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,

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 $\pm$ 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 $\leq$ 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^{o}C for 20 $\pm$5 minutes. Remove from oven and allow to cool on baking tray for 30 minutes.

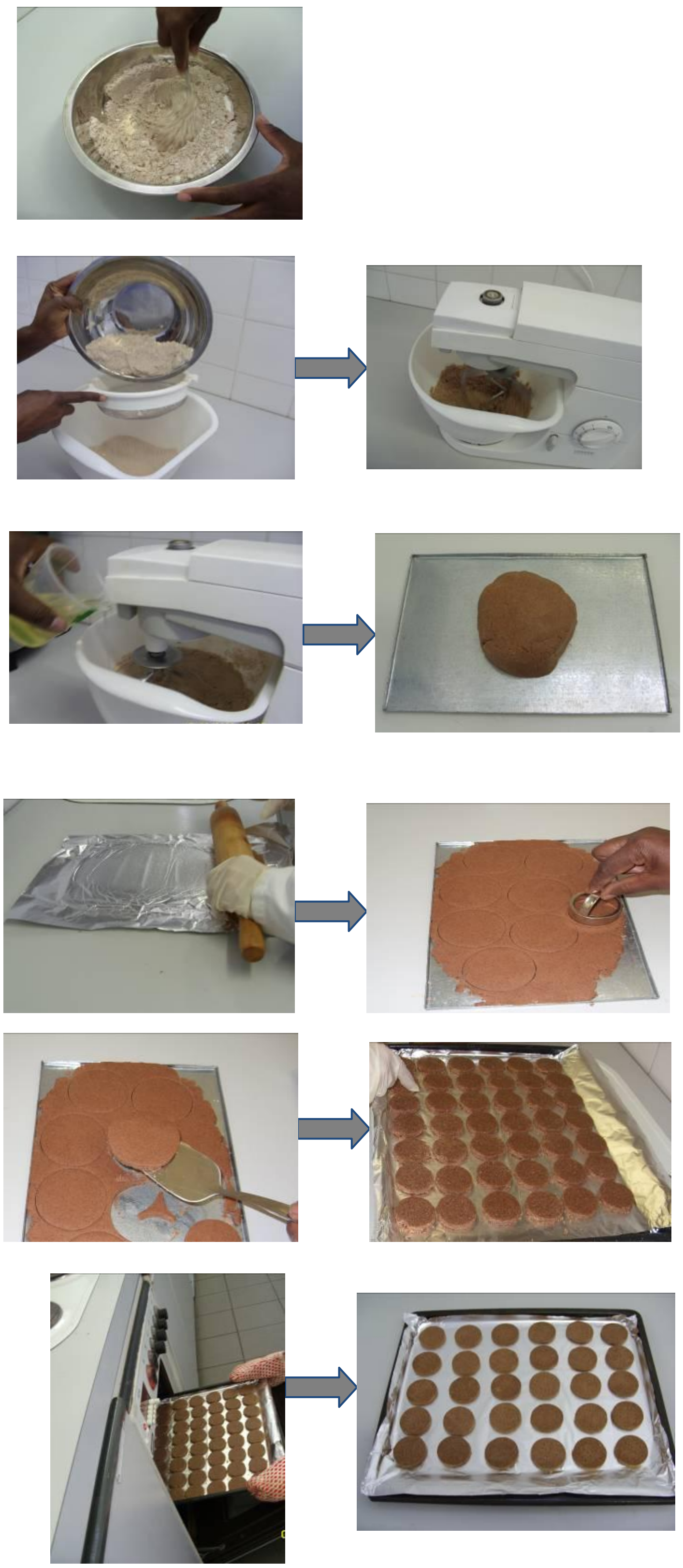

Figure 4.1.1 Flow diagram for preparation of soy fortified sorghum or bread wheat biscuits.

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^{o}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^{o}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	$11.8^{g}\pm0.3$	$11.4^{c}\pm0.1$	$3.1^{c}\pm0.6$	$1.4^{c}\pm0.0$	$2.0^{c}\pm0.0$	$70.3^{j}\pm0.2$	1504^{b}
Wheat flour	$11.3^{g}\pm0.2$	$13.4^{d}\pm0.0$	$1.4^{b}\pm0.1$	$0.7^{b}\pm0.0$	$0.2^{a}\pm0.0$	$73.2^{k}\pm0.1$	1520^{c}
Soy flour	$6.2^{de}\pm0.4$	$50.1^{l}\pm0.1$	$0.5^{a}\pm0.0$	$6.2^{k}\pm0.1$	$7.5^{j}\pm0.1$	$29.5^{b}\pm0.1$	1372^{a}
Sorghum /Soy biscuit							
100:0	$3.2^{a}\pm0.4$	$9.2^{a}\pm0.1$	$21.0^{e}\pm0.3$	$1.4^{c}\pm0.0$	$1.7^{b}\pm0.4$	$63.5^{i}\pm0.4$	2013^{j}
71.4: 28.6	$4.9^{bc}\pm0.2$	$17.9^{e}\pm0.4$	$20.5^{e}\pm0.1$	$2.1^{e}\pm0.1$	$2.8^{d}\pm0.1$	$51.8^{g}\pm0.4$	1943^{h}
50:50	$3.8^{ab}\pm0.6$	$24.7^{g}\pm0.4$	$19.7^{d}\pm0.6$	$2.8^{g}\pm0.1$	$3.7^{f}\pm0.2$	$45.6^{e}\pm0.3$	1924^{gh}
28.6:71.4	$4.9^{bc}\pm0.2$	$30.7^{i}\pm0.3$	$19.7^{d}\pm0.2$	$3.3^{i}\pm0.0$	$4.7^{h}\pm0.2$	$36.6^{c}\pm0.2$	1873^{ef}
Wheat/ Soy biscuits							
100:0	$7.2^{ef}\pm0.7$	$10.8^{b}\pm0.2$	$20.7^{e}\pm0.2$	$0.5^{a}\pm0.0$	$0.2^{a}\pm0.0$	$60.6^{h}\pm0.4$	1980^{i}
71.4: 28.6	$7.4^{f}\pm1.5$	$19.5^{f}\pm0.2$	$19.8^{d}\pm0.9$	$1.5^{d}\pm0.0$	$2.0^{c}\pm0.0$	$49.8^{f}\pm0.8$	1910^{g}
50:50	$6.4^{def}\pm0.4$	$25.8^{h}\pm0.4$	$19.3^{d}\pm0.5$	$2.4^{f}\pm0.0$	$3.3^{e}\pm0.1$	$42.8^{d}\pm0.5$	1880^{f}
28.6:71.4	$6.0^{cd}\pm0.4$	$31.9^{j}\pm0.2$	$19.4^{d}\pm0.1$	$3.1^{h}\pm0.0$	$4.4^{g}\pm0.2$	$35.2^{b}\pm0.3$	1859^{de}
Soy biscuit 100%	$4.6^{b}\pm1.2$	$39.9^{k}\pm0.3$	$19.3^{d}\pm0.5$	$4.2^{j}\pm0.1$	$5.5^{i}\pm0.0$	$26.5^{a}\pm0.4$	1842^{d}

Values are means ±standard deviations. Values in a column followed by different letter superscripts are significantly different at $P\leq0.05$ 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} ±0.05[1]	8.28^{g} ±0.39	1.37^{a} ±0.12	3.93^{c} ±0.38	4.54^{d} ±0.38	4.78^{d} ±0.26	5.13^{d} ±0.03
	(0.23^{b} ±0.01)	(4.15^{g} ±0.20)	(0.13^{ab} ±0.01)	(0.70^{c} ±0.06)	(1.12^{d} ±0.09)	(1.47^{e} ±0.07)	(2.05^{f} ±0.01)
Wheat: Soy	2.48^{b} ±0.00[2]	8.28^{f} ±0.39	1.86^{a} ±0.19	4.20^{c} ±0.17	4.69^{d} ±0.19	4.92^{de} ±0.09	5.13^{e} ±0.03
	(0.33^{b} ±0.00)	(4.15^{g} ±0.20)	(0.20^{ab} ±0.0)	(0.82^{c} ±0.03)	(1.21^{d} ±0.03)	(1.57^{e} ±0.04)	(2.05^{f} ±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} ±0.20[1]	3.68^{f} ±0.08	0.70^{a} ±0.40	2.10^{c} ±0.17	2.50^{cd} ±0.44	2.83^{de} ±0.07	3.01^{e} ±0.13
	(0.16^{a} ±0.02)	(1.85^{f} ±0.04)	(0.05^{a} ±0.03)	(0.30^{b} ±0.03)	(0.47^{c} ±0.09)	(0.72^{d} ±0.01)	(1.97^{e} ±0.03)
Wheat: Soy	1.89^{a} ± 0.17[2]	3.68^{d} ±0.08	2.25^{b} ±0.05	2.29^{b} ±0.18	2.33^{b} ±0.56	2.42^{b} ±0.15	3.01^{c} ±0.13
	(0.27^{ab} ±0.03)	(1.85^{f} ±0.04)	(0.19^{a} ±0.00)	(0.35^{b} ± 0.03)	(0.52^{c} ±0.11)	(0.63^{d} ±0.05)	(1.97^{e} ±0.03)

Values are mean ±SD. Values followed by different letter superscripts in a row are significantly different at $P \leq 0.05$ 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	$56.0^{b}\pm1.1^{1}$	$97.5^{f}\pm0.0$	$30.0^{a}\pm3.3$	$74.3^{c}\pm1.6$	$81.1^{d}\pm2.3$	$87.3^{e}\pm3.5$	$88.1^{e}\pm3.7$
Wheat: Soy	$97.3^{cd}\pm1.5^{2}$	$97.5^{d}\pm0.0$	$96.7^{cd}\pm1.5$	$95.5^{cd}\pm1.1$	$94.2^{bc}\pm0.5$	$91.4^{b}\pm2.5$	$88.1^{a}\pm3.7$

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^{o}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^o 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^{o}C 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.

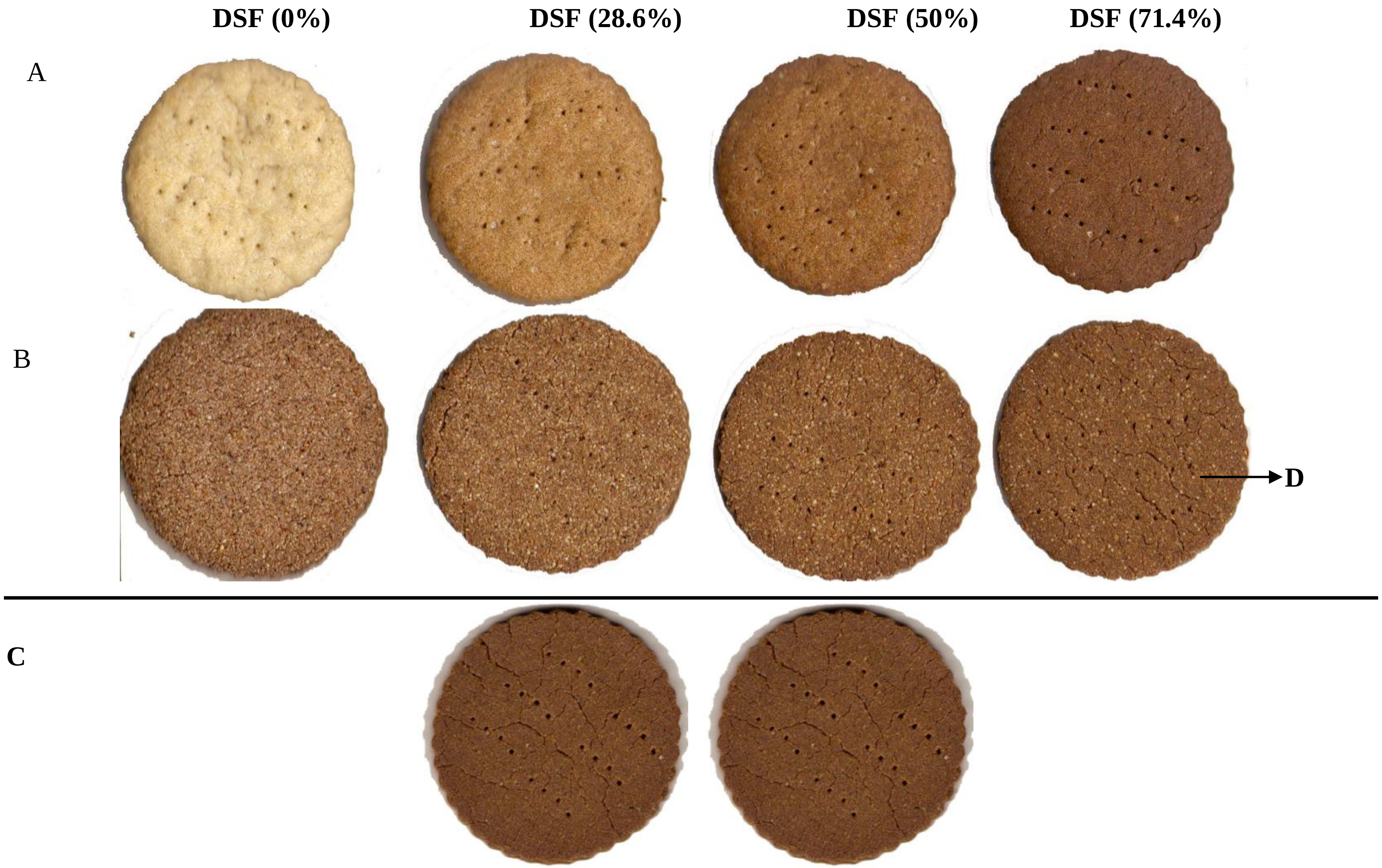


Figure 4.2.1 Soy fortified sorghum and bread wheat biscuits. DSF= Defatted Soy Flour; A= bread-wheat biscuits; B= sorghum biscuits; C= defatted soy flour 100% biscuits; D= visible white specks in sorghum biscuits.

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

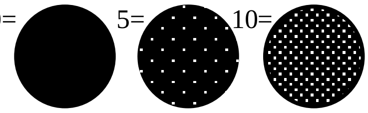
Sensory Attribute	Definitions	References to clarify and anchor sensory attributes	Rating scale
Appearance			
Surface colour intensity	Colour intensity ranging from light cream to dark brown	Mantelli shortbread (light) =0. Oreo chocolate biscuits (dark) = 10	Not dark = 0 Very dark = 10
Quantity of visible 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^{o}C 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^{o}C 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^{o}C) 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^{o}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^{o}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^{o}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^{o}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/cm^3)
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 $P \leq 0.05$ 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 $P \leq 0.05$ 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	$46.6^{e}\pm1.1$	$13.0^{c}\pm0.4$	$7.1^{bc}\pm0.9$	$14.8^{bc}\pm0.8$	$28.3^{b}\pm3.2$
71.4: 28.6	$45.4^{de}\pm0.8$	$12.8^{c}\pm0.1$	$8.6^{c}\pm0.5$	$15.5^{bc}\pm0.4$	$34.1^{d}\pm1.4$
50:50	$43.6^{cd}\pm0.4$	$13.1^{c}\pm0.2$	$8.7^{c}\pm0.2$	$15.8^{bc}\pm0.5$	$33.7^{cd}\pm0.6$
28.6:71.4	$40.8^{ab}\pm0.9$	$12.7^{bc}\pm0.3$	$6.6^{b}\pm0.8$	$14.3^{ab}\pm0.6$	$27.6^{b}\pm2.5$
Wheat/ Soy biscuits					
100:0	$69.0^{g}\pm1.2$	$6.9^{a}\pm0.6$	$21.0^{f}\pm1.7$	$22.1^{e}\pm1.6$	$71.7^{f}\pm2.0$
71.4: 28.6	$48.9^{f}\pm2.6$	$12.5^{bc}\pm0.5$	$13.4^{e}\pm1.7$	$18.4^{d}\pm1.5$	$46.8^{e}\pm3.2$
50:50	$44.2^{d}\pm0.2$	$14.2^{d}\pm0.2$	$10.7^{d}\pm0.8$	$17.8^{d}\pm0.6$	$37.1^{d}\pm1.8$
28.6:71.4	$41.7^{bc}\pm1.2$	$13.8^{d}\pm0.8$	$8.0^{bc}\pm1.0$	$15.9^{bc}\pm0.7$	$30.1^{bc}\pm2.6$
Soy biscuit 100%	$38.8^{a}\pm0.3$	$12.1^{b}\pm0.0$	$4.9^{a}\pm0.2$	$13.1^{a}\pm0.1$	$22.0^{a}\pm0.8$

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 $p \leq 0.05$ as assessed by Fisher's least significant test.
[1]Significant at *** $p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$, ns, not significant.

Table 4.2.5 Continued.

Attributes	Sorghum biscuits				Wheat biscuit				Soy biscuit	Sample effects	Panelists effects
	Soy level (%)				Soy level (%)						
	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 $p \leq 0.05$
[1]Significant at *** $p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$, ns, not significant.

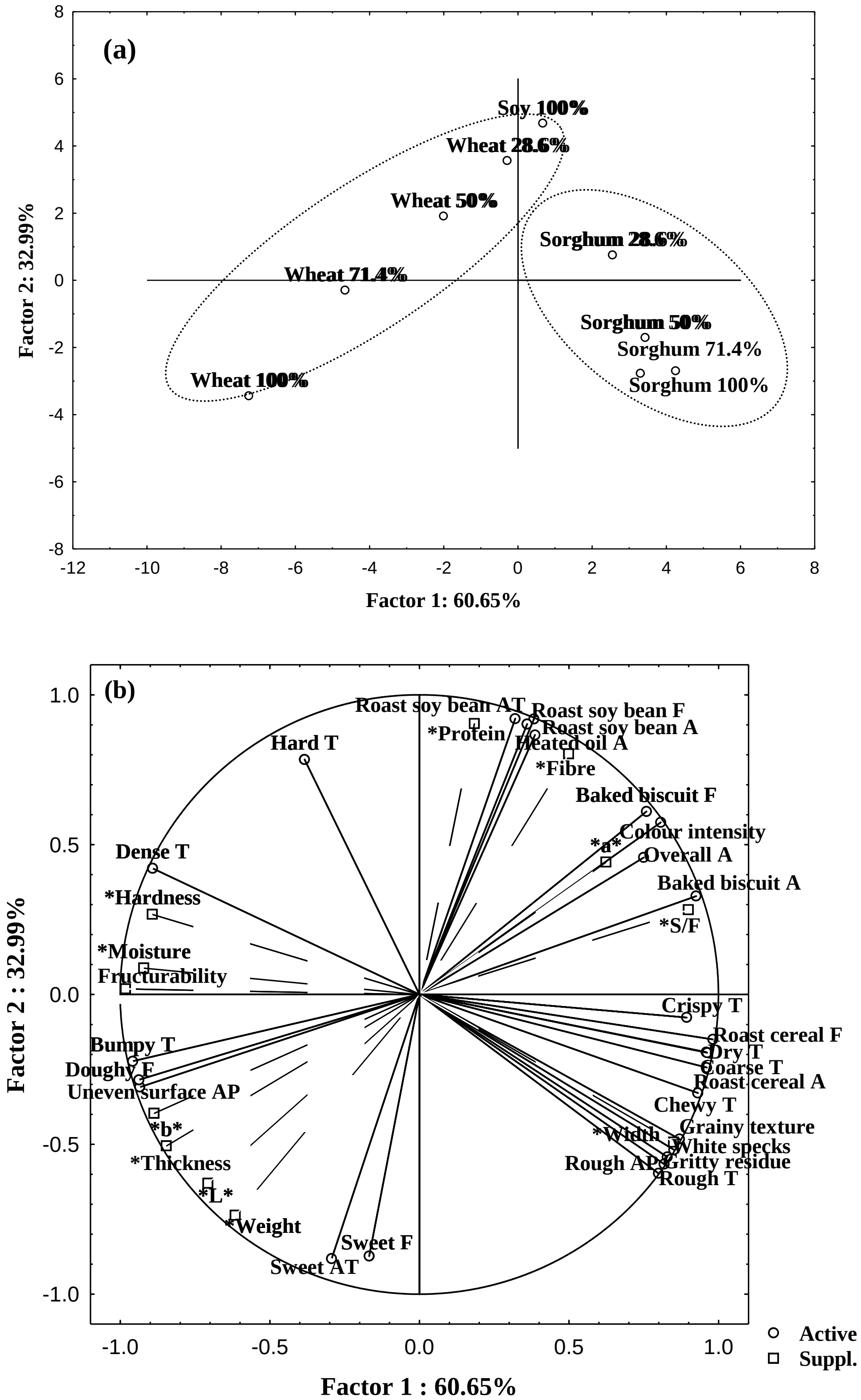


Figure 4.2.4 Principal component analysis (correlation matrix) of sorghum and bread wheat biscuits composited with soy at levels of 0, 28.6, 50, 71.4 and 100%. (a) Plot of the first two principal component scores of the sorghum and bread wheat biscuits, (b) Plot of the first two principal component loading 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.

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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

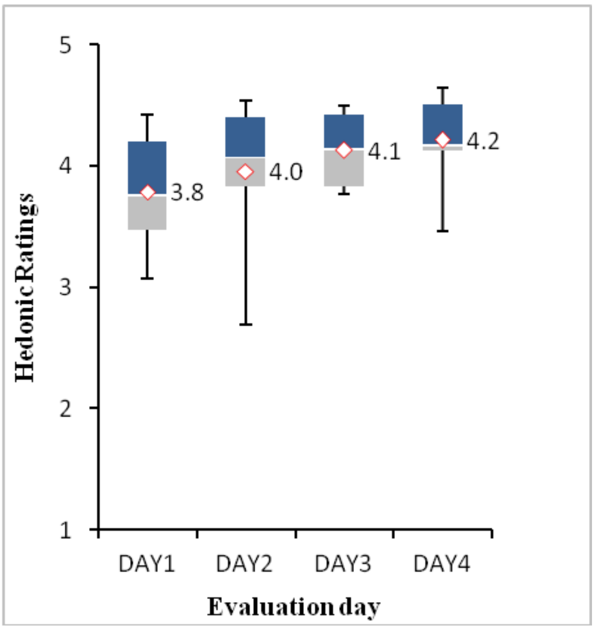
Source of variation	Sums of Squares (SS)	Degrees of freedom (d.f)	Mean squares (MS)	F	P
Group	53.71	3	17.90	0.99	ns*
Biscuit	16.09	3	5.36	3.35	<0.05
Biscuit/group	15.08	9	1.68	1.05	ns
Day	17.32	3	5.77	4.21	<0.01
Day/group	9.11	9	1.01	0.74	ns
Session	34.34	1	34.34	18.71	<0.001
Session/group	25.27	3	8.42	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.

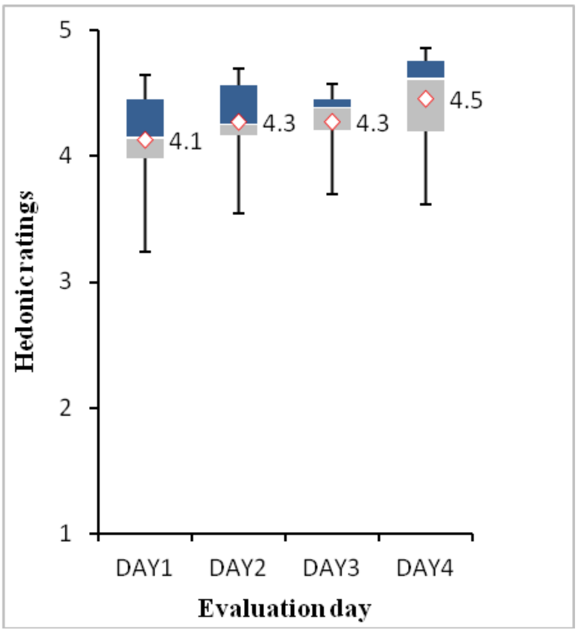
Table 4.2.7 The effect of compositing sorghum and bread wheat with DSF on overall liking of biscuits by 8 to 9 year old children

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)	$4.19^{ab} \pm 0.10$

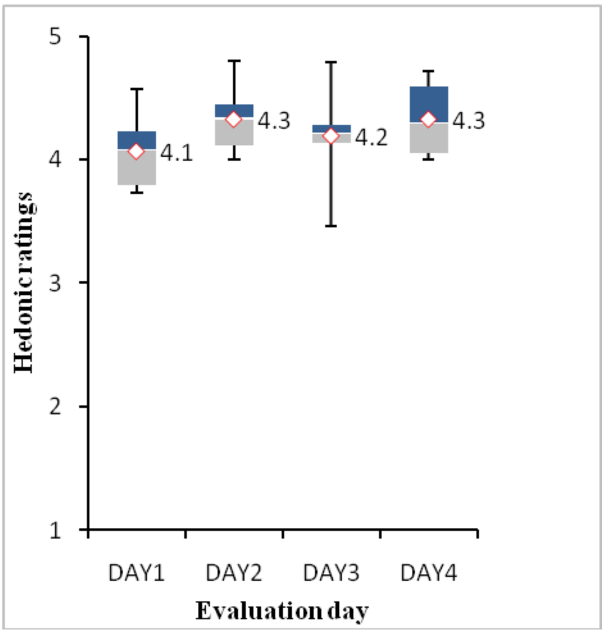
Values are mean±SD. Values followed by different letter superscripts in a column are significantly different at P≤0.05 as assessed by Fisher's least significant test. Overall liking ratings 1= dislike very much, 2= dislike a little, 3= not sure, 4= like a little, 5= like very much. Consumers n=60.



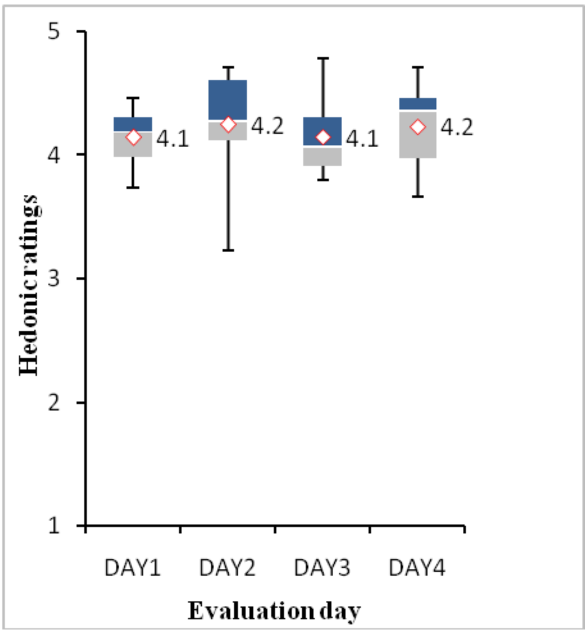
a)Sorghum: (100)



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 $p \leq 0.05$. 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).

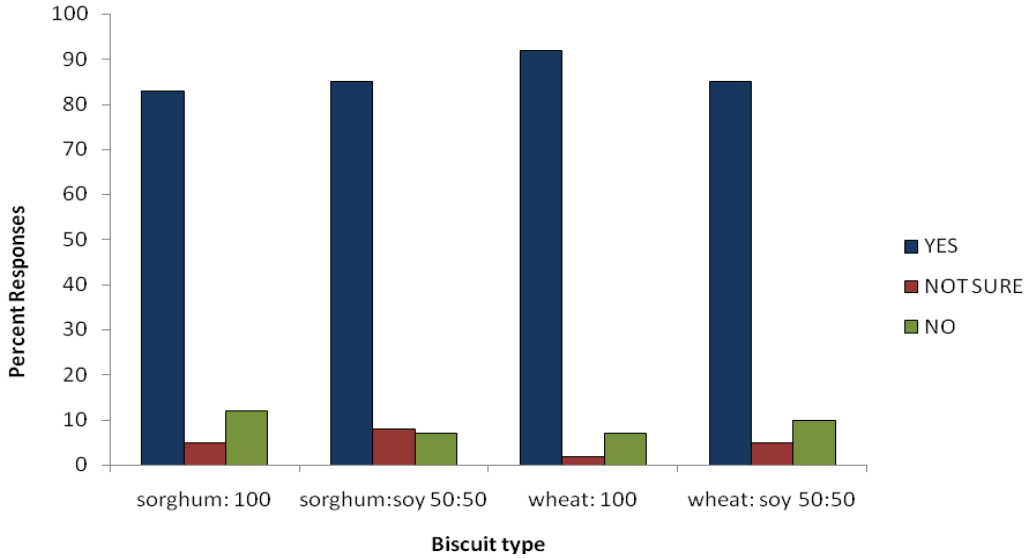


Figure 4.2.6 The effect of compositing sorghum and bread wheat with DSF on children's (n=60) desire to consume biscuits again.

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 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

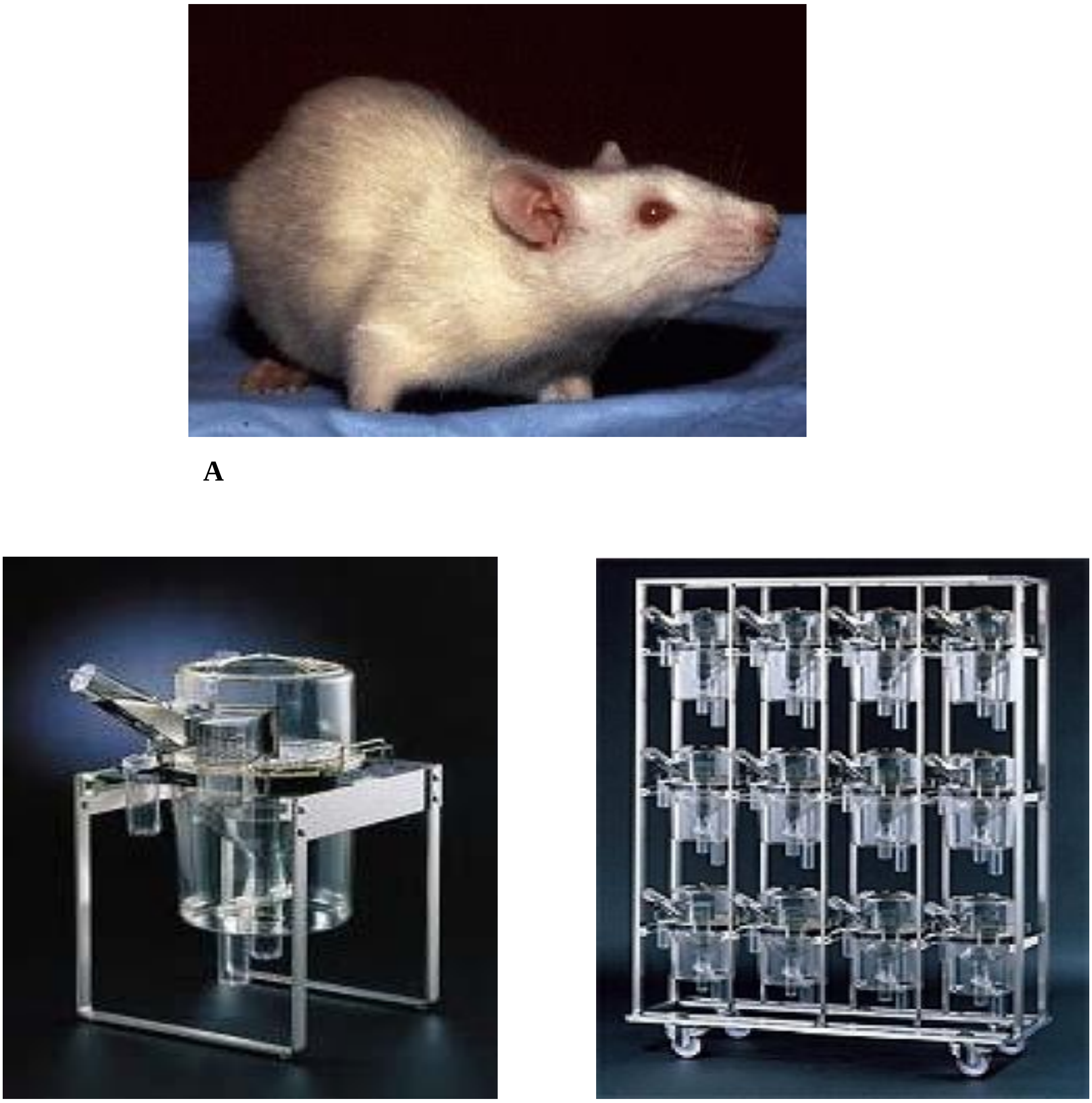
Table 4.3.2 Formulation of the four experimental diets (g/kg dry basis)

	Diets			
Ingredients	**Basal (protein-free)**	**Casein (reference)**	**Sorghum: soy (50:50)**	**Sorghum: (100:0)**
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^{o} 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^{o} 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 indespensable 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 $P \leq 0.05$ 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.

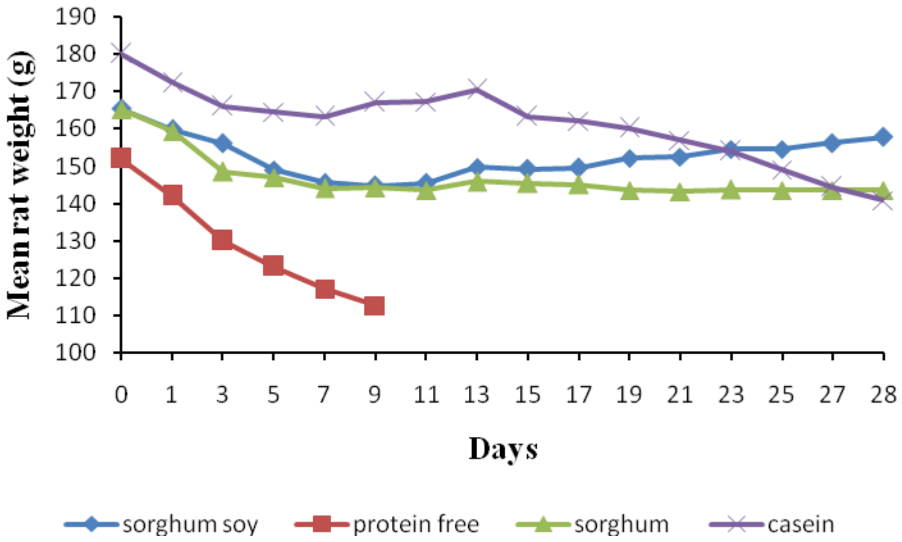


Figure 4.3.2 Average weight of the rats on the four diets for the 28 day period of the feeding trial.

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)	$54.55^{b} \pm 8.35$	$48.53^{b} \pm 5.14$	$49.54^{b} \pm 1.88$	$24.57^{a} \pm 3.50$
Protein intake (g)	$4.36^{a} \pm 0.67$	$3.88^{a} \pm 0.41$	$3.96^{a} \pm 0.15$	nd
Faecal excretion (g)	$3.14^{b} \pm 0.37$	$2.89^{b} \pm 0.32$	$4.70^{c} \pm 0.57$	$1.62^{a} \pm 0.26$
Faecal protein output (g)	$0.46^{b} \pm 0.05$	$0.56^{c} \pm 0.06$	$0.72^{d} \pm 0.09$	$0.23^{a} \pm 0.04$
Faecal protein (%)	$5.25^{a} \pm 1.19$	$8.64^{b} \pm 2.03$	$12.25^{c} \pm 1.87$	nd
Urine excretion (g)	$20.00^{ab} \pm 2.90$	$22.60^{b} \pm 2.97$	$16.00^{ab} \pm 4.08$	$15.25^{a} \pm 8.05$
Urine protein output (g)	$1.21^{b} \pm 0.17$	$1.29^{b} \pm 0.17$	$0.68^{a} \pm 0.71$	$0.72^{a} \pm 0.42$
Urine protein (%)	$11.58^{b} \pm 5.04$	$14.92^{b} \pm 4.81$	$-1.11^{a} \pm 4.2$	nd
Protein (N) Balance (g)	$3.65^{a} \pm 0.74$	$2.98^{a} \pm 0.52$	$3.52^{a} \pm 0.24$	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 (%)	$89.38^{c} \pm 1.55$	$85.38^{b} \pm 2.45$	$81.94^{a} \pm 1.76$
True protein digestibility (%)	$94.75^{c} \pm 1.19$	$91.36^{b} \pm 2.04$	$87.75^{a} \pm 1.87$
Apparent protein BV[1] (%)	$68.19^{a} \pm 8.01$	$60.43^{a} \pm 7.99$	$79.10^{b} \pm 5.48$
True protein BV (%)	$89.51^{a} \pm 5.18$	$85.62^{a} \pm 5.23$	$103.25^{b} \pm 4.83$
Apparent NPU[2] (%)	$61.00^{ab} \pm 7.13$	$51.71^{a} \pm 7.97$	$64.85^{b} \pm 5.41$
True NPU (%)	$84.81^{ab} \pm 4.93$	$78.26^{a} \pm 5.79$	$90.63^{b} \pm 5.52$

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 $P \leq 0.05$ 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.

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