5 GENERAL DISCUSSION

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

5.1 METHODOLOGIES

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

For consumer evaluation, the 100% sorghum and 50% DSF substituted sorghum biscuits were prepared using the basic procedure described in Chapter 4.1. However, based on the sensory characterization results, the flour was milled to a finer 500 µm particle size to improve the coarse and gritty texture and rough appearance of the biscuits. The limitation
with the change in particle size is that the dough became difficult to manage due to the reasons explained above, requiring double the amount of water, 18.1 and 33.2% per 225 g flour formulation for sorghum and sorghum-soy, respectively to make it workable. The higher water requirement of the re-milled flour can be attributed to increased surface area exposing more flour particles for interaction with water and increased damaged starch that absorbs high amounts of water (Bushuk 1998). This is comparable to forming damaged starch when milling bread wheat flour to increase its water absorption properties.

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

Differences in thickness, among the nine treatments of biscuits are a limitation because this may have introduced another source of variation to the descriptive sensory evaluation for crispness. The increase in crispness of biscuits with increase in soy as perceived by panelists may have been due to reduced thickness of biscuits as well. This is indicated by the fact that there was a significant panelist effect for this variable (Chapter 4.2, Table 4.2.5) and thickness was negatively correlated with protein content which increased when DSF was increased (Chapter 4.2, Figure 4.2.5). The effect of differences in thickness between treatments was eliminated for instrumental sensory evaluation values of biscuits because
method D 790-03 (ASTM International 2003), which was used to determine stress and strain, used formulae that took into account the thickness/height of each treatment.

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

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

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

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

Repeated exposure was used to determine long-term acceptability of biscuits over four days in eight sessions of two sessions a day. Consumer exposure tests normally consist of repeated consumption of products over several days or weeks (Wiejzen et al 2008). Studies with children have shown that repeated exposure can increase liking of food products. For instance, Birch and Marlin (1982) demonstrated that preference for cheese or new fruit increased with repeated exposure. Liking can also be reduced as reported by Hetherington et al (2002) for chocolate after 22 days of exposure. A drawback in the present study, however,
is that 4 days may have been too short to determine long-term acceptability by repeated consumption. Results showed that though all biscuits were moderately liked, there was no change in liking over time (Chapter 2, Figure 4.2.6) indicating that four days may have been inadequate to elicit change in liking. A better approach may have been to have one day of two sessions a week for 4 weeks. For instance, Sulmonte-Rossé et al (2008) exposed study participants to drinks over 24 times in six sessions of 30 minutes each but the interval between sessions was one week. Another reason for the lack of change over time is that the protocol was too involved and may have exceeded the children’s span of attention. According to ASTM International (2003), children of this age have a limited span of attention but have the capability to master complex tasks. It should, however, be appreciated that there are few documented studies that determine acceptability of food by children using repeated exposure and that the results in this study are similar to results from other studies using repeated exposure for staple foods, such as Hetherington et al (2002) for bread and butter and by Siegel and Pilgrim (1958) for dairy products and bread.

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

School children were used in this study to determine consumer acceptability of fortified biscuits as they are the target population. Eight to nine year old children were selected because they are considered semi-literate, most can read at this level, self administer hedonic scale questionnaires and are more discriminating than younger children (Kroll 1990). Additionally, as stated earlier, previous studies showed that children in this age group were consistent when using hedonic categorization (Leon et al 1999). However, the weakness of using this age range is that there is tremendous variation in skills among children of the same
age range (Conlin, Gathercole and Adams 2005). It has been shown that the age at which 10% of children can master a task compared to 90% of children doing the same task can vary by as much as four years (Popper and Kroll 2003). The study treated all children as equally intelligent, a factor that may have affected responses given by the slow learners.

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

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

When the study was restarted, the animals on the protein-free diet again lost weight very rapidly and had to be euthanized after 10 days. Their weight loss was higher than loss from the animals fed the sorghum-soy and casein diets. Therefore NPR values were negative for all the diets. Rats require at least 5% protein in their diet for maintenance (National Research Council 1995). Addition of a low level of protein in the protein-free diet may have reduced
their rate of weight loss. For instance, Mosha and Bennink (2004) instead of using a protein-
free diet used a low-protein diet with 20 g lactalbumin per kg diet to estimate the endogenous
nitrogen excretion of rats.

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

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

An important criticism with respect to determination of protein digestibility as done in this
investigation may be possible microbial modification of undigested and absorbed nitrogenous
residues in the rat large intestine. The Faecal Index method only accounts for nitrogen
consumed and voided in faeces but not the modifying effect of microbes in the hind gut of the
animal (Zhang, Qiao, Chen, Wang, Xing and Yin 2005). It has been demonstrated that the pattern of nitrogen excretion is modified by microflora in the hindgut for foods that have uncooked starch and undigested proteins (Beames and Eggum 1981) and sorghum foods because of the starch-kafrin complex (Bach Knudsen et al 1988a, Bach Knudsen et al (1988b). In this study it was clearly shown that the pure sorghum biscuit diet that could not support growth had a very high Biological Value (Chapter 4.3, Table 4.3.5) and Net Protein Utilization compared to the casein and sorghum-soy biscuit diets because no nitrogen was excreted in the urine. A possible approach would have been to analyze digesta collected at the end of the small intestine (terminal ileum) to increase sensitivity of the digestibility assay. Rutherfurd and Moughan (1998) used this method to determine digestibility of protein from milk and soy products by sampling digesta of Sprague-Dowley male rats at the end of the small intestine.

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

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

5.2 RESEARCH FINDINGS

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

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

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

FAO/WHO (1994) Recommendations

<table>
<thead>
<tr>
<th>Protein/Energy ratio</th>
<th>≥1674</th>
<th>≥15</th>
<th>≥0.65</th>
<th>≥0.70</th>
<th>≥1</th>
</tr>
</thead>
</table>

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

<sup>5</sup>Essential amino acids index (EAAI) = \( \sqrt[n]{\text{Product of the ratio of each EAA in the test food to the EAA of 3 to 10 year old children in reference pattern.}} \)
The results in this study established from the rat bioassay that 100% sorghum biscuits had zero PER, hence cannot support growth of rats and by extrapolation humans. It was also found that the effect of complementing sorghum with soy protein on growth is the same as the casein reference (Chapter 4.3, Table 4.3.3). The reason that sorghum protein did not support growth is the deficiency in lysine, which as explained earlier is a characteristic of all cereals (review, Chapter 2, Section 2.1.2.3). This was indicated by the low Relative Protein Efficiency Ratio value of 5% and 100% for sorghum and sorghum-soy diets, respectively. Waggle et al (1966) found that the deficiency of lysine in a high protein sorghum grain (11.8%) resulted in lower growth of rats than a low protein sorghum grain (7.9%) with higher lysine content. This indicates that deficiency of one essential amino acid is enough to cause the failure of an entire diet. This suggests that in developing countries where a single cereal staple can contribute 70 to 90% of total dietary protein (Lasztity 1984), incidences of PEM could be high. The ability of the sorghum-soy biscuits to support growth was due to increased lysine content in the biscuits (Chapter 4.1, Table 4.1.3). This indicates that the potential to enhance growth by sorghum and other cereals can be achieved through complementation with soy and other legumes.

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

The major reason for reduced protein digestibility of cooked sorghum foods is disulphide mediated polymerisation of sorghum proteins, making them less susceptible to enzymatic
attack (Hamaker et al 1987, Duodu et al 2003). This indicates that compositing sorghum with DSF is advantageous because higher digestibility of the proteins in the biscuits means the children can ingest higher amounts of high quality proteins with improved amino acid profiles and higher lysine and reactive lysine contents (Chapter 4.1, Tables 4.1.4 and 4.1.6).

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

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

Compositing with defatted soy flour at levels above 50% resulted in a beany flavour of the biscuits (Chapter 4.2, Table 4.2.5). The cause for beany flavour from soy products is due to autoxidation of linolenic acid to the cis and trans 2-(1-pentenyl) furan (Chang 1979). It should be noted that in studies where soy flour is used (McWaters 1978, Mashayekh et al...
2008, Mohsen et al 2009) food products were only acceptable at levels below 30%. Above this level, consumers reported an objectionable beany flavour. In this study biscuits with 50% DSF were acceptable to school age children. A reason for this is that during the baking process, it is likely that furans were released as volatiles which reduced the beany flavour. Mohsen et al (2009) identified two furans, 2-ethyl-5-methylfuran and 2-pentylfuran which are lipid derived volatile compounds from soy fortified wheat cookies. Another possible explanation is that flavouring with vanilla essence helped to mask the beany flavour so that a higher amount of DSF could be added to biscuits. Acceptability studies by Marrero, Payumo Aguinaldo and Homma (1988) using mung beans and cowpeas reported that consumers preferred gruels flavoured with fruit essence, vanilla, chocolate and ginger. Of importance to this study, is that it was possible to increase the protein density of biscuits by substituting 50% cereal flour with DSF without them being objectionable to school children.

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

For the bread wheat biscuits there was increased percentage stress (hardness) and reduced percentage strain (more brittle) as DSF increased (Chapter 4.2, Table 4.2.3 and 4.2.4). Evaporation of water in the high soy biscuits resulted in thinner biscuits that were more brittle. Another reason for increase in brittleness is weakening of the gluten network by replacement with soy protein as explained earlier (Chapter 4.2, section 4.2.3.2).
The findings from consumer acceptability of the fortified sorghum and bread wheat biscuits showed that biscuits were liked by school children and liking was sustained over 8 consumption occasions (Chapter 4.2, Table 4.2.7 and Figure 4.2.6). A reason for this is that biscuits are popular food products among children because they are sweet (Sudha et al 2007). Another reason is that as stated earlier (Chapter 4.2, section 4.2.3.5), soy protein imparted positive sensory characteristics associated with biscuits such as reduced hardness, density and chewiness and increased crispness in biscuits, which children could identify with from previous consumption of biscuits. A third reason is that biscuits were tested during morning break at 10.00 and the children were hungry and this made them like the biscuits.

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

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

The most recent estimates by the Food and Agriculture Organization (2009) show that more than one billion people worldwide are undernourished, and most exist on starchy staples which are poor sources of protein. It was also suggested by FAO (2009) that school feeding programmes could be designed to stimulate local economies by increasing agriculture and local value added food production. Purchase of locally produced grain such as sorghum, bread wheat and soy beans for school meals could generate income and guarantee markets for small holder farmers. In Africa, the potential demand for school feeding is a total number of 114 million children who are enrolled in primary school (2007). Of these, 70 million are
currently attending school in hunger stricken areas in sub-Saharan Africa (World Food Programme 2007). In the developing world, there are 66 million primary school age children who are undernourished and 23 million of these live in Africa (World Food Programme 2009).

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

A challenge to producing the sorghum and bread wheat biscuits by different groups of people is assuring that the minimum nutritional standards are maintained. For instance, using the right type of sorghum, (non-tannin sorghum) and compositing sorghum and bread wheat with soy in the right proportions and maintaining a constant supply of grain for biscuit production. A possible approach could be for specific millers to buy grain from farmers, prepare a pre-mix and supply it to the community bakeries with already trained personnel. A similar approach was used for a school feeding programme in Malawi, where a pilot project supports
five community bakeries to manufacture and deliver fortified scones to schools using a pre-mix that is delivered to them by the World Food Programme (World Food Programme 2009). Lack of basic infrastructure such as water, kitchens, storage facilities, cooking equipment and manpower does not facilitate the successful preparation and provision of meals to children in rural Africa. For instance, a school lunch programme in Kenya that provided maize and beans involved more than four hours of preparation time (UNESCO 2004). In South Africa, bread trucks could not reach some rural schools due to impassable roads during the rainy season and children got diarrhoea because unsafe water was used to reconstitute milk shake (Sizwe and Nikiwe 2010). High transportation costs were incurred because the foods had a short shelf-life. The advantage of a snack food, such as the fortified sorghum and bread wheat biscuits is that preparation time is eliminated, they are shelf stable so large amounts can be stored in the school and are unlikely to substitute family meals as the school meal should be an addition to the diet.

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

Two soy fortified sorghum or bread wheat biscuits of 28 g each providing 14 g of protein per day are within the range for protein requirements for school children of 3 to 10 years. Two biscuits of 28 g would provide 1077 kJ and 1053 kJ from fortified sorghum and bread wheat biscuits, respectively. This translates into slightly below half the minimum energy requirements for 3 to 10 year olds. An assessment of nine school feeding studies in developing countries showed that the daily ration provided energy ranging from 815 kJ to 2500 kJ (Galloway, Kristjansson, Gelli, Meir, Espejo and Bundy 2009). This suggests that the fortified sorghum and bread wheat biscuits would make a substantial contribution to the protein and energy needs of 3 to 10 year old children’s school feeding programmes.
The typical nutritional composition of high-energy fortified biscuits offered by WFP for school feeding is 12 g protein and 1883 kJ energy per 100 g of biscuits (Bundy et al 2009) and weigh between 20 and 40 g (World Food Programme, 2000). Sorghum-soy and bread wheat-soy biscuits at 1:1 ratio of 100 g contain 25 g protein and 1924 kJ energy and 26 g protein and 1880 kJ energy, respectively (Chapter 4.1, Table 4.1.2). This indicates that compared to the World Food Programme biscuits, the fortified biscuits in the current study have double the protein content and similar energy content and could have a higher impact on alleviating PEM.

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

A study by Galloway et al (2009) on costs of school feeding in African countries Malawi, Kenya, Lesotho and Gambia reported that for a 200 day school year, the cost ranged from US$28 to US$63 per child per year. An estimation of the cost of feeding a child with two biscuits a year for the same length of time would probably be approximately US$40 if the
Table 5.2 Estimation of costs of soy fortified sorghum and bread wheat biscuits for 56 g ration/day/year and comparison to low priced commercial biscuits

<table>
<thead>
<tr>
<th>Ingredients and cost</th>
<th>Sorghum-soy biscuits (355 g dm)</th>
<th>Bread wheat-soy biscuits (355 g dm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum flour (112.5 g) @ R7.99/kg</td>
<td>R 0.89</td>
<td>R 1.01</td>
</tr>
<tr>
<td>Bread wheat flour (112.5 g) @ R8.99/kg</td>
<td>R 1.22</td>
<td>R 1.22</td>
</tr>
<tr>
<td>Defatted soy flour (112.5 g) @ R10.83/kg</td>
<td>R 0.50</td>
<td>R 0.50</td>
</tr>
<tr>
<td>Sugar (56 g) @ R8.99/kg</td>
<td>R 0.80</td>
<td>R 0.80</td>
</tr>
<tr>
<td>Sunflower oil (66 g) @ R12.20/kg</td>
<td>R 0.66</td>
<td>R 0.66</td>
</tr>
<tr>
<td>Vanilla essence (13.5 g) @ R24.49/500 g</td>
<td>R 0.10</td>
<td>R 0.06</td>
</tr>
<tr>
<td>Baking powder: sorghum (1.5 g) @ R13.99/200 g</td>
<td>R 0.10</td>
<td></td>
</tr>
<tr>
<td>Baking Powder: wheat (1 g) @ R13.99/200 g</td>
<td></td>
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</tbody>
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<table>
<thead>
<tr>
<th></th>
<th>Sorghum-soy biscuits</th>
<th>Bread wheat-soy biscuits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cost</td>
<td>R 4.17</td>
<td>R 4.19</td>
</tr>
<tr>
<td>Cost including 14% VAT</td>
<td>R 4.75</td>
<td>R 4.78</td>
</tr>
<tr>
<td>¹Estimated cost of biscuits including manufacturing cost (cost of ingredients x 2)</td>
<td>R 9.50</td>
<td>R 9.56</td>
</tr>
<tr>
<td>Cost of 2 biscuits 28 g each (56 g) child/day (ingredients only)</td>
<td>R 0.75</td>
<td>R 0.75</td>
</tr>
<tr>
<td>Cost of 2 biscuits 28 g each (56 g) child/day (ingredients + manufacturing cost)</td>
<td>R 1.50</td>
<td>R 1.50</td>
</tr>
<tr>
<td>Cost/child/200 day school year (ingredients only)</td>
<td>R²150 (US$20)</td>
<td>R 150 (US$20)</td>
</tr>
<tr>
<td>Cost/child/200 day school year (ingredients + manufacturing cost)</td>
<td>R 300 (US$40)</td>
<td>R 300 (US$40)</td>
</tr>
<tr>
<td>Cost of 200 g biscuits (ingredients only)</td>
<td>R 2.68</td>
<td>R 2.69</td>
</tr>
<tr>
<td>Cost of 200 g biscuits (ingredients + manufacturing cost)</td>
<td>R 5.35</td>
<td>R 5.39</td>
</tr>
<tr>
<td>³Cost of 200 g Marie or Rich Tea biscuits (commercial)</td>
<td>R 5.99</td>
<td></td>
</tr>
<tr>
<td>Cost/child/200 day school year (56 g – commercial/day)</td>
<td>R 335</td>
<td></td>
</tr>
</tbody>
</table>

¹Cost of manufacturing estimated as equivalent to cost of ingredients
²South African Rand (R), conversion of Rand to US$ based on 1US$ = 7.5R
³Low priced commercial biscuits on South African Market.
Prices of ingredients are retail prices from Pick and Pay, a South African chain store.
cost of ingredients for biscuits was doubled to include the costs of production. Therefore, the
cost of feeding children with the fortified biscuits would be comparable to the general costs
incurred for school feeding programmes in Africa but with the added nutritional value as a
protein-rich food supplement. According to the World Food Programme (2002) fortified
biscuits provided to schools cost US$1250 per ton. The estimated cost of feeding a child on
such biscuits per year is 13US$. However, most grains used by the World food Programme
are donations hence the lower cost of production and as stated earlier the fortified biscuits
have only half the protein content of biscuits in the current study.
6 CONCLUSIONS AND RECOMMENDATIONS

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

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

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

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

Further studies should be conducted to determine the True Protein Digestibility of sorghum biscuits using either a pig model or a clinical study because results obtained from the rat bioassay were too high and cannot be compared to sorghum protein digestibility in children. Further studies should be carried out to develop biscuits using composites of sorghum, bread wheat and other cereals such as maize, rice, millet and teff, with soy and indigenous African legumes and oil seeds such as marama bean, cowpea, bambara nut, cashew nuts and others. This will enable production of fortified biscuits for school feeding using cereals and
legume/oilseeds that are locally available and sustainable within their ecological zone. Local purchase of such grains for school feeding will be a force multiplier, benefiting children by preventing PEM and uplifting rural economies by providing an income to smallholder farmers in low income communities in Africa through the Home Grown School Feeding Programme.