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Development of soy fortified sorghum and bread wheat biscuits as a supplementary food to combat Protein Energy Malnutrition in young children

By

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DECLARATION

I hereby declare that the thesis submitted at the University of Pretoria for the award of PhD degree is my work and has not been submitted by me for a degree at any other university or institution of higher learning.

Charlotte Atsango Serrem

DEDICATION

This thesis is dedicated to my loving husband Dr. Cornelius Kibet Serrem for understanding and sharing my dreams, believing in my ability to achieve them and for the support and sacrifice to enable me achieve them.

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ABSTRACT

Development of soy fortified sorghum and bread wheat biscuits as a supplementary food to combat Protein Energy Malnutrition in young children

By

Charlotte Atsango Serrem

Supervisor: Prof J. R. N. Taylor

Co-supervisors: Prof A. Oelofse

Dr H. L. de Kock

Protein Energy Malnutrition (PEM) due to under nutrition is a major public health problem among children in Africa and other developing countries. Sorghum and bread wheat, which are important dietary staples in the semi-arid tropics of Africa, are suitable vehicles for delivering proteins to alleviate PEM. Formulation of foods from these low-lysine staples fortified with legumes is a sustainable approach to improve the protein nutritional quality of foods for young children. Biscuits offer a valuable vehicle for fortification as they are nutrient dense, ready-to-eat, have a long shelf-life and are popular. Therefore, this study investigated the effect of complementing sorghum and bread wheat with defatted soy flour on the nutritional and sensory quality and consumer acceptability of biscuits.

Biscuits were formulated and developed by compositing sorghum and bread wheat flours with defatted soy flour at different ratios. To establish the nutritional characteristics of biscuits, proximate composition, lysine and reactive lysine contents and in vitro protein digestibility were determined. Protein Efficiency Ratio (PER), Food Efficiency Ratio (FER) True Digestibility and Biological Value (BV) of sorghum biscuits were determined using Sprague Dowley weanling male rats. The sensory characteristics of biscuits were evaluated using a descriptive panel and instrumental texture analysis. Acceptability was evaluated using eight to nine year old school children.

Compared to the 100% cereal biscuits, sorghum-soy and bread wheat-soy composite biscuits in a 1:1 ratio had at least double the protein, mineral and crude fibre contents. The lysine contents of biscuits increased by 500-700%. For the sorghum-soy biscuits, in vitro protein digestibility increased by 170% and Protein Digestibility Corrected Amino Acid Score (PDCAAS) was 8 times higher. Two such biscuits of 28 g each could provide 50% of the recommended daily protein intake for 3 to 10 year olds. In the animal study, PER and FER for sorghum-soy biscuits were equivalent to the reference casein. True Digestibility was high for all diets, 85 to 95% and BV of sorghum biscuits was higher than sorghum-soy diet by 20%.

Principal Component Analysis (PCA) revealed that 61% and a further 33% of the variation in sensory properties was due to the type of cereal and concentration of soy in biscuits, respectively. Maximum stress increased by 39% and 34% in sorghum-soy and bread wheat-soy biscuits, respectively at 1:1 ratio. Spread factor of biscuits increased by 7 to 32%. Biscuits were darker in colour (reduced L* value) by 14 to 56% and hardness increased by 84% in sorghum biscuits. Positive hedonic scores by 8 to 9 year old school children for fortified biscuits were sustained above 80% through 8 consumption occasions. This data shows that fortifying with defatted soy flour imparts positive sensory characteristics associated with biscuits to sorghum and bread wheat biscuits and the acceptance of such biscuits may be sustained over an extended period of time.

This study indicates that soy fortified sorghum and bread wheat biscuits have high nutrient density, protein quality, positive sensory properties and high acceptability if consumed over an extended period. Hence, the biscuits have great potential as protein-rich supplementary foods to alleviate PEM among children and to provide an income to small holder farmers in rural African communities through purchase of grain for the Home Grown School Feeding Programme.

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1   INTRODUCTION

Protein Energy Malnutrition (PEM) continues to be the major nutritional problem resulting from undernutrition that affects children in most of the developing world (Muller and Krawinkel 2005). The most recent estimates show that more than one billion people worldwide are undernourished (Food and Agriculture Organization (FAO) 2009). Africa is home to over 70 million undernourished children (World Food Programme (WFP) 2008). In this region, poverty causes food shortages and most vulnerable populations survive predominantly on starchy staples such as maize, wheat, rice, sorghum, millet and cassava, with little or no meat and dairy products (Mayer, Pfeiffer and Beyer 2008). The protein nutritional quality of these staple foods is poor and lysine is the most limiting amino acid (United States Department of Agriculture (USDA) 2008).

The health consequences most pronounced in children suffering from PEM include higher susceptibility to infectious and metabolic diseases, impaired physical and cognitive development and increased mortality rates because of their higher nutritional requirements due to high growth velocities (Stipanuk 2006). The problem is further compounded by the Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) epidemic that has increased the number of vulnerable children. An estimated 91% of new infections among children worldwide and 14.1 million AIDS orphans are in sub-Saharan Africa (Joint United Nations Programme on HIV/AIDS/ World Health Organization (UNAIDS/WHO) 2009).

Strategies that have been used to address protein deficiencies include food diversification (FAO 1997), fortification of food with indispensable amino acids, supplementation with good quality protein, improvemement of protein quality by plant breeding and genetic engineering, and minimising the damage to the nutritional value of protein during food processing and storage (Friedman 2004)

Cereals constitute the most suitable vehicle for delivering proteins to at-risk populations because of their widespread consumption, stability and versatility (Bulusu, Laviolette, Mannar and Reddy 2007). In developing countries where a single cereal is often the primary staple, they contribute 70 to 90% of the total dietary protein (Lasztity 1984). The production

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of novel cereal-based food products designed to provide additional proteins to the daily diet has increased (Vitali, Dragojevic and Sebecic 2008). These products include nutritionally improved biscuits designed to reduce the risk of developing nutrient deficiency diseases. Some reasons for their increased popularity are their low cost compared to other processed foods, varied taste, ease of availability and longer shelf-life (Sudha, Vetrimani and Leelavathi 2007). To augment the protein quality, the concept of cereal-legume complementation by blending cereal and legume flours can be applied (Hooda and Jood 2005, FAO/WHO 1994).

Wheat flour is the principal component of virtually all biscuits because when mixed with water, it forms a unique visco-elastic dough (Kent and Evers 1994). However, good quality biscuits can be prepared using non-wheat flours. The problem facing the bakery industry is the non-availability and or cost of wheat creating a need to substitute wheat flour with other cereal flours such as sorghum (El-Khalifa and El-Tinay 2002), which are cheaper and more sustainable in their ecological zone. Sorghum is an important source of energy and protein for a large segment of the human population in the semi-arid and arid tropics (ICRISAT 2009) where it is too hot and dry for successful wheat and maize production (Bennet, Tucker and Maunder 1990, Doggett 1988). Hard, bread-type wheat, which is cultivated in some 33 countries in Africa, is also another important cereal staple in the semi-arid tropics of Africa (Taylor 2004).

The protein content and quality of sorghum and bread wheat can be improved by using them in protein-rich supplementary foods such as biscuits, to alleviate PEM among children in the semi-arid and arid regions of Africa where they are dietary staples. There is a need to determine the effect of such improvement on protein nutrition. Therefore this study investigated the effect of fortifying sorghum and bread wheat with soy on the nutritional and sensory characteristics and consumer acceptability of such composite biscuits.

2 LITERATURE REVIEW

This review explores the potential use of sorghum and bread wheat in legume composite biscuits to alleviating PEM. The issues addressed are the protein nutritional quality of sorghum, bread wheat and soy in relation to human requirements, processes that negatively affect lysine availability in cereals including sorghum and bread wheat and studies aimed at developing low cost cereal based supplementary foods in Africa, including biscuits aimed at alleviating PEM. Also reviewed are methods for evaluating protein and sensory quality and long-term acceptability of new food products.

2.1 SORGHUM AND BREAD WHEAT

Sorghum [*Sorghum bicolor* (L) Moench] is a drought-resistant crop of African origin (reviewed by Dahlberg 2000). Sorghum is the second most important cereal food in Africa after maize (Taylor 2004). It is of great nutritional significance in the diets of millions of rural poor people in the semi-arid and arid tropics because it constitutes their major source of protein and energy (ICRISAT 2009). Wheat, mainly hard, bread-type wheat is another important cereal staple in the semi-arid tropics of Africa, being cultivated in some 33 countries (Taylor 2004).

2.1.1 Sorghum grain morphology

The sorghum kernel is generally spherical with an average size of 4 mm long, 2 mm wide and 2.5 mm thick and 1000 kernel weight of 25-35 g (Rooney and Miller 1982). The sorghum kernel is naked, like wheat, and has three distinct anatomical components: the pericarp (outer layer), germ (embryo) and endosperm (storage tissue), shown in Figure 2.1. (Taylor and Belton 2002). The average proportions in the sorghum kernel are 6, 10 and 84% for the pericarp, germ and endosperm, respectively (Rooney and Serna-Saldivar 2003).
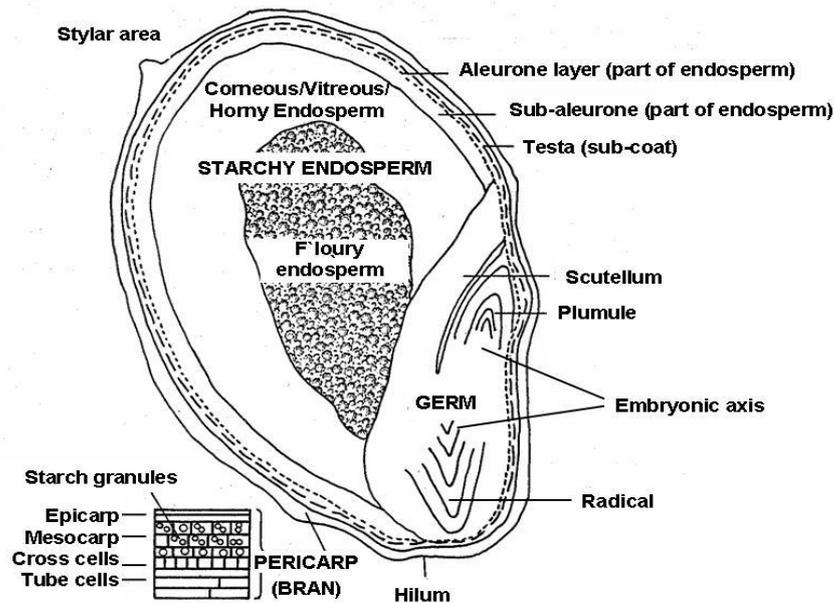
Figure 2.1 Cross-section of sorghum grain (adapted from Taylor and Belton 2002)

2.1.1.1 Pericarp

In their review of the structure of sorghum, Waniska and Rooney (2000) state that the pericarp is formed from the ovary wall with a thickness range of 8 to 160 µm and is divided into three distinctive parts, the epicarp, mesocarp and the endocarp. High levels of tannins in the pericarp of some sorghum cultivars inhibit protein digestibility (Rooney and Miller 1982).

The seed coat (testa) is derived from the ovule and has a thickness ranging from 8 to 40 µm (Rooney and Miller 1982). Sorghum grains can be classified according to the pigmentation of the testa. Type I sorghums with no testa do not contain tannins (proanthocyanidins), type II have condensed tannins and type III have the highest tannin content (Serna-Saldivar and Rooney 1995). The high level of tannins in type III sorghums makes them bird-resistant but as stated inhibits protein digestibility (Rooney and Miller 1982).

2.1.1.2 Endosperm

In their review of the sorghum structure, Waniska and Rooney (2000) state that the sorghum endosperm is composed of the aleurone layer, peripheral, and corneous and floury portions. The aleurone, the outer cover consists of a single layer of rectangular cells, adjacent to the testa. The cells have a thick cell wall, large amounts of proteins (protein bodies, enzymes),

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ash (phytin) bodies, and oil, (spherosomes) as well as minerals and water soluble vitamins. The peripheral endosperm directly beneath the aleurone layer consists of blocky cells with starch granules embedded in a dense proteinacious matrix of glutelin proteins and prolamin protein bodies (Rooney and Miller 1982). The protein bodies and matrix retard enzyme hydrolysis of starch making the starch unavailable for utilization. The corneous endosperm has a continuous interface between starch and protein. To release the starch granules for digestion processing of the kernel should disrupt the starch granules. Both the peripheral and corneous areas appear translucent and affect processing and nutrient digestibility (Serna-Saldivar and Rooney 1995).

The corneous and floury endosperm cells contain starch granules, protein matrix, protein bodies and cell walls rich in cellulose, arabinoxylans and hemicelluloses (Rooney and Serna-Saldivar 2003). Taylor, Novellie and Liebenberg (1984) found that the protein bodies are largely circular and have a diameter that varies from 0.4 to 2.0 μm. The starch granules which are often dented from the protein bodies are polygonal in shape and 4 to 25 μm in size. Granules in the corneous endosperm are smaller and angular while those in the floury endosperm are larger and spherical. The opaque, floury endosperm located near the centre of the caryopsis has a discontinuous protein phase, air spaces and loosely packaged round starch granules (Serna-Saldivar and Rooney 1995). The protein content of the floury endosperm within a kernel is lower than the corneous endosperm so the availability is improved.

In their review, Rooney and Miller (1982) defined sorghum endosperm texture as the relative proportion of the corneous to floury endosperm within a sorghum kernel. Texture affects the processing properties of the grain because in sorghums with a higher percentage of corneous, the pericarp (bran) is more readily separated from the intact starchy endosperm. When the peripheral and corneous cells remain intact during milling, there is grittiness in the flour. This can affect the acceptability of food products made using such flours.

2.1.1.3 Germ

The germ consists of two major parts, the embryonic axis and the scutellum, as shown in Figure 2.1. The scutellum, is the germ reserve tissue containing large amounts of oil, protein,

enzymes and minerals and serves as the bridge between the endosperm and germ (reviewed by Waniska and Rooney 2000).

2.1.2 Chemical composition of the sorghum grain

Starch the major component of the sorghum grain is 75 to 79% of grain weight and is composed of 70 to 80% amylopectin and 20 to 30% amylose (Waniska, Rooney and MacDonough 2004). Protein is the second most abundant nutrient 9.0 to 14.1% (Rooney and Serna-Saldivar 2003). The protein content and composition in the sorghum grain varies because of agronomic conditions such as water availability, soil fertility, temperatures and environmental conditions during grain development and genotype (Lasztity 1984). Nitrogen fertilization significantly increases kafirin accumulation and protein content (Waniska et al 2004). Kafirins contain cross-linked polypeptides that slow digestibility of the protein. Table 2.1 shows the chemical composition of the sorghum grain and its anatomical tissues (Waniska and Rooney, 2000). This review will focus on sorghum proteins since improvement of sorghum protein quality is the aim of the study.

Table 2.1 Chemical composition (%) of sorghum and its anatomical tissues

	Caryopsis	**Endosperm**	**Germ**	**Pericarp**
Caryopsis	100	84.2	9.4	6.5
Protein	7.3-15.6 (100)	8.7-13.0 (80.9)	17.8-19.2 (14.9)	4.3-8.7 (4.0)
Crude fibre	1.2-6.6 (100)			
Lipid	0.5-5.2 (100)	0.4-0.8 (13.2)	26.9-30.6 (76.2)	3.7-6.0 (10.6)
Ash (minerals)	1.1-2.5 (100)	0.3-0.4 (20.6)	10.4 (68.6)	2.0 (10.8)
Starch	55.6-75.2 (100)	81.3-83.0 (94.4)	13.4 (1.8)	34.6 (3.8)

Adapted from Waniska and Rooney (2000). Figures in parentheses are percentages of nutrient in the specific tissue.

2.1.3 Distribution of proteins in the sorghum grain

The amount and distribution of protein in the endosperm, germ and pericarp of the sorghum caryopsis differs. A comprehensive study on the distribution of the different types of proteins in the anatomical parts of the sorghum grain was carried out by Taylor and Schussler (1986),

using two non-tannin sorghum cultivars. The endosperm contains 80% of the sorghum nitrogen. The endosperm has the highest proportion of prolamins accounting for 67 to 69% of the total nitrogen and is rich in the amino acids, glutamine, proline, alanine, and leucine and low in lysine, a typical characteristic of prolamin proteins. The endosperm also contained a lower proportion of low molecular weight nitrogen (LMWN) of 1.5 to 2.5 and albumins plus globulins in comparison to whole grain, pericarp and germ. The G_3-glutelin is the second largest protein fraction in the endosperm accounting for 13.6 to 17.3%. It was also found to be poor in glutamine, 11.2% and rich in lysine 6.1%. These workers also postulated that the G_3-glutelins comprise the glutelin matrix that surrounds the protein bodies in sorghum starchy endosperm. The germ contained 16% of the grain nitrogen. Low molecular weight nitrogen (LMWN) was the most abundant nitrogenous fraction accounting for 40.1 to 48%, comprising amino acids, peptides and nucleotides. Albumin and globulin proteins were also present in substantial amounts, 32 to 34% of the proteins in the germ, and were rich in lysine. The pericarp contained the least amount, only 3% of the grain protein and it was inextractible using the modified Osborne fractionation procedure of Landry and Moureaux (1970). Taylor and Schussler (1986) suggested that this could be because it was associated with the cell wall.

2.1.4   Kafirin proteins

The kafirins are the main storage proteins in the sorghum caryopsis. Shull, Watterson, and Kirleis (1991) classified the kafirins on the basis of solubility, molecular weight and structure into three classes, α-kafirin (M_r 24,000 and 26,000), β-kafirins (M_r 20,000, 18,000 and 16,000) and γ-kafirins (M_r 28,000). Alpha-kafirin is the highest in content, 50 to 70%, followed by γ-kafirins 15% and β-kafirins, 5% (Rooney and Serna-Saldivar 2003). In a review by Belton, Delgadillo, Halford and Shewry (2006), the identification of a fourth kafirin at the gene and transcript level, δ-kafirin is described.

Kafirin content also differs with the type of grain or endosperm. Hamaker, Mohamed, Habben, Huang and Larkins (1995) found that kafirin content in whole grain ranged from 68 to 73% and 77 to 82% in the endosperm. In their investigation of differences in protein composition of vitreous and opaque endosperms of sorghum, Watterson, Shull and Kirleis (1993) reported that the vitreous endosperm contained up to two times more total protein than opaque endosperms of the same variety. The vitreous endosperm also had a higher kafirin

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content, 5.8 to 8.5% compared to 2.0 to 2.4% in opaque endosperm. The opaque endosperm had higher amounts of albumin and globulin proteins. The same workers also found that α-kafirins constitutes 66 to 71% and 80 to 84% of total kafirins in the floury and vitreous sections respectively, β-kafirins, 10-13% and 7 to 8%, and γ-kafirins 19-21% and 9-12%.

Kafirins are located in the protein bodies, which are well defined structures in the sorghum starchy endosperm. The investigation by Watterson, Shull and Kirleis (1993) also found that in the internal sorghum protein body structures, α-kafirins are encapsulated by β- and γ-kafirins which exist in a disulphide-bound polymeric network. Most of the α-kafirins are located in the interior of the protein body, while the β- and γ-kafirins are in the periphery. All three groups of kafirins were found to be low in the essential amino acid lysine supporting earlier findings that sorghum only has approximately 2 g/100 g protein.

2.1.5 Amino acid composition of sorghum protein

The composition of the indispensable amino acids of a protein is an important indicator of its protein nutritional value. Like other cereals, lysine is the first limiting amino acid in sorghum relative to the WHO (2007) reference pattern and is much lower compared to egg protein as shown in Table 2.2. Sorghum has the lowest lysine content of approximately 2.1% when compared to the other major cereals rice, wheat and maize with lysine contents of approximately 3.5%, 3.0% and 3.4%, respectively (Young and Pellet 1985). Further, in the sorghum kernel, the lysine-rich albumin and globulin predominate in the germ and pericarp and debranning sorghum reduces a substantial amount of the lysine content (Taylor and Schussler 1986). Waggle, Parrish and Deyoe (1966) compared the nutritive value of protein of two sorghum grain cultivars containing 7.9% and 11.8% protein, respectively on the basis of their ability to support rat growth. The high protein sorghum grain with lower lysine content caused significantly lower growth than the low protein sorghum grain with higher lysine content. These workers concluded that deficiency of one indispensable amino acid is enough to cause failure of an entire diet. Sorghum foods therefore have to be eaten with legumes, vegetables and animal proteins so that the protein can be utilized nutritionally (Munck 1995). Table 2.2 shows the amino acid profile of sorghum, wheat and soy grains compared to animal source protein and amino acid requirements for different age groups.

Table 2.2 Indispensable amino acid composition (mg/g protein) of whole grain sorghum, wheat and soy compared with pattern for amino acid requirements (mg/g crude protein) for infants, school age children and adults and amino acid composition of high quality protein

Amino acid	Sorghum[a]	Wheat[b]	Soy[c]	Egg[d]	Amino acid requirements[e]		
					Infants	3-10 yrs	Adults
Lysine	23	28	63	70	57	48	45
Leucine	142	68	85	86	66	61	59
Phenylalanine + Tyrosine	51	64	96	47	52	41	38
Valine	54	39	49	66	43	40	39
Tryptophan	10	11	11	47	8.5	6.6	6
Methionine + Cysteine	10	35	68	93	28	24	22
Threonine	34	29	38	47	31	25	23
Histidine	21	15	25	22	20	16	15
Isoleucine	41	37	47	54	32	31	30

[a] Values based on crude protein content of 11.3% USDA (2008).
[b] Values from Shewry (2009).
[c] Values from Friedman (1996).
[d] FAO/WHO/UNU (1985).
[e] Amino acid requirements for selected age groups male and female combined (WHO 2007).

2.1.6 Digestibility of sorghum proteins

The nutritional quality of a protein is also indicated by the digestibility in the protein with the resultant provision of amino acids for utilization by the body. Both in vitro and in vivo studies show that sorghum proteins are less digestible than those of other cereals. The first documented evidence was by Kurien, Narayaranao, Swaminathan and Subramanyan (1960) who found that when protein in rice with 75% digestibility was progressively replaced with sorghum, the apparent digestibility was reduced to 55%. Similar results were obtained in related human studies by Daniel, Leela, Doroiswamy, Jajalakshmi, Rao, Swaminathan, and Parpia (1966) in young girls fed a sorghum diet. In a study by Elkin, Arthur, Hamaker, Axtell, Douglas and Parsons (2002), sorghum mutant cultivars with high in vitro protein digestibility and two normal sorghums were all inferior to maize in chick feeding trials. Chicks fed maize gained mean weight of 60 g compared to the highest in sorghum of 44 g.

2.1.6.1 Effect of cooking on protein digestibility

Studies have shown that sorghum protein becomes less digestible when cooked. A study by MacLean, De Romana, Placko and Graham (1981) on Peruvian children showed apparent protein digestibility in cooked sorghum porridge from four non-tannin cultivars to be only 46% compared to wheat, maize and rice with values of 81%, 73% and 66%, respectively. In vitro pepsin digestibility studies have also demonstrated significant reduction in protein digestibility when sorghum is wet cooked. Axtell, Kirleis, Hassen, De Croz-Mason, Mertz and Munck (1981) found significant reduction in protein digestibility after cooking sorghum, an indication of probable alteration of sorghum protein making them indigestible from 88.6% (raw) to 45.3% (cooked) for whole grain and 78.6% (raw) to 37.1% (cooked) for dehulled kernels.

Hamaker, Allen, Mertz and Axtell (1986) reported that cooking sorghum altered the solubility properties of the kafirins (fractions II and III) from 42 to 6%, which was more than maize prolamins, the zeins. An examination using polyacrylamide gel electrophoresis established that the predominant proteins in the indigestible residue of pepsin-indigestible proteins were α- and β-kafirins. These workers suggested that high levels of disulphide crosslinked kafirin proteins might be responsible for low sorghum protein digestibility after cooking.

Studies have been carried out to understand how kafirins, the least digestible proteins in sorghum, are altered by cooking. Hamaker, Kirleis, Butler, Axtell and Mertz (1987) found that sorghum cooked in the presence of 2-mercaptoethanol or other reducing agents had a significant increase (25 %) in protein digestibility compared to sorghum cooked in water alone. These authors proposed that the reducing agents open up the protein matrix through the cleavage of disulphide bonds allowing the digestive enzymes more accessibility to the protein bodies. This was confirmed by Ezeogu, Duodu, Emmambux and Taylor (2008) who found that cooking floury and vitreous sorghum endosperm flours in the presence of 2-mercaptoethanol prevented a collapse of the protein matrix, an indication of reduced disulphide cross-linking.

Inaccessibility of the endosperm protein bodies in vitro to enzymes for digestion may also be a cause of poor pepsin digestibility. In a study by Taylor, Taylor, Belton and Minnaar (2009), the high digestibility of microparticles prepared from kafirin was attributed to the large surface area available for pepsin attack. Chandrashekar and Kirleis (1988) found that protein bodies remain intact after cooking and are present after pepsin digestion. Close proximity of starch granules to protein bodies in the grain may reduce accessibility of proteolytic enzymes to the protein bodies when gelatinization takes place, or bind the digestive enzymes in the gastrointestinal tract reducing protein digestibility (Duodu, Nunes, Delgadillo, Parker, Mills, Belton and Taylor 2002). Oria, Hamaker and Schull (1995) demonstrated that disulphide bonded complexes form extensively in γ-kafirins and somewhat less in the β-kafirins late in grain development. They suggested that the decrease in grain moisture content as the grain matures is the cause of reduced protein digestibility in both uncooked and cooked protein of low tannin sorghums. This can be accounted for by kafirin hydrophobicity and possibly racemisation (reviewed by Duodu, Taylor, Belton and Hamaker 2003).

Tannins present in sorghum have been reported to have a negative impact on sorghum digestibility. Emmambux and Taylor (2003) investigated sorghum-kafirin-phenolic compound interaction and found that 30 to 40% of sorghum-condensed tannins bound to kafirin. They concluded that complexation may be involved in decreased digestibility of high tannin sorghums. Mukuru, Butler, Rogler, Kirleis, Ejeta, Axtell and Mertz (1992) demonstrated that in vitro digestibility of high tannin sorghum untreated with wood ash was only 9% compared to treated high tannin sorghum with digestibility of up to 70%. Tannins have a high affinity for kafirins in sorghum and form complexes making them unavailable to the body (Butler, Riedl, Lebryk and Blytt 1984). They also bind the protein digesting enzymes and inhibit protein digestion in humans. The digestibility of sorghum proteins can be improved by varied processing applications.

2.2 WHEAT

Ninety five percent of the world's wheat (*Triticum* spp.) is the common hexaploid type botanically *Triticum aestivum* L.em Thell (Shewry 2009). The tetraploid durum constitutes the remaining 5%. Today, wheat is among the big three cereal crops and comes third in world production after maize and rice with a harvest of over 600 million tons annually (FAO 2007).

2.2.1 Morphology of the wheat grain

The wheat kernel (Figure 2.2) is a naked kernel with an average size of 8 mm in length and weight of 35 mg (Hoseney 1994). The kernel can be divided into three parts, the endosperm, pericarp and germ that form 83%, 14% and 3% of the grain, respectively (Bushuk and Scanlon 1993).
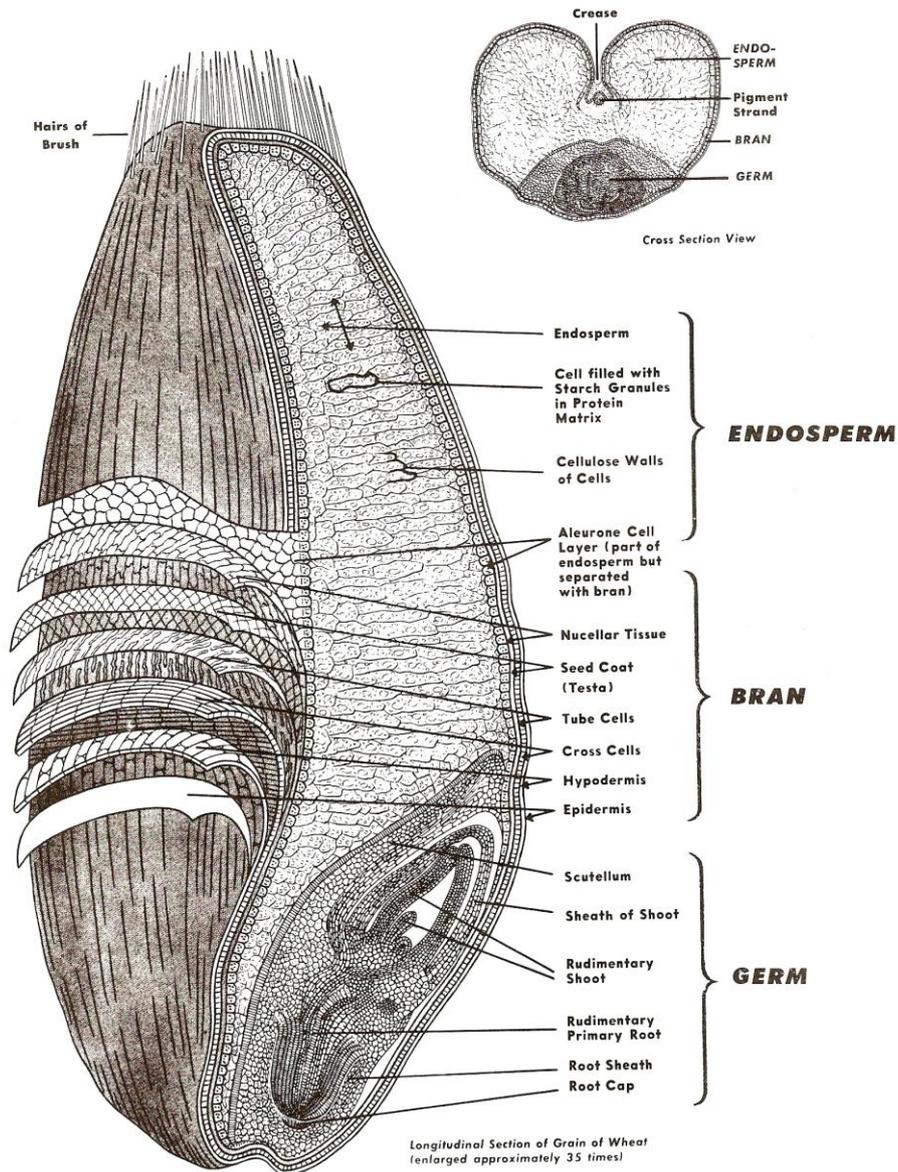
Figure 2.2 Longitudinal and cross sections of a wheat kernel (Potter and Hotchkiss 1998).

2.2.2 Chemical composition of wheat grain

The three morphological parts of the wheat kernel differ markedly in chemical composition (Table 2.3), depending on the class of wheat, area of growth, climate and wheat variety (Bushuk and Scanlon 1993). The protein content of wheat varies over a wide range from 6 to 21% and is influenced more by the soil and climatic conditions. Graybosch, Peterson, Baenziger and Shelton (1995) measured the environmental modification of flour protein content in 30 hard red winter wheat cultivars and found that gluten and gliadin content declined with exposure to greater number of hours of elevated temperature during grain filling. Soft wheats all have low protein content of 8 to 11%, a soft endosperm, weak gluten properties and are used for flatbreads, cakes, pastries, crackers, cookies, quick breads muffins and snack foods (Lukow 2006). Hard wheats are characterized by hard kernels, mill to high flour extraction, have medium to high protein content of 10 to 15%, strong gluten properties and are normally referred to as bread wheats.

Table 2.3 Composition of whole wheat grain, endosperm, bran and germ

Constituent	Whole grain (%)	Endosperm (%)	Pericarp (%)	Germ (%)
Dry matter	100	(82)[b]	(15)	(3)
Carbohydrate	82.7	86.4(85)	70.0(13)	50.6(2)
Protein (N x 5.7)	12.8	11.2(72)	16.7(20)	32.4(8)
Fat	2.5	1.6(52)	5.4(32)	11.9(16)
Ash (minerals)	2.0	0.8(34)	7.4(58)	5.1(8)

Adapted from Bushuk and Scanlon (1993).
Figures in parentheses are percentages of total in the grain.

2.2.3 Types of protein in wheat

Wheat is unique among cereals because of the properties of its dough, which allow it to be processed into bread and other baked products such as cakes and biscuits, pasta and a range of other processed foods. These properties are derived from the structures and interaction of the grain storage proteins which together form the gluten fraction (Shewry 2009). Wheat proteins are comprised of albumin 15% (water soluble), globulin, 5% (soluble in 0.5 M NCl),

gliadin 33% (soluble in 70% ethanol), soluble glutenin 14% and insoluble glutenin 33% (soluble in 0.05 M Acetic acid) (Bushuk and Scanlon 1993).

Gluten proteins are formed by two fractions, glutenins and gliadins (Shewry 2006). Gliadins comprise the sulphur-poor ω-type with M_r 30-80,000, the sulphur-rich α-type and γ-type gliadins with M_r 30-55,000. They are monomeric and interact by hydrogen bonding and hydrophobic interactions (Shewry, Tatham, Farde, Kreis and Miflin 1986). Gliadins impart extensibility to wheat doughs. Glutenins are polymeric and are separated by inter-chain disulphide bonds. They can be divided into the high molecular weight (HMW) subunits, M_r 65-90,000 and S-rich LMW subunits of glutenin. This classification is important for the functional importance of the fractions.

2.2.4 Amino acid composition and protein digestibility of wheat

Comparison of amino acid requirements of infants, school children and adult man with those of whole wheat grain in Table 2.2 shows that wheat is only deficient in lysine. Mossé and Huet (1990) found that there was relative decrease of lysine in wheat of high protein content and increases of up to 30 mg per 100 g protein in low protein grain. The decrease in lysine results from proportional increases in the lysine-poor gluten proteins and also results in lower lysine content in flour because gluten proteins are located in the starchy endosperm tissue (Shewry 2009), the part of the grain made into flour.

Eggum, Kreft and Javornic (1981) determined the amino acid composition and compared protein quality of buckwheat (a lysine-rich pseudo cereal) with the values of wheat using a rat bioassay. Wheat was deficient in lysine 2.3 g/100 g protein compared to buckwheat, which had 5.0 to 5.2 g/100 g protein, close to the ideal level of 5.2 g/100 g protein for infants. The true protein digestibility of wheat was 96%. However, the biological value and net protein utilization were 55% and 53%, respectively compared to buckwheat with true digestibility of 79 to 80% and biological value and net protein utilization of 90 to 93% and 71 to 74%. These workers concluded that the high digestibility in wheat is a result of low crude fibre and absence of tannins, and biological value is low because of the low lysine content. Studies by Axtell et al (1981) showed that wheat had the highest protein digestibility among four

cereals, 81% compared to maize, rice and sorghum which had values of 73%, 66% and 46%, respectively.

2.3 PROTEIN ENERGY DEFICIENCY IN CHILDREN

Protein Energy Malnutrition (PEM) refers to a group of diseases that result from under nutrition and is a major public health problem in developing countries. It is a macronutrient deficiency disease resulting from an inadequate intake and/or utilization of protein and energy, and affects children most because of their higher needs for protein and energy per kilogram body weight compared to adults (Stipanuk 2006). It is estimated that approximately 27% of children younger than five years in developing countries are underweight and in sub-Saharan Africa 38% have stunted growth while 28% are underweight (UNICEF 2007). PEM is associated with the deaths of approximately 5 million children each year (WHO 2000). The main cause of PEM in developing countries is dependence on a single starchy staple for virtually all the protein and energy requirements (Onis and Blossner 1997).

The symptom of mild to moderate forms of PEM in children is inadequate growth (Shetty 2006). The classic clinical syndromes of severe forms of PEM are Kwashiorkor, Marasmus and the mixed condition of Marasmic Kwashiorkor. Kwashiorkor arises from low protein intake and adequate energy consumption leading to reduced synthesis of visceral proteins. Hypoalbuminaemia develops because of short term protein deficiency and causes oedema (Furham, Charney and Mueller 2004). The combination of a fatty and enlarged liver, because of impaired synthesis of hepatic proteins and fluid accumulation distends the stomach and disguises weight loss (Stipanuk 2006). Other symptoms commonly observed are anaemia, hair discolouration, dry or peeling skin, diarrhoea, and fluid and electrolyte disorders. Marasmus is a result of chronic deficiency of both protein and energy leading to protein loss in the skeletal muscle and adipose tissue (Gibney, Vorster and Kok 2002). There is absence of oedema, severe muscle wasting, and shrivelled skin. The consequences are stunted brain development, depressed metabolism, stunted physical growth and development, anaemia, impaired immune system and fluid and electrolyte imbalance. Marasmic Kwashiorkor occurs when a child has wasted muscles and fat characteristic of marasmus, and oedema as in Kwashiorkor. Untreated Marasmus can result in death from heart failure and dehydration (Thompson, Manore and Vaughan 2008).

2.3.1   Functions of proteins in human nutrition

2.3.1.1 Contribution of protein to children's health

Proteins contribute to cell growth, repair and maintenance, act as enzymes and hormones, maintain fluid, electrolyte and acid base balance and also maintain a strong immune system (Thompson et al 2008). When fats and carbohydrates are not provided in adequate amounts in the diet, proteins also serve as an energy source, limiting their availability for the functions stated earlier (Gibson 2005). Additionally, proteins act as carriers for other nutrients that include lipids, Vitamin A, iron, sodium and potassium. Consequently, protein deficiency in children is also accompanied by other nutrient deficiencies including micronutrient deficiency (Muller and Krawinkel 2005).

Acute malnutrition causes wasting, low weight-for-height, while chronic malnutrition causes stunting, low height-for-age. Underweight, low weight-for-age reflects both stunting and wasting (Gibson 2005). It has been shown that when school children consume animal source proteins, there is a positive impact on weight gain and increased lean body mass (Grillenberger 2006). Protein helps maintain a strong immune system by supporting the increased production of antibodies in response to common infections such as colds, flu or allergic reactions (Thompson et al 2008). Children who have PEM have greatly increased susceptibility to life-threatening infectious diseases such as HIV/AIDS, tuberculosis and malaria (Schaible and Kaufmann 2007). There is also evidence that chronic PEM in 5 to 10 year olds impairs cognitive development (Kar, Rao and Chandramouli 2008).

2.3.1.2 Importance of lysine in the diet

L-lysine was discovered as an indispensable amino acid by Osborne and Mendel using a rat model as a measure of nutritional adequacy in 1914 (Stipanuk 2006). These workers also showed that rats required lysine for growth by using wheat gliadin as the protein source in place of casein. The biological functions of lysine include; synthesis of connective tissues such as bone, skin, collagen, and elastin; synthesis of carnitine and resultant conversion of fatty acids to energy; support for healthy growth and development and maintenance of healthy immune function, particularly with regard to antiviral activity. The structure of L-

lysine characterized by the presence of an amino group at the end of a 4-carbon aliphatic side chain [-(CH2)4-NH31] makes it a relatively reactive component in different chemical reactions including carbonyl-amine interactions (Walsh 2002).

2.4   FOOD PROCESSING AND LYSINE AVAILABILITY IN FOOD

A variety of methods are used to process cereals aimed at making them more edible, improve flavour, texture, extend shelf life and to destroy microorganisms and toxins (Friedman 2004). Unfortunately, processing often leads to loss of nutritional value and formation of anti-nutritional and toxic compounds because of molecular interactions among nutrients and with other food ingredients.

2.4.1   Milling of cereal grains

In milling, the protein nutritive value of the cereal is reduced. Taylor and Schussler (1986) found that the germ and the pericarp, the parts normally removed during processing were three to four times richer in lysine than the endosperm. In fact, they noted that with the exception of leucine, the protein composition of the germ conformed to high quality protein. The high lysine content of the germ accounts for the fact that debranning sorghum leads to a product with reduced lysine content (Eggum, Bach Knudsen, Munck, Axtell and Mukuru 1982).

2.4.2   Thermal Processing

Heat treatments involved in the production and processing of foods such as baking, roller drying, frying and grilling, or storage in relative humidities of 30-70% enhance a basic reaction making lysine unavailable to the body (Erbersdobler and Faist 2001).   The Maillard reaction is a general term used to describe a complex series of reactions between free amino acids of proteins, and reactive carbonyl groups of reducing sugars, such as glucose (Alais and Linden 1991).   The resultant brown compounds formed impart colour and a desirable flavour distinct to particular food products such as bread crust, fried potatoes, baked cakes, and biscuits. However, this reaction is also responsible for the specific loss of nutritional value of foods resulting in decrease of protein digestibility and lysine bioavailability (Charissou, Ait-
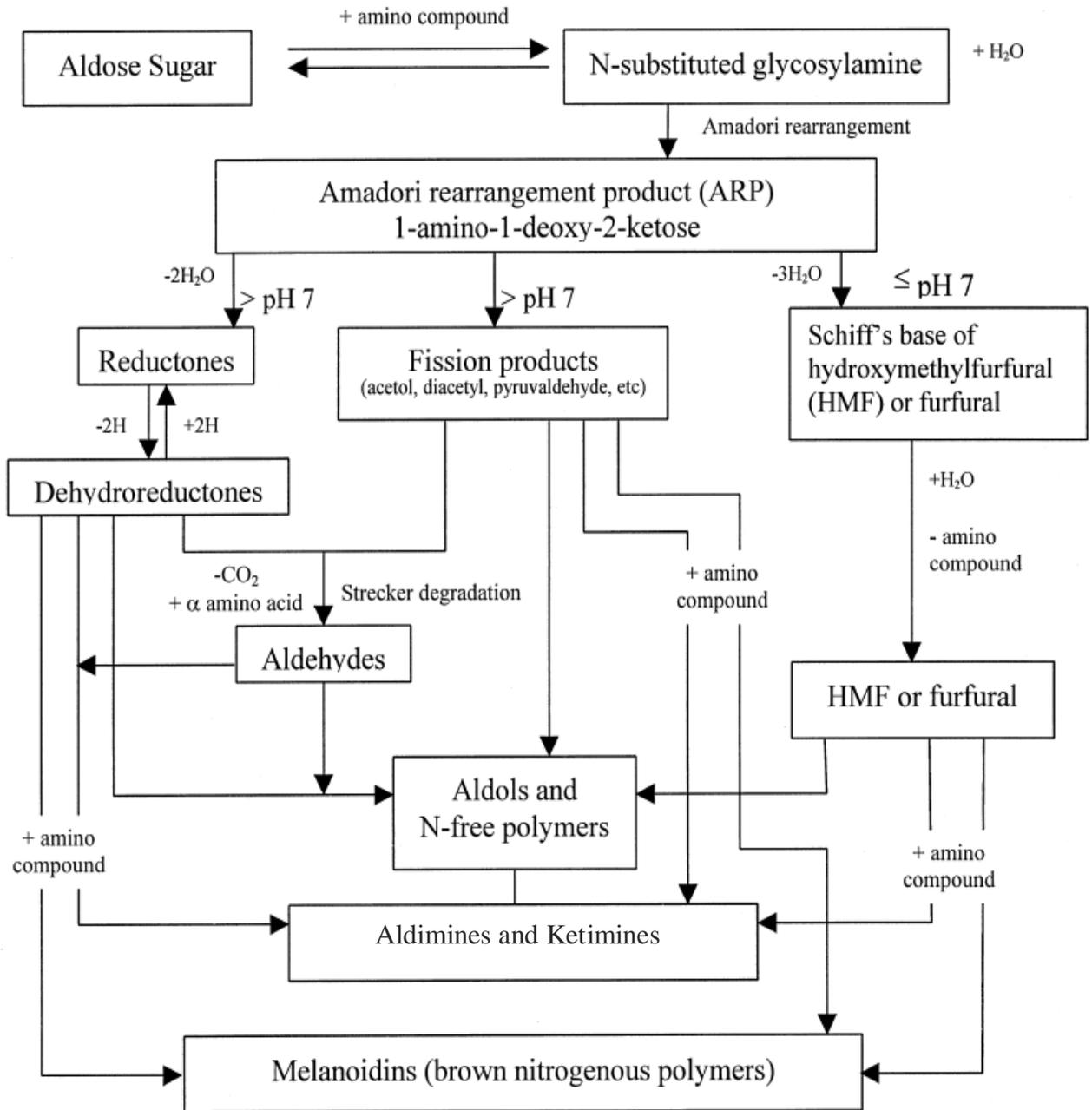
Figure 2.3: Maillard Reaction scheme adapted from Hodge (1953).

Ameur and Birloeuz-Aragon 2007). In cereal products destruction of lysine, the most limiting amino acid can greatly reduce the protein nutritional value.

The general scheme of the Maillard reaction has been described by Alais and Linden (1991) shown in Figure 2.3 (Hodge 1953). The first step is the reaction of a reducing sugar such as glucose with an amino acid frequently the ε-amino group of lysine to form a condensation product N-substituted glycosylamine which rearranges to form the Amadori compound. Lysine is blocked in reactions with aldoses and ketoses causing darker colours, while cysteine with a sulphur group causes specific flavours but less colour intensity (De Mann 1999). The Amadori compound easily isomerizes into three different structures and the next step differs depending on the isomer of the Amadori compound. The amino acid may be removed to produce reactive compounds that are finally degraded to the important flavour components furfural and hydroxymethyl furfural (HMF). The other reaction is the Amadori-rearrangement, which is the starting point of the main browning reactions. After the Amadori-rearrangement, three different main pathways can be distinguished: dehydration reactions, fission with production of diacetyl, and pyruvaldehyde or Strecker degradation with amino acids which are condensated to aldols. These three main pathways result in complex mixtures including flavour compounds and brown high molecular weight pigments called melanoidins (Nursten 1980).

The nature of lysine loss varies depending on the food process or processing temperature. High temperatures such as those in extrusion cooking, baking, roller drying and toasting negatively affect available lysine in processed cereal products. Horvatic and Eres (2002) carried out a comparative investigation of the changes in available lysine content and protein nutritive quality during industrial production of dietetic and standard biscuits. They found that lysine content in all biscuits was significantly lowered during baking within a range of 27 to 47% and more lysine is lost with high temperatures and long baking time.

The type of sugar also influences the extent of loss of available lysine. Charissou et al (2007) evaluated the effect of formulation and baking temperature in model cookies containing glucose, fructose and sucrose. The percentage of lysine blocked as fructose lysine (furosine) was maximal in the presence of glucose accounting for at least 50%, and the lowest in fructose. They concluded that the reason for fructose being associated with less fructoselysine

was because fructose forms the hynes product during the early steps of the Maillard reaction which gives no furosine after acid hydrolysis. The same workers also demonstrated that at temperatures below 200°C majority of lysine blockage was due to products other than fructose and sucrose. Charissou et al (2007) suggested that lipid peroxidation is one of the causes of lysine blockage in food products at lower temperatures with the production of carboxymethyllysine (CML).

The low moisture content of food products that are baked or extruded further exacerbates the extent of the lysine loss. Ait-Ameur, Mathieu, Lalanne, Trystram and Birloeuz-Aragon (2007), found that the increase in temperature in the core of model biscuits, caused water to evaporate and water activity decreased to a critical value of 0.5-0.7, allowing the formation of HMF from reducing hexoses. Fernandes-Artigas, Garcia-Villanova and Guerra-Hernandez (1999) showed that the decrease in available lysine is higher when the available lysine in total proteins is greater. The lysine losses in roller dried rice-maize-soy blends of infant cereal products was greatest with a 53% decrease in lysine content, compared to unblended toasted flour with losses of 14 to 29%. Storage of food products at higher temperatures and lower moisture can also promote the Maillard reaction derivatives. Ramirez-Jimenez, Guerra-Hernandes, and Garcia-Villanova (2003) measured the furosine content during twelve months storage of an infant rice cereal. They found that available lysine losses were higher (25%) at a high storage temperature of 55°C compared to 7% at 32°C.

2.4.2.1 Effects of Maillard products on protein digestibility

Human and animal studies have demonstrated the effects of Maillard products on protein availability. The decrease in biological usability of lysine starts with the formation of Amadori products at the initial stage of the Maillard reaction. A study by Erbersdobler and Faist (2001) on the metabolic transit of Amadori products found that urinary excretion after ingestion of a test meal showed a rapid elimination of the absorbed part suggesting a low absorption rate of fructoselysine. With continued heating these intermediates react further and form insoluble protein complexes with extensive lysine arginine cross-links. These advanced glycation end-products have low solubility and extensive chemical modifications of their amino acid constituents makes them have extremely low bioavailability and food value. The loss of protein digestibility can be explained by the resistance of such cross linked

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compounds to intestinal protease (De Mann 1999). Heating can also promote the cross-linking of L-alanine with lysine in food proteins to generate lysineoalanine and racemisation to D-lysine, which cannot be utilized by the human body and is possibly toxic (Friedman 1999).

2.4.3 Soy beans

Food legumes are seeds of leguminous plants that belong to the leguminosae family (Uebersax and Occena 2003). The storage proteins in food legumes are predominantly globulin fraction and the metabolic proteins are primarily the albumin fraction. Most food legumes contain 20 to 25% protein, but the soy bean typically contains 30 to 45%, with an average of 35.5% at 13% moisture (Hammond, Murphy and Johnson 2003)

The Soy bean (*Glycine max* (L) Merr.) originated from China where it has been part of the diet for thousands of years (Fehily 2003). Soy bean products widely used in Eastern Asia include: soy sauce, miso (fermented soy beans), tempeh (fermented and deep-fried whole beans), sufu (fermented soy bean protein curd), kinako (roasted soy bean flour), tofu (soybean protein curd), and abura age (soy bean protein film made from soy milk) (Fukushima 1991). More recently, soy bean is being widely used to produce ingredients such as soy flour and protein, which are used in meat products and vegetarian alternatives. They are also used for soy oil, soy lecithin, soy milk and infant formulas for lactose intolerant infants (Fehily 2003).

2.4.3.1 Soy bean proteins

Soy proteins have four major water extractible fractions 2S, 7S, 11S, and 15S, isolated on the basis of their sedimentation coefficient (Snyder 2003). The 7S, β-conglycinin a storage protein and 11S, glycinin represent the majority of the protein fractions in soy bean. This was confirmed in a study by Riblett, Herald, Schmidt and Tilley (2001) who isolated the soy protein fractions β- conglycinin and 11S glycinin from four soy genotypes grown under controlled environments. The β-conglycinin is a trimer and constitutes 85% of the protein with sub-units α, α⁻, and β with molecular weights of 84,000, 72,000 and 51,000, respectively. In different combinations, the three sub-units give six distinct β-conglycinin

sub-units (Snyder 2003). The remaining 15% is glycinin with acidic and basic sub-units of molecular weight range, 36,000 to 40,000 and 18,000 to 20,000, respectively. Glycinin is a hexamer and each of the six sub-units consists of acidic and basic polypeptides (Snyder 2003). The 15S fraction is a dimer of glycinin.

The 2S fraction contains low molecular weight proteins that include the Bowman-Kirk and Kunitz trypsin inhibitors which inhibit growth in young animals (Snyder 2003). Lectins (haemmaglutinnins) are part of the 7S fraction and are known to cause agglutination of the red blood cells. Vasconcelos, Maia, Siebra, Oliveira, Carvalho. Melo, Carlini and Castelar (2001) demonstrated that heat treatment inactivates the antinutrients, when raw soy bean had a digestibility of 50.9 to 59.7%, while the cooked had a 78.3% showing that antinutrients were inactivated. Heat treatment, can also affect lysine content. Mao, Lee and Erbersdobler (1993), found that heat treatment of soy with glucose decreased total lysine by 7 to 13%.

2.4.3.2 Soy amino acid composition and digestibility

Soy bean proteins are relatively high in essential amino acids, in particular lysine, threonine, isoleucine, leucine, phenylalanine and valine. In their study, Vasconcelos et al (2001), determined amino acid content of two soy bean cultivars and compared to the FAO/WHO/UNU (1985) pattern of amino acid requirements for children (2 to 5 years and 10 to 12 years). Except for tryptophan the meals contained higher levels of indispensable amino acids than the requirements. The lysine content was an average of 7 g/100 g protein, higher than the requirement. Table 2.2 shows that compared to meat and egg, soy bean is lower in sulphur containing amino acids methionine and cysteine. Fernandes-Quintela, Macarulla, del Barrio and Martinez (1997) determined the amino acid content of soy bean and found methionine limiting.

Human studies suggest that soy bean is nutritionally equivalent to animal proteins such as egg, milk, fish and beef. Istfan, Murray, Janghorbani and Young (1983) compared the nutritional value of soy protein concentrate with milk protein in young men taking a mean daily intake of 95 mg N/kg body weight per day. They found that soy protein supported nitrogen equilibrium as well as the animal protein. Where present, antinutrients can reduce the digestibility of soy bean. Vasconcelos et al (2001) found that weight gain was higher in

rats fed toxin-free soy diets than those fed toxin-containing diets, and rats fed egg white diet had a higher weight increase compared to all those fed raw soy diets. The pancreas was also enlarged. These workers concluded that trypsin inhibitors in soy cause poor digestibility of dietary protein, leading to growth inhibition and pancreatic hypertrophy.

2.5 STRATEGIES TO PREVENT PROTEIN DEFICIENCIES

2.5.1 Dietary diversification

Dietary diversification is an approach to enhance the availability, access and utilization of foods with high content and bioavailability of nutrients (FAO 1997). In protein nutrition, it includes increased commercial production of protein-lysine rich foods for example, meat, poultry, and dairy products, fish farming, keeping small livestock, and home gardening of legumes (Gershoff, McGandy, Suttapreyasri, Nondasuta, Pisolyabutra and Tantiwongse 1975). Nutrition education complements dietary diversification by providing people with adequate information, skills and motivation to procure and consume appropriate diets (FAO 1997).

2.5.2 Amino acid fortification of cereals

The aim of food fortification is to add the protein to a dietary item (food or condiment), that is regularly consumed by the targeted population at a level that will control an existing dietary deficiency, without posing risks of overdosing those who habitually consume large quantities of the fortified product (FAO/WHO 1994). The dietary item is called the vehicle. Technical barriers to food fortification include adverse effects on the sensory quality of foods, nutrient-nutrient interactions, poor bioavailability of some fortificants, and the difficulty of fortifying some staple foods such as rice (Allen, Rosado and Casterline 1995).

Historically, there was interest in fortifying bread and other grain based foods with lysine to improve the biological value of cereal proteins for populations with lysine-poor diets. Consequently lysine has been widely used in fortification of cereals by addition either as a protein such as milk, fish or soy or as the free amino acid lysine monohydrochloride (Jansen 1969). Recent studies indicate that lysine fortification of wheat flour can significantly

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improve some indicators of the nutritional status and immune function in people consuming wheat-based diets. In China, significant gain in weight and height in children, and increase in the numbers of immune cells was reported in a community that consumed fortified wheat flour (Zhao, Zhai, Zhang, An, Liu, He, Ge, and Scrimshaw 2004). In Pakistan, families provided with wheat flour fortified with 3 g lysine per kg all showed significant increases in C3, C4 and C8 T-cells compared to those that received unfortified flour (Hussain, Abbas, Khan and Scrimshaw 2004).

2.5.3   Plant breeding and biofortification

The prolamin proteins, which are dominant in cereals, are very low in lysine, a major factor that contributes to the low protein quality of cereals. Efforts have been made to develop cereals such as sorghum with elevated lysine levels by chemically induced mutation, which radically suppresses the synthesis of some of the kafirin storage proteins (Mertz, Axtell, Ejeta and Hamaker 1993). The lysine content of the mutant is 60% higher than normal varieties.

2.5.4   The principle of complementation

The term complementation is used with respect to proteins when the relative deficiency of an amino acid in one is compensated by a surplus from another protein consumed at the same time (Bender 2005). A combination of cereals and legumes where one supplements the other with the deficient amino acid creates mutual balance resulting in nutritional complementation (Young and Pellet 1994).   The protein quality is greater than either protein source alone. This principle can be utilized in the development of high protein-energy supplementary foods to alleviate PEM.

The nutritional response of combining two proteins has been classified into four groups using rat bioassay by Bressani, Elias and Gomez-Brenes (1972). Type I involves two proteins with similar lysine deficiency such as maize and peanuts. There is no complementary effect. In type II, the two protein sources have the same limiting amino acids but in different proportions such as maize and cotton seed flour. Type III has the true complementary effect because one protein source has considerably higher concentration of the most limiting amino acid in the other, producing a synergistic effect such as maize and soy or sorghum and soy. In

type IV, both protein sources have common amino acid deficiencies and the one with the higher provides the one without, such as soy proteins and beef.

2.5.4.1 Cereal-legume complemented foods used for supplementary feeding

The World Food Programme of the United Nations provides food to millions of hungry people in less developed countries (WFP 2002). To improve the nutritional value of cereal grains aimed to prevent malnutrition in children and pregnant and lactating mothers, blended food supplements are formulated using soy as a versatile source of high quality low cost protein (Bookwalter 1981). The fortified blended foods are a mixture of cereals such as maize, wheat, sorghum, millet, and soy as the legume but sometimes chickpeas are used. The blended flour is prepared by milling, extrusion or roasting and may be fortified with mineral and vitamin premixes (WFP 2002). They are precooked but not instant food products and need cooking. Different types of foods can be prepared from fortified blended foods. They include thin and thick porridge, roasted blended drinks, soups, flat breads, sweets, cakes, fritters, dumplings, samosas, sweet-balls and biscuits.

The formulated foods are intended to serve as supplements but not replace the diet of young children, school children, pregnant and lactating mothers and emergency feeding in adults (Bookwalter 1981). They include Corn (maize) Soy Blend (CSB), Wheat Soy Blend (WSB), Soy Fortified Bulgur (SFB), Soy Fortified Sorghum Grits (SFSG) and Soy Fortified Rolled Oats (SFRO) (WFP 2002). Bookwalter, Kirleis and Mertz (1987) determined the protein content and digestibility in processed cereals and soy-cereal blends utilized in international feeding programmes. Addition of 15% soy flour to sorghum flours increased protein content from a range of 10 to 11.5% to a range of 16 to 18%. For digestibility, the ranges were 75.1 for sorghum to 98.6 for wheat before cooking, and 51.9% for sorghum to 94.5% for wheat after cooking.

2.5.4.2 Development of low cost cereal legume supplements

The FAO/WHO (1994) Codex Alimentarius Committee proposed that formulation of foods from low lysine staples fortified with legumes could be used as a means to improve the protein nutritional quality of foods for children in developing countries. The staple foods in

many developing countries affected by PEM are cereal based. Consequently, many researchers in these countries have identified cereals as the most suitable vehicles for delivering proteins to at-risk populations (Bulusu et al 2007). Efforts are also being made to develop low cost supplementary foods by complementing cereal and legume proteins using locally available plant foods that are sustainable within the ecological zones.

Several researchers have developed foods intended for complementary feeding of infants and young children. In a recent study Osundahunsi and Aworh (2003) enriched maize-based complementary foods with soy bean and cowpea tempe. The protein content doubled to 18.6-19.7% compared to the unfortified food with 9%. Biological evaluation of the protein quality showed no significant differences between rats fed the enriched food and those fed a casein diet. Soy milk was also used to fortify wheat, maize and rice in the production of protein-based baby foods and the protein content of 25-28.5% was not significantly different from a pure casein diet (Wadud, Abid, Ara, Kosar and Shah 2004). In a similar study, Mensa-Wilmot, Phillips and Hargrove (2001) demonstrated that extruded soy and cowpea fortified maize-based foods had acceptable protein quality with protein efficiency ratios of 2.1 to 2.4 and a true protein digestibility of 87.4 to 92.1.

Other foods that are not weaning foods have also been developed. El-Adawy (1997) made bread by replacing wheat flour with 14, 16, 18 and 20% protein levels of sesame products in the form of sesame meal, roasted and autoclaved sesame meals and sesame protein isolate and concentrate. Protein content of the products increased by 27 to 46% and in vitro protein digestibility by 5 to 13% compared to the pure wheat bread control. Mashayekh, Mahmoodi and Enterazi (2008) substituted wheat with 3, 7 and 12% defatted soy flour in wheat bread and reported an increase in protein content of 21.4 and 29.1% for the 3 and 7% replacement levels, respectively. Additionally, these workers found that the 3% soy substituted bread had the most preferred sensory characteristics.

In the process of developing legume fortified cereal foods, there is a need to recognize that there are problems with rural and urban low income groups who live in environments that lack infrastructure for distribution, time, clean water, and facilities to prepare foods from fortified blends. High nutrient dense and compact ready to eat products are more suitable for such conditions and include products such as biscuits and snacks. Such products can be

developed. Asare, Sefa-Dedeh, Sakyi-Dawson, and Afoaku (2004) developed a model for the production of a puffed snack with enhanced spongy structure from rice-cowpea-groundnut blend with low moisture content produced by extrusion cooking. The optimal process variables were low feed moisture of 14 to 20% and maximum additions of 20% cowpea and 10% groundnut. Baskaran, Mahadevamma, Maleshi, Jayaprakeshan and Lokesh (2001) formulated eight supplementary foods using cereals, sorghum, finger millet, pearl millet and wheat flour popped at 300 to 350°C composited with soy bean and green gram. The ready-to eat supplement which had cereal flour, soy flour, green gram and cane syrup, in proportions of 40, 10, 10, and 35% respectively, with the remainder made up of vitamins and minerals, was heated to 100 ^{o}C and pelletized in 25 g pieces. The protein content was 10.4 to 12.5% with moisture content of 10%.

2.5.4.3 Sorghum use in developing cereal-legume supplementary foods

Sorghum has been used in the production of legume fortified cereal foods. In the Food for Peace Program, it was used in the form of sorghum grits, and also fortified with 12% soy protein (Bookwalter 1981). A study by Bookwalter et al (1987) showed that the protein content of sorghum grits improved from 10.8 to 17.7% when the grits were fortified with 15% soy flour. The workers showed that the 75% protein digestibility of sorghum grits was lower than that of other major cereals such as wheat, bulgur, rolled oats and maize, with 99, 86, 88 and 80%, respectively. The digestibility was further reduced by cooking. However, the addition of soy flour improved the digestibility to 84% in cooked and 72% in the uncooked products. Uruakpa (1996) determined that optimum blending ratio for sorghum-African yam bean blends for maximum benefit is 71.7% sorghum and 28.3% African yam bean. Awadelkareem, Mustafa and El Tinay (2008) reported an increase in lysine content in soy fortified sorghum meal from 106 in pure sorghum flour to 252 and 510 mg/100 g, of 18% protein and 22% protein, respectively.

2.6   TECHNOLOGY OF BISCUIT PRODUCTION

The term "biscuit" actually means twice baked, first to set the structure, then to reduce the moisture content (Hazelton, Des Rochers and Walker 2004). Normal biscuits however are only baked once. Biscuits can be distinguished from other baked goods by their low moisture

content of less than 5%, which makes them have a low risk of microbial spoilage and long shelf life (Zydembos and Humphrey-Taylor 2004). They are made with soft wheat flour with low protein content of 8-10%, using gentle mixing to avoid development of gluten in the dough. Biscuits contain high levels of sugar and shortening (fat) relative to flour, and may include other ingredients like baking powder, salt, eggs, flavouring and milk (Hoseney 1994).

Industrially produced biscuits can be categorized as rotary mould, cutting machine and wire cut biscuits based on the way the biscuit dough is divided (Menjivar and Faridi 1994). Hoseney (1994) explained that rotary mould biscuits are made using visco-plastic dough that is high in sugar and fat and about 10% moisture forced into moulds on a rotating roll. The spread and rise of the biscuits is minimized because the dough is dry, crumbly and stiff with no elastic properties and is only cohesive when the pressure is applied. Cutting machine biscuits have water content of about 20%. This allows some gluten to develop during mixing and sheeting and as a consequence the dough is cut out. Partial development of gluten prevents the biscuit from spreading and makes it hard. Wire cut biscuits are made by extruding soft dough containing high levels of sugar, shortening and eggs through an orifice and then a cutting disk-shapes biscuits by a wire. The biscuits spread and increase in size during baking.

2.6.1 Cereal-legume blend biscuits

The beneficial effect on protein content and quality, of complementing cereals and legumes has led to attempts to fortify biscuits using this principle. Wheat biscuits have been fortified using soy, cowpea, great northern bean, faba bean, navy bean, lupin, chick pea, field pea and green gram. Patel and Rao (1995) substituted wheat flour with different levels of untreated, roasted and germinated black gram flours. Protein content increased from 8.5 to 12% in the germinated flour. Biscuits with roasted gram flour at 5 and 10% were not significantly different in acceptability from the control wheat biscuit. Sugar snap cookies prepared with wheat and substituted with up to 20% navy bean seed flour had acceptable organoleptic characteristics (Hoojjat and Zabik 1984). However, substitution at 30% resulted in scores as low as 2.6 on a 7 point scale. In a similar study by McWatters (1978), wheat flour in sugar cookies was replaced with peanut, soybean and field pea. At a 30% replacement level for all the three legumes in cookies, protein content was double that of plain wheat biscuits.

Non-wheat flours have also been fortified with legumes to improve protein content and quality. Eneche (1999) produced biscuits from millet flour and pigeon pea blends. The range of protein content in blended biscuits was 12.1 to 15.2% compared to the 7.2% for the pure millet biscuits. There was no significant difference in acceptability of sensory characteristics of all the biscuits and Nasco, a locally available shortcake biscuit in Nigeria.

There have been attempts to make pure sorghum biscuits, a term that corresponds to the term cookie in this investigation. Badi and Hoseney (1976) reported that cookies made of sorghum and pearl millet were dense, compact, mealy, gritty, and did not spread during baking. Addition of unrefined soy lecithin improved spread characteristics and an increase of the pH of the dough reduced grittiness. Wheat flour in the biscuits made the biscuits less fragile. Similar results were found by Leon-Chapa (1999). This worker also reported that reduced particle size increased damaged starch significantly, reduced fragility and increased cookie acceptability. Addition of 5% pregelatinised maize starch also reduced grittiness, dryness and fragility.

Sorghum biscuits have also been made with addition of other wheat and legume flours. Geervani, Vimala, Predeep and Devi (1996) developed biscuits for supplementary feeding. Sweet and salt biscuits were prepared using decorticated sorghum, pearl millet and finger millet in combination with dehulled chickpea and green gram flours at a ratio of 4:1. The sorghum biscuits had a Net Protein Utilization (NPU) of 57.8 without legume and 70.4 with legume, and for the millet biscuits the NPU increased from 62.7 to 78.6. The authors concluded that the baking process did not have any adverse effect on protein quality of biscuits made using cereal-legume composite flours.

Biscuits are a popular snack food, a type of food not meant to be eaten as a main meal, but one intended to assuage hunger between meals, providing a brief supply of energy for the body. They are popular snacks among children. In developing countries as well as being available on the market for consumption, biscuits are distributed in schools for school feeding by the WFP as an energy dense snack (WFP 2002). Additionally, a study by van Stuijvenburg, Kvalsvig, Faber, Kruger, Kenoyer and Benade (1999) showed that iron, iodine and β-carotene fortified biscuits significantly improved the micronutrient status of school children in South Africa. From the foregoing, it appears possible to use sorghum and bread

wheat in biscuit products. This study is aimed at developing lysine fortified sorghum and bread wheat biscuits for supplementary feeding in schools and elsewhere to alleviate PEM.

2.7 ANALYTICAL METHODS FOR PROTEIN QUALITY IN CEREAL-LEGUME COMPLEMENTED FOODS

The ideal and most sensitive assessment of protein quality is achieved by clinical human studies, or animal assays that measure growth or metabolic indicators (Boutrif 1991). However, for reasons of cost, time and ethics particularly in humans where some procedures are invasive, cause pain and have negative health consequences, in vitro techniques using chemicals and enzymes have also been developed.

2.7.1 Bioassays for protein quality

2.7.1.1 Protein Efficiency Ratio (PER)

The Protein Efficiency Ratio (PER) method as described in AOAC (2000) Method 960.48 utilizes an in vivo assay to estimate protein quality by measuring rat growth as weight gain per gram of fed protein (Smith 2003). It is used when determining protein as a percent of the daily value on the nutrition label of foods intended for consumption by infants and determination of protein quality in new food products. It is limited in application because the requirements of a weanling rat are similar only to a human infant but not to other age groups. It is considered a time consuming method and does not account for the maintenance requirement of the weanling rat. A protein that produces no weight gain in the assay has a zero score. PER is calculated as follows:

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Weight gain of animals on test diet (g)}}{\text{Weight protein consumed by animal on test diet (g)}}$$

2.7.1.2 Net Protein Ratio (NPR) and relative NPR

The NPR and relative NPR indices are an improvement of PER and account for the maintenance requirement of the weanling rat, unlike the PER index. They are calculated as follows:

$$\text{Net Protein Ratio(NPR)} = \frac{\text{g of weight gain test diet} + \text{g of weight loss in protein free diet}}{\text{Protein (N x 6.25) consumed by test animal}}$$

$$\text{Relative NPR} = \frac{\text{(mean NPR of test protein) X 100}}{\text{Mean NPR of reference protein}}$$

2.7.1.3 True Protein Digestibility (TPD)

True Protein Digestibility (TPD), Method 99.29 (AOAC 2000) is a rat bioassay. According to the AOAC (2000) description, in this method, protein hydrolysis begins in the stomach and is catalyzed by proteases and peptidase before absorption. The indigestible food excreted in the faeces from the colon includes small amounts of metabolic nitrogen. To determine true digestibility, corrections must made for metabolic nitrogen estimated as the amount of faecal nitrogen excreted when the animal is consuming a protein free diet. According to WHO (2007) in the TPD assay, apparent protein digestibility is obtained from the difference between the nitrogen ingested and nitrogen excreted in faeces. The drawback is that information on how much of the absorbed nitrogen is retained or utilized by the body is not provided. TPD has been the method of choice for many researchers to determine *in vivo* protein digestibility and has become more important because it forms part of the required measurement to compute Protein Digestibility Corrected Amino Acid Score (PDCAAS). TPD is calculated as follows:

$$\text{True digestibility (TD\%)} = \frac{\text{Nitrogen intake of test animals} - (F-F_m) \text{ X } 100}{\text{Nitrogen intake of test animals}}$$

F= faecal nitrogen output by test animals.

F_m = Faecal nitrogen output by "protein free" animals or endogenous (metabolic) nitrogen.

2.7.1.4 Biological Value

It is assumed that the effectiveness with which the absorbed nitrogen can be utilized is determined by the amino acid profile and is defined in terms of the Biological Value (WHO 2007).

$$\text{True protein (N) biological value (\%)} = \frac{\text{Nitrogen intake} - (F - F_m) - (U - U_m) \times 100}{\text{Nitrogen intake} - (F - F_m)}$$

U = urinary nitrogen loss on the test diet.

U_m = urinary nitrogen loss on a protein-free diet.

2.7.1.5 Protein Digestibility Corrected Amino Acid Score (PDCAAS)

The FAO/WHO (1991) expert group replaced the rat growth assay Protein Efficiency Ratio (PER) with Protein Digestibility Corrected Amino Acid Score (PDCAAS). The PDCAAS is based on human amino acid requirements and is more appropriate for the estimation of protein quality than an animal assay (Smith 2003). The parameters it takes into consideration critical to quality evaluation of a protein source are indespensable amino acid profile of the test protein, its digestibility and ability to supply the amino acid in sufficient quantity (WHO 2007). In a review Smith (2003) states that the PDCAAS method estimates protein nutritional quality by combining information from a calculation comparing the amount of the first-limiting amino acid in a protein to the amount of that amino acid in a reference protein and in vivo measure of digestibility of the protein by rats. The Codex Alimentarious Committee on vegetable proteins (CCVP) recommended the use of the amino acid requirement for 2-5 year old child (FAO/WHO/UNU 1985) as a reference pattern, also endorsed by the FAO/WHO (1991) expert committee. The limitation is that only information about the limiting amino acid is included, disregarding other indespensable amino acids (Smith 2003). The calculation for PDCAAS is as follows:

PDCAAS = TPD x Lowest amino acid score

2.7.2 In vitro methods

2.7.2.1 Amino acid composition

Amino acid analysis quantitatively determines the amino acid composition of protein foods (Smith 2003). The protein sample is hydrolyzed to liberate amino acids in 6 M HCl at 110°C for 20 to 96 hr, separated using chromatographic methods and quantified. The purpose for the determination of amino acid composition in complemented foods is to assure the correct balance of amino acids and establish the effects of processing on the protein quality (Aristoy and Toldra 2004).

Ion exchange chromatography amino acid analysis was developed by Moore and Stein (1951) using synthetic amino acids to the composition of protein hydrolysates and a sequence of buffers. The yield was a single chromatogram and effluent curve with every component as a discrete peak. The method was revised by Moore, Spackman and Stein (1958) and has since been automated. This method is known to produce accurate results in samples and has been used extensively in determination of amino acids in a variety of foods (Hurrell, Lerman and Carpenter 1979). Ion exchange chromatography is the "gold standard". However, the shortcoming is the length of time taken to analyze samples, the high cost of the ion exchange amino acid analyzer and maintenance and complex composition of the mobile phase (Aristoy and Toldra 2004).

Aristoy and Toldra (2004) explain that Reversed Phase High Performance Liquid Chromatography (RP-HPLC) is widely used in the determination of amino acids in many kinds of matrices. Amino acid derivatization is necessary to confer hydrophobicity to the amino acid molecule to be effectively partitioned using chromatography. The most used column packaging is alkyl-bonded silica particles. RP-HPLC is more rapid than ion exchange chromatography.

2.7.2.2 Lysine availability

Available lysine refers to protein bound lysine in which the end amino acid is free so that after enzyme hydrolysis and hence the lysine is available for absorption (Bender 1998).

Lysine availability assays measure the nutritional quality of heat processed products because the free amino group in lysine may be bound to a reducing sugar in the Maillard reaction, or other linkages as reviewed earlier and cannot be hydrolysed during digestion. Such structural changes to lysine cannot be detected by amino acid analysis and total lysine values include damaged proteins that are not available (Moughan and Rutherfurd 2008). Alternative methods have been developed to determine "reactive lysine" that has an unblocked ε-amino group and is nutritionally available.

1-fluoro-2, 4-dinitrobenzene was the first reagent used to determine available lysine and was reported by Carpenter (1960). In the Fluorodinitrobenzene (FDNB) method (AOAC International 2000) Method 975.44, the Sanger reaction is used to convert lysine to yellow dinitrophenyl (DNP)-lysine, which can be extracted and measured spectrophometrically or by HPLC. In a review, Hurrell and Carpenter (1981) rated the FDNB method among the best in detecting early Maillard reaction derivatives. FDNB, however, is not soluble in water and affects people with sensitive skin, ε-DNP is destroyed during the hydrolysis step of the assay particularly in high carbohydrate foods and correction factors need to be applied (Moughan and Rutherfurd 2008).

The rapid dye-binding lysine procedure described by Hurrell et al (1979) was developed to overcome the shortcomings of the FDNB method in quantification of reactive lysine. In this method a dye such as Acid Orange 12 is used as an indicator of protein quality. The procedure requires two measurements of dye binding capacity (DBC). The first measurement of the unmodified sample gives histidine + arginine + lysine. The second measurement from a sample modified with propionic anhydride to block the lysine gives only histidine and arginine. The difference between the two is the measure of lysine. Hurrell et al (1979) compared the efficiency of FDNB and dye-binding and concluded that it is a convenient replacement of methods of determining reactive lysine that are laborious because of hydrolysis and is a sensitive indicator of the first 15% of nutritional damage to foods, the region of practical importance. However, it can underestimate the extent of nutritional damage more than the FDNB method.

2.7.2.3 Protein digestibility

In vitro protein digestibility provides information on how efficiently a protein is digested and detects protein induced changes in protein quality (Damodaran 1996). In vitro protein digestibility may be carried out by multi-enzyme or single-enzyme assays.

The pH-shift and pH-stat are both multi-enzyme assays that use commercial proteolytic enzymes under standardized conditions to estimate the digestibility of protein by measuring the extent of protein hydrolysis upon reaction (Smith 2003). Digestibility is determined using two three or four of trypsin, chymotrypsin, peptidase and bacterial protease to simulate human digestion.

Hsu, Vavac, Satterlee and Miller (1977) developed a multi-enzyme assay using trypsin, chymotrypsin and peptidase. The pH of the digest after 10 minutes highly correlated with *in vivo* digestibility of rats. This method is a pH shift method in which the pH of the protein solution drops when hydrogen ions are liberated following hydrolysis. Serna-Saldivar, Abril-Dominguez, Lopez-Ahumada and Ortega-Ramirez (1999) used this method to determine digestibility in soy fortified bread. A modified version by addition of a fourth enzyme protease from *Streptomyces griseous* was used by Osman (2004). This method best correlates with rat protein digestibility after 15 minutes of enzymatic treatment (Wolzak, Bressani and Brenes 1981). The limitation of this method is that the pH may drop to a level that is not optimum for the digestive enzyme (Smith 2003).

Two-enzyme methods have also been used by researchers. Saunders, Connor, Booth, Bickoff and Kohler (1973), compared digestibility by papain, pepsin-pancreatin and pepsin-trypsin and found that the two systems in which pepsin was used correlated well with digestibility in rats. The digestibility of Indian and Sudanese sorghum cultivars was tested using the pepsin-panceatin assay by Awadelkareem, Muralikrishna, El Tinay and Mustafa (2009) and the digestibility reduced after cooking, as previously established by other workers.

The single enzyme method has been used extensively by researchers to determine protein digestibility in cereals. Axtell et al (1981) used pepsin to determine digestibility of ground whole or dehulled sorghum samples cooked under the same conditions used by MacLean et al

(1981). They found that the range of values for reduced digestibility were similar to those found in children, 45-57% for whole kernel and 37-43% for dehulled. Chibber, Mertz and Axtell (1981) compared the efficacy of trypsin-chymotrypsin combination to pepsin in digesting sorghum proteins and found that pepsin was more efficient than the trypsin-chymotrypsin mixture.

2.8   EVALUATION OF SENSORY CHARACTERISTICS IN BISCUITS

In descriptive sensory evaluation, an instrument is developed to measure a set of attributes in the food under study to complement results from traditional physical and chemical instrumental analysis, using a highly trained panel (Rousseau 2004).   Sensory evaluation has been widely used in the evaluation of biscuits. For example, Akinwande, Ade-Omowaye, Olaniyan and Akintaro (2008) used a trained sensory panel of 15 to evaluate soy fortified cassava biscuits developed for possible school feeding. The sensory attributes evaluated included colour, crispness, crumbliness, hardness, aroma and taste using a 7 point scale. It was found that Incorperation of ginger spice reduced the beany soy flavour. Consumer acceptability testing involves measurement of hedonic responses from subjects to determine which product is liked or disliked the most as well as relative liking of the sample. Consumer sensory evaluation is also very useful for the evaluation of biscuits. For example, Brown and Braxton (2000) evaluated consumer perception of texture and preference for biscuits in relation to breakdown during eating.

2.8.1   Methods for evaluation with children

Food products developed specifically for children must be tested with children (Guinard 2001). The sensory perception of children is not very widely researched on as most of the research has been done by adults. The limitation with studies involving children is availability of methodology to measure their food preferences. Methods must be simple enough to be understood but robust enough to measure preference reliably (Leon, Couronne, Marcuz, and Koster 1999). Some studies have compared the different methods in terms of discrimination, repeatability and validity. Leon et al (1999) investigated three non verbal methods, paired comparison, ranking by elimination and hedonic categorization to assess

food preference in children aged 4 to 10 years. Five biscuits dressed with different types of jams were used. They found that the products were more discriminated with hedonic categorization than the comparative methods. They concluded that the reliability of a method is linked to the age of the child and the more distinguishable the product, the more reliable the method.

2.8.2 Evaluating long term acceptability of foods

According to Vickers and Holton (1998), a food with long term acceptability can be eaten repeatedly even when other acceptable foods are available to the consumer. Researchers have found that repeated exposure can change acceptance showing decreased, increased or sustained acceptance (Mela 2000). The acceptance of novel foods may increase with repeated exposure, while acceptance of familiar foods may decrease with repeated exposure resulting in boredom (Wiejzen, Zandstra, Alfieri and de Graaf 2008). Vickers and Holton (1998) found that intensity level of stimuli influenced acceptance over repeated exposure. Tea with low flavour intensity was gradually preferred to tea with higher intensity over 20 consumptions. Chung and Vickers (2007) using repeated exposure found that the liking of tea with lower sweetness was preferred over time to low sweet tea.

Long-term acceptance of food products can be established by a consumer exposure test of several days or weeks (Wiejzen at al 2008). Vickers, Holton and Wang (1997) proposed that sensory specific satiety (SSS) could serve as a rapid method of measuring long-term acceptability of food. SSS is the relative change in liking for a food due to consuming it. A typical SSS test protocol involves subjects who taste and rate their liking of several samples of food, including the test food, eat a serving of the test food, then finally re-taste and re-rate their liking of the set of foods (Rolls 1986). Both the amount of test food consumed and the change in liking of the test food from before to after consumption have the potential to serve as indicators of long term acceptability, because they include some aspects of ingestion adaptation and habituation (Johnson and Vickers 1992). These researchers found a trend for less liked test meals to drop more in liking than well liked test meals. This observation was similar to the longer term acceptability study by Schutz and Pilgrim (1958) that showed well liked foods did not change in liking with repeated exposure while liking ratings decreased for

less liked foods. Vickers et al (1997) found that sensory satiety extends to foods with stimuli characteristics similar to eaten foods. After consuming highly sweet yoghurt there was a significant drop in liking of chocolate bars and canned peaches. Also, a sweeter yoghurt elicited stronger sensory specific satiety than a less sweet yoghurt.

2.9   CONCLUSIONS

PEM malnutrition is the major nutritional deficiency disease among children in Africa and other developing countries. Sorghum is the second most important cereal crop in Africa and grows where maize cannot grow, and bread wheat is also widely cultivated in several African countries. These cereals are therefore suitable vehicles for proteins where they are staple foods. Protein fortified sorghum and bread wheat biscuits could be a very effective method of delivering proteins to children and other vulnerable groups for the alleviation of PEM. They are ready-to-eat, protein-and energy-dense, are shelf stable and are made of ingredients that are sustainable within the ecological zones. They are also a very popular snack among children. Developing legume fortified sorghum and bread wheat biscuits and determining their protein and sensory quality and acceptability among the target vulnerable population is a necessary step toward the fight against PEM among children in Africa and other developing countries.

3 HYPOTHESES AND OBJECTIVES

3.1 HYPOTHESES

1. Biscuits made of sorghum and bread wheat composited with Defatted Soy Flour (DSF) will have significantly improved nutritional value in terms of protein, lysine and indispensable amino acids and mineral content compared to unfortified biscuits. Defatted soy flour has a better nutrient composition with respect to protein, lysine and indispensable amino acids and minerals than sorghum and bread wheat (USDA 2008).

2. Fortified sorghum and bread wheat biscuits will have significantly higher levels of bioavailable protein and lysine compared to unfortified biscuits. Protein and lysine that are deficient in sorghum and bread wheat and are adversely affected when sorghum is processed (reviewed by Taylor and Belton 2002), will be increased by addition of DSF to the biscuits.

3. Soy fortified sorghum biscuits will have higher true protein digestibility and improve growth rate in rats compared to unfortified biscuits. The added soy proteins have an amino acid profile that is superior to sorghum protein amino acid profile and higher lysine content (USDA 2008). Complementing sorghum with legumes improves growth and apparent protein digestibility in rats (Nnam 2001) and increasing lysine content in rat diet increases growth (Ashley and Anderson 1975).

4. Sorghum flour can be used to make biscuits that are similar in texture and sensory properties to wheat-based biscuits. Wheat flour is the principal component of virtually all biscuits because when mixed with water it forms a unique visco-elastic dough (Kent and Evers 1994). However, good quality biscuits can be prepared using non-wheat flours because biscuits do not require high gluten flours.

5. Defatted soy flour fortified sorghum and bread wheat biscuits will be liked by school children over an extended period of time. Biscuits are popular snack foods among children (Sudha et al 2007) because they are sweet and sorghum and bread wheat are staples that African children are familiar with.

3.2 OBJECTIVES

1. To formulate, develop and standardise sorghum-soy and bread wheat-soy biscuit formulae that include fortified levels of protein to meet half the Recommended Dietary Allowance for 3 to 10 year old children according to Institute of Medicine, Food and Nutrition Board (2005) and to double the lysine content.

2. To determine the effect of fortifying sorghum and bread wheat with defatted soy flour on proximate composition, protein quality with reference to amino acid (lysine) content and lysine availability and in vitro protein digestibility of biscuits.

3. To determine the effect of fortifying sorghum and bread wheat with soy on the physical and sensory characteristics of biscuits.

4. To determine the effect of compositing sorghum and bread wheat on school children's overall liking and long term acceptability of biscuits.

5. To determine the protein nutritional quality and effect on growth of sorghum-soy composite biscuits compared to unfortified sorghum biscuits using a rat bioassay.

4 RESEARCH

4.1 Effect of fortifying sorghum and bread wheat with soy protein on the nutritional properties of biscuits

ABSTRACT

Protein Energy Malnutrition (PEM) is the most important nutritional problem among children in developing countries. Biscuits are a useful supplementary food as they are ready-to-eat, nutrient dense and have high acceptability. Biscuits were formulated and developed by compositing sorghum and bread wheat flours with defatted soya flour at different ratios. To establish the nutritional characteristics of biscuits, proximate composition, lysine and reactive lysine contents and in vitro protein digestibility were determined. Compared to the 100% cereal biscuits, sorghum-soy and bread wheat-soy 1:1 ratio composite biscuits had at least double the protein, mineral and crude fibre contents. The lysine contents of the biscuits increased by 500-700%. For the sorghum-soy biscuits in vitro protein digestibility increased by 170% and Protein Digestibility Corrected Amino Acid Score was 8 times higher. Two such biscuits of 28 g each could provide 50% of the recommended daily protein intake for 3 to 10 year olds. Hence, sorghum- and bread wheat-soy composite biscuits have considerable potential as protein-rich supplementary foods to alleviate PEM in children.

4.1.1 INTRODUCTION

Protein Energy Malnutrition (PEM) is the most important nutritional problem facing children in developing countries (Muller and Krawinkel 2005). Affected children have higher susceptibility to infectious diseases, impaired physical and cognitive development and increased mortality rates (Stipanuk 2006). Cereals such as sorghum, wheat and maize, which are principal sources of protein and energy in their diet, are the most suitable vehicles for delivering protein to prevent PEM (Bulusu et al 2007).

Sorghum [*Sorghum bicolor* (L) Moench], an indigenous African cereal, is unique because it is a drought-tolerant staple food for over 500 million people living in arid and semi arid tropics where maize cannot grow (Doggett 1988, ICRISAT 2009). Wheat, mainly hard, bread type wheat is also another important cereal staple in the semi arid tropics of Africa, being cultivated in some 33 countries (Taylor 2004). Like other cereals, sorghum and wheat have poor protein quality because they are limiting in the indispensable amino acid lysine with only approximately 2 g per 100 g protein (Taylor and Schussler 1986, Shewry 2009). Lysine is further rendered unavailable to the body through thermal processing and storage following the Maillard and other reactions because of its highly reactive ε-amino group (Erbersdobler and Faist 2001). Additionally, the problem is compounded by the reduction in sorghum protein digestibility on wet cooking (Duodu et al 2002), which is apparently unique to sorghum. In a nutritional study MacLean et al (1981) demonstrated that nitrogen from a sorghum diet did not support growth in 6 to 30 month old children because of poor absorption and retention.

The protein quality of sorghum and bread wheat can be upgraded to meet human physiological requirements by compositing with legume flours using the principle of complementation (Young and Pellet 1994). Formulation of foods from low-lysine staples fortified with legumes has been proposed as a most practical and sustainable approach to improving the protein nutritional value of foods for young children in developing countries (WHO/UNICEF 1998, FAO/WHO 1994). Soy bean (*Glycine max* L. *Merr*) typically contains 30 to 45% protein (Hammond et al 2003) and is a good source of all indespensable amino acids (Karr-Lilienthal, Bauer, Zinn, Frazier, Parsons and Fahey 2006). Vasconcelos et al (2001) established that the indispensable amino acid profile of soy beans was comparable to

the FAO/WHO/UNU (1985) reference pattern for children of 2 to 5 years and 10 to 12 years and had an average lysine content of 7 g/100 g. Bodwell and Marable (1981) found 75 to 97% and 84 to 95% true nitrogen digestibilities of soy protein in adults and children, respectively. The high lysine content and high digestibility of soy protein makes it a good complement to sorghum and wheat.

Biscuits are the largest category of snack foods among baked products worldwide (Ait-Ameur et al 2008). They offer a valuable vehicle of supplementation with protein because of their popularity, relatively low cost, varied taste, ease of availability, high nutrient density and long shelf-life (Sudha et al 2007). Soft wheat flours are normally used for biscuit preparation, but they can be made from composites of wheat and other flours and non-wheat flours (Dendy 1993). Studies on fortification of wheat biscuits with soy protein have been conducted. For example, Mohsen, Fadel, Bekhit, Edris and Ahmed (2009), achieved a 12 to 20% increase in protein content by substituting wheat with 5 to 20% soy protein isolate. Similarly, Singh, Singh and Chauhan (2000) and McWatters (1978) doubled protein content in wheat biscuits by substituting 20 and 30%, respectively, wheat flour with defatted soy flour (DSF). There are also reports of biscuits made using sorghum flour (Badi and Hoseney 1976) or pre-gelatinised sorghum flour dough (Dendy 1993). Findings of these two studies showed that biscuits were gritty and fragile. However, grittiness could be reduced by an increase of dough pH (Badi and Hoseney 1976) and fragility reduced by addition of wheat flour (El Khalifa and El Tinay 2002) or reduced flour particle size (Leon-Chapa 1999).

The production of sorghum-legume composite biscuits has been reported by Hikeezi (1994) who supplemented sorghum with peanut or sunflower flours raising the protein content to 16%. Mridula, Gupta and Manikantan (2007) showed that biscuits of acceptable quality can be made using wheat-sorghum composites with 10 to 50% sorghum and 5% DSF. However, soy complementation of sorghum only biscuits has not been reported. Therefore, the objective of this study was to formulate, develop, and determine the nutritive value of biscuits made from sorghum and bread wheat, cereals widely grown in the semi-arid tropics of Africa, composited with DSF for use as a protein-rich supplementary food for PEM vulnerable children.

4.1.2   MATERIALS AND METHODS

4.1.2.1 Biscuit ingredients

All ingredients used were commercially available and purchased in Pretoria, South Africa. These were: Sorghum "Fine Mabele Meal" (roughly decorticated red, non tannin sorghum) and white bread wheat flour "Golden Cloud" (Tiger Consumer Brands Ltd, Bryanston, South Africa), pure white sugar "Selati" (TSB Sugar, Malelane, South Africa), defatted soy flour "Toasted Flour" (Nedan Oil Mills Ltd, Potgietersrus, South Africa), sunflower oil "Sunfoil" (Willowton Oil, Pietermaritzburg, South Africa), "Bokomo-Moirs" baking powder and vanilla essence both from (Pioneer Foods Ltd, Cape Town, South Africa).

4.1.2.2 Biscuit formulation

Formulation of the biscuit was based on providing at least half the daily protein requirement for school children aged between 3 and 10 years. The scoring pattern for 3 to 10 year olds is recommended when judging protein quality for school children and adolescents (WHO 2007). The Acceptable Macronutrient Distribution Range (AMDR) for protein-energy for prevention of chronic diseases such as PEM for this age group is 10 to 30 g protein per day (Institute of Medicine, Food and Nutrition Board 2005). The study aimed at providing at least half, 14 g of protein per day with 7 g provided in one 28 g weight biscuit, and to double the lysine content in sorghum.

The 100% sorghum, 100% bread-wheat and 100% soy biscuits' basic formulation comprised 225 g flour, 56 g sugar, 66 g sunflower oil and 13.5 g vanilla essence. Water was dependent on the treatment and ranged from 10% (100% sorghum biscuits) to 30.7% (100% soy biscuits) of total weight of ingredients, as was baking powder, 0.25 g in 100% bread wheat biscuits to 1.5 g in the sorghum biscuits. The amounts added were based on results of preceding experiments which revealed that substitution with DSF made doughs dry crumbly and difficult to manage requiring more water. For the bread wheat biscuit dough, incorporating the same amount of baking powder as sorghum made the dough pieces rise excessively on baking due to the strength of the flour. In the various formulations, 28.6, 50,

45

Table 4.1.1 Formulation of the sorghum, bread wheat, soy and composite biscuit doughs

Ingredients	Sorghum: Soy				Soy	Wheat: Soy			
	100:0	71.4:28.6	50:50	28.6:71.4	100:0	100:0	71.4:28.6	50:50	28.6:71.4
Defatted Soy flour (g)	0	64 (14.9)	112.5(24.9)	161(33.1)	225(43.1)	0	64(14.2)	112.5(24.4)	161(33.2)
Sorghum flour (g)	225(55.9)	161(37.2)	112.5(24.9)	64(13.0)	0	0	0	0	0
Wheat flour (g)	0	0	0	0	0	225(53.4)	161(35.7)	112.5(24.4)	64(13.0)
Sugar (g)	56(13.9)	56 (13.0)	56(12.4)	56(11.5)	56(10.7)	56(13.3)	56(12.4)	56(12.1)	56(11.5)
Sunflower oil (g)	66(16.4)	66 (15.3)	66(14.6)	66(13.6)	66(12.6)	66(15.7)	66(14.6)	66(14.3)	66(13.6)
Baking powder (g)	1.5(0.4)	1.5(0.3)	1.5(0.3)	1.5(0.3)	1.5(0.3)	0.25(0.1)	0.5(0.1)	1(0.2)	1(0.2)
Vanilla essence (g)	13.5(3.4)	13.5 (3.1)	13.5(3.0)	13.5(2.8)	13.5(2.6)	13.5(3.2)	13.5(3.0)	13.5(3.0)	13.5(2.8)
Water (g)	40,80*(10.0)	70 (16.2)	90,180*(19.9)	125(25.7)	160(30.7)	60(14.3)	90(20.0)	100(21.6)	125(25.7)
Total dough weight (g)	402(100)	432(100)	452(100)	487(100)	522(100)	421(100)	451(100)	462(100)	487(100)

Figures in parentheses are percentages
*Maximum particle size for sorghum flour reduced to 500 μm for sorghum: soy biscuits ratios 100:0 and 50:50 for consumer study and water content of dough doubled to make it workable.

71.4 and 100% DSF replaced sorghum or bread wheat flours on a weight by weight basis (Table 4.1.1). The proportions were based on a basic formulation in which a maximum of 161 g DSF (50 g protein/ 100 g) and 64 g cereal (12 g protein/100 g) in a 225 g composite could produce 12 biscuits each containing approximately 7 g protein.

4.1.2.3 Biscuit preparation

The dry ingredients: flour, sugar and baking powder, were sieved into a mixing bowl and mixed by hand for 3 minutes. Oil and water were added gradually and the mixture kneaded for 2 minutes at medium speed in an electric mixer, to a firm dough. The dough was manually sheeted on a steel tray to a height of 5 mm using a wooden rolling pin and cut into circular shapes using a 6.3 cm diameter biscuit cutter. Aluminium foil was used to prevent dough sticking to the rolling pin. The cut dough pieces were transferred onto a baking sheet lined with aluminium foil. The biscuits were baked in a preheated air circulation oven at $180^{o}C \pm 2^{o}C$ for 20 ± 5 minutes and cooled for 30 minutes at ambient temperature. Biscuits were vacuum packed in polyethylene bags and stored in a cold room at $10^{o}C$. Three batches of 10 biscuits each were prepared for each experimental treatment. For chemical analyses, biscuits were ground using a mortar and pestle to a particle size of ≤ 1 mm before storage. The procedure for biscuit preparation is illustrated in Figure 4.1.1.

Sorghum or wheat flour or their composites with defatted soy flour.

Mix all dry ingredients, 225 g flour, 56 g sugar and baking powder.

Sieve into mixing bowl and mix for 2 minutes in electric mixer.

Gradually add 66 g sunflower oil, 13.5 g vanilla essence and water. Mix at medium speed (2) for 3 minutes to firm dough.

Cover crumbly dough with aluminium foil and sheet on a 5 mm height steel tray. Cut into circular shapes with a 6.3 cm diameter biscuit cutter.

Transfer the cut dough pieces onto a baking sheet lined with aluminium foil.

Bake in a preheated oven at 180°C for 20 ± 5 minutes. Remove from oven and allow to cool on baking tray for 30 minutes.

Figure 4.1.1 Flow diagram for preparation of soy fortified sorghum or bread wheat biscuits.

4.1.2.4 Proximate analyses

Moisture

Moisture content of biscuits and raw flour was determined by the one stage air oven procedure (AACC International 2000) Method 44-15A. Samples of 3 g weight were dried at $130^{o}C$ for 3 hours and moisture content was obtained by calculating loss in moisture as a percentage of the original wet weight of the sample.

Ash

Ash mineral content was determined by the AACC International (2000) Method 08-01. Samples were heated at $550^{o}C$ for 5 hours or to constant weight. Ash content was obtained by calculating the weight of the residue as a percentage of the original sample weight.

Oil

Oil content was determined by the soxhlet extraction method (AACC International 2000) Method 30-25. Samples of 3 g were weighed into an extraction thimble and fat extracted for 1 hour using petroleum ether $(40-60^{o}C)$. The petroleum ether extract was dried in an oven at $103^{o}C$ for 30 minutes. Total fat content was obtained by calculating weight of extract as a percentage of the original sample weight.

Crude fibre

Crude fibre content was determined by the AACC International (2000) Method 32-10 using a Fibertec apparatus (Tecator, Hoganas, Sweden). Defatted samples of 1 g weight were digested using 0.127 M sulphuric acid and 0.313M sodium hydroxide solutions. The residue was dried overnight in an oven at $105^{o}C$ and ashed at $550^{o}C$ for 5 hours. Crude fibre content was obtained by calculating weight loss on ignition of dried residue as a percentage of the original sample weight.

Carbohydrate

Percentage of carbohydrate was calculated by difference by summing up the weight in grams of determined values of protein, fat, ash, crude fibre and moisture from the total weight of the food (FAO 2003).

Energy

Energy content was calculated using Atwater calorie conversion factors, based on assumptions that each gram of carbohydrate, fat and protein will yield 17 kJ (4.0 kcal), 37 kJ 9.0 kcal and 17 kJ (4.0 kcal), respectively (FAO 2003). The values were expressed in kJ.

Protein content

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

4.1.2.5 Protein quality analyses

Amino acid content

The content of lysine and other amino acids in the samples was determined using the Pico-Tag method (Bidlingmeyer, Cohen and Tarvin 1984). Protein and peptides are hydrolyzed with 6 M hydrochloric acid, pre-column derivatised and analysed using reverse phase HPLC.

Reactive Lysine

Reactive lysine content was determined using a rapid dye-binding lysine (DBL) method of Kim, Kim, Ma and Chung (2007) using Crocein Orange G dye (70% dye content) (Fluka grade 27965: Sigma-Aldrich, Buchs, Switzerland). The mass of sample weights for treatments A and B were calculated depending on their protein content using the following equation of Anyango (2009):

Treatment A: y= -0.026x + 0.784

Treatment B: y= -0.026x + 0.982

Where y= mass of sample weighed (g)

x= protein content (%) (wb)

Accurate amounts of the calculated mass for treatments A and B were weighed and reacted with 5 ml 16% sodium acetate for 15 minutes. For the B samples, 0.2 ml propionic anhydride was added to block the reactive ε-amino group of lysine. The 12 ml dye solution of Crocein Orange G (70% dye content) used for dye-binding, was added to the samples which were shaken for two hours, diluted and the absorbance measured at 482 nm. Reactive lysine (dye bound lysine) was obtained by calculating the difference between (treatment B) the sample treated with propionic anhydride to give histidine and arginine by blocking lysine and (treatment A) the untreated sample with histidine, arginine and lysine.

In vitro protein digestibility

In vitro protein digestibility was determined by a pepsin digestion method based on that of Hamaker et al (1986). Accurately weighed samples (200 mg) were digested with P7000-100G porcine pepsin, activity 863 units/mg protein (Sigma-Aldrich, St. Louis, MO) for 2 h at 37°C. The supernatant was pipetted off using a Pasteur pipette, the residue washed and the clear supernatant pipetted off again. Residues were dried overnight in an oven at 100°C. Protein content in the dried residue was determined by the Dumas combustion method (AACC International 2000) method 46-30. Digestibility was calculated by obtaining the difference between total protein and residual protein and expressed as a percentage of the total protein.

4.1.2.6 Statistical analyses

Three independent batches of biscuits were made and analyses were repeated three times. The data generated included: (9 biscuits x 3 batches x 3 replicates) + (3 flours x 3 replicates x 3 repetitions) = 108 samples analyzed for each parameter. Data were analysed using one way analysis of variance (ANOVA) and means compared using Fisher's least significant difference (LSD) test. The statistical program used was Statgraphics Centurion XV (Stat Point, Herndon, VA).

4.1.3 RESULTS AND DISCUSSION

Ingredients that are locally available and sustainable were selected for biscuit preparation. Red non-tannin sorghum, bread wheat and defatted soy flours and sunflower oil are readily available on the South African market. DFS is one of the legume flours recommended by the Codex Alimentarius Commission (FAO/WHO 1994) for use as a source of protein in foods for young children. Complementing cereal and DSF, high temperature and low moisture baking conditions, enhance the Maillard and other chemical reactions that affect protein quality (Charissou et al 2007, Friedman 1996). It was therefore necessary to assess the protein nutritional quality of the final baked products (biscuits).

4.1.3.1 Proximate composition

The proximate composition of biscuits and flours is shown in Table 4.2.2. Sorghum biscuits had half the moisture content of wheat biscuits that had a range of 7.15 to 5.98%. The hydrophobic nature of sorghum kafirins compared to hydrophilic wheat proteins (Duodu et al 2003) may explain this finding. Kafirins probably expelled water as the temperature increased while wheat prolamins absorbed water (Belton et al 2006). The high moisture content was probably due to damaged starch, high protein and pentosans in bread wheat which absorb once, twice and ten times, their weights in water, respectively (Kent and Evers 1994).

Fortification with DSF ranging from 28.6 to 71.4% relative to cereal flour increased ash (mineral) content of sorghum-and bread wheat-based biscuits by 50 to 136% and 200 to 520%, respectively compared to the 100% cereal biscuits. Similar results were reported by Shrestha and Noomhorm (2002) when they compared total ash and acid insoluble ash contents in wheat-soy composite flour biscuits and plain wheat biscuits. The increase in ash content through complementation with DSF is because soy flour has a higher mineral content than the two cereals (USDA 2008). Defatted soy flour contains high potassium, moderate levels of calcium, phosphorus and magnesium and traces of selenium, manganese, copper, iron, sodium and zinc.

Table 4.1.2 The effect of compositing sorghum and bread wheat with defatted soy flour on proximate composition (g/100 g)

Flour / Biscuits	Moisture	Protein (N x 6.25)	Fat	Ash	Crude fibre	Carbohydrate[1]	Energy[2] (kJ/g 100 g)
Flour							
Sorghum flour	11.8[g]±0.3	11.4[c]±0.1	3.1[c]±0.6	1.4[c]±0.0	2.0[c]±0.0	70.3[j]±0.2	1504[b]
Wheat flour	11.3[g]±0.2	13.4[d]±0.0	1.4[b]±0.1	0.7[b]±0.0	0.2[a]±0.0	73.2[k]±0.1	1520[c]
Soy flour	6.2[de]±0.4	50.1[l]±0.1	0.5[a]±0.0	6.2[k]±0.1	7.5[j]±0.1	29.5[b]±0.1	1372[a]
Sorghum /Soy biscuit							
100:0	3.2[a]±0.4	9.2[a]±0.1	21.0[e]±0.3	1.4[c]±0.0	1.7[b]±0.4	63.5[i]±0.4	2013[j]
71.4: 28.6	4.9[bc]±0.2	17.9[e]±0.4	20.5[e]±0.1	2.1[e]±0.1	2.8[d]±0.1	51.8[g]±0.4	1943[h]
50:50	3.8[ab]±0.6	24.7[g]±0.4	19.7[d]±0.6	2.8[g]±0.1	3.7[f]±0.2	45.6[e]±0.3	1924[gh]
28.6:71.4	4.9[bc]±0.2	30.7[i]±0.3	19.7[d]±0.2	3.3[i]±0.0	4.7[h]±0.2	36.6[c]±0.2	1873[ef]
Wheat/ Soy biscuits							
100:0	7.2[ef]±0.7	10.8[b]±0.2	20.7[e]±0.2	0.5[a]±0.0	0.2[a]±0.0	60.6[h]±0.4	1980[i]
71.4: 28.6	7.4[f]±1.5	19.5[f]±0.2	19.8[d]±0.9	1.5[d]±0.0	2.0[c]±0.0	49.8[f]±0.8	1910[g]
50:50	6.4[def]±0.4	25.8[h]±0.4	19.3[d]±0.5	2.4[f]±0.0	3.3[e]±0.1	42.8[d]±0.5	1880[f]
28.6:71.4	6.0[cd]±0.4	31.9[j]±0.2	19.4[d]±0.1	3.1[h]±0.0	4.4[g]±0.2	35.2[b]±0.3	1859[de]
Soy biscuit 100%	4.6[b]±1.2	39.9[k]±0.3	19.3[d]±0.5	4.2[j]±0.1	5.5[i]±0.0	26.5[a]±0.4	1842[d]

Values are means ±standard deviations. Values in a column followed by different letter superscripts are significantly different at P≤0.05 as assessed by Fisher's least significant difference.

[1]Calculated as total carbohydrate by difference]. 100-(weight in grams [moisture + fat + protein + ash + fibre] in 100 g of food.

[2]Calculated using the following factors: protein 17 kJ/g, fat 37 kJ/g, and carbohydrates 17 kJ/g.

The fat content in sorghum and bread wheat biscuits increased 7 and 15 times, respectively compared to their flours. The increase is due to inclusion of 20% sunflower oil to the biscuit formulae. Consequently, there were no significant differences in fat content among the bread wheat biscuits. However, there was a slight reduction, of 7% fat content in sorghum biscuits as soy increased to 50 and 71.4% because the fat content was 6 times higher in sorghum than the DSF. Fat is an important ingredient used to raise energy density in formulation of Fortified Blended Foods for vulnerable populations (Hoppe, Anderson, Jacobsen, Molgaard, Friis, Sangild and Michaelsen 2008, FAO/WHO1994). Fats that provide most of the energy in the form of essential fatty acids such as sunflower oil used in this study are recommended because they promote growth, cognitive development and immune function (Hoppe et al 2008). The oil content in the fortified biscuits of 20 to 21% in sorghum biscuits and 19 to 20% for bread wheat biscuits, was within the FAO/WHO (1994) recommended range of 10 to 25 g oil per 100 g of food for supplementary feeding of young children.

The carbohydrate content decreased substantially by 22 to 73% in both sorghum and bread wheat biscuits, respectively compared to the 100% cereal biscuits as the level of DSF increased from 28.6 to 71.4%. This decrease can be explained by the low carbohydrate content of DFS (30%). Soy bean stores energy as approximately 20% oil and 9 % of carbohydrate concentration is classified as fibre, while sorghum and wheat store 72 to 75% energy as carbohydrate (starch) (USDA 2008). Compositing with soy diluted the carbohydrate content of sorghum and bread wheat biscuits. It also increased their crude fibre content threefold and twenty two fold, respectively. Some researchers have also reported similar results when cereals were blended with legumes. For instance, Mohsen et al (2009) found a decrease in carbohydrate content on addition of isolated soy protein to biscuits. Kayitesi (2010) also reported reduction of carbohydrate content in sorghum porridge through addition of marama bean, which is low in carbohydrate content. The FAO/WHO (1994) Codex Committee recommends that foods for preschool children should contain no more than 5 g dietary fibre and other non-absorbable carbohydrates per 100 g of dry matter. The crude fibre content of biscuits in this study which ranged from 2.8 to 4.7 g/100 g in sorghum-based biscuits and 2 to 4.8 g/100 g in bread wheat-based biscuits was probably within the recommended range. High fibre density has been reported to increase bulk and reduce protein intake in diets for young children (Hofvander and Underwood 1987).

The energy density of biscuits in this study was enhanced by inclusion of fat in the formulation. The fortified biscuits contained about 1873 to 1943 kJ and 1859 to 1910 kJ for sorghum and bread wheat, respectively, which meet the recommended minimum value of 1,674 kJ/100 g (FAO/WHO 1994) for supplementary foods for young children. High dietary energy is important for sparing protein for body building and repairing body tissues avoiding diversion to provide energy (Stipanuk 2006). The FAO/WHO (1994) Codex Alimentarius Commission recommended that protein-energy in foods for pre-school children should not be less than 15%. Hence, two biscuits of 28 g would provide some 14% of the energy requirements of a 5 to 7 year old child, which are approximately 7500 kJ (FAO/WHO/UNU 1985).

Incorporation of DSF in biscuits substantially increased the protein content of the biscuits. Replacement of cereal flour with 28.6, 50 and 71.4% DSF increased protein content by 95, 168, and 234%, respectively, in sorghum-based biscuits and 81, 139 and 195%, respectively, in bread wheat-based biscuits compared to the 100% cereal biscuits. The increase was due to the high protein content of DSF flour (50 g/100 g flour). Several workers have reported similar results on substituting cereal with legume flours. Bookwalter, Warner and Anderson (1977) found increased quantities of protein in flour blends as toasted DSF increased and sorghum reduced. Substitution of wheat with 30% (McWatters 1978) and 20% (Singh et al 2000) DSF achieved a 100 and a 115% increase in protein content, respectively. Similarly, Taha, Majdi and Khalil (2006) realised a 36% increase in protein content in wheat biscuits supplemented with 12% isolated soy protein. Likewise, Awadelkareem et al (2008) reported that sorghum-soy composite meals had increased protein contents of 18 to 26% after adding soy concentrate at between 4 to 12% levels.

According to the WHO (2007) Expert Consultation, the protein requirements of children aged 1 to 2 years, 3 to 10 years and 10 to 18 years, are 1.12 g/kg/day, 0.73 g/kg/day and 0.7 g/kg/day. Based on FAO (2004) weight for age values, the daily protein requirements for these children translates to 12 to 13 g/day for 1 to 2 year olds, 11 to 22 g/day for 3 to 10 year olds and 24 to 40 g/day for 10 to 18 year olds. The protein content of biscuits fortified with 24.6 to 71.4% DSF in this study was 18 to 31% for sorghum-based biscuits and 20 to 32% for bread wheat-based biscuits. The biscuits with a 1:1 ratio of sorghum or bread wheat with soy flour met the target of providing 7 g protein in 28 g biscuit weight. Consumption of 1, 2 and 3

biscuits would provide half the protein intake for children aged 1 to 2, 3 to 10, and 10 to 18 years, respectively (IOM 2005). Similarly, Mohsen et al (2009) reported that 100 g of wheat biscuits supplemented with 20% isolated soy protein would provide half the recommended daily requirement for protein according to WHO (2007).

4.1.3.2 Lysine and reactive lysine content

Compositing sorghum and bread wheat with DSF increased the lysine contents of biscuits made from both cereals (Table 4.1.3). The protein lysine content in DSF, which was more than three times that of sorghum and bread wheat flours, is related to the high levels of the globulin fraction in soy bean, which is rich in lysine (Marcone and Kakuda 1999). Consequently, protein lysine content of sorghum and bread wheat biscuits with DSF at levels of 28.6, 50 and 71.4% increased by 186, 231 and 378% and 126, 152 and 165%, respectively compared to the 100% cereal biscuits. Studies by a number of workers are in agreement with the findings from this study. For example, Lindell and Walker (1984) achieved a 27% increase in lysine content of sorghum and wheat flours used for the preparation of chapattis by addition of DSF and it represented 77% of the WHO (2007) pattern. The improvement in lysine content of the biscuits themselves was even more dramatic. At a 1:1 cereal to soy ratio, there was 500 to 700% increase.

Compositing sorghum and bread wheat with DSF at levels of 28.6 to 71.4% increased reactive lysine content in protein by 200 to 300% in sorghum biscuits and 4.5 to 9% in bread wheat biscuits compared to the 100% cereal biscuits (Table 4.1.4). Reactive lysine is the amount of lysine that can be absorbed in a structural form for potential use in body protein synthesis (Hendricks, Moughan, Boer and Van der Poel 1994). Similar results were reported by Bookwalter et al (1977) using 15% DSF to fortify sorghum meal.

It was also observed in this study that generally the increase of reactive lysine content in the composite biscuits was not proportional to the increase in lysine and protein. This indicates substantial loss of reactive lysine during baking when DFS flour increased. It is likely that the Maillard reaction, enhanced by high soy protein, low moisture content and high baking temperature caused these losses as was also observed by Villamiel (2006). Evidence of Maillard reaction derivatives in soy-cereal composite products has been reported. Working on

Table 4.1.3 The effect of compositing sorghum and bread wheat with defatted soy flour on the lysine content of flours and biscuits (g/100 g protein) and (g/100 g biscuits and flour)

Biscuit type	Flour		Sorghum or Wheat: Soy flour (%)				
	Cereal flour	Soy flour	100:0	71.4: 28.6	50:50	28.6:71.4	0:100
Sorghum: Soy	$2.98^b \pm 0.05^1$	$8.28^g \pm 0.39$	$1.37^a \pm 0.12$	$3.93^c \pm 0.38$	$4.54^d \pm 0.38$	$4.78^d \pm 0.26$	$5.13^d \pm 0.03$
	$(0.23^b \pm 0.01)$	$(4.15^g \pm 0.20)$	$(0.13^{ab} \pm 0.01)$	$(0.70^c \pm 0.06)$	$(1.12^d \pm 0.09)$	$(1.47^e \pm 0.07)$	$(2.05^f \pm 0.01)$
Wheat: Soy	$2.48^b \pm 0.00^2$	$8.28^f \pm 0.39$	$1.86^a \pm 0.19$	$4.20^c \pm 0.17$	$4.69^d \pm 0.19$	$4.92^{de} \pm 0.09$	$5.13^e \pm 0.03$
	$(0.33^b \pm 0.00)$	$(4.15^g \pm 0.20)$	$(0.20^{ab} \pm 0.0)$	$(0.82^c \pm 0.03)$	$(1.21^d \pm 0.03)$	$(1.57^e \pm 0.04)$	$(2.05^f \pm 0.01)$

Values are mean±SD. Values followed by different letter superscripts in a row are significantly different at P≤0.05 as assessed by Fisher's least significant difference.

Figures in parentheses are lysine content (g/100 g) biscuits or flour.

[1]Sorghum flour.

[2]Wheat flour.

Table 4.1.4 The effect of compositing sorghum and bread wheat with defatted soy flour on reactive lysine content of flours and biscuits (g/100 g protein)

Biscuit type	Flour		Sorghum or Wheat: Soy flour (%)				
	Cereal flour	Soy flour	100:0	71.4: 28.6	50:50	28.6:71.4	0:100
Sorghum: Soy	$1.46^b \pm 0.20^1$	$3.68^f \pm 0.08$	$0.70^a \pm 0.40$	$2.10^c \pm 0.17$	$2.50^{cd} \pm 0.44$	$2.83^{de} \pm 0.07$	$3.01^e \pm 0.13$
	$(0.16^a \pm 0.02)$	$(1.85^f \pm 0.04)$	$(0.05^a \pm 0.03)$	$(0.30^b \pm 0.03)$	$(0.47^c \pm 0.09)$	$(0.72^d \pm 0.01)$	$(1.97^e \pm 0.03)$
Wheat: Soy	$1.89^a \pm 0.17^2$	$3.68^d \pm 0.08$	$2.25^b \pm 0.05$	$2.29^b \pm 0.18$	$2.33^b \pm 0.56$	$2.42^b \pm 0.15$	$3.01^c \pm 0.13$
	$(0.27^{ab} \pm 0.03)$	$(1.85^f \pm 0.04)$	$(0.19^a \pm 0.00)$	$(0.35^b \pm 0.03)$	$(0.52^c \pm 0.11)$	$(0.63^d \pm 0.05)$	$(1.97^e \pm 0.03)$

Values are mean ±SD. Values followed by different letter superscripts in a row are significantly different at P≤0.05 as assessed by Fisher's least significant difference.

Figures in parentheses are reactive lysine content (g/100 g) biscuits and flour.

[1]Sorghum flour.

[2]Wheat flour.

baby cereals, Guerra-Hernandes and Carzo (1996) found higher furosine, levels of 1010 mg/100 g in soy fortified samples compared to 293 mg/100 g protein in unfortified samples. It is also possible that the presence of DSF flour with higher levels of reactive lysine in the flour mixtures increased losses of reactive lysine. This suggests that losses are higher when the available lysine content in the proteins is greater as was noted by Fernandes-Artigas et al (1999). The results in the present study also agree with the findings of Horvatic and Eres (2002) who reported 27 to 47% loss of available lysine during production of dietetic biscuits.

The 100% bread wheat biscuit apparently had reactive lysine content 53% higher than raw bread wheat flour. This inconsistency may be attributed partly to inefficiency of the lysine dye binding method in determining the lysine damage in low lysine products (Hendricks et al 1994). Equal amounts of Crocein Orange G dye are used for both high protein foods such as legume-cereal complements and low protein uncomplemented cereal foods, with lower values of histidine, arginine and lysine. Excess dye in the reaction in low lysine foods may have caused an overestimation of the reactive lysine content (Hurrell et al 1979). Similar findings were noted by Anyango (2009) who reported inconsistent reactive lysine values in cooked pure sorghum foods and their uncooked flours. It is also possible that a proportion of the early Maillard products reverted back to lysine during acid hydrolysis of lysine determination. A similar trend of results was observed by Rutherfurd and Moughan (2007) who reported slightly higher reactive lysine content in dry cat food than lysine content. The high moisture content in wheat biscuits may also have prevented lysine reactivity and increased reactive lysine content (Hendricks et al 1994). A similar observation was made by Bjorck, Noguchi, Asp, Cheftel and Dahlqvist (1983) who postulated that high moisture spared lysine reactivity.

4.1.3.3 In vitro protein digestibility

Table 4.1.5 shows that compositing sorghum with DSF substantially increased in vitro protein digestibility (IVPD) of biscuits compared to the 100% sorghum biscuit. Replacement of sorghum flour with 28.6, 50, and 71.4% DSF increased IVPD by 148, 170 and 191%, respectively. The increase in digestibility could be attributed to

Table 4.1.5 The effect of compositing sorghum and bread wheat with soy on in vitro protein digestibility (%) of biscuits and flour

Biscuit type	Flour		Sorghum or Wheat: Soy composite				
	Cereal flour	Soy flour	100:0	71.4: 28.6	50:50	28.6:71.4	0:100
Sorghum: Soy	56.0[b]±1.1[1]	97.5[f] ±0.0	30.0[a]±3.3	74.3[c]±1.6	81.1[d] ±2.3	87.3[e]±3.5	88.1[e]±3.7
Wheat: Soy	97.3[cd]±1.5[2]	97.5[d] ±0.0	96.7[cd]±1.5	95.5[cd] ± 1.1	94.2[bc] ±0.5	91.4[b]±2.5	88.1[a]±3.7

Values are mean±SD. Values followed by same letter superscripts in a row are not significantly different at P≤0.05 as assessed by Fisher's least significant difference.

[1]Sorghum flour.

[2]Wheat flour.

dilution of the less digestible sorghum kafirins with the more soluble soy bean globulins. Improved digestibility suggests potentially improved protein absorption and retention in humans. Similar results have been reported after complementing cereals with DSF. For instance, Bookwalter et al (1987) found a 13% increase in IVPD of sorghum grits on inclusion of 15% soy grits.

The 100% sorghum biscuit was 87% lower in IVPD compared to raw sorghum flour with 56%. Duodu et al (2002) also reported large reductions in IVPD (96%) for red non tannin sorghum after cooking. This may be explained by formation of high levels of disulphide cross-linked kafirins (Hamaker et al 1986). It is also likely that an exogenous factor such as the high amount of lipids in the biscuits resulted in formation of protein-lipid complexes that were resistant to attack by enzymes as described by Duodu et al (2002).

The bread wheat biscuits exhibited a slight reduction of 6% in IVPD when DSF increased to 71.4% (Table 4.1.5). Heating wheat biscuit dough at high temperatures for a prolonged period of time may have rendered the gluten less soluble and therefore less accessible to proteolysis. The results in this study agree with the findings from several other studies that demonstrate the behaviour of protein when subjected to heat. For example, Weegels, de Groot, Verhoek and Hamer (1994) reported reduced solubility of gluten proteins in urea and guanidine HCl when subjected to high temperatures. They proposed that this could be a result of increased cross-linking of proteins. Similarly, Erbersdobler and Faist (2001) linked Maillard reaction derivatives to reduced digestibility when proteins are heated. The high oil content in biscuits probably reduced the IVPD of soy flour. A similar study conducted by Taha and Mohamed (2004) demonstrated that denaturing protein by heating at high temperatures in the presence of oxidised oil caused lipid-protein complexes that reduced in vitro protein digestibility of DSF.

The results from this study showed that IVPD of sorghum flour was half that of wheat flour. Fortified sorghum biscuits were also 4 to 22% lower in IVPD than fortified bread wheat biscuits with the same levels of DFS flour of 28.6 to 71.4% (Table 4.1.5). This was due to higher IVPD of wheat than sorghum. Addition of soy in sorghum-soy

formulations may simply have compensated for decreased IVPD resulting from cooking sorghum as was also observed by Bookwalter et al (1987). Studies that show similar differences have been conducted by Mertz, Hassen, Cairns-Whittern, Kirleis, Tu and Axtell (1984), who reported 86% IVPD for wheat, compared to 56% for sorghum. MacLean et al (1981) reported 46% and 81% apparent digestibility for sorghum and wheat, respectively in young children.

4.1.3.4 Amino acids

Tables 4.1.6 and 4.1.7 show that compositing sorghum and bread wheat with DSF markedly improved the lysine scores of biscuits. Lysine contents of 100% cereal biscuits were less than half the 48 and 52 mg/100 g protein requirement for 3 to 10 and 1 to 2 year old children, respectively (WHO 2007). However, on replacement of 28.6, 50 and 71.4% cereal flour with DSF, the lysine content of the biscuits improved to 82, 95 and 100%, respectively in sorghum biscuits and 88, 98 and 107%, respectively in wheat biscuits of the WHO (2007) requirement for 3 to 10 year olds. Additionally, all the fortified products had amino acid scores above the 65% minimum recommended by FAO/WHO (1994) for supplementary foods for young children. The increase can be explained by the higher lysine contained in DSF (106% of the recommended levels) for 3 to 10 year old children. Lindell and Walker (1984) conducted a similar study in which red sorghum and wheat flours were fortified with DSF in chapattis and obtained 75% of the WHO (2007) recommended level for lysine. Similar results were reported by Asma, El Fadil and El Tinay (2006) who found that supplementing sorghum meal with cowpea and pigeon pea improved the lysine scores by 15 to 45%.

The amount of indispensable amino acids in biscuits increased when DSF was replaced at 28.6 to 71.4% levels by 39 to 41% and 14 to 42% in sorghum and wheat biscuits, respectively compared to the total recommended amount by WHO (2007) for 3 to 10 year olds of 293 mg/g (Table 4.1.7). Some researchers have reported improved total indispensable amino acid content in legume fortified cereal products. For example, Mosha and Vicent (2005) reported a 2.9 to 19.8% increase in total amino acids of SUA-90 bean and peanut fortified supplementary foods. Similarly, Serna-

Table 4.1.6 The effect of compositing sorghum and bread wheat with defatted soy flour on amino acid composition of biscuits and flour

	Indispensable amino acids								Dispensable amino acids							
	Hist	Thr	Val	Met[a]	Isoleu	Leu	Phe[b]	Lys	Asp	Glu	Ser	Gly	Arg	Ala	Pro	Tyr
Flours																
Soy flour	1.24[c]	0.31	2.29	0.69	2.19	3.51	2.33	0.23	4.15	8.18	2.28	2.03	3.57	2.13	2.52	1.79
	24[d]; 1.5[e]	32; 1.3	46; 1.2	89; 3.7	44; 1.4	70;1.1.	50; 2.1	83; 1.7	83	163	46	41	71	43	30	36
Sorghum flour	0.23	0.31	0.55	0.18	0.44	1.41	0.55	4.15	0.49	2.08	0.46	0.34	0.41	1.00	0.91	0.42
	20; 1.3	27; 1.1	48; 1.2	29; 1.1	39; 1.3	124; 2.0	85; 3.5	20; 0.4	43	183	41	30	36	88	80	37
Wheat flour	0.24	0.60	0.51	0.20	0.43	0.84	0.60	0.33	0.33	3.94	0.57	0.46	0.49	0.38	1.51	0.40
	18; 1.1	23; 0.9	38; 1.0	42; 1.8	32; 1.0	63; 1.03	75; 1.8	25; 0.5	25	295	43	43	37	29	113	30
Sorghum/Soy biscuits																
100:0	0.17	0.53	0.43	0.14	0.35	1.17	0.45	0.13	0.33	1.70	0.36	0.26	0.28	0.82	0.26	0.33
	19; 1.2	21; 0.9	47; 1.2	28; 1.2	38; 1.2	127; 2.1	84; 2.1	14; 0.3	36	184	39	28	30	89	83	35
71.4:28.6	0.41	0.69	0.87	0.26	0.80	1.67	0.87	0.70	1.26	3.28	0.83	0.69	1.06	1.09	1.14	0.64
	23; 1.4	22; 1.2	48; 1.2	45; 1.1	44; 1.4	93; 1.5	85; 2.1	39; 0.8	71	18	46	38	59	61	64	36
50:50	0.59	0.69	1.20	0.35	1.12	2.05	1.20	1.12	2.12	4.42	1.16	1.00	1.61	1.25	1.38	0.85
	23; 1.5	28; 1.1	48; 1.2	58; 0.9	45; 1.5	83; 1.4	83; 2.0	45; 1.0	86	179	47	41	65	50	56	34
28.6:71.4	0.71	0.84	1.41	0.42	1.35	2.35	1.44	1.47	2.46	5.22	1.39	1.21	2.02	1.39	1.58	1.03
	23; 1.5	27; 1.1	46; 1.2	71; 0.7	44; 1.4	76; 1.3	81; 2.0	48; 1.0	80	169	45	39	66	45	52	34
0:100	0.96	1.25	1.82	1.38	1.77	2.88	1.89	2.05	3.45	6.70	1.85	1.64	2.73	1.69	1.98	1.31
	24; 1.5	31;1.9	46;1.1	89; 1.7	44; 1.4	72; 1.2	80; 2.0	51; 1.0	87	169	46	41	69	42	50	33
Wheat/Soy biscuits																
100:0	0.19	0.16	0.41	0.15	0.35	0.67	0.45	0.20	0.26	3.18	0.42	0.35	0.39	0.31	1.16	0.29
	18; 1.1	15; 0.6	38; 1.0	42; 1.7	33; 1.1	62; 1.0	64; 1.6	19; 0.4	245	295	39	32	36	29	107	27
71.4:28.6	0.43	0.48	0.87	0.28	0.81	1.39	0.92	0.82	1.17	4.34	0.88	0.75	1.14	0.75	1.43	0.62
	22; 1.4	25; 1.0	45; 1.1	55; 1.4	42; 1.4	71; 1.2	79; 1.9	42; 0.9	60	223	45	38	59	39	74	32
50:50	0.58	0.70	1.16	0.37	1.12	1.86	1.23	1.21	1.80	5.04	1.19	1.03	1.64	1.04	1.59	0.85
	23; 1.4	27; 1.1	45; 1,1	66; 1.6	43; 1.4	72; 1.2	81; 2.0	47; 1.0	70	195	46	40	64	40	62	33
28.6:71.4	0.74	0.95	1.45	0.45	1.41	2.30	1.50	1.57	2.59	5.76	1.47	1.29	2.10	1.31	1.73	1.05
	23; 1.4	30; 1.2	46; 1.1	76; 0.9	44;1.4	72; 1.2	80; 1.9	49; 1.0	81	180	46	40	66	41	54	33
Reference pattern[f]	16	25	40	24	31	61	41	48								

[a] Methionine + cystine; [b] Phenylalanine + tyrosine; [c] Amino acid content (g/100 g, db); [d] Amino acid concentration (mg/g protein; rounded off to a whole number)
[e] Amino acid score= mg amino acid in 1 g protein of test sample/ mg amino acid in requirement pattern (WHO 2007) for children 3-10 year. [f] Pattern for amino acid requirements for 3-10 year old children (WHO 2007).

Table 4.1.7 Indispensable amino acid composition (mg/g protein) of soy fortified sorghum and bread wheat biscuits compared with the pattern for amino acid requirements (mg/g crude protein children 3-10 years (1-2 years)

Amino acid	WHO reference pattern[1]	Sorghum: Soy				Soy	Wheat: Soy			
		100:0	71.4:28.6	50:50	28.6:71.4	100:0	100:0	71.4:28.6	50:50	28.6:71.4
Lysine	48 (52)	14*	39	45	48	51	19*	42	47	49
Leucine	61 (63)	127	94	83	76	72	62	71	72	72
Phenylalanine + tyrosine	41 (46)	84	85	83	80	81	64	69	81	80
Valine	40 (42)	47	48	48	46	46	38	45	45	46
Tryptophan[3]	6.6 (7.4)	12	28	40	52	68	14	30	41	53
Methionine + Cysteine[4]	24 (26)	28	45	58	71	89	41	55	66	76
Threonine	25 (27)	21	30	28	27	31	15	25	27	30
Histidine	16 (18)	19	23	24	23	24	18	22	23	23
Isoleucine	31 (31)	38	44	45	44	44	33	42	43	44
Total	293 (312.4)	380	410	415	416	439	280	335	398	417
Lysine score (3-10)[5]		0.29	0.82	0.95	1.00	1.07	0.39	0.88	0.98	1.03
Lysine score (1-2)		0.26	0.76	0.87	0.92	0.99	0.36	0.81	0.90	0.95
PDCAAS (3-10)[6]		0.09	0.61	0.77	0.87	0.94	0.38	0.84	0.92	0.93
PDCAAS (1-2)		0.08	0.56	0.71	0.80	0.87	0.34	0.78	0.85	0.86

[1]Amino acid reference patterns for children 3-10 years and 1-2 years in parentheses (WHO, 2007); [3]Tryptophan values calculated from USDA (2008) for sorghum, defatted soy flour and bread wheat; [4]Cystine values calculated from USDA (2008); [e]Lysine score= mg lysine in 1 g protein of test sample/ mg lysine in requirement pattern (WHO 2007) for children 3-10 year; [5]Protein Digestibility Corrected Amino Acid Score (lysine score x IVPD); *=Most limiting amino acid.

Saldivar et al (1999) reported an increase in the number of indispensable amino acids in bread fortified with DSF. The increase in indispensable amino acids may be linked to the higher protein content from DSF with higher lysine and indispensable amino acids (USDA 2008).

The dramatic improvement in protein digestibility and amino acid score also markedly improved the Protein Digestibility Corrected Amino Acid Score (PDCAAS) in DSF composite biscuits compared to 100% cereal biscuits (Table 4.1.7). Replacing sorghum and bread wheat flours with DSF at 28.6 to 71.4% levels increased PDCAAS by 7 to 10 times and 2 to 2.5 times, respectively in biscuits. The PDCAAS is the internationally accepted measure of food protein quality and is used to assess the protein quality of both dietary mixtures and individual protein food sources (WHO 2007).

4.1.4   CONCLUSIONS

Fortifying sorghum or bread wheat with DSF dramatically improves the protein content and quality of biscuits. At a 1:1 cereal: DSF ratio, one and two biscuits of 28 g provide 50% of the protein requirements for 1 to 2 and 3-10 and year old children, respectively with greatly improved PDCAAS. Hence, composite sorghum or bread wheat biscuits have considerable potential for use as protein and indispensable amino acid-rich supplementary food in semi arid tropical countries to prevent PEM in school and pre-school children.

It is recommended that further study be carried out to determine the protein nutritional quality of biscuits using a small animal assay as this is the standard method of determining protein nutritional quality in foods.

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4.2 Effects of compositing sorghum and bread wheat with soy on the sensory characteristics and consumer acceptability of biscuits

ABSTRACT

Protein Energy Malnutrition (PEM) remains a major nutritional problem affecting children in Africa. Sorghum biscuits with improved protein quality through complementation with defatted soy flour could be effective in alleviating PEM. The aim of this study was to evaluate the effect of compositing sorghum and bread wheat flours with soy on sensory characteristics and acceptability of biscuits. Sorghum and bread wheat biscuits with ratios of 0, 28.6, 50 and 71.4% substituted with defatted soy flour were evaluated. Compositing sorghum and bread wheat with defatted soy flour at 1:1 ratio reduced biscuit weight by 16 to 26%, and thickness by 11 to 28%, respectively, compared to 100% cereal biscuits because of reduction in dry matter content. Spread factor increased by 7 to 32%. Biscuits were darker in colour (reduced $L*$ value) by 14 to 56% and hardness increased by 84% in sorghum biscuits. Principal component analysis (PCA) for 26 attributes for biscuits rated by a descriptive sensory panel revealed that 61% of the variation explained by the first principal component was due to the type of cereals, sorghum or bread wheat, while an additional 33% (PC2) was due to the concentration of soy in the biscuits. Positive hedonic scores for fortified sorghum and bread wheat biscuits by 8 to 9 year olds were sustained above 80% through eight consumption occasions. Sorghum and bread wheat biscuits fortified with defatted soy flour have positive characteristics associated with biscuits such as crispy texture, roasted cereal flavour, and improved spread factor and appear to retain high acceptance over time as a protein rich supplementary food.

4.2.1 INTRODUCTION

Protein Energy Malnutrition (PEM) continues to be an important nutritional problem affecting children in most developing countries (Muller and Krawinkel 2005). Sorghum is an important staple food in the semi arid and arid tropics of Africa (ICRISAT 2009) as is hard bread wheat that is cultivated in at least 33 African countries (Taylor 2004). Biscuits made from sorghum or bread wheat fortified with defatted soy flour to improve the protein content and quality could serve as a vehicle for protein to alleviate PEM among vulnerable children in Africa. In Chapter 4.1, Section 4.1.2.2, biscuits were developed using sorghum and bread wheat, substituted with 0, 28.6, 50, 71.4% and 100% defatted soy flour (DSF) to improve the protein content and quality. At 1:1 DFS replacement level, two sorghum or bread wheat biscuits of 28 g each could provide 50% of the daily protein requirement for 3 to 10 year old children.

Soy proteins have functional properties such as water-holding, heat coagulability and emulsifying capacities (Marcone and Kakuda 1999) that affect the quality of foods. Therefore, substitution with soy protein (Vittadini and Vodovotz 2003) and thermal processing, influence textural, physico-chemical and flavour characteristics of baked cereal products, which are important for consumer acceptance (Sablani, Marcotte and Baik 1998). Some studies have shown the effects of addition of soy proteins to food products. For instance, Perez, Ribotta, Steffolani and Leon (2008) demonstrated that addition of soy protein to wheat flour weakened dough by interacting with glutenin, which enhanced gluten depolymerisation. Singh and Mohamed (2007) reported higher farinograph water absorption in flours with 70% soy protein substitution compared to 100% cereal flour.

Thermal processing of soy proteins using dry heat has been shown to induce changes in wheat biscuits that can be explained by the Maillard reaction, caramelisation and lipid peroxidation (Mohsen et al 2009). These workers identified volatile compounds that included pyrazines, associated with roasted flavours and aldehydes and ketones with the beany flavour, which discourages consumption of soy beans (Boge, Boylston and Wilson 2009). Singh and Mohamed (2007) noted that every increment in soy protein isolate resulted in darker top colour of cookies. Sensory characterization is therefore necessary to identify both desirable and undesirable characteristics related to biscuits that may influence consumer acceptability.

Food products developed specifically for children must be tested by children (Guinard 2001). Methods used to measure food preference by children should be simple enough to be understood, but robust enough to measure preference reliably (Leon et al 1999). An acceptability study by Leon et al (1999) with 4 to 10 year old children of five biscuits established that the products were discriminated more using hedonic categorization than paired comparison and ranking by elimination. Additionally, children aged 8 to 9 years were the most consistent. However, hedonic ratings do not always predict long term acceptability (Goldman 1994), a factor that is important in introducing new food products. A consumer exposure test of several days can be used to determine long term acceptance of new food products (Wiejzen et al 2008).

Studies on soy fortified sorghum and wheat biscuits have only focused on nutritional quality and consumer acceptability. There are no reported studies on comprehensive descriptive sensory characterization of soy fortified sorghum and bread wheat biscuits using a trained panel. There are also no reported studies using children in Africa to establish the long-term acceptability of new foods by using the repeated exposure test. Therefore the objectives of this study were to determine the effect of compositing sorghum and bread-wheat with soy on the sensory characteristics of biscuits, to develop a lexicon for fortified sorghum and bread wheat biscuits, to determine 8 to 9 year old children's liking of sorghum-soy and bread wheat-soy composite biscuits using hedonic categorization and to determine long term acceptability using a repeated exposure test.

4.2.2   MATERIALS AND METHODS

4.2.2.1 Biscuit Sample Preparation

Sorghum and bread-wheat biscuits were prepared using the basic formulation and procedure described in Chapter 4.1, Sections 4.1.2.2 and 4.1.2.3. After sheeting the dough, biscuits were cut using a 4.5 cm diameter biscuit cutter for sensory evaluation and 6.3 cm for physical evaluation. Measurements of weight, width and height were taken for physical evaluation after baking and cooling biscuits at ambient temperature for 30 minutes. Biscuits were vacuum packed and stored at 10°C until further analyses.

4.2.2.2 Physical evaluation

Biscuit width, thickness and spread factor were determined by the baking quality of cookie flour method (AACC International 2000) Method 10-50D. Measurements were taken using a vernier caliper. Six biscuits were placed edge to edge and the width measured. They were rotated 90° and re-measured to obtain average width (W) in mm. The thickness (T) of biscuits was measured after stacking six biscuits on top of one another, re-stacking in a different order and re-measuring to get average thickness. These measurements were read to the nearest 0.5 mm. Spread factor (SF) was calculated as SF = W/T. Mean weight of six biscuits was also noted. Volume was calculated as radius (r^2) x (T) x 3.14 and density of biscuits was calculated (mass/volume) and expressed as g per cm^3.

4.2.2.3 Instrumental colour measurement

Colour of biscuit samples was measured using a CR210 Minolta chromameter (Model CR-400, Osaka, Japan), and recorded using the L* a* b* colour system. The chromameter was calibrated using a standard white plate (CIE L* = 97.58, a* = -0.17, b* = 1.88). Two readings of the L* a* b* values were taken at two positions on the top sides of 3 randomly selected biscuits from each treatment and the mean values recorded. The hue angle ($\tan^{-1}$ b*/a*) and chroma $[(a^*)^2 + (b^*)^2]^{0.5}$ (McGuire 1992) were calculated.

4.2.2.4 Instrumental texture analyses

Texture analysis of biscuits made from sorghum and bread wheat flours composited with DSF was performed using a TA-XT2 Texture Analyser (Stable Micro Systems, Godalming, UK). The nine treatments of biscuits were measured for maximum force ("Hardness") and distance compressed before breaking ("Fracturability") using a three point bend rig attachment at a cross-head speed of 3.0 mm/ sec for a distance of 5 mm and load cell of 5 kg. The temperature was 25^oC and relative humidity 70%. The force and distance required to break a single biscuit was recorded and the average value of 9 replicates is reported for each treatment. Due to varying thickness among biscuit treatments, the fracture properties were further determined according to the following expressions (ASTM International 2003, Baltsavias and Jurgens 1997, Zoulias, Oreopoulou and Tzia 2002).

$$\sigma = \frac{3FL}{2bh^2}$$

$$\varepsilon = \frac{6hY}{L^2}$$

Brittleness = σ/ε

where σ is the stress at midpoint (MPa), ε is the strain, F is the force at the beam centre (N), L is the distance between supports or span length (mm), b is the biscuit width (mm), h is the biscuit thickness (mm) and Y is the deformation/deflection at the beam centre under the load (mm). Stress was expressed as kPa and strain as a percentage.

4.2.2.5 Descriptive Sensory Analysis

Recruitment and screening

The descriptive sensory panel comprised nine women and two men, aged between 20 and 41 years who were students of the University of Pretoria, South Africa. They were selected from nineteen applicants after undergoing screening tests. Ethical approval to conduct the study was granted by the University of Pretoria Ethics Committee of the Faculty of Agricultural and Natural Sciences. Before engaging in the sensory exercise the panelists signed a consent form informing them of the nature of the biscuit samples they would evaluate. Three types of screening tests used in this study included the basic taste test as described by Lawless and Heyman (1998), an aroma identification test and an excercise to describe differences in attributes related to appearance, texture, odour and flavour among biscuits. The aroma compounds used in the identification test were mushroom, smoked ham, vanilla, cheese, onion, lime and strawberry, all on smelling strips. The five basic tastes, bitter, sweet, sour, salt and umami were presented to panelists as taste solution impregnated filter papers of different shapes. Panelists that could not identify bitterness, an attribute characteristic of sorghum were eliminated. The biscuits used were digestive whole meal biscuits and Scottish shortbread biscuits "Eet-Sum-More", (Baker's Biscuits, Rivonia, South Africa), Pyotts cream crackers (National Brands, Bryanston, South Africa), and fruit and nut delights (Georgio's Biscuit Factory, Port Elizabeth, South Africa), all available on the South African market.

Panel training

Descriptive sensory profiling of the nine biscuit samples was performed using the generic descriptive method described by Einstein (1991). The descriptive sensory panel was trained in 10 sessions of 2 hours each per day over a three week period. During the training sessions, the panelists were acquainted with the experimental biscuits and had to identify differences in attributes related to appearance, texture, flavour and aftertaste. A written procedure and practical demonstration on determining texture characteristics in biscuits as described by Munoz, Szcesniak, Einstein and Schwartz (1992) was included. Reference samples, mainly food items as shown in Table 4.2.1 were used to clarify the sensory attributes of biscuits among panelists. Panelists also learnt how to use and evaluate samples using a computer system and the computer software program (Compusense® Five release 4.6 [1986-2003] Guelph, Ontario Canada). Following discussions, the panel generated and reached consensus on 28 descriptors with definitions, reference standards to anchor the scale ends and the sequence of descriptors on the ballot (Table 4.2.1).

Evaluation of biscuits

Evaluation of the biscuits was carried out over a period of three days in three sessions of 1.5 hours each a day following a randomized complete block design. During each session all nine biscuits were randomly presented to each panelist. To avoid fatigue, panelists first evaluated a set of 5 biscuits, followed by a 20 minute break before evaluating a second set of 4 biscuits. Each sample was presented as ½ a biscuit in a transparent polyethylene zip-lock type bag of 100 mm x 80 mm, identified with random three digit codes. Panelists assessed the samples seated in individual sensory booths under red light. Each panelist was also provided with a glass of deionised water and raw carrot slices to cleanse the palate before and between tasting of samples. Additionally, each panelist received written methodology of assessment and the list of descriptors with definitions. Reference samples were availed to panelists throughout the evaluation sessions. The sensory evaluation laboratory was maintained at a temperature of 20^0C. Using the 28 descriptors, each of the 9 biscuit samples was rated for appearance, texture, flavour and aftertaste on 100-mm line scales (0-10). Responses were collected using the Compusense software.
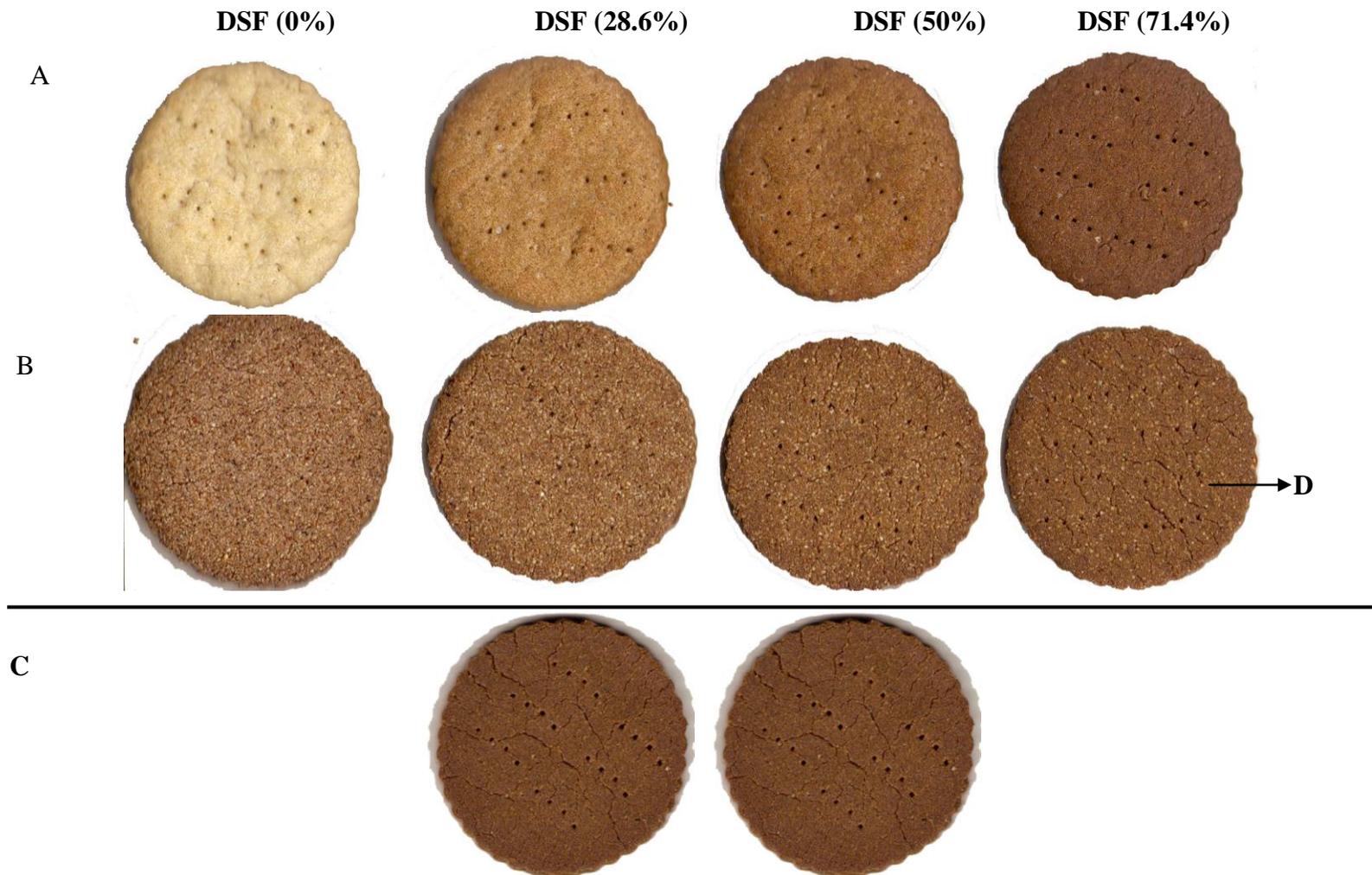
Figure 4.2.1 Soy fortified sorghum and bread wheat biscuits. DSF= Defatted Soy Flour; A= bread-wheat biscuits; B= sorghum biscuits; C= defatted soy flour 100% biscuits; D= visible white specks in sorghum biscuits.

Table 4.2.1 Descriptive sensory attributes used by the trained panel to evaluate fortified biscuits

Sensory Attribute	Definitions	References to clarify and anchor sensory attributes	Rating scale
Appearance			
Surface colour intensity	Colour intensity ranging from light cream to dark brown	Mantelli shortbread (light) =0. Oreo chocolate biscuits (dark) = 10	Not dark = 0 Very dark = 10
Quantity of visible white specks	Quantity of white specks visible on the surface of a biscuit	No specks = 0 Moderate number of specks = 5 Many specks = 10 0= 5= 10=	No white specks = 10. Many white specks = 10
Roughness of top surface	Degree to which roughness could be perceived on the top surface of a biscuit	Bakers Scottish shortbread (smooth) = 0. Da Vinci's cookies, fruit and nut delight (rough) = 10	Not rough (smooth) = 0 Very rough = 10
Evenness of surface	Degree of evenness on top surface	Bakers Scottish shortbread (smooth) = 0. Pyott's cream cracker (uneven) = 10	Not uneven = 0 Very uneven = 10
Aroma/smell			
Overall aroma strength	Overall intensity of aroma of the biscuit		No aroma = 0, Intense aroma = 10
Baked biscuit aroma	Intensity of aroma associated with basic sugar biscuit	Marie biscuit = 5 Over-baked biscuit = 10	No baked aroma = 0, Intense baked aroma = 10
Roasted cereal flour aroma	Intensity of aroma associated with cereal flour sufficiently heated to caramelize some sugars and starches	Uncooked cereal, maize, sorghum or wheat flour = 0. Oven roasted cereal, maize sorghum or wheat (180^oC for 10 minutes) = 10	No roasted cereal flour aroma = 0. Intense roasted cereal flour aroma = 10
Roasted soy bean aroma	Intensity of aroma associated with roasted soy bean.	Unroasted soy bean = 0 Oven roasted soy bean (180^oC for 15 minutes) = 10.	No roasted soy bean aroma = 0 Intense roasted soy bean aroma = 10
Heated oil aroma	Intensity of aroma associated with heated oil	Fresh sunflower oil = 0 Sunflower oil (heated 20 minutes at 180^oC) and cooled = 10	No heated oil aroma = 0 Intense heated oil aroma = 10

Table 4.2.1 continued

Sensory Attribute	Definitions	References to clarify and anchor sensory attributes	Rating scale
Texture			
Roughness	Degree of abrasiveness of products surface perceived by the lips and tongue before chewing.	Bakers Scottish shortbread (smooth) = 0. Da Vinci's cookies, fruit and nut delight (rough) = 10	Not rough (smooth) = 0 Very rough = 10
Bumpy texture	Overall amount of large bumpy areas on surface perceived by lips and tongue	Bakers Scottish shortbread (smooth) = 0. Pyott's cream cracker (bumpy) = 10	Not bumpy = 0 Very bumpy = 10
Hardness	Force required to compress a biscuit between molar teeth	Mantelli shortbread (soft) = 0. Oreo chocolate biscuits (hard) = 10	Not hard = 0 Very hard = 10
Crispness	Force and sound with which the sample raptures	Mantelli shortbread (soft) = 0, Bakers ginger biscuits (crispy) = 10	Not crispy = 0 Very crispy = 10
Denseness	Degree of compactness of cross section of sample after biting completely through.	Bakers ginger biscuits (not dense) = 0. Bakers Scottish shortbread (dense) = 10.	Not dense = 0 Very dense = 10
Dry	Degree to which the sample feels dry or absorbs saliva in mouth.	Mantelli shortbread (not dry) = 0. Pyott's cream cracker (dry) = 10	Not dry (moist) = 0 Very dry = 10
Graininess	Amount of small particles perceived by the tongue when the mass is gently compressed between the tongue and palate.	Bakers Scottish shortbread (not grainy) = 0 Bakers digestive biscuits (grainy) = 5	Not grainy = 0 Very grainy = 10
Coarseness	Degree to which the mass feels coarse or abrasive during product mastication.	Bakers Scottish shortbread (not coarse) = 0 Nice biscuits (coarse) = 10	Not coarse = 0 Very coarse = 10
Chewiness	Number of chews (at 1 chew/sec) needed to masticate the sample to consistency suitable for swallowing.	Mantelli shortbread (not chewy) = 0. Bakers digestive biscuits (chewy) = 5	Not chewy = 0 Very chewy = 10
Flavour			
Sweet	Fundamental taste sensation associated with sugars.	Spring water without sucrose (not sweet) = 0 5% sucrose in spring water (sweet) = 10	No sweet taste = 0 Intense sweet taste = 10
Roasted soy bean flavour	Intensity of flavour associated with roasted soy bean	Unroasted soy bean = 0 Oven roasted soy bean (180°C for 15 minutes) = 10	No roasted soy bean flavour = 0 Intense roasted soy bean flavour = 10

Table 4.2.1 continued

Sensory Attribute	Definitions	References to clarify and anchor sensory attributes	Rating scale
Flavour			
Roasted cereal flavour	Intensity of flavour associated with cereal flour sufficiently heated to caramelize some of the starches and sugars	Uncooked cereal, maize, sorghum or wheat flour = 0. Oven roasted cereal, maize sorghum or wheat (180°C for 10 minutes) = 10	No roasted cereal flour flavour = 0. Intense roasted cereal flour flavour = 10
Doughy flavour	Intensity of flavour associated with uncooked dough	Bakers digestive biscuits (not doughy) = 0 Uncooked dough (doughy) = 10	No doughy flavour = 0. Intense doughy flavour = 10
Heated oil flavour	Intensity of flavour associated with heated oil	Fresh sunflower oil = 0 Sunflower oil (heated 20 minutes at 180°C) and cooled = 10	No heated oil flavour = 0. Intense heated oil flavour = 10
Baked biscuit flavour	Intensity of flavour associated with basic sugar biscuit	Marie biscuits = 5 Over-baked biscuit = 10	No baked flavour = 0, Intense baked flavour = 10
Aftertaste			
Sweet aftertaste	Fundamental taste sensation associated with sugars.	Spring water without sucrose (not sweet) = 0 5% sucrose in spring water (sweet) = 10	No sweet taste = 0 Intense sweet taste = 10
Gritty residue in mouth	Degree to which mouth contains small particles after all of the sample has been swallowed	Mantelli shortbread (not gritty) = 0. Bakers digestive biscuits (gritty) = 5	Not gritty = 0 Very gritty = 10
Roasted soy bean flavour aftertaste	Intensity of flavour associated with roasted soy bean	Unroasted soy bean = 0 Oven roasted soy bean (180°C for 15 minutes) = 10	No roasted soy bean flavour = 0 Intense roasted soy bean flavour = 10
Heated oil flavour	Intensity of flavour associated with heated oil	Fresh sunflower oil = 0 Sunflower oil (heated 20 minutes at 180°C) and cooled = 10	No heated oil flavour = 0 Intense heated oil flavour = 10

[Bakers Scottish shortbread biscuits, "EET-SUM-MOR; Marie biscuit Bakers "Blue Label"; Bakers digestive whole wheat biscuits; Bakers ginger biscuits (Bakers Biscuit, Rivonia, South Africa)]; Da Vinci's cookies, fruit and nut delight (Georgio Biscuit Factory, Port Elizabeth, South Africa); Pyott's cream cracker (National Brands, Bryanston, South Africa); Oreo chocolate sandwich cookies (Kraft Foods, Gallo Manor, South Africa); Mantelli's handmade shortbread rounds (Mantelli's, Cape Town, South Africa).

4.2.2.6 Consumer acceptability

Biscuit sample preparation

Four variations of biscuits were used in this study. The different biscuits were prepared from: 100% sorghum, 100% wheat, 50% sorghum and 50% DSF, 50% wheat and 50% DSF flours. The 100% wheat biscuit was the control. Selection of composited biscuits was based on results obtained from Chapter 4.1, Section 4.1.3.1, that 1 biscuit of 28 g provided ≤ 7 g protein and results from the descriptive sensory panel that inclusion of DFS should not exceed 50% as higher levels imparted a detectable beany flavour to the biscuits. The basic formulation and procedure described in Chapter 4.1, Sections 4.1.2.2 and 4.1.2.3 was used to prepare biscuits. Sorghum flour was milled to a maximum 500 μm particle size to improve the coarse and gritty texture and rough appearance of biscuits based on findings of sensory characterization. The visual differences between biscuits made from re-milled and flour not re-milled is shown in Figure 4.2.2. Reduced particle size increased the requirement for water because of the higher absorption rates as a result of a larger surface area. Water was increased to 80 ml and 180 ml/225 g flour for 100% sorghum and sorghum-soy composite biscuits, respectively to make the dough workable (Chapter 4.1, Table 4.1.1). A 4.5 cm diameter biscuit cutter was used to cut dough.

| A | B |
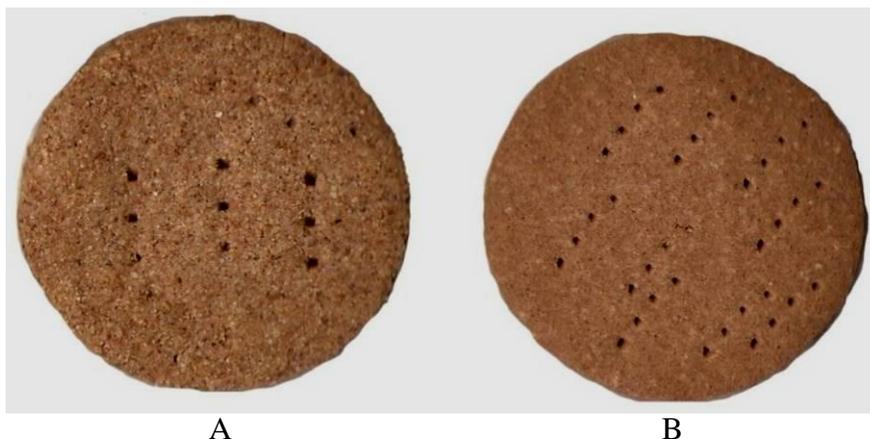
Figure 4.2.2 Biscuits made using 100% sorghum flour. A= without re-milling. B= with re-milling to maximum 500 μm particle size.

Recruitment and screening

Consumers were 60 children (26 boys and 34 girls) who attended Zakhele Primary School in Mamelodi near Pretoria, South Africa. The study was age specific so the screening selected for 8 to 9 year old children who consumed biscuits and did not have allergies. Ethical approval was granted by the University of Pretoria, Faculty of Agricultural and Natural Sciences Ethics Committee. Policy guidelines by the Faculty of Education of the University of Pretoria (Human-Vogel 2007) on the inclusion of minor children in research investigations were observed. Permission to carry out the study was granted by the principal of Zakhele Primary School. The childrens' parents were informed about the purpose, procedures, activities, risks and benefits of the study their children would be involved in, in a letter and verbally by the School Principal. Only children who voluntarily consented and whose parents signed the consent form allowing them to participate were included.

Orientation

A one hour orientation session was carried out on the first day of the 5 day study in the school hall, during tea-break at 10:00. The purpose of orientation was to familiarize and teach the children how to use the score card, a five point scale with stylized faces (Figure. 4.2.3). The sitting arrangement was designed to divide the 60 children into 4 groups of 15 each. Each group was allocated a red, yellow, green or blue colour. The children sat randomly at one of 60 stations with set trays containing evaluation samples and name tags with the child's number and group colour.

Attribute	Dislike very much	Dislike a little	Not sure	Like a little	Like very much
Rating	1	2	3	4	5

Figure 4.2.3 Five point facial scale used by school children for hedonic categorization of sorghum and bread wheat biscuits

Two research assistants, from the University of Pretoria that can speak both English and the mother tongue languages of the children were allocated to each group. It was explained to the children that the faces meant that they liked very much, liked a little, were not sure, disliked a little or disliked very much what they were eating. Two types of apples, one that children generally liked (sweet, red) and one they generally did not like (sour, green) labeled with 3 digit blind codes were used as test examples. The children were instructed to remove the label from the apple and place it above the face corresponding to how they felt about the apple they had just tasted, with the liked apple's label on a happy face and the disliked apple's label on a sad face. Bottled water to cleanse the pallet before and in between tasting was provided. The session was conducted using both English and mother tongue languages. The children were also informed that they could withdraw from the study at any point if they wanted to.

Procedure for evaluation

Evaluation of biscuits was carried out over a four day period in the children's classrooms with two groups in each class. Two half-hour evaluation sessions were conducted each day. During the first session, the children were randomly presented with a set of the four types of biscuits with three digit blinding codes on removable labels. Each ½ biscuit was in a transparent polyethylene zip-lock type bag of 100 x 80 mm. The five point facial scale was used for hedonic categorization. The children tasted each biscuit, starting from the biscuit on the left to the right, removed the coding label and placed it on the score card A above the face that corresponded with their feelings. The completed score card and the left over biscuits were withdrawn. Each child was then provided with 2 whole biscuits of one of the four types of biscuits, which was the test sample for the day and asked to eat and complete or eat as much as they wished. Each of the groups received a different test biscuit. Immediately after they finished consuming the biscuits, the left over test biscuits were withdrawn. The children were then asked by the research assistants if they would like to eat the specific test biscuit again the next day, to which they could answer yes, not sure or no. This was entered by the research assistant into the B score card that had provision for this at the bottom and provided with a second tray for the second session containing a new set of the four types of biscuits. The children evaluated these using the same procedure and entered their responses on score card B. The same procedure was repeated for four days at the same time, except that the test biscuit was different for each group every day. At the end of the 4 days, each child had

evaluated all four types of test biscuits once and the set of four biscuits 8 times. On each of the four days before the tests commenced, the procedure for evaluation was demonstrated to the children by the research assistant. At the end of the study each child was rewarded with a football, sweets and motivational cards. Additionally, the children were given a lesson on sensory evaluation and the use of their five senses.

4.2.2.7 Statistical analysis

Data for physical and instrumental texture and colour measurements were analyzed by one way analysis of variance (ANOVA). The statistical program used was Statgraphics Centurion XV (Stat Point, Herndon, VA). The descriptive panels' mean scores for sensory attributes were determined by two-way analysis of variance ANOVA with samples as fixed effects and panelists as random effects, using Statistica software Version 8.0 (Statsoft, Tulsa, OK). Principal Component Analysis (PCA) of the significant sensory attributes from means across panelists was performed using a correlation matrix with biscuit samples in rows and descriptors in columns. Physical and instrumental texture and colour characteristic and chemical properties (Chapter 4.1, Section 4.1.2.4) were included as supplementary variables in PCA to establish their relationship with sensory attributes. The data for consumer evaluation were analyzed using repeated measures analysis of variance (ANOVA). Means for all analyses were compared using Fisher's least significant difference (LSD). Box and whisker plots were used to illustrate consumer hedonic score distributions for the biscuits.

4.2.3   RESULTS AND DISCUSSION

4.2.3.1 Physical Evaluation

In Chapter 4.1 Section 4.1.2.2 it was observed that sorghum and bread wheat doughs required more water with increasing levels of DSF substitution between 28.6 and 71.4%. This may be attributed to the ability of soy proteins to absorb high amounts of water. Similar results were reported by Perez et al (2008) who attributed increased water absorptive properties of doughs from gluten-soy bean blends to hydrophilic soy proteins.

Table 4.2.2 Effect of compositing sorghum and bread wheat with soy on the physical characteristics of the biscuits

Biscuit type	Biscuit weight (g)	Width (mm)	Thickness (mm)	Spread factor	Density (g/cm³)
Sorghum /Soy biscuit					
100:0	25.9^e ±0.1	69.4^e ±0.7	8.3^d ±0.0	8.3^d±0.1	0.81
71.4: 28.6	23.7^d ±0.5	68.2^d ±0.3	7.7^{bc} ±0.2	8.9^e ±0.3	0.84
50:50	22.4^c ±0.4	66.8^c ±0.3	7.5^{bc} ±0.1	8.9^e ±0.1	0.84
28.6:71.4	21.2^b ±0.7	65.8^c ±0.9	7.4^b±0.1	8.9^e ±0.1	0.84
Wheat/ Soy biscuits					
100:0	30.7^g ±0.3	62.7^{ab} ±0.7	13.2^g±0.3	4.7^a ±0.1	0.74
71.4: 28.6	27.4^f ±0.5	62.5^a ±0.2	11.6^f ±0.6	5.4^b ±0.3	0.77
50:50	24.2^d ±0.7	63.5^{ab} ±0.3	10.3^e ±0.1	6.2^c ±0.1	0.74
28.6:71.4	21.2^b± 0.5	63.6^b ±1.0	7.9^c ±0.3	8.1^d±0.4	0.84
Soy biscuit 100%	18.2^a ±0.8	63.6^b±0.3	6.7^a ±0.1	9.1^f ±0.1	0.85

Values are means ±Standard deviations. Values in a column followed by different letter superscripts are significantly different at P≤0.05 as assessed by Fisher's least significant test.

Table 4.2.2 shows that increased substitution of sorghum and bread wheat flours with DSF from 28.6 to 71.4% reduced weights of biscuits by 9 to 22% and 12 to 45%, respectively, compared to the 100% cereal biscuits. The highest percentage (69%) weight loss was by the 100% soy biscuits. This may be explained by the high hydrophilicity of soy proteins (Marcone and Kakuda 1999) which may have caused greater hydration of soy proteins that made doughs dry and crumbly requiring more water to make them workable as DSF increased (Chapter 4.1, Table 4.1.1). This may have resulted in a reduction of total solids in such dough and the biscuits baked to lower weight because they had less dry matter. The results in this study are similar to findings by Akubor and Ukwuru (2003) who reported a reduction in weight of cassava-soy composite biscuits as DSF increased. It is also possible that thermal processing increased hydrophobicity of soy proteins causing loss of high amounts of water absorbed during dough mixing as DSF increased.

Compositing sorghum flour with 28.6 to 71.4% DSF reduced the width of sorghum biscuits between 2% and 5.4% compared to the 100% sorghum biscuit, which had the highest overall width (Table 4.2.2). It is possible that lateral expansion of biscuits was impeded by denaturation of protein (Slade and Levine 1998) in DSF early in the baking process reducing biscuit width. Similar results were reported by Maache-Rezzoug, Bouvier, Allaf and Patras (1998) who studying biscuit dough rheology and biscuit quality, found that an increase in protein tends to reduce the length of biscuits after baking. There was no consistent trend for width of bread wheat biscuits.

Bread wheat biscuits had higher thickness than sorghum biscuits by between 2 and 78%. This may partly be attributed to air which was trapped by the gluten structure developed during mixing and kneading of the dough (Stauffer 2007) and raised biscuits during baking increasing the thickness. Similar findings were reported by Singh and Mohammed (2007) for reduced carbohydrate cookies with gluten-soy protein blends. Sheeting bread wheat dough may also have increased elastic recovery of dough pieces because of gluten development (Manohar and Rao 2002) and this may have reduced width and spread factor and increased thickness as described by Slade and Levine (1994). Addition of soy flour diluted gluten protein, reduced elasticity and thickness and increased the spread factor. It is also possible that reduction in dry matter as DSF increased resulted in smaller biscuits that were less thick and wide.

4.2.3.2 Instrumental texture analyses

Table 4.2.3 shows that replacement of 50% and 71.4% sorghum flour with DSF increased the maximum stress of biscuits by between 38% and 83%, and the stress/strain ratio by 86% and111% respectively, compared to the 100% sorghum biscuit. It is possible that increasing maximum stress and stress/strain ratio of sorghum-DSF composite biscuits was due to formation of relatively strong protein-protein interactions when DSF was thermally processed. Similarly, Marcone and Kakuda (1999) reported increased aggregation of soy bean globulin protein when it was heated. Maximum stress is an important index of biscuit texture because it has been correlated with hardness of biscuits (Zoulias et al 2002). Biscuits with high maximum stress values are hard, an unpleasant characteristic for biscuits. The stress/strain ratio is important because it is indicative of brittleness (Jackson, Bourne and Barnard 1996), a pleasant sensorial characteristic in biscuits.

Low hardness and high fracturability (strain) of 100% sorghum biscuits may be ascribed to absence of gluten in sorghum flour. Gluten which is developed during dough mixing and coagulated into a foam with a fibrelike network is responsible for the mechanical structure of baked products (Goeseart, Brijs, Veraverbeke, Courtin, Grubruers and Delcour 2005). Additionally, the high fibre content of 100% sorghum biscuits may have introduced coarse particles which interfered with the homogeneity of doughs and biscuit structure, resulting in lower stress and strain values. Saleem, Wildman, Huntley and Whitworth (2005) showed that large inhomogeneities decreased stress and strain of semi-sweet biscuits. Similarly, Badi and Hoseney (1976) reported high crumbliness and fragility of sorghum biscuits. Chiremba, Taylor and Duodu (2009) also reported that fragility was more pronounced in wholemeal sorghum biscuits with high bran content which were difficult to handle.

Table 4.2.3 shows that the 100% bread wheat biscuits were hard and the least fracturable of all the biscuits. Substitution with 28.6% DSF reduced maximum stress by 20% but addition of 50% and 71.4% DSF increased maximum stress by 34% to 194%, respectively. However, fracture strain reduced by 13% to 195% when 28.6% to 71.4% DSF replaced bread wheat flour. Brittleness substantially increased by 815% when 71.4% DSF replaced bread wheat flour. Hardness in 100% bread wheat flour biscuits may be explained by the nature of the

Table 4.2.3: Effect of compositing sorghum and bread wheat with soy on stress and strain of biscuits

Biscuit type	Fracturability (mm)	Hardness (N)	Stress (kPa)	Strain (%)	Stress/strain (kPa)
Sorghum/Soy biscuit					
100:0	0.63^{ab}±0.1	10.4^{a}±2.0	163.3^{a}±0.02	3.56^{ab}±0.00	4.63^{bc}±0.9
71.4: 28.6	0.60^{a}±0.1	12.3^{a}±3.0	154.9^{a}±0.02	2.86^{a}±0.00	5.60^{c}±1.4
50:50	0.70^{ab}±0.2	18.9^{ab}±5.0	226.8^{ab}±0.06	2.73^{a}±0.00	8.62^{d}±3.1
28.6:71.4	0.63^{ab}±0.1	24.0^{ab}±6.5	299.3^{cd}±0.08	3.04^{a}±0.01	9.80^{d}±2.1
Wheat/ Soy biscuits					
100:0	2.03^{d}±0.3	64.0^{d}±8.9	263.5^{bc}±0.04	16.65^{f}±0.02	1.59^{a}±0.2
71.4: 28.6	1.76^{cd}±0.2	40.7^{c}±5.1	220.5^{ab}±0.03	14.74^{e}±0.03	1.54^{ab}±0.4
50:50	1.56^{c}±0.2	53.2^{c}±8.7	353.4^{d}±0.06	12.59^{d}±0.01	2.85^{ab}±0.7
28.6:71.4	1.06^{b}±0.2	65.5^{cd}±9.0	774.6^{f}±0.11	5.64^{c}±0.01	13.91^{e}±1.6
Soy biscuit 100%	1.06^{b}±0.3	32.3^{b}±7.8	560.0^{e}±0.12	4.72^{bc}±0.02	12.41^{e}±4.2

Values are means ±Standard deviations. Values in a column followed by different letter superscripts are significantly different at P≤0.05 as assessed by Fisher's least significant test

bread wheat flour. Bread wheat has broken starch granules that absorb high amounts of water during mixing (Kent and Evers 1994). It is possible that gluten in bread wheat flour, which is an amorphous rubbery fluid during dough mixing (Hoseney, Zelezak and Lai 1986), crosslinked to form a thermoset gel network. The gel retained water during baking and on cooling to room temperature, existed as a hard thermoset matrix due to expansion of starch granules (Slade and Levine 1994).

Increase in hardness and brittleness of bread wheat biscuits was probably because soy protein that lacks the viscoelastic properties of bread wheat, disrupted the gluten network and formed new interactions with wheat proteins that were weaker than 100% wheat flour gluten. Similarly, Perez et al (2008) working on the effect of soy protein in soy-wheat flour composite doughs, found that soy proteins in doughs were associated with wheat proteins through physical interaction and covalent bonds increasing solubility of gluten proteins which caused gluten depolymerization and network weakening. Additionally, soy protein has high absorptive (Marcone and Kakuda 1999) properties and may have competed with wheat proteins for water denying wheat proteins water for gluten development.

4.2.3.3 Instrumental colour evaluation

The biscuits became darker as DFS replaced sorghum and bread wheat in composite flours at levels of 28.6 to 71.4% compared to the 100% cereal biscuits (Figure 4.2.1 and Table 4.2.4). The L* (lightness) values decreased in sorghum and bread wheat biscuits by 3 to 14% and 41 to 65%, respectively as DFS increased. In bread wheat biscuits the a* (redness) values increased by 81 to 100% and b* (Yellowness) values decreased by 57 to 163% compared to the 100% bread wheat biscuit. The decrease in L* values may be an indication of higher protein content in the biscuits with higher DSF, which may be involved in Maillard reaction to generate the brown colour in biscuits.

Similar results were reported by Mohamed, Rayas-Duarte and Xu (2008) who found that bread made using flours with high protein content had darker crusts compared to flours with lower protein content. The dark colour of sorghum biscuits was partly because of red non tannin sorghum. Dark colour of biscuits has been reported by several researchers working with sorghum flours. For example, Chiremba et al (2009) found that wholemeal sorghum

Table 4.2.4 Effect of compositing sorghum and bread-wheat with defatted soy flour on the instrumental colour parameters of biscuits

Biscuit type	L*	a*	b*	C*(chroma)	h* (hue)
Sorghum/Soy biscuit					
100:0	46.6[e]±1.1	13.0[c]±0.4	7.1[bc]±0.9	14.8[bc]±0.8	28.3[b]±3.2
71.4: 28.6	45.4[de]±0.8	12.8[c]±0.1	8.6[c]±0.5	15.5[bc]±0.4	34.1[d]±1.4
50:50	43.6[cd]±0.4	13.1[c]±0.2	8.7[c]±0.2	15.8[bc]±0.5	33.7[cd]±0.6
28.6:71.4	40.8[ab]±0.9	12.7[bc]±0.3	6.6[b]±0.8	14.3[ab]±0.6	27.6[b]±2.5
Wheat/ Soy biscuits					
100:0	69.0[g]±1.2	6.9[a]±0.6	21.0[f]±1.7	22.1[e]±1.6	71.7[f]±2.0
71.4: 28.6	48.9[f]±2.6	12.5[bc]±0.5	13.4[e]±1.7	18.4[d]±1.5	46.8[e]±3.2
50:50	44.2[d]±0.2	14.2[d]±0.2	10.7[d]±0.8	17.8[d]±0.6	37.1[d]±1.8
28.6:71.4	41.7[bc]±1.2	13.8[d]±0.8	8.0[bc]±1.0	15.9[bc]±0.7	30.1[bc]±2.6
Soy biscuit 100%	38.8[a]±0.3	12.1[b]±0.0	4.9[a]±0.2	13.1[a]±0.1	22.0[a]±0.8

Values are means±Standard deviations. Values in a column followed by different letter superscripts are significantly different at P≤0.05 as assessed by Fisher's least significant test.
[1]L* = Lightness where "0" indicates darkness and "100" lightness.
[2]a* = Redness where positive a* = redness and negative a* = greenness
[3]b* = Yellowness where positive b* = yellowness and negative b* = blueness

biscuits had dark colour. Similarly, Mridula et al (2007) observed that increasing proportions of sorghum in sorghum-wheat composite flour darkened biscuits, which resulted in decreased L* values while a* values increased.

4.2.3.4 Descriptive Sensory Analysis

Analysis of variance *F*- values of the biscuits profile data of the 28 attributes scored by the descriptive sensory panel showed significant differences (P≤0.05) between the biscuit types for 26 attributes (Table 4.2.5). The data were further analyzed by principal component analysis (PCA) to determine the systematic variation and underlying relationships among physical, instrumental colour and texture and sensory attributes of the biscuits made from composite flours of varying cereal-legume ratios. The first two principal components explained 94% of the total variation (Figure 4.2.4). Figure 4.2.4a shows the first two principal

Table 4.2.5 Mean scores for sensory attributes of soy composited sorghum and wheat biscuits as evaluated by a trained descriptive sensory panel (n=9)

| Attributes | Sorghum biscuits | | | | Wheat biscuits | | | | Soy biscuit | Sample effects | Panelist effects |
| | Soy level (%) | | | | Soy level (%) | | | | | | |
	0	28.6	50	71.4	0	28.6	50	71.4	100	F-values[1]	
Colour intensity	6.3^{ef}±0.9	5.7^{cd}±0.9	5.9^{de}±1.1	6.8^{fg}±0.8	0.5^a±0.4	3.9^b±1.4	5.4^c±1.1	6.9^{gh}±1.2	7.3^h±1.5	130.1***	3.64***
Visible white specks	8.6^d±1.6	8.6^d±0.7	8.3^d±0.9	6.5^c±1.1	0.2^a±0.3	0.9^b±1.3	0.7^{ab}±0.7	0.9^b±1.7	0.7^{ab}±0.7	318.1***	0.6ns
Roughness of surface	8.1^c±1.7	8.0^c±1.0	7.7^c±0.9	5.3^b±1.7	1.8^a±1.3	1.6^a±1.2	1.5^a±1.1	1.9^a±1.8	1.6^a±1.4	134.4***	1.2ns
Evenness of surface	1.5^a±1.0	1.1^a±0.8	1.3^a±0.8	1.1^a±1.1	8.0^d±1.1	5.3^c±2.3	4.4^b±2.1	1.7^a±1.6	1.1^a±1.7	99.4***	3.9***
Overall aroma strength	7.1^b±1.1	6.6^{ab}±1.5	7.2^b±0.9	7.1^b±1.0	6.4^a±1.3	6.4^a±1.2	6.8^{ab}±1.0	7.2^b±1.1	7.1^b±1.2	6.9*	20.6***
Baked biscuit aroma	6.4^d±1.1	5.9^{cd}±0.8	6.0^{cd}±1.1	6.0^{cd}±1.2	4.2^a±2.0	4.8^{ab}±1.7	5.5^{bc}±1.7	6.2^{cd}±1.2	6.0^{cd}±1.6	9.5***	4.9***
Roasted cereal aroma	6.5^e±1.6	6.0^e±1.0	6.1^e±2.2	5.4^{de}±2.1	2.5^a±2.1	3.4^{ab}±2.4	4.4^{bcd}±2.3	4.7^{cd}±2.5	4.1^{bc}±2.7	16.9***	7.5***
Roasted soy aroma	2.4^b±2.5	2.4^b±2.2	3.1^{bc}±2.6	4.2^{cd}±2.5	0.4^a±0.7	2.4^b±2.2	4.1^{cd}±2.7	5.1^{de}±2.1	5.5^e±2.3	2.7***	6.6***
Heated oil aroma	2.5^{bc}±2.2	2.2^b±1.8	2.4^b±2.0	2.7^{bc}±1.9	1.4^a±1.3	2.2^b±2.0	3.1^{bc}±2.0	3.4^{cd}±2.1	3.5^d±1.8	9.2***	16.6***
Roughness	6.7^d±2.4	6.5^{cd}±2.2	5.8^c±2.3	4.2^b±1.9	1.9^a±1.1	1.7^a±1.0	1.7^a±1.0	1.9^a±1.2	2.4^a±1.5	44.5***	1.2ns
Bumpy texture	2.0^{ab}±1.5	1.4^a±1.1	1.5^a±1.1	1.6^a±1.6	7.2^d±1.8	5.1^c±1.8	4.3^c±2.3	2.6^b±2.1	2.0^{ab}±2.5	42.2***	3.3***
Hard texture	3.8^b±2.0	2.2^a±1.3	2.6^a±1.4	4.0^b±1.6	4.6^b±1.7	3.8^b±1.6	5.6^c±1.6	6.4^c±1.5	6.0^c±1.7	26 8***	3.2***
Crispy texture	6.9^f±1.6	5.8^e±1.9	5.6^{de}±1.6	6.1^e±1.1	3.8^{ab}±1.5	3.1^a±1.7	4.9^{cd}±1.4	4.5^{bc}±1.9	4.6^{de}±1.7	16.8***	2.1**
Dense texture	6.8^d±1.6	2.7^a±1.4	2.7^a±1.4	4.1^b±1.2	6.7^d±1.5	6.8^d±0.9	6.3^d±0.9	6.3^d±0.9	5.3^c±1.5	84.1***	6.7***

Values are means±standard deviations. Values in a row followed by different letter notations [a - h] are significantly different at p ≤ 0.05 as assessed by Fisher's least significant test.
[1]Significant at *** p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05, ns, not significant.

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Table 4.2.5 Continued.

Attributes	Sorghum biscuits Soy level (%)				Wheat biscuit Soy level (%)				Soy biscuit	Sample effects	Panelists effects
	0	28.6	50	71.4	0	28.6	50	71.4	100	*F*-values[1]	
Dry	7.0^e ±1.1	6.6^e ±1.3	6.3^{de}±1.4	5.6^{cd}±1.2	2.9^a±1.4	2.9^a±1.6	4.2^b±1.5	4.3^b±1.1	5.2^c±1.2	51 9***	5.2***
Grainy	8.4^d±1.6	8.2^d±0.8	7.8^d±1.0	6.4^c±1.1	0.6^a±0.7	0.8^a±1.3	1.2^{ab}±1.1	1.8^b±1.9	1.8^b±1.6	200.0***	1.6*
Coarse	6.2^e±2.4	5.7^e±2.0	5.9^e±2.0	5.3^{de}±1.8	2.3^a±1.6	2.5^{ab}±1.7	3.5^c±1.8	3.5^{bc}±1.9	4.3^{cd}±1.6	25.4***	5.6***
Chewy	7.5^f±1.0	6.9^e±1.1	6.5^e±1.0	5.6 ^{d}±1.2	3.5^a±1.7	3.7^{ab}±2.0	4.5^{bc}±1.4	4.7^c±1.9	4.9^{cd}±1.2	27.5***	1.56*
Overall flavour strength	6.9^b±1.0	6.4^{ab}±1.0	6.7^{ab}±1.1	6.6^{ab}±1.0	6.5^{ab}±1.0	6.3^a±1.1	6.4^{ab}±1.0	6.6^{ab}±1.0	6.7^{ab}±0.8	0.9ns	16.9***
Sweet flavor	7.0^c±0.9	6.6^{bc}±1.0	6.7^{bc}±1.0	6.3^a ±1.0	7.1^c±0.9	6.5^b ±1.2	6.5^b ±1.1	6.3^b ±1.1	5.6^a±1.0	11.9***	14.3***
Baked biscuit flavour	5.7^c±1.4	5.3^{bc}±1.4	5.4^{bc}±0.9	5.5^{bc}±1.6	4.2^a±2.0	4.8^{ab}±1.3	5.4^{bc}±1.3	5.7^c±1.0	5.9^c±1.7	5.3***	6.7***
Roasted cereal flavour	6.3^d ±1.4	6.0^d±1.7	5.7^d ±1.9	5.3^{cd}±2.3	2.4^a±2.1	3.3^{ab}±2.2	4.1^b±2.2	4.5^{bc}±2.3	4.4^{bc}±2.7	20.2***	10.2***
Roasted soy flavour	1.4^{ab}±1.7	1.6^{bc}±1.8	2.6^{cd}±2.5	3.8^{ef} ±2.4	0.3^a ±0.4	1.7^{bc}±2.0	3.2^{de}±2.3	4.2^{ef}±2.2	4.5^f ±2.7	19.4***	5.7***
Doughy flavor	0.6^a ±1.0	0.5^a ±0.6	0.5^a±0.6	0.7^a ±0.8	6.9^d±2.3	3.6^c±2.7	2.7^b ±2.2	1.1^a ±1.0	1.1^a ±1.8	59.1***	3.3***
Sweet aftertaste	5.9^{bc}±1.6	5.7^{bc}±1.6	5.7^{bc}±1.4	5.6^{ab}±1.5	6.4^c ±1.5	5.6^{ab}±1.9	5.6^{ab}±1.7	5.4^{ab}±1.8	4.7^a ±1.4	8.5*	28***
Gritty residue in mouth	8.1^e±1.7	7.9 ^{e}±1.0	7.1^d ±1.2	5.7^c±1.7	0.5^a±0.5	0.8^{ab}±1.4	1.2^b ±1.0	1.4^b ±1.8	1.2^b ±1.1	178***	1.45*
Roasted soy aftertaste	1.0^{ab}±1.1	1.6^{bc}±1.9	2.1^{cd}±2.2	3.4^{fg}±2.1	0.2^a±0.3	1.4^{bc}±1.9	2.5^{de}±2.2	3.1^{ef}±2.6	3.7^g ±2.5	13.1***	5.2***
Heated oil aftertaste	1.6^{ab}±1.9	1.4^a ±1.4	1.8^{ab}± 1.8	2.0^{ab}±1.7	1.2^a± 1.2	1.3^a± 1.5	1.9^{ab}±1.7	2.3^b± 1.9	2.4^b±1.8	0.7ns	26.0***

Values are means±standard deviations. Values in a row followed by different letter notations $^{(a-h)}$ are significantly different at p ≤ 0.05
[1]Significant at *** p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05, ns, not significant.
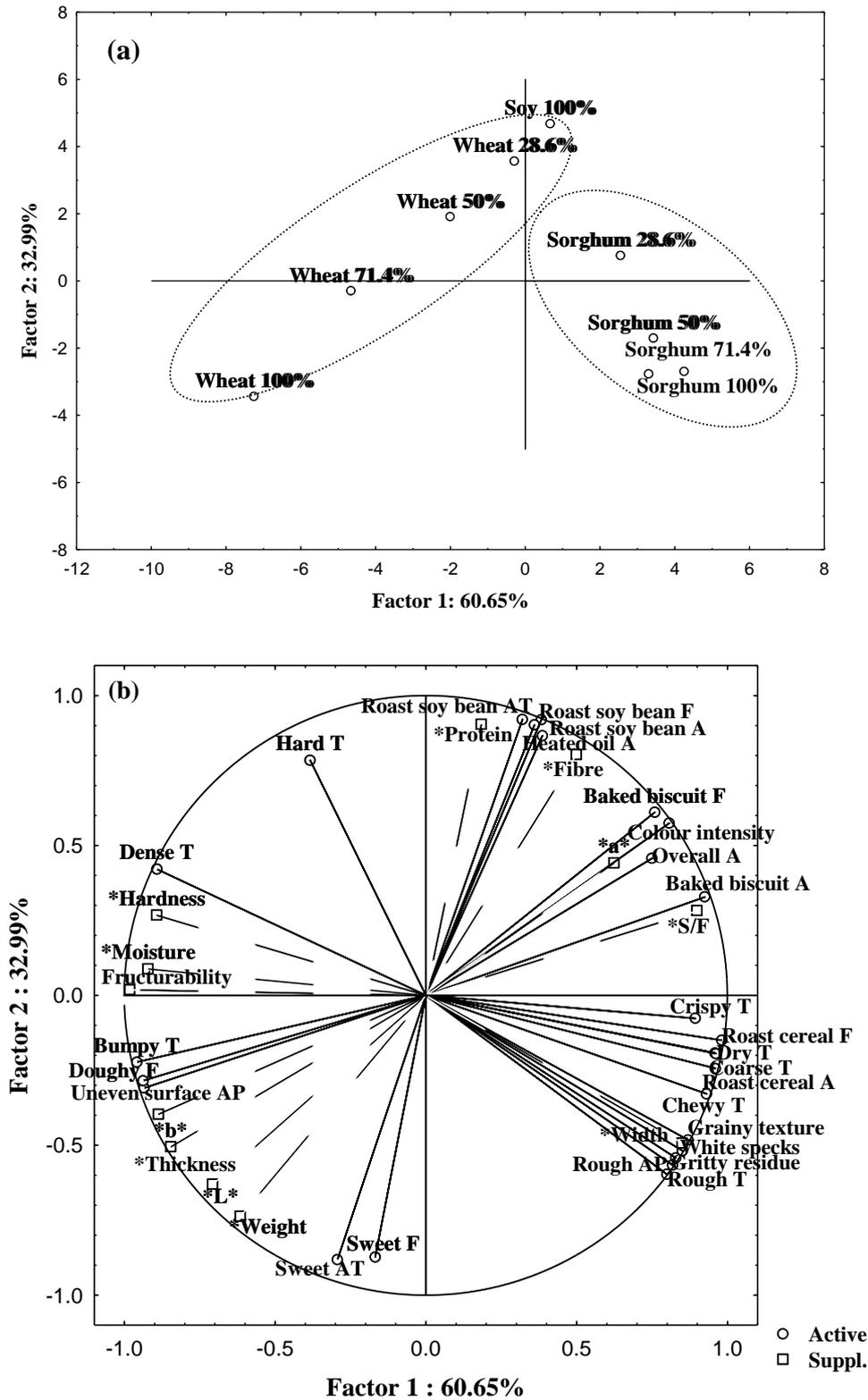
**Figure 4.2.4** Principal component analysis (correlation matrix) of sorghum and bread wheat biscuits composited with soy at levels of 0, 28.6, 50, 71.4 and 100%. (a) Plot of the first two principal component scores of the sorghum and bread wheat biscuits, (b) Plot of the first two principal component loading projections of sensory attributes, A=aroma, T= texture. F= flavour, AP= appearance, AT=aftertaste.

component scores of the sorghum and wheat biscuits. PC1 explained 61% of the total variation and separated the biscuits based on their cereal component with the wheat biscuits to the left and the sorghum biscuits to the right. PC2 accounted for 33% of the total variation and separated soy and cereals, with high soy content biscuits at the top and high cereal content biscuits at the bottom.

The attribute loadings for the first two principal components (Figure 4.2.4b), show the relationship between the sensory attributes, physical, instrumental colour and texture characteristics of the biscuits. The 100% DFS and sorghum and bread wheat biscuits substituted with 71.4% DFS were associated with the roasted soy bean flavour, aroma and aftertaste, baked biscuit flavour and aroma, heated oil aroma and overall aroma intensity which were positively correlated and were also correlated with sensory colour intensity, instrumental colour a* value, protein and crude fibre content. All these characteristics were negatively correlated with sweet flavour and aftertaste, L* and b* colour values and thickness.

Beany flavour is commonly associated with food legumes. In soy beans, enzymatic breakdown by lipoxygenases or autoxidation of linoleic and linolenic acid produces hydroperoxides such as ketones, aldehydes and alcohols that may be responsible for the beany, grassy, painty, or cardboardy flavour which discourages soy consumption (Boge et al 2009). Examples of such compounds have been identified by some researchers in soy products. For instance, Mohsen et al (2009) categorized furans identified in wheat biscuits fortified with isolated soy protein as 2-ethyl-5methylfurans and 2-pentylfurans. 2-pentyl furans were also identified in soy bean oil by Chang (1979). Sorghum biscuits with DSF content above 50% had burnt flavour and odour notes described by panelists as baked biscuit. It is possible that the high level of protein contributed by DSF readily reacted with sugar in the Maillard reaction, hence the positive correlation with protein content and negative correlation with sweet flavour and aftertaste in this study. Similarly, Mohsen et al (2009) reported biscuit-like and burnt odour in biscuits when 2-ethyl-5-methylpyrazine increased after substituting wheat with 20% soy protein isolate.

High intensity of browning was perceived in biscuits with high DSF and was positively correlated with a* (redness) colour values and negatively correlated with L* (lightness) and

b* (yellowness) values. The dark colour on the surface of the biscuits may have been caused by Maillard reaction that produces brown polymers, which contribute to the surface colouration of biscuits (Manley 1991) as explained earlier. Additionally, 5-hydroxymethylfurfural, a compound formed from degradation of the Amadori product leading to formation of brown polymers, was identified in a model cookie/biscuit during baking by Ait-Ameur, Rega, Giampaoli, Trystram and Birlouez-Aragon (2008). The sorghum biscuits also scored high on sensory colour intensity (Table 4.2.5) because of red non-tannin sorghum flour as already explained. Similarly, working on traditional African sorghum foods, Anyango (2009) reported dark colour intensity of ugali, a stiff porridge made from red sorghum flour.

The sorghum biscuits were characterized with rough appearance, gritty, grainy, coarse and chewy texture, which were positively correlated with each other and also positively correlated with roasted cereal flavour and aroma, crispy and dry textures and white specks. These attributes were negatively correlated with moisture content, instrumental texture (hardness and fracturability). The roasted cereal flavour and aroma associated with sorghum biscuits with DSF replacement of 50% and below may be attributed to derivatives of the Maillard reaction favoured by the high temperature and low moisture conditions of baking. Similar findings were reported by Bredie, Mottram, Hassell and Guy (1998) who noted the development of roasted/toasted flavour in thermally treated maize and wheat flours when pyrazines increased.

The rough characteristics for sorghum biscuits may be due to the hard, corneous endosperm cells of sorghum grain that remain intact during milling (Rooney and Miller 1982). After baking, it might be a result of sorghum starch granules that are encapsulated by hydrophobic cross-linked kafirins (Munck 1995), which do not absorb adequate water for expansion and are perceived as gritty (Kebakile, Rooney, De Kock and Taylor 2008). Similar results have been reported by researchers working on sorghum biscuits. For instance, Badi and Hoseney (1976) reported that cookies from sorghum and pearl millet flours were mealy and gritty but demonstrated that grittiness could be reduced by increasing dough pH using sodium carbonate. Chiremba et al (2009) also reported that grittiness was more pronounced in biscuits made from tannin sorghum than non-tannin sorghum flours. The white specks also visible in Figure 4.2.1, which were positively correlated with the rough and coarse

characteristics, may have been contributed by the bran fragments that were not completely milled. Kebakile et al (2008) also reported the presence of coloured specks in porridge made using sorghum flour which they attributed to the pericarp of the sorghum kernel.

The sorghum biscuits were also associated with crispiness and dry texture which were negatively correlated with instrumental texture characteristics of hardness and fracturability. Fracturability, the panel's descriptor for crispness was explained earlier. The dry texture may be ascribed to the sorghum doughs absorbing the least amount of water compared to all other composite doughs (Chapter 4.1, Table 4.4.1). This may be due to the hydrophobic nature of kafirin proteins of the endosperm (Duodu et al 2003, Munck 1995), which probably expelled most of the water as the temperature, increased during baking (Belton et al 2006).

Crispness in sorghum biscuits was negatively correlated with moisture content, while in wheat biscuits moisture was negatively correlated with crisp texture. The sensory panel scores (Table 4.2.5) also show that sorghum biscuits with lower moisture content were 123% more crisp than wheat biscuits. The increase of crispness scores by 58% when bread wheat was substituted with 50% DSF was probably due to dilution of gluten with soy protein hence reduced water holding capacity, as explained earlier. Sensitivity of crispness to moisture has been reported by researchers working on biscuits. Piazza and Masi (1997) using a trained panel reported that the sensory scores of crispness decreased continuously as the moisture content of cookies increased, similar to the findings of this study.

The bread wheat biscuits were described as doughy and dense, attributes that were positively correlated with moisture content of the biscuits. The doughy texture was probably caused by the damaged starch and high protein content of bread wheat absorbing high amounts of water during dough mixing as described by Kent and Evers (1994). Retention of moisture during baking, may have imparted the dense and doughy texture. These findings are in agreement with Slade and Levine (1994), who reported that biscuits made from flours with increased damaged starch baked to higher moisture content. The hard texture was probably due to the stronger gluten of hard wheat flours, which impart toughness to biscuits (Hoseney 1994).

4.2.3.5 Consumer acceptability

Repeated measures analysis of variance, an application which simultaneously compares repeated measurements on the same subjects revealed a significant effect for biscuits, day, session, and session x group (Table 4.2.6). Table 4.2.7 shows that biscuits made from sorghum substituted with 50% DSF and 100% bread wheat were slightly higher in overall liking (5 to 6%) than the 100% sorghum biscuits when they were evaluated by 8 to 9 year old school children. There was no difference in liking between 50% DSF substituted bread wheat biscuits and 100% bread wheat, 100% sorghum and 50% DSF substituted sorghum biscuits. This may be explained by the fact that the children were not familiar with sorghum as a basic ingredient in biscuits as biscuits on the market are made using wheat flour. A study by Delk and Vickers (2006) showed that children preferred refined wheat bread, which they were more familiar with, to whole wheat bread. It is also likely that compositing with DSF imparted positive characteristics that improved liking scores of 1:1 sorghum: DSF biscuits compared to the 100% sorghum biscuits. Results from the descriptive sensory panel (Table 4.2.5) show that the 50% DSF substituted sorghum biscuits were less hard, dense and chewy and more crisp than the 100% sorghum biscuits by 46, 151, 27 and 19%, respectively.

Figure 4.2.5 shows that all the biscuits had moderately high initial mean hedonic scores of 80% and above and these were sustained during the entire study period. This is an indication that repeated exposure did not change the children's liking of biscuits over time. A possible explanation is that biscuits were made from staple foods, sorghum and bread wheat, which have sustained acceptance. Findings from previous studies have demonstrated that time preference curves for staple foods, which are moderately liked are flat because there is no significant decrease following repeated servings (Hetherington, Pirie and Nabbs 2002). For instance, a pioneer repeated exposure study conducted by Siegel and Pilgrim (1958) found that palatability of staple foods such as dairy products, and bread did not change over time, while foods that are not staples such as vegetables declined showing that staple foods are more resistant to boredom than other foods. A similar study by Hetherington et al (2002) confirmed that a staple food, bread and butter was tolerated well and there was no change in liking over a 3 week exposure period compared to chocolate, a food that is not a staple that declined in liking.

Table 4.2.6 Repeated Measures Analysis of Variance (ANOVA) of 8 to 9 year old children's ratings for sorghum and bread wheat biscuits composited with DSF

Source of variation	Sums of Squares (SS)	Degrees of freedom (d.f)	Mean squares (MS)	F	P
Group	53.71	3	17.90	0.99	ns*
Biscuit	16.09	3	5.36	3.35	<0.05
Biscuit/group	15.08	9	1.68	1.05	ns
Day	17.32	3	5.77	4.21	<0.01
Day/group	9.11	9	1.01	0.74	ns
Session	34.34	1	34.34	18.71	<0.001
Session/group	25.27	3	8.42	4.59	<0.01
Biscuit/day	7.06	9	0.78	0.69	ns
Biscuit/day/group	28.39	27	1.05	0.92	ns
Biscuit/session	0.39	3	0.13	0.10	ns
Biscuit/session/group	10.55	9	1.17	0.90	ns
Day/session	6.43	3	2.14	2.20	ns
Day/session/group	10.81	9	1.20	1.23	ns
Biscuit/day/session	4.33	9	0.48	0.46	ns
Biscuit/day/session/group	30.60	27	1.13	1.08	ns

*not significant.

Table 4.2.7 The effect of compositing sorghum and bread wheat with DSF on overall liking of biscuits by 8 to 9 year old children

Biscuit type	Hedonic score
Sorghum: (100)	$4.03^{a} \pm 0.46$
Sorghum: Soy (50:50)	$4.28^{b} \pm 0.39$
Wheat: (100)	$4.23^{b} \pm 0.31$
Wheat: Soy (50:50)	$4.19^{ab} \pm 0.10$

Values are mean±SD. Values followed by different letter superscripts in a column are significantly different at P≤0.05 as assessed by Fisher's least significant test. Overall liking ratings 1= dislike very much, 2= dislike a little, 3= not sure, 4= like a little, 5= like very much. Consumers n=60.
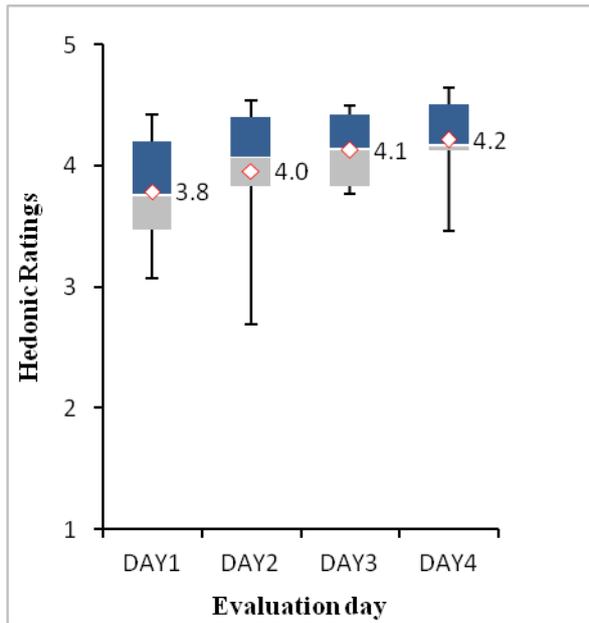
a) Sorghum: (100)
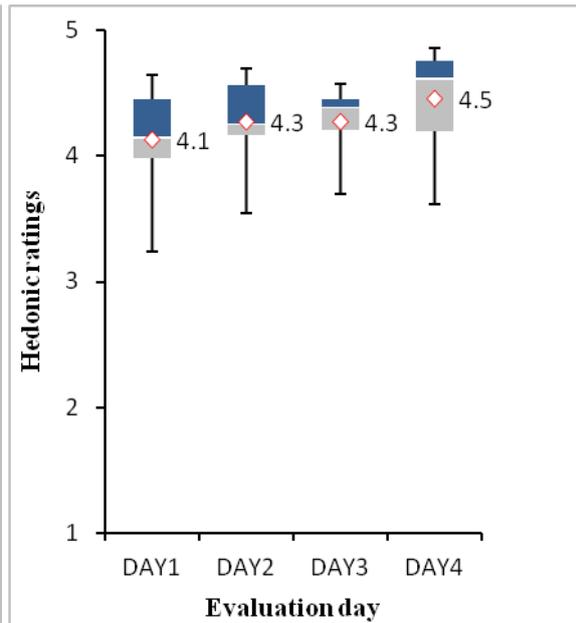
b) Sorghum: soy (50:50)
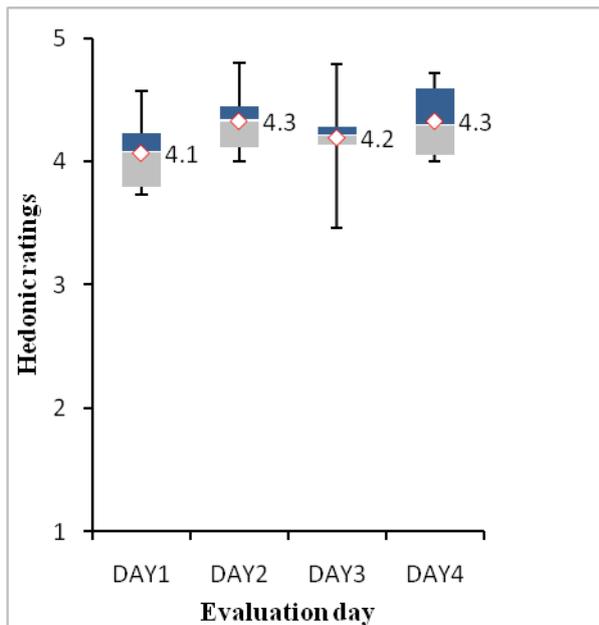
c) Wheat: (100)
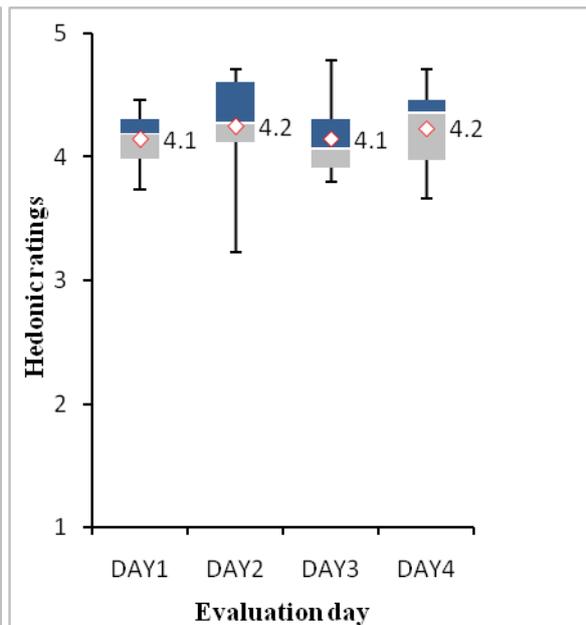
d) Wheat: soy (50:50)

Figure 4.2.5 The effect of compositing sorghum and bread wheat with DSF on 8 to 9 year old children's (n=60) ratings of biscuits over time. Means and standard deviations: means in all graphs are not significantly different at p≤0.05. The dark shaded area is the higher percentile and represents the value above which 75% of the ratings fell. The light shaded area is the lower percentile and represents the area where 25% of the ratings fell. The median is the thin line between the two shaded areas where 50% of the values fell above and 50% below. Hedonic rating scale, 1= dislike very much, 2= dislike a little, 3= not sure, 4= like a little, 5= like very much.

It is also likely that the biscuits were sustainably liked by the children. A study by Stubenitsky, Aaron, Catt and Mela (1999) demonstrated that consumer hedonic ratings for pork sausages and milk chocolate snack bars did not show any change in preference and did not decrease with time, suggesting a generally high and sustained consumer acceptance over an extended period.

It is possible that initial hedonic ratings of the biscuits may have predicted long-term acceptability of the biscuits. Similarly, Goldman (1994) conducted a repeated consumption test of breakfast cereals and the hedonic scores were consistent over the 5 day test. Kramer, Lesher and Meiselman (2001) also observed that for food items that were eaten repeatedly, the difference between ratings for the first time the food was eaten was minimal compared to subsequent ratings.

The distribution along the bar line of the graph explains agreement among consumers (Figure 4.2.5). The short distribution of scores along the bar graphs for this study is also an indication that generally there was agreement among the children over the scores for all the biscuits and the results obtained were generally consistent. These results agree with the findings of Leon et al (1999) for a study using five varieties of biscuits dressed with different types of jams, which showed that children aged 8 to 10 years are consistent when using hedonic categorization to evaluate food samples. Additionally, most research protocols expose children only once to a novel food given in small amounts which are not representative of the product (Popper and Kroll 2003). In this study, children were exposed to the product nine times including the test biscuit making it more repeatable and it is likely that the results are a true reflection of the product's potential.

Figure 4.2.6 shows that more than 80% of the children expressed the desire to consume all the types of biscuits again. Similarly, consumers in a study by Chen, Weingartner and Brewer (2003) expressed positive attitudes about soy foods after evaluating soy ingredient containing cookies, indicating that they were not bored with the biscuits. Measuring desire to consume a food again is a method of determining whether consumers are bored with a product (Zandstra, Weegels, Van Spronsen and Klerk 2004).
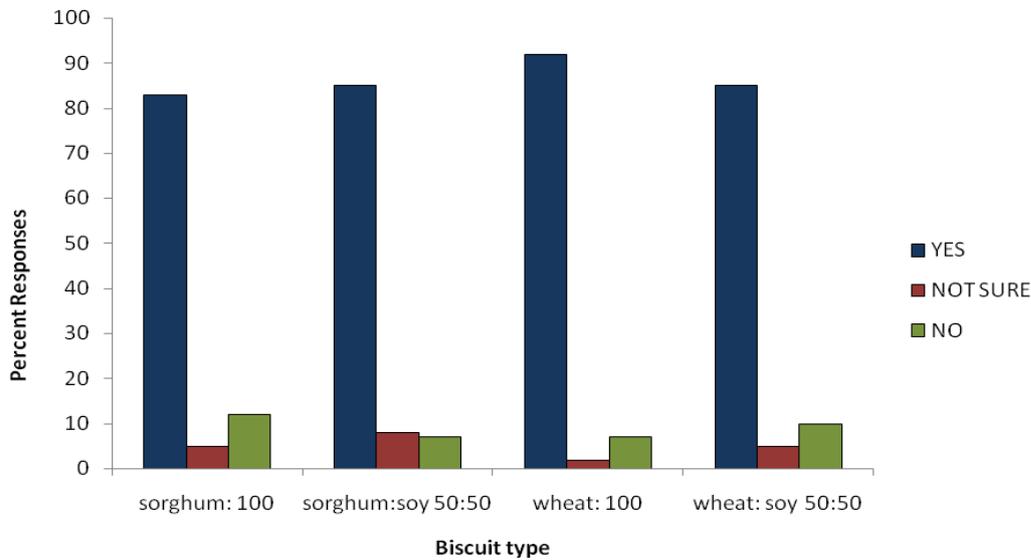
Figure 4.2.6 The effect of compositing sorghum and bread wheat with DSF on children's (n=60) desire to consume biscuits again.

Table 4.2.6 shows there was a significant (p= ≤0.001) session effect. This is because the first session had higher scores than the second (data not shown). It may be that short lived fatigue caused by the monotony and length of the experimental procedure caused the change because the same biscuits were always rated higher the following day during the first session. In a similar study, Sulmont-Rossé, Chabanet, Issanchou and Koster (2008) demonstrated that repeated exposure can produce experimental boredom leading to low scores.

4.2.4   CONCLUSIONS

Compositing sorghum and bread wheat with defatted soy flour at 1:1 ratio imparts positive characteristics associated with biscuits such as increased spread factor and crispy texture and reduced hard, dense and chewy texture, but higher proportions of soy add the beany flavour. Sorghum and bread wheat biscuits, made from staples that African children are familiar with, fortified with defatted soy flour at a 1:1 ratio have a moderately high acceptability to 8 to 9 year old school children and may retain their acceptance over conditions of repeated use. Hence, they have the potential to be protein rich supplementary foods to alleviate PEM malnutrition in Africa.

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4.3 **Effect of compositing with soy on the protein nutritional quality of sorghum biscuits determined by rat bioassay**

ABSTRACT

This study was conducted to evaluate the protein nutritional quality of sorghum-soy composite biscuits and their potential to support growth, compared to unfortified sorghum biscuits. Three isonitrogenous diets of 8% protein made from the two types of sorghum biscuits and casein as a reference were fed to male Sprague Dowley weanling rats. The indices of protein quality determined were Protein Efficiency Ratio (PER), Food Efficiency Ratio (FER), True and Apparent Protein Digestibility, Biological Value (BV), Net Protein Utilization (NPU) and Protein Digestibility Corrected Amino Acid Score (PDCAAS). The PER and FER for the fortified sorghum biscuit diet were the same as the casein diet and zero for the 100% sorghum diet. Faecal bulk for the 100% sorghum diet was 1.5 times higher and faecal protein 1.5 to 2.4 times higher than the fortified sorghum and casein diets. True digestibility for all the diets was high, between 88 and 95%. BVs for 100% sorghum biscuit were 15 to 20% higher than fortified sorghum and casein diets and NPU value was 16% higher than fortified sorghum. Casein had PDCAAS of 1.0 and fortified sorghum biscuits 0.80 to 0.87, considered acceptable for pre-school and school children. The rat is a poor model for determination of sorghum protein digestibility because it digests sorghum protein very efficiently. It appears that 100% sorghum biscuits do not support growth in rats. However, fortification with soy substantially improves PER of sorghum biscuits and by extrapolation, could support growth of school age children if used as a supplementary food.

4.3.1 INTRODUCTION

The most serious deficiency diseases resulting from under-nutrition among children in developing countries are the various forms of Protein Energy Malnutrition (PEM) (Walker, 1990, Muller and Krawinkel 2005). The Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) epidemic has aggravated the situation in sub-Saharan Africa, which accounts for 91% of new infections among children worldwide and has an estimated 14.1 million AIDS orphans (Joint United Nations Programme on HIV/AIDS/ World Health Organization (UNAIDS/WHO) 2009). A dietary staple such as sorghum that is readily available in sufficient quantities in the semi-arid and arid zones found in most of Africa (ICRISAT 2009) could be used in protein-rich supplementary foods for children such as sorghum-legume composite biscuits to help alleviate PEM.

In Chapter 4.1 Section 4.1.4 it was shown that substituting sorghum or bread wheat with 50% defatted soy flour dramatically improved the protein quality of biscuits. For example, compared to the 100% sorghum biscuits, the protein content and in vitro protein digestibility were 168%, and 170% higher. Lysine and reactive lysine contents increased by 221% and 257% per 100 g protein compared to the 100% sorghum biscuit. However, it has been stated that the most sensitive assessment of protein quality is achieved by clinical studies or animal assays that measure growth or metabolic indicators (Boutrif 1991). The WHO (2007) has given high priority to the use of animal models for protein quality research because animals can be controlled more effectively than humans, over longer periods and results can be extrapolated to human requirements. Additionally, the nitrogen balance method using animals is necessary for predicting true digestibility of proteins in humans for use in computing the Protein Digestibility Corrected Amino Acid Score (PDCAAS) (FAO/WHO 1991, WHO 2007).

Studies on sorghum protein quality have been conducted using in vivo assays in both humans and animals and shown that the overall effect of cooking sorghum is reduction in digestibility and enlargement of faecal volume. For example, a study by MacLean et al (1981) demonstrated that cooked whole grain sorghum fed to preschool children did not support growth, had apparent digestibility of 46% and gave stool weight 3 times higher than the control casein diet. Eggum, Monowar, Bach Knudsen, Munck and Axtell (1983) using a rat

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study also reported a reduction in true protein digestibility (TPD) and increase in biological value (BV) of products made from cooked compared to uncooked sorghum foods. However, high apparent protein digestibility of 81% and 74% were achieved when sorghum grain was decorticated and extrusion cooked (MacLean, De Romana, Placko and Graham 1983) and fermented (Graham, MacLean, Morales, Hamaker, Kirleis, Mertz and Axtell 1986), respectively.

Studies have shown that the young rat is more efficient in digesting sorghum protein than children. Evaluating the digestibility of sorghum proteins, Axtell et al (1981) showed that whole grain sorghum had 80% digestibility in rats. Bach Knudsen, Kirleis, Eggum and Munck (1988a) further established that white non-tannin sorghum had 60% higher true digestibility than red tannin sorghum. Soy protein quality was also determined using a rat bioassay by Vasconcelos et al (2001) who demonstrated that heat treatment inactivates antinutrients in soy bean which had a true digestibility of 51 to 60% when raw and 78.3% after cooking.

Notwithstanding possible drawbacks, it appears using a rat model is necessary to determine the efficacy of the formulated soy fortified sorghum biscuits as protein supplements for alleviate PEM. The objective of this study was therefore to determine the protein nutritional quality and effect on growth of sorghum-soy composite biscuits compared to unfortified sorghum biscuits using a rat bioassay.

4.3.2   MATERIALS AND METHODS

4.3.2.1 Biscuit Samples

Two types of sorghum biscuits were used in this study, one made of 100% sorghum and the other with 50% defatted soy flour (DSF) substituting sorghum. Biscuits were prepared according to the procedure described in Chapter 4.1, Sections 4.1.2.2 and 4.1.2.3, except that the sorghum flour was milled to a particle size of not more than 500 μm using a laboratory hammer mill (Falling Number 3100, Huddinge, Sweden). After preparation, biscuits were stored at 10°C until required.

4.3.2.2 Diet preparation

The biscuit samples used for preparation of the diets were pulverized using a Waring Commercial® laboratory blender (New Harford, CT), set at medium speed for 2 minutes. Three isonitrogenous diets were prepared from the two types of sorghum biscuits and Animal Nutrition Research Council Reference Casein (ANRC) casein (calcium caseinate, Fonterra, Co-operative Group, New Zealand) to provide 8% crude protein in the final diet on a dry weight basis. The percentages of ingredients in the diet formulation were calculated based on the composition of the test protein (Table 4.3.1). The casein diet was the reference.

The biscuits and casein were incorporated into the basal diet (Table 4.3.2) at the expense of corn starch-sucrose mixture of 1:1 ratio. The diets also contained 1% vitamin and 5% mineral fortification mixes, both from ADVIT Animal Nutrition, Kempton Park, South Africa. A protein-free diet was prepared in which the test food was replaced by the corn starch-sucrose mixture in the basal diet. The purpose of the protein-free diet was to estimate the endogenous nitrogen excretion by the rats. The oil content in all four diets was adjusted to 9% using sunflower oil. Formulation of the diets is shown in Table 4.3.2.

Table 4.3.1 Proximate composition of the sorghum biscuits and reference casein (g/100 g)

	Protein	Fat	Moisture	Carbohydrates	Ash (minerals)	Crude fibre
Casein	90.10	1.00	4.10	0.10	4.70	0.00
Sorghum :100	9.23	21.00	3.25	63.50	1.39	1.61
Sorghum-Soy:50:50	24.71	19.68	3.82	45.60	2.78	3.66

Table 4.3.2 Formulation of the four experimental diets (g/kg dry basis)

Ingredients	Basal (protein-free)	Casein (reference)	Sorghum: soy (50:50)	Sorghum: (100:0)
			Diets	
Casein[1]	0	90.0	0	0
Sorghum-soy biscuits	0	0	332.0	0
Sorghum biscuits	0	0	0	850.0
Sunflower oil[2]	90.0	90.0	90.0	90.0
Mineral mixture[3]	50.0	50.0	50.0	50.0
Vitamin mixture[4]	10.0	10.0	10.0	10.0
Cellulose powder[5]	10.0	10.0	10.0	0
Corn starch[6]	420.0	375.0	508.0	0
Sucrose	420.0	375.0	249.0	0
Total	1000	1000	1000	1000

[1]Casein – New Zealand Milk Products (Clover Fonterra, Johannesburg, South Africa); [2]Sunflower oil "Sunfoil" (Willowton Oil, Pietermaritzburg, South Africa); [3]Mineral mixture and [4]Vitamin mixture composition according to AOAC method 960.48 (ADVIT Animal Nutrition S. A, BASF, Kempton Park, South Africa); [5]Cellulose powder (W& R, Balston, England); [6]Corn starch (Tongaat Hulett, Johannesburg, South Africa).

4.3.2.3 Animals and housing

Twenty four weanling male Sprague Dowley rats (South African Vaccine Producers, Johannesburg, South Africa) four to five weeks old from the same colony, weighing 80 to 120 g were used for this study (Figure 4.3.1)

A

B

C

Figure 4.3.1 Type of rats and housing used in the study. A= male weanling Sprague Dowley rats (Wikipedia 2010); B= Tecniplast® metabolic rat cage and; C= storage rack for 12 metabolic rat cages (Tecniplast Group 2010).

Pretoria, according to AOAC International (2000) method 960-48 with modifications. Approval by the University of Pretoria Ethics Committee was granted for this study. Maintenance of animals was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council 1996). The animals were housed individually in stainless steel metabolic cages (Tecniplast®, Tecniplast Group, Varese, Italy) equipped to separate urine and faeces. The metabolic cages were stored on two racks each holding 12 cages. Temperature was maintained at 21 to 24° C with alternate periods of light and dark of 12 hours and relative humidity between 40 to 70%. Figure 4.3.1 shows the type of rat breed, metabolic unit and storage racks used in the study.

4.3.2.4 Growth study

On arrival, the animals were fed on standardized laboratory irradiated rat pellets (EPOL, Pretoria, South Africa) for an acclimatization period of 3 days. After acclimatization, the animals were randomly distributed into 4 groups of 6 rats each with the average weight of rats in any one group on the first day of the assay not exceeding 5 g average weight of rats in any other group. One group of rats was fed the protein-free diet, another, the casein, and the third and fourth groups the 100% sorghum and sorghum-soy 50:50 diets, respectively (Table 4.3.2.) Food and sterilized water were provided ad libitum for the entire study period. Records of food intake for each rat were maintained daily. Rats were weighed on alternate days including the first and last days of the study.

The growth study lasted 28 days. Net Protein Ratio (NPR) was measured on the 10th day of the study. The rats fed the protein-free diet were euthanized on the 10th day after the protein digestibility and NPR studies due to their rapid weight loss and the remaining three groups of rats at the end of the PER study by placing them in an inhalation chamber containing a high dose of anaesthetic (isoflurane). NPR, PER and Feed Efficiency Ratio (FER) were calculated according to FAO/WHO (1991). The PER and NPR in this study were computed for 5 days (days 8 to 13 of the study) because the rats on the casein diet unexpectedly lost weight after the 13th day.

4.3.2.5 Protein digestibility study

The protein digestibility study started on the 5th day, days 5 to 9 of the growth study and lasted 5 days. During the study, the food consumed was calculated from the food supplied minus the weight of uneaten food daily. Faeces for each rat were collected into polyethylene type paper bags daily, as was urine into plastic containers containing 1 ml 0.5 M sulphuric acid. Faeces and urine were frozen at -20° C until required.

4.3.2.6 Computations

The following protein quality indices were calculated from the data collected (FAO/WHO 1991, WHO 2007):

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{g of weight gain}}{\text{g of protein consumed}}$$

$$\text{Net Protein Retention Ratio} = \frac{\text{g of weight gain + g of weight loss in protein free diet}}{\text{g of protein consumed}}$$

$$\text{Food Efficiency Ratio} = \frac{\text{g of weight gain}}{\text{g of food consumed}}$$

$$\text{Apparent Protein (N) Digestibility (\%)} = \frac{I - F \times 100}{I}$$

$$\text{True Protein (N) Digestibility (\%)} = \frac{I - (F - F_0) \times 100}{I}$$

$$\text{Apparent Protein (N) Biological value (\%)} = \frac{(I - F - U) \times 100}{I - F}$$

$$\text{True Protein (N) Biological Value (\%)} = \frac{I - (F - F_0) - (U - U_0) \times 100}{I - (F - F_0)}$$

$$\text{Faecal Protein (\%)} = \frac{F - F_0 \times 100}{I}$$

$$\text{Urine Protein (\%)} = \frac{U - U_0 \times 100}{I}$$

$$\text{Protein (N) Balance} = I - (F - F_0) - (U - U_0)$$

Apparent Net Protein (N) Utilization = % Apparent digestibility x % biological value

True Protein (N) Utilization = % True digestibility x % biological value

Where I = nitrogen intake of the test diet, F = faecal nitrogen loss on the test diet, F_0 = faecal nitrogen loss on a protein-free diet, U = urinary nitrogen and U_0 = urinary nitrogen loss on protein-free diet.

4.3.2.7 Chemical analyses

The total faeces from each rat were dried overnight at 100° C in an air circulation oven, weighed and pulverized using a laboratory blender. The pulverized faeces from six rats fed the same diet were pooled. The urine samples from each rat were pooled and freeze dried. Nitrogen in the faeces and urine was determined by Dumas combustion (AACC International, 2000) Method 46-30 and protein content (N x 6.25) calculated. The moisture content of the food and faeces was determined by a one stage air oven procedure (AACC International 2000) Method 44-15A. Faecal and urine nitrogen of rats fed the protein-free diet was used to calculate endogenous nitrogen loss. Six individual values per diet for true protein digestibility (TD), biological value (BV) and net protein utilization (NPU) were computed from nitrogen intake, faecal nitrogen, urinary nitrogen and endogenous fecal and urinary nitrogen (FAO/WHO 1991). Apparent PD, BV and NPU were also computed excluding the endogenous nitrogen.

4.3.2.8 Protein Digestibility Corrected Amino Acid Score (PDCAAS) determination

The PDCAAS is the official method for predicting protein quality for food based on human amino acid requirements (WHO 2007). The parameters it takes into consideration critical to quality evaluation of a protein source are indispensable amino acid profile of the test protein, its digestibility and ability to supply the amino acid in sufficient quantity (WHO 2007). In this study, amino acid composition data for the test products (100% sorghum and sorghum-soy biscuits), previously reported in Chapter 4.1 and true digestibility values determined in this investigation were used to compute the PDCAAS using the following equation (WHO 2007):

$$\text{Amino acid score} = \frac{\text{mg of amino acid in 1 g test protein}}{\text{mg of amino acid in requirement pattern (3-10 or 1-2 yr olds)}}$$

PDCAAS = True Digestibility x Lysine score (or the amino acid with the lowest ratio).

Amino acid scores for 9 indispensable amino acids, lysine, leucine, phenylalanine + tyrosine, valine, tryptophan, methionine + cysteine, threonine, histidine and isoleucine, were computed using a human pattern for amino acid requirements for preschool (1 to 2) and school age (3 to 10) years (WHO 2007) and a rat growth pattern of amino acid requirements National Research Council (NRC) (NRC 1995) as the reference proteins. Indispensable amino acid profiles were determined using the Pico-Tag method (Bidlingmeyer et al 1984) by acid hydrolysis, separation and quantification by reverse phase HPLC (Chapter 4.1). Three sets of PDCAAS values were computed.

4.3.2.9 Statistical analysis

Results were presented as mean values and standard deviations. Data was subjected to one-way analysis of variance (ANOVA) using Statistica Software version 8.0 (Statsoft, Tulsa, OK). Means were separated using Fisher's Least Significant Difference test (LSD).

4.3.3 RESULTS AND DISCUSSION

4.3.3.1 Growth study

The animals arrived on 15 October 2009. They were fed on a laboratory diet for 7 days (15 to 21 October). On 22 October when the rats started feeding on the experimental diets, they rapidly lost weight up to 28 October. The laboratory diet was restarted to rehabilitate the rats on 28 October and was continued to 2 November 2009. As a result of the rehabilitation period, when the PER and digestibility studies started on 2 November considered day 0, the animals on the casein diet had approximately 15 g higher mean weight than the nearest two groups. Protein content of urine and faeces for 7 to 11 November (days 5 to 9 of the study) was used for the digestibility study).

The four groups of animals all lost weight again from 3 November, The protein-free diet fed rats were euthanized on 11 November (day 10 of the PER study). The remaining three groups fed on sorghum-soy, sorghum and casein diets, started gaining weight again on 10 November but after 15 November (day 13), the casein diet fed rats started losing weight and this continued until 30 November 2009 (day 28) the last day of the study. However, the sorghum-soy diet fed rats steadily gained weight until day 28, while the sorghum diet fed rats did not gain weight. Consequently, the PER was calculated using data from the 5 days, 10 to 15 November (days 8 to 13) when the casein diet fed rats gained weight.

The food and protein intake and rat growth data needed to determine PER and NPR are shown in Table 4.3.3. The food intake of the protein-free diet was less than half of the other two diets. The higher food intake for casein, sorghum-soy and sorghum biscuit diets may have been influenced by the quality and type of protein. According to the National Research Council (1995), low protein diets result in reduced food intake, and protein deficiency in weanling rats causes reduced growth, muscular wasting, emaciation and death if sufficiently severe. Previous studies have shown that low and/or imbalanced dietary protein suppresses food intake. For instance, Mosha and Benink (2004) reported low intake by rats (5.21 g/ day) for a 100% maize meal supplementary food compared to 11.43 g per day for a maize-bean-sardine meal. Food intake influences both energy and protein intake which are critical for growth.

Despite the problems with the unexpected loss in weight by the casein reference group, the results nevertheless indicate that the rats fed the soy fortified sorghum biscuit diet gained the same amount of weight as rats fed the reference diet, compared to the rats fed the 100% sorghum biscuit diet which did not gain weight in 5 days (Table 4.3.3). Figure 4.3.2 shows that during the 5 days used to compute PER, rats fed the casein diet had a mean weight gain of 1.25 g, sorghum-soy 1.17 g, 100% sorghum 0.06 g and protein-free -3.80 g. The PER of the sorghum-soy biscuit diet fed rats was also the same as the PER of the reference casein compared to the 100% sorghum biscuit diet fed rats that had a zero value for PER. Table 4.3.3 and Figure 4.3.2 further show that during the last 20 days of the study, the sorghum-soy diet fed rats gained 10% higher weight than their original weight compared to the sorghum biscuit diet fed rats that did not gain any weight. It is possible that the increased growth rate of the sorghum-soy diet fed rats may have been partly due to higher lysine content in the legume protein.

In a similar study, Ashley and Anderson (1975) demonstrated that increasing the lysine content of a wheat gluten diet for rats resulted in 56% higher growth rate than rats fed a pure gluten diet. Mensa-Wilmot et al (2001) and Joseph and Swanson (1993) reported PER values were 84 to 96% of a casein diet for maize-soybean-cowpea-peanut composite weaning foods and relative PER values of up to 80% for bean-rice diets compared to 67% for rice a only diet. Nnam (2001) demonstrated that rats fed a diet of sorghum flour substituted with 46% bambara groundnut with 19% protein caused the same growth rate as rats fed a reference casein diet.

Some earlier studies also showed that cereal only diets do not support growth. For example, Mosha and Bennink (2004) showed that a maize meal only diet fed to rats caused weight loss. Also, as stated, MacLean et al (1981) reported dramatic slowing of weight gain or weight loss of approximately 5.9 g/kg body weight per day in children fed whole grain sorghum gruel and ascribed it to inadequate quality and quantity of dietary protein similar to the findings in this study. On the contrary, Kavithaparna, Geervani and Sumathi (1988) reported slight increase of 0.25 kg in mean body weight for children fed on decorticated sorghum diets. However, the higher energy and protein in the experimental diet compared to the pre-experimental diet may have contributed to the weight increase due to the catch up growth phenomenon for these children who were undernourished.

Table 4.3.3 Growth of rats, protein efficiency ratio (PER) and food efficiency ratio (FER) values for 100% sorghum and sorghum-soy biscuit diets

Protein Quality Indices	Diet groups			
	Casein (reference)	**Sorghum-soy**	**Sorghum 100%**	**(Basal) Protein-free**
Food intake (g)	54.55[b]±8.35	48.53[b]±5.14	49.54[b]±1.88	24.57[a]±3.50
Protein intake (g)	4.36[a]±0.67	3.88[a]±0.41	3.96[a]±0.15	nd
Initial weight (g)	164.25[b]±13.65	143.83[a]±10.00	145.00[a]±17.58	142.00[a]±8.60
Weight after 5 days (g)	170.50[c]±15.06	149.67[b]±9.20	145.33[b]±18.20	123.00[a]±7.91
Weight gain after 5 days (g)	6.25[c]±5.51	5.83[c]±3.63	0.33[b]±1.50	-19.00[a]±2.00
Weight after 20 days	143.25[b]±12.09	157.83[c]±11.27	143.50[b]±16.4	112.50[a]±7.53
Weight gain after 20 days (g)	-21.00[a]±11.21	14.00[c]±3.32	-1.50[b]±3.21	-29.5±2.60**
PER	1.49[b]±0.23	1.49[b]±0.21	0.08[a]±0.37	nd
Corrected PER	2.50[b]±0.00*	2.49[b]±0.80	0.13[a]±0.63	nd
Relative PER (%)	100.00[b]±0.00*	99.79[b]±12.42	5.17[a]±25.26	nd
FER	0.12[b]±0.01	0.12[b]±0.02	0[a]±0	nd
NPR (%)	5.79	6.40	4.42	nd

Values are means±standard deviations for 6 rats fed for 5days. Values in a row followed by different letter superscripts are significantly different at P≤0.05 as assessed by Fisher's least significant difference; nd= not determined; *= values of 2.5 and 100% are assumed values for casein; **=weight loss for protein-free diet fed rats for 9 days.

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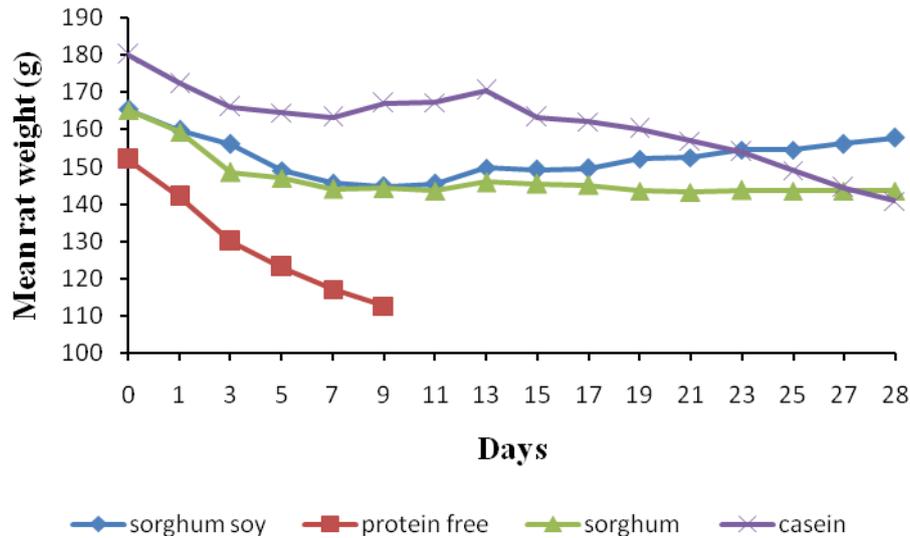
Figure 4.3.2 Average weight of the rats on the four diets for the 28 day period of the feeding trial.

Table 4.3.3 also shows that the 50:50 sorghum: DFS diet had the highest NPR. However, the NPRs of all three diets were quite similar, as a result of the large weight loss in the protein-free group. In contrast to PER, NPR takes into account the weight loss of the rats ont he protein-free diet (FAO/WHO 1991). The NPR of the 50:50 sorghum: DFS diet was slightly higher than that of the casein diet because of the slightly, but not significantly, lower protein/food intake of the former group.

The Food Efficiency Ratio (FER) for the sorghum-soy biscuit diet was equivalent (99.8%) to the reference casein compared to the 100% sorghum biscuit diet that had a zero Food Efficiency Ratio (Table 4.3.4). Mosha and Bennink (2004) also reported a negative food efficiency ratio for a pure maize meal supplementary food product. A high Food Efficiency Ratio is an important quality attribute for cereal-based supplementary foods of high dietary bulk which may limit the quantity of food consumed by children (Lungqvist, Mellander and Svanberg 1981) to meet their nutritional needs.

Table 4.3.4 Effect of consumption of 100% sorghum and sorghum-defatted soy flour biscuit diets on protein intake, output and retention of rats for 5 days

Quality Indices	Diet groups			
	Casein (control)	**Sorghum-soy**	**Pure sorghum**	**Protein free**
Food intake (g)	54.55[b]±8.35	48.53[b]±5.14	49.54[b]±1.88	24.57[a]±3.50
Protein intake (g)	4.36[a]±0.67	3.88[a]±0.41	3.96[a]±0.15	nd
Faecal excretion (g)	3.14[b]±0.37	2.89[b]±0.32	4.70[c]±0.57	1.62[a]±0.26
Faecal protein output (g)	0.46[b]±0.05	0.56[c]±0.06	0.72[d]±0.09	0.23[a]±0.04
Faecal protein (%)	5.25[a]±1.19	8.64[b]±2.03	12.25[c]±1.87	nd
Urine excretion (g)	20.00[ab]±2.90	22.60[b]±2.97	16.00[ab]±4.08	15.25[a]±8.05
Urine protein output (g)	1.21[b]±0.17	1.29[b]±0.17	0.68[a]±0.71	0.72[a]±0.42
Urine protein (%)	11.58[b]±5.04	14.92[b]±4.81	-1.11[a]±4.2	nd
Protein (N) Balance (g)	3.65[a]±0.74	2.98[a]±0.52	3.52[a]±0.24	nd

Values are means±standard deviations based on data for 6 rats. Values in a row followed by different letter superscripts are significantly different at P≤0.05 as assessed by Fisher's least significant difference. nd= not determined. Food and protein intake, and faecal excretion and faecal protein output (dry basis). Urine excretion, and urine protein output (as is).

4.3.3.2 Digestibility study

Nitrogen excretion and retention

The faecal bulk for rats fed the 100% sorghum biscuit diet was 52% and 62% higher than for the casein and sorghum-soy composite biscuit diets, respectively (Table 4.3.4). This may be attributed to the formation of enzyme-resistant starch and unavailable kafirin during thermal processing of sorghum. High stool volume for rats fed on a sorghum diet were reported by Bach Knudsen et al (1988a) who recovered 26 to 74% of starch content of the diet fed to rats in the faeces. MacLean et al (1981) also reported stool volumes 2.5 times higher than the control for children fed on a sorghum diet and Kurien et al (1960) reported doubling of stool weights when sorghum replaced rice in the diets of 10 to 15 year old boys.

The faecal nitrogen expressed as a percentage of nitrogen intake for rats fed the 100% sorghum biscuit diet was some 54% and 17% higher than the casein and sorghum-soy composite biscuit diets, respectively (Table 4.3.4). The higher faecal nitrogen content may be accounted for by the unavailable sorghum endosperm proteins, the kafirins. Previous studies have shown that thermal processing reduces protein digestibility of sorghum proteins. For example, Hamaker et al (1986) attributed reduced digestibility of cooked sorghum proteins to disulphide mediated polymerization making them less susceptible to attack by proteolytic enzymes. Most faecal nitrogen is from undigested food, and the rest from cells shed from intestinal mucosa and residues of digestive juices (Kavithaparna et al 1988).

The faecal nitrogen loss of rats fed the sorghum-soy biscuit diet was 37% higher than the loss from rats fed the reference casein diet (Table 4.3.4). It is possible that the higher soy content in this diet elicited nitrogen excretion from sources other than undigested soy protein. For instance, Fairweather-Tait, Gee and Johnson (1983) comparing faecal nitrogen excretion from rats fed a casein control diet and a bean diet attributed the higher nitrogen excretion of the bean diet to increased microbial activity in the intestines, utilizing indigestible carbohydrates and proteins from bean substrates. In a similar study, Bender and Mohammadiha (1981) ascribed the increase to enhancement of mucosal cell turnover through consumption of a bean diet. The results also show that the animals fed all the three diets were in positive nitrogen balance.

Protein digestibility, bioavailability and net utilization

Table 4.3.5 shows that apparent and true protein digestibility of all the diets were high (82% and above) with only slight differences between diets. The casein diet was higher than the sorghum-soy and 100% sorghum biscuit diets by 5% and 9%, respectively. The 100% workers concluded that sorghum is a poor source of dietary protein for infants and children. Studies conducted by Kavithaparna et al (1988) reported apparent protein digestibility of 69% and 66% for sorghum roti and cooked decorticated sorghum diets, respectively by preschool children and Kurien et al (1960) found apparent digestibility of 54% for teenage boys consuming a high sorghum diet.

The slightly higher protein digestibility of 4% for sorghum-soy diet compared to the 100% sorghum biscuit diet may be due to replacement of the less digestible kafirins with the more digestible soy proteins the globulins. Nnam (2001) similarly reported apparent protein digestibility of 89% for sorghum substituted with 46% bambara ground nut fed to rats. The true digestibility value of the sorghum-soy biscuit diet is comparable to results from two studies by Mensa-Wilmot et al (2001) and Edem, Ayatse and Itam (2001) who reported 91% true digestibility for cowpea-soy-maize weaning food and soy protein supplemented cassava gari, respectively.

Table 4.3.5 Indices of protein quality for 100% sorghum and soy fortified sorghum biscuits

Protein quality indices	Diet groups		
	Casein (control)	Sorghum-soy	Sorghum 100%
Apparent protein digestibility (%)	89.38[c]±1.55	85.38[b]±2.45	81.94[a]±1.76
True protein digestibility (%)	94.75[c] ±1.19	91.36[b]± 2.04	87.75[a] ±1.87
Apparent protein BV[1] (%)	68.19[a]±8.01	60.43[a]±7.99	79.10[b]±5.48
True protein BV (%)	89.51[a]±5.18	85.62[a]±5.23	103.25[b]±4.83
Apparent NPU[2] (%)	61.00[ab]±7.13	51.71[a]±7.97	64.85[b]±5.41
True NPU (%)	84.81[ab]± 4.93	78.26[a]±5.79	90.63[b]± 5.52

Values are means±standard deviations for 6 rats fed for 5 days. Values in a row followed by different letter superscripts are significantly different at P≤0.05 as assessed by Fisher's least significant difference. [1]BV = Biological Value; [2]NPU = Net Protein Utilization

The high BVs for the 100% sorghum biscuit diet may be explained by the fact that no dietary nitrogen was excreted in the urine partly because microbial fermentation of uncooked starch in the sorghum biscuits may have changed the routes of nitrogen excretion from urine to the faeces (Bach Knudsen, Munck and Eggum 1988b). Sorghum starch granules are encapsulated by hydrophobic cross-linked kafirins (Ezeogu et al 2008), which do not absorb adequate water for expansion and probably remained uncooked in the biscuits. Beames and Eggum (1981) who added raw potato starch to diets fed to rats reported increased BV and reduced digestibility. These workers suggested that less nitrogen was secreted in the urine and more in faeces due to fermentation of raw starch in the lower intestine and microflora obtain nitrogen from urea diffusing from blood to the caecum and colon.

Bach Knudsen et al (1988a) and Bach Knudsen et al (1988b) who evaluated stiff porridges, tuwo and ugali, made from tannin and non-tannin sorghum, respectively, using rat balance studies attributed the lower digestibility and high biological value to the resistant starch-kafirin complex formed due to thermal processing, that passes through the small intestine without being hydrolyzed by endogenous enzymes. It is fermented by microorganisms in the hindgut which utilize energy from resistant starch and nitrogen from kafirin. These workers proposed that low true sorghum protein digestibility in man may be caused by this phenomenon.

4.3.3.3 Protein Digestibility Corrected Amino Acid Score

Table 4.3.6 shows the quantities of the indispensable amino acids in the casein and experimental food products relative to the WHO (2007) reference patterns for 3 to 10 and 1 to 2 year old children, and the National Research Council (1995) reference pattern for growing rats. The PDCAAS index reflects the estimated ability for the food product to meet the protein needs of an individual. The 3 to 10 year old amino acid scoring pattern is recommended by WHO (2007) for judging protein quality for school children and adolescents.

The casein diet had an amino acid pattern that is considered adequate for both preschool and school children. Fortification of sorghum with 50% defatted soy flour improved the PDCAAS by 248% compared to the unfortified biscuit and the most limiting indispensable

Table 4.3.6 Comparison of amino acid composition mg/g protein of diet protein sources with WHO requirement pattern for preschool and school children and NRC recommended pattern for rats

| | Diet protein sources | | | | | |
Amino acid	Casein[1]	Sorghum: soy	Sorghum	WHO[2]	WHO[3]	NRC[4]
Lysine	73	45	14	48	52	61
Leucine	92	83	127	61	63	71
Phenylalanine + tyrosine	102	83	84	41	46	68
Valine	64	48	47	40	42	49
Tryptophan	16	40	12	6.6	7.4	13
Methionine + Cysteine	33	58	28	24	26	65
Threonine	39	28	21	25	27	41
Histidine	33	24	19	16	18	19
Isoleucine	50	45	38	31	31	41
Total	502	415	380	293	312	430
True protein digestibility %	94.75	91.36	87.75			
Lysine score (3-10 yrs)	1.52	0.95	0.29			
PDCAAS[5] (3-10 yrs)	1.0	0.87	0.26			
Limiting AA (3-10 yrs)	NIL	Lysine	Lysine			
Lysine score (1-2 yrs)	1.40	0.87	0.26			
PDCAAS (1-2 yrs)	1.0	0.80	0.24			
Limiting AA (1-2 yrs)	NIL	Lysine	Lysine			
Lysine score (rat)	1.20	0.73	0.23			
PDCAAS (rat)	(1.0)	0.67	0.20			
Limiting AA (rat)	M+C[6]	Threonine	Lysine			

[1]Casein amino acid profile as determined for calcium caseinate of New Zealand Milk Board (Rutherfurd and Moughan 1998).
[2]Amino acid reference pattern for children aged 3 to 10 years (WHO 2007).
[3]Amino acid reference pattern for children aged 1to 2 years (WHO 2007).
[4]National Research Council (NRC) amino acid reference pattern for rats (NRC 1995).
[5]Protein digestibility-corrected amino acid score (PDCAAS) based on lysine score even when limiting amino acid is different.
[6]M+C= Methionine +Cysteine.

amino acid was lysine for both pre-school and school children. However, the PDCAAS for the sorghum-soy diet was higher than the minimum score of 70% of casein as recommended by FAO/WHO (1994) and (FAO/WHO 2009) Codex Alimentarius Committees. The 100% sorghum biscuit diet was deficient of the indispensable amino acid lysine, required for school and pre-school age children, and the PDCAAS was 161% and 192% lower than the recommended minimum for school and preschool children, respectively (FAO/WHO 1994, WHO 2007).

Several researchers have conducted similar studies on supplementary foods and used the PDCAAS index to provide information about the complementation potential of processed plant food protein sources. For instance, Kannan, Nielsen and Mason (2001) reported a 34% increase in PDCAAS of a rice-bean infant weaning food compared to beans alone. Similarly, Mensa-Wilmot et al (2001) reported acceptable PDCAAS values ranging between 0.72 and 0.82 for extruded cowpea-soy-maize foods for 2 to 5 year olds. Values ranging from 0.77 to 0.90 were reported by Mosha and Bennink (2004) for varying composites of rice, maize, bean and sardine meals for preschool children.

According to the National Research Council (1995), growing rats have much higher requirements for lysine, sulphur amino acids and all other indispensable amino acids than school and preschool children (Table 4.3.6). Although the casein diet fully provided the lysine requirements of the rat which may explain the high growth pattern of the casein fed rats compared to the 100% sorghum biscuit diets, it was probably limiting in the sulphur amino acids shown by the low PDCAAS of 0.47 based on the most limiting amino acids. Rats require greater amounts of sulphured amino acids to sustain hair growth (Augustin and Munoz 2006). The results also show that the indispensable amino acid pattern for the sorghum-soy biscuit diet was utilized as well as the casein pattern in synthesis of body proteins hence the comparable growth rate to casein. The sorghum-soy biscuit diet had a value of 0.67 essentially meeting the minimum 0.70 recommended by FAO/WHO (1994). The results in this study imply that since the amino acid requirements of the rat are much higher than those of children, growth patterns observed in this study would probably be higher in preschool and school children if they consumed the fortified sorghum biscuit. Mosha and Bennink (2004) also suggested that a protein source that supports modest growth in rats would support optimal growth in children.

4.3.4 CONCLUSIONS

Compositing sorghum with 50% defatted soy flour produces a protein that is similar to animal protein in PER and has the potential for use in a protein supplement food for school age children. It appears that the fortified sorghum biscuits could support growth in rats and possibly children. The rat is a poor model for determination of sorghum protein digestibility because it digests sorghum proteins very efficiently.

4.3.5 ACKNOWLEDGEMENTS

4.3.5 REFERENCES

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5   GENERAL DISCUSSION

The discussion first critiques the methodologies as applied in this study and suggests ways of future improvement. It then discusses the main findings of this investigation with reference to the effect of fortifying sorghum and bread wheat with defatted soy flour on protein quality, sensory characteristics and consumer acceptability of biscuits. Finally, it examines the possible integration of the fortified biscuits into school feeding programmes in Africa and gives recommendations for further research.

5.1   METHODOLOGIES

During formulation of the sorghum and bread wheat biscuits, the quantities of ingredients used for 225 g flour in all the treatments were kept constant except water, which is a possible limitation in this study. Preliminary test baking trials revealed that all nine treatments could not be prepared using the same amount of water, because the increase in DSF in the formulations made the dough dry, crumbly and difficult to manage, requiring more water to be workable. The high amount of water-soluble proteins, 70 to 80% in DSF (Senthil, Ravi, Bhat and Seethalakshmi 2002) and high hydrophilicity of soy proteins (Marcone and Kakuda 1999) presumably contributed to greater hydration capacity of DSF, compared to the sorghum kafirins which are hydrophobic (Duodu et al 2003) and water insoluble wheat gluten (Senthil et al 2002). Additionally, the sorghum flour required less water initially to form dough than the bread wheat flour which absorbed higher amounts of water likely due to damaged starch (Kent and Evers 1994). The optimum water contents for workable biscuit dough for 0, 28.6, 50 and 71.4% DSF substitution for sorghum and bread wheat was found to be 10, 16.2, 19.9 and 25.7% , and 14.3, 20.0, 21.6 and 25.7 %, respectively per 225 g flour blend formulation by measuring the added water. Consequently, the final dough weights for each treatment increased as the DSF increased and each treatment had a different dough weight (Chapter 4.1, Table 4.1.1).

For consumer evaluation, the 100% sorghum and 50% DSF substituted sorghum biscuits were prepared using the basic procedure described in Chapter 4.1. However, based on the sensory characterization results, the flour was milled to a finer 500 μm particle size to improve the coarse and gritty texture and rough appearance of the biscuits. The limitation

with the change in particle size is that the dough became difficult to manage due to the reasons explained above, requiring double the amount of water, 18.1 and 33.2% per 225 g flour formulation for sorghum and sorghum-soy, respectively to make it workable. The higher water requirement of the re-milled flour can be attributed to increased surface area exposing more flour particles for interaction with water and increased damaged starch that absorbs high amounts of water (Bushuk 1998). This is comparable to forming damaged starch when milling bread wheat flour to increase its water absorption properties.

Biscuit dough pieces from all the nine treatments were cut to the same volume using a 5 mm height steel tray and 6.3 cm diameter biscuit cutter. The major drawback with this approach is that after baking, the weights and heights of biscuits reduced with increasing substitution of DSF (Chapter 4.2, Table 4.2.2). This was due to reduction in total solids content in the dough pieces as the water increased in the dough piece with DSF addition. When water evaporated during baking, the biscuits with low dry matter were lower in weight and height. McWaters (1978) cut biscuits to 9 mm height and 3.8 cm diameter and reported lower height for wheat-soy composite biscuits with 1:1 flour:water compared to a 2:1ratio. Akubor and Ukwuru (2003) also reported a reduction in weight of cassava-soy composite flour biscuits as soy flour increased. If the dry matter content for all the dough pieces in all the treatments had been kept constant, all the biscuits would have baked to similar weights and heights. This could have been achieved by dividing all dough from each of the nine treatments to the same number of dough pieces of weight according to treatment. This approach was used by Hikeezi (1994) who divided each dough into 12 dough pieces of equal weight in a study on sorghum, peanut, and sunflower flour composite biscuits.

Differences in thickness, among the nine treatments of biscuits are a limitation because this may have introduced another source of variation to the descriptive sensory evaluation for crispness. The increase in crispness of biscuits with increase in soy as perceived by panelists may have been due to reduced thickness of biscuits as well. This is indicated by the fact that there was a significant panelist effect for this variable (Chapter 4.2, Table 4.2.5) and thickness was negatively correlated with protein content which increased when DSF was increased (Chapter 4.2, Figure 4.2.5). The effect of differences in thickness between treatments was eliminated for instrumental sensory evaluation values of biscuits because

method D 790-03 (ASTM International 2003), which was used to determine stress and strain, used formulae that took into account the thickness/height of each treatment.

Descriptive sensory evaluation was used for sensory characterization of the nine biscuits. According to Einstein (1991), descriptive sensory evaluation is the identification, description and quantification of the sensory attributes of food material or products using human subjects who have been specifically trained for this purpose. The panelists in this study clearly differentiated the sorghum from bread wheat biscuits and high legume from high cereal ones (Chapter 4.2, Figure 4.2.5). However, a possible criticism is that ANOVA showed that there were significant panelist effects for a number of attributes. Panelist effects in descriptive sensory studies are not uncommon. In this study, it could partly be a result of panelist fatigue due to the relatively high number of samples they had to evaluate, though first five and then four samples were assessed with a 20 minute break in between.

Another cause of the significant panelist effect for some attributes may have been psychological errors associated with human judgment (Stone and Sidel 2004) like the degree of understanding or perception of certain attributes, definitions and individual scaling behaviour. For example, the biscuits had extremes in colour from white to dark brown (Chapter 4.2, Figure 4.2.1) and errors could have been made in judging the extent of light or darkness. Differences among panelists due to routine use of either the upper or lower part of the scale as observed by Kobue-Lekalake (2008) in a study on the effect of sorghum phenolics on sensory properties could also have introduced significant panelist effects. To minimize such errors of judgment, panelists had access to reference samples throughout the evaluation period and were also provided with the list of attributes with definitions and scale anchors to refer to. An additional cause may be individual physiological differences between panelists. For example, Brown and Braxton (2000) found that the perception of texture and preference for rich tea biscuits was affected by differences in sequence and duration of chewing and salivary production among panelists. In the present study it is possible that panelists may have differed in the extent to which the food was masticated before swallowing with some swallowing larger particle sizes chewed in a shorter time.

The five point facial hedonic scale used to determine the children's liking of the biscuits in this study was considered appropriate because it has been successfully used in other studies

involving school children of the same age. For example, Delk and Vickers (2006) and Zandstra and de Graaf (1998) determined the liking for whole wheat bread and sensory perception of orange beverages, respectively using five point scales. It is also an approved ASTM International (2003) standard method E 2299-03 for sensory evaluation of products by children aged 8 to 9 years. However, the relative mean scale differences found among the biscuits for overall liking were slight. Though it is likely that this was the children's true perception of the biscuits, it is possible that a longer hedonic scale might have captured greater differences in liking. It has been suggested that longer scales could create confusion (Kroll 1990), but there is evidence that 7 and 9 point hedonic scales can be more discriminating and produce more reliable results when used for children. For example, Kroll (1990) showed that 8 to 10 year old children in the United States of America (USA) discriminated better using a 9 point than a 7 point facial scale and reducing the scale length did not offer any advantage.

During the orientation, the children were familiarized with the use of the scale and a demonstration was conducted on each of the four days before the study commenced. Words from the traditional 9 point hedonic scale (Peryam and Guidardot 1952) were used with the facial scale (Chapter 4.2, Figure 4.2.3). Kroll (1990) developed a scale for use in a study with words that children in the USA used such as super good, good and bad, which are equivalent to like extremely, like moderately and dislike in the traditional scale and got better responses. The children in the present study were taught both in English and their mother tongue, but it was observed that out of class they spoke their mother tongue. Though the children properly translated their feelings of the biscuits from the facial scale, the scale might have been more discriminative if child oriented mother tongue words had been used.

Repeated exposure was used to determine long-term acceptability of biscuits over four days in eight sessions of two sessions a day. Consumer exposure tests normally consist of repeated consumption of products over several days or weeks (Wiejzen et al 2008). Studies with children have shown that repeated exposure can increase liking of food products. For instance, Birch and Marlin (1982) demonstrated that preference for cheese or new fruit increased with repeated exposure. Liking can also be reduced as reported by Hetherington et al (2002) for chocolate after 22 days of exposure. A drawback in the present study, however,

is that 4 days may have been too short to determine long-term acceptability by repeated consumption. Results showed that though all biscuits were moderately liked, there was no change in liking over time (Chapter 2, Figure 4.2.6) indicating that four days may have been inadequate to elicit change in liking. A better approach may have been to have one day of two sessions a week for 4 weeks. For instance, Sulmonte-Rossé et al (2008) exposed study participants to drinks over 24 times in six sessions of 30 minutes each but the interval between sessions was one week. Another reason for the lack of change over time is that the protocol was too involved and may have exceeded the children's span of attention. According to ASTM International (2003), children of this age have a limited span of attention but have the capability to master complex tasks. It should, however, be appreciated that there are few documented studies that determine acceptability of food by children using repeated exposure and that the results in this study are similar to results from other studies using repeated exposure for staple foods, such as Hetherington et al (2002) for bread and butter and by Siegel and Pilgrim (1958) for dairy products and bread.

The sensory evaluation sessions were conducted in the children's classrooms, with two groups of 15 learners each seated in each class room. A possible limitation of conducting the study in a school classroom instead of individual cubicles is peer influence. Friendship among some children could have influenced the study results even though efforts were made to separate children who appeared to be friends. Birch (1980) showed that children could change their preference for food depending on what they see other children eat and the shift in change could be sustained weeks after, even in the absence of their peers. However, it unlikely that this had an influence on the final results because as explained earlier there was agreement among the children over the scores and results were consistent (Chapter 4.2, Figure 4.2.6).

School children were used in this study to determine consumer acceptability of fortified biscuits as they are the target population. Eight to nine year old children were selected because they are considered semi-literate, most can read at this level, self administer hedonic scale questionnaires and are more discriminating than younger children (Kroll 1990). Additionally, as stated earlier, previous studies showed that children in this age group were consistent when using hedonic categorization (Leon et al 1999). However, the weakness of using this age range is that there is tremendous variation in skills among children of the same

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age range (Conlin, Gathercole and Adams 2005). It has been shown that the age at which 10% of children can master a task compared to 90% of children doing the same task can vary by as much as four years (Popper and Kroll 2003). The study treated all children as equally intelligent, a factor that may have affected responses given by the slow learners.

The rat bioassay method was used to determine protein digestibility and effect on growth of soy fortified sorghum biscuits. According to FAO/WHO (1991) and WHO (2007), rats are the standard animal model for predicting protein digestibility for humans. Although the PDCAAS has officially replaced the PER as a measure for protein quality, the Faecal Index method in which the nitrogen voided is subtracted from the amount ingested using a rat model was necessary to determine the true protein digestibility which was required to compute the PDCAAS.

When the animals arrived, they were fed on a laboratory (commercial) diet for seven days but when they were given the experimental diet, they lost weight very rapidly and had to be rehabilitated. The weight loss problem may have been caused by the low protein content (8%) of the experimental diet. According to National Research Council (1995), rats need approximately 15% dietary protein for growth of approximately 5 g a day. The laboratory (commercial) diet of 18% protein made some rats gain up to 6 to 10 g a day. The experimental diet had only 8% protein and animals initially lost weight before they adapted to the low protein diet and started gaining weight again. According to National Research Council (1995) animals will first experience rapid weight loss before adapting to a less nutritious diet. Additionally, it was realized that the hopper in the metabolic unit was too deep for the rats to reach food when it was little. Thereafter, the hoppers were filled with food and the food consumed was calculated from the food supplied minus the weight of uneaten food. The acclimatization period could have been reduced to 3 days to limit prolonged effects of the laboratory (commercial) diet.

When the study was restarted, the animals on the protein-free diet again lost weight very rapidly and had to be euthanized after 10 days. Their weight loss was higher than loss from the animals fed the sorghum-soy and casein diets. Therefore NPR values were negative for all the diets. Rats require at least 5% protein in their diet for maintenance (National Research Council 1995). Addition of a low level of protein in the protein-free diet may have reduced

their rate of weight loss. For instance, Mosha and Bennink (2004) instead of using a protein-free diet used a low-protein diet with 20 g lactalbumin per kg diet to estimate the endogenous nitrogen excretion of rats.

A major drawback in the study was that the casein diet fed rats showed reduced food intake from the 14th day of the study and started losing weight. This trend continued until the end of the study. A possible cause of this could have been a deficiency of a nutrient, an imbalance of amino acids or toxic proportions of a specific nutrient. It has been found that the effect of imbalances can be considerable in diets that contain sub-optimal concentrations of protein and the immediate response is decreased food intake (Harper 1974). As noted, the animals in this study were already on a low protein diet of 8% relative to their growth requirements of 15%. The reduced food intake and unexpected weight loss might have been prevented by analyzing for the amino acids, minerals as well as proximate composition of all the diets after preparation. Although this is not normally done in most studies, possibly because it is costly, Babji and Letchumanan (1988) carried out analysis of rat diets of soy-beef hamburgers to ensure the nutrients were in the right proportions.

Another limitation that may have affected the results is the age of the rats. According to AOAC International (2000) Method 960-48, rats that are less than 28 days old should be used. Because of their weight loss and rehabilitation period, the study commenced when rats were 8 weeks old. PER was computed from 9 week old rats and data showing growth rate was from 8 to 12 weeks. A study conducted by Bender, Mahammadiha and Kauser Almas (1978) showed that nitrogen digestibility of cooked haricot beans with 5, 10 and 20% protein was 80, 74 and 67% , respectively in 23 day old rats, but reduced to 63, 55 and 51% , respectively in 63 day old rats. Another study by Gilani and Sepehr (2003) also found that protein digestibility of 20 week old rats was 7 to 17% lower than that of 5 week old rats and that the differences were higher when there were toxic factors. The age of the rats may also have compounded the weight loss of the casein-diet rats.

An important criticism with respect to determination of protein digestibility as done in this investigation may be possible microbial modification of undigested and absorbed nitrogenous residues in the rat large intestine. The Faecal Index method only accounts for nitrogen consumed and voided in faeces but not the modifying effect of microbes in the hind gut of the

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animal (Zhang, Qiao, Chen, Wang, Xing and Yin 2005). It has been demonstrated that the pattern of nitrogen excretion is modified by microflora in the hindgut for foods that have uncooked starch and undigested proteins (Beames and Eggum 1981) and sorghum foods because of the starch-kafirin complex (Bach Knudsen et al 1988a, Bach Knudsen et al (1988b). In this study it was clearly shown that the pure sorghum biscuit diet that could not support growth had a very high Biological Value (Chapter 4.3, Table 4.3.5) and Net Protein Utilization compared to the casein and sorghum-soy biscuit diets because no nitrogen was excreted in the urine. A possible approach would have been to analyze digesta collected at the end of the small intestine (terminal ileum) to increase sensitivity of the digestibility assay. Rutherfurd and Moughan (1998) used this method to determine digestibility of protein from milk and soy products by sampling digesta of Sprague-Dowley male rats at the end of the small intestine.

Generally, digestibility of protein was very high for all diets. This appears to be a weakness of using a rat model specifically for evaluating sorghum proteins because rats have been found to be very efficient in their digestibility of sorghum proteins (Axtell et al 1981, Bach Knudsen et al 1988a). However, generally the ability of rats and humans to digest a variety of foods are similar (FAO/WHO 1991). The pig is commonly used as a model animal for studying human nutrition (Rowan, Moughan, Wilson, Maher and Tasman-Jones 1994) and to address the problem, could have been used instead of the rat bioassay to determine the protein digestibility of the sorghum biscuits. Mitaru, Reichert and Blair (1984) investigated the nature of protein binding by sorghum tannins during digestion using a pig model and found that tannin-associated proteins were more hydrophobic than dietary protein.

The PDCAAS method used to determine protein quality is considered a good approximation of the bioavailability of amino acids of mixed diets and properly processed foods that contain minimal amounts of anti-nutritional factors (WHO 2007). The protein digestibility value of 95% obtained for the casein reference diet is similar to those from the studies by Joseph and Swanson (1993) and Mensa-Wilmot et al (2001) who obtained 94% and 96%, respectively. However, the high protein digestibility values obtained in this rat study limit the PDCAAS indices from the sorghum diets because they are not a true reflection of human sorghum protein digestibility. As stated, using an animal such as a pig may have provided digestibility

values that could be used to determine the PDCAAS for sorghum and soy fortified sorghum biscuits.

5.2 RESEARCH FINDINGS

As stated (Chapter 4.1, Table 4.1.2) this study shows that the overall nutritional value of sorghum and bread wheat biscuits was improved by compositing with DSF at different levels. This is reflected in the higher mineral (ash), fibre, protein and amino acids content for all fortified biscuits compared to the 100% cereal biscuits. The improvement can be attributed to the better nutrient composition of soy beans with respect to protein quality (USDA 2008). Additionally, the removal of fat to approximately 1% when DSF is processed (Lusas and Riaz 1995) further concentrated the nutrients. Making biscuits concentrated the solids content and increased overall nutrient density. High dietary bulk caused by high water content in foods such as porridge reduces the protein and energy intake by young children (Ljungqvist et al 1981) and contributes to PEM.

The findings of this study (Chapter 4.1, Tables. 4.1.3, 4.1.4 and 4.1.5) indicate that the protein quality of soy fortified biscuits increased substantially compared to the 100% cereal biscuits. The adequacy of protein from a dietary source is judged by the pattern of amino acids in relation to body requirements, the quantity of food and its protein density and digestibility that avails the food for utilization (Millward and Jackson 2004). Of great importance is the fact that, optimal utilization of protein is only possible when dietary energy intakes satisfy energy needs (WHO 2007). The improved protein quality is a result of complementing soy globulins with superior indispensable amino acid profile (USDA 2008), which exceed the amino acid requirements of children (Chapter 4.1, Table 4.1.6) and which are more digestible than kafirin proteins. This is indicated by increased lysine and other indispensable amino acids, reactive lysine and in vitro protein digestibility. All other parameters that measure protein quality including Protein/Energy Ratio that measures protein density, Protein Digestibility Corrected Amino Acid Score, and Essential Amino Acid Index, were within the minimum recommended values of the Codex Alimentarius Committee (FAO/WHO 1994, FAO/WHO 2009), as shown in Table 5.1. Additionally, the predicted amount of available protein in relation to the energy content of biscuits increased as shown by the PDCAAS-adjusted Protein/Energy ratio.

Table 5.1 Protein quality and energy parameters for soy fortified sorghum and bread wheat biscuits compared to FAO/WHO (1994) recommendations

Flour / Biscuits	Energy (kJ/g 100 g)	Protein (N x 6.25)	P/E Ratio[1]	Lysine[2] score	IVPD	PDCAAS[3]	PDCAAS-adjusted P/E ratio[4]	EAAI	No of biscuits for 14 g protein
Flour									
Sorghum flour	1504	11.4	12.9	0.42	56.0	0.24	0.03	1.37	
Wheat flour	1520	13.4	15.0	0.52	97.0	0.50	0.08	1.25	
Soy flour	1372	50.1	62.1	1.73	98.0	1.00	0.62	2.70	
Sorghum /Soy biscuit									
100:0	2013	9.2	7.8	0.29	30.0	0.09	0.01	1.32	6
71.4: 28.6	1943	17.9	15.7	0.82	74.0	0.61	0.10	1.76	3
50:50	1924	24.7	21.8	0.95	81.0	0.77	0.17	2.01	2
28.6:71.4	1873	30.7	27.9	1.00	87.0	0.87	0.24	2.24	2
Wheat/ Soy biscuits									
100:0	1980	10.8	9.3	0.39	97.0	0.38	0.04	1.17	5
71.4: 28.6	1910	19.5	17.4	0.88	96.0	0.84	0.15	1.71	3
50:50	1880	25.8	23.3	0.98	94.0	0.92	0.21	2.01	2
28.6:71.4	1859	31.9	29.2	1.03	91.0	0.94	0.27	2.28	2
Soy biscuit 100%	1842	39.9	36.8	1.07	88.0	0.94	0.35	2.62	2
FAO/WHO (1994) Recommendations	≥1674		≥15	≥0.65		≥0.70		≥1	

[1]Protein /Energy ratio = (protein g/100 g x 17 kJ)/energy kJ/g 100 g x 100; [2]Lysine score = mg lysine in 1 g protein of test sample/ mg lysine in requirement pattern (WHO 2007) for children 3-10 year; Protein Digestibility Corrected Amino Acid Score (PDCAAS) = Lysine x in vitro protein digestibility (IVPD); [4]PDCAAS adjusted P/E ratio = PDCAAS x P/E ratio.

[5]Essential amino acids index (EAAI) = $\sqrt[n]{\dfrac{\text{Product of the ratio of each EAA in the test food to the EAA of 3 to 10 year old children in reference pattern.}}{}}$

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The results in this study established from the rat bioassay that 100% sorghum biscuits had zero PER, hence cannot support growth of rats and by extrapolation humans. It was also found that the effect of complementing sorghum with soy protein on growth is the same as the casein reference (Chapter 4.3, Table 4.3.3). The reason that sorghum protein did not support growth is the deficiency in lysine, which as explained earlier is a characteristic of all cereals (review, Chapter 2, Section 2.1.2.3). This was indicated by the low Relative Protein Efficiency Ratio value of 5% and 100% for sorghum and sorghum-soy diets, respectively. Waggle et al (1966) found that the deficiency of lysine in a high protein sorghum grain (11.8%) resulted in lower growth of rats than a low protein sorghum grain (7.9%) with higher lysine content. This indicates that deficiency of one essential amino acid is enough to cause the failure of an entire diet. This suggests that in developing countries where a single cereal staple can contribute 70 to 90% of total dietary protein (Lasztity 1984), incidences of PEM could be high. The ability of the sorghum-soy biscuits to support growth was due to increased lysine content in the biscuits (Chapter 4.1, Table 4.1.3). This indicates that the potential to enhance growth by sorghum and other cereals can be achieved through complementation with soy and other legumes.

The results from the rat bioassay also indicate that true protein digestibility of sorghum and sorghum-soy biscuits were similar (Chapter 3, Table 4.3.5). The sorghum and sorghum-soy biscuits had true digestibilities of 82% and 85%, respectively. The reason for this is that as stated, the rat is very efficient in digesting sorghum proteins (Axtell et al 1981) and is likely to have overestimated the true protein digestibility of sorghum containing biscuits. In vitro protein digestibility results (Chapter 1, Table 4.1.5) show that sorghum and sorghum-soy biscuits had digestibility of 30% and 81%, respectively. The pepsin digestion method used in this study reportedly simulates the digestion values found in children for sorghum, wheat, maize, rice and pearl millet (Mertz et al 1984). Additionally, the few clinical studies carried out show that apparent protein digestibility of sorghum ranges from 46 to 69% (MacLean et al 1981, Kavithaparna et al 1988, Kurien et al 1960). Therefore, true digestibility values determined by the rat bioassay and any values derived from them such as the PDCAAS are limited because the rat is very efficient in digesting sorghum proteins.

The major reason for reduced protein digestibility of cooked sorghum foods is disulphide mediated polymerisation of sorghum proteins, making them less susceptible to enzymatic

attack (Hamaker et al 1987, Duodu et al 2003). This indicates that compositing sorghum with DSF is advantageous because higher digestibility of the proteins in the biscuits means the children can ingest higher amounts of high quality proteins with improved amino acid profiles and higher lysine and reactive lysine contents (Chapter 4.1, Tables 4.1.4 and 4.1.6).

The findings in this study indicate that losses of reactive lysine were enhanced by addition of DSF because the increase of available lysine content in the composite biscuits was not proportional to the increase of lysine and protein (Chapter 4.1, Table 4.1.4). The reason for this is that lysine is the most chemically reactive amino acid because of its ε-amino group and the increase in protein level by addition of DSF could influence the rate of the Maillard reaction either by hydrolysis or deamination of bound amino acids (Pozo-Bayon, Guchard and Cayot 2006). A condensation reaction between the carbonyl group of a reducing sugar and the ε-amino group of lysine form a schiff's base which undergoes irreversible rearrangement to produce the Amadori product, ε-N-deoxyketosyllysine, that is biologically unavailable (Rutherfurd and Moughan 2007, Hurrell and Carpenter 1981). The rather severe heat treatments during baking and low moisture content of biscuits enhances the Maillard reaction (Ait-Ameur et al 2008) that may have exacerbated the loss of lysine.

The findings in this study also indicate that the Maillard reaction enhanced the colour, flavour and aroma characteristics of sorghum and bread wheat biscuits. This is suggested by the positive correlation between protein content and colour intensity and roasted flavour (Chapter 2, Figure 4.2.5). Colour development occurs during the final stage of the Maillard reaction and involves the conversion of carbonyl compounds which may be furfurals, dehydroreductones or aldehydes to high molecular weight brown nitrogenous polymers called melanoidins (Nursten 1981). For the flavour and aroma characteristics, volatile compounds from soy products are related to different chemical classes that include Strecker aldehydes, diketones, pyrazines, furans, pyrolles, lactones, pyranone, fatty acids alcohols and esters (Mohsen et al 2009).

Compositing with defatted soy flour at levels above 50% resulted in a beany flavour of the biscuits (Chapter 4.2, Table 4.2.5). The cause for beany flavour from soy products is due to autoxidation of linolenic acid to the *cis* and *trans* 2-(1-pentenyl) furan (Chang 1979). It should be noted that in studies where soy flour is used (McWaters 1978, Mashayekh et al

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2008, Mohsen et al 2009) food products were only acceptable at levels below 30%. Above this level, consumers reported an objectionable beany flavour. In this study biscuits with 50% DSF were acceptable to school age children. A reason for this is that during the baking process, it is likely that furans were released as volatiles which reduced the beany flavour. Mohsen et al (2009) identified two furans, 2-ethyl-5-methylfuran and 2-pentylfuran which are lipid derived volatile compounds from soy fortified wheat cookies. Another possible explanation is that flavouring with vanilla essence helped to mask the beany flavour so that a higher amount of DSF could be added to biscuits. Acceptability studies by Marrero, Payumo Aguinaldo and Homma (1988) using mung beans and cowpeas reported that consumers preferred gruels flavoured with fruit essence, vanilla, chocolate and ginger. Of importance to this study, is that it was possible to increase the protein density of biscuits by substituting 50% cereal flour with DSF without them being objectionable to school children.

The results in this study suggest that the functional properties of soy protein influenced biscuit geometry and instrumental texture characteristics of sorghum biscuits and bread wheat biscuits. The weight, width, and height of sorghum-soy biscuits and weight and height of bread wheat biscuits reduced as DSF was increased in the formulae (Chapter 4.2, Table 4.2.2). This can be explained by the biscuit dough that had high DSF which absorbed high amounts of water due to reasons explained in section 5.1. Therefore, the dough pieces had a higher amount of water and less solid matter compared to those with less DSF. Consequently, the biscuits baked to reduced weight, width and thickness/height when water evaporated. Soy protein has the ability to form protein-protein interactions when heated leading to aggregation (Marcone and Kakuda 1999) which could have increased the hardness of sorghum biscuits indicated by increase in stress (Chapter 4.2, Table 4.2.3). Therefore, the level of fragility such as that reported in a study by Chiremba et al (2009) in which sorghum biscuits were difficult to handle by consumers because they were too crumbly was not observed in this study.

For the bread wheat biscuits there was increased percentage stress (hardness) and reduced percentage strain (more brittle) as DSF increased (Chapter 4.2, Table 4.2.3 and 4.2.4). Evaporation of water in the high soy biscuits resulted in thinner biscuits that were more brittle. Another reason for increase in brittleness is weakening of the gluten network by replacement with soy protein as explained earlier (Chapter 4.2, section 4.2.3.2).

The findings from consumer acceptability of the fortified sorghum and bread wheat biscuits showed that biscuits were liked by school children and liking was sustained over 8 consumption occasions (Chapter 4.2, Table 4.2.7 and Figure 4.2.6). A reason for this is that biscuits are popular food products among children because they are sweet (Sudha et al 2007). Another reason is that as stated earlier (Chapter 4.2, section 4.2.3.5), soy protein imparted positive sensory characteristics associated with biscuits such as reduced hardness, density and chewiness and increased crispness in biscuits, which children could identify with from previous consumption of biscuits. A third reason is that biscuits were tested during morning break at 10.00 and the children were hungry and this made them like the biscuits.

5.3 INTEGRATING FORTIFIED SORGHUM AND BREAD WHEAT BISCUITS INTO SCHOOL FEEDING PROGRAMMES IN AFRICA

The consequences of PEM described earlier (Chapter 1) indicate that children need adequate protein and energy in their diet for optimal growth, cognitive development, and general well-being. School feeding programmes worldwide are designed to alleviate short term hunger, address nutrient deficiencies and provide incentives for children to attend school (Del Rosso 1999). School meals constitute breakfast or lunch in school, (with meals prepared in schools, the community or centralized kitchens), or high energy biscuits or snacks (World Food Programme 2009). School feeding programmes target children individually, or use schools as distribution points for all children enrolled in it. They can also reach children affected by HIV/AIDS, orphans and the disabled. Therefore, soy fortified sorghum and bread wheat biscuits are appropriate as protein rich supplements to prevent PEM in Africa and other developing countries through school feeding.

The most recent estimates by the Food and Agriculture Organization (2009) show that more than one billion people worldwide are undernourished, and most exist on starchy staples which are poor sources of protein. It was also suggested by FAO (2009) that school feeding programmes could be designed to stimulate local economies by increasing agriculture and local value added food production. Purchase of locally produced grain such as sorghum, bread wheat and soy beans for school meals could generate income and guarantee markets for small holder farmers. In Africa, the potential demand for school feeding is a total number of 114 million children who are enrolled in primary school (2007). Of these, 70 million are

currently attending school in hunger stricken areas in sub-Saharan Africa (World Food Programme 2007). In the developing world, there are 66 million primary school age children who are undernourished and 23 million of these live in Africa (World Food Programme 2009).

In 2003, African governments endorsed the Home Grown School Feeding (HGSF) Programme of the Comprehensive Africa Development Programme (CAADP) in an effort to restore food security, adequate nutrition levels and rural development in Africa. The HGSF is a programme that offers foods produced and purchased within a country (World Food Programme 2009). Since then, the World Food Programme and other agencies have taken up this initiative to increase children's well-being and promote local agricultural production and development by providing an ongoing market for smallholder farmers particularly in rural areas of low agricultural productivity and high chronic malnutrition (World Food Programme 2009). The soy fortified sorghum and bread wheat biscuits could be integrated into the HGSF programme in countries where this initiative has been implemented such as Congo, Ethiopia, Ghana, Kenya, Malawi, Mali, Mozambique, Nigeria, Senegal, Uganda and Zambia. The World Food Programme and country governments could provide small grants and training to community-driven food security projects to develop the capacity to produce and market the biscuits. Schools would then be provided with grants to purchase the biscuits. Alternatively, where HGSF programmes have not been implemented, schools could make arrangements with community development women groups or street vendors to produce and supply the biscuits to schools as income generating projects. For example, in South Africa, women are encouraged to form small businesses that provide for school feeding programmes (Bundy, Burbano, Gosh, Gelli, Jukes, and Drake 2009).

A challenge to producing the sorghum and bread wheat biscuits by different groups of people is assuring that the minimum nutritional standards are maintained. For instance, using the right type of sorghum, (non-tannin sorghum) and compositing sorghum and bread wheat with soy in the right proportions and maintaining a constant supply of grain for biscuit production. A possible approach could be for specific millers to buy grain from farmers, prepare a pre-mix and supply it to the community bakeries with already trained personnel. A similar approach was used for a school feeding programme in Malawi, where a pilot project supports

five community bakeries to manufacture and deliver fortified scones to schools using a pre-mix that is delivered to them by the World Food Programme (World Food Programme 2009). Lack of basic infrastructure such as water, kitchens, storage facilities, cooking equipment and manpower does not facilitate the successful preparation and provision of meals to children in rural Africa. For instance, a school lunch programme in Kenya that provided maize and beans involved more than four hours of preparation time (UNESCO 2004). In South Africa, bread trucks could not reach some rural schools due to impassable roads during the rainy season and children got diarrhoea because unsafe water was used to reconstitute milk shake (Sizwe and Nikiwe 2010). High transportation costs were incurred because the foods had a short shelf-life. The advantage of a snack food, such as the fortified sorghum and bread wheat biscuits is that preparation time is eliminated, they are shelf stable so large amounts can be stored in the school and are unlikely to substitute family meals as the school meal should be an addition to the diet.

School meals or snacks usually provide one third to one half of the RDA for protein and energy for the targeted age group (UNESCO 2004). Based on WHO (2007), 3, 4 to 6 and 7 to 10 year olds require 13, 17 and 26 g protein per day, respectively. Therefore, acceptable ranges in the school feeding programme would be 4 to 9 g, 6 to 11 g and 9 to 17 g for 3, 4 to 6 and 7 to 9 year olds, respectively. For energy, based on FAO/WHO (1985), requirements would be 2370 kJ to 4742 kJ for 3 to 5 year olds and 2650 to 5300 kJ for 6 to 10 year olds. The general energy content of meals for school feeding for primary school children $\leq$ 12 years provided by the World Food Programme is 1883 to 3473 kJ for half day and 4,644 to 5803 kJ for full day (Bundy et al 2009).

Two soy fortified sorghum or bread wheat biscuits of 28 g each providing 14 g of protein per day are within the range for protein requirements for school children of 3 to 10 years. Two biscuits of 28 g would provide 1077 kJ and 1053 kJ from fortified sorghum and bread wheat biscuits, respectively. This translates into slightly below half the minimum energy requirements for 3 to 10 year olds. An assessment of nine school feeding studies in developing countries showed that the daily ration provided energy ranging from 815 kJ to 2500 kJ (Galloway, Kristjansson, Gelli, Meir, Espejo and Bundy 2009). This suggests that the fortified sorghum and bread wheat biscuits would make a substantial contribution to the protein and energy needs of 3 to 10 year old children's school feeding programmes.

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The typical nutritional composition of high-energy fortified biscuits offered by WFP for school feeding is 12 g protein and 1883 kJ energy per 100 g of biscuits (Bundy et al 2009) and weigh between 20 and 40 g (World Food Programme, 2000). Sorghum-soy and bread wheat-soy biscuits at 1;1 ratio of 100 g contain 25 g protein and 1924 kJ energy and 26 g protein and 1880 kJ energy, respectively (Chapter 4.1, Table 4.1.2). This indicates that compared to the World Food Programme biscuits, the fortified biscuits in the current study have double the protein content and similar energy content and could have a higher impact on alleviating PEM.

Table 5.2 shows the estimated costs of ingredients for soy fortified sorghum and bread wheat biscuits. A comparison of the costs shows that there would be no difference in the costs of ingredients for fortified sorghum and bread wheat biscuits. Comparison of the cost of cake flour normally used for making biscuits with bread flour used in this study shows that in some cases, cake flour may be cheaper than bread flour in South Africa. However, an investigation of retail prices of cake flour compared to bread flour show that in three African countries, Kenya, Zambia and Namibia, cake flour is more expensive than bread wheat flour (Personal communication). This suggests that use of bread flour in these countries will reduce the costs of production of biscuits. Additionally, value addition by substitution with DFS only increases the cost of production of the fortified biscuits by 8% compared to production of the 100% cereal biscuits. The cost of ingredients for production of 200 g fortified biscuits is approximately half the cost of low priced commercial biscuits, Marie and Rich Tea. This suggests that when labour, energy, equipment and their depreciation, packaging and transportation are included, chances are that the overall cost of the fortified biscuits may be almost double. However, the procedure for preparation is simple and the time shorter compared to baked products used for school feeding such as bread in South Africa (Sizwe and Nikiwe 2010) or scones in Malawi (World Food Programme 2009).

A study by Galloway et al (2009) on costs of school feeding in African countries Malawi, Kenya, Lesotho and Gambia reported that for a 200 day school year, the cost ranged from US$28 to US$63 per child per year. An estimation of the cost of feeding a child with two biscuits a year for the same length of time would probably be approximately US$40 if the

Table 5.2 Estimation of costs of soy fortified sorghum and bread wheat biscuits for 56 g ration/day/year and comparison to low priced commercial biscuits

Ingredients and cost	Sorghum-soy biscuits (355 g dm)	Bread wheat-soy biscuits (355 g dm)
Sorghum flour (112.5 g) @ R7.99/ kg	R 0.89	
Bread wheat flour (112.5 g) @ R8.99/kg		R 1.01
Defatted soy flour (112.5 g) @ R10.83/kg	R1.22	R1.22
Sugar (56 g) @ R8.99/kg	R 0.50	R 0.50
Sunflower oil (66 g) @ R12.20/kg	R 0.80	R 0.80
Vanilla essence (13. 5 g) @ R24.49/500 g	R 0.66	R 0.66
Baking powder: sorghum (1.5 g) @ R13.99/200 g	R 0.10	
Baking Powder: wheat (1 g) @ R13.99/200 g		R 0.06
Total cost	R 4.17	R 4.19
Cost including 14% VAT	R 4.75	R 4.78
[1]Estimated cost of biscuits including manufacturing cost (cost of ingredients x 2)	R 9.50	R 9.56
Cost of 2 biscuits 28 g each (56 g) child/day (ingredients only)	R 0.75	R 0.75
Cost of 2 biscuits 28 g each (56 g) child/day (ingredients + manufacturing cost)	R 1.50	R 1.50
Cost/child/200 day school year (ingredients only)	R[2]150 (US$20)	R150 (US$20)
Cost/child/200 day school year (ingredients + manufacturing cost)	R 300 (US$40)	R 300 (US$40)
Cost of 200 g biscuits (ingredients only)	R 2.68	R 2.69
Cost of 200 g biscuits (ingredients + manufacturing cost)	R 5.35	R 5.39
[3]Cost of 200 g Marie or Rich Tea biscuits (commercial)	R 5.99	
Cost /child/200 day school year (56 g – commercial/day)	R 335	

[1]Cost of manufacturing estimated as equivalent to cost of ingredients

[2]South African Rand (R), conversion of Rand to US$ based on 1US$ = 7.5R

[3]Low priced commercial biscuits on South African Market.

Prices of ingredients are retail prices from Pick and-Pay, a South African chain store.

cost of ingredients for biscuits was doubled to include the costs of production. Therefore, the cost of feeding children with the fortified biscuits would be comparable to the general costs incurred for school feeding programmes in Africa but with the added nutritional value as a protein-rich food supplement. According to the World Food Programme (2002) fortified biscuits provided to schools cost US$1250 per ton. The estimated cost of feeding a child on such biscuits per year is 13US$. However, most grains used by the World food Programme are donations hence the lower cost of production and as stated earlier the fortified biscuits have only half the protein content of biscuits in the current study.

6   CONCLUSIONS AND RECOMMENDATIONS

Complementing sorghum and bread wheat with defatted soy flour at different levels improves the nutrient density with respect to ash (minerals), fibre, protein and amino acid content and protein quality in terms of lysine and reactive lysine contents, amino acid profile and protein digestibility. The increase is due to the better nutrient composition of soy beans and soy globulins that have higher lysine content and are more soluble and digestible.

Biscuits made from soy fortified sorghum flour can support growth of rats and by extrapolation human children as effectively as the casein reference. However, the rat is not a good model for determining sorghum protein digestibility. This is because it is very efficient in its digestion of sorghum proteins.

Compositing with defatted soy flour imparts positive sensory characteristics associated with biscuits such as increased spread factor, crisp texture and roasted flavour and reduces hard dense and chewy texture. Soy fortified sorghum and bread wheat biscuits are liked by school children and the biscuits show promise of sustained consumption over a prolonged period of time.

This study established that soy fortified sorghum and bread wheat biscuits are easily prepared simple food products made from cereals that children are familiar with, have high protein quality and nutrient density, positive sensory characteristics associated with biscuits, are liked by school age children. Hence, the fortified biscuits have great potential to be used as a protein-rich supplementary food, to prevent Protein Energy Malnutrition among school children in rural Africa.

Further studies should be conducted to determine the True Protein Digestibility of sorghum biscuits using either a pig model or a clinical study because results obtained from the rat bioassay were too high and cannot be compared to sorghum protein digestibility in children. Further studies should be carried out to develop biscuits using composites of sorghum, bread wheat and other cereals such as maize, rice, millet and teff, with soy and indigenous African legumes and oil seeds such as marama bean, cowpea, bambara nut, cashew nuts and others. This will enable production of fortified biscuits for school feeding using cereals and

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legume/oilseeds that are locally available and sustainable within their ecological zone. Local purchase of such grains for school feeding will be a force multiplier, benefiting children by preventing PEM and uplifting rural economies by providing an income to smallholder farmers in low income communities in Africa through the Home Grown School Feeding Programme.

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8 APPENDIX

Publications and presentations from this work

Scientific Papers

Serrem, C. A., de Kock, H. L., Taylor, J. R. N. 2011. Nutritional quality, sensory quality and consumer acceptability of sorghum and bread wheat biscuits fortified with defatted soy flour. Int. J. Food Sci. Technol. 46:74-83.

Serrem, C. A., de Kock, H. L., Oelofse, A., Taylor, J. R. N. 2011. Rat bioassay of the protein nutritional quality of soy fortified sorghum biscuits for supplementary feeding of school-age children. In Press, J. Sci. Food Agric.

Conference posters

Serrem, C. A., de Kock, H. L., Oelofse, A., Taylor, J. R. N. 2010. The effect of compositing with soy on the sensory characteristic of sorghum biscuits. Cereal Science and Technology – South Africa (CST-SA) – International Association of Cereal Science and Technology (ICC) International Grains Symposium on Quality and Safety of Grain Crops and Foods. 3-5 February 2010, Pretoria, South Africa. Won 2nd Prize for student posters.

Also includes article:

Serrem, C. A., de Kock, H. L., Oelofse, A., Taylor, J. R. N. 2010. Effect of compositing with soy on the sensory characteristics of sorghum biscuits. Pages 263-267 In: Proceedings of the CST-SA-ICC International Grains Symposium on Quality and Safety of Grain Crops and Foods. M. Labuschagne, and K. G, Duodu, eds. 3-5 February 2010, Pretoria, South Africa. ISBN: 978 0 86886.

Serrem, C. A., de Kock, H. L., Oelofse, A., Taylor, J. R. N. 2010. Protein quality of soy fortified sorghum biscuits. 15th IUFOST World Congress of Food Science and Technology. 22-26th August 2010, Cape Town, South Africa.

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Workshop presentation

Serrem, C. A., demonstrated preparation procedure for soy fortified sorghum biscuits at a two day Practical Workshop on Small-scale Processing and Marketing of Oilseeds and Legumes, 25-26 February 2010**,** Pretoria, South Africa.