Factors affecting sorghum protein digestibility

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Received 8 July 2002; accepted 17 January 2003

Abstract

In the semi-arid tropics worldwide, sorghum (Sorghum bicolor (L.) Moench) is cultivated by farmers on a subsistence level and consumed as food by humans. A nutritional limitation to its use is the poor digestibility of sorghum protein when wet cooked. The factors affecting wet cooked sorghum protein digestibility may be categorised into two main groups: exogenous factors (grain organisational structure, polyphenols, phytic acid, starch and non-starch polysaccharides) and endogenous factors (disulphide and non-disulphide crosslinking, kafirin hydrophobicity and changes in protein secondary structure). All these factors have been shown to influence sorghum protein digestibility. More than one factor may be at play at any time depending on the nature or the state in which the sorghum grain is; that is whether whole grain, endosperm, protein body preparation, high-tannin or condensed-tannin-free. It is proposed that protein crosslinking may be the greatest factor that influences sorghum protein digestibility. This may be between γ- and β-kafirin proteins at the protein body periphery, which may impede digestion of the centrally located major storage protein, α-kafirin, or between γ- or β-kafirin and α-kafirin.

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Keywords: Sorghum; Maize; Kafirin; Zein; Protein digestibility; Disulphide crosslinking; α-helical conformation; β-sheet conformation

1. Introduction

Sorghum (Sorghum bicolor (L.) Moench) is an important food cereal in many parts of Africa, Asia and the semi-arid tropics worldwide. It has the distinct advantage (compared to other major cereals) of being drought-resistant and many subsistence farmers in these regions cultivate sorghum as a staple food crop for consumption at home (Murty and Kumar, 1995). Therefore sorghum acts as a principal source of energy, protein, vitamins and minerals for millions of the poorest people living in these regions (Klopfenstein and Hoseney, 1995). In this way, sorghum plays a crucial role in the world food economy as it contributes to rural household food security (International Crops Research Institute for the Semi-Arid Tropics, 1996).

The food uses of sorghum are still mostly traditional and the methods of processing may involve the use of wet or dry heat (Murty and Kumar, 1995). Porridges appear to be the most common types of food prepared from sorghum. A range of porridges of varying consistencies (soft or thick) may be prepared from fermented or non-fermented sorghum meal (Murty and Kumar, 1995). Porridge preparation involves cooking the meal with boiling water and the process varies considerably depending on the type of porridge being produced (Taylor et al., 1997). Flat breads and alcoholic beverages are also produced from sorghum. Sorghum grains are also popped and consumed as snacks or delicacies.

A nutritional constraint to the use of sorghum as food is the poor digestibility of sorghum proteins on cooking. Digestibility may be used as an indicator of protein availability. It is essentially a measure of the susceptibility of a protein to proteolysis. A protein with high digestibility is potentially of better nutritional value than one of low digestibility because it would provide more amino acids for absorption on proteolysis. In mixed diets containing marginal or low protein contents and where the percentage of sorghum is high, increased protein digestibility would provide much needed protein to the consumer. The protein digestibility of sorghum in comparison with other cereals has been a subject of extensive research. In vivo studies using pepsin (Maclean et al., 1981), and in vitro studies (Axtell et al., 1981) (Table 1) show that the proteins of wet
Table 1
Effect of grain type, grain fraction, wet cooking and treatment with reducing agent on the in vitro protein digestibility of sorghum and maize

<table>
<thead>
<tr>
<th>Grain</th>
<th>Fraction and treatment</th>
<th>Digestibility (%)</th>
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<tbody>
<tr>
<td>Condensed-tannin-free sorghum (Axtell et al., 1981)</td>
<td>Whole grain flour; cooking</td>
<td>88.6–93.0 (uncooked, P)</td>
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<td>45.3–56.7 (cooked, P)</td>
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<td>Dehulled flour; cooking</td>
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<td>37.1–43.0 (cooked, P)</td>
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<tr>
<td>Condensed-tannin-free sorghum (Hamaker et al., 1986)</td>
<td>Whole grain flour; cooking</td>
<td>80.7 (uncooked, P)</td>
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<td>64.8 (cooked, P)</td>
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<td>72.7 (uncooked, TC)</td>
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<td>57.1 (cooked, TC)</td>
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<td>87.6 (uncooked, P-TC)</td>
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<td>70.5 (cooked, P-TC)</td>
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<td>Yellow dent maize (Hamaker et al., 1986)</td>
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<td>81.5 (uncooked, P)</td>
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<td>81.9 (cooked, P)</td>
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<td>88.3 (uncooked, P-TC)</td>
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<td>90.7 (cooked, P-TC)</td>
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<tr>
<td>Condensed-tannin-free sorghum (Rom et al., 1992)</td>
<td>Whole grain flour; cooking and treatment with reducing agent</td>
<td>79.0 (uncooked, P)</td>
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<td>58.0 (cooked, P)</td>
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<td>96.0 (uncooked, RA, P)</td>
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<td>79.0 (cooked, RA, P)</td>
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<td>Condensed-tannin-free sorghum (Oria et al., 1995b)</td>
<td>Decorticated flour; cooking and treatment with reducing agent</td>
<td>69.2 (uncooked, P)</td>
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<td>55.2 (cooked, P)</td>
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<tr>
<td>Condensed tannin-free sorghum (Arbab and El Tinay, 1997)</td>
<td>Whole grain flour; cooking and treatment with reducing agent</td>
<td>40 (uncooked, P)</td>
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<td>18 (cooked, P)</td>
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<td>25 (cooked, RA, P)</td>
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<td>Condensed tannin sorghum (Arbab and El Tinay, 1997)</td>
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<td>31 (uncooked, P)</td>
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<td>12 (cooked, P)</td>
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<td>15 (cooked, RA, P)</td>
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<tr>
<td>Condensed-tannin-free red sorghum (Duodu et al., 2002)</td>
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<td>59.1 (uncooked, P)</td>
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<td>30.5 (cooked, P)</td>
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<td>Endosperm flour; cooking</td>
<td>65.7 (uncooked, P)</td>
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<td>35.9 (cooked, P)</td>
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<td>Protein body preparations; cooking</td>
<td>72.8 (uncooked, P)</td>
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<td>44.2 (cooked, P)</td>
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<tr>
<td>Condensed-tannin-free white sorghum (Duodu et al., 2002)</td>
<td>Whole grain flour; cooking</td>
<td>55.8 (uncooked, P)</td>
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<td>36.6 (cooked, P)</td>
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<td>Endosperm flour; cooking</td>
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<td>Protein body preparations; cooking</td>
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<td>63.5 (cooked, P)</td>
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<td>White dent maize (Duodu et al., 2002)</td>
<td>Whole grain flour; cooking</td>
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<td>Endosperm flour; cooking</td>
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<td>Protein body preparations; cooking</td>
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<td>67.4 (cooked, P)</td>
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cooked sorghum are significantly less digestible than the proteins of other similarly cooked cereals like wheat and maize. It has been shown that the in vitro pepsin digestibility of sorghum proteins correlates with in vivo digestibility (Maclean et al., 1981), which indicates the physiological relevance of the problem.

The improvement of sorghum nutrient availability is critical for food security. Cereal scientists and sorghum food processors are thus faced with the challenge of identifying the factors that adversely affect, and developing processing procedures that improve sorghum protein digestibility. A great deal of research has been conducted by different workers into the possible reasons for this poor quality characteristic of sorghum protein. It is not surprising therefore, that diverse hypotheses have been proposed.

The factors that contribute to the poor protein digestibility of sorghum may be divided into two broad categories:

Exogenous factors: These refer to factors that arise out of the interaction of sorghum proteins with non-protein components like polyphenols, non-starch polysaccharides, starch, phytates and lipids.

Endogenous factors: These refer to factors that arise out of changes within the sorghum proteins themselves and do not involve interaction of the proteins with non-protein components.

The involvement of these factors is set in motion when the grain is milled and cooked. During this processing proteins may interact with non-protein components and the proteins themselves may undergo changes. Both of these events may affect their digestibility.

2. Exogenous factors

2.1. Grain organisational structure

One of the main factors that affects sorghum protein digestibility is the organisational structure of the sorghum grain. Three main levels of grain organisational structure that include protein may be envisaged; whole grain, endosperm and protein bodies. A recent study has shown that the protein digestibility of sorghum varies at these different levels (Duodu et al., 2002) (Table 1). Fundamentally, sorghum protein digestibility depends on the form in which the grain is provided and there is a need for this to be well defined. In vitro protein digestibility assays have been conducted on either whole grain (Axtell et al., 1981; Duodu et al., 2002; Hamaker et al., 1986, 1987; Rom et al., 1992; Oria et al., 1995a), decorticated grain (Axtell et al., 1981; Mertz et al., 1984; Oria et al., 1995b; Weaver et al., 1998; Chibber et al., 1980), endosperm (Duodu et al., 2002) (Table 1) or some undefined commercial grain fraction (Bookwalter et al., 1987). These different types of grain material have differing proportions of pericarp, endosperm and germ and also different types of protein. The prolamin proteins of sorghum (kafrin) are similar to the prolamins of maize (zein), and these are the major storage proteins of these grains. The proteins are located within the starchy endosperm and make up about 70 and 60% of the sorghum and maize total grain protein, respectively (Paulis and Wall, 1979; Lending et al., 1988). A general picture which emerges is that in vitro protein digestibility of sorghum is improved as the proportion of pericarp and germ material becomes less (Duodu et al., 2002; Chibber et al., 1980). In other words, as the grain is taken apart, moving from whole grain, to endosperm and further on to the protein bodies, protein digestibility improves. Similar results have been reported in which an improvement in protein digestibility from whole grain to endosperm was observed in rice (Bradbury et al., 1984). Sorghum protein bodies, however, still have comparatively low cooked protein digestibility. In contrast, the situation in maize seems different in that the protein digestibilities of uncooked and cooked maize at all three levels of organisation appear to be similar (Duodu et al., 2002) (Table 1).

Improvement of sorghum protein digestibility with change in organisational level suggests that some exogenous factors interfere with the protein digestibility. These may be polyphenols in the pericarp, phytate in the pericarp and germ, non-starch polysaccharides in the pericarp and endosperm cell walls and starch in the endosperm. The mechanism by which these interfering factors interact with proteins to reduce digestibility may be two-fold:

- The interfering factor may be involved in a chemical interaction with the protein. The products of such interactions may be indigestible.
- The interfering factor may form a physical barrier and prevent access of proteases to the protein.

2.2. Polyphenols

Phenolic compounds in sorghum may be divided into three major categories: phenolic acids, flavonoids and tannins (Hahn et al., 1984). The fact that some sorghum cultivars produce large quantities of tannins makes it unique among the cereals (Serna-Saldivar and Rooney, 1995). However, not all sorghum varieties contain condensed tannin. Barley (Gupta and Haslam, 1978) and rye (Butler et al., 1984) have also been reported to contain small quantities of tannins. Whilst tannins protect the grain against insects, birds and fungal attack, this agronomic advantage is accompanied with nutritional disadvantages and reduced food quality (Serna-Saldivar and Rooney, 1995). It is believed that under optimal conditions, sorghum tannin is capable of binding and precipitating at least 12 times its own weight of protein and the tannin-protein interaction in sorghum is thought to involve hydrogen bonding and non-polar hydrophobic associations (Butler et al., 1984). Sorghum grain contains approximately 10% protein and therefore in theory, high-tannin cultivars would contain more than enough...
tannin (2–4%) to bind all the seed protein (Butler et al., 1984). Lower protein yields were obtained for high-tannin compared with low-tannin (condensed tannin-free) sorghum on subjecting both grains to the Landry–Moureaux protein fractionation procedure (Daiber and Taylor, 1982). This was due to interactions between tannin and the albumin, globulin and prolamin proteins, rendering most of the proteins insoluble. Furthermore, electrophoresis indicated that proteins extracted from high-tannin sorghum were bound to tannins. Generally, the characteristics of proteins that bind strongly to sorghum tannin are that they are relatively large, have a loose, open structure and are rich in proline (Butler et al., 1984).

In high-tannin sorghum varieties, formation of indigestible protein–tannin complexes is a major limiting factor to protein utilisation (Chibber et al., 1980). In vivo (Armstrong et al., 1973; Rostagno et al., 1973; Armstrong et al., 1974a) and in vitro (Butler et al., 1984; Armstrong et al., 1974b; Schaffert et al., 1974) studies have demonstrated this antinutritional effect of tannins in uncooked and cooked sorghum. Electrophoresis indicated that the indigestible residue of high-tannin sorghum consisted mainly of prolamins (Butler et al., 1984). This is also true of condensed-tannin-free sorghums (Duodu et al., 2002).

Sorghum tannins are known to inhibit enzymes such as amylases (Daiber, 1975). However, it has been suggested that the antinutritional effect of sorghum tannins lies in their ability to form less digestible complexes with dietary protein and not by inhibition of digestive enzymes (Butler et al., 1984). Grinding, cooking and other processing methods of high-tannin sorghum enhance the opportunity for interaction of tannin with dietary protein before it encounters digestive enzymes (Butler et al., 1984).

Due to their hydroxyl groups, tannins may interact with and form complexes with proteins, which may lead to precipitation because of the large size of the tannins. However, it may not be this precipitation per se which causes reduction in protein digestibility. Denaturation of proteins (sometimes characterised by protein precipitation) may actually lead to improvement in protein digestion (Damodaran, 1996). One of the main determinants of protein digestibility is its conformation and to what extent this allows access of enzymes to the protein. In addition to possibly causing a change in protein conformation (which may not favour enzyme accessibility), the tannins may also exert steric effects (due to their large size) and prevent enzymes access to the proteins. While the occurrence of protein–tannin interactions is not in doubt, it is not clear whether this occurs mostly before or after cooking.

It has been shown that the protein digestibility of sorghum is not dependent on tannin content alone. The protein digestibilities of sorghum cultivars with similar tannin contents may show great variability (Elkin et al., 1996). Furthermore, lowering of sorghum protein digestibility on cooking also occurs with condensed-tannin-free varieties, in vivo (Maclean et al., 1981) and in vitro (Mertz et al., 1984).

Flavonoids and phenolic acids contain hydroxyl groups and therefore may also interact with and form complexes with proteins. However, unlike the tannins, there has not been any conclusive evidence that such interactions cause a reduction in protein digestibility. Although flavonoids and phenolic acids have been reported to hinder iron absorption in the gastro-intestinal lumen (Brune et al., 1989), the current school of thought is that they are not known to have any adverse effects on protein digestibility (Serna-Saldívar and Rooney, 1995; Bravo, 1998). However, this hypothesis may not be discounted totally. It has been suggested that plant polyphenols may be oxidised to quinones by molecular oxygen at neutral to alkaline pH (Damodaran, 1996). The quinones may then go on to form peroxides that are highly reactive oxidising agents and could bring about oxidation of several amino acid residues and polymerisation of proteins (Damodaran, 1996). This could be a mechanism by which flavonoids and phenolic acids hinder protein digestion in sorghum.

2.3. Phytic acid

Phytic acid (myo-inositol hexaphosphoric acid) usually occurs in seeds as mixed potassium, magnesium and calcium salts (phytins or phytate) (Ryden and Selvendran, 1993). The phytate content of sorghum and maize is variable and appears to be dependent on cultivar. Phytate values of 0.27% (Elkhalil et al., 2001), 0.3% (Mahgoub and Elhag, 1998), 0.886% (Marfo et al., 1990), and 1% (García-Estepa et al., 1999) have been reported for sorghum and 0.734%, 0.686% (Marfo et al., 1990) and 1% (García-Estepa et al., 1999) for maize. In sorghum and maize, the highest phytate concentration is found in the germ (Hulse et al., 1980; Ali and Harland, 1991). Phytic acid is also associated with the bran (pericarp) of cereals (García-Estepa et al., 1999). The phytate molecule is highly charged with six phosphate groups and so is an excellent chelator, forming insoluble complexes with mineral cations and proteins (Ryden and Selvendran, 1993). This leads to reduced bioavailability of trace minerals and reduced protein digestibility. The inhibitory effect of phytate on protein digestibility has been demonstrated in experiments with casein and bovine serum albumin (Knuckles et al., 1985). It was observed that phytate significantly decreased in vitro pepsin digestion of both casein and bovine serum albumin. This observation was attributed to the possible formation of a phytate–protein complex, which is less susceptible to enzymatic attack (Knuckles et al., 1985). Similar results showing the inhibitory effect of phytate on protein digestibility have been reported for lactalbumin, soybean protein isolate and maize zein (Carnovale et al., 1988).

The possible formation of a complex between phytate and sorghum proteins, which could lead to reduced protein digestibility, has not been studied in sorghum, but
processing treatments have been shown to result in reduced phytic acid content and enhanced protein digestibility of various legumes (Katariya et al., 1989; Vijayakumari et al., 1998; Alonso et al., 2000; Elsheikh et al., 2000; Abd El-Moneim et al., 2000) and cereals such as pearl millet (Kheterpaul and Chauhan, 1991; Kumar and Chauhan, 1993) and sorghum (Elkalil et al., 2001). However, although the addition of phytate to phaseolin (bean protein) resulted in an initial decrease in rate of proteolysis, it was considered unlikely by the authors that this would be of nutritional significance (Sathe and Sze-tao, 1997). It has also been observed that the inhibitory effect of phytate on digestive proteases is dependent on factors such as pH and the presence of metal cations such as Ca$^{2+}$ and Mg$^{2+}$ (Deshpande and Damodaran, 1989; Vaintraub and Bulmaga, 1991).

In a study on the effect of microbial phytase on ileal amino acid digestibilities of some cereals including sorghum, it was observed that addition of phytase led to an improvement in protein and amino acid digestibilities (Ravindran et al., 1999). However, there was no significant correlation between the percentage improvements in protein digestibility and dietary total phytic acid concentration. It was suggested that the observed effects of phytase addition might be due to structural or chemical properties of both the phytic acid and the protein rather than the total concentration of phytic acid (Ravindran et al., 1999). These structural or chemical properties determine the degree of phytate–protein binding, which may then influence the protein and amino acid responses to phytase addition (Ravindran et al., 1999). Many questions remain unanswered regarding the effect of phytate on proteins in general and sorghum proteins in particular. At present, the possibility of phytic acid inhibiting protein digestibility in sorghum, or for that matter, in any other grains, may not be ruled out.

2.4. Cell wall components

An association has been reported between protein and the pericarp or endosperm cell walls in barley (Gram, 1982) and sorghum (Glennie, 1984; Bach Knudsen and Munck, 1985). It is possible that such an association could lower protein digestibility either by reducing the accessibility to enzymes or the formation of indigestible complexes. Within sorghum endosperm, starch granules and protein bodies are surrounded by cell walls (Shull et al., 1990) and it has been observed that isolated sorghum endosperm cell walls had 46% protein associated with them (Glennie, 1984). Furthermore, significant amounts of protein were associated with total dietary fibre and acid detergent fibre (residue obtained after extraction of starch, protein and hemicelluloses with an acid detergent solution) fractions in uncooked and cooked sorghum (Bach Knudsen and Munck, 1985). The amino acid composition of the sorghum proteins associated with acid detergent fibre resembled that of kafirins. These results indicate that proteins are able to bind to dietary fibre or more specifically, cell wall components.

Dietary fibre refers to the polysaccharide fraction of plant foods, particularly cereals, which is not digested by the human alimentary tract (Johnson and Southgate, 1994). In cereals, this fraction is mainly derived from the pericarp and endosperm cell walls and the major constituents are cellulose and non-cellulosic polysaccharides mainly, heteroxylans (mainly glucuronoxarabinoxylans) and (1→3; 1→4)-β-D-glucans (Johnson and Southgate, 1994; Verbruggen, 1996). The heteroxylans may be esterified with phenolic acids such as p-coumaric and ferulic acid (Fry, 1988; Bacic et al., 1988; Brett and Walldron, 1990). In immature sorghum grains, the prolamins are located in membrane-bound protein bodies (Taylor et al., 1985). Therefore, if prolamin-cell wall attachment does occur, it must happen as the grain dries out or during cooking as the organelle integrity in the cell is destroyed. Two main modes of attachment may be proposed to explain the nature of the protein-cell wall adhesion.

Firstly, the binding of protein to the non-starch polysaccharide components is believed to be one of the factors that impairs protein digestion (Damodaran, 1996). In other words, protein-cell wall adhesion could be by direct attachment of the protein to carbohydrate moieties. Cell walls are known to contain a variety of different proteins and most of these are glycosylated (Brett and Walldron, 1990). The best characterised are the structural cell wall glycoproteins known as hydroxyproline-rich glycoproteins (HRGs) or extensins and their presence in sorghum has been reported (Raz et al., 1991). Sorghum extensins are rich in hydroxyproline, proline, lysine, tyrosine and threonine (Raz, 1991). The hydroxyproline residues normally serve as attachment points for arabinose oligosaccharides (Kielszewski and Lamport, 1987; Kielszewski et al., 1992). The polypeptide–carbohydrate linkage is thought to be an O-glycosidic linkage in which the reducing terminus of the carbohydrate is attached to an –OH group on the polypeptide (Fry, 1988). These structural glycoproteins are highly resistant to most proteases, especially when the oligoarabinose side chains are still attached. By analogy, it may be suggested that such enzyme-resistant protein–carbohydrate linkages may be formed in sorghum on cooking through formation of O-glycosidic linkage between hydroxyproline residues of sorghum proteins and the arabinose residues of the cell wall.

A second mode of attachment of proteins to cell walls may be by ferulic acid-mediated crosslinking. Phenolic acids have been identified as being involved in crosslinking within the cell wall where it is important in maintaining the integrity (Fry, 1988). Ferulic acid has the ability to couple oxidatively to another ferulic acid-bearing arabinoxylan to form a diferulate crosslink. The formation of such phenolic crosslinks is dependent on the synthesis of the phenol-bearing arabinoxylan, the presence of peroxidase enzyme and a supply of hydrogen peroxide or an equivalent oxidising agent (Fry, 1988). Therefore, it may be said that in general, oxidising conditions could promote
the formation of phenolic or specifically, ferulic crosslinks. In a similar manner, it has been suggested that dimerisation may occur between tyrosine residues in proteins and ferulic acid residues on arabinoxylans (Bacic et al., 1988). It could be hypothesised therefore, that the cooking process, which is conducted in the presence of oxygen, could lead to the formation of such tyrosyl–feruloyl crosslinks between proteins and arabinoxylans and, in so doing, bring about adhesion between proteins and the cell wall. Removing the outer layers of the grain would reduce the amount of cell wall material and hence, also reduce protein-cell wall adhesion and improve protein digestibility.

However, there is no reason why these two modes of attachment may not occur in both maize and sorghum. Extensins (Hood et al., 1991) and phenolic acids also occur in maize cell walls (Fry, 1988; Bacic et al., 1988; Brett and Waldron, 1990; Huisman et al., 2000). Therefore, it is possible that protein–carbohydrate interactions and feruloyl–tyrosyl crosslinks may occur in maize on cooking and so may not be the unique factor that explains the differences observed between the digestibility of wet-cooked sorghum and maize proteins.

2.5. Starch

In sorghum and maize endosperm, starch granules and protein bodies are in very close association with each other. The largely polygonal, tightly packed starch granules are surrounded with numerous, largely spherical protein bodies embedded in a protein matrix (Shull et al., 1990; Seckinger and Wolf, 1973; Watson, 1987). The implication of such a close association between starch and protein may be that the starch, especially when gelatinised after cooking, could reduce the accessibility of proteolytic enzymes to the protein bodies and therefore reduce protein digestibility.

Information in the literature on the role of starch in sorghum protein digestibility appear to be conflicting. The in vitro protein digestibility of decorticated sorghum flour cooked with heat-stable alpha-amylase was approximately the same as that cooked without (Oria et al., 1995b). However, treating cooked sorghum and maize samples with alpha-amylase prior to incubation with pepsin led to an improvement in in vitro protein digestibility (Duodu et al., 2002).

It certainly appears that in sorghum, the protein has an effect on starch gelatinisation and starch digestibility. Interaction between protein and starch has been identified as a factor that influences sorghum starch digestibility. These interactions may reduce the susceptibility of native and processed starch to enzyme hydrolysis (Rooney and Pflugfelder, 1986). Pronase treatment significantly increased the rate of in vitro starch hydrolysis in sorghum (Rooney and Pflugfelder, 1986). This was due to hydrolysis of the protein matrix by the pronase enzyme resulting in increased surface area of starch in contact with amylglucosidase (Rooney and Pflugfelder, 1986). It has been observed that sorghum grains with lower capacities for starch gelatinisation contained more kafirin-containing protein bodies (Chandrashekar and Kirleis, 1988). Additionally, the manner in which protein bodies are organised around the starch granule (Shull et al., 1990) appears to act as a barrier to starch gelatinisation. In rice, it has been reported that addition of a reducing agent (2-mercaptoethanol) to the cooking medium increased the degree of gelatinisation of the starch (Hamaker and Griffin, 1993). The reducing agent presumably cleaved disulphide bonds linking protein polymers surrounding the starch granules thus leading to an increase in degree of starch gelatinisation. In sorghum, treating flour with pepsin before cooking or cooking with a reducing agent (dithiothreitol) led to an increase in starch digestibility, suggesting that protein may act as a barrier to starch digestion (Zhang and Hamaker, 1998).

Resistant starch possibly reduces protein digestibility in cooked sorghum. It has been hypothesised that cooking cooked porridge leads to formation of resistant starch which may form complexes with kafirin proteins which are less susceptible to enzyme attack (Bach Knudsen, 1988; Bach Knudsen et al., 1988a, 1988b).

The fact that the arrangement of starch granules and protein bodies within maize endosperm is similar to sorghum suggests that starch may also influence maize protein digestibility. Treating cooked maize whole grain and endosperm flours with alpha-amylase prior to pepsin digestion led to an improvement in protein digestibility (Duodu et al., 2002).

3. Endogenous factors

3.1. Protein crosslinking

During processing, the physical and chemical conditions proteins encountered can result in changes ranging from subtle changes in the hydration of the protein to thermal destruction (pyrolysis) with potential formation of mutagens (Finley, 1989). The main chemical reactions that occur are the formation of derivatives of special amino acids or their covalent crosslinking with other amino acids in the same or in another protein molecule (Erbersdobler, 1989). Such protein crosslinks may bring about decreases in the digestibility and biological value of the food proteins. Two main types of protein crosslinking will be discussed here; isopeptide crosslinking and disulphide crosslinking.

3.2. Racemization and isopeptide formation

The process whereby L-amino acids are converted to the D form is known as racemization. This conversion is of importance nutritionally because D-amino acids are absorbed more slowly than the corresponding L-form and
even if digested and absorbed, most D isomers of essential amino acids are not utilised by humans (Liardon and Hurrell, 1983). In addition, L-D, D-L, and D-D-peptide bonds introduced during the racemization process would resist attack by proteolytic enzymes, which function best with l-L bonds (Friedman et al., 1981). Amino acid racemization occurs most readily after alkaline treatments (Liardon and Hurrell, 1983; Masters and Friedman, 1979; Jenkins et al., 1984), to a lesser extent in acid conditions (Manning, 1970; Jacobson et al., 1974), and during severe heat treatment and roasting of proteins (Liardon and Hurrell, 1983; Hayase et al., 1975). Racemization of amino acids is believed to be a prelude to the formation of isopeptide bonds (Bunjapamai et al., 1982). The likelihood of amino acid crosslinks may decrease the digestibility and bioavailability of proteins. However, it is considered unlikely that conventional processing or cooking methods will cause extensive racemization of protein amino acids in foods (Bunjapamai et al., 1982). The likelihood of amino acid racemization and its extent cooked sorghum porridge have not been investigated. It may be speculated that if it occurs in sorghum porridge, it is not likely to be extensive. Perhaps during cooking, the likelihood of racemization is greatest at the bottom of the cooking vessel where moisture is driven out and the porridge becomes dry, reaching temperatures in excess of 100°C.

3.3. Disulphide crosslinking

A general observation is that in uncooked sorghum, Landry–Moureaux fraction 3 proteins (kafirin 2, soluble in aqueous alcohol plus reducing agent) are more abundant than fraction 2 (kafirin 1, soluble in aqueous alcohol alone) (Hamaker et al., 1986; Jambunathan and Mertz, 1973; Guiragossian et al., 1978; Vivas et al., 1992; Hamaker et al., 1994) whilst the opposite is the case for the zein 1 and zein 2 fractions of uncooked maize (Hamaker et al., 1986, 1994; Vivas et al., 1992; Landry and Moureaux, 1980) (Table 2). On cooking, the kafirin proteins tend to become less soluble and there is a further increase in the proportion of Landry–Moureaux fraction 3 proteins (Hamaker et al., 1986; Taylor and Taylor, unpublished data). There also appears to be a shift in alcohol-soluble proteins (fractions 2 and 3) to the higher fractions, namely fraction 5 (extracted with pH 10 buffer, 2-mercaptoethanol and sodium dodecyl sulphate) and fraction 6 (defined as non-extractable) (Hamaker et al., 1986). It was therefore suggested that there could be a potential relationship between kafirin solubility and protein digestibility. This was based on the observation that in cooked sorghum, the amount of indigestible protein was significantly larger than in uncooked while there was essentially no difference in cooked and uncooked maize (Hamaker et al., 1986). The observed lowering of kafirin solubility on cooking appears to be a result of disulphide crosslinking.

The kafirins may be classified into α-kafirins (M, 24,000 and 26,000), β-kafirins (M, 20,000, 18,000 and 16,000) and γ-kafirins (M, 28,000) (Shull et al., 1991) and the zeins, α-zeins (M, 22,000 and 19,000), β-zeins (M, 16,000 and 14,000) and γ-zeins (M, 27,000) (Esen, 1986; Esen, 1987). The α-prolamins is the major storage protein of the grains. After synthesis, kafirins and zeins are translocated to the lumen of the rough endoplasmic reticulum where they accumulate and are packaged into discrete protein bodies about 1 μm in diameter (Taylor et al., 1985; Miflin et al., 1981). Protein bodies are structured such that α-prolamins are located centrally with most of the γ-prolamins and some β-prolamins at the body periphery in sorghum and maize (Lending et al., 1988; Shull et al., 1992). However, what is not clear is the location of the different α-prolamins in the protein body and whether there is differential deposition of the protein.

Related to the potential of these individual protein classes to disulphide crosslink, α-prolamins contain about

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Proportions of Landry–Moureaux protein fractions 2 and 3 in uncooked and cooked sorghum and maize</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uncooked</strong></td>
<td></td>
</tr>
<tr>
<td>Sorghum</td>
<td>Maize</td>
</tr>
<tr>
<td>Kafirin 1</td>
<td>Kafirin 2</td>
</tr>
<tr>
<td>17.3 (Hamaker et al., 1986)</td>
<td>24.5 (Hamaker et al., 1986)</td>
</tr>
<tr>
<td>9.9* (Guiragossian et al., 1978)</td>
<td>15.3* (Guiragossian et al., 1978)</td>
</tr>
</tbody>
</table>

Results are expressed as % total nitrogen except those indicated * which are % total protein.
1 mol% Cys, α-prolamins about 5 mol% Cys, and γ-prolamins about 7 mol% Cys (Shull et al., 1992; Landry and Moureaux, 1980). In uncooked sorghum, disulphide crosslinked protein oligomers comprising γ-(Mr 29,000), α1-(Mr 26,000) and α2-(Mr 24,000) kafirins were found. However, β-kafirin (Mr 18,000) was absent from these oligomers (El Nour et al., 1998). The β-kafirin was found only as a component of higher molecular weight polymers, which also consisted of γ- and α1-kafirins. These polymers were devoid of α2-kafirin. It was hypothesised that β-kafirin (which is rich in cysteine) could act as a chain extender by linking together oligomers of γ- and α1-kafirin by disulphide bridges to form high molecular weight polymers (El Nour et al., 1998). On the other hand, α2-kafirin (comparatively lower in cysteine) could act as a chain terminator by preventing the possibility of bonding with β-kafirin and formation of high molecular weight polymers (El Nour et al., 1998).

It appears that on cooking, more of such disulphide crosslinked protein oligomers and polymers are formed. When sorghum is cooked, enzymatically resistant protein polymers are formed through disulphide bonding of the β- and γ-kafirins (Rom et al., 1992; Oria et al., 1995b; Hamaker et al., 1994), and possibly other proteins which are located on the outside of the protein body. The disulphide crosslinked proteins thus formed would then prevent access to and restrict digestion of the more digestible and centrally located α-kafirin within the protein body (Rom et al., 1992; Oria et al., 1995b; Hamaker et al., 1994). Various in vitro studies indicate that cooking sorghum with reducing agents improves its protein digestibility (Hamaker et al., 1987; Rom et al., 1992; Oria et al., 1995; Arbab and El Tinay, 1997). It is suggested that on cooking, a disulphide-crosslinked protein coat may be formed by proteins surrounding the protein body and this could reduce accessibility of the protein bodies to enzymatic attack (Hamaker et al., 1987). Scanning electron micrographs of uncooked sorghum flour subjected to pepsin treatment showed that the protein bodies were digested by pitting from the outer surface (Rom et al., 1992), as has been observed during germination (Taylor et al., 1985b). However, on cooking, sorghum protein bodies were not pitted after pepsin treatment (Rom et al., 1992). When cooked samples were treated with reducing agent, pits were observed on the protein bodies after pepsin treatment (Rom et al., 1992). These observations suggest a reversal of the reactions that may occur during cooking (disulphide crosslinking) after treating cooked samples with a reducing agent. Work done on some sorghum mutants with high uncooked and cooked protein bodies after pepsin treatment (Rom et al., 1992). The hypothesis suggests a reversal of the reactions that may occur during cooking (disulphide crosslinking) after treating cooked samples with a reducing agent. Work done on some sorghum mutants with high uncooked and cooked protein bodies after pepsin treatment (Rom et al., 1992). Protein bodies of the highly digestible mutants are highly invaginated and contain deep folds rather than a typical spherical shape (Fig. 1). Gamma-kafirin is located at the base of the folds in protein bodies of the highly digestible mutants (Fig. 2) as opposed to the periphery in normal protein bodies. As a result, α-kafirin in the highly digestible sorghum is more exposed to digestive enzymes than in normal protein bodies and this improved accessibility accounts for the overall higher protein digestibility (Weaver et al., 1998; Oria et al., 2000). All these observations indicate that kafirin packaging (location of various kafirins within the protein body) and kafirin type do affect sorghum protein digestibility (Hicks et al., 2001).

However, disulphide crosslinking of proteins on cooking does not happen with sorghum alone. Electrophoretic analysis has shown that during cooking of maize there is disulphide-mediated polymerisation of α-zein (Batterman-Azcona and Hamaker, 1998) and also the β- and γ-zeins (Duodu et al., 2002). In spite of this, cooking does not reduce the protein digestibility of maize to the same extent as it does with sorghum.
sorghum. Perhaps one of the shortcomings of the disulphide bonding hypothesis, as presented, is that it does not explain this observed difference between sorghum and maize. Recent results obtained from SDS–PAGE of uncooked and cooked sorghum and maize protein body preparations (Fig. 3) (Duodu et al., 2002) appear to offer some clues. More disulphide-bonded protein oligomers were found in sorghum than in maize, suggesting that oligomer formation on cooking may be more extensive in sorghum compared to maize. Therefore, more enzyme-resistant protein oligomers would be formed in sorghum than in maize and this may explain the lower digestibility of sorghum proteins (Duodu et al., 2002). It appears that production of enzyme-resistant proteins on cooking also occurs in rice. Examination of poorly digested cooked rice protein bodies showed that the major polypeptide within this fraction had a high sulphur-containing amino acid content and had a $M_r$ of about 13,000 (Resurreccion et al., 1993). A cooked rice diet fed to mice (a monogastric animal) produced more faecal protein particles than an uncooked rice diet (Collier et al., 1998).

It is interesting that the use of a reducing agent during cooking does not appear to completely reverse the effect of lowered sorghum protein digestibility on cooking. Cooking sorghum flour with a reducing agent did improve protein digestibility but not to the level of uncooked sorghum flour (Oria et al., 1995b). Similar results have been reported from SDS–PAGE analyses of pepsin-indigestible residues of sorghum protein body preparations (Fig. 3) (Duodu et al., 2002). On electrophoretic analyses in the presence of excess reducing agent, reduction-resistant protein oligomers were found in cooked sorghum (Fig. 3) (Duodu et al., 2002). If protein crosslinking on cooking occurred exclusively through the formation of disulphide bridges, it would be expected that cooking sorghum flour with a reducing agent would almost completely eliminate the problem of lowered protein digestibility on cooking. Furthermore, one would not expect to find reduction-resistant oligomers in cooked sorghum. Two possible reasons have been proposed to explain these observations. Firstly, it is suggested that there might be disulphide bonds that are inaccessible to the reducing agent (Oria et al., 1995b). Perhaps the reduction-resistant oligomers may have conformations that do not allow easy access of reducing agent to disulphide bonds (Duodu et al., 2002). Secondly, the possibility of formation of non-disulphide crosslinks has been proposed (Duodu, 2000). It is suggested that the oxidising conditions of the cooking process could lead to oxidative coupling of tyrosine residues (Duodu, 2000) which could result in the formation of dimers (isodityrosine) (Fry, 1982; Epstein and Lamport, 1984; Biggs and Fry, 1990) or tetramers (di-isodityrosine) (Brady et al., 1996). Such tyrosine dimers and tetramers would then become the source of non-disulphide inter-polypeptide crosslinks (Fry, 1982; Epstein and Lamport, 1984; Biggs and Fry, 1990; Brady et al., 1996). In vitro protein digestibility experiments involving alkylation of uncooked and cooked kafirins and zeins also seem to point to the possible formation of non-disulphide crosslinks (Duodu, 2000). In the alkylation process, a reducing agent is used to cleave disulphide bonds thus generating free thiols which are trapped with an alkyllating agent such as 4-vinylpyridine to prevent re-oxidation (Hollecker, 1997). It was observed that alkylated kafirin and zein samples (uncooked and cooked) were more digestible than unalkylated. However, alkylated and cooked kafirin still had much lower digestibility than alkylated and uncooked (Duodu, 2000). This suggests the possibility of formation of non-disulphide crosslinks otherwise it would be expected that alkylation would very significantly improve the protein digestibility of cooked kafirin.

3.4. Kafirin and zein hydrophobicity

The kafirins and the zeins are known to be hydrophobic proteins. It has also been suggested that the kafirins are less soluble (therefore more hydrophobic) than the zeins (Wall and Paulis, 1978). Enzymes function in an aqueous environment. Therefore, if the kafirins are indeed more hydrophobic, they may be generally less accessible to enzymes and hence less digestible than the zeins.

One way by which the relative hydrophobicities of the kafirins and zeins may be determined is by calculating their free energies of hydration. This may be done if their amino acid sequences and the free energy of hydration of each amino acid are known. The higher and more negative the free energy of hydration, the less hydrophobic the protein.

Table 3 shows the free energies of hydration calculated for $\alpha$-zein and $\alpha$-kafirin and $\gamma$-zein and $\gamma$-kafirin based on

![Fig. 3. SDS–PAGE of pepsin-indigestible residues of uncooked (U) and cooked (C) sorghum and maize protein body samples under non-reducing (NR) and reducing (R) conditions (Duodu et al., 2002).](image-url)
<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Free energy of hydration (kcal/mol) (Shewry et al., 2002)</th>
<th>mol % amino acid in 22 kDa α-zein (Woo et al., 2001)</th>
<th>mol % amino acid in α-kafirin (<a href="http://ars">http://ars</a>)</th>
<th>mol % amino acid in glutelin 2 precursor (27 kDa γ-zein) (Prat et al., 1985; Wang and Esen, 1986; Prat et al., 1987)</th>
<th>mol % amino acid in γ-kafirin preprotein (De Freitas et al., 1994)</th>
<th>Free energy of hydration for 22 kDa α-zein (kcal/mol)</th>
<th>Free energy of hydration for α-kafirin (kcal/mol)</th>
<th>Free energy of hydration for glutelin 2 precursor (27 kDa γ-zein) (kcal/mol)</th>
<th>Free energy of hydration for γ-kafirin preprotein (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>-3.11</td>
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<td>5.91</td>
<td>0.00</td>
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<td>-17.23</td>
<td>-18.38</td>
<td>-6.52</td>
<td>-7.03</td>
</tr>
<tr>
<td>Thr</td>
<td>-1.69</td>
<td>3.54</td>
<td>2.82</td>
<td>3.86</td>
<td>4.16</td>
<td>-5.98</td>
<td>-4.77</td>
<td>-8.94</td>
<td>-11.54</td>
</tr>
<tr>
<td>Gln</td>
<td>-3.15</td>
<td>22.58</td>
<td>23.80</td>
<td>17.89</td>
<td>14.18</td>
<td>-71.13</td>
<td>-74.97</td>
<td>-56.35</td>
<td>-44.67</td>
</tr>
<tr>
<td>Pro</td>
<td>0.23</td>
<td>7.52</td>
<td>7.50</td>
<td>21.14</td>
<td>19.65</td>
<td>1.73</td>
<td>1.73</td>
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<tr>
<td>Gly</td>
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<td>0.67</td>
<td>3.52</td>
<td>4.95</td>
<td>-0.15</td>
<td>-0.15</td>
<td>-0.81</td>
<td>-1.14</td>
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<tr>
<td>Cys</td>
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<td>0.36</td>
<td>6.54</td>
<td>7.05</td>
<td>-0.10</td>
<td>-0.10</td>
<td>-1.77</td>
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<tr>
<td>Val</td>
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<td>6.36</td>
<td>0.21</td>
<td>0.21</td>
<td>0.29</td>
<td>0.25</td>
</tr>
<tr>
<td>Met</td>
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<td>1.77</td>
<td>1.07</td>
<td>1.74</td>
<td>-0.22</td>
<td>-0.18</td>
<td>-0.11</td>
<td>-0.17</td>
</tr>
<tr>
<td>Ile</td>
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<td>5.07</td>
<td>4.66</td>
<td>1.89</td>
<td>2.55</td>
<td>0.35</td>
<td>0.33</td>
<td>0.13</td>
<td>0.18</td>
</tr>
<tr>
<td>Leu</td>
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<tr>
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<tr>
<td>Lys</td>
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<td>0.87</td>
<td>0.00</td>
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</tr>
<tr>
<td>His</td>
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<td>2.30</td>
<td>8.94</td>
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<td>2.70</td>
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<td>-3.56</td>
<td>-25.76</td>
<td>-18.50</td>
</tr>
<tr>
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<td>0.00</td>
<td>-0.54</td>
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</tr>
</tbody>
</table>

Totals -139.78 -140.36 -124.52 -113.63

Calculations for β-kafirin and zein are not included. The amino acid sequence for β-kafirin is not available and so could not be compared with β-zein.
their amino acid sequences. The hydration free energy values obtained for the kafirins and the zeins are less negative than similarly calculated values for wheat gliadins (−159.794 kcal/mol) (Shewry et al., 2003) and glutenins (−165.817 kcal/mol) (Shewry et al., 2003). This indicates that the kafirins of sorghum and the zeins of maize are more hydrophobic than the gluten proteins of wheat. The hydrophilic wheat proteins are able to absorb water to form the viscoelastic gluten.

The α-prolamins of sorghum and maize have virtually the same level of hydrophobicity (Table 3). In contrast, the value obtained for γ-kafirin indicates that it is more hydrophobic than the γ-zein. These results appear to confirm the earlier suggestion that the kafirins are generally more hydrophobic than the zeins (Wall and Paulis, 1978). Perhaps the relatively more hydrophobic nature of the kafirins may be a contributing factor to the observed lower digestibility of cooked sorghum compared to cooked maize and wheat (Mertz et al., 1984).

A closer examination of the γ-zein and γ-kafirin primary structures reveals two main differences. Firstly, γ-zein has eight tandem repeats (occurring in succession) of the sequence PPPVHL from residues 31 to 78. A variant, PPPVHV occurs at residues 67–72. In contrast, γ-kafirin has only four tandem repeats (occurring in succession) of the sequence PPPVHL from residues 34 to 57. The PPPVHV variant occurs at residues 52–57. Secondly, γ-zein has two tandem repeats (occurring in succession) of the sequence QPHPCPCQ from residues 97 to 112. A variant QPHPSPCQ occurs at residues 105–112. In contrast, γ-kafirin does not have either of the repeat sequences QPHPCPCQ or QPHPSPCQ (Fig. 4). These differences in the primary structures of γ-zein and γ-kafirin may have a bearing on the differences observed in the protein digestibilities of maize and sorghum on cooking.

3.5. Change in protein secondary structure

The effects of wet and dry cooking on proteins of sorghum, maize and highly digestible sorghum mutants have been studied using Fourier transform infrared (FTIR) (Kretschmer, 1957; Duodu et al., 2001) and nuclear magnetic resonance (NMR) spectroscopic techniques (Duodu et al., 2001). Both spectroscopic techniques gave similar results—a small change in protein secondary structure occurred on cooking, from an α-helical to antiparallel, intermolecular β-sheet conformation in all samples studied (Kretschmer, 1957; Duodu et al., 2001). However, the cooked and popped samples still contained significant amounts of proteins with an α-helical conformation (Duodu et al., 2001), thus indicating that the structure of the unprocessed protein is not altered to a great extent on processing. The change in secondary structure appeared to occur to a greater extent in wet cooked than in popped samples, an observation which shows some correlation with results obtained from in vitro protein digestibility assays (Duodu et al., 2001; Parker et al., 1999). Comparing sorghum with maize, there seemed to be greater changes

![Fig. 4. Comparison of the primary structures of γ-zein and γ-kafirin showing relative differences in number of tandem repeats.](image-url)
in the secondary structure of the sorghum proteins. However it is difficult to attribute the differences between the digestibilities of sorghum and maize proteins to the apparent greater secondary structural changes in sorghum due to the similar overall trends in both cereals. To explain the observed change in secondary structure, it is proposed that the application of heat energy during the cooking process may break hydrogen bonds, which stabilise the α-helical conformation (Duodu et al., 2001). The polypeptides would then become unravelled and aligned next to each other to form the observed intermolecular β-sheet conformation (Duodu et al., 2001) stabilised by disulphide and possibly also non-disulphide crosslinks between polypeptides.

4. Conclusions

The causes of the poor digestibility of sorghum proteins appear to be multi-factorial. Depending on the nature of the sorghum used (whole grain, endosperm, protein body preparations, high-tannin grain or condensed-tannin-free grain), different factors may contribute with some being more important than others. The relatively higher hydrophobicity of sorghum kafirins compared to maize zeins may have a bearing on their relative digestibilities. It appears that the phobicity of sorghum kafirins compared to maize zeins may be more important than others. The relatively higher hydrophobicity of the α-kafirin, may have the greatest influence on sorghum protein digestibility. However, notwithstanding the wealth of knowledge and understanding gained from research into this phenomenon, the exact reason for the difference in protein digestibility between sorghum and maize is yet to be fully understood. It seems that a greater amount of protein crosslinking occurs on wet cooking in sorghum than in maize and this may explain the poorer digestibility of wet cooked sorghum proteins.

The probable causes of reduced protein digestibility indicate that a number of currently used processing technologies may be applied to improve sorghum protein digestibility. These include dry cooking (popping) (Duodu et al., 2001; Parker et al., 1999), extrusion (Hamaker et al., 1994; Fapojuwo et al., 1987; Maclean et al., 1983), malting (Elmaki et al., 1999; Elkhalfa and Chandrashekar, 1999), fermentation (Kazanas and Fields, 1981; Lorri and Svanberg, 1993; Taylor and Taylor, 2002), flaking (Chen et al., 1994, 1995) and grain refinement (Duodu et al., 2002; Chibber et al., 1980; Maclean et al., 1983), the latter reducing the levels of phytate and polyphenols.

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