Whole grain sorghum and whole grain cowpea biscuits as a complementary food for improved child nutrition

By

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Dissertation

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DECLARATION

I declare that the dissertation herewith submitted for the degree MSc Food Science at the University of Pretoria, has not previously been submitted by me for a degree at any other university or institution of higher education.

Koya Ange Pamela Dovi

Date: 22/11/2013
DEDICATION

This dissertation is dedicated to God for the gift of life and making this possible. It is also dedicated to my family for their unfailing love, support and encouragements.
ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and acknowledgements to the following people and institutions for their support and assistance with this research:

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My family and friends for their encouragements, prayers and support in various ways;

Above all, to God who made all possible.
ABSTRACT

Whole grain sorghum and cowpea biscuits as a complementary food for improved child nutrition

By

Koya Ange Pamela Dovi

Supervisor: Prof J. R. N. Taylor

Co-supervisors: Prof H. L. de Kock

Dr C. Chiremba

Protein-energy malnutrition (PEM) among children remains a huge burden in Africa. Due to poverty, such children rely on the same starchy staples, such as sorghum, consumed in the household, as both their source of energy and protein. However, sorghum has a low protein quality, particularly with respect to the indispensable amino acid lysine and also protein digestibility. Local pulses such as cowpea are important vehicles to address PEM. Biscuits are favoured as means of fortification because they are palatable, nutrient-dense, in ready-to-eat form and have a long shelf-life. Therefore, the objectives of this study were to develop and evaluate the effect of fortifying whole grain sorghum with whole grain cowpea on the nutritional quality, instrumental, sensory characteristics and consumer evaluation of the biscuits.

Composite biscuits were produced from two types of whole grain sorghums, white tan-plant, non-tannin (WTP) and red non-tannin (RNT) composited with whole grain cowpea at 60:40 ratio. These were compared with commercial economic wheat biscuits.

Sorghum-cowpea biscuits had 50-60% higher protein content than 100% sorghum biscuits but were the same as that of wheat biscuits. The mineral content of sorghum-cowpea biscuits was 27-29% and 37% higher than that of 100% sorghum and wheat biscuits, respectively. The pepsin in-vitro protein digestibility (IVPD) of the sorghum-cowpea biscuits was 71-81% higher than that of 100% biscuits due to inclusion of the more digestible cowpea globulin proteins. However, the average pepsin IVPD of the sorghum and sorghum-cowpea biscuits was 211% and 76% lower than that of wheat biscuits, respectively. There was no trypsin
inhibitor activity in the sorghum-cowpea biscuits due to the dilution of the trypsin inhibitors in cowpea. The total phenolic content of the sorghum-cowpea biscuits was 30-45% and 70% higher than that of 100% sorghum and wheat biscuits, respectively.

Sorghum-cowpea biscuits were stronger than 100% sorghum biscuits due to water soluble-globulin proteins from cowpea. Correspondence analysis (CA) revealed that 64% of the variation in terms of texture and flavour of the biscuits was due to type of cereal (sorghum or wheat) and 23% was due to the presence of cowpea in biscuits, respectively. Standard wheat biscuits were the most liked. However, using cluster analysis, individual overall liking of consumers varied and four different clusters of consumers with similar liking of the biscuits were identified. Two clusters with substantial percentage of consumers (41%) liked the sorghum-cowpea biscuits. These findings suggest that sorghum-cowpea composite biscuits could well serve as an acceptable high quality protein-rich complementary food to alleviate PEM, and generate income for smallholder farmers in rural areas of Africa where sorghum and cowpea are produced and consumed as staples.
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1 INTRODUCTION

Protein-energy malnutrition (PEM) among children remains a huge burden in Africa. Based on the most recent estimate from the United Nations Children’s Fund (UNICEF, 2013), 162 million children worldwide are undernourished and sub-Saharan Africa accounts for 40% of undernourished children. The underlying cause of undernutrition in this region is poverty, which results in low food availability and poor nutrition (FAO/WHO, 1997). Due to poverty, many children rely only on the same starchy staple foods, such as sorghum as consumed in the household for both their source of energy and protein (reviewed by Anglani, 1998, Serna-Saldivar, 2010).

PEM has a significant impact on children because of lack of energy and protein, which are required during their most rapid physical and mental growth, and development (FAO/WHO, 1997). As a result, affected children suffer from delayed growth and development, and impaired learning capacity. In addition, undernutrition lowers children’s ability to fight infectious diseases, leading to poor health, severe limitation of development of human potential and reduction in overall quality of life. This reduces the resources and earning capacity of households that are already poor, thus compounding their social and economic problems (FAO/WHO, 1997).

In sub-Saharan Africa, sorghum is a major food crop because of its unique ability of being drought-tolerant, serving as a dietary staple for several millions of people (ICRISAT, 2011). However, sorghum has a low protein quality because the storage proteins, the prolamins are poor in the indispensable amino acid lysine, with approximately 2.6% (Taylor and Schüssler, 1986). This is very low compared to the 4.8% required by 3 to 10 year old children (WHO/FAO/UNU Expert Consultation, 2007). Also, a unique feature of sorghum that contributes partly to its low protein quality is that its protein digestibility is reduced upon wet cooking to make foods, such as porridge (Duodu et al., 2003).

Cereal-legume complementation remains by far the most practical and sustainable approach to improve sorghum protein quality, and also address PEM among children (FAO, 1995). Cowpea, an important drought-tolerant and inexpensive source of protein, indigenous to Africa (Mensa-Wilmot et al., 2001, Singh et al., 2003) is a potential vehicle to improve sorghum protein quality and address PEM. The cowpea storage proteins, globulins are rich in
lysine with approximately 4.6% (Anyango, 2009). In addition, cowpea proteins are readily digestible after cooking (Lima et al., 2004).

Biscuits have been suggested as a better vehicle for compositing with legumes than bread because they are nutrient dense, in a ready-to-eat form, low cost, palatable and have a long shelf-life (McWatters et al., 2003). Serrem et al. (2011) formulated and developed biscuits by compositing sorghum with defatted soy flour (DSF) as a protein-rich supplementary food.

Although soybean has been widely used to complement cereal-based foods, the technology involved to defat soybean prior to its use in supplementary value-added products is based on solvent extraction, which is a large-scale process involving heavy capital investment and large volumes of raw materials. Such technology has not found wide application in developing countries and thus keep the production of value-added cereal-based supplementary foods out of reach of the most needy segment of many populations (Wijeratne and Nelson, 1991). Due to this limitation of DSF, the use of cowpeas in value-added cereal-based supplementary foods could be a valuable vehicle to address PEM.

Most research has focused on the use of refined sorghum grain to make biscuits of acceptable quality (Badi and Hoseney, 1976, Chiremba et al., 2009). This is notwithstanding the fact that whole grains are rich in dietary fibre, B vitamins, minerals, essential fatty acids, and also health promoting phytochemicals (reviewed by Slavin, 2004), and they are straightforward to process.

Therefore, the objectives of this study are to develop and evaluate the effect of fortifying whole grain sorghum with whole grain cowpea on the nutritional quality, instrumental, sensory characteristics and consumer evaluation of the biscuits. The aim is to determine their potential as a low-cost high quality protein-rich complementary food to alleviate PEM among children in rural areas of Africa where these crops are produced and consumed as staples.
2 LITERATURE REVIEW

In this review, focus is placed on the protein nutritional quality of whole grain sorghum and cowpea in relation to child requirements, and their potential use in high quality protein-rich value-added complementary foods, such as biscuits to alleviate and/or prevent PEM. Sensory characterization, rapid methods for consumer evaluation and in vitro assays for evaluating protein quality are also reviewed.

2.1 Nutrients

2.1.1 Nutrient chemical composition of whole grain sorghum and cowpea

Starch is the most abundant chemical component in sorghum, it forms 50% to 75% of the grain weight. It comprises 23% to 30% amylose and the rest is amylopectin (Serna-Saldívar& Rooney, 1995). Protein is the second chemical component at about 11% (Taylor et al., 1984).

The protein content of cowpea is much higher, about twice than that of sorghum, approximately 25% protein (Lima et al., 2004). The starch content of cowpea is lower than that of sorghum and ranges between 31.5 and 48.0% (reviewed by Phillips et al., 2003). Cowpea also contains about 7.0% oligosaccharides (Longe, 1980). Although the oligosaccharides, which comprise of raffinose, starchyose, verbascose, cause gas flatulence in human, they have beneficial metabolic effects, such as reducing gastric transit time and promoting the growth of beneficial bacteria (Medeiros and Wildman, 2000, reviewed by Ofuya and Akhidue, 2006).

According to The Whole Grains Council (2004), ‘whole grains, such as sorghum comprise the intact or ground caryopsis’. The caryopsis consists of three distinctive anatomical components: pericarp or bran, germ and endosperm. The relative chemical composition of the different anatomical components varies and depends to a large extent on the degree of milling. Table 2.1 shows the average chemical composition of whole grain sorghum and cowpea and their anatomical parts.
## Table 2.1 Chemical composition (g/100 g) of whole grain sorghum and cowpea and their anatomical parts on dry basis

<table>
<thead>
<tr>
<th></th>
<th>Whole grain</th>
<th>Endosperm</th>
<th>Germ</th>
<th>Pericarp</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sorghum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>12.3</td>
<td>10.5</td>
<td>18.4</td>
<td>6.0</td>
</tr>
<tr>
<td>Fat</td>
<td>3.6</td>
<td>0.6</td>
<td>28.1</td>
<td>4.9</td>
</tr>
<tr>
<td>Minerals (ash)</td>
<td>1.6</td>
<td>0.4</td>
<td>10.4</td>
<td>2.0</td>
</tr>
<tr>
<td>Starch</td>
<td>73.8</td>
<td>82.5</td>
<td>13.4</td>
<td>34.6</td>
</tr>
<tr>
<td>Dietary fibre</td>
<td>6.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Cowpea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>24.9</td>
<td>25.8</td>
<td>-</td>
<td>10.9</td>
</tr>
<tr>
<td>Fat</td>
<td>1.4</td>
<td>1.6</td>
<td>-</td>
<td>0.3</td>
</tr>
<tr>
<td>Minerals (ash)</td>
<td>3.3</td>
<td>3.3</td>
<td>-</td>
<td>2.3</td>
</tr>
<tr>
<td>Starch</td>
<td>44.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dietary fibre</td>
<td>10.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

1 Adapted from Phillips (1982)
2 Adapted from Serna-Saldivar and Rooney (1995)
3 Adapted from USDA (2008).
4 (-) means no values were obtained from the literature.
Milling of sorghum and cowpea to transform them into more palatable and more desirable foods, generally involves recovery of the main anatomical component, the endosperm and cotyledons, respectively, and concomitant removal of the pericarp or seed coat and the germ (in case of sorghum) (Taylor, 2003, Wood and Malcolmson, 2011). However, as shown in Table 2.1, the pericarp and the germ of sorghum are relatively rich in protein, minerals and dietary fibre (Serna-Saldívar and Rooney, 1995, USDA, 2008), as well as B vitamins and essential fatty acids (reviewed by Slavin, 2004). Thus, when decorticated, the separated endosperm is lower in these components than the original whole grain. In addition, whole grains contain high levels of phenolic compounds, which have high antioxidant activity (reviewed by Awika and Rooney, 2004). Phenolic compounds are able to react with free radicals, which would otherwise attack the deoxyribonucleic acid (DNA), lipids and proteins, thereby leading to several chronic diseases (reviewed by Slavin, 2004). Chiremba et al. (2009) found that the total phenolic content of whole grain sorghum was approximately 0.3 g catechin equivalents/100 g and 0.13 g catechin equivalents/100 g in flour produced at 70% extraction rate. Adebooye and Singh (2007) investigating the effect of decortication on the total phenolics in two cowpea varieties found a reduction of 24% to 54%.

The focus of this review is thus mainly on the protein nutritional quality of whole grain sorghum and cowpea flours.

2.1.2 Protein and amino acid composition

A study by Taylor et al. (1984) on 38 sorghum cultivars found that the average protein content was 11%. By fractionating these proteins, the authors found prolams as the most abundant protein fraction (48%), followed by glutelins (24.6%), albumins plus globulins (16.4%) and low molecular weight nitrogen (LMWN), which accounted 6.6%. Like in most other cereals, the prolams, which in sorghum are called kafirins, are the main storage proteins, whose function serves as a nitrogen store for the growing plant during germination (Taylor and Schüssler, 1986). Cowpea seeds are rich in protein. Lima et al. (2004) found that the cowpea protein content was approximately 25%, which varied between cultivars. Similar results were reported by Chan and Phillips (1994). These authors found that the globulin fraction was the major cowpea seed protein fraction comprising 66% of the total protein, followed by albumins, 25% and glutelins and prolams composed of 5-7% and 1% of the total protein, respectively.
Protein nutritional quality refers to the proportion of indispensable amino acids in the protein compared with the needs of human cells (Medeiros and Wildman, 2000). Indispensable amino acids are amino acids that cannot be synthesised by human cells, either at all or in adequate amounts to meet the needs of cells to synthesize proteins for the body’s growth and maintenance (Medeiros and Wildman, 2000). As reviewed by Shewry (2007) such amino acids must be provided in the diet. There are 20 amino acids which are constituents of proteins and eight to nine of them are strictly indispensable. These are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and additionally histidine for infants (Medeiros and Wildman, 2000). If one of these amino acids is limiting, the others will be metabolised and excreted resulting in loss of nitrogen required by the body for protein synthesis, and hence poor growth of children (Medeiros and Wildman, 2000).

According to WHO/FAO/UNU Expert Consultation (2007), the average protein requirement for children aged between 3 to 10 years is 0.73 g protein/kg body weight per day. The average body weight of children between 3 and 10 years is 15 to 30 kg. Thus, the average protein requirement for these children translates to 11 to 22 g protein/day. Based on WHO/FAO/UNU Expert Consultation (2007) requirement of indispensable amino acids for children aged between 3 to 10 years in Table 2.2, the composition of whole grain sorghum is particularly low in lysine (Taylor and Schüssler, 1986), but adequate in whole grain cowpea (Anyango, 2009). On the other hand, however, sorghum like other cereals has a higher amount of the indispensable amino acids, methionine and cysteine, which seems to be substantially low in cowpea.
<table>
<thead>
<tr>
<th>Indispensable amino acids</th>
<th>Whole grain sorghum</th>
<th>Whole grain cowpea</th>
<th>WHO/FAO standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>2.6</td>
<td>2.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.0</td>
<td>3.5</td>
<td>3.1</td>
</tr>
<tr>
<td>Leucine</td>
<td>13.1</td>
<td>6.0</td>
<td>6.1</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.6</td>
<td>4.6</td>
<td>4.8</td>
</tr>
<tr>
<td>Methionine + Cysteine</td>
<td>1.7</td>
<td>1.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Phenylalanine + Tyrosine</td>
<td>4.4</td>
<td>4.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Threonine</td>
<td>3.5</td>
<td>3.1</td>
<td>2.5</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>ND</td>
<td>ND</td>
<td>6.6</td>
</tr>
<tr>
<td>Valine</td>
<td>5.5</td>
<td>4.2</td>
<td>4.0</td>
</tr>
</tbody>
</table>

1 Adapted from Taylor and Schüssler (1986).
2 Adapted from WHO/FAO/UNU Expert Consultation (2007). The scoring pattern (requirement for each indispensable amino acid for 3 to 10 years old children).
3 Adapted from Anyango (2009).
4 ND: Not Determined.
Therefore, complementation with lysine-rich cowpea could be favourable for improvement of sorghum protein quality. Anyango et al. (2011b) found that the addition of cowpea to traditional African sorghum foods at a ratio of 70:30 increased the protein contents of flours by between 32% and 57%, whereas the lysine contents of the protein in all the composite foods produced uji (unfermented porridge), ugali (unfermented thick porridge) and injera (fermented flatbread) improved more, by between 67% and 139%.

2.2 Protein antinutritional factors in cowpea seeds

Although a complemented diet of sorghum with cowpea may provide a good source of protein, cowpea like other legumes have non-toxic heat-labile antinutritional factors, which can nonetheless cause adverse physiological responses, and, unless destroyed can reduce the full nutritional potential of legumes (Liener, 1981, Rangel et al, 2004). Protease inhibitors, commonly referred to as trypsin inhibitors, are the best known and studied of such antinutritional factors. Protein inhibitors are known to interfere with digestion of dietary protein by binding to proteolytic enzymes present in the intestinal tract (Liener, 1981), thereby reducing protein digestibility, and thus dietary protein quality.

According to Ologhobo and Fetuga (1983) trypsin inhibitor is the only one in cowpea of importance. Trypsin inhibitors are low molecular weight proteins capable of binding the digestive protease enzyme, trypsin (Hefnawy, 2011), forming stable complexes and as a consequence inhibiting its ability to degrade dietary proteins. This in turn limits the intake of amino acids needed to synthesise new proteins (Rangel et al., 2004, Sreerama et al., 2012). A study by Ologhobo and Fetuga (1983) on the antinutritional factors in 10 varieties of cowpea revealed approximately 24 trypsin inhibitor units/mg protein. Similar results were reported by Ene-obongo (1995), where the trypsin inhibitor content of four different varieties of cowpea ranged between 9.8 and 20.5 trypsin inhibitor units/mg protein.

2.3 Effect of thermal processing on the protein quality of whole grain sorghum and cowpea flours and their end products

In order to preserve foods, make them palatable or add to their variety or novelty, they undergo many processes, of which heat is the most important because of its effect on protein quality. Heating can be both beneficial and detrimental to the nutrient in foods. For example, the protein quality of most, if not all legumes, is improved by heating. This is partly due to
the destruction of trypsin inhibitors and toxic substances and partly to improved digestibility (reviewed by Bender, 1972). However, as reviewed by Bender (1972) heat may also induce losses of the essential amino acids.

2.3.1 Effect on proteins

Heat can enhance protein denaturation and expose free amino acid residues such as the reactive ε-amino group of lysine. If the food contains reducing sugars such as, glucose, fructose and lactose, these may react with the ε-amino group of lysine in the non-enzymatic (Maillard) browning reaction, and form an indigestible complex (Hardy et al., 1999). Such reactions reduce the availability of free amino acids, and hence the protein quality. For example, working on compositing traditional African sorghum foods, Anyango et al. (2011b), reported that the increase in reactive lysine (chemically available lysine) was lower in foods compared to the corresponding raw flours. Similar results were reported by Serrem et al. (2011) who found that the reactive lysine of composite sorghum-defatted soy biscuits was considerably lower than their total lysine content. During cooking of sorghum-DSF biscuits (Serrem et al., 2011) and sorghum-cowpea traditional African foods (Anyango et al., 2011b) heat may have enhanced protein denaturation, thereby exposing the reactive ε-amino group of lysine. This possibly reacted with the reducing sugars in the non-enzymatic (Maillard) browning reaction, and form an indigestible complex (Hardy et al., 1999), thus decreasing the reactive lysine content. Although, the results reported by both Anyango et al. (2011b) and Serrem et al. (2011) showed a decrease in reactive lysine content of the composite processed foods, the decrease was not as marked as with sorghum foods alone, indicating that compositing with legumes compensated for the loss.

2.3.2 Effect on protein digestibility

Many researchers have investigated the effect of cooking on the in vitro protein digestibility of sorghum. Axtell et al. (1981) investigated the digestibility difference between uncooked and cooked sorghum using a pepsin digestion method. The authors found that uncooked sorghum had a protein digestibility of 89-93%, which dropped to 45-57% after wet cooking. In other studies, Mertz et al. (1984) and Hamaker et al. (1986) compared the digestibility of sorghum protein and other cereals using the pepsin assay, and found that the digestibility of cooked sorghum was approximately 25% lower than that of wheat, maize and rice.
According to Duodu et al. (2003), there are several causes of the reduction in sorghum protein digestibility but the major cause is the disulphide cross-linking of the kafirins. The disulphide bonding seems to specifically involve the cysteine-rich γ- and β-kafirins which are concentrated near the surface of the kafirin protein bodies. This cross-linking appears to render the major kafirin sub-class, the α-kafirins, which are located more in the centre of the protein bodies, less accessible to protein hydrolysis. Furthermore, cooking reduces digestibility by inducing conformational changes in the kafirin polypeptides, from α-helical to β-sheet conformation, which could facilitate formation of disulphide-linked polymers (Emmambux and Taylor, 2009).

Unlike sorghum, the digestibility of cowpea proteins is limited mainly by protein structure and the presence of antinutritional factors (Chau and Cheung, 1997). A study by Lima et al. (2004) compared the in vitro digestibility of unheated and heated globulins from cowpea seeds using bovine trypsin. The authors found that globulins from uncooked cowpea seeds were resistant to trypsin attack, whereas the heat treated seeds were completely digested. It was suggested that heat treatment denatured the globulins by opening up their structure, which in turn enhanced the accessibility of the proteins to enzymatic attack.

Many researchers have reported evidence concerning improvement of sorghum protein digestibility by compositing with legumes such as cowpea. Anyango et al. (2011b) reported that addition of cowpea to traditional African sorghum foods (uji, injera and ugali) improved in their pepsin digestibility by 13 to 62%. The improvement was attributed to the increased content of more digestible globulin proteins, and decrease in the less digestible kafirins. Furthermore, cowpea globulins become more digestible after cooking because of reduction of trypsin inhibitors. A study by (Khattab and Arntfield, 2009) investigated the effect of various heat treatments on the antinutritional factors of cowpea, kidney bean and pea. They found that heat treatments destroyed the heat labile trypsin inhibitors, which in addition contributed to improved protein digestibility. Olapade et al. (2011) investigated the trypsin inhibition activity of biscuits made from a blend of fonio and cowpea flours at a ratio of 80:20 baked at 180°C for 12 minutes. They found that trypsin inhibition activity of the blends increased with proportions of cowpea flour. However, significant reduction in trypsin inhibition activity was noted in the biscuits, from 4.01 trypsin inhibitor units (TIU)/g to 0.09 TIU/g. This inactivation was attributed to the high temperature of baking.
2.4 Biscuit formulation

2.4.1 Science and technology

Biscuits are unleavened crisp, sweet pastry made from soft wheat flour (reviewed by Hoseney, 1994), with a low protein content, ranging from 8 to 10% (Yener, 2008). Biscuits are characterised by a formula high in shortening (fat), sugar, and low in water. They may contain other ingredients such as baking powder, salt, flavourings, eggs and milk (reviewed by Hoseney, 1994). The sugar restricts the development of gluten by competing for water, which would otherwise be absorbed by the gluten. The fat inhibits formation of a gluten network by coating the flour particles before they can be hydrated (Gallagher, 2008).

In industrial practice, biscuits are baked in long tunnel ovens with a baking zone of about 1 m wide and 100-150 m long (reviewed by Hoseney, 1994). They are generally baked on a solid steel band that conveys the product through the oven at a rate that will produce the desired bake time. Biscuits are classified by the way the dough is placed on the baking band, rotary mould, cutting machine or wire cut biscuits (reviewed by Hoseney, 1994).

To produce rotary mould biscuits, the dough is forced into moulds on a rotating roll, extracted from the cavity after rolling a half turn and placed on the band for baking. Formulations for rotary mould biscuits are characterized by high sugar and shortening levels and very low amounts of water. High levels of fat result in a dough that is cohesive and plastic, but lack extensibility and elasticity, due to limited gluten network development. The flour is given very little mixing time to minimize the development of the protein network. Cutting machine biscuit doughs are made into continuous sheet and the products are cut out from it. The formulation of such biscuits comprises relatively low levels of fat and sugar but much more water than that of rotary mould biscuits. The gluten is developed in the system, which stops during baking of cutting machine biscuits. With wire cut biscuits a relative soft dough is extruded through an orifice and cut to size by a reciprocating wire. The dough must be cohesive enough to hold together, yet non-cohesive enough to separate cleanly when cut by wire. A typical formulation may contain 50-75% sugar, 50-60% shortening, and up to 15% eggs. Wire cut biscuits rise and spread as they are baked and their final size is determined by the formulation and flour used.

Because of minimal gluten network that is formed, the texture of the baked biscuits is attributable mostly to starch gelatinization and supercooled sugar, rather than a protein
network. The development of the gluten network required for biscuit production is minimal and only serves to provide cohesion for handling and subsequent shaping (Gallagher, 2008). Therefore, substitution of wheat with sorghum, which is a gluten-free cereal and a major dietary source of both energy and protein for at risk communities in areas like sub-Saharan Africa, has been found desirable (FAO, 1995).

Biscuits from sorghum flour were produced by Badi & Hoseney (1976), but these were described as tough, hard, gritty and mealy. The reason for this low quality was attributed in part to the lack of polar lipids in sorghum when compared to those from wheat. Improvement was made by combining sorghum flour and wheat to produce biscuits with acceptable quality. In another study, Chiremba et al. (2009) evaluated consumer acceptability of sorghum biscuits and also reported that biscuits made from whole grain sorghum flour were very fragile, difficult to handle and judged not suitable for consumers. The reason for the observed textural properties of sorghum biscuits could be due to the higher level of bran, which may have resulted in coarse flour particles causing discontinuities in the dough and biscuit matrix, thus weakening the biscuit structure. In addition, the absence of gluten also contributed to their fragility.

However, Chiremba et al. (2009) found that by altering the extraction rate, sorghum biscuits produced from 90% extraction flour were equally liked as wheat biscuits. The authors concluded from this finding that sorghum biscuits have potential for a large-scale production. Though acceptable biscuits were made from sorghum, a possible technology that can use the whole grain may be more advantageous for such production in order to prevent nutrient loss by removing the bran and germ fractions.

2.4.2 Cereal-legume biscuits as complementary food to prevent and/or alleviate protein-energy deficiency

Among strategies which have been used to address PEM among young children, as stated cereal-legume complementation remains by far the most practical and sustainable approach to improve cereal protein content and quality (FAO, 1995). Because of the outstanding features of cereal-legume complementation (Philips, 1993), attempts have been made to produce biscuits by complementing sorghum with legumes.

As stated, Serrem et al. (2011) formulated and developed biscuits by compositing sorghum and DSF as a protein-rich supplementary food to alleviate PEM in children. They found that
sorghum-soy composite biscuits with 1:1 sorghum:DSF ratio had more than double the protein content of 100% sorghum flour. The protein lysine content markedly improved by 231%, whereas the reactive (chemically available) lysine by 261%. The in vitro protein digestibility was 170% higher compared to 100% sorghum biscuits. The increase of protein content was attributed to the 50% protein content of DSF, whereas the increase of protein and reactive lysine was because DSF protein has three times higher lysine than the sorghum protein (Serrem, 2011). Moreover, the increase of in vitro protein digestibility was attributed to dilution of the less digestible sorghum kafirin with more available soy globulins.

As explained, due to limited availability of DSF, the use of local pulses, such as cowpea in producing value-added complementary foods may be an important vehicle to address PEM. This is because cowpea natural fat content is low, about 1.5%, which makes the flour more stable. As mentioned, Anyango et al. (2011b) showed that addition of cowpea to traditional African sorghum foods (uji, injera and ugali) improved their protein quality.

Notwithstanding the fact research has been done to improve the nutritional quality of sorghum using inexpensive sources of proteins in several products, including biscuits, little has been done on how to develop useful technology for production of biscuits on a commercial scale (Fig 2.1A). For example, the method of biscuit dough cutting used by Chiremba et al. (2009) and Serrem et al. (2011) made the process laborious and time-consuming. The dough was manually rolled out on a steel tray to a thickness of approximately 5 to 6 mm and cut into circular biscuits using a cutter of 50 mm diameter and excess dough was removed (Fig 2.1B). In order to improve production so as to make a large number of biscuits at a time, there is need to use comparative simple and inexpensive processing technology. This would also result in other advantages such as biscuit dough piece with identical height and shape resulting in a generally improved biscuit quality.
Figure 2.1 Industrial and “home” methods of cutting biscuit doughs A: on an industrial level using a rotary cutter and B: manually using a circular cutter by Serrem (2011)
2.5 Consumer tests suitable for biscuits and evaluating their liking

While fortified whole grain sorghum-cowpea biscuits may increase the nutritional quality of sorghum, determination of consumer acceptability of such biscuits is critical. Consumer acceptability tests are related to consumer likes or dislikes of a product. Such tests are mostly achieved though hedonic rating tests where consumers are asked to indicate the extent of liking of the product from extreme dislike to extreme like (Lyon et al., 1992). A popular test used for this purpose is the 9-point hedonic scale (Peryam and Pilgrim, 1957). Consumer acceptability has been widely used in the evaluation of biscuits. For example, Chiremba et al. (2009) used 59 consumers between 18 and 45 years to evaluate sorghum biscuits developed as source of antioxidants with potential health benefit for school children. Consumer acceptance of sorghum biscuits was compared to wheat biscuits using a 9-point hedonic scale and biscuits made from the red tannin-free sorghum flour were equally liked as wheat flour biscuits, except texture.

According to Guinard (2001) foods developed specifically for children should be tested with children. This is because children have lower taste detection thresholds than adults (reviewed by Guinard, 2001), and thus may perceive food differently. Serrem et al. (2011) used school children to evaluate the acceptability of sorghum biscuits fortified with DSF, which were found to be equally liked to whole wheat biscuits. However, as reviewed by Guinard (2001) children have a limitation in cognitive abilities pertinent to sensory testing, including lack of verbal skills, short attention span and difficulties in task comprehension. To overcome such limitations, for example in the sensory evaluation with children, Serrem et al. (2011) used a simple 5-point facial scale for hedonic categorization of sorghum biscuits. In addition, the children (60) were divided into four groups of 15 with two assistants per group, and they were oriented to familiarize and teach them how to use the scale.

Consumers have been considered only capable of hedonic judgments to indicate the degrees of dislike to like (Meilgaard et al., 1999) and information about how they perceive the sensory characteristics of a product is not gathered. Thus, detailed sensory characteristics of the product are obtained from trained panel. However, according to Ten Kleij and Musters (2003) trained panellists may describe the product differently to consumers or take into account attributes that may be irrelevant to consumers. To gather information of the reason for any preference or rejection, consumer studies usually include additional questions about the product’s sensory attributes about how consumers perceive the sensory characteristics of
a food product (Meilgaard et al., 1999). Check-all-that-apply (CATA) question is a novel methodology developed to obtain sensory information from consumers to determine which sensory attributes they perceive in a food product (reviewed by Varela and Ares, 2012). A CATA question consists of a list of attributes or terms from which the respondents should select all attributes they consider appropriate to describe a food product. The terms are generated either based on previous research or by trained panellists, and/or by a focus group. An advantage of the CATA question method is that it allows consideration of terms related to sensory characteristics, and it is a very fast and spontaneous method, minimizing the amount of time and cognitive effort, and also it is regarded as very appropriate method to use with naïve consumers (Lado et al., 2010, reviewed by Varela and Ares, 2012). (Ares et al., 2010) used the CATA question method in the development of chocolate milk deserts. In another study Lado et al. (2010) used it to study consumers’ perception of new strawberry cultivars. However, the interest in purchase intent has not been widely researched as most research has been done on consumer evaluation (Lyon et al., 1992).

2.6 In vitro assays for protein quality of complementary foods

When predicting the protein nutritional quality of foods, information on protein bioavailability is of utmost importance. However, in vivo determinations are time-consuming, costly and there are ethical issues, which is why effort has been devoted to the development of in vitro procedures. In vitro techniques generally use specific enzymes either to give maximal protein digestibility values or to measure the initial rate of hydrolysis (Boisen and Eggum, 1991).

2.6.1 Pepsin protein digestibility

Axtell et al. (1981) developed the pepsin digestion method to determine the digestibility difference between uncooked and cooked sorghum. The method involves first determining the protein content of the food, then digesting the protein in the food with pepsin under specific conditions. The amount of the undigested protein in the food is then determined and the in vitro protein digestibility is calculated by difference, and expressed as a percentage of the total protein (Hamaker et al., 1986). In their study, Axtell et al. (1981) found that uncooked sorghum protein had a high digestibility of 78-93% for whole grain and 78-86% for decorticated grain, which dropped to 45-55% for whole grain and 37-43% for decorticated grain after cooking. These values paralleled results obtained from young children recovering from PEM who received a sorghum-based diet (MacLean et al., 1981). The pepsin method
has been widely used to determine and differentiate the protein digestibility of sorghum and other cereals. Mertz et al. (1984) found that the pepsin digestibility of protein in processed whole grain sorghum was 60%, which was substantially lower than that of wheat, maize and rice, 86, 85 and 84%, respectively.

2.6.2. Multienzyme protein digestibility

Hsu et al. (1977) developed an in vitro method using a multienzyme system for the estimation of protein digestibility. The multienzyme system consists of pancreatic trypsin, chymotrypsin and peptidase. It involves the measurement of the pH drop after the digestion has proceeded for 10 minutes. The pH drop depends on the principle that during proteolysis, protons are released from the cleaved peptide bonds, resulting in a decrease in pH of the suspension (reviewed by Moughan, 1999). A correlation is assumed between the initial (10 minute) rate of pH drop and protein digestibility (reviewed by Moughan, 1999). Hsu et al. (1977) reported that the pH drop at 10 minutes after enzyme addition highly correlated with rat in vivo apparent digestibility. The authors also reported that the multienzyme system gave a better approximation of protein digestibility than a single enzyme system. This is because a single enzyme attacks specific peptide bonds and may give different results for proteins containing different concentrations of specific amino acids. An advantage of this in vitro method is that it can be completed within an hour and is sufficiently sensitive to detect the effects of processing and the presence of protease inhibitors (Hsu et al., 1977).

2.7 Trypsin inhibitor activity

The trypsin inhibitor activity of processed foods can be determined by measuring the loss of activity of added trypsin under standard conditions (AACC International, 2000). According to this method, trypsin inhibitor activity is determined by incubating a sample extract with a specific enzyme substrate benzoyl-DL arginine-\(p\)-nitroanilide (BAPA), and bovine trypsin. Trypsin activity is indicated by an increase in absorbance at 410 nm due to hydrolysis of BAPA by the trypsin, thereby releasing the \(p\)-nitroanilide. Inhibition of trypsin by the inhibitor present in the sample decreases the absorbance increase.
2.8 Conclusions

PEM among children remains a huge burden in Africa. As a result of poverty, many children rely on sorghum as a dietary staple as both their source of energy and protein. However, sorghum has low protein quality and low protein digestibility when wet cooked into foods. Local pulses such as cowpea are important vehicles to improve sorghum nutritional quality and address PEM. Sorghum-cowpea composite biscuits could be favourable as a protein-rich complementary food because they are palatable, nutrient-dense, ready-to-eat and have a long shelf-life. Whole grain products have additional nutritional benefits in terms of minerals, dietary fibre and health promoting phytochemicals. There is therefore need to develop whole grain sorghum biscuits fortified with whole grain cowpea and evaluate their nutritional quality, sensory characteristics, and consumer evaluation of the biscuits among the targeted socio-economic group.
3 HYPOTHESES AND OBJECTIVES

3.1 Hypotheses

1. Whole grain sorghum biscuits composited with whole grain cowpea flour will have substantially improved nutritional quality, in terms of protein, mineral and in-vitro protein digestibility compared to unfortified sorghum biscuits. This is because cowpea seeds are pulses and have high mineral content (USDA, 2008, Oomah et al., 2011). Additionally, as whole grains sorghum and cowpea will also contribute to mineral content (Serna-Saldívar, 1995, USDA, 2008). The biscuit protein digestibility will be high due to addition of cowpea globulin proteins (Lima et al., 2004), which will decrease the less digestible sorghum kafirin proteins (Duodu et al., 2003).

2. Whole grain sorghum biscuits fortified with whole grain cowpea will have no trypsin inhibitor activity. This is because cowpea seed contain low levels of trypsin inhibitors (Ologhobo and Fetuga, 1983), which will be further diluted by the sorghum. Furthermore, the high temperature used during baking will inactivate the heat labile trypsin inhibitors (Liener, 1981).

3. Biscuits made of whole grain sorghum and cowpea flours will have similar sensory characteristics and be equally acceptable as local commercial economic wheat biscuits. This is because good quality biscuits (nice tender bite) do not require much gluten development (Gallagher, 2008), and thus can be prepared from gluten-free flours.
3.2 Objectives

1. To determine the effect of compositing whole grain sorghum with whole grain cowpea on the nutritional quality of biscuits, in terms of protein and mineral contents, in-vitro protein digestibility and trypsin inhibitor activity with the aim of improving sorghum nutritional quality and alleviating PEM among children in rural areas of Africa where sorghum and cowpea are produced and consumed.

2. To determine the instrumental, sensory characteristics and consumer evaluation of whole grain sorghum and whole grain cowpea composite biscuits compared to local commercial economic wheat biscuits. Acceptable biscuits from these commonly consumed crops may serve as a low-cost high quality protein-rich complementary food to alleviate PEM among children in rural areas of Africa.
4 Complementing whole grain sorghum with whole grain cowpea in biscuits on their nutritional quality, instrumental, sensory characteristics and consumer evaluation
Abstract

Protein-energy malnutrition (PEM) among children remains a huge burden in Africa. Biscuits are favoured as means of fortification because they are palatable, nutrient dense, ready-to-eat and have long shelf-life. Composite biscuits were produced from two types of whole grain sorghum, white tan-plant, non-tannin (WTP) and red non-tannin (RNT) complemented with whole grain cowpea at a ratio of 60:40. The nutritional quality, instrumental, sensory characteristics and consumer evaluation of the composite biscuits were compared to that of existing commercial economical wheat biscuits. Sorghum-cowpea composite biscuits had 50-60% higher protein than 100% sorghum but the same as wheat biscuits. The mineral content was 27-29% and 37% higher than that of sorghum and wheat biscuits, respectively. The pepsin in vitro protein digestibility (IVPD) of the sorghum-cowpea biscuits was also 71-81% higher and with no trypsin inhibitor activity. Further, the total phenolic content of the sorghum-cowpea biscuits was 30-45% and 70% higher than that of the sorghum and wheat biscuits, respectively. Sorghum-cowpea biscuits were stronger than 100% sorghum biscuits due to water soluble-globulin protein from cowpea. Correspondence analysis (CA) revealed that 64% of the variation in terms of texture and flavour of the biscuits was due to type of cereal and 23% was due to the presence of cowpea in biscuits, respectively. The wheat biscuits were the most liked. However, using cluster analysis a substantial percentage of consumers (41%) liked the sorghum-cowpea biscuits. This work shows that sorghum-cowpea composite biscuits could well serve as an acceptable high quality protein-rich complementary food to alleviate PEM in rural areas of Africa where these crops are produced and consumed as staples.
4.1 INTRODUCTION

Sorghum forms a major source of energy and proteins in the diets of many children in Africa (reviewed by Anglani, 1998, Serna-Saldivar, 2010). However, sorghum protein quality is poor. For example, in a study on protein composition of different parts of sorghum, Taylor and Schüssler (1986) found that the storage proteins in sorghum, called kafirins contain only approximately 2.6% of the indispensable amino acid lysine. This is very low compared to 4.8% required for 3 to 10 year old children (WHO/FAO/UNU Expert Consultation, 2007). Also, a unique feature of sorghum which contributes partly to its low protein quality is that its protein digestibility is reduced upon wet cooking to make foods such as porridge (Duodu et al., 2003).

The sorghum caryopsis consists of the endosperm, pericarp (bran) and germ (Taylor and Dewar, 2001). Taylor and Schüssler (1986) found that the germ and pericarp are three to four times richer in lysine than endosperm. In addition, the pericarp and the germ are highly rich in many other nutrients, including dietary fibre, B vitamins, minerals, essentially fatty acids, and also health-promoting phytochemicals (reviewed by Slavin, 2004). However, production of most sorghum foods involves firstly milling the grain, where the pericarp and the germ are removed, and the endosperm is reduced into flour (Taylor, 2003). This process further reduces the nutritional quality of sorghum that is already poor. Therefore, there is need to use and complement whole grain sorghum to improve its nutritional quality.

Cereal-legume complementation is the most practical and sustainable approach to improve sorghum protein quality, and also address protein-energy malnutrition (PEM) among children (FAO, 1995). This is because of the use of inexpensive sources of protein such as cowpea, which are derived from dietary staples and are readily available in sufficient quantity to the target population (Mensa-Wilmot et al., 2001). The protein content of cowpea is approximately 25% (Lima et al., 2004) and the cowpea storage proteins are rich in lysine with approximately 4.6% (Anyango, 2009). In addition, cowpea proteins are readily digestible after cooking (Lima et al., 2004). Work by Anyango et al. (2011b) showed that addition of 30% cowpea to traditional African sorghum foods improved their protein quality, mostly the lysine and protein digestibility.

Biscuits have been suggested as a better vehicle for fortification than bread because they are nutrient dense, in a ready-to-eat form, fairly low cost, palatable and have long shelf-life (McWatters et al., 2003). Serrem et al. (2011) formulated and developed biscuits by
compositing sorghum and defatted soy flour (DSF) as a protein-rich supplementary food for school feeding programmes in Africa. Although whole grains have been found to be rich in nutrients and health-promoting phytochemicals (reviewed by Slavin, 2004), most research has focused on the use of refined sorghum grain to make biscuits of acceptable quality (Badi and Hoseney, 1976, Chiremba et al., 2009).

Therefore, the objectives of this study were to develop and evaluate the effect of fortifying whole grain sorghum with whole grain cowpea on the nutritional quality, instrumental, sensory characteristics and consumer evaluation of the biscuits. The aim was to determine the potential of whole grain sorghum-cowpea composite biscuits as a low-cost high quality protein-rich complementary food to alleviate PEM among children in rural areas of Africa where sorghum and cowpea are produced and consumed.
4.2 MATERIALS AND METHODS

4.2.1 Preparation of whole grain flours and biscuit ingredients

Grains of two sorghum cultivars and one cowpea variety were used. White tan plant, non-tannin sorghum (WTP) and red, non-tannin sorghum (RNT) produced at the Agricultural Research Council, Potchefstroom, North West Province, South Africa and cowpea (Bechuana white) in Delareyville, North West Province, South Africa. The grains were separately milled using a laboratory hammer mill (Falling Number 3100, Huddinge, Sweden) fitted with a 500 µm opening screen to give whole grain flours. These were then stored at 10°C prior to biscuit preparation and other analysis. Other ingredients were obtained from retail outlets. These included pure white sugar, sunflower oil, baking powder and vanilla essence. Two types of commercial economic wheat biscuits Shortbread and Original (Casa Mia Biscuits, Nancefield, South Africa) were used as standards.

Biscuit formulation of whole grain sorghum-cowpea composite biscuits

The average protein content of sorghum is approximately 11% (Taylor et al., 1984) and that of cowpea is approximately 25% (Lima et al., 2004). Thus, a ratio of 60:40 whole grain sorghum to cowpea was chosen. This ratio would improve the protein content of the composite flour to approximately 16.6%. In addition, as the lysine content of sorghum is approximately 2.6% (Taylor and Schüssler, 1986) and about 4.6% for cowpea (Anyango, 2009), thus the lysine content of the protein would be approximately 4%.

For the 100% whole grain sorghum biscuits, the formulation comprised 450 g sorghum flour, 112 g sugar, 132 g sunflower oil, 3 g baking powder, 27 g vanilla essence and 157.5 g water (Table 4.1). The 60:40 whole grain sorghum-cowpea composite biscuits comprised 270 g sorghum flour, 180 g cowpea flour, 112 g sugar, 132 g sunflower oil, 3 g baking powder, 27 g vanilla essence and 103.5 g water (the formulation was based on that developed by Serrem, 2011). The lower amount of water used to make sorghum-cowpea composite biscuits was based on preliminary experiments, which showed that doughs with added cowpea flour required less water to form a firm dough.
Table 4.1 Composition of whole grain sorghum and whole grain sorghum-cowpea composites biscuits

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Sorghum: Cowpea ratio</th>
<th>Sorghum</th>
<th>Sorghum: Cowpea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole grain sorghum flour (g)</td>
<td>100:00</td>
<td>450 (51.0)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>270 (32.6)</td>
</tr>
<tr>
<td>Whole grain cowpea flour (g)</td>
<td>100:00</td>
<td>0</td>
<td>180 (21.8)</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>100:00</td>
<td>112 (12.7)</td>
<td>112 (13.5)</td>
</tr>
<tr>
<td>Sunflower oil (g)</td>
<td>100:00</td>
<td>132 (15.0)</td>
<td>132 (16.0)</td>
</tr>
<tr>
<td>Baking powder (g)</td>
<td>100:00</td>
<td>3 (0.3)</td>
<td>3 (0.4)</td>
</tr>
<tr>
<td>Vanilla essence (g)</td>
<td>100:00</td>
<td>27 (3.1)</td>
<td>27 (3.2)</td>
</tr>
<tr>
<td>Water (g)</td>
<td>100:00</td>
<td>157.5 (17.9)</td>
<td>103.5 (12.5)</td>
</tr>
<tr>
<td><strong>Total dough weight (g)</strong></td>
<td>100:00</td>
<td>881.5 (100)</td>
<td>827.5 (100)</td>
</tr>
</tbody>
</table>

<sup>1</sup>Figures in brackets are percentages of total dough weight.

**Biscuit preparation**

The sugar and the sunflower oil were creamed starting at minimum speed and gradually increased at a speed of 3 for three minutes using an electric mixer (Kenwood Electronic Chef Excel, Maraisburg, South Africa) with a k-beater attachment (Fig 4.1). The dry ingredients (flour and baking powder) were mixed in a bowl, and then added to the creamed mixture. This was then mixed for 1 minute, and then vanilla essence and water were added and mixed for 3 minutes at a speed of 3 to obtain a firm dough. The total dough weight was divided into half, and then rolled manually using a wooden rolling pin on a lightly greased aluminium foil to a thickness of 5±1 mm. The sheeted dough was then cut into square shapes by pressing with a multi-square wire cutter (Fig 4.2) to obtain 35-48 biscuit dough pieces. Excess dough was removed and added to the next batch dough to be rolled. Each dough piece was then pricked with six holes using a knife to release moisture and prevent the biscuits from cracking during baking. The aluminium foil with the biscuit dough pieces were transferred onto a baking tray and baked in a pre-heated steam convection oven (Unox, Padova, Italy) for approximately 20±5 minutes at 190°C±5°C. The typical baked aroma of baked goods was used as indication that the biscuits were properly baked. After baking, the biscuits were cooled for 30 minutes at ambient temperature (25°C). The average weight of each biscuit was 10 g. After cooling, the biscuits were packaged in polyethylene zip-lock type of bags, and stored at 6°C. For the chemical analyses, the biscuits were first ground into flour using a mortar and pestle to a particle size ≤ 500 µm.
Cream sugar with sunflower oil and mix for 3 minutes at a low speed (3). Add to the creamed mixture mixed dry ingredients (450 g flour mixed with 3 g baking powder). Mix for 1 minute at a low speed.

Add vanilla essence and water and mix for 3 minutes to form a firm dough. Then roll half of the dough piece using a wooden rolling pin on a lightly greased aluminium foil to a thickness of 5±1 mm.

Cut the sheeted dough into square shapes by pressing with a multi-square wire cutter. Then, prick each biscuit dough piece with six holes using a knife.

Transfer the aluminium foil with the biscuit dough pieces by sliding it onto a baking tray. Then bake in a pre-heated convection oven for 20±5 minutes at 190°C±5°C.

After baking allow to cool for 30 minutes at ambient temperature.

Figure 4.1 Flow diagram for preparation of whole grain sorghum and whole grain sorghum-cowpea composite biscuits
The multi-square wire cutter (Fig 4.2) used to cut and shape the sheeted biscuit dough had stiff wires of 4 mm diameter. The length and the width of the whole cutter were 37.5 cm and 20 cm, respectively and it consisted of 28 single squares. Each square was approximately 50 × 50 mm.

Figure 4.2 Multi square cutter used to cut and shape the sorghum and sorghum-cowpea biscuits
4.2.2 Proximate analyses

**Moisture**

The moisture contents of the sorghum and cowpea flours, their composites, biscuits made from these flours and standard wheat biscuits was determined according to AACC International (2000) Method 44-15A (One stage). Accurately weighed samples of approximately 2 g were dried in a 103°C forced draught oven for 3 hours.

**Ash**

Ash (minerals) content was determined according to AACC International (2000) Method 08-01. Samples were heated at 550°C for 5 hours until the samples were uniform light grey.

**Protein**

Protein contents (N × 6.25) for sorghum and cowpea flours, their composites and biscuits made from these flours and (N × 5.70) for standard wheat biscuits were determined by a Dumas combustion method according to AACC International (2000) Method 46-30.

**Crude fat**

Fat contents of the above mentioned samples were determined by a Soxhlet extraction method according to AACC International(2000) Method 30-25. Samples were weighed into extraction thimbles and fat extraction was carried out for 1 hour using petroleum ether (40-60°C boiling point). The petroleum ether extract was then dried at 103°C in a forced draught oven for 30 minutes.

**Starch**

Total starch content of the above mentioned samples was determined according to the Amyloglucosidase/Amylose Method, K-TSTA 07/11 from Megazyme International (2011). Samples were cooked in presence of thermostable α-amylase, which hydrolysed the starch into maltodextrins followed by amyloglucosidase, which quantitatively hydrolyzed maltodextrins to D-glucose. The D-glucose produced was measured using glucose oxidase/peroxidase reagent.
**Dietary fibre**

The dietary fibre content of the flours was calculated by difference from the sum of the weights of ash, protein, fat and starch contents. For the biscuits, the dietary fibre content was determined by difference by summing up the weights as above plus that of added sugar.

**Energy**

Energy content was calculated using Atwater calorie conversion factors, based on the assumption that each gram of protein, fat and carbohydrate yield 17 kJ, 37 kJ and 17 kJ, respectively (FAO, 2003).

**4.2.3 In vitro assays for protein quality**

**Pepsin protein digestibility**

The pepsin protein digestibility assay was based on the method of Hamaker et al. (1986). Samples were digested with porcine pepsin P7000-100 G Pepsin (Sigma-Aldrich, St Louis, MO) for 2 hours at 37°C. The products of digestion were carefully pipetted off using a Pasteur pipette. The contents were then washed with distilled water, centrifuged and the clear supernatant pipetted off once again. The residues were dried at 100°C overnight in a forced draft oven and the protein content of the residual material was determined by Dumas combustion. In-vitro protein digestibility was calculated by the difference between the initial total weight of the protein and the residual weight of the protein after pepsin digestion, and expressed as percentage of the total protein.

**Multienzyme protein digestibility**

The multienzyme protein digestibility assay used was based on that of Hsu et al. (1977). Sample suspensions were adjusted to pH 8.0 with 0.1 M HCl and/or NaOH. A multienzyme solution was prepared containing bovine trypsin (T0303-1G), bovine chymotrypsin (C4129-1G) and protease (P5747-5G) from *Streptomyces griseus* (Sigma- Aldrich). The multienzyme solution was then added to food suspensions which were incubated at 37°C and the pH of the product of digestion recorded every 2 minutes for 10 minutes. In-vitro protein digestibility was calculated by using the equation of Hsu et al. (1977). \[ Y = 210.46 - 18.10x \]

where \( Y \) is the in vitro protein digestibility (%), \( x \) is the pH of the product of digestion after 10 minutes. The multienzyme solution was freshly prepared and its activity was standardized using casein, which has a known in vivo apparent digestibility.
4.2.4 Trypsin inhibitor activity

Trypsin inhibitor activity was determined according to AACC International (2001) Method 22-40. Sample extracts were prepared for 1 hour in distilled water and adjusted to pH 7.6 with tris buffer. The extracts were centrifuged and further diluted in distilled water, then mixed with the pre-warmed N α-Benzoyl-DL-arginine 4-nitroanalide hypochloride (BAPA) (Sigma-Aldrich) and porcine trypsin (Sigma-Aldrich). Exactly 10 minutes after incubation, the reaction was stopped by adding acetic acid (30 %, v/v) and the absorbance read at 410 nm against the blank. Trypsin inhibitor activity was expressed as one trypsin inhibitor unit (TIU).

Since the plot TIU/ml extract versus volume of portions of extract analysed did not give a linear correlation, then TIU/g sample was calculated by multiplying the averaged values obtained for each volume extract TIU/ml with the dilution factors. A defatted sample of whole grain soybean was used as a standard.

4.2.5 Total phenolics

A modified Folin-Ciocalteu method was used (Waterman and Mole, 1994). The method is based on the reducing power of the phenolic hydroxyl groups, which react with the Folin-Ciocalteu phenol reagent to form chromogens that can be detected spectrophotometrically. Phenolic extracts were prepared in 30 ml acidified methanol (1% concentrated HCl in methanol, v/v) from 3 g samples. Centrifuged extracts were mixed with Folin-Ciocalteu phenol reagent and sodium carbonate (20%, w/v) solution was added within 2 minutes from addition of the phenolic reagent. The contents were left to stand for 2 hours, after which absorbance was read at 760 nm. Catechin was used as a standard.

4.2.6 Instrumental colour analysis

The colour of biscuit samples was measured using a CR 210 Minolta Chromameter model CR-400(Osaka, Japan) and the recorded using the L* a* b* colour system. The Chromameter was calibrated using a standard white plate. Three readings of the L* a * and b* values were taken at three positions on the top sides and middle side of three randomly selected biscuits from each treatment.

4.2.7 Instrumental textural analysis

Textural analysis of biscuit samples was performed using a TA.XT2 Texture Analyser (Stable Microsystems, Godalming, UK). The maximum peak force during first compression
(hardness) and the distance compressed before breaking (fracturability) were measured using a three point bend rig (HDP/3PB) attachment, comprising an upper blade and one rig base plate with two adjustable supports adjusted to 28 mm. A vertical force was applied using the upper blade on a biscuit placed horizontally like a bridge over the two supports at a cross-head pre-test speed of 1.0 mm/sec, test speed of 3.0 mm/sec, post-test speed of 10.0 mm/sec, distance of 7 mm. The maximum peak force and the distance compressed before breaking a single biscuit were measured. An average of 3 replicate analyses were performed and reported for each treatment. The fracture properties of the biscuits were further determined using the following:

$$\sigma = \frac{3FL}{2bh^2}, \quad \varepsilon = \frac{6h}{L^2}$$ (Batsavias and Jurgens, 1997)

Where $\sigma$ is the Stress at midpoint (Mpa), $\varepsilon$ is the Strain, $F$ the force at the beam centre (N), $L$ is the distance between the supports (mm), $b$ is the biscuit width (mm) and $h$ is the biscuit thickness (mm). Stress was expressed as kPa and Strain as percentage.

### 4.2.8 Consumer evaluation

A consumer study was carried out in a lecture hall at the Mamelodi Campus of the University of Pretoria, South Africa. Low income consumers were randomly selected from the local community (Mashigo, 2012) based on their interest to participate in this study. A total of 97 consumers (74% female and 23% male), ranging between 18 to 55 years participated in the study. Six biscuit samples were presented at a time to each consumer, each one in a closed zip-lock type of bag 100 mm × 80 mm, labeled with three-digit random numbers, at ambient temperature (approximately 25°C). Water was served as a palate cleanser to rinse in between samples. Consumers were asked to evaluate the biscuit samples and answer a check-all-that-apply (CATA) question with 38 terms related to sensory characteristics of biscuits. The terms were a combination of aroma, texture and flavour, and these were generated from previous research by Anyango (2009), Serrem (2011) and Kayitesi et al. (2012). Consumers were asked to select all the attributes they considered to describe best each biscuit. Consumers were also asked to score their overall liking on a 9-point hedonic scale, with dislike extremely as 1 and like extremely as 9, and additionally to score their intent to buy on a 5-point hedonic scale, with 1 as “Definitely would not buy”, and 5 as “Definitely would buy”, as indicated in the evaluation form (Table 4.2).
Table 4.2 Sensory evaluation form used by consumers to evaluate whole grain sorghum, whole grain sorghum-cowpea composite and wheat biscuits

Biscuit: 懔懔懔懔

1. Before eating, smell and select/tick all the attributes that you think describe this biscuit.

- Baked biscuit
- Butter
- Sweet
- Heated oil
- Mabele (sorghum)
- Plant/grass
- Beany
- Spices
- Herbs

2. Taste and select/tick all the attributes that you think describe this biscuit.

- Rough
- Soft
- Hard
- Crunchy
- Dense
- Coarse
- Moist
- Dry
- Crumbly
- Chewy
- Bitter
- Burnt
- Nutty
- Roasted
- Soy, Pronutro
- Sweet
- Vanilla
- Caramel
- Ginger
- Cinnamon
- Coconut
- Spices
- Herbs
- Medicinal
- Doughy
- Beany
- Peanut butter
- Bland
- Gritty, grainy, sandy
Table 4.2 Continued

3. How much do you like or dislike this biscuit? Select the number that describes best your opinion.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dislike extremely</td>
<td>Like extremely</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. If this biscuit was available in your local shop, will you buy it or not? Select the number that describes best your opinion.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Definitely would not buy</td>
<td>Probably would not buy</td>
<td>Might or might not buy</td>
<td>Probably would buy</td>
<td>Definitely would buy</td>
</tr>
</tbody>
</table>
4.2.9 Data analysis

One way analysis of variance (ANOVA) was used to analyse the effect of compositing on the nutritional quality, the instrumental, as well as the consumer overall liking and their intent to buy of the biscuits. Means for all analyses were compared at p < 0.05 using Fisher’s Least Significant Difference (LSD) test.

In order to identify groups of consumers with similar preference patterns, a cluster analysis using k-means using the within group variance was performed on the overall liking data (Tomlins et al., 2005). Frequency of mention of each term of the CATA question was determined by counting the number of consumers who used that term to describe each biscuit sample. To evaluate whether the CATA question was able to detect differences in the consumers’ perception of each biscuit, Friedman’s test was performed for each term, considering biscuit sample and consumer as source of variation (Lado et al., 2010). To describe the correspondence between the evaluated biscuits and consumers perception, Correspondence Analysis (CA) was performed on the frequency tables containing the response to the CATA question (Valentin and Abdi, 2012). Consumer average overall liking scores, average intent to buy scores, colour and instrumental textural analyses were also considered. Statistica Software version 11.0 (StatSoft, Tulsa, USA) was used for ANOVA, Fisher’s test, k-means cluster analysis, and CA. XLSTAT Software version 8.0 (Addinsoft, New York City, USA) was used for Friedman’s test.
4.3 RESULTS AND DISCUSSION

4.3.1 Proximate composition

As expected, biscuit baking drastically reduced the moisture contents of the biscuits compared to their corresponding flours (Table 4.3) due to water evaporation because of the high temperature used (190°C). The moisture content of wheat biscuits was nearly four times higher than that of the sorghum and sorghum-cowpea composite biscuits. This may have been due to the nature of low density polyethylene bag which was used as the packaging material. Such packaging is a poor moisture barrier (Barnetson, 1996), and may have led to moisture absorption of the wheat biscuits over time. Also, as the wheat biscuit bag was not completely sealed once opened, this probably allowed additional moisture absorption.

The ash (minerals) content of cowpea flour was more than twice to that of sorghum flours (Table 4.3). The high value in cowpea is due to its higher levels of iron, potassium, calcium and sodium than sorghum (USDA, 2008). Compositing sorghum flours with cowpea flour resulted in them being intermediate in mineral content. Biscuit making resulted in a substantial decrease of the mineral contents of the sorghum and the sorghum-cowpea composite biscuits compared to their corresponding flours. This was due to the addition of 16.4% sunflower and 13.9% sugar, which diluted the minerals. Despite the decrease of mineral content, the mineral content of the composite biscuits was 27-29% higher than that of sorghum biscuits, indicating that whole grain cowpea addition improved further the mineral content. The mineral contents of wheat biscuit standards were 37% lower compared to that of sorghum-cowpea composite biscuits. This is because both whole grain sorghum and whole grain cowpea have a higher mineral content compared to refined wheat flour (USDA, 2008).

The protein content of cowpea flour was more than twice of the sorghum flours (Table 4.3). This is because cowpea seeds are pulses (Oohmah et al., 2011). Similar results have been found by other workers. Lima et al. (2004) reported that the cowpea protein content was approximately 25 g/100 g. Compositing sorghum flours with cowpea flour resulted in them being intermediate in protein content. Similar findings were reported by Pelembe et al. (2002), where the authors found a proportional increase in protein content of extruded sorghum cowpea composite instant porridge with increasing amount of added cowpea. Furthermore, Anyango et al. (2011b) found that compositing sorghum with cowpea at a ratio of 70:30 increased the protein contents of traditional sorghum foods by up to 57%.
Biscuit making resulted in substantial decrease of the protein content of the biscuits compared to their corresponding flours. This was due to the inclusion of sunflower oil and sugar, which diluted the protein. However, the protein contents of the sorghum-cowpea composite biscuits were 50-60% higher than that of the sorghum biscuits due to addition of the cowpea. The sorghum-cowpea composite and wheat biscuits did not differ significantly with respect to their protein content. This is because wheat has higher protein content than sorghum (USDA, 2008).

The daily protein requirements for children of age of 3, 4 to 6 and 7 to 10 years old are 13, 17 and 26 g protein per day, respectively (WHO/FAO/UNU Expert Consultation, 2007). The protein content of sorghum biscuits fortified with cowpea at a ratio of 60:40 was 9 g/100g, suggesting that 7, 8 and 14, respectively of such biscuits of 10 g each would provide 50% of the children daily protein requirement and the rest may be provided in other foods. The number of sorghum-cowpea biscuits required to deliver 50% of the daily protein requirement is 3-7 times higher than that suggested by Serrem et al. (2011) working on sorghum-soy biscuits. The reason for this is due to the 50% protein content of DSF used and the smaller size of the biscuits.
Table 4.3 Proximate composition of whole grain sorghum and cowpea flours, their composites and biscuits made from these flours (g/100 g)

<table>
<thead>
<tr>
<th>Flours/Biscuits</th>
<th>Composite ratio</th>
<th>Moisture</th>
<th>Ash</th>
<th>Protein (^2)</th>
<th>Fat</th>
<th>Starch</th>
<th>Dietary fibre (^3)</th>
<th>Energy (^4) (kJ/g 100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cereal grains</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White sorghum</td>
<td>100:0</td>
<td>12.27 (\pm) 0.11</td>
<td>1.59 (\pm) 0.06 (1.82)</td>
<td>7.60 (\pm) 0.20 (8.67)</td>
<td>3.04 (\pm) 0.05 (3.46)</td>
<td>65.0 (\pm) 1.7 (74.1)</td>
<td>11.9</td>
<td>1535</td>
</tr>
<tr>
<td>Red sorghum</td>
<td>100:0</td>
<td>10.52 (\pm) 0.06</td>
<td>1.29 (\pm) 0.11 (1.45)</td>
<td>8.96 (\pm) 0.05 (10.01)</td>
<td>3.21 (\pm) 0.01 (3.59)</td>
<td>65.5 (\pm) 2.6 (73.2)</td>
<td>11.8</td>
<td>1547</td>
</tr>
<tr>
<td><strong>Legume seed</strong></td>
<td></td>
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</tr>
<tr>
<td>Cowpea</td>
<td>0:100</td>
<td>11.60 (\pm) 0.04</td>
<td>3.45 (\pm) 0.07 (3.91)</td>
<td>19.78 (\pm) 0.12 (22.37)</td>
<td>0.92 (\pm) 0.01 (1.04)</td>
<td>39.6 (\pm) 1.6 (44.8)</td>
<td>27.9</td>
<td>1180</td>
</tr>
<tr>
<td><strong>Composite flours</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>White sorghum/Cowpea</td>
<td>60:40</td>
<td>12.02 (\pm) 0.07</td>
<td>2.47 (\pm) 0.05 (2.81)</td>
<td>12.99 (\pm) 0.07 (14.76)</td>
<td>2.39 (\pm) 0.10 (2.71)</td>
<td>54.8 (\pm) 1.8 (62.2)</td>
<td>17.5</td>
<td>1409</td>
</tr>
<tr>
<td>Red sorghum/Cowpea</td>
<td>60:40</td>
<td>11.02 (\pm) 0.07</td>
<td>2.36 (\pm) 0.15 (2.66)</td>
<td>13.17 (\pm) 0.03 (14.80)</td>
<td>2.12 (\pm) 0.11 (2.39)</td>
<td>55.2 (\pm) 1.2 (62.1)</td>
<td>18</td>
<td>1396</td>
</tr>
<tr>
<td><strong>Sorghum biscuits</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>White sorghum</td>
<td>100:0</td>
<td>1.71 (\pm) 0.05</td>
<td>1.34 (\pm) 0.03 (1.36)</td>
<td>5.21 (\pm) 0.05 (5.30)</td>
<td>20.55 (\pm) 0.06 (20.91)</td>
<td>45.9 (\pm) 0.2 (46.7)</td>
<td>11.8</td>
<td>1658</td>
</tr>
<tr>
<td>Red sorghum</td>
<td>100:0</td>
<td>1.72 (\pm) 0.03</td>
<td>1.11 (\pm) 0.04 (1.31)</td>
<td>6.06 (\pm) 0.02 (6.16)</td>
<td>20.39 (\pm) 0.39 (20.75)</td>
<td>44.2 (\pm) 2.7 (45.0)</td>
<td>13.3</td>
<td>1637</td>
</tr>
<tr>
<td><strong>Composite biscuits</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White sorghum/Cowpea</td>
<td>60:40</td>
<td>1.31 (\pm) 0.01</td>
<td>1.73 (\pm) 0.03 (1.76)</td>
<td>8.51 (\pm) 0.11 (8.61)</td>
<td>20.66 (\pm) 0.11 (20.93)</td>
<td>40.6 (\pm) 0.4 (41.2)</td>
<td>13.6</td>
<td>1621</td>
</tr>
<tr>
<td>Red sorghum/Cowpea</td>
<td>60:40</td>
<td>1.61 (\pm) 0.01</td>
<td>1.65 (\pm) 0.01 (1.67)</td>
<td>9.08 (\pm) 0.11 (9.23)</td>
<td>21.77 (\pm) 0.12 (21.11)</td>
<td>39.8 (\pm) 0.2 (40.4)</td>
<td>13.7</td>
<td>1625</td>
</tr>
<tr>
<td><strong>Wheat biscuits standards</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Short bread</td>
<td></td>
<td>6.10 (\pm) 0.03</td>
<td>1.20 (\pm) 0.01 (1.28)</td>
<td>8.93 (\pm) 0.16 (9.51)</td>
<td>15.06 (\pm) 0.04 (16.04)</td>
<td>39.1 (\pm) 0.3 (41.7)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Original</td>
<td></td>
<td>6.29 (\pm) 0.04</td>
<td>1.15 (\pm) 0.03 (1.22)</td>
<td>7.92 (\pm) 0.14 (8.45)</td>
<td>18.07 (\pm) 0.07 (19.29)</td>
<td>40.1 (\pm) 0.7 (42.8)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

\(^{1}\)Values are mean ± standard deviation, (mean on dry basis). \(n = 3\). Values in a column followed by different letter superscripts are significantly different (\(p < 0.05\)).

\(^{2}\)Calculated using \(N \times 6.25\) for sorghum and cowpea and \(N \times 5.70\) for wheat.

\(^{3}\)Calculated by difference [for flours = 100-(ash + protein + fat + starch) and for biscuits = 100-(ash + protein + fat + starch + sugar)].

\(^{4}\)Calculated using the following conversion factors: protein 17 kJ/g, fat 37 kJ/g and carbohydrates 17 kJ/g.

\(^{5}\)NA: Not Available.
The fat contents of the sorghum flours were more than twice that of the cowpea flour (Table 4.3). Sorghum grain has a fat content of approximately 3.6 g/100g (Serna-Saldivar and Rooney, 1995), whereas the fat content in the whole seed cowpea is approximately 1.4 g/100 g (Phillips, 1982). Biscuit making greatly increased the fat content of the sorghum and sorghum-cowpea composite biscuits. This was due to the inclusion of the sunflower oil. The energy requirements for 3 to 5 and 6 to 10 year olds based on FAO/WHO (1985) are 2370 kJ to 4742 kJ and 2650 kJ to 5300 kJ, respectively. Thus, seven to 14 sorghum-cowpea biscuits of 10 g each would provide 1211 kJ to 2422 kJ, which is approximately half of the required kilojoules for 3 to 10 year olds.

The starch content of sorghum flour was substantially higher than that of cowpea flour (Table 4.2). This is because cereals primarily store their energy in form of starch (Serna-Saldivar and Rooney, 1995). The starch content of sorghum biscuits was higher than that of the composite biscuits. This was due to the dilution effect of the cowpea flour. The starch content of the sorghum-cowpea composite biscuits was slightly lower than that of wheat biscuits, due to the cowpea in the composite biscuits.

Cowpea had more than double of the dietary fibre than that of sorghum (Table 4.3), due to its higher dietary fibre (USDA, 2008). The dietary fibre content of the composite flours was intermediate. Biscuit making decreased the dietary fibre content of all sorghum biscuits, due to the dilution effect of the fat and sugar. The dietary fibre content of the sorghum-cowpea biscuits was slightly higher than that of sorghum biscuits, presumably because of the dietary fibre from whole grain cowpea.

4.3.2 In-vitro protein digestibility

The in-vitro pepsin protein digestibility (IVPD) of the cowpea flour was substantially higher than that of sorghum flours (Table 4.4). This can be attributed to the presence of the more soluble globulin proteins (Chan and Phillips, 1994). The pepsin IVPD of whole grain 100% WTP flour and biscuits was slightly higher than of whole grain 100% RNT flour and biscuits. This is possibly due to higher phenolics content in the RNT flour (Table 4.7). The phenols may have interacted with proteins (as reviewed by Slavin, 2004), forming complexes, which are less susceptible to proteolytic attack, thereby decreasing protein digestibility. Compositing the
sorghum flours with cowpea flour resulted in them being intermediate in pepsin IVPD. This finding is similar to that of Anyango et al. (2011b) working on the effect of compositing sorghum with cowpea on the in-vitro protein digestibility of traditional sorghum foods. Biscuit making decreased the pepsin IVPD of the 100% sorghum biscuits by half, whereas that of the sorghum-cowpea biscuits decreased to a lesser extent. The pepsin IVPD of sorghum-cowpea biscuits was 71-81% higher than that of 100% sorghum biscuits. This is presumably because the cowpea globulins prevented disulphide cross-linking, which occurs with sorghum kafirin proteins (Duodu et al., 2003). However, the pepsin IVPD of the sorghum and sorghum-cowpea biscuits was 211% and 76% lower than that of the wheat biscuits, respectively. Mertz et al. (1986) also reported that the protein digestibility of sorghum was approximately 25% lower than that of wheat. The lower protein digestibility of the sorghum biscuits is presumably due to the kafirin cross-linking (Duodu et al., 2003).
Table 4.4 The pepsin in-vitro protein digestibility of whole grain sorghum and cowpea flours, their composites and biscuits made from these flours

<table>
<thead>
<tr>
<th>Cereal grains</th>
<th>Composite ratio</th>
<th>Flours</th>
<th>Biscuits</th>
</tr>
</thead>
<tbody>
<tr>
<td>White sorghum</td>
<td>100:0</td>
<td>74.4&lt;sup&gt;i&lt;/sup&gt; ± 0.6&lt;sup&gt;1&lt;/sup&gt;</td>
<td>33.9&lt;sup&gt;b&lt;/sup&gt; ± 0.9</td>
</tr>
<tr>
<td>Red sorghum</td>
<td>100:0</td>
<td>65.4&lt;sup&gt;e&lt;/sup&gt; ± 4.0</td>
<td>27.8&lt;sup&gt;a&lt;/sup&gt; ± 0.9</td>
</tr>
<tr>
<td><strong>Legume seed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cowpea</td>
<td>0:100</td>
<td>94.8&lt;sup&gt;i&lt;/sup&gt; ± 0.2</td>
<td>NA&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Composites</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White sorghum/Cowpea</td>
<td>60:40</td>
<td>85.9&lt;sup&gt;b&lt;/sup&gt; ± 0.8</td>
<td>58.0&lt;sup&gt;d&lt;/sup&gt; ± 0.0</td>
</tr>
<tr>
<td>Red sorghum/Cowpea</td>
<td>60:40</td>
<td>79.6&lt;sup&gt;e&lt;/sup&gt; ± 2.3</td>
<td>50.3&lt;sup&gt;c&lt;/sup&gt; ± 0.1</td>
</tr>
<tr>
<td><strong>Wheat biscuit standards</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shortbread</td>
<td></td>
<td>96.4&lt;sup&gt;i&lt;/sup&gt; ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td></td>
<td>95.4&lt;sup&gt;i&lt;/sup&gt; ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are mean ± standard deviation, n = 2. Means followed by different letter superscripts are significantly different (p < 0.05).

<sup>2</sup>NA: Not Available.
The multi-enzyme IVPD of cowpea was higher than that of sorghum (Table 4.5), as found with the pepsin IVPD assay (Table 4.4). As stated, this is because of the presence of more soluble globulin proteins in cowpea (Chan and Phillips, 1994). As with pepsin IVPD, biscuit making decreased the multi-enzyme IVPD of all sorghum and sorghum-cowpea biscuits. The multi-enzyme IVPD of the sorghum and cowpea flours, their composites, the resulting biscuits and the wheat biscuits was lower than that of casein standard. This could be due to the presence of other non-protein compounds, such as starch, fibre, fat and minerals. Such compounds may have hindered the proteolytic enzymes. This can prevent their access to the protein and reducing their hydrolysis (Damodaran, 1996). The multi-enzyme IVPD of the sorghum and sorghum-cowpea biscuits were lower than that of wheat biscuits. As with pepsin IVPD, this could have been due to the kafirin cross-linking (Duodu et al., 2003).

The multi-enzyme IVPD of the sorghum flours and all the biscuits was higher (Table 4.5) than that of pepsin IVPD (Table 4.4). This could be due to the use of three proteolytic enzymes (trypsin, chymotrypsin and protease) rather than pepsin alone. Similar findings were reported by Hsu et al. (1977) while developing the multi-enzyme technique for estimating protein digestibility. The authors found that the multi-enzyme preparation created the largest pH drop after a 10 min hydrolysis which correlated to high digestibility, while the individual enzymes created the least drop. The higher digestibility of the multi-enzyme preparation may be due to the different specificities of the three enzymes used (Boisen and Eggum, 1991). These enzymes may have hydrolyzed several different peptide bonds compared to the use of a single enzyme which attacks a specific peptide bond (Hsu et al., 1977). Pepsin stimulates hydrolysis at peptide bonds involving the aromatic amino acids, such as phenylalanine, tryptophan and tyrosine. On the other hand, when partially broken down protein products enter the small intestine, trypsin hydrolyzes the peptide linkages involving groups of arginine and lysine, chymotrypsin acts on linkages involving carboxyl ends of phenylalanine, tyrosine and tryptophan, and carboxypeptidase which in this study was used as protease, has two forms A and B. Carboxypeptidase A hydrolyzes terminal residues that possess aromatic and aliphatic linkages and the B form acts on terminal arginine or lysine (Medeiros and Wildman, 2000).

The multi-enzyme IVPD of cowpea flour was lower than that of pepsin IVPD. Similarly Hsu et al. (1977) found that when excessive amounts of trypsin inhibitor were added to a casein
suspension, trypsin activity was greatly reduced. This suggests that the trypsin inhibitors present in the raw cowpea (Table 4.6) may have bound to the trypsin enzyme reducing its activity and the protein digestibility.
**Table 4.5 Multi-enzyme in-vitro protein digestibility of whole grain sorghum and cowpea flours, their composites and biscuits made from these flours**

<table>
<thead>
<tr>
<th>Cereal grains</th>
<th>Composite ratio</th>
<th>Flours</th>
<th>Biscuits</th>
</tr>
</thead>
<tbody>
<tr>
<td>White sorghum</td>
<td>100:0</td>
<td>73.1(^b) ± 0.9(^l)</td>
<td>70.9(^a) ± 1.0</td>
</tr>
<tr>
<td>Red sorghum</td>
<td>100:0</td>
<td>71.7(^a) ± 0.9</td>
<td>75.0(^bc) ± 0.8</td>
</tr>
</tbody>
</table>

**Legume seed**

<table>
<thead>
<tr>
<th>Legume seed</th>
<th>Composite ratio</th>
<th>Flours</th>
<th>Biscuits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cowpea</td>
<td>0:100</td>
<td>78.4(^e) ± 0.6</td>
<td>NA(^2)</td>
</tr>
</tbody>
</table>

**Casein standard**

<table>
<thead>
<tr>
<th>Casein standard</th>
<th>Flours</th>
<th>Biscuits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>85.9(^f) ± 2.4</td>
<td></td>
</tr>
</tbody>
</table>

**Composites**

<table>
<thead>
<tr>
<th>Composites</th>
<th>Composite ratio</th>
<th>Flours</th>
<th>Biscuits</th>
</tr>
</thead>
<tbody>
<tr>
<td>White sorghum/Cowpea</td>
<td>60:40</td>
<td>74.2(^bc) ± 2.3</td>
<td>73.9(^b) ± 0.4</td>
</tr>
<tr>
<td>Red sorghum/Cowpea</td>
<td>60:40</td>
<td>76.2(^c) ± 1.0</td>
<td>74.4(^bc) ± 0.9</td>
</tr>
</tbody>
</table>

**Wheat biscuit standards**

<table>
<thead>
<tr>
<th>Wheat biscuit standards</th>
<th>Flours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Shortbread</td>
<td></td>
</tr>
<tr>
<td></td>
<td>77.7(^c) ± 1.1</td>
</tr>
<tr>
<td>Original</td>
<td></td>
</tr>
<tr>
<td></td>
<td>78.9(^ed) ± 1.1</td>
</tr>
</tbody>
</table>

\(^1\) Values are mean ± standard deviation, n = 3. Means followed by different letter superscripts are significantly different (p < 0.05).

\(^2\) NA: Not applicable.
4.3.3 Trypsin inhibitor activity

Table 4.6 shows that there was no trypsin inhibitor activity in the sorghum flours and their biscuits and the wheat biscuits. This finding is similar to very low levels of trypsin inhibitor activity reported by Filho (1974) in extracts of sorghum grain powder. There was a low level trypsin inhibitor activity in the cowpea flour (6857 TIU/g), which was some thirteen-fold less than that of defatted soybean flour (86588 TIU/g) used as a standard. It has been reported that cowpea contains lower trypsin inhibitor activity than soy (Ologhobo and Fetuga, 1983). Compositing whole sorghum with cowpea resulted in no detectable trypsin inhibitor activity in the composite flours, and the resulting biscuits, probably due to dilution of the already low level of trypsin inhibitor in cowpea by the sorghum.
Table 4.6 The trypsin inhibitor activity (TIU) of whole grain sorghum and cowpea flours, their composites and biscuits made from these flours

<table>
<thead>
<tr>
<th>Cereal grains</th>
<th>Composite ratio</th>
<th>Flours</th>
<th>Biscuits</th>
</tr>
</thead>
<tbody>
<tr>
<td>White sorghum</td>
<td>100:0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Red sorghum</td>
<td>100:0</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Legume seed**

| Cowpea                      | 0:100           | \(6857 \pm 1330\)\(^1\) | NA\(^3\) |

**Soy flour standard**

| Defatted raw soybean        | 0:100           | \(86588 \pm 3514\)   | NA\(^3\) |

**Composites**

| White sorghum/Cowpea       | 60:40           | ND\(^2\)            | ND       |
| Red sorghum/Cowpea         | 60:40           | ND                  | ND       |

**Wheat biscuit standards**

| Shortbread                 |                 | ND                  |          |
| Original                   |                 | ND                  |          |

\(^1\)Values are mean ± standard deviation.  \(n = 3\).
\(^2\)ND: Not detected.
\(^3\)NA: Not applicable.
4.3.4 Total phenolics

Red non tannin flour had substantially higher total phenolics than the white tan-plant variety (Table 4.7). This is due to the higher anthocyanin content of the red sorghum bran (reviewed by Awika and Rooney, 2004) than the white tan-plant sorghum, which is very low in phenols. The total phenolic content of the cowpea flour was twice than that of the sorghum flour, indicating that cowpea is richer in phenolics than sorghum. Similar results showing that cowpea is rich in phenolics were reported by Kayitesi (2013). By compositing sorghum with cowpea, the total phenolic content of the resulting composite flours and biscuits increased by at least 50%. Biscuit making reduced the total phenolic contents by nearly 50% in all sorghum and sorghum-cowpea composite biscuits compared to their corresponding flours. This is in part due to the dilution effect by the sugar and fat ingredients. In addition, the sorghum and cowpea phenolics may have complexed with cell wall materials (Awika et al., 2003), forming insoluble complexes that could not be extracted and measured. Although the total phenolic contents of all sorghum and sorghum-cowpea composite biscuits decreased upon biscuit making, the phenolic content of the composite biscuits was 30-45% higher than that of sorghum biscuits. Also, the phenolic content of sorghum-cowpea composite biscuits was 70% higher than that of wheat biscuits. This was due to the fact that sorghum and cowpea were used as whole grains and the phenolics are highly concentrated in the outer layers of the grain, the pericarp, seed coat and aleurone layers (Dykes and Rooney, 2007; Makgope, 2007; Sikwese and Duodu, 2007). In contrast, when wheat is milled into white flour, only the endosperm is used and it is separated from the bran and germ (Kent and Evers, 1994).
Table 4.7 The total phenolic content of whole grain sorghum and cowpea flours, their composite and biscuits made from these flours

<table>
<thead>
<tr>
<th>Cereal grains</th>
<th>Composite ratio</th>
<th>Flours</th>
<th>Biscuits</th>
</tr>
</thead>
<tbody>
<tr>
<td>White sorghum</td>
<td>100:0</td>
<td>239 ± 1 (273)(^{d,l})</td>
<td>196 ± 6 (199)(^{b})</td>
</tr>
<tr>
<td>Red sorghum</td>
<td>100:0</td>
<td>299 ± 2 (334)(^{f})</td>
<td>229 ± 2 (233)(^{c})</td>
</tr>
<tr>
<td><strong>Legume seed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cowpea</td>
<td>0:100</td>
<td>668 ± 26 (755)(^{h})</td>
<td>NA(^{2})</td>
</tr>
<tr>
<td><strong>Composites</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White sorghum/Cowpea</td>
<td>60:40</td>
<td>503 ± 7 (571)(^{g})</td>
<td>286 ± 6 (289)(^{de})</td>
</tr>
<tr>
<td>Red sorghum/Cowpea</td>
<td>60:40</td>
<td>508 ± 8 (571)(^{g})</td>
<td>299 ± 5 (304)(^{f})</td>
</tr>
<tr>
<td><strong>Wheat biscuit standards</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short bread</td>
<td></td>
<td></td>
<td>172 ± 3 (183)(^{ab})</td>
</tr>
<tr>
<td>Original</td>
<td></td>
<td></td>
<td>155 ± 5 (165)(^{a})</td>
</tr>
</tbody>
</table>

\(^{1}\) Values are mean ± standard deviation (mean on dry basis-db), n = 3. Means on db followed by different letter superscripts in a column are significantly different (p < 0.05)

\(^{2}\) NA: Not applicable
4.3.5 Instrumental colour analysis

Red sorghum biscuits had lower L* values (darker) and higher a* values (redness) than white sorghum biscuits (Table 4.8). Similar results were reported by Chiremba et al. (2009), comparing red non-tannin to white non-tannin cultivars. The lower lightness and higher redness can be attributed to the pigmentation that occurs in the pericarp of the red non-tannin cultivars (reviewed by Awika and Rooney, 2004). As expected, the sorghum-cowpea composite biscuits were slightly lighter with decreased redness than the sorghum biscuits. No trend was observed in change in b* values of the composite biscuits. Sorghum and sorghum-cowpea biscuits were darker and had higher b* values than the wheat biscuits. This was related to the pericarp pigmentation from whole grains sorghum. Also, the higher lightness of the wheat biscuits may be due to the use of only wheat endosperm (Kent and Evers, 1994).
Table 4.8 Colour of whole grain sorghum and whole grain sorghum-cowpea composite biscuits

<table>
<thead>
<tr>
<th>Colour parameters</th>
<th>Sorghum biscuits</th>
<th>Composite ratio</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>White sorghum</td>
<td>100:0</td>
<td></td>
<td>50.4&lt;sup&gt;b&lt;/sup&gt; ± 0.2&lt;sup&gt;1&lt;/sup&gt;</td>
<td>6.6&lt;sup&gt;b&lt;/sup&gt; ± 0.0</td>
<td>17.7&lt;sup&gt;b&lt;/sup&gt; ± 0.2</td>
</tr>
<tr>
<td>Red sorghum</td>
<td>100:0</td>
<td></td>
<td>48.7&lt;sup&gt;a&lt;/sup&gt; ± 0.4</td>
<td>12.5&lt;sup&gt;f&lt;/sup&gt; ± 0.3</td>
<td>16.4&lt;sup&gt;a&lt;/sup&gt; ± 0.4</td>
</tr>
<tr>
<td><strong>Composite biscuits</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White sorghum/Cowpea</td>
<td>60:40</td>
<td></td>
<td>55.5&lt;sup&gt;d&lt;/sup&gt; ± 0.1</td>
<td>5.4&lt;sup&gt;c&lt;/sup&gt; ± 0.3</td>
<td>18.8&lt;sup&gt;c&lt;/sup&gt; ± 0.4</td>
</tr>
<tr>
<td>Red sorghum/Cowpea</td>
<td>60:40</td>
<td></td>
<td>53.5&lt;sup&gt;c&lt;/sup&gt; ± 0.5</td>
<td>8.6&lt;sup&gt;c&lt;/sup&gt; ± 0.1</td>
<td>17.2&lt;sup&gt;ab&lt;/sup&gt; ± 0.3</td>
</tr>
<tr>
<td><strong>Wheat biscuit standards</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short bread</td>
<td></td>
<td></td>
<td>66.3&lt;sup&gt;e&lt;/sup&gt; ± 0.8</td>
<td>10.7&lt;sup&gt;e&lt;/sup&gt; ±0.5</td>
<td>31.1&lt;sup&gt;e&lt;/sup&gt; ± 0.8</td>
</tr>
<tr>
<td>Original</td>
<td></td>
<td></td>
<td>66.8&lt;sup&gt;e&lt;/sup&gt; ± 0.2</td>
<td>9.3&lt;sup&gt;d&lt;/sup&gt; ±2.4</td>
<td>29.7&lt;sup&gt;d&lt;/sup&gt; ± 0.5</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are mean ± standard deviation, n = 3. Means followed by different letter superscripts in the same column are significantly different (p < 0.05).

L*: indicates lightness where 0 = darkness and 100 = lightness.

a*: indicates redness where –a* = greenness and +a* = redness.

b*: indicates yellowness where –b* = blue and +b* = yellow.
4.3.6 Instrumental textural properties

Sorghum biscuits made with baking margarine were extremely soft (Fig 4.3 and Table 4.9), which was also indicated by their open structure (Fig 4.4A). In contrast, sorghum biscuits made with cooking sunflower oil were strong, which was indicated by their compact structure (Fig 4.4B). The weakness of the biscuits made with margarine was due to the baking margarine entrapping air bubbles during creaming (Campbell and Mougeot, 1999). Consequently, as sorghum is gluten-free, its dough was not able to expand to form a permanent network during baking, and instead resulted in a soft biscuit with an open structure. Biscuits made from the red sorghum were nearly twice as strong as those from white sorghum biscuits, as indicated by the higher stress (Table 4.9). This can be attributed to the texture of the red sorghum grain. Adetunji (2012) found that red non-tannin sorghum grain was more corny than white tan-plant sorghum variety. In addition, the hardness of the biscuits made from red sorghum may be due to the interaction of the anthocyanins with proteins through hydrophobic interactions, as described by Hagerman and Butler (1981). Sorghum-cowpea composite biscuits showed a high wide range of stress and were generally stronger than the wheat biscuits. This could be due to the soluble globulin protein in cowpea. McWatters et al. (2003) found that biscuits made from 50% fonio/50% cowpeas were stronger than 100% wheat biscuits. It was suggested that the soluble globulins of cowpea with fonio components may have contributed to the stronger texture during dough development and baking. The sorghum and sorghum-cowpea composite biscuits showed lower strain than the wheat biscuits. This is probably due to the absence of gluten in both sorghum and cowpea.
Figure 4.3 Typical Force-distance graphs showing the effect of the baking margarine and cooking sunflower oil on the texture of biscuits made from white sorghum

Sorghum biscuits made with cooking sunflower oil
Sorghum biscuits made with Baking margarine

Figure 4.4 Influence of (A) baking margarine and (B) cooking sunflower oil on the physical structure of biscuits made from white sorghum compared to that of (C) wheat biscuits
Table 4.9 Texture properties of whole grain sorghum and sorghum-cowpea composite biscuits

<table>
<thead>
<tr>
<th>Texture parameters</th>
<th>Sorghum biscuits</th>
<th>Composite ratio</th>
<th>Hardness (N)</th>
<th>Fracturability (mm)</th>
<th>Stress (kPa)</th>
<th>Strain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White sorghum made with margarine(^1)</td>
<td>100:0</td>
<td>1.6(^a) ± 0.0(^3)</td>
<td>0.5(^a) ± 0.0</td>
<td>32.6(^a) ± 0.4</td>
<td>1.9(^a) ± 0.9</td>
<td></td>
</tr>
<tr>
<td>White sorghum(^2)</td>
<td>100:0</td>
<td>7.2(^b) ± 0.2</td>
<td>0.6(^ab) ± 0.2</td>
<td>417.3(^i) ± 9.9</td>
<td>2.6(^ab) ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Red sorghum(^2)</td>
<td>100:0</td>
<td>17.3(^c) ± 6.2</td>
<td>0.9(^cd) ± 0.1</td>
<td>767.8(^e) ± 61.0</td>
<td>2.9(^ab) ± 0.3</td>
<td></td>
</tr>
</tbody>
</table>

**Composite biscuits**

| Composite ratio | White sorghum/Cowpea\(^2\) | 60:40 | 30.8\(^d\) ± 0.1 | 1.1\(^de\) ± 0.2 | 1616.9\(^f\) ± 52.2 | 3.8\(^b\) ± 0.8 |
| Red sorghum/Cowpea\(^2\) | 60:40 | 18.9\(^c\) ± 0.4 | 0.8\(^bc\) ± 0.1 | 627.3\(^d\) ± 20.6 | 2.9\(^ab\) ± 0.6 |

**Wheat biscuit standards**

<table>
<thead>
<tr>
<th>Wheat biscuit standards</th>
<th>Short bread</th>
<th>Original</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23.6(^d) ± 1.1</td>
<td>36.4(^d) ± 0.0</td>
</tr>
<tr>
<td></td>
<td>1.7(^f) ± 0.0</td>
<td>1.3(^e) ± 0.0</td>
</tr>
<tr>
<td></td>
<td>278.3(^b) ± 17.3</td>
<td>412.9(^c) ± 79.3</td>
</tr>
<tr>
<td></td>
<td>12.9(^d) ± 0.3</td>
<td>9.1(^c) ± 0.7</td>
</tr>
</tbody>
</table>

\(^2\) Biscuits made with cooking sunflower oil

\(^3\) Values are mean ± standard deviation, n = 3. Means followed by different letter superscripts in the same column are significantly different (p < 0.05).
4.3.7 Consumer evaluation

The mean overall consumer liking scores of the evaluated biscuits ranged from 4.5 to 7.1 on a 9-point hedonic scale (Table 4.10). The sorghum and sorghum-cowpea biscuits did not differ significantly and their average overall liking scores were positioned in the middle part of the 1-9 hedonic scale, corresponding to biscuits that were neither liked nor disliked. The most liked biscuits were the wheat shortbread and original biscuits, types that are commonly consumed by children and adults, with an average overall liking score of 7. Similarly, in a study Sabbe et al. (2009) investigated the overall liking among consumers who are not familiar with açai-based fruit juices (i.e. juices produced from small black-purple coloured berries grown in Brazil). The authors found that the juices with higher açai concentrations had lower overall liking than those with lower concentrations. It was suggested that the unique and unfamiliar flavour of açai was assumed to be more pronounced and perceived by consumers, which resulted in lower overall liking scores. This suggests that the unique and unfamiliar flavour of the sorghum and sorghum-cowpea biscuits could probably be the reason of their lower overall liking scores. Additionally, the darker colour of sorghum and sorghum-cowpea biscuits than wheat biscuits (Table 4.8) possibly contributed to their lower overall liking scores.

Significant differences were found in the consumers’ intent to buy. The mean intent to buy ranged from 2.9 to 4.2 on a 5-point scale. The sorghum biscuits showed the lowest mean intent to buy scores, indicating that consumers’ willingness to buy them is less. Sorghum-cowpea biscuits had a slightly higher average intent to buy scores than sorghum biscuits, which was positioned in the middle part of the 1-5 scale, meaning that consumers might or might not buy such biscuits. The wheat biscuits had the highest intent to buy scores, in agreement with their higher average overall liking scores. The relative frequency for the intent to buy gave similar results 10-12%, 14-15% and 23-25% for 100% sorghum, sorghum-cowpea and wheat biscuits, respectively. While studying the female intent to buy for both familiar and unfamiliar cheeses, Arvola et al. (1999) reported that neophobic consumers (consumers with tendency to avoid novel foods) rated the unfamiliar cheese lower. It was suggested that such consumers avoid novel foods. Because the sorghum and sorghum-cowpea biscuits were unfamiliar to consumers, this could possibly be the reason for their lower scores of intent to buy compared to wheat biscuits. However, the slightly higher intent to buy of the
white sorghum-cowpea biscuits may be due to the familiar nutty flavour perceived by a group of consumers as indicated in Table 4.10 and Fig 4.5.
### Table 4.10 Mean consumers’ overall liking and intent to buy of the sorghum, sorghum-cowpea and wheat biscuits (n = 97 consumers)

<table>
<thead>
<tr>
<th>Biscuits</th>
<th>Composite ratio</th>
<th>Overall liking&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Intent to buy&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sorghum biscuits</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White sorghum</td>
<td>100:0</td>
<td>4.7&lt;sup&gt;a&lt;/sup&gt; (3.0)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2.9&lt;sup&gt;a&lt;/sup&gt; (1.4)</td>
</tr>
<tr>
<td>Red sorghum</td>
<td>100:0</td>
<td>4.5&lt;sup&gt;a&lt;/sup&gt; (3.1)</td>
<td>2.9&lt;sup&gt;a&lt;/sup&gt; (1.4)</td>
</tr>
<tr>
<td><strong>Composites</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White sorghum/cowpea</td>
<td>60:40</td>
<td>5.1&lt;sup&gt;a&lt;/sup&gt; (2.9)</td>
<td>3.3&lt;sup&gt;b&lt;/sup&gt; (1.4)</td>
</tr>
<tr>
<td>Red sorghum/cowpea</td>
<td>60:40</td>
<td>4.7&lt;sup&gt;a&lt;/sup&gt; (3.0)</td>
<td>3.1&lt;sup&gt;ab&lt;/sup&gt; (1.4)</td>
</tr>
<tr>
<td><strong>Wheat biscuit standards</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short bread</td>
<td></td>
<td>6.8&lt;sup&gt;b&lt;/sup&gt; (2.6)</td>
<td>4.1&lt;sup&gt;c&lt;/sup&gt; (1.1)</td>
</tr>
<tr>
<td>Original</td>
<td></td>
<td>7.1&lt;sup&gt;b&lt;/sup&gt; (2.3)</td>
<td>4.2&lt;sup&gt;c&lt;/sup&gt; (1.0)</td>
</tr>
</tbody>
</table>

<sup>1</sup>Overall consumer liking scores evaluated on a 9-point hedonic scale (where 1 = dislike extremely and 9 = like extremely)

<sup>2</sup>Intent to buy evaluated on a 5-point scale (where 1 = definitely would not buy and 5 = definitely would buy)

<sup>3</sup>Values are means (standard deviations). Different letters within a column for overall liking and intent to buy indicate significance differences (p< 0.05)
Using k-means cluster analysis, a grouping method that considers individual overall liking (Tomlins et al., 2005), the overall liking of the 97 consumers varied and four different groups of consumers with similar liking of the biscuits were identified (Fig 4.5). The liking of each biscuit differed significantly (\(p<0.001\)) within different clusters. The largest cluster of consumers (cluster 4) represented by 25% consumers liked moderately to very much original and shortbread wheat biscuits, but disliked sorghum and sorghum-cowpea biscuits. The next largest group, cluster 3 with 23% consumers liked moderately to extremely all biscuits. Cluster 1 with 21% consumers disliked all biscuits with exception of the original wheat biscuits, which they neither liked nor disliked. Cluster 2 comprised the smallest group of consumers (18%) and they liked moderately to very much the sorghum-cowpea biscuits and wheat biscuits, respectively.

As indicated in Figure 4.5, most consumers (66%) from the three clusters (2, 3 and 4) liked the shortbread and original wheat biscuits. A study by Tuorila et al. (2001) relating responses to familiar and unfamiliar foods, found that most consumers consume food designed to be familiar. This may explain the high liking of wheat biscuits. In contrast, an interesting result from the clustering is that there were two clusters (clusters 2 and 3), with a substantial percentage of consumers (41%) that liked the sorghum-cowpea biscuits. As stated above, with wheat biscuits the high liking of sorghum-cowpea biscuits by certain consumers was possibly due to their familiarity with sorghum (mabele) and cowpea (dinawa). Additionally, a possible explanation could be that these consumers liked the nutty flavour (Table 4.11 and Figure 4.5), which they associated to sorghum-cowpea biscuits. Yadav et al. (2012) reported that wheat biscuit fortified with 15% de-oiled peanut meal flour were highly acceptable by consumers. As reviewed by Singh and Singh (1991), peanuts have a distinct pleasant aroma and nutty flavour that places them above all other legumes.
Figure 4.5 Mean consumers’ overall liking by clusters comprised of consumers with similar liking pattern for the sorghum, sorghum-cowpea and wheat biscuits. Vertical bars = ± standard deviation. Scores were evaluated on a 9-point hedonic scale (where 1 = dislike extremely and 9 = like extremely)
Table 4.11 shows frequencies in which each term/attribute of the check-all-that-apply (CATA) question was used to describe the evaluated biscuits. The most frequently used aroma terms were baked biscuit, sweet, butter, heated oil and mabele (sorghum). The most used texture and flavour terms used were hard, dry, crunchy, rough, chewy and mabele (sorghum) flavour and sweet flavour. The least used flavour terms were medicinal, doughy, spice and herbs.

Significant differences were found in the frequencies of 22 out of the 38 terms of the CATA question, mainly for terms related to the flavour of the biscuits. For example, consumers were able to distinguish differences in the sensory characteristic of different type of biscuits in terms of butter aroma, sweet aroma, mabele (sorghum) aroma, rough texture, hard texture, nutty flavour and beany flavour. This indicated that consumers perceived differences in the sensory characteristics of the sorghum, sorghum-cowpea and wheat biscuits. Lado et al. (2010) and Ares et al. (2010) also found that consumers were able to differentiate terms of the CATA question when evaluating new strawberry cultivars and chocolate milk desserts, respectively. However, no significant differences between the biscuits were found for baked biscuits aroma, plant/grass aroma, crunchy texture, dense texture, crumbly texture, chewy texture, bitter flavour, burnt flavour, roasted flavour, soy, Pronutro flavour (an instant porridge made of cereal and soy), caramel flavour, medicinal flavour and doughy flavour. It may be that biscuits did not truly differ with regards to these attributes or that consumers may have not understood their meaning to differentiate between the biscuits. Terms like baked biscuit aroma, crunchy texture and chewy texture were used for almost all biscuits, which indicates that these particular attributes were common for all the biscuits.
Table 4.11 Frequency of mention for each term of check-all-that-apply (CATA) question determined by counting the number of consumers who used that term to describe each biscuit evaluated (n = 97 consumers)

<table>
<thead>
<tr>
<th>Terms</th>
<th>Sorghum biscuits</th>
<th>Composite biscuits (sorghum/cowpea)</th>
<th>Standard wheat biscuits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole grain white sorghum</td>
<td>Whole grain red sorghum</td>
<td>Whole grain white sorghum/Cowpea</td>
</tr>
<tr>
<td>Aroma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baked biscuits n.s.</td>
<td>73</td>
<td>72</td>
<td>74</td>
</tr>
<tr>
<td>Butter *</td>
<td>42</td>
<td>36</td>
<td>47</td>
</tr>
<tr>
<td>Sweet *</td>
<td>43</td>
<td>39</td>
<td>46</td>
</tr>
<tr>
<td>Heated oil *</td>
<td>39</td>
<td>42</td>
<td>32</td>
</tr>
<tr>
<td>Mabele (sorghum) *</td>
<td>46</td>
<td>47</td>
<td>40</td>
</tr>
<tr>
<td>Plant/grass n.s.</td>
<td>13</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Beany *</td>
<td>11</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td>Spices *</td>
<td>9</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Herbs *</td>
<td>15</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Texture and flavour</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rough *</td>
<td>52</td>
<td>51</td>
<td>44</td>
</tr>
<tr>
<td>Soft *</td>
<td>4</td>
<td>12</td>
<td>43</td>
</tr>
<tr>
<td>Hard *</td>
<td>72</td>
<td>66</td>
<td>32</td>
</tr>
<tr>
<td>Crunchy n.s.</td>
<td>47</td>
<td>46</td>
<td>50</td>
</tr>
<tr>
<td>Dense, thick, compact n.s.</td>
<td>20</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>Moist n.s.</td>
<td>9</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Dry *</td>
<td>72</td>
<td>68</td>
<td>57</td>
</tr>
<tr>
<td>Crumbly n.s.</td>
<td>27</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>Chewy n.s.</td>
<td>31</td>
<td>35</td>
<td>37</td>
</tr>
<tr>
<td>Bitter n.s.</td>
<td>10</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Burnt n.s.</td>
<td>16</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Terms</td>
<td>Sorghum biscuits</td>
<td>Composites biscuits (sorghum/cowpea)</td>
<td>Standard wheat biscuits</td>
</tr>
<tr>
<td>------------------------</td>
<td>------------------</td>
<td>-------------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td></td>
<td>Whole grain white sorghum</td>
<td>Whole grain red sorghum</td>
<td>Whole grain white sorghum/Cowpea</td>
</tr>
<tr>
<td>Mabele (sorghum) *</td>
<td>43</td>
<td>45</td>
<td>34</td>
</tr>
<tr>
<td>Nutty *</td>
<td>12</td>
<td>18</td>
<td>41</td>
</tr>
<tr>
<td>Roasted n.s.</td>
<td>26</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td>Soy, Pronutro n.s.</td>
<td>21</td>
<td>14</td>
<td>22</td>
</tr>
<tr>
<td>Sweet *</td>
<td>27</td>
<td>41</td>
<td>43</td>
</tr>
<tr>
<td>Vanilla *</td>
<td>18</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>Caramel n.s.</td>
<td>11</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>Ginger *</td>
<td>10</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Cinnamon *</td>
<td>11</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Coconut n.s.</td>
<td>22</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Spices n.s.</td>
<td>7</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Herbs *</td>
<td>9</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>Medicinal n.s.</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Doughy (dough) n.s.</td>
<td>11</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Beany (beans) *</td>
<td>11</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Peanut butter *</td>
<td>17</td>
<td>16</td>
<td>27</td>
</tr>
<tr>
<td>Bland (no taste) *</td>
<td>20</td>
<td>23</td>
<td>15</td>
</tr>
<tr>
<td>Gritty, grainy, sandy *</td>
<td>23</td>
<td>24</td>
<td>27</td>
</tr>
</tbody>
</table>

1n.s. means no significant difference (p ≥ 0.05).
2 * indicates significant difference (p < 0.05) according to Friedman’s test.
3 Pronutro: an instant porridge made of cereal and soy.
Correspondence Analysis (CA) was carried out only on attributes with significant differences for both the aroma, texture and flavour terms from the CATA counts (Table 4.11). Figure 4.6 shows the representation of the six evaluated biscuit types in the first two CA dimensions with respect to the aroma terms. The first two dimensions of the CA accounted 94% of the variance in the aroma of the biscuits. Dimension 1 explained 75% of the total variation and separated the biscuits based on the type of cereal with the sorghum and sorghum-cowpea biscuits to the right and wheat biscuits to the left. Dimension 2 represented 19% of the total variation and separated biscuits based on the presence or absence of cowpea. Also, dimension 2 clearly separated shortbread from original wheat biscuits. The amount of variation accounted in dimension 2 did not have a significant explanatory variation for the sorghum and sorghum-cowpea composite biscuits. These biscuits correlated to the beany, heated oil and mabele attributes, indicating that both sorghum and sorghum-cowpea had similar aroma characteristics. According to Lawless and Heyman (2010) aroma is better perceived through the dual role of the olfaction system, by smelling through the external sensory system and in the mouth through internal sensory system, where the aroma rises and passes up into the nasal cavity from the rear direction. This may explain that smelling the biscuits alone did not reveal much of the difference between sorghum and sorghum-cowpea biscuits. The shortbread wheat biscuits was characterised by butter and sweet aroma attributes, and these were negatively correlated with beany and mabele (sorghum) attributes. The spices and herbs aroma attributes were the most discriminating and related to original biscuits. The difference between the two wheat biscuits may be due to the difference in their formulation.
Figure 4.6 Representation of the biscuits (sorghum, sorghum-cowpea and wheat standards) and the aroma terms in the first two dimensions of the correspondence analysis (CA) performed on consumers’ responses to check-all-that-apply (CATA) questions
Figure 4.7 shows the representation of the six biscuit types evaluated with respect to the flavour and texture terms, as well as the texture and colour instrumental, average overall liking and intent to buy data. The first two dimensions of the CA accounted for 87% of the variance in the flavour and texture of the biscuits. Dimension 1 explained 64% of the total variation and separated the biscuits based on the type of cereal, with the wheat biscuits to the left and the sorghum and sorghum-cowpea biscuits to the right. Dimension 2 explained a further 23% of the total variation and in contrast to aroma separated biscuits based on presence or absence of cowpea. Sorghum biscuits on the second dimension corresponded to hard, rough, dry, mabele (sorghum), gritty and bland texture and flavour characteristics. Badi and Hoseney (1976) similarly reported that sorghum biscuits had a hard texture compared to wheat biscuits and thus was attributed in part to the lack of polar lipids in sorghum. The gritty and rough characteristic of sorghum biscuits was also reported by Badi and Hoseney (1976), Chiremba et al. (2009) and Serrem et al. (2011). The grittiness in sorghum biscuits was presumably due to the high temperature of sorghum starch gelatinisation, leaving more starch ungelatinised (as reviewed by Taylor et al., 2006). Serrem et al. (2011) also noted the dry texture of the sorghum biscuits. This may be due to the hydrophobic nature of kafirin proteins of the endosperm (Duodu et al., 2003), which possibly expelled most of the water as the temperature increased during baking.

Sorghum-cowpea biscuits on the second dimension corresponded particularly with the nutty flavour. As indicated in Table 4.11 and Figure 4.7, consumers also related the beany and peanut butter flavour to the sorghum and sorghum-cowpea biscuits. Consumers may have perceived the beany and peanut butter flavour in both the sorghum and sorghum-cowpea biscuits due to unfamiliarity with such biscuits. The beany flavour, also described as peanut butter, by consumers in this study is associated with legume foods and has been reported by other researchers. Anyango et al. (2011a) reported a beany flavour in sorghum ugali (thick porridge) made with cowpea. Also, Serrem et al. (2011) reported that biscuits made with soy were characterised by a beany flavour. In another study, Mohsen (2009) identified furans in wheat cookies substituted with soy protein isolate (SPI). This flavour is possibly attributable to products of autoxidation of linolenic acid that are responsible for the beany flavour in legumes (furans) released as volatiles compounds during baking, which reduced the beany flavour and gave a perception of a nutty or peanut butter flavour (Chang, 1979).
Ginger and herbs flavours discriminated original wheat biscuits from wheat shortbread biscuits. In contrast, vanilla flavour discriminated the short bread wheat biscuits from the original wheat biscuits as well as the sorghum and sorghum-cowpea biscuits (Figure 4.7 and Table 4.11). The separation of the two wheat biscuit types by consumers based on their flavours was probably due to different ingredients used in their formulations.

Using CA, some relationships between sensory characteristics and instrumental variables were identified. The colour attributes L* and b* were related the wheat biscuits, whereas the a* was related to the red sorghum biscuits. These results were in agreement with the instrumental data (Table 4.8). No correlation was observed between the instrumental variable hardness and the CATA texture attribute hard. This was probably due to the difference of how the hard texture was perceived by the instrument and consumers. Hardness as measured instrumentally is defined as the maximum peak force during the first compression, i.e. the force required to snap a biscuit (first bite) (Stable Microsystems, Godalming, UK), which was found to be higher in the wheat biscuits as indicated in Table 4.9. However, the hard texture attribute as described by consumers is not only described by the first bite but rather by a combination of other oral tactile sensations, among which ingestion by lips and chewing of the biscuits are important to describe their texture (Lawless and Heyman, 2010). During ingestion the lips of the consumers may have signalled the gritty texture of the sorghum biscuits and perceived them as hard. According to Lawless and Heyman (2010), hardness is also positively correlated with the length of chewing cycles and buttering decreases the number of chewing cycles prior to swallowing.

From Figure 4.7 vanilla, sweet and cinnamon attributes were positively correlated with overall liking and intent to buy. Also, the instrumental data, lightness (L*), yellowness (b*) and fracturability correlated with overall liking and buying. These attributes may possibly be the reason the highest liking of the wheat biscuits, and could thus be considered as drivers of overall liking and intent to buy.
Figure 4.7 Representation of the biscuits (sorghum, sorghum-cowpea, wheat standards) and the texture and flavour terms in the first two dimensions of the correspondence analysis (CA) performed on consumers’ responses to check-all-that-apply (CATA) questions, including instrumental data, consumers’ overall liking and intent to buy data. Texture terms: rough, soft, hard and dry. Flavour terms: mabele (sorghum), nutty, sweet, vanilla, ginger, cinnamon, herbs, beany (beans), peanut butter, bland and gritty. Instrumental data included texture and colour data. Instrumental data: hardness (force), fracturability, stress and strain. Colour data: L*, a* and b*.
4.4 CONCLUSIONS

Sorghum-cowpea composite biscuits have improved nutritional quality in terms of protein content, mineral content, in-vitro protein digestibility, and have no trypsin inhibitor activity. In addition, the composite biscuits have a high phenolic content. Correspondence Analysis (CA) shows that variation in terms of aroma, texture and flavour attributes is due to type of cereal and the presence of cowpea in biscuits. Standard wheat biscuits are the most liked by consumers. However, cluster analysis shows a substantial high percentage of consumers that liked sorghum-cowpea composite biscuits.

These findings show that whole grain sorghum-cowpea biscuits could well serve as an acceptable high quality protein-rich complementary value-added food to improve child nutrition and alleviate PEM in rural areas of sub-Saharan Africa where sorghum and cowpea crops are produced and consumed as staples.
5 GENERAL DISCUSSION

This chapter is divided into two sections. The first section will critically discuss the methodologies used and the second section will evaluate the major findings, the potential for whole grain sorghum-cowpea biscuits and transfer of technology to communities in rural areas of Africa.

5.1 Critical review of the methodologies

Two types of non-tannin sorghum, white tan-plant and red non-tannin were used in this study to compare their effects on the nutritional and sensory characteristics of biscuits. Red sorghum varieties are known to have agronomic advantages because they contain anthocyanans (Kambal and Bate-Smith, 1976), which makes birds dislike such sorghums. Despite their agronomic advantage, non-tannin red sorghums have been reported to have low protein digestibility (Axtell et al., 1981). This was also shown in this study where all red sorghum flours and biscuits yielded slightly lower pepsin and multienzyme IVPD than the white sorghum counterparts (Table 4.4 and 4.5). These results suggest that biscuits made from red sorghum have slightly lower nutritional value compare to ones from white sorghum.

Sorghum and cowpea were intentionally used as whole grain to replicate the simple manner of milling technology which is used in many rural areas of Africa. In addition, as reviewed in Section 2.1 whole grain has high nutritional and health benefits compared to refined grain, suggesting that biscuits made from whole grain should be highly nutritious and healthy. However, such as whole grain, refined sorghum and cowpea flours and biscuits were not compared in this study. This would have provided a direct comparison concerning the nutritional and sensory characteristics of the whole grain and refined flours and biscuits.

Other researchers, Schober et al. (2003) and Chiremba et al. (2009) reported sticky dough during gluten-free bread and sorghum biscuit production, respectively. The use of sunflower oil instead of baking margarine in the biscuit recipe had a very beneficial effect, enhancing dough firmness and enabling the production of whole grain sorghum-cowpea biscuits that are much less fragile. It would have been useful to have systematically analysed the causes of the improved biscuit texture.
Consumer evaluation was used to determine the sensory characteristics, consumer acceptability and intent to buy of sorghum and sorghum-cowpea biscuits compared to existing commercial economic wheat biscuits. Consumers used in this study were low-income consumers between the ages of 18 and 55 years. This age group was chosen because consumers in this range do not require parental consent and ethical approval, which are required with children. Although the consumers represented the target socio-economic group, a drawback was that the biscuits were targeted for children and should have been tested by them to provide the best representation of their choices. Notwithstanding this issue it is known that what children eat is influenced by their parents (reviewed by Heather et al., 2005). This suggests that if parents are aware of the nutritional benefit of these biscuits, it would impart positively on the children’s liking and consuming of the biscuits. Additional problem with sensory evaluation by children is that they show a wide range of cognitive abilities and attention spans, which should be taken into consideration when preparing food sensory testing with them, as it may be more time-consuming and expensive than traditional sensory tests with adult consumers (reviewed by Guinard, 2001).

The low level of English literacy among some of the consumers was a limitation because it was observed that some of the consumers might not have fully understood the task. Thus mistakes could have been made when they were evaluating. This problem probably did not affect the overall finding because of the large number of consumers used in the study. However, for future studies where such low-income consumers are used for sensory testing, translation of the evaluation form in the vernaculars language may be more valuable. In addition, the use of visual animations for demonstration of the evaluation task could be helpful. Six biscuit samples, 38 CATA attributes and liking and buying intention was an intensive evaluation for consumers, and in the future studies fewer samples and attributes would be more efficient.

Evaluation was carried out in a conference hall, which had benefits and drawbacks compared to a sensory laboratory. The hall was advantageous as consumers did not feel isolated because they could see everyone and this probably helped them to be relaxed. The major problem was that the consumers were not separated enough from each other and some may have looked at their neighbour’s answer. For future studies, it would better to ensure enough space between consumers and create a more natural environment.
5.2 Potential production of whole grain sorghum-cowpea biscuits and transfer of technology to local communities

As stated (Chapter 1), a major cause of PEM among children is consumption of starchy foods that have low protein content and quality. Whole grain sorghum-cowpea biscuits are relatively rich in protein and minerals and the inclusion of oil in these biscuit gives them a high energy density (Table 4.3). In addition, their protein digestibility is high compared to 100% whole grain sorghum biscuits (Table 4.4 and 4.5). Therefore, whole grain sorghum-cowpea biscuits have potential to alleviate PEM. According WHO/FAO/UNU Expert Consultation (2007), children of the age of 3, 4 to 6 and 7 to 10 year old require 13, 17 and 26 g protein per day, respectively. To provide at least half of the required protein 7, 8 and 14 biscuits of 10 g each per day would be needed by 3, 4 to 6 and 7 to 10 year olds, respectively. The energy requirements for 3 to 5 and 6 to 10 year olds based on FAO/WHO (1985) are 2370 kJ to 4742 kJ and 2650 kJ to 5300 kJ, respectively. Thus, seven to 14 biscuits of 10 g each would provide 1211 kJ to 2422 kJ, which is approximately half of the required kilojoules for 3 to 10 year olds. These intakes could be achieved through school feeding programmes by serving 2 to 3 biscuits as a morning snack, after lunch and afternoon snack. However a challenge to school feeding programmes is that at risk children in rural areas do not attend school (WFP, 2004).

In order to reach all children, especially those in need, training of mothers to use the simple biscuit making technology developed in this study may provide the solution. The procedure to make whole grain sorghum-cowpea biscuits is straight forward, uses inexpensive equipment and locally produced and readily available ingredients. Table 5.1 shows the estimated cost of whole grain sorghum-cowpea biscuits compared to that of existing commercial economic wheat biscuits. The cost of ingredients including manufacturing for production of 1 kg whole grain sorghum-cowpea biscuits is half the cost of 1 kg of the wheat biscuits. Thus, the 50% difference can be considered as a profit for the biscuit producer. According to the South African Labour Department (2012), the wage of the least paid domestic worker is roughly SA Rand 100 per day. This may translate to the income for a smallholder farmer in a rural area. To make a profit of SA Rand 100 per day, if the profit is SA Rand 35.36 per kg of biscuits, then he or she would need to make and sell 3 kg of biscuits. Since one biscuit weighs 10 g, he or she would need to produce 300 biscuits per day, which is feasible with the use of a multi square wire-cutter (Fig 4.2).
The wheat shortbread and original biscuits, which are commonly consumed, were most liked by consumers. Compositing biscuits with 40% whole grain cowpea resulted in a beany flavour described as nutty or peanut butter by the consumers. However, as explained, the cluster analysis revealed a substantial percentage (41%) of consumers who liked the sorghum-cowpea biscuits. Serrem et al. (2011) using DSF-composite biscuits reported that biscuits with 50% DSF were acceptable to school age children. As indicated, a reason for this could be that furans (products of autoxidation of linolenic acid that are responsible for the beany flavour in legumes) (Chang, 1979) were released as volatiles compounds during baking, which reduced the beany flavour and gave a perception of a nutty or peanut butter flavour. In addition, the vanilla essence may have masked the beany flavour.

To improve the palatability of the sorghum-cowpea biscuits and further improve their protein quality, the inclusion of peanut should be investigated in future studies. Because peanut is rich in protein, 22-30% (Yadav et al., 2012) and oil, 50% (Raheja et al., 1987), it would probably contribute to more protein than cowpea and also reduce the amount of fat required in the biscuit formulation. As observed in this study, even with a 60% sorghum: 40% cowpea composite ratio, biscuit making substantially diluted the protein content of the composite biscuits compared to their corresponding flours. If peanut masks the cowpea flavour, it may be possible to use 50% cowpea to substantially improve the protein content of the resulting biscuits. This would have an advantage of reducing the number of biscuits needed to be eaten by children.
Table 5.1 Estimation of the cost in South African Rand of whole grain sorghum-cowpea biscuits compared to existing commercial economic wheat biscuits

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Cost whole grain sorghum-cowpea biscuits (75 biscuits per 830 g total weight dough)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum (270 g) @ R5/kg</td>
<td>1.35</td>
</tr>
<tr>
<td>Cowpea (180) @ R5/kg</td>
<td>0.9</td>
</tr>
<tr>
<td>Sugar (112 g) @ R12.50/kg</td>
<td>1.4</td>
</tr>
<tr>
<td>Sunflower oil (132 g) @ 17.50/kg</td>
<td>2.63</td>
</tr>
<tr>
<td>Baking powder (3 g) @ R10/100 g</td>
<td>0.3</td>
</tr>
<tr>
<td>Vanilla essence (27 g) @ R30/500 g</td>
<td>1.62</td>
</tr>
<tr>
<td><strong>Total cost</strong></td>
<td><strong>8.2</strong></td>
</tr>
<tr>
<td>Cost of 1 biscuit (10 g each)</td>
<td>0.11</td>
</tr>
<tr>
<td>Cost of 1 biscuit (6 g each) relating to the weight of 1 Shortbread or Original wheat biscuit</td>
<td>0.07</td>
</tr>
<tr>
<td>Cost of 200 g biscuits, if 1 biscuit is 6 g</td>
<td>2.3</td>
</tr>
<tr>
<td>Cost of 1 kg biscuits, if 1 biscuit is 6 g</td>
<td>11.5</td>
</tr>
<tr>
<td>Estimated cost of 1 kg biscuits including manufacturing cost¹ (cost of ingredient × 3)</td>
<td>34.5</td>
</tr>
<tr>
<td>Cost of 1 kg biscuits including manufacturing cost and 14% VAT</td>
<td>34.64</td>
</tr>
<tr>
<td>Cost of 200 g Shortbread or Original wheat biscuits²</td>
<td>14</td>
</tr>
<tr>
<td>Cost of 1 kg Shortbread or Original biscuits</td>
<td>70</td>
</tr>
</tbody>
</table>

¹ Cost of ingredients estimated as a third of estimated cost of manufacturing.
² Existing commercial economic wheat biscuits.
6 CONCLUSIONS AND RECOMMENDATIONS

Whole grain sorghum-cowpea flours and biscuits have improved nutritional quality in terms of protein content and quality, and mineral content compared to whole grain sorghum flours and biscuits. Protein digestibility of the composite flours and biscuits is high compared to that of whole grain sorghum flours and biscuits due to dilution of the less digestible sorghum kafirin proteins by the addition of more digestible globulin proteins from cowpea. Compositing whole grain sorghums with cowpea diluted the already low level of trypsin inhibitors in cowpea, resulting in no detectable trypsin inhibitor activity in the composite flours and biscuits. Studies on the amino acid composition of the biscuits are required in future to evaluate particularly the indispensable amino acid lysine. Whole grain sorghum-cowpea flours and biscuits have higher phenolic content compared to whole grain sorghum flours and biscuits, suggesting that such biscuits may not only be nutritious but also healthy. For further studies, refined sorghum and cowpea flours and biscuits may be investigated to provide direct comparison on the nutritional and sensory characteristics of the flours and biscuits.

Compositing whole grain sorghum with cowpea improved the strength of the composite biscuits due to soluble globulin protein from cowpea. In addition, the use of sunflower oil instead of baking margarine in biscuits, results in biscuits that are much less fragile. This has an advantage that it could allow biscuit production on large-scale and easy transport for a smallholder farmer producer, reducing the number of broken biscuits, thus increasing their shelf-life and making it a viable business. However, for further studies it would be useful to determine the causes of the improved texture of sorghum-cowpea composite biscuits with sunflower oil. In addition, determining shelf-life of such biscuits may also be useful for future studies.

The commercial wheat biscuits that are commonly consumed were most liked by consumers. However, cluster analysis, revealed a group of consumers who liked sorghum-cowpea biscuits. This is probably due to the volatile compounds released from furans during baking, that gave the perception of nutty or peanut butter flavour, which was preferred by a group of consumers. To improve the palatability by eliminating the beany flavour, the addition of peanut in the formulation should be considered. If peanut masks the cowpea flavour, the use of 50% cowpea to
substantially improve the protein content and protein digestibility should be investigated in future studies.

Therefore, whole grain sorghum-cowpea composite biscuits could well serve as an acceptable high quality protein-rich complementary food to improve child nutrition and alleviate PEM in rural areas of Africa. In addition, the estimated cost of production of these biscuits shows that they have potential as a viable business to generate income for smallholder farmers in rural areas of sub-Saharan Africa where sorghum and cowpea are produced and consumed as staples.
7 REFERENCES


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APPENDIX

Poster presentation from research:

Dovi, K.A.P., de Kock, H. L., Chiremba, C. & Taylor, J. R. N. Whole grain sorghum and whole grain cowpea biscuits as a complementary food for improved child nutrition. 20th SAAFoST Biennial Congress and Exhibition, Pretoria, South Africa.