# 1 INTRODUCTION

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

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

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

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

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

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

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

# 2 LITERATURE REVIEW

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

## 2.1 SORGHUM AND BREAD WHEAT

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

### 2.1.1 Sorghum grain morphology

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

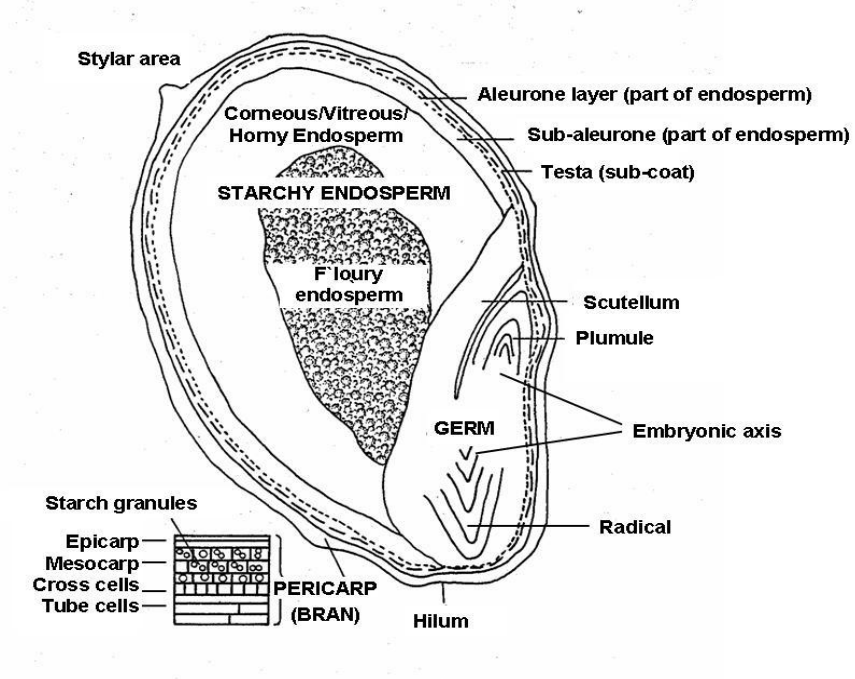


**Figure 2.1** Cross-section of sorghum grain (adapted from Taylor and Belton 2002)

### 2.1.1.1 Pericarp

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

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

### 2.1.1.2 Endosperm

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

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

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

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

#### 2.1.1.3 Germ

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

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

### 2.1.2 Chemical composition of the sorghum grain

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

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

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

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

### 2.1.3 Distribution of proteins in the sorghum grain

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

using two non-tannin sorghum cultivars. The endosperm contains 80% of the sorghum nitrogen. The endosperm has the highest proportion of prolamins accounting for 67 to 69% of the total nitrogen and is rich in the amino acids, glutamine, proline, alanine, and leucine and low in lysine, a typical characteristic of prolamin proteins. The endosperm also contained a lower proportion of low molecular weight nitrogen (LMWN) of 1.5 to 2.5 and albumins plus globulins in comparison to whole grain, pericarp and germ. The $G_3$-glutelin is the second largest protein fraction in the endosperm accounting for13.6 to 17.3%. It was also found to be poor in glutamine, 11.2% and rich in lysine 6.1%. These workers also postulated that the $G_3$-glutelins comprise the glutelin matrix that surrounds the protein bodies in sorghum starchy endosperm. The germ contained 16% of the grain nitrogen. Low molecular weight nitrogen (LMWN) was the most abundant nitrogenous fraction accounting for 40.1 to 48%, comprising amino acids, peptides and nucleotides. Albumin and globulin proteins were also present in substantial amounts, 32 to 34% of the proteins in the germ, and were rich in lysine. The pericarp contained the least amount, only 3% of the grain protein and it was inextractible using the modified Osborne fractionation procedure of Landry and Moureaux (1970). Taylor and Schussler (1986) suggested that this could be because it was associated with the cell wall.

### 2.1.4 Kafirin proteins

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

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

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

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

### 2.1.5 Amino acid composition of sorghum protein

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

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

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

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

### 2.1.6 Digestibility of sorghum proteins

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

### 2.1.6.1 Effect of cooking on protein digestibility

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

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

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

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

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

## 2.2 WHEAT

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

### 2.2.1 Morphology of the wheat grain

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

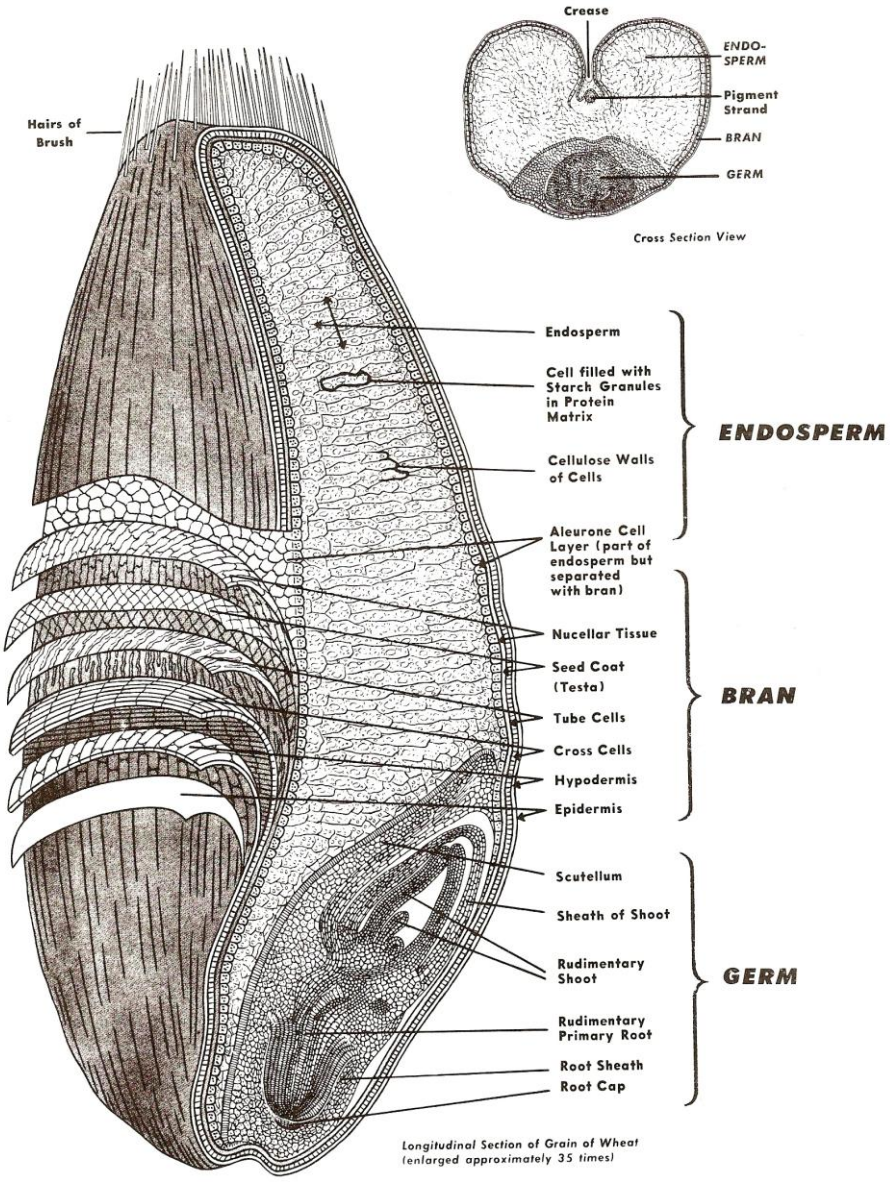


**Figure 2.2** Longitudinal and cross sections of a wheat kernel (Potter and Hotchkiss 1998).

### 2.2.2 Chemical composition of wheat grain

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

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

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

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

### 2.2.3 Types of protein in wheat

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

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

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

### 2.2.4 Amino acid composition and protein digestibility of wheat

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

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

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

## 2.3 PROTEIN ENERGY DEFICIENCY IN CHILDREN

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

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

### 2.3.1 Functions of proteins in human nutrition

#### 2.3.1.1 Contribution of protein to children's health

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

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

#### 2.3.1.2 Importance of lysine in the diet

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

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

## 2.4 FOOD PROCESSING AND LYSINE AVAILABILITY IN FOOD

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

### 2.4.1 Milling of cereal grains

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

### 2.4.2 Thermal Processing

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

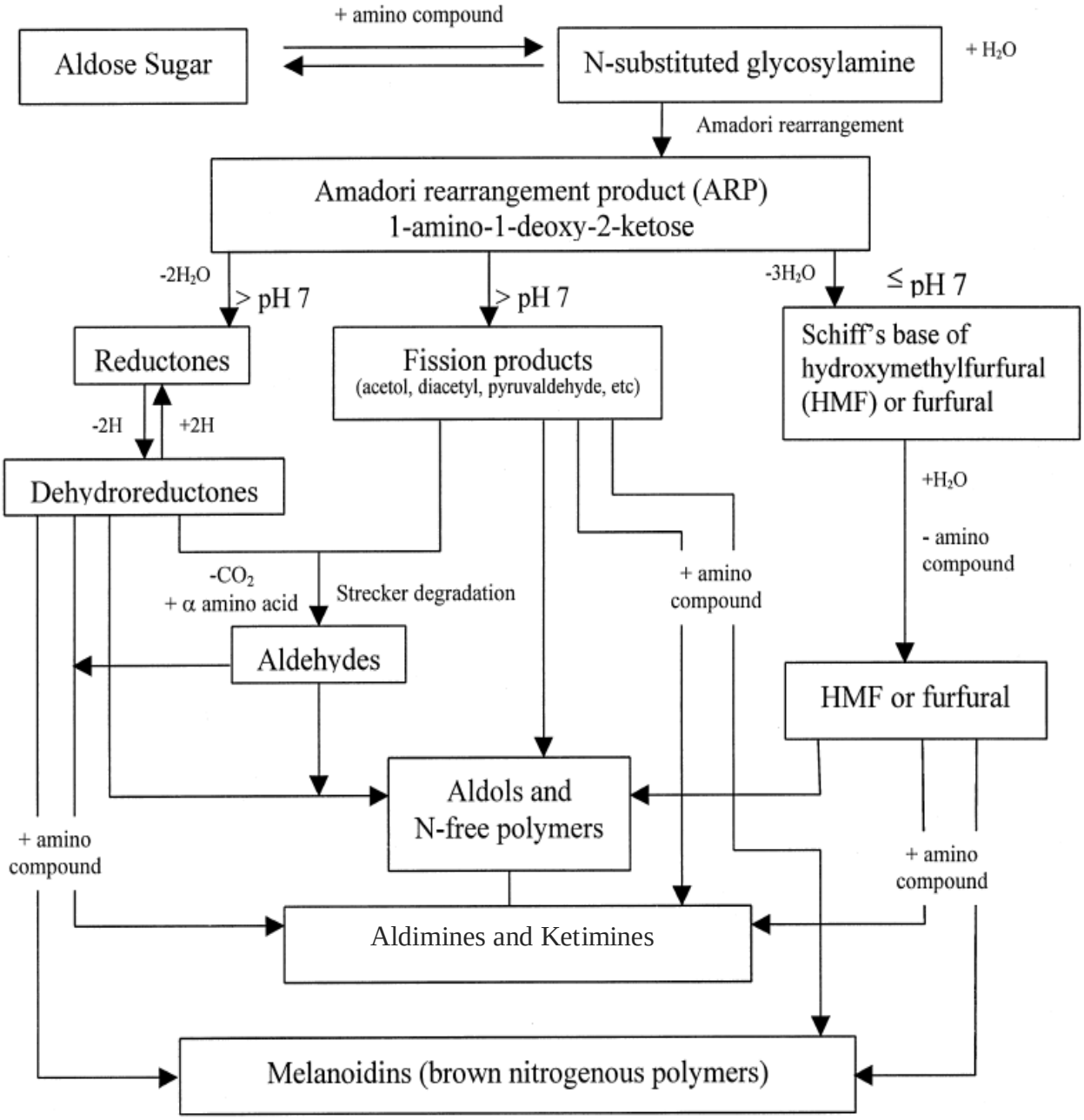


**Figure 2.3:** Maillard Reaction scheme adapted from Hodge (1953).

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

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

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

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

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

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

#### 2.4.2.1 Effects of Maillard products on protein digestibility

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

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

### 2.4.3 Soy beans

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

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

#### 2.4.3.1 Soy bean proteins

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

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

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

#### 2.4.3.2 Soy amino acid composition and digestibility

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

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

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

## 2.5 STRATEGIES TO PREVENT PROTEIN DEFICIENCIES

### 2.5.1 Dietary diversification

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

### 2.5.2 Amino acid fortification of cereals

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

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

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

### 2.5.3 Plant breeding and biofortification

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

### 2.5.4 The principle of complementation

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

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

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

### 2.5.4.1 Cereal-legume complemented foods used for supplementary feeding

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

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

### 2.5.4.2 Development of low cost cereal legume supplements

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

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

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

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

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

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

#### 2.5.4.3 Sorghum use in developing cereal-legume supplementary foods

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

## 2.6 TECHNOLOGY OF BISCUIT PRODUCTION

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

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

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

### 2.6.1 Cereal-legume blend biscuits

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

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

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

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

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

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

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

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

### 2.7.1 Bioassays for protein quality

#### 2.7.1.1 Protein Efficiency Ratio (PER)

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

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

### 2.7.1.2 Net Protein Ratio (NPR) and relative NPR

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

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

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

### 2.7.1.3 True Protein Digestibility (TPD)

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

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

F= faecal nitrogen output by test animals.

$F_m$ = Faecal nitrogen output by “protein free” animals or endogenous (metabolic) nitrogen.

### 2.7.1.4 Biological Value

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

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

U = urinary nitrogen loss on the test diet.
$U_m$ = urinary nitrogen loss on a protein-free diet.

### 2.7.1.5 Protein Digestibility Corrected Amino Acid Score (PDCAAS)

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

PDCAAS = TPD x Lowest amino acid score

### 2.7.2 In vitro methods

#### 2.7.2.1 Amino acid composition

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

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

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

#### 2.7.2.2 Lysine availability

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

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

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

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

### 2.7.2.3 Protein digestibility

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

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

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

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

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

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

## 2.8 EVALUATION OF SENSORY CHARACTERISTICS IN BISCUITS

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

### 2.8.1 Methods for evaluation with children

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

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

### 2.8.2 Evaluating long term acceptability of foods

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

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

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

## 2.9 CONCLUSIONS

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