors influence the protein quality of sorghum foods. The low lysine content of sorghum protein and poor protein digestibility, especially when wet cooked, appear to be the main reasons for low lysine quality of sorghum foods. In addition, intrinsic sorghum grain quality such as presence of antinutrients such as tannins worsens this situation in sorghum foods as it affects protein digestibility.

As cowpea has higher protein content and better protein quality than sorghum, it may be used to improve the protein quality of traditional sorghum foods. However, lysine can be destroyed easily even under mild processing conditions because of its highly reactive ε-amino group. Therefore, to improve protein quality of sorghum foods through compositing with a legume, food preparation techniques may need to be taken into account.

The functional quality of sorghum foods is influenced by the physico-chemical properties of the sorghum grain such as grain endosperm texture and tannin content, other food nutritional ingredients including proteins and starch, as well as the food preparation technique used. The functional quality in turn affects the consumption of sorghum foods, thereby influencing the ability of the foods to supply the required nutrients.

Since both sorghum and cowpea are relatively resistant to marginal climatic conditions, it appears worthwhile to investigate the effects of compositing with cowpea on the protein quality and functional properties of traditional sorghum foods. This will provide information on how such foods might be utilized optimally to alleviate lysine deficiency in the arid and semi-arid tropics of the developing world where a majority of the poor populations rely on sorghum as their main staple food.

3 HYPOTHESES AND OBJECTIVES

3.1 HYPOTHESES

a) Sorghum foods composited with cowpeas will have improved protein quality, as the lysine-rich proteins from cowpea (USDA 2008) will supplement sorghum proteins, which are poor in lysine (Taylor and Schüssler 1986) and poorly digestible (Hamaker et al 1986).

b) The functional properties of sorghum foods, like other cereals products, are affected by interactions among the chemical constituents of the foods and are influenced by the food preparation techniques used (Bushuk 1998). Hence, compositing sorghum with cowpea in the preparation of traditional sorghum foods will affect their functional quality differently, depending on the particular chemical composition of the sorghum grain.

3.2 OBJECTIVES

The primary objective of the research was to determine the effects of compositing sorghum with an indigenous legume, cowpea, on the protein and functional quality of traditional sorghum foods. The specific objectives were:

a) To determine the effects of compositing with cowpea on the lysine content and lysine availability of proteins in traditional sorghum foods.
b) To determine the effects of compositing with cowpea on the protein digestibility of traditional sorghum foods.
c) To determine the effects of compositing with cowpea on the texture and other sensory properties of traditional sorghum foods.

4 RESEARCH

4.1. Effects of compositing with cowpea on the protein quality of traditional sorghum foods

ABSTRACT

Sorghum protein quality is poor because of its low lysine content and poor digestibility. A way of improving this situation is through compositing sorghum foods with lysine-rich legumes. The efficacy of compositing with cowpea, a locally available legume, to improve the protein quality of traditional sorghum foods was determined by measuring their protein contents, total lysine and reactive lysine contents, and in vitro protein digestibility. Two sorghum cultivars, a red, tannin type (NS 5511) and a white tan plant, non-tannin type (Orbit) composited with cowpea at 70:30 ratio, were used to prepare three sorghum foods: ugali (unfermented thick porridge), uji (fermented thin porridge) and injera (fermented flatbread). Compositing with cowpea increased the protein contents of the foods by up to 35% and 57% for NS 5511 and Orbit foods, respectively. Lysine contents of the food proteins increased by between 67% and 139%. Reactive lysine content, increased by between 10% and 75%. Protein digestibility of NS 5511 and Orbit foods increased by up to 62% and 13%, respectively. Orbit sorghum foods had higher protein quality than NS 5511 foods probably because of tannins, which bind proteins thereby inhibiting their digestibility. Compositing with cowpea improves the protein quality of traditional sorghum foods.

4.1.1 INTRODUCTION

The nutrient content of sorghum grain is generally similar to other cereals (FAO 1995). However, studies have shown that the storage proteins in sorghum (kafirins) are particularly deficient in the essential amino acid, lysine. For example, Taylor and Schüssler (1986), studying protein composition of different parts of sorghum grain, reported an average of about 2% lysine in sorghum prolamins. This value (2% lysine) is quite low compared to 5.2% recommended for a 1 to 2 year-old child (WHO 2007). In addition, Eggum et al (1983), studying protein quality of sorghum and sorghum foods from Sudan using rats as test animals, reported low lysine content and biological value. Similarly, MacLean et al (1981) using a nitrogen balance study on 13 children, found that lysine was the most limiting amino acid in sorghum. The sorghum protein quality is also low in part because of the low digestibility of its proteins, which decreases especially on wet cooking (reviewed by Duodu et al 2003). Therefore, sorghum foods require fortification to enhance their protein nutritional value.

Cowpea is an important legume to millions of people in less developed countries of the tropics, particularly in Africa (AATF 2005). It is used in various food preparations such as akara (a fried cowpea paste), moin moin (a steamed cowpea paste) (Phillips et al 2003) and kpejigaou (a griddled cowpea-paste) (Amonsou et al 2008). With an average of 24 g protein per 100 g and about 7 g lysine per 100 g protein (USDA 2008), cowpea is a major source of protein. Because of the ability of sorghum and cowpea to survive in the tropical and subtropical climates (Doggett 1988; Chavan et al 1989), sorghum-cowpea composite foods appear to be good candidates in the alleviation of protein malnutrition in populations living in such areas.

Most research aimed at improving protein quality of sorghum foods such as ogi (Akinyele and Fasaye 1988), instant porridge (Bookwalter et al 1987; Pelembe et al 2002), and fermented sorghum slurry (Chavan et al 1988) through compositing with cowpea or other legumes has focused on the protein content, amino acid composition and digestibility. However, as explained by Moughan and Rutherfurd (2008), total lysine values from amino acid analysis do not always reflect available to the body for metabolism. This is because the ε-amino group of lysine can undergo reaction with many compounds in food including reducing sugars, fats and their oxidation products, polyphenols, vitamins, food additives and

other amino acids rendering the lysine unavailable nutritionally, as explained by Hurrell and Carpenter (1981). The present study determined the effects of compositing with cowpea as a means of enhancing the protein quality of traditional sorghum foods. The information obtained will be useful in alleviating lysine deficiency among poor people living in arid and semi-arid tropics who rely on sorghum as their staple food.

4.1.2 MATERIALS AND METHODS

4.1.2.1 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) was a year 2007 harvest grown in the Free State Province, South Africa; Orbit (white tan plant, non-tannin sorghum) was a year 2005 harvest from Agricultural Research Council, Potchefstroom, South Africa, and cowpea (Bechuana white variety) was a year 2007 harvest, grown in Delareyville, North West Province, South Africa. The NS 5511 sorghum, Orbit sorghum and cowpea grains had protein contents (N x 6.25) of 11.0, 8.4 and 23.5 g per 100 g (db), respectively. 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^{o}C prior to food preparation and other treatments. Composite flours were prepared by mixing sorghum and cowpea flours at a ratio of 70:30 (w/w) in a plastic bucket.

4.1.2.2 Food preparation procedures

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

Preparation of ugali

Tap water (40 mL) 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.

Preparation and maintenance of natural inoculum

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 and 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^{o}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^{o}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^{o}C for 3 days by which time the pH dropped to 3.7.

Preparation of uji

A slurry prepared with 30 g flour and 60 mL tap water was inoculated with 10 mL starter culture and incubated in a closed plastic bucket at 25^{o}C for 24 hr. The fermented slurry was added to 160 mL boiling water and cooked while stirring (for 2 min) until a smooth product formed.

Preparation of injera

Injera was prepared according to Yetnerberk et al (2004) with modification (Figure 4). A mixture of 50 g flour and 65 mL tap water was thoroughly kneaded in a plastic bucket until the flour was uniformly hydrated. To initiate the first fermentation, the slurry was inoculated with 10 mL starter culture and incubated for 24 hr. Approximately one third (38 g) of the fermented dough was mixed with 10 mL water and added to 40 mL boiling tap water and cooked for 2 min while stirring to avoid formation of lumps. The cooked portion was cooled to 45^{o}C. To initiate the second fermentation, 0.5 g commercial instant dried baker's yeast (NCP, Modderfontein, South Africa) and 1.5 g sugar was added to the rest of the fermented batter and stirred thoroughly to obtain 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 hr in a water bath at 35^{o}C. The actively fermenting dough was stirred to obtain a 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 sec) until it formed honeycombed structured surface (‘eyes’).

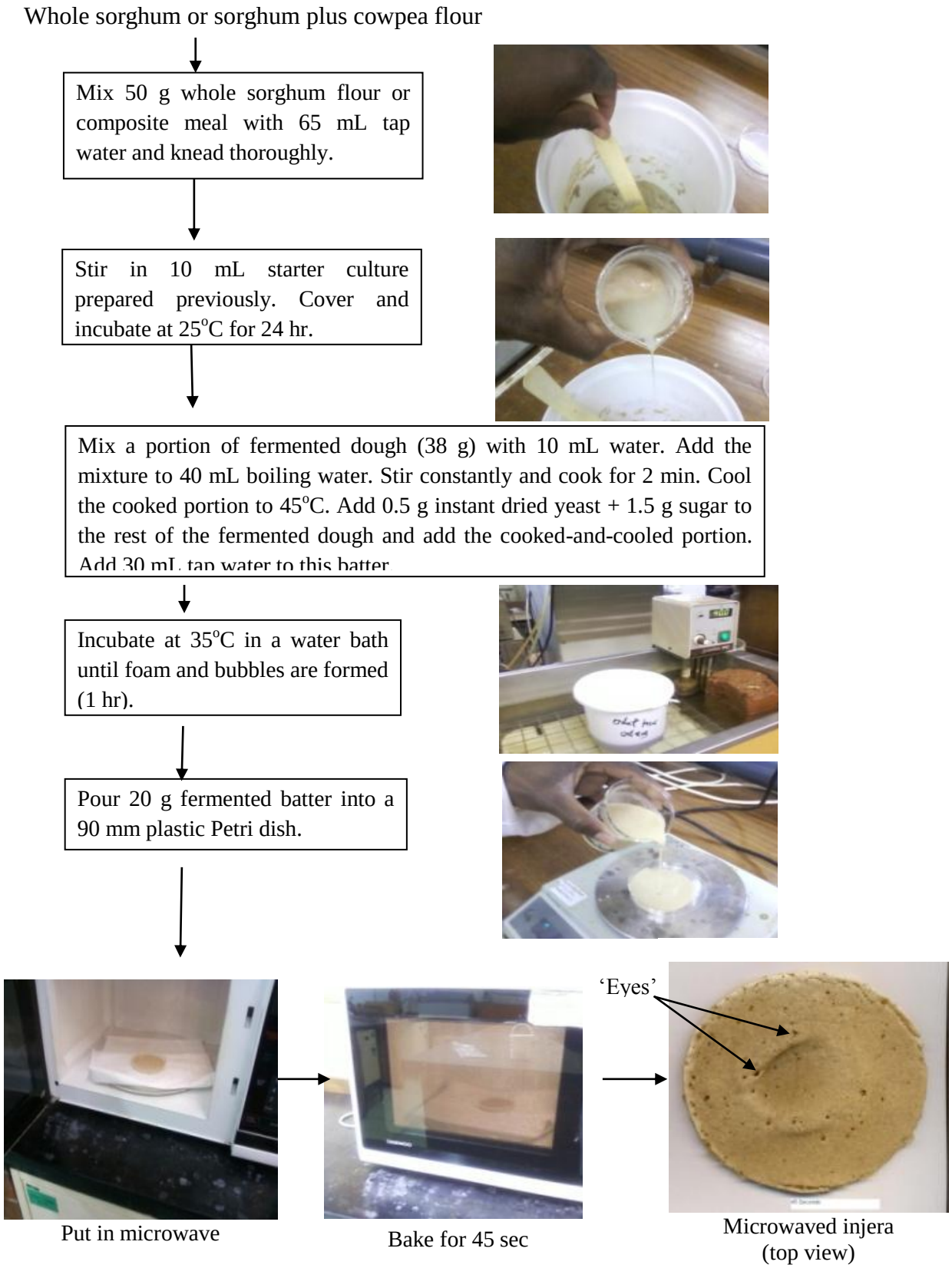

Figure 4: Flow diagram for preparation of microwaved sorghum injera

4.1.2.3 Analyses

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 sec.

Moisture content

Moisture content was determined by oven drying (American Association of Cereal Chemists (AACC) International 2000) Method 44-15A. Samples were subjected to single stage air oven drying at 103°C for 3 hr, then the moisture content calculated as the loss in weight expressed as a percent of the original weight of the sample.

Tannin content

Tannin content was determined using a modified Vanillin-HCl method (Price et al 1978). Phenolic compounds were extracted from the samples using 1% concentrated HCl in methanol and reacted with vanillin at room temperature (25°C). Extract blanks were prepared to compensate for highly coloured samples, where colour was not only due to tannins. Catechin standard (Sigma-Aldrich, St. Louis, MO) was used to calculate values of catechin equivalents determined for samples and blanks. Results were calculated with blank correction, then tannin concentration expressed as catechin equivalents (CE).

Protein content

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

In vitro protein digestibility

The pepsin digestion method was used, based on that of Hamaker et al (1987). Accurately weighed samples (200 mg) were digested with P7000-100G pepsin, activity 863 units/mg protein (Sigma-Aldrich, St. Louis, MO) for 2 hr at 37°C and products of digestion were pipetted off using a Pasteur pipette. The residues were washed with distilled water and clear supernatant pipetted off. The residues were dried in an oven at 100°C overnight. The residual protein was determined by the Dumas combustion method (AACC International 2000) Method 46-30. 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.

Lysine content

Lysine contents of the samples were determined by Pico-Tag method, which is a reverse phase HPLC method (Bidlingmeyer et al 1984). This method involves three main steps: hydrolysis of the protein and peptides with 6 M HCl to yield free amino acids, pre-column derivatization of sample and analysis by reverse phase HPLC.

Reactive lysine

Reactive lysine content was determined using a rapid dye-binding lysine (DBL) method (Kim et al 2007). Accurate amounts samples were weighed depending on their protein contents. The amounts of samples weighed were calculated for treatments A and B using these equations developed from preliminary studies:

Treatment A: $y = -0.026x + 0.782$

Treatment B: $y = -0.026x + 0.982$

Where y= mass of sample weighed (g)

x= protein content (%) (wb)

All samples, used in treatments A and B, were reacted with 5 mL 16% sodium acetate for 15 min. For treatment B, 0.2 mL propionic anhydride was added to block the reactive ε-amino group of lysine. Dye binding was carried out by adding 12 mL Crocein Orange G dye (70% dye content) (Fluka grade 27965: Sigma-Aldrich, Buchs, Switzerland) then shaking for 2 hr at ambient temperature before dilution and absorbance measurement. Using the property that lysine does not bind to the Crocein Orange G dye after being treated with propionic anhydride, the reactive lysine in the samples were determined by calculating the difference between the amount of dye bound to the sample treated with propionic anhydride (treatment B) and the amount of dye bound to an untreated sample (treatment A).

4.1.2.4 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).

4.1.3 RESULTS AND DISCUSSION

A number of protein quality parameters were determined to establish the effect of compositing of sorghum with cowpea on the protein quality of the foods. They included protein content, amino acid profile, reactive lysine and in vitro protein digestibility. Tannin content was determined, as it is known that tannins bind with sorghum protein (Butler et al 1984; Emmambux and Taylor 2003; Taylor et al 2007) thereby reducing protein quality (Serna-Saldivar and Rooney 1995). Additionally, it has been established that various cooking methods affect sorghum protein quality differently (Duodu et al 2003; Hamaker et al 1986; Lorri and Svanberg 1993b; Taylor and Taylor 2002). Therefore, the combined effects of compositing with cowpea and cooking, on the protein quality was determined.

4.1.3.1 Tannin content

As expected, higher tannin contents were obtained for foods prepared from tannin sorghum (NS 5511) than those from non-tannin sorghum (Orbit) (Table V). Cooking resulted in decreases in assayable tannin contents by between 18% and 69%. These reductions are in agreement with reports by previous workers. For example Dlamini et al (2007) working on the effects of sorghum type and different preparation technologies of traditional African foods on tannin content, found substantial reductions in tannin contents after cooking. The reductions in assayable tannin after cooking may be due to thermally induced degradation of tannins (Awika et al 2003) or interaction with other grain components such as proteins forming insoluble complexes (Butler et al 1984; Emmambux and Taylor 2003), thus lowering tannin extractability and therefore reducing the amount assayed.

Cooking resulted in a proportionately greater reduction in the tannin content of foods prepared from NS 5511 sorghum than in the foods prepared from NS 5511 sorghum plus cowpea. This may be due to the higher tannin content of NS 5511 and increase in protein

content through compositing with the protein-rich cowpea, probably enhancing tannin-protein interactions, thereby lowering extractability of the tannins in composite foods. These results are in agreement with findings of Emmambux and Taylor (2003) who studied the affinity of tannins for sorghum kafirin proteins. These workers reported a linear increase of tannins bound with increase in tannin content. As the cowpea had a higher tannin content than the Orbit sorghum (Table V), compositing increased the tannin content proportionately. However, the levels of tannin were within the group I category sorghum (less than 1 g CE per 100 g) according to a classification by Maxson and Rooney (1972). Therefore, they would not be expected to have significant effect on protein quality.

Table V: Effects of compositing sorghum with cowpea on the tannin content (catechin equivalents g/100 g, db) of raw flour and traditional sorghum foods

Grain	Flour		Food		
		Raw flour	Ugali	Uji	Injera
Cowpea	Cowpea	2.30^{j}±0.01	NA	NA	NA
NS 5511 (red, tannin)	Sorghum	5.62^{m}±0.04	1.79^{h}±0.01	1.78^{g}±0.01	1.75^{g}±0.02
	Sorghum+Cowpea	4.71^{l}±0.03	2.11^{i}±0.01	2.37^{k}±0.02	2.07^{i}±0.01
Orbit (white-tan plant, non-tannin)	Sorghum	0.23^{b}±0.01	0.08^{a}±0.01	0.11^{a}±0.02	0.10^{a}±0.02
	Sorghum+Cowpea	0.76^{f}±0.02	0.45^{c}±0.01	0.62^{e}±0.02	0.56^{d}±0.01

Values are means ±standard deviations. Values followed by different superscript letters are significantly different at $p \leq 0.05$.

NA – Not applicable

4.1.3.2 Protein content

Table VI shows that compositing of sorghum with cowpea at a 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. The increase in protein content of the composite foods is due to the relative high protein content of the cowpea (Table VI). Pelembe et al (2002) also reported a proportionate increase in protein content of extruded sorghum-cowpea composite instant porridge with increasing amounts of cowpea added. The highest increase in protein content was recorded for injera, 42% and 69%, from NS 5511 and Orbit sorghum, respectively. The higher increase with injera is probably mainly because the yeast added during preparation of the food is protein-rich containing about 38 g protein/100 g db (USDA 2008). Additionally, it can be attributed to the decrease of carbon ratio in the total mass during fermentation. During fermentation, microorganisms utilize carbohydrates as an energy source and produce carbon dioxide as a by-product (Jay 2000). This causes the nitrogen in the fermented slurry to be concentrated and thus the proportion of protein in the total mass increases. Onyango et al (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 and Taylor 2002). In the present study, it was noted that uji (fermented porridge) prepared form 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 the tannin in the sorghum. Tannins have been reported to be bacteriostatic and/or bactericidal for many bacteria species (reviewed by Chung et al 1998; Scalbert 1991). Scalbert (1991) 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.

Except for injera, there were no substantial differences in protein content between the raw flours and corresponding food products. This may be attributed to the fact that protein content was measured as nitrogen (N x 6.25). Hence, the protein content was not affected by cooking, as nitrogen is not affected by heat treatment. Similar observations were made by Pelembe et al (2002), working on extruded sorghum-cowpea composite porridges. These authors noted that different extrusion temperatures did not affect the protein contents of the extrudates.

Table VI: Effects of compositing sorghum with cowpea on the protein content (g/100 g, db) of raw flour and traditional sorghum foods

Grain	Flour		Food		
		Raw flour	Ugali	Uji	Injera
Cowpea	Cowpea	23.5^{k}±0.2	NA	NA	NA
NS 5511 (red, tannin)	Sorghum	11.0^{d}±0.1	11.2^{d}±0.0	11.2^{d}±0.1	11.6^{e}±0.1
	Sorghum+Cowpea	14.9^{h}±0.1	14.8^{h}±0.1	15.1^{i}±0.0	15.6^{j}±0.0
Orbit (white-tan plant, non-tannin)	Sorghum	8.4^{a}±0.2	8.6^{a}±0.0	8.8^{b}±0.1	10.5^{c}±0.1
	Sorghum+Cowpea	13.2^{f}±0.1	13.2^{f}±0.0	13.3^{f}±0.1	14.2^{g}±0.0

Values are means ±standard deviations. Values followed by different superscript letters are significantly different at $p \leq 0.05$.

NA – Not applicable

4.1.3.3 Lysine and reactive lysine content

Compositing with cowpea increased the lysine contents of the proteins in all the foods by between 67% and 139% (Table VII). An increase was expected because the high lysine content of cowpea. However, contrary to expectations, there were inconsistencies in the increase of the lysine contents of the food proteins through cowpea addition. As shown in Table VII, the lysine content of proteins in NS 5511 ugali, uji and injera increased by 113%, 139% and 81%, respectively. On the other hand, lysine contents of proteins in Orbit foods increased by 67%, 87% and 72% for ugali, uji and injera, respectively. 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 and 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. In addition, the HPLC method used in the determination of lysine content may be somewhat inaccurate, especially in the case of very low lysine foods as in the present study. This is because a very small quantity of sample is drawn for analysis (0.1 to 5 µg samples injected) (Bidlingmeyer et al 1984), which may not properly represent the entire food sample.

Compositing with cowpea increased the level of reactive lysine, an estimate of lysine availability (Hurrell et al 1979), by between 10% and 75% (Table VII). This may be due to the corresponding increase in lysine content through compositing. Similar findings have been reported by other workers. For example, in vivo studies, Van Barneveld et al (1994) and Copelin et al (1978) found a positive correlation between lysine content and lysine availability. Cooking had mixed effects on the reactive lysine contents. Ugali and uji proteins had 0 to 4% and 0 to 15% lower reactive lysine contents, respectively, compared to the corresponding raw flour. On the other hand, injera proteins had 0 to 6% higher reactive lysine content than the corresponding raw flour. A reduction in reactive lysine content was expected because of the effect of heat on lysine (Nursten 1981). The apparent inconsistencies in the results for ugali and uji may be due to the specificity of protein-polyphenol interactions as explained above.

Table VII: Effects of compositing sorghum with cowpea on the lysine and reactive lysine contents (g/100 g protein, db) of raw flour and traditional sorghum foods

Grain	Flour	Lysine content				Reactive lysine content			
		Raw flour	Ugali	Uji	Injera	Raw flour	Ugali	Uji	Injera
Cowpea	Cowpea	4.56^{h}±0.18	NA	NA	NA	4.89^{h}±0.24	NA	NA	NA
NS 5511	Sorghum	1.62^{b}±0.14	1.58^{b}±0.00	1.25^{a}±0.20	1.88^{c}±0.00	1.77^{b}±0.16	1.75^{ab}±0.29	1.51^{a}±0.22	1.88^{b}±0.17
	Sorghum+Cowpea	3.43^{f}±0.11	3.37^{f}±0.00	2.99^{e}±0.05	3.40^{f}±0.19	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	2.05^{cd}±0.00	1.59^{b}±0.08	2.28^{d}±0.14	2.40^{cd}±0.13	2.35^{c}±0.26	2.52^{cde}±0.20	2.33^{c}±0.26
	Sorghum+Cowpea	3.45^{f}±0.18	3.43^{f}±0.17	2.97^{e}±0.11	3.92^{g}±0.05	2.82^{g}±0.18	2.71^{ef}±0.28	2.76^{ef}±0.07	2.82^{g}±0.16

Values are means ±standard deviations. Values of a parameter, followed by different superscript letters are significantly different at $p \leq 0.05$.

NA – Not applicable

Injera had higher reactive lysine than the other foods probably as result of the lysine-rich proteins from the added yeast, which contains about 8 g lysine/100 g protein (USDA 2008). There were generally little effects of cooking on the reactive lysine content of proteins in some of the foods, such as ugali and uji. It seems that short time, moist heat cooking used during preparation of ugali and uji, had minimal effect on the reactive lysine. This finding is in agreement with a report by Undi et al (1996) which showed an inverse correlation between autoclaving time and the availability of lysine in canola meal proteins. These workers noted that short time heat treatment (15 min autoclaving) had little effect (7% reduction) on the lysine availability of canola proteins, measured by an in vivo analysis.

There were inconsistencies in some of the reactive lysine data, as some of the values of reactive lysine contents were higher than the corresponding lysine contents particularly for proteins in sorghum-only foods. These apparent inconsistencies may be explained by considering the principle of the DBL method used. In this method, it is known that the ratio of dye to the basic amino acids influences the amount of dye bound (Hurrell et al 1979). Because proteins from the sorghum-only foods contained lower values of the basic amino acids (histidine, arginine and lysine) compared to their composite food counterparts (Table VIII), it is possible that there was excess dye in reactions involving sorghum-only foods as the equal amount of Crocein Orange G dye was used for to all the foods. This may have caused an overestimation of the reactive lysine content of the proteins in the food samples as stated by Hurrell et al (1979). These authors noted a steady increase in the dye bound as the excess dye increased even within the concentration limits considered acceptable for the working procedure. Hendriks et al (1994) working on the effects of extrusion of soya bean meal and peas on dye binding lysine, FDNB-reactive lysine and total lysine, made a similar observation. These workers found that the DBL procedure overestimated the reactive lysine content in the samples studied and the dye binding capacity after propionylation of lysine was higher than combined values of histidine and arginine. Therefore, the method appears unsuitable for quantitative determination of reactive lysine content when the amount of dye added to achieve a binding ratio of dye and basic amino acids of 1:1, is unknown. Hurrell and Carpenter (1981) also reviewed the methods of estimation of available lysine in foods after Maillard reactions. They noted that because the major early Maillard reaction product (Amadori product), deoxyketosyl derivative, is slightly basic in character, it may pose an analytical challenge to the DBL technique, thereby giving results that are not in line with

those from in vivo assays. However, after advanced Maillard reaction, little deoxyketosyl lysine remains in the food and in vitro methods generally give similar results. In the present study, presumably the main Maillard reaction products present in foods were the Amadori products as the heating conditions may not have been severe enough for the production of advanced Maillard products. This may further explain the inconsistencies noted.

There was no significant difference in lysine contents of proteins in ugali compared to corresponding raw flours (Table VII). Proteins in uji had the lowest lysine content of all the foods studied with about 13% to 23% lower lysine content than the corresponding raw flour samples. 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 principle 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 appear to explain the reductions in lysine content of proteins in uji as well as the increase in lysine content of proteins in injera.

In general, NS 5511 foods had lower lysine and reactive lysine content than the corresponding Orbit foods. This is because tannins are capable of binding and precipitating protein (Butler et al 1984; Schofield et al 2001), thus reducing the assayable lysine. It has been suggested that tannins react covalently with ε-amino groups of lysyl residues thereby inhibiting enzymic cleavage of the lysyl peptide bond (Damodaran 1996a). In addition, by precipitating with protein and forming insoluble tannin-protein complexes (Mangan 1988), tannins contributes substantially to the reduction in reactive lysine contents of the proteins (Hurrell and Carpenter 1981).

Compositing with cowpea had little effect on contents of other essential amino acids of the food proteins, except leucine (Table VIII). This was expected because apart from lysine and leucine, generally there are little differences in the contents of other essential amino acids between sorghum and cowpea proteins (USDA 2008). The reduction in leucine in the composite foods may be nutritionally beneficial, as its high level in sorghum has been implicated in niacin deficiency in several studies (reviewed by Klopfenstein and Hoseney 1995).

Table VIII: Effects of compositing sorghum with cowpea on amino acid content (g/100 g protein) of raw flour and traditional sorghum foods

Amino acid	Cowpea	NS 5511 Sorghum				NS 5511 Sorghum+Cowpea			
	Raw flour	Raw flour	Ugali	Uji	Injera	Raw flour	Ugali	Uji	Injera
Alanine	$3.77^{a}\pm0.07$	$8.09^{d}\pm0.14$	$7.89^{cd}\pm0.26$	$7.24^{c}\pm0.26$	$7.57^{cd}\pm0.57$	$6.15^{b}\pm0.16$	$5.93^{b}\pm0.45$	$6.20^{b}\pm0.49$	$5.93^{b}\pm0.09$
Arginine	$5.33^{c}\pm0.39$	$2.93^{a}\pm0.00$	$2.88^{a}\pm0.13$	$2.60^{a}\pm0.26$	$4.03^{b}\pm0.89$	$4.51^{b}\pm0.37$	$4.46^{b}\pm0.15$	$3.99^{b}\pm0.19$	$4.20^{b}\pm0.19$
Aspartic acid	$8.25^{e}\pm0.03$	$4.85^{bc}\pm0.14$	$4.27^{ab}\pm0.26$	$3.39^{a}\pm0.33$	$3.23^{a}\pm0.00$	$6.30^{d}\pm0.79$	$5.90^{cd}\pm0.40$	$4.96^{bcd}\pm0.19$	$4.86b^{c}\pm1.32$
Glutamic acid	$14.14^{a}\pm0.16$	$17.29^{f}\pm0.14$	$16.66^{de}\pm0.02$	$15.22^{b}\pm0.13$	$16.80^{ef}\pm0.44$	$16.11^{cd}\pm0.11$	$16.58^{de}\pm0.10$	$15.70^{bc}\pm0.10$	$14.63^{a}\pm0.42$
Glycine	$3.70^{d}\pm0.03$	$2.73^{a}\pm0.00$	$2.65^{a}\pm0.07$	$2.83^{ab}\pm0.46$	$2.82^{ab}\pm0.32$	$3.36^{c}\pm0.00$	$3.27^{bc}\pm0.05$	$3.30^{c}\pm0.00$	$3.27^{bc}\pm0.00$
Histidine	$2.44^{c}\pm0.13$	$1.72^{a}\pm0.00$	$1.72^{a}\pm0.07$	$1.76^{a}\pm0.26$	$1.93^{ab}\pm0.32$	$2.20^{bc}\pm0.05$	$2.25b^{c}\pm0.10$	$2.34^{c}\pm0.00$	$2.13^{bc}\pm0.09$
Isoleucine	$3.47^{bc}\pm0.23$	$3.49^{bc}\pm0.07$	$3.29^{ab}\pm0.07$	$3.11^{a}\pm0.33$	$3.45^{abc}\pm0.06$	$3.73^{c}\pm0.11$	$3.76^{c}\pm0.05$	$3.27^{ab}\pm0.05$	$3.50^{bc}\pm0.24$
Leucine	$5.95^{a}\pm0.29$	$11.02^{e}\pm0.29$	$10.86^{e}\pm0.26$	$9.84^{cd}\pm0.79$	$9.95^{d}\pm0.51$	$9.03^{bc}\pm0.11$	$8.96^{bc}\pm0.25$	$8.57^{b}\pm0.24$	$8.63^{b}\pm0.14$
Lysine	$4.56^{f}\pm0.18$	$1.62^{b}\pm0.14$	$1.58^{b}\pm0.00$	$1.25^{a}\pm0.20$	$1.88^{c}\pm0.00$	$3.43^{e}\pm0.11$	$3.37^{e}\pm0.00$	$2.99^{d}\pm0.05$	$3.40^{e}\pm0.19$
Methionine	$1.15^{a}\pm0.07$	$1.42^{c}\pm0.00$	$1.44^{c}\pm0.07$	$1.35^{bc}\pm0.07$	$1.75^{d}\pm0.19$	$1.42^{c}\pm0.00$	$1.44^{c}\pm0.05$	$1.31^{bc}\pm0.00$	$1.23^{b}\pm0.05$
Phenylalanine	$4.21^{bc}\pm0.29$	$4.10^{ab}\pm0.07$	$4.13^{b}\pm0.07$	$3.76^{a}\pm0.33$	$4.08^{ab}\pm0.19$	$4.59^{c}\pm0.05$	$4.35^{bc}\pm0.10$	$4.34^{bc}\pm0.10$	$4.33^{bc}\pm0.09$
Proline	$3.68^{a}\pm0.00$	$7.38^{c}\pm0.29$	$7.29^{c}\pm0.46$	$6.96^{c}\pm0.26$	$7.53^{c}\pm0.63$	$5.74^{b}\pm0.32$	$5.79^{b}\pm0.55$	$5.61^{b}\pm0.44$	$5.56^{b}\pm0.24$
Serine	$4.23^{b}\pm0.00$	$3.84^{a}\pm0.00$	$3.81^{a}\pm0.00$	$3.85^{a}\pm0.46$	$3.85^{a}\pm0.51$	$4.18^{a}\pm0.00$	$4.11^{a}\pm0.15$	$4.16^{a}\pm0.05$	$3.96^{a}\pm0.14$
Threonine	$3.13^{b}\pm0.07$	$2.78^{a}\pm0.07$	$2.65^{a}\pm0.07$	$2.55^{a}\pm0.07$	$3.36^{b}\pm0.57$	$2.98^{ab}\pm0.11$	$2.99^{ab}\pm0.15$	$2.99^{ab}\pm0.15$	$2.93^{ab}\pm0.09$
Tyrosine	$3.15^{ab}\pm0.29$	$3.19^{b}\pm0.07$	$3.20^{bc}\pm0.20$	$2.92^{a}\pm0.33$	$3.63^{c}\pm0.32$	$3.54b^{c}\pm0.16$	$3.62b^{c}\pm0.15$	$3.17^{ab}\pm0.10$	$3.23^{bc}\pm0.05$
Valine	$4.16^{b}\pm0.16$	$4.35^{bc}\pm0.14$	$4.18^{abc}\pm0.13$	$3.85^{a}\pm0.20$	$4.35^{bc}\pm0.19$	$4.48^{c}\pm0.11$	$4.32^{bc}\pm0.15$	$4.10^{ab}\pm0.05$	$4.10^{ab}\pm0.14$

Values are means ±standard deviations. Values in a row followed by different superscript letters are significantly different at $p\leq0.05$

Table VIII continued

Amino acid	Orbit Sorghum				Orbit Sorghum+Cowpea			
	Raw flour	Ugali	Uji	Injera	Raw composite flour	Ugali	Uji	Injera
Alanine	7.94^{d}±0.29	7.68^{cd}±0.26	7.56^{cd}±0.00	7.33^{c}±0.28	5.75^{b}±0.06	5.08^{a}±0.06	5.67^{b}±0.06	5.60^{b}±0.05
Arginine	3.26^{ab}±0.00	3.32^{ab}±0.09	2.66^{a}±0.08	5.20^{de}±0.77	4.56^{cd}±0.18	4.10^{bc}±0.11	3.87^{bc}±0.61	5.89^{e}±0.78
Aspartic acid	4.27^{b}±0.48	3.02^{a}±0.34	3.90^{b}±0.17	3.86^{b}±0.42	6.44^{d}±0.18	5.95^{cd}±0.06	5.51^{c}±0.17	5.82^{cd}±0.05
Glutamic acid	16.62^{d}±0.29	14.87^{bc}±0.51	14.77^{bc}±0.17	14.80^{bc}±0.49	15.68^{cd}±0.36	14.21^{b}±0.06	13.17^{a}±0.61	14.57^{b}±0.83
Glycine	3.05^{ab}±0.10	2.90^{a}±0.00	3.13^{ab}±0.25	3.86^{d}±0.42	3.41^{bc}±0.00	3.19^{ab}±0.06	3.09^{ab}±0.17	3.73^{cd}±0.00
Histidine	1.90^{a}±0.00	1.81^{a}±0.00	2.24^{ab}±0.67	4.90^{c}±0.33	2.17^{ab}±0.06	2.05^{a}±0.00	1.84^{a}±0.17	3.04^{b}±0.16
Isoleucine	3.53^{c}±0.00	3.44^{bc}±0.09	3.19^{a}±0.00	3.12^{a}±0.07	3.54^{c}±0.06	3.19^{a}±0.06	3.17^{a}±0.06	3.26^{ab}±0.26
Leucine	10.78^{d}±0.10	10.46^{d}±0.26	9.57^{c}±0.17	9.01^{c}±0.00	8.10^{b}±0.12	7.21^{a}±0.06	7.23^{a}±0.17	7.80^{b}±0.57
Lysine	1.90^{b}±0.00	2.05^{bc}±0.00	1.59^{a}±0.08	2.28^{c}±0.14	3.45^{e}±0.18	3.43^{e}±0.17	2.97^{d}±0.11	3.92^{f}±0.05
Methionine	1.56^{ab}±0.10	1.51^{ab}±0.09	1.30^{a}±0.00	1.73^{b}±0.07	1.45^{ab}±0.00	1.38^{a}±0.17	1.49^{ab}±0.11	1.28^{a}±0.26
Phenylalanine	4.14^{ab}±0.10	4.05^{ab}±0.09	3.84^{a}±0.25	3.76^{a}±0.14	4.30^{b}±0.18	3.98^{ab}±0.28	4.30^{b}±0.00	4.28^{b}±0.26
Proline	7.80^{b}±0.29	7.74^{b}±0.00	7.68^{b}±0.67	7.62^{b}±0.42	5.67^{a}±0.06	5.55^{a}±0.28	5.67^{a}±0.28	5.31^{a}±0.05
Serine	4.00^{ab}±0.10	3.69^{a}±0.09	3.84^{ab}±0.42	4.65^{c}±0.42	4.22^{bc}±0.06	3.82^{ab}±0.06	3.63^{a}±0.17	4.32^{bc}±0.00
Threonine	2.85^{a}±0.19	2.78^{a}±0.00	2.95^{a}±0.33	3.81^{c}±0.49	3.28^{ab}±0.06	2.99^{a}±0.22	3.24^{ab}±0.06	2.96^{a}±0.05
Tyrosine	3.32^{bc}±0.10	3.38^{bc}±0.00	2.95^{a}±0.17	3.56^{c}±0.14	3.41^{bc}±0.12	3.15^{ab}±0.22	3.28^{abc}±0.22	3.29^{abc}±0.10
Valine	4.41^{cd}±0.10	4.17^{abc}±0.26	4.02^{a}±0.00	4.60^{d}±0.21	4.35^{bcd}±0.12	3.90^{a}±0.06	4.06^{ab}±0.00	4.21^{abc}±0.16

Values are means ±standard deviations. Values in a row followed by different superscript letters are significantly different at $p \leq 0.05$

4.1.3.4 In vitro protein digestibility

Compositing increased the in vitro protein digestibility (IVPD) of the NS 5511 and Orbit sorghum foods by about 54% to 74% and 4% to 13%, respectively (Table IX). A reason for the improvement in IVPD is that compositing increased the content of more digestible globulin proteins from the cowpea with concomitant decrease in less digestible kafirin proteins from sorghum. This is possible because cowpea is rich in globulins (reviewed by Chavan et al 1989), which become more digestible after cooking because of denaturation as opposed to the poorly digestible sorghum proteins (kafirins) (Hamaker et al 1987). Additionally, by reducing tannin content of the foods, as in the case of NS 5511 (Table V), foods made from NS 5511 plus cowpea had relatively less proteins-tannin complexing, which probably improved their IVPD. This is in agreement with the report by Emmambux and Taylor (2003) on the affinity of tannins to bind with kafirin proteins. These workers reported an increase in protein bound with increasing tannin concentration. Similarly, Nguz and Huyghebaert (1998) working on eight sorghum cultivars with different colours of pericarp and tannin contents found that a high level of tannins in sorghum grain reduces their protein digestibility.

NS 5511 sorghum foods had lower IVPD than Orbit sorghum foods (Table IX). This may be attributed to the higher tannin contents of the NS 5511 sorghum (Table V). As has been explained previously, tannins are known to bind protein and precipitate proteins thereby reducing their digestibility (Bach-Knudsen et al 1988; Serna-Saldivar and Rooney 1995; Taylor et al 2007). Cooking decreased the IVPD of NS 5511, NS 5511-cowpea composite, Orbit and Orbit-cowpea composite foods by approximately 34% to 47%, 18% to 25%, 5% to 20%, and 7% to 16%, respectively (Table IX). Duodu et al (2003) reviewing the effects of cooking on IVPD of sorghum foods, observed that more than one factor may influence the sorghum protein digestibility depending on the nature or the state of the sorghum grain. They also proposed that protein cross-linking (probably between γ- and β-kafirin proteins at the protein body periphery) may be the greatest factor that influences sorghum protein digestibility. According to a theory proposed by Hamaker et al (1987), the reduction in protein digestibility after cooking is probably due oxidative formation of disulphide bonds (cross-linking) during the cooking process creating the less digestible polymeric protein units bound by intermolecular disulphide bonds. The polymers may be less susceptible to digestion because of their poor accessibility by protease enzymes.

Table IX: Effects of compositing sorghum with cowpea on the in vitro protein digestibility (%) of raw flour and traditional sorghum foods

Grain	Flour		Food		
		Raw flour	Ugali	Uji	Injera
Cowpea	Cowpea	91.3^{l}±2.3	NA	NA	NA
NS 5511 (red, tannin sorghum)	Sorghum	61.8^{e}±0.2	32.6^{a}±0.8	38.8^{b}±0.1	40.7^{c}±0.2
	Sorghum+Cowpea	76.0^{i}±0.2	56.7^{d}±0.6	61.0^{e}±0.1	62.5^{e}±0.3
Orbit (white-tan plant, non-tannin sorghum)	Sorghum	80.6^{j}±0.9	64.6^{f}±0.3	68.3^{g}±1.9	76.4^{i}±1.2
	Sorghum+Cowpea	85.8^{k}±0.2	72.2^{h}±0.4	77.0^{i}±0.7	79.8^{j}±1.1

Values are means ±standard deviations. Values followed by different superscript letters are significantly different at $p \leq 0.05$

NA- Not applicable

The fermented foods, uji and injera, had relatively higher IVPD than the straight cooked ugali. This agrees with the report by Taylor and 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. Chavan et al (1988) investigating the effect of fermentation on soluble proteins and IVPD of sorghum, green gram and sorghum-green gram blends, also reported similar findings. In addition, Khetarpaul and Chauhan (1990) working on pearl millet, suggested that IVPD may increase after fermentation because of partial degradation of complex storage proteins by endogenous and microbial proteolytic enzymes into soluble products. Injera had the highest IVPD compared to the other foods. It is possible that as the yeast added proliferated, more protein was added to the food system (Table VI). This higher protein content probably resulted in higher IVPD. A similar suggestion was made by Taylor and Taylor (2002) when they found that sorghum with a relatively higher protein content gave a higher IVPD value than the sorghum of lower protein content.

4.1.4 CONCLUSIONS

The lysine contents and lysine availability of proteins from traditional sorghum foods are increased through compositing with cowpea. This is mainly because the major storage proteins in cowpea (globulins) are richer in lysine than kafirins in sorghum. The protein contents and protein digestibility of the foods substantially increased through compositing as the cowpea added has higher content of more digestible protein than the sorghum. Improvement of the protein quality of sorghum foods through compositing with cowpea is adversely affected by the tannins in tannin type sorghum cultivar used. This is attributed to protein-tannin complexing, which reduces protein digestibility. Based on these observations, it can be concluded that, with an appropriate choice of sorghum cultivar, compositing with cowpea, a relatively drought-resistant legume, is a viable potential for improving the protein quality of traditional sorghum foods in the semi arid tropics. This offers consumers, who are dependent on sorghum as their staple food, a way of alleviating the problem of protein malnutrition.

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4.2 Effects of compositing with cowpea on the textural and other sensory properties of traditional sorghum foods

ABSTRACT

To understand the influence of compositing with cowpea on the functional quality of traditional sorghum foods, sensory properties of three African foods were determined using instrumental texture analysis and a trained sensory panel. Flours from two sorghum cultivars, NS 5511 (tannin-sorghum) and Orbit (non-tannin sorghum), each composited with cowpea flour at 70:30 ratio, were used to prepare ugali (unfermented stiff porridge), uji (fermented thin porridge) and injera (fermented flatbread). Compositing with cowpea reduced pasting peak viscosity and cool paste viscosity of uji by 6% to 23%, and 6% to 12%, respectively. These reductions probably resulted from decreased uji starch content. Principal component analysis (PCA) of ratings for 17 sensory attributes of ugali showed that most of the variation in sensory attributes (59%) explained by the first two principal components, was due to the sorghum cultivar. Compositing with cowpea contributed 38% of variation in the sensory properties of ugali. Ugali prepared from sorghum-plus-cowpea was associated with cowpea flavour. Compositing increased the stiffness of NS 5511 injera by up to 25%. Orbit injera stiffness reduced by up to 12%. These differences in stiffness suggested a weakening effect of interactions involving tannins and other food polymers such as proteins. Apart from imparting cowpea flavour, compositing with cowpea has no substantial effect on functional properties of the sorghum foods.

4.2.1 INTRODUCTION

In Chapter 4.1, it was established that compositing important traditional African sorghum foods with cowpea improves their protein content and quality. Many workers have made similar observations. For example, Pelembe et al (2002) developed a protein-rich extruded sorghum-cowpea porridge, which had protein quality similar to commercial instant maize-soya porridge product. Compositing with legumes has been identified as an important way to improve the protein quality of sorghum foods (reviewed by Klopfenstein and Hoseney 1995). However, compositing with cowpea has been shown to influence the acceptability of traditional sorghum foods, which in turn is influenced by their functional properties. For example, Akinyele and Fasaye (1988) found that compositing of sorghum with cowpea at high proportions had a negative effect on consumer acceptability of ogi (a Nigerian malted and fermented sorghum thin porridge). It is therefore important to find out how addition of this tropical legume, which appears promising for improving protein quality of sorghum foods, affects the functional properties of important traditional African foods. Therefore, this study determined the effects of compositing cowpea with sorghum on textural and other sensory attributes of traditional sorghum foods.

4.2.2 MATERIALS AND METHODS

4.2.2.1 Preparation of flour samples

Whole grain flour of two sorghum cultivars, NS 5511, a red, tannin-sorghum and Orbit, a white tan plant sorghum, and one cowpea variety, Bechuana white, prepared as previously described in Chapter 4.1, were used in this study. Longitudinal sections of kernels of the sorghums showing their endosperm texture are given in Figure 5. The flours were used to prepare three traditional African sorghum foods, uji (fermented thin porridge), injera (fermented flatbread) and ugali (unfermented stiff porridge).

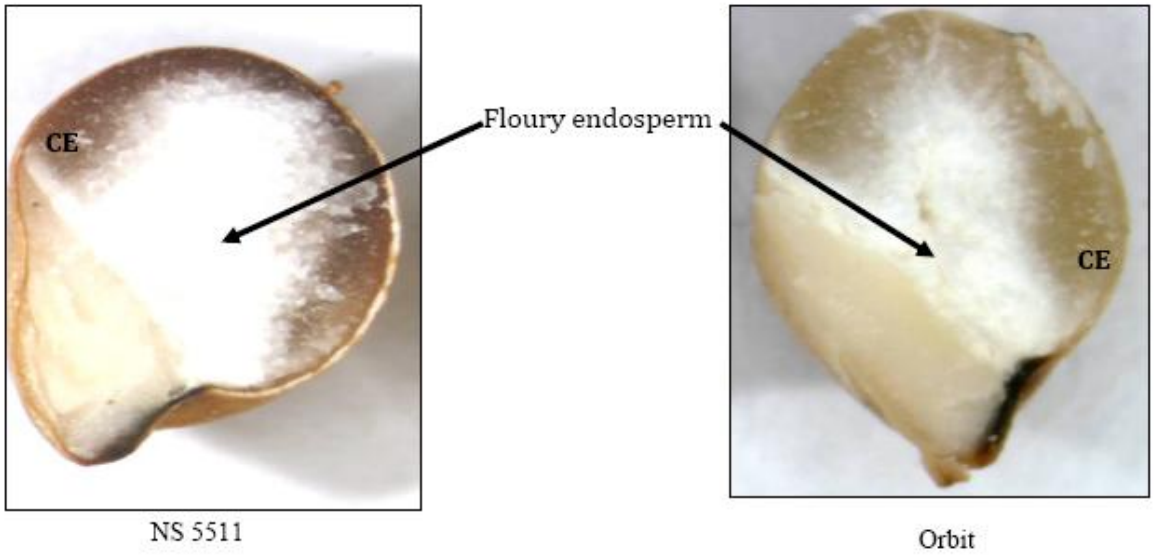


Figure 5: Longitudinal sections of NS 5511, a floury endosperm, red, tannin-sorghum and Orbit sorghum, an intermediate endosperm, white tan plant, non-tannin sorghum grains showing the differences in endosperm texture of the two cultivars. CE (corneous endosperm). Photographs courtesy of Ms H. Jacobs (MSc. student, University of Pretoria)

4.2.2.2 Preparation of uji and measurement of texture

Fermented uji slurries were prepared from sorghum and sorghum plus cowpea using the method described in Chapter 4.1. Flour (30 g) and 60 mL tap water was inoculated with 10 mL starter culture in a closed plastic tub and incubated at 25^{o}C for 24 hr. Tap water (160 mL) was added to the fermented slurry to make a uniform diluted suspension (30 g solids/250 g). The fermented suspensions were used to prepare uji and study their pasting properties using a Rapid Visco Analyser (RVA) (Model 3 D) (Newport Scientific, Warriewood, Australia). The RVA was programmed to rapidly stir each freshly mounted suspension at 960 rpm for 10 sec, then decrease and hold shear rate constant at 160 rpm for the remainder of the test period. The temperature profile involved holding initially at 50ºC for 2 min, then increasing to 91ºC over 4 min and holding at 91ºC for 8 min before finally cooling to 50ºC over 4 min and holding constant for 3 min. The peak viscosity (PV) and the cool paste viscosity (CPV) were determined for each suspension from the RVA plots. Each sample was analysed three times.

4.2.2.3 Preparation of injera and measurement of texture

Injera were prepared from the sorghum and sorghum-cowpea composite flours using the method described in Chapter 4.1. After baking, injera were cooled for 45 min at room temperature. The injera were then prepared for texture analysis using a similar protocol to

that of Yetneberk et al (2004) with some modifications. Each injera was put into a separate ziplock-type polythene bag and stored at 25^{o}C in an incubator for 1 hr, 24 hr and 48 hr. For texture analysis, each injera was cut when fresh using a 65 mm diameter cookie cutter, to obtain uniform size samples. The texture of the injera were analysed using a TA-XT2 Texture Analyser (Stable Micro Systems, Godalming, UK). During each texture analysis session, three pieces of injera per treatment were analysed for maximum bending force using a three-point bending rig attachment. The following test parameters were used: Mode was Measure Force in Compression; Option was Return to Start; Pre-Test Speed was 1.0 mm/sec; Test Speed was 3.0 mm/sec; Post-Test Speed was 10.0 mm/sec; Distance was 5 mm; Trigger type was auto 0.049 N; and the two adjustable supports of rig base plate were set 30 mm apart. Each injera type was analysed in triplicate.

4.2.2.4 Preparation of ugali and measurement of texture

Ugali for instrumental texture analysis were prepared from sorghum and sorghum plus cowpea using the method described in Chapter 4.1. To obtain ugali with solid content that could be uniformly filled into sample tubes, the ratio of flour to water was adjusted to 1:3. The cooked ugali were filled immediately into 50 mL glass sample tubes, diameter 30 mm. Each of the tubes containing ugali was then covered with aluminium foil, and maintained in an oven at 50^{o}C for 90 min. To analyse the ugali, the aluminium foil was removed and the surface of the sample scraped off. The ugali were analysed immediately for maximum penetration force (firmness) and stickiness using a TA-XT2 Texture Analyser as described by Kebakile (2008). The following texture analyser settings were used: Mode was Measure Force in Compression; Option was Return to Start; Pre-Test Speed was 2.0 mm/sec; Test Speed was 2.0 mm/sec; Post-Test Speed was 10 mm/sec; Penetration Distance was 10 mm; Trigger type was auto, 0.049 N. A cylindrical Perspex probe, diameter 20 mm, was used.

4.2.2.5 Descriptive sensory evaluation of ugali

For sensory evaluation, four treatments as indicated above were prepared as described in Chapter 4.1. The cooking process involved first bringing to boil 400 mL tap water in a 1.9 L stainless steel cooking pan. Flour (200 g) was then added to the boiling water and vigorously mixed to form a uniform product while heating on a hot plate maintained at medium heat for 10 min. This product was then allowed to stiffen by covering it in the cooking pan with a lid and heating on a hot plate set at medium heat for an additional 3 min. Ugali (40 g) was served

in glass ramekins using a 40 mL ice-cream scoop (Figure 6). The ramekin of ugali was immediately covered with aluminium foil and maintained at 50^{o}C on a food warmer before serving to the panellists. For each tasting session, four ugali samples representing each of the four flour samples were freshly cooked.

Descriptive sensory profiling of the ugali was performed based on the generic descriptive method described by Einstein (1991). A trained panel of eight female students aged between 24 and 48 years analyzed the ugali. Five of the panellists had at least 24 hr experience with descriptive sensory evaluation of other sorghum-based food products. Before participating in the sensory analysis, the panellists signed a consent form, which informed them about the nature of the food they would evaluate and the duration of the exercise. To prepare for sensory evaluation, the panellists participated in seven training sessions each lasting for 2 hr, during which they were familiarized with the product and developed descriptive terms and evaluation scales. Agreement was reached on 17 attributes, which described and differentiated the ugali types. These attributes described the appearance, aroma, texture, flavour and sensations after swallowing of the products (Table X). Sensory evaluation was done in two sessions. During each session, the four ugali types were blind-labelled with random 3-digit codes, and presented in a random order to each panellist in transparent glass ramekins. Deionised water and slices of carrots were provided to the panellists for cleansing their palates. The panellists handled the ugali with their fingers, the way ugali is normally eaten. Finger feel is an important quality parameter for ugali. Panellists assessed samples under red light in individual booths. Responses were entered directly into a computer system using Compusense software (Compusense® Five release 4.6, Compusense, Guelph, Ontario, Canada). Each ugali treatment was evaluated twice by each panellist giving 16 data points for each attribute per treatment.

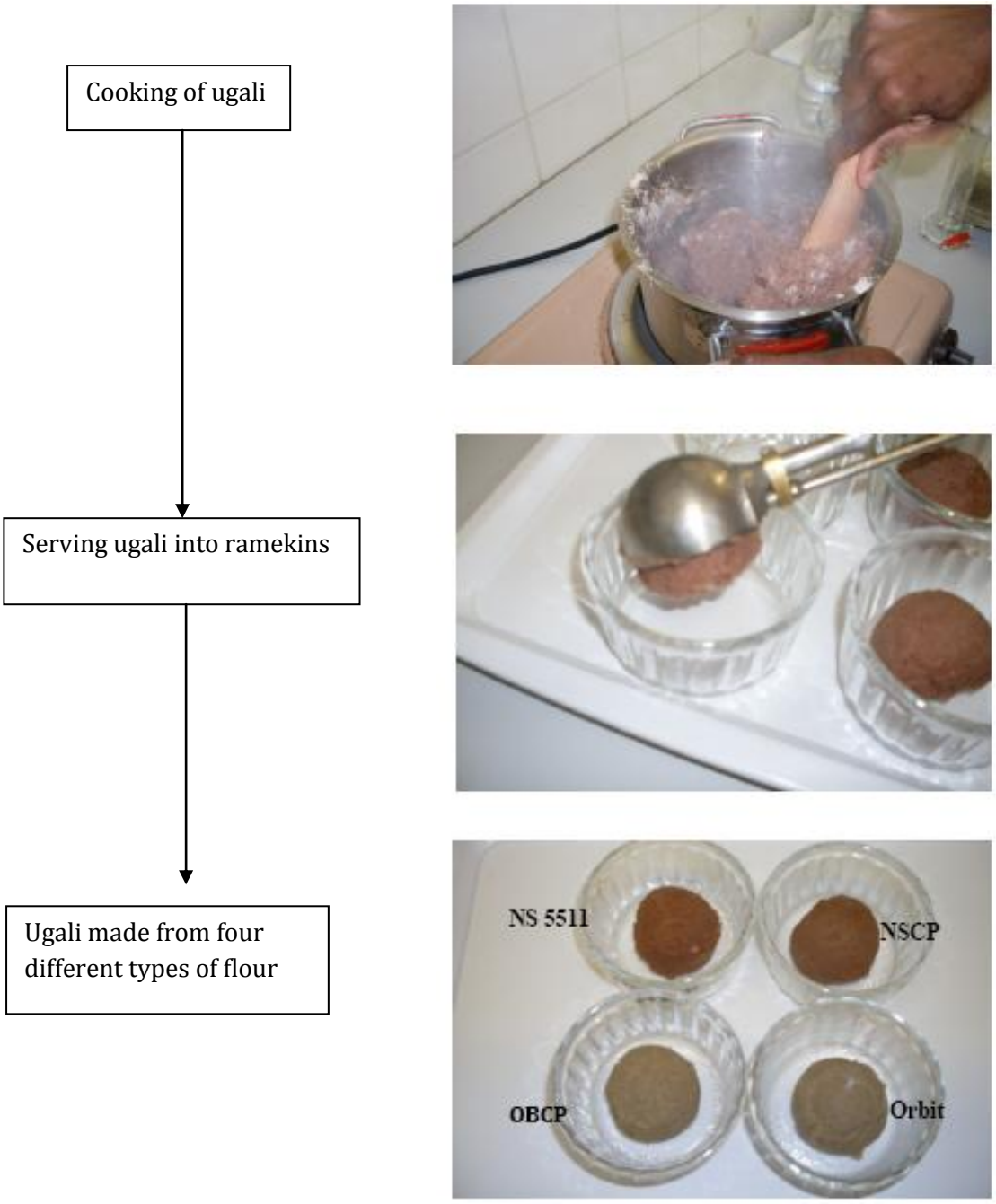


Figure 6: Flow diagram for preparation of ugali for sensory evaluation. NSCP, NS 5511+ Cowpea; OBCP, Orbit+Cowpea

Table X: Descriptive sensory lexicon used by a trained panel to evaluate ugali

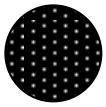
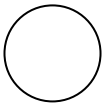
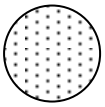
Sensory attribute	Definition	References to clarify and anchor sensory attributes	Rating scale
Appearance			
Colour intensity	Ugali colour ranging from cream white to dark amber/brown	0= thick white maize meal porridge (25% solids) (Super Sun, Premier Foods, Sandton, South Africa). 9 = thick red sorghum porridge (25% solids) (Fine Mabele, Tiger Consumer Brands, Bryanston, South Africa).	0 = Light 10 = Dark
White specks	Quantity of white specks visible on porridge	0=  10= 	0= None 10 = Many
Dark specks	Quantity of dark specks visible on porridge	0=  10= 	0= None 10 = Many
Roughness	The degree to which roughness could be perceived on the surface of ugali	0 = smooth peanut butter (Black Cat Tiger Consumer Brands, Bryanston, South Africa). 10 = porridge (coarse red sorghum porridge) (33% solids) (Coarse Mabele, Pride Milling, Nigel, South Africa).	0 = Not rough 10 = Very rough
Aroma			
Overall aroma intensity	Intensity of the aroma of the ugali		0= Not intense 10 = Very intense
Sorghum porridge aroma	Intensity of the aroma of cooked sorghum	10 = thick red sorghum porridge) (25% solids) (Fine Mabele, Tiger Consumer Brands, Bryanston, South Africa).	0= Not intense 10 = Very intense
Cooked-cowpea aroma	Intensity of the aroma of cooked cowpea	10 = Boiled whole cowpea (boiled for 80 min in excess water) (Bechuana white variety)	0=Not intense 10 =Very intense
Texture			
Firmness	Force required to compress a lump of porridge with fingers	0 = thin white maize meal porridge (10% solids) (Super Sun, Premier Foods, Sandton, South Africa). 10 = stiff red sorghum porridge (33% solids) (Fine Mabele, Tiger Consumer Brands, Bryanston, South Africa).	0 = Not firm 10 = Very firm

Table X continued

Sensory attribute	Definition	References to clarify and anchor sensory attributes	Rating scale
Springiness	Degree to which ugali sample returned to its original shape after compression with fingers	2 = thick white maize meal porridge (25% solids) (Super Sun, Premier Foods, Sandton, South Africa). 10 = white bread wheat dough (100 g flour + 75 mL water developed for 10 min) (Snowflake, Premier Foods, Sandton, South Africa).	0 =Not springy 10 =Very springy
Stickiness	The extent to which material adhered to fingers during normal handling	1= thick maize meal porridge (25% solids) (Super Sun, Premier Foods, Sandton, South Africa). 10= Cooked starch (25% solids) (40^{o}C) (Maizena, Bokomo Foods, Cape Town, South Africa)	0 = Not sticky 10 = Very sticky
Rough texture	The degree to which roughness could be perceived in the mouth while eating ugali	0 = smooth peanut butter (Black Cat, Tiger Consumer Brands, Bryanston, South Africa). 10= coarse red sorghum porridge (33% solids) (Coarse Mabele, Pride Milling, Nigel, South Africa).	0= Not rough 10= Very rough
Flavour			
Overall flavour intensity	Overall flavour intensity of ugali		0= Not intense 10 = Very intense
Cooked-cowpea flavour	Intensity of the flavour of cooked cowpea	10 = Boiled whole cowpea (boiled for 80 min in excess water) (Bechuana white variety)	0= Not intense 10= Very intense
Sorghum porridge flavour	Intensity of flavour of cooked sorghum	10 = thick red sorghum porridge (25% solids) (Fine Mabele, Tiger Consumer Brands, Bryanston, South Africa).	0=Not intense 10 =Very intense
Sensations after swallowing the sample			
Sorghum aftertaste	The intensity of cooked sorghum porridge flavour perceived in the mouth after swallowing.	10 = thick red sorghum porridge) (25% solids) (Fine Mabele, Tiger Consumer Brands, Bryanston, South Africa).	0= Not intense 10 = Very intense
Cowpea aftertaste	The intensity of a cooked cowpea flavour perceived in the mouth after swallowing.	10 = Boiled whole cowpea (boiled for 80 min in excess water) (Bechuana white variety)	0= Not intense 10 = Very intense
Powdery residue	The extent to which particles of the pericarp felt dry in the mouth after swallowing	10 = uncooked sorghum flour (Fine Mabele, Tiger Consumer Brands, Bryanston, South Africa).	0= None 10= Very much

4.2.2.6 Statistical analysis

Instrumental textural measurements were subjected to one-way analysis of variance (ANOVA) using flour type as the independent variable and textural parameters as the dependent variables. Panel mean scores of the attributes were subjected to a two-way ANOVA with samples and panellists as independent variables and ratings for ugali attributes as the dependent variables. Fisher's least significant differences (LSD) were used for comparison of means using Statistica software Version 8.0 (StatSoft, Tulsa, OK). Principal component analysis (PCA) was used to summarise and give more explanation of the variation in the sensory attributes of the stiff porridge (ugali).

4.2.3 RESULTS AND DISCUSSION

4.2.3.1 Textural properties of uji and ugali

Table XI shows PV (marking the equilibrium point between starch granule swelling and rupture) and the CPV (usually caused by retrogradation of amylose) of uji prepared from the different flours used in this study. Figure 7 shows the pasting profiles of fermented sorghum and sorghum plus cowpea slurries (uji). Compositing with cowpea reduced PV of NS 5511 uji and Orbit uji pastes by 6% and 23%, respectively. The reduction in PV is probably due to the increase in protein content with a concomitant decrease in starch content. As the increase in viscosity during heating is associated with pasting of starch (Batey and Curtin 2000), sorghum-cowpea composite uji was expected to have a lower PV than sorghum-only uji because of a decrease in starch content. In addition, protein content has been shown to influence the rheological changes of other cereals such as rice (Teo et al 2000). These authors noted that rice flour containing 8% protein (db), had a 21% lower PV than rice starch isolate with 0.5% protein (db).

Table XI: Effects of compositing sorghum with cowpea on the peak and cool paste viscosities of uji as measured using a Rapid Visco Analyser

Sorghum cultivar	Flour	Peak viscosity (centipoises)	Cool paste viscosity (centipoises)
NS 5511 (red, tannin sorghum)	Sorghum	1865^{c}±14	2227^{c}±13
	Sorghum+Cowpea	1749^{b}±20	1965^{a}±20
Orbit (white-tan plant, non-tannin sorghum)	Sorghum	2168^{d}±20	2073^{b}±14
	Sorghum+Cowpea	1664^{a}±10	1946^{a}±12

Values are means ±standard deviations. Values in a column followed by different superscript letters are significantly different at $p \leq 0.05$.

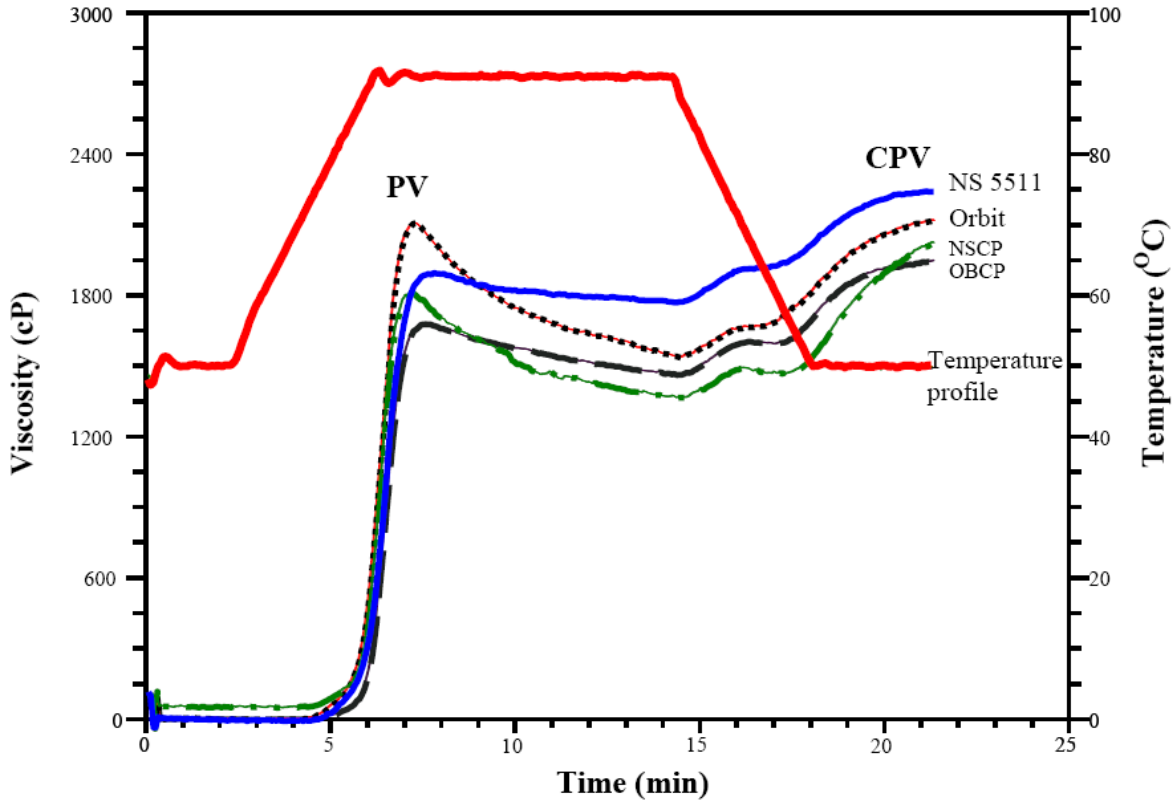


Figure 7: Effects of compositing sorghum with cowpea on the pasting properties of fermented uji slurries as measured using a RVA. Curves are representative of each type of uji. NSCP, NS 5511+Cowpea; OBCP, Orbit+Cowpea; PV, peak viscosity; CPV, cool paste viscosity.

It appears that there was inconsistency in the reduction paste PV of uji after compositing the two sorghum cultivars, not reflecting the fact that equal proportions of cowpea were used in the preparation of the composite flours. This observation appears to suggest that starch content alone may not explain differences in viscosity of food samples, an opinion shared by other workers on starch. For example, Hansen et al (1991) studying the rheological properties of starch-water systems, found differences in rheological characteristics even within starch-water systems of equal concentrations. In the present study, the variations in the food matrices of the different fermented flour suspensions may have played a role in the differences in observed PV. This is because the two sorghum cultivars possessed different physicochemical qualities such as grain endosperm texture (Figure 4), tannins (Table V) and protein (Table VI) contents.

Compositing reduced CPV of NS 5511 uji and Orbit uji by 12% and 6%, respectively, probably because of increase in protein content on addition of cowpea, which is rich in protein. Protein content has been shown to affect CPV of starch. For example, Zhang and Hamaker (2003) investigating a three way interaction based on a model starch pasting system where normal sorghum starch was the primary component and protein and free fatty acids were the minor components, reported a lower CPV from pasting profiles of starch in the presence of whey protein compared to starch-only. It is believed that the proteins may interact with the C-2 and C-3 hydroxyl groups of glucose units through H-bonding and prevent intermeshing of amylose and amylopectin helices (reviewed by Preston 1998). This theory was supported by Eliasson (1983), who used differential scanning calorimetry (DSC) to show that addition of gluten to gelatinized starch decreased starch crystallization during ageing at 21^{o}C.

Table XII: Effects of compositing with cowpea on the firmness and stickiness of ugali as determined by a TA-XT2 Texture Analyser and descriptive sensory panel (DSP)

Sorghum cultivar	Flour	Firmness		Stickiness	
		TA-XT2 (N)	DSP (Score)	TA-XT2 (N)	DSP (Score)
NS 5511 (red, tannin sorghum)	Sorghum	14.43^{d}±0.84	6.25^{c}±1.28	2.17^{b}±0.76	4.17^{a}±1.98
	Sorghum+Cowpea	11.56^{c}±0.74	6.46^{c}±1.10	0.96^{a}±0.16	3.92^{a}±1.77
Orbit (white-tan plant, non-tannin sorghum)	Sorghum	6.22^{a}±0.30	4.88^{a}±1.52	3.62^{c}±0.01	5.88^{b}±1.69
	Sorghum+Cowpea	9.04^{b}±0.14	5.70^{b}±1.31	3.57^{c}±0.30	5.28^{b}±1.61

The values are Means ±Standard deviations. Values in a column followed by different superscript letters are significantly different at $p \leq 0.05$

DSP score Firmness 0 = Not firm; 10 = Very firm

Stickiness 0 = Not sticky; 10 = Very sticky

Table XII shows that except for the NS 5511 ugali texture data from the TA-XT2 Texture Analyser, compositing increased ugali firmness. The firmness of Orbit ugali was increased by 45% and 14% as assessed by a texture analyser and a sensory panel, respectively. However, compositing Orbit ugali had no substantial effect on stickiness. Increase in firmness of ugali after compositing appears to be inconsistent with the CPV reduction noted for uji prepared from sorghum-plus-cowpea. The apparent inconsistency may be explained by two factors. Firstly, the difference in protein contents of the foods, which may be attributed to the differences in solid contents of the foods (about 10% solids in uji compared to 25% and 33% solids in ugali for instrumental and sensory panel texture assessment, respectively). To form a self-supporting protein network, a minimum protein concentration is required (Damodaran 1996b; Acton et al 1981). Below the minimum protein concentration, instead of forming an ordered network, the thermally unfolded polypeptide chains may not form extensive protein network. In the present study, it appears that the low solid content in uji probably resulted in a dilute mixture with low protein concentration that could not form an extensive protein network. Therefore CPV of uji probably depended mostly on amount of retrograded starch. The opposite case may have occurred in ugali because of its relatively higher solid content and hence higher protein concentration. Significance of the difference in solid content between uji and ugali on texture may also be explained by viscosity cross-over phenomenon. This theory was proposed by Steeneken (1989) when studying the rheological properties of aqueous suspensions of swollen starch granules. According to this theory, in dilute systems, the viscosity is proportional to the volume fraction of the swollen particles, which depends on the swelling capacity, whereas, in concentrated systems, the viscosity is determined by particle rigidity. However, network density and gel rigidity are linearly related while there is an inverse relationship between network density (and particle rigidity) and swelling capacity. Therefore, in general, in the dilute regime the higher swelling starches are the more viscous, whereas in the concentrated regime the lower swelling starches are more viscous.

Secondly, is the issue of the differences in pH of the different foods. As explained by Damodaran (1996b) only at optimum pH, which permits an optimum balance of protein-protein and protein-solvent interactions, may uniform strong extensive networks be formed. For most proteins, the optimum pH is about 7 to 8. Because uji was fermented giving it acidic pH, its proteins would assume a net positive charge. This net positive charge may inhibit extensive protein network formation by electrostatic repulsion therefore, probably inhibiting the role of protein network formation in contributing to CPV of uji.

4.2.3.2 Other sensory properties of ugali

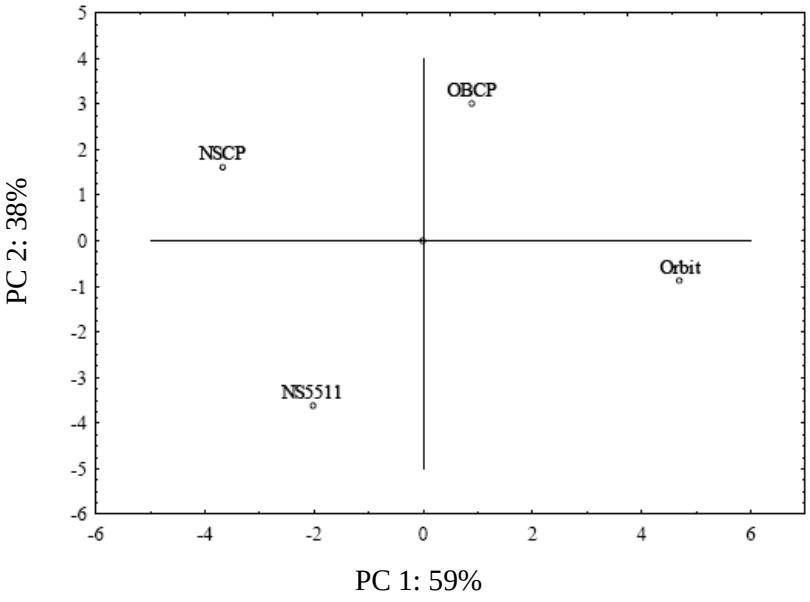
Analysis of variance (ANOVA) F-values were significant ($p<0.05$) for all the 17 sensory attributes ugali (Table XIII), indicating that the panellists were able to differentiate ugali prepared from the different types of flour using the descriptive terms selected.

Principal component analysis (PCA) (Figure 8) was used to summarise and give more explanation of the variation in the sensory attributes of the ugali prepared from different types of flour. The first two principal components (PC1 and PC2) accounted for about 97% of the total variation in the 17 sensory attributes of ugali. These were used to explain relationships between the variables. PC1, which accounted for 59% of the variation, separated ugali samples in terms of the sorghum cultivar used in their preparation. Ugali made from red, tannin sorghum (NS 5511 and NSCP) (Figure 8a) were perceived as darker in colour, stiffer, more cohesive, less sticky, springier, rough textured, more strongly flavoured, with more white specks and had more powdery residue (Figure 8b). Ugali types displayed on the right of the score plot (Figure 8a), prepared from Orbit and Orbit-plus-cowpea flour, were characterised by stickiness and dark specks, and were generally less firm and lighter in colour (Figure 8b). These sensory texture results for firmness and stickiness are consistent with findings from instrumental texture analysis, which indicated that ugali prepared from NS 5511, and NSCP flour were less sticky but more firm than Orbit and OBCP ugali (Table XII).

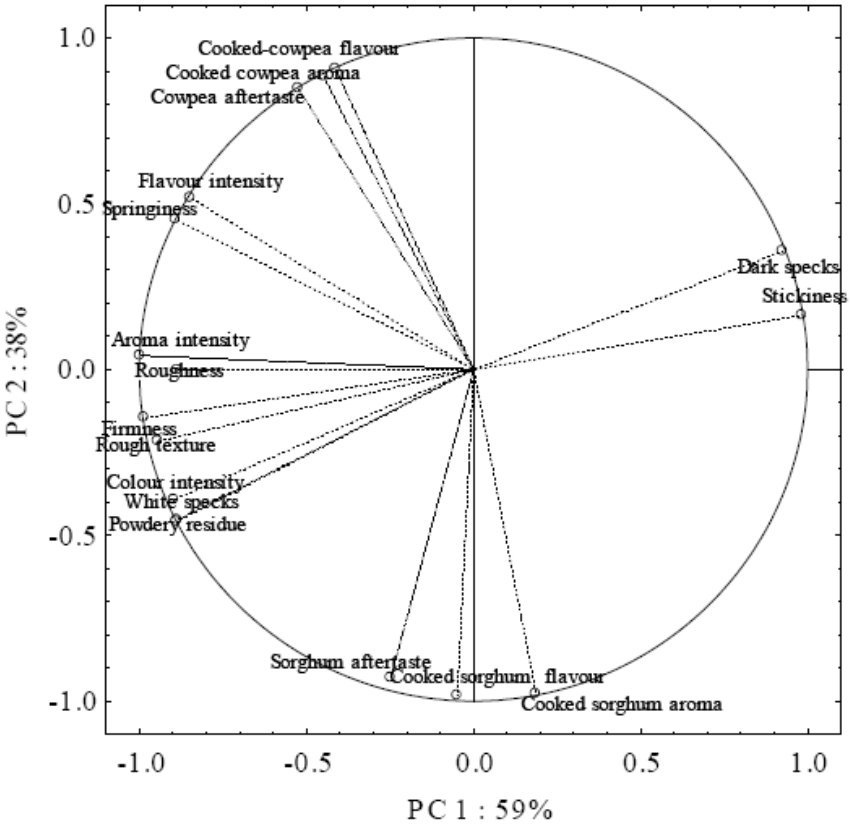
Table XIII: Effects of compositing sorghum with cowpea on the mean scores of sensory attributes of ugali as evaluated by a trained sensory panel

Attribute	Sorghum cultivar				Sample effect[#]	Panellist effect[#]
	NS 5511(red, tannin)		Orbit (white-tan, plant)			
	Sorghum flour	Sorghum+Cowpea flour	Sorghum flour	Sorghum+Cowpea flour		
Colour intensity	7.2^{b} ±1.2	7.3^{b}±1.0	3.9^{a}±0.9	4.0^{a}±0.9	165.5*	8.4*
White specks	4.6^{b}±1.5	4.4^{b}±1.6	2.2^{a}±1.6	2.4^{a}±1.4	10.6*	0.7
Dark specks	1.9^{a} ±1.2	2.3^{a}±1.9	4.9^{c}±1.7	3.9^{b}±2.0	19.0*	4.8*
Roughness	4.5^{ab} ±1.0	5.0^{b}±0.9	4.1^{a}±1.0	4.1^{a}±1.0	3.6*	1.3
Aroma intensity	6.4^{bc}±0.9	6.6^{c}±0.7	5.2^{a}±1.5	5.9^{b}±1.4	8.6*	3.8*
Sorghum porridge aroma	6.0^{b}±0.9	5.0^{a}±1.1	5.7^{b}±1.0	4. 8^{a}±1.1	6.0*	1.7
Cooked cowpea aroma	2.1^{a} ±1.5	5.0^{b}±2.0	2.0^{a}±1.4	5.1^{b}±1.8	19.3*	1.5
Firmness	6.2^{c}±1.3	6.4^{c}±1.1	4.9^{a}±1.5	5.7^{b}±1.3	14.7*	9.8*
Springiness	4.9^{b} ±1.8	5.9^{c}±1.2	4.0^{a}±1.7	5.1^{bc}±1.5	7.7*	5.2*
Stickiness	4.2^{a} ±2.0	3.9^{a}±1.8	5.9^{b}±1.7	5.3^{b}±1.6	8.4*	4.6*
Rough texture	4.9^{c}±1.0	4.6^{c}±1.2	3.1^{a}±0.7	4.0^{b}±1.2	14.2*	2.9*
Overall flavour intensity	5.7^{b} ±1.2	6.8^{c}±0.6	4.5^{a}±1.7	6.3^{bc}±1.2	18.9*	4.5*
Sorghum porridge flavour	6.4^{c}±0.8	4.9^{ab} ±1.3	5.5^{b}±1.3	4.8^{a}±1.2	9.4*	2.8*
Cooked cowpea flavour	2.0^{a} ±1.3	5.5^{b}±2.0	1.6^{a}±1.1	5.8^{b}±1.4	46.9*	2.0*
Sorghum aftertaste	6.1^{b}±1.2	4.3^{a}±1.7	4.6^{a}±1.4	4.1^{a}±1.5	12.1*	4.7*
Cowpea aftertaste	1.7^{a}±1.2	4.8^{b}±2.1	1.2^{a}±0.8	4.7^{b}±1.6	34.1*	2.8*
Powdery residue	5.0^{b}±1.6	4.4^{b}±1.7	2.0^{a}±1.0	2.4^{a}±1.1	18.3*	0.9

Values are means±standard deviations. Values in a row followed by different letter notations are significantly different at $p \leq 0.05$
[#]F-value
*$p<0.05$



(a)



(b)

Figure 8: Principal component analysis of ugali samples prepared from four different flours: NS 5511, a red, tannin sorghum; NSCP, NS 5511+Cowpea; Orbit, a white tan plant, non-tannin sorghum; OBCP, Orbit+cowpea. (a) Plot of the first two principal component scores of ugali (b) Plot of the first two principal component loading vectors for sensory attributes of ugali

PC2, which accounted for 38% of the variation, separated the samples based on presence or absence of cowpea in the ugali. Ugali prepared from sorghum-plus-cowpea (NSCP and OBCP) were associated with springiness, more intense cooked cowpea flavour and aroma, cowpea aftertaste, lower cooked sorghum flavour and stronger overall flavour intensity. The beany flavour (described by panellists as cowpea flavour) appeared to be the most important attribute characterizing ugali made from sorghum plus cowpea. Beany flavour is typical of many legumes. It may be attributed to the action of lipoxygenase enzyme, which catalyzes the formation of odorous carbonyl compounds (pentyl furans) from components containing a cis-1,4-pentadiene system (reviewed by Okaka and Potter 1979). An example of such pentyls identified in soybean is 2-pentenyl furan (Chang 1979). The dark colour intensity of NS 5511 foods is probably due to staining of the porridges by phenolic pigments (anthocyanins) present in the pericarp of red sorghum grain (Hahn et al 1984; Beta et al 1999). Sorghum grain colour is associated with pigmented testa (if present) and the pericarp of the sorghum kernel, which varies in thickness and pigmentation colour depending on the sorghum type (Rooney and Miller 1982; Awika et al 2005). An attribute that was uniquely more prominent in ugali made from NS 5511 was the perception of powdery residue. High scores for this attribute probably indicate mouth-puckering (dry sensation) effect of tannins (Prinz and Lucas 2000). As explained by these authors, tannins reduce the lubricating qualities of human saliva by both decreasing its viscosity and increasing friction. These changes probably occurred in the NS 5511 (tannin-sorghum) ugali causing the powdery and dry sensations noted by the panellists.

4.2.3.3 Textural properties of injera

Compositing increased the stiffness measured as bending force, of NS 5511, while it reduced stiffness of Orbit injera (Table XIV and Figure 9). These differences in the effects of compositing with cowpea on stiffness of injera may be explained by the differences in tannin contents of the two sorghum cultivars. Tannins have a high propensity to complex with proteins (Hagerman and Butler 1980) and it has been shown that tannins interact with proteins through weaker bonds H-bonds (Orliac et al 2002). Compositing with cowpea, which had higher protein content and low tannin content, reduced the tannin content of the NS 5511 flour by 16% (Table V), thus probably minimizing the protein network-weakening effect of tannins. In the case of Orbit injera, an important difference between sorghum-cowpea and sorghum-only injera is probably their protein contents. As compositing increased

the protein content of the injera, this may have inhibited the role of starch, a primary component in staling (particularly amylose molecules), and hence lowering maximum bending force of the Orbit-cowpea composite injera. This explanation accords with observations by Kim and D'Appolonia (1977) in the context of their study on the effect of protein content on the role of starch in bread staling. These authors observed that the effect of amylose on bread staling diminishes as the flour protein content increases.

Table XIV: Effects of compositing sorghum with cowpea on the maximum force (N) required for bending injera stored at 25°C over a period of two days as measured using a TA-XT2 Texture Analyser

Sorghum cultivar	Flour	Storage time		
		1 hr	24 hr	48 hr
NS 5511 (red, tannin sorghum)	Sorghum	0.83^{ab}±0.01	1.08^{d}±0.02	1.22^{e}±0.09
	Sorghum+Cowpea	0.87^{b}±0.05 (5)	1.33^{f}±0.03 (23)	1.53^{g}±0.05 (20)
Orbit (white-tan plant, non-tannin sorghum)	Sorghum	0.99^{c}±0.07	1.58^{g}±0.03	1.77^{i}±0.03
	Sorghum+Cowpea	0.77^{a}±0.02 (-22)	1.37^{f}±0.07 (-13)	1.66^{h}±0.03 (-6)

Values are Means ±Standard deviations. Values followed by different superscript letters are significantly different at $p \leq 0.05$.

Values in the brackets are percent increases in maximum bending force. Negative values indicate decreases

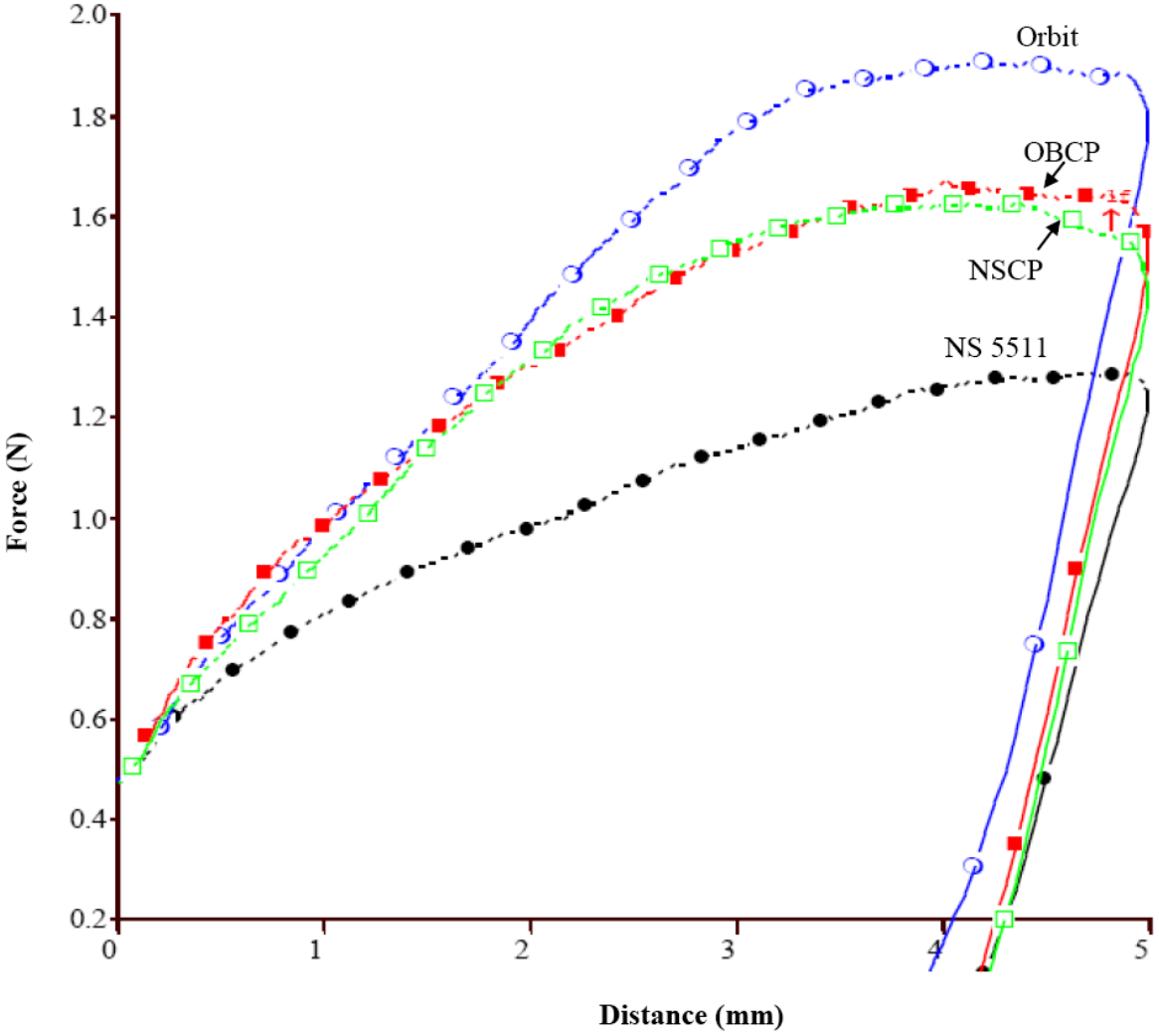


Figure 9: Effects of compositing with cowpea on the force required to bend injera stored at 25°C for 48 hr measured using a TA-XT2 Texture Analyser. Each curve is an average of six plots. NSCP, NS 5511+Cowpea; OBCP, Orbit+Cowpea

As expected there was an increase in stiffness of injera over time due to staling. Similar observations were made by Yetneberk et al (2004). The beginning of staling is normally associated with retrogradation of gelatinized starch (Kulp and Ponte 1981). In addition, Martin et al (1991) working on a model for bread firming incorporating roles of gluten and starch as influencing factors, suggested that cross-linking (H-bonding) between the protein matrix and the discontinuous remnants of starch granules during storage could contribute to bread firming. Martin and Hoseney (1991) made a similar observation when they evaluated the role of starch hydrolysis on bread firming. It was noted that Orbit injera was stiffer than the NS 5511 injera as depicted by the maximum bending force (Figure 9). A possible explanation may be the differences in tannins contents, which as explained previously, has a weakening effect on protein networks.

4.2.4 CONCLUSIONS

The textural properties of traditional sorghum foods are not substantially affected by compositing with cowpea at 70:30 ratio. These attributes are largely dependent on the sorghum grain endosperm texture and chemical composition, which are characteristic of the sorghum cultivar used in the food preparation.

Consumers of traditional sorghum foods are normally used to particular sorghum flavours and colours of the sorghum foods. Therefore, to improve the protein quality of such sorghum foods through compositing with cowpea, consumers need to be willing to accept cowpea flavour as part of their sorghum-based diets as it strongly characterizes foods composited with cowpea. Alternatively, it might be important to devise mechanisms of masking or eliminating such legume flavour in a manner that minimizes its sensation in traditional sorghum food products without compromising their protein quality.

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5 GENERAL DISCUSSION

5.1 METHODOLOGY: A CRITICAL REVIEW

Two sorghum cultivars with different tannin contents were used in the research for comparison because tannins are known to bind proteins thus reduce the nutritional quality of sorghum foods (Butler et al 1984; Serna-Saldivar and Rooney 1995). In addition, tannins have been shown to affect sensory properties of sorghum products such as injera (Yetneberk et al 2005); sorghum bran infusions and sorghum rice (Kobue-Lekalake et al 2007). Furthermore, some communities consume tannin sorghum foods (Aboubacar et al 1999), therefore making the use of tannin sorghum in this study to be of practical relevance.

A possible limitation in the research was that only sorghum from a single season per cultivar was used. Therefore, the study could not establish any possible effects of seasonal variations on the quality of foods prepared from the different sorghum cultivars. Effects of environmental conditions and seasons may be important factors that affect sorghum food quality as was explained by Rooney and Miller (1982). These authors noted that grain characteristics substantially depend on the growing environment. However, the effects of seasonal variations may affect absolute values but not the trends. For example, a study by Yetneberk et al (2004) on the effects of sorghum cultivar on injera quality showed similarities in the trends for the more important sensory attributes across two growing seasons.

Three different types of traditional sorghum foods were selected based on their importance in the diets of the target populations and the differences in cooking methods. Cooking methods are known to affect the protein nutritional quality and functional properties of sorghum foods, as reviewed in sections 2.3 and 2.6, respectively. Most sorghum for human use is consumed as flatbreads and porridges (FAO 1995), justifying the choice of the three types of foods used in the present study.

A possible area of contention is that preparation of injera was slightly modified from the typical protocol mainly by the addition of small amounts of sugar and yeast to enhance fermentation. This appeared to alter the chemical composition of the product thus comparing injera with the other foods may not be ideal. However, these modifications were considered appropriate offering a good control of injera preparation process. In addition, by preparing

injera from different types of flour using the same procedure, it was considered that any differences noted in quality would be mainly attributed to the effects of type of flour used.

Another possible area of concern may be differences in time of cooking for ugali used for protein quality assessment (1 min) and for sensory evaluation (13 min), as cooking time is known to affect protein quality (Undi et al 1996). However, the values obtained for protein quality parameters are an indication of effects of compositing with cowpea on the quality of the foods and are not necessarily absolute.

Not all foods were subjected to sensory evaluation using a trained panel, which could have provided more information on interactions between compositing with cowpea and cooking methods on sensory properties of a wider range of sorghum foods. However, because of importance porridge in the menu of many communities in sub-Saharan Africa (FAO 1995) and simplicity of its preparation, unfermented stiff porridge (ugali) was considered an appropriate choice that would provide information on food quality that may be of relevance to a large population.

Reactive lysine content was measured as a means for determining lysine availability of the foods, which is regarded to reflect closely the quantity of lysine available for utilization by the body. The dye binding lysine (DBL) method was used to determine the reactive lysine contents of the foods. This method is generally more rapid, convenient for routine use and less expensive than other chemical methods (section 2.5.2). The method's drawback, however, lies in differences in affinity of a dye for several proteins that react or bind differently depending on their structural characteristics (reviewed by Kolakowski 2005). This virtually requires that the method be adjusted to a given sample each time it is applied, ruling out its use as a universal procedure for reactive lysine assay. Howver, as stated in Chapter 4.1, preliminary tests were done to determine the suitable amounts of each sample to be used for analysis.

Protein digestibility may be used as an indicator of protein quality as it is a measure of the susceptibility of a protein to proteolysis during digestion. As discussed previously, a protein with high digestibility is potentially of better nutritional value than one of low digestibility because it would provide more amino acids for absorption after proteolysis. The in vitro pepsin digestion method was used in this research. It is considered reasonably reliable, as it

appears to provide results, which parallel those in human protein digestibility studies (Maclean et al 1981; Mertz et al 1984).

Having established that compositing improved the protein quality of the foods, it was deemed vital to assess the influence this approach would have on the functional characteristics of the foods. The primary focus in functional quality assessment was the texture of the foods, which is considered a major sensory attribute that affects consumer acceptance of sorghum foods (Aboubacar et al 1999). The parameters for assessing textural properties of the foods were different as they depend on the type of food. This is because different types of traditional sorghum foods have their unique quality attributes depending on the preferences of target communities (Murty and Kumar 1995; Rooney and Waniska 2000). Generally, a stiff porridge such as ugali is preferred when it is firm and not sticky (Kebakile 2008; Bello et al 1990; Da et al 1982). A semi-solid consistency is preferred for thin porridges such as uji especially when intended for children (Lorri and Svanberg 1993a). A flatbread such as injera is preferred when it is soft, rollable and fluffy (Yetneberk et al 2004). In addition, flavour and colour may play significant roles in acceptability of sorghum foods (Zegeye 1997).

As explained in Chapter 4.2, PV and CPV were determined using a RVA. PV is often correlated with final product quality parameters such as water binding capacity and an indication of the viscous load likely to be encountered by mixing during cooking (Newport Scientific 1995). In this work, CPV was an indication of the capacity of the uji to form a viscous paste after cooking and cooling, and may be useful in assessing the textural quality of the uji at the point of consumption. The use of RVA has an advantage of being a rapid method that cooks the product while the viscosity profile at every cooking stage is displayed and recorded simultaneously. However, its drawback may be that it is not an ideal equipment for determining absolute viscosity of a gruel such as uji. As explained by Mouquet and Trèche (2001), in a gruel, which is a non-Newtonian fluid, viscosity can vary as a function of shear time and shear rate and so it is the apparent viscosity which is linked to the measurement conditions. However, the RVA serves well to provide a qualitative indication of effects of treatments used in this study on viscosity of uji.

Stiffness of injera was assessed using a TA-XT2 Texture Analyser with a three point bending rig. This method is a quick way of determining the effect of compositing with cowpea on softness of injera. It does not, however, provide more information on the actual mechanical

strength of injera which affects the handling of injera. Injera may be soft but breaks easily therefore not rollable. Rollability is a fundamental functional quality of injera (Yetneberk et al 2004).

Firmness and stickiness of ugali were measured using a TA-XT2 Texture Analyser with a Perspex cylindrical probe attachment. There was an enormous challenge using this method as penetration force depends on how uniform the ugali is filled in the sample tube. Getting repeatable results was thus quite tricky. The ugali assessed using instrumental texture analysis had to be of lower solid content (25% solid) compared to those analysed by a trained sensory panel (33% solid), in order to obtain reasonably repeatable readings. A general drawback of all instrumental measurements is that they are restrictive as there are always a limited number of parameters involved, whereas sensorial assessments are based on visual and tactile impressions of the food products prior to and during their consumption (Mouquet and Trèche 2001). Descriptive sensory evaluation was used to determine the sensory attributes of ugali. This method has the advantage of being a specialized form of sensory analysis that may include all sensory parameters of the food or it can be limited to certain aspects of interest as in flavour or texture profiling (Einstein 1991). It is, however, time consuming and expensive to train and maintain a trained panel. Furthermore, the performance of a trained panel depends on the individual abilities which are physiologically governed (Fischer et al 1994).

ANOVA showed that there were significant panellist effects for most of the attributes evaluated (Table XIII). This was expected and it accords with other sensory studies. For example, Kebakile et al (2007) investigating sensory properties of thick porridges from different sorghum types milled by different milling methods made a similar observation. Similarly, Lapveteläinen and Rannikko (2000) studying changes in the sensory properties of oatmeal due to cooking conditions, noted a substantial effect of panellists on the results. A possible reason for the significant effects of panellists as explained by Fischer et al (1994) is that panellists have individual physiological qualities, which influence their perception of stimuli. As an example, these authors noted that salivary flow rate affects the perception of stimuli taste by titration, dilution, or precipitation of stimuli. Thus, individuals who have low-flow would take a longer time to reach maximum intensity and may take a longer duration perceiving the same intensity than high-flow subjects. Panellist effects are also due to the relative differences in the use of different parts of the scale as was found by Kobue-Lekalake

(2008), studying the effects of phenolics in sorghum on its sensory properties. This author noted that some panellists routinely use the upper part of the scale while others use the lower end. This results in large standard deviations therefore affecting the results. Nonetheless, it was noted that, despite human errors, the sensory panel used in the current study, clearly differentiated the ugali samples. Therefore, panellist effects would not nullify the results.

5.2 COMPARATIVE EFFECTS OF COMPOSITING WITH COWPEA ON SORGHUM FOOD QUALITY

As reported in Chapter 4.1, compositing sorghum with cowpea at 70:30 ratio produced different levels of improvements in the protein quality of the foods. Protein quality improvement was indicated by increase in lysine content, lysine availability, protein digestibility and protein content. The differences noted were dependent on the sorghum cultivar. A reason for the improvements was suggested to be addition of lysine-rich globulins from cowpea. As cowpea is richer in protein than sorghum (Table VI), compositing was also accompanied by increase in protein content of the foods evaluated. This increase in protein content may be important in the protein nutritional quality of the food as more protein would be available for digestion. Protein digestibility is paramount in the realization of the goals of achieving lysine sufficiency in the diet (Schaafsma 2000).

Another reason for the improvement in protein quality after compositing may be that it was accompanied by significant reductions in measurable tannin content, particularly in case of NS 5511 foods (Table V). This may be important, as tannins are known to bind proteins thus reducing their protein nutritional quality by making them inaccessible to proteolytic enzymes, was explained previously. The tannin content of sorghum grain appeared as an important factor that contributed to the results on protein quality because it was a main fundamental difference between the two sorghum cultivars used in this study. Foods that were processed from tannin sorghum type (NS 5511) showed lower levels of protein quality than those prepared from tannin free sorghum type. This was indicated by the higher PDCAAS of Orbit foods (0.21 to 0.60) compared to the corresponding NS 5511 foods (0.09 to 0.41) (Table XV). In addition, compositing with cowpea was accompanied by a decrease in the content of amino acid, proline (Table VIII). Proline has been associated with enhancement of protein-tannin complexing (Butler et al 1984; Emmambux and Taylor 2003) therefore inhibiting protein digestibility. As explained by Butler et al (1984), proline residues disrupt the α-helix,

in which the peptide carbonyl oxygens and amide hydrogens are all internally hydrogen bonded. Therefore, proline-rich peptides tend to form open structures with carbonyl and amide groups extending into the solvent thus enhancing hydrogen bonding between tannin molecules and the peptide backbone of the protein and nonpolar interactions. In addition, a study by Emmambux and Taylor (2003) showed a linear increase for tannins bound to proteins with increase in tannin content. Therefore, the reduction in tannin content of NS 5511 foods after compositing with cowpea may have greatly reduced protein-tannin complexing thus increasing their protein digestibility. These arguments are supported by the apparently higher percent increase in PDCAAS values of NS 5511 foods after compositing with cowpea than Orbit foods (Table XV).

Because of similar short cooking times used in the present study, foods composited with cowpea did not show substantial differences in protein quality among themselves. However, there were substantial effects of cooking on protein quality indicated by changes in PDCAAS values compared to corresponding raw flours (Table XV). For example, cooking reduced the PDCAAS of NS 5511 foods by between 18% and 52%. Except for injera, the PDCAAS of Orbit foods were reduced by between 14% and 29%. This may be because cooking is known to reduce the available lysine, as was found by Smith and Friedman (1984) when they modelled different cooking conditions to study their effects on available lysine in casein and carbohydrates mixtures. Lysine is the essential amino acid most sensitive to heat because of its highly reactive ε-amino group (Moughan and Rutherfurd 2008). The involvement of an ε-amino group in a chemical reaction such as Maillard reaction, in which this functional group is utilized, is regarded as a main way through which the nutritional quality of lysine is lost (Nursten 1981). With regard to foods made from NS 5511 sorghum, the presence of tannin may have reduced the PDCAAS further, as tannins inhibit protein digestibility (Butler et al 1984).

As was explained in Chapter 4.1, cooking also reduces protein quality of sorghum foods by reducing protein digestibility mainly through disulphide cross-linking of sorghum proteins into polymeric units making them less susceptible to enzymic attack (Hamaker et al 1987; reviewed by Duodu et al 2003). It was noted that the effect of compositing sorghum uji and ugali with cowpea on their protein quality, as indicated by PDCAAS (Table XV), was virtually the same. Compositing with cowpea increased the PDCAAS of NS 5511 ugali and uji by 271% and 276%, respectively. The PDCAAS of Orbit ugali and uji increased by 93%

and 87%, respectively after compositing. These apparent similarities are probably because a possible nutritional gain through fermentation may be lost by preferential utilization of lysine by lactic acid bacteria for growth and metabolism, in a similar manner to that proposed by Onyango et al (2004).

Table XV: Effects of compositing sorghum with cowpea on the protein quality of sorghum ugali, uji and injera

Cultivar	Food / Raw sample	Flour	Lysine content (mg/g protein)	Protein Digestibility (%)	Lysine score*	PDCAAS	PDCAAS increase after compositing (%)	PDCAAS (% of raw) #
NS 5511	Raw	Sorghum	16.2	61.8	0.31	0.19	160	
		Sorghum+Cowpea	34.3	76.0	0.66	0.50		
	Ugali	Sorghum	15.8	32.6	0.30	0.10	271	51
		Sorghum+Cowpea	33.7	56.7	0.65	0.37		73
	Uji	Sorghum	12.5	38.8	0.24	0.09	276	48
		Sorghum+Cowpea	29.9	61.0	0.58	0.35		70
	Injera	Sorghum	18.8	40.7	0.36	0.15	178	76
		Sorghum+Cowpea	34.0	62.5	0.65	0.41		82
Orbit	Raw	Sorghum	19.0	80.6	0.37	0.29	93	
		Sorghum+Cowpea	34.5	85.8	0.66	0.57		
	Ugali	Sorghum	20.5	64.6	0.39	0.25	87	86
		Sorghum+Cowpea	34.3	72.2	0.66	0.48		84
	Uji	Sorghum	15.9	68.3	0.31	0.21	111	71
		Sorghum+Cowpea	29.7	77.0	0.57	0.44		77
	Injera	Sorghum	22.8	76.4	0.44	0.33	80	114
		Sorghum+Cowpea	39.2	79.8	0.75	0.60		106

* Based on 52 mg/g protein requirement for a 1 to 2 year-old child (WHO 2007)

PDCAAS – Protein Digestibility Corrected Amino Acid Score

PDCAAS of food as percent of PDCAAS of corresponding raw flour.

Injera appeared to have better protein quality than the other types of traditional foods used in the present study. This is probably due to the addition of yeast during the second stage of fermentation in the preparation of injera. As explained previously, yeasts are rich in high quality protein (USDA 2008), and they may increase the protein quality of the foods in which they are utilised.

Compositing reduced the paste PV and CPV of uji by 6.2% in NS 5511 and 23% in Orbit. The CPVs were reduced by 12% and 6% for NS 5511 and Orbit uji, respectively. The reduction in PV, as explained in Chapter 4.2, was probably due to a reduction in starch content by addition of protein-rich cowpea. This would reduce the amount of starch granule swelling during pasting. It is also possible that high protein content enhanced protein-starch interactions thus lowering the peak viscosity, as was suggested by Teo et al (2000). In addition, Sun et al (2008) studying the influence of protein on the rheological properties of rice, suggested that proteins may compete with the starch in binding with water and decrease the swelling and collapsing of the starch granules and the leaching of amylose thus affecting pasting properties of starch. The paste PV of NS 5511 uji was 14% less than that of Orbit sorghum uji. The differences may be explained by two reasons. Probably most important is the effect of the high tannin content of NS 5511. Tannins, like similar phenolic compounds, are known to reduce pasting viscosity and cold paste viscosity of cereal starch, as was reported by Zhu et al (2008). These authors suggested that the functional groups (methoxy and hydroxyl) of phenolic compounds may interact with amylose and amylopectin through hydrogen bonding and van der Waal's forces, reducing the ability of starch granules to swell. In addition, these authors proposed that as phenolic compounds compete with starch in binding water molecules to form their hydrated forms, they probably reduce the water content of the system, thereby affecting starch gelatinization.

The differences in paste PV of uji may also be attributed to the differences in protein contents of the two sorghums. NS 5511 uji had higher protein content (11.2 g/100 g), than Orbit (8.8 g/100 g). Protein content has been shown to influence the rheological changes associated with aging of rice (Teo et al 2000). CPV of NS 5511 uji was slightly higher (7.4%) than that of Orbit uji (Table XI) The difference may be attributed to the differences in protein content of the two sorghum cultivars. It appears that the relatively higher protein content of NS 5511 may have enhanced cross-linking by H-bonding between the protein matrix and the discontinuous remnants of starch granules during cooling in a similar way explained by

Martin et al (1991), causing higher CPV. The fact that CPV values of uji made from sorghum-plus-cowpea are apparently slightly lower than corresponding sorghum-only uji indicates that compositing sorghum uji with cowpea would have little effect on the uji viscosity at consumption. High viscosity is considered a negative attribute in a thin porridge such as uji because it affects its consumption by children (Ljungqvist et al 1981). This study shows that, there would be some slight reduction in viscosity of uji after compositing with cowpea, which may offer a slight advantage for feeding young children. As was noted by Stephenson et al (1994) in their study on the effects of weaning-food viscosity and energy density on consumption and energy intake, reduction of viscosity may be advantageous. This is because it may facilitate feeding of children on such foods thus improving the nutrient intake. Based on these observations, compositing uji with cowpea seems suitable for improving its protein quality. This may help in mitigating protein malnutrition associated with consumption of sorghum foods.

Concerning the effects of compositing on textural and other sensory properties of ugali, it was noted from the PCA of the ugali sensory ratings, that 38% of the sensory differences was contributed by compositing sorghum with cowpea. Ugali made from sorghum-plus-cowpea were associated with cowpea flavour. Like other beans, the cowpea flavour is probably due to lipoxygenase activity, which is responsible for the beany flavour in many bean products (Fu et al 1987). It has been reported in a legume such as soybean, that linolenic acid can autoxidise to form *cis* and *trans* 2-(1-pentenyl) furans responsible for beany flavour (Chang 1979). The cowpea flavour may be eliminated by pre-treatments such as soaking in an acid and/or heat treatment such as boiling, which have been used to remove the beany flavour (Alobo 1999). These pre-treatments probably inactivate lipoxygenase, responsible for the beany flavour (reviewed by Okaka and Potter 1979). It was noted, however, that most variations in sensory attributes (59%) were due to the grain quality characteristics of the sorghum cultivars.

As indicated previously, finger feel is an important quality parameter for ugali as it is normally eaten by hand. Two important textural attributes of ugali that characterize finger feel are firmness and stickiness. The firmness of Orbit ugali was increased by compositing with cowpea, while no substantial difference was noted for NS 5511 ugali. In the case of Orbit sorghum, it is likely that the increase in protein enhanced protein-protein and or protein-starch interactions thus forming stronger extensive networks, as explained previously.

On the other hand, because of interference of the tannins in these interactions, this may have affected the role of such interactions on the firmness of NS 5511 ugali. This implies that compositing of NS 5511 ugali with 30% cowpea may not change its functional properties substantially. However, in the case of tannin-free sorghum (Orbit) ugali, compositing with cowpea at this proportion may change the textural properties of the food because there is no tannin interference. The textural properties of Orbit foods would depend mainly on the proportions and interactions of proteins and starch in the food matrix. It is important to note though, that acceptability of such a product would depend on the consistency range a particular community regards as suitable, as this often reflects differences in cultural norms (Stephenson et al 1994). As sensory evaluation showed a 17% difference in firmness of Orbit ugali due to compositing with cowpea (Table XIII), which was within the error margin, this change might not be substantial to affect the acceptability of the ugali by consumers. Furthermore, the preferred porridge texture varies among different consumers and communities (Murty and Kumar 1995). Thus, it may be concluded that in general, compositing sorghum ugali with cowpea at 70:30 ratio improves its protein quality, while the food still maintains a normal firmness. It was also noted that compositing did not significantly affect stickiness of ugali ($p>0.05$) as assessed by the sensory panel.

The effects of compositing on stiffness of injera (represented as maximum bending force) depended on the sorghum cultivar. Measurement of stiffness of injera is important as consumers roll up the injera with stew inside when they eat it (Zegeye 1997). Compositing increased the maximum bending force of NS 5511 injera by 4.8% to 25% after storage at 25^{o}C, for a period between 1 hr to 48 hr (Table XIV). However, the stiffness of Orbit injera was reduced by 6% to 22%. These differences are probably due to the differences in tannin contents of the two sorghum cultivars. As was shown previously in Chapter 4.1, addition of cowpea to NS 5511 reduced the tannin content of its flour. High tannin content beyond the quantity that can be bound by the protein network thought to result in weaker mechanical strength (Orliac et al 2002). These authors suggested that the weakening effects of tannins arise from introduction of weaker H-bonds between the proteins and the tannins. In addition, these authors noted that, because of the large size of tannins (Figure 3), their inclusion in a network may result in steric hindrance. This may create general incoherence within the networks formed thus causing weakness.

Another suggestion to explain this weakening effect of tannins proposed by Siebert et al (1996) focuses on the ratio between the protein and tannin molecules. By studying the nature of protein-polyphenol complex formation, these authors proposed a model that may account for the differences that occur in protein quality and mechanical strength of the sorghum foods with different levels of tannins. According to this model, each tannin molecule is considered to have a fixed number of binding ends, and each protein is viewed as having a fixed number of tannin binding sites. Therefore, a situation in which the number of tannin ends equals the number of protein binding sites should produce the largest network resulting in the largest particles and the greatest mechanical strength. With an excess of protein molecules relative to tannin molecules, each tannin molecule should be able to bridge between two protein molecules, but it would be unlikely that these proteins would be further bridged to others. This would result mainly in protein dimers and smaller aggregates, which would be more accessible to pepsin digestion as in the current study. With excess tannin relative to protein, all of the protein binding sites would be occupied, but the likelihood that bridging would occur would be low because each free tannin end would have a limited chance of finding a free binding site on a protein molecule. In such a situation, protein digestibility would be hindered probably because of the binding between the excess tannins and the digestive enzymes, which are proteins in nature. These proposed interactions may help in explaining the observations noted in the protein quality and textural properties of the foods made from tannin sorghum.

6 CONCLUSIONS AND RECOMMENDATIONS

Protein quality of traditional African sorghum foods in terms of protein content and essential amino acid is increased through cowpea addition. This is mainly because cowpea has higher protein content than sorghum and the major storage proteins in cowpea (globulins) are richer in the first limiting essential amino acid, lysine, than kafirins in sorghum. Protein digestibility and PDCAAS of the foods are substantially increased through addition of cowpea as this African legume has higher content of more digestible protein than the sorghum. The tannins in tannin-type sorghum somewhat adversely affect improvement in the protein of sorghum foods through addition of cowpea.

Compositing with cowpea has little effect on texture of traditional sorghum foods. Most functional properties of foods made from sorghum plus cowpea depend on the sorghum chemical composition. The absence or presence of tannin in the sorghum grains affects the textural properties of such foods.

Compositing with cowpea imparts a distinctive cowpea flavour to unfermented stiff porridge. This cowpea flavour appears to be a main sensory difference between stiff porridge made from sorghum plus cowpea and porridge made from sorghum only. Therefore, it may be important to find a way to eliminate the characteristic cowpea flavour if the cowpea composited sorghum food is to be eaten by consumers to whom the flavour is objectionable. A suggestion for eliminating the cowpea flavour would be to apply pre-treatments such as soaking, boiling and drying of the cowpea grains before milling. These pre-treatments can inactivate inherent lipoxygenase enzyme, responsible for the beany flavour. For future research, use of an appropriate plant breeding (conventional or genetic modification) technique that might suppress lipoxygenase production in cowpea could be explored. A genetic approach has been applied in soybean to remove beany flavour from soybean oil. This latter approach may lessen the processing requirements.

This study has established that with an appropriate choice of sorghum cultivar, addition of cowpea is a viable option for improving the protein quality of traditional sorghum foods in Africa and other semi arid tropics without substantially affecting their textural properties. This information will be useful in solving the problem of lysine deficiency faced by the poor dwellers of the arid and semi-arid tropics where sorghum and cowpea are important food crops.

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