LOW PROTEIN DIGESTIBILITY OF COOKED SORGHUM – CAUSES AND NEEDS FOR FURTHER RESEARCH

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Sorghum (*Sorghum bicolor* (L.) Moench) is cultivated by farmers on a subsistence level in the semi-arid tropics worldwide and consumed as a food staple by humans. Sorghum proteins have poor digestibility when wet cooked and this constitutes a nutritional limitation to its use as food. The factors affecting wet cooked sorghum protein digestibility may be categorised into 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). Depending on the nature or state of the sorghum grain, namely whole grain, endosperm, protein bodies, high tannin or condensed-tannin-free, more than one factor may be important at any time. Protein crosslinking may be the greatest factor that influences sorghum protein digestibility.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is an important cereal crop grown in the semi-arid tropics of Africa and Asia due to its drought tolerance. It is a staple food crop cultivated on a subsistence level by farmers in these areas for human consumption[1] and therefore plays an important role in food security.

In vivo[2] and *in vitro*[3,4] studies indicate that the proteins of wet cooked sorghum are significantly less digestible than the proteins of other similarly cooked cereals such as wheat and maize[3,4]. Factors contributing to this quality characteristic of sorghum proteins have been reviewed recently[5,6]. Reduction in sorghum protein digestibility on cooking is a nutritional constraint to its use as food and has implications for food security in these semi-arid areas. In this paper the major outcomes of sorghum protein digestibility studies are reviewed and some areas for further research are outlined.

Exogenous factors

Grain organisational structure

Sorghum protein digestibility depends on the form in which the grain is provided. *In vitro* protein digestibility assays have been reported for various forms of sorghum grain including whole grain[7,8], decorticated grain[7] and endosperm[8]. These different

types of grain material have differing proportions of pericarp, endosperm and germ and also different types of protein. Results indicate that *in vitro* protein digestibility of sorghum is improved as the proportion of pericarp and germ material becomes less[8] (see Table I). As the sorghum grain is taken apart along the three main levels of organisational structure, namely whole grain, endosperm and protein bodies, protein digestibility improves[8]. This demonstrates clearly that grain organisational structure influences sorghum 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[8] (see Table I).

Variety	Treatment	Whole grain flour		Endosperm flour		Pb-enriched sample	
		PD[2]	% of uncooked	PD	% of uncooked	PD	% of uncooked
NK 283 sorghum	Uncooked	59.1 c[3] ± 3.7[4]	100	65.7 c ± 0.9	100	72.8 b ± 2.5	100
	Cooked	30.5 a ± 1.6	52	35.9 a ± 5.1	55	44.2 a ± 3.2	61
	Cooked/ *alpha*-amylase	36.5 b ± 1.8	62	49.0 b ± 4.3	75	45.3 a ± 3.4	62
KAT 369 sorghum	Uncooked	55.8 c ± 0.9	100	67.4 c ± 1.2	100	74.3 b ± 4.7	100
	Cooked	36.6 a ± 2.8	66	39.4 a ± 4.4	58	63.5 a ± 1.7	85
	Cooked/ *alpha*-amylase	42.2 b ± 2.0	76	43.7 b ± 2.9	65	62.7 a ± 3.9	84
PAN 6043 maize	Uncooked	66.6 b ± 1.3	100	67.4 a ± 1.2	100	68.8 a ± 2.3	100
	Cooked	62.0 a ± 3.2	93	63.6 a ± 2.3	94	67.4 a ± 4.1	98
	Cooked/ *alpha*-amylase	72.5 c ± 3.3	109	72.2 b ± 2.3	107	68.2 a ± 3.8	99

[1] Protein body [2] *In vitro* protein digestibility. [3] For each grain variety, mean values in the same column with different letters are significantly different from each other ($p < 0.05$). [4] Standard deviation.

Table I. Effect of cooking and addition of *alpha*-amylase after cooking on percentage *in vitro* protein digestibility of whole grain flour, endosperm flour and protein body (Pb[1])-enriched samples of sorghum (NK 283 and KAT 369) and maize (PAN 6043) varieties

Polyphenols

The antinutritional effect of tannins in sorghum has been demonstrated clearly. In high-tannin sorghum varieties, formation of indigestible protein-tannin complexes is a major limiting factor to protein utilisation[9]. This has been shown through *in vivo*[10,11] and *in vitro*[12,13] studies conducted on uncooked and cooked sorghum grain. The tannin-protein interaction in sorghum involves hydrogen bonding and hydrophobic interactions[12]. Sorghum prolamins (proline-rich proteins) therefore bind strongly to sorghum tannins and this results in reduced protein digestibility. However, lowering

of sorghum protein digestibility on cooking also occurs with condensed-tannin-free varieties, *in vivo*[14] and *in vitro*[8]. This suggests that the non-tannin phenolic compounds in sorghum such as the flavonoids and phenolic acids may play a role though there has not been any conclusive evidence to support this. Currently, it is generally accepted that flavonoids and phenolic acids are not known to have any adverse effects on protein digestibility[15,16]. However, it has been proposed that oxidation of plant polyphenols leads to formation of quinones and highly reactive peroxides, which are oxidising agents. These peroxides may then bring about oxidation of amino acid residues and subsequently, polymerisation of proteins. This may then lead to reduced protein digestibility[5].

Phytic acid

The phytate molecule, containing six phosphate groups, is highly charged. This makes it an excellent chelator and it can form insoluble complexes with proteins[17] leading to reduced digestibility. The inhibitory effect of phytate on protein digestibility has been demonstrated in experiments with casein, bovine serum albumin[18], lactalbumin, soybean protein isolate and maize zein[19]. In sorghum, procedures such as pre-treatment of flour with malt[20] or microbial phytase[21], that have produced reduced phytic acid content have led to enhanced protein digestibility. However, the observed effects of phytase addition is believed to be due to structural or chemical properties of both the phytic acid and the protein rather than the total concentration of phytic acid[21]. These structural or chemical properties determine the degree of phytate-protein binding.

Cell wall components

Proteins have been shown to associate with pericarp or endosperm cell walls in sorghum[22,23]. It is suggested that such an association could lower protein digestibility either by reducing the accessibility to enzymes or the formation of indigestible complexes. It has been observed that the amino acid composition of sorghum proteins associated with acid detergent fibre resemble that of kafirins[23], in other words, they are prolamin-like proteins. Due to the location of prolamins in membrane-bound protein bodies in immature sorghum grains[24], it is suggested that prolamin-cell wall attachment could occur as the grain dries out or during cooking as the organelle integrity in the cell is destroyed[5]. Two main modes of attachment have been proposed to explain the nature of the protein-cell wall adhesion. These are by the direct attachment of proteins to non-starch polysaccharide components in the cell wall and by ferulic acid-mediated crosslinking[5].

Starch

It has been shown that treating cooked sorghum samples with *alpha*-amylase prior to incubation with pepsin leads to an improvement in *in vitro* protein digesitibility[8] (see Table I). This is an indication that gelatinised starch could reduce the accessibility of proteolytic enzymes to protein bodies and therefore reduce protein digestibility. Protein in turn, does affect starch gelatinisation and starch digestibility. This is perhaps not surprising given the very close association starch granules and protein bodies are with each other in their arrangement in sorghum endosperm. It has also

been hypothesised that resistant starch in cooled, cooked porridge may form complexes with kafirin proteins which are less susceptible to enzyme attack[25,26].

ENDOGENOUS FACTORS

Racemization and isopeptide formation

Racemization, the process whereby L-amino acids are converted to the D form is of nutritional importance because D-amino acids are absorbed more slowly than the corresponding L form. Even if digested and absorbed, most D isomers of essential amino acids are not utilised by humans[27]. Racemization also leads to the formation of isopeptide crosslinks, which may decrease the digestibility of proteins. Alkaline[27] and to a lesser extent, acid conditions[28] and severe heat treatments such as in roasting of proteins[27] enhance amino acid racemization of proteins. Therefore, it is considered unlikely that conventional processing or cooking methods used during preparation of sorghum porridge will cause extensive racemization of amino acids[5].

Disulphide crosslinking

When sorghum is cooked, enzymatically resistant protein polymers are formed through disulphide bonding of *beta*- and *gamma*- kafirins[29-31]. This is perhaps one of the most important factors contributing to reduced protein digestibility of cooked sorghum. These disulphide cross-linked proteins prevent access to and restrict digestion of the more digestible and centrally located *alpha*-kafirin within the protein body[29-31]. The role of disulphide crosslinking has been demonstrated in various *in vitro* studies that show that cooking sorghum with reducing agents improves its protein digestibility[29,30]. Work done on some sorghum mutants with high uncooked and cooked *in vitro* protein digestibility indicates that kafirin packaging (location of various kafirins within the protein body) and kafirin type (which indicates propensity for disulphide crosslinking) do affect sorghum protein digestibility[32,33]. The highly digestible mutants have highly invaginated protein bodies rather than a typical spherical shape in normal protein bodies (see Figure 1). As a result, *alpha*-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[32,33].

However, disulphide crosslinking of proteins on cooking also happens in maize[8,34] though this does not appear to reduce maize protein digestibility. The inability to explain the observed difference in digestibility with maize appears to be a shortcoming of the disulphide bonding hypothesis. Recent results obtained from SDS-PAGE of uncooked and cooked sorghum and maize protein body preparations and prolamin fractions[8] (see Figure 2) indicate that more disulphide-bonded protein oligomers appear to be formed in sorghum than in maize. This may explain the lower digestibility of sorghum proteins[8]. However, the use of a reducing agent during cooking does not appear to completely reverse the effect of lowered sorghum protein digestibility on cooking[30]. Furthermore, reduction-resistant protein oligomers occur in cooked sorghum[8] (see Figure 2).

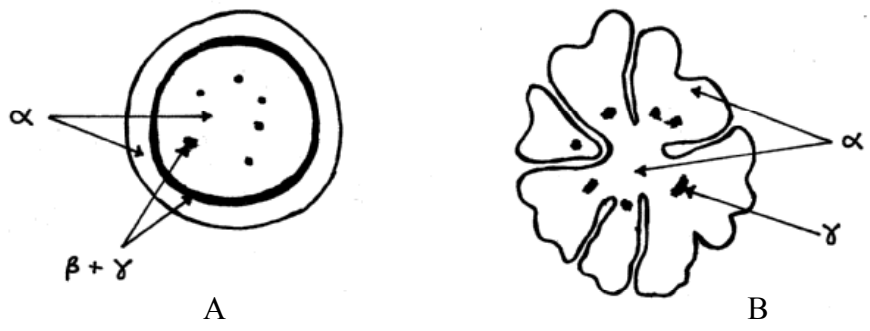

Figure 1: Diagrammatic representation of section through A) a normal sorghum protein body and B) protein body of a highly digestible sorghum mutant showing differential location of *alpha*-, *beta*- and *gamma*-kafirins. Note highly invaginated structure of highly digestible mutant protein body.

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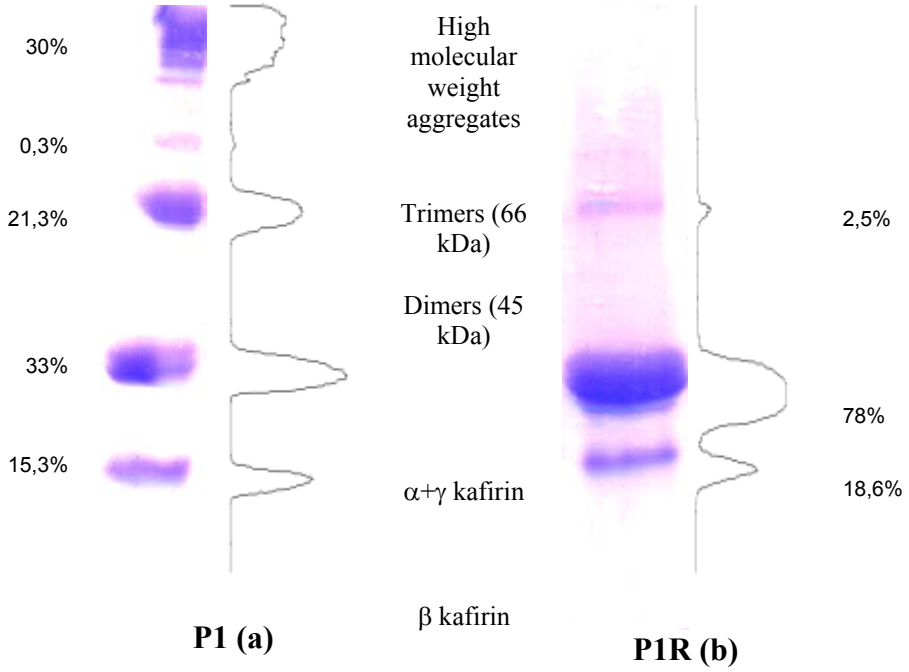


Figure 2A Electrophoretic profile of prolamin 1 (P1) fraction extracted from uncooked NK 283 sorghum. (a) Non reduced and (b) Reduced.

It is suggested that this may be due to oligomers in such a conformation that does not allow easy access of disulphide bonds to reducing agents. The possibility of formation of non-disulphide crosslinks through oxidative coupling of tyrosine residues has also been proposed[5].

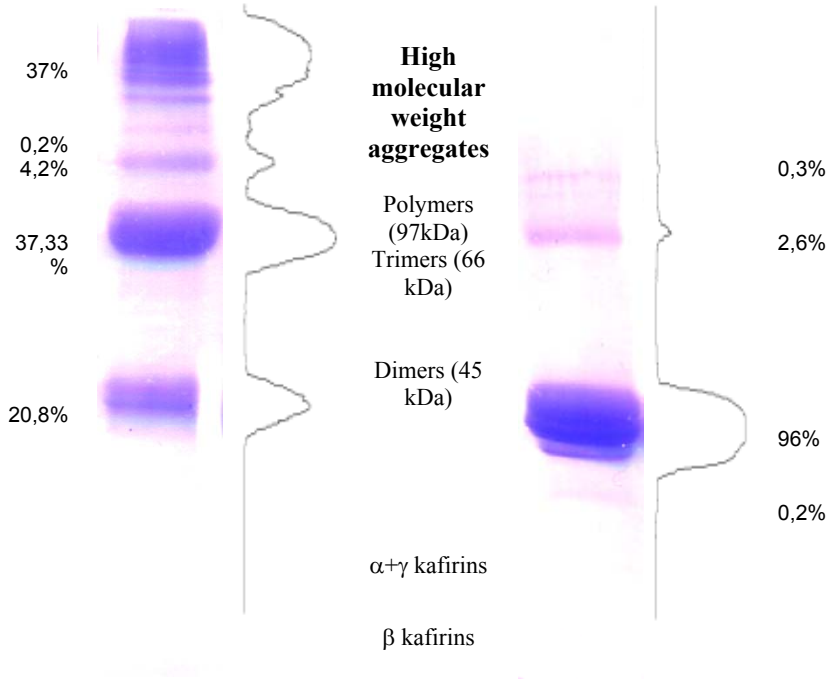


Figure 2B Electrophoretic profile of prolamin 1 (P1) fraction extracted from cooked NK 283 sorghum. (a) Non reduced and (b) Reduced.

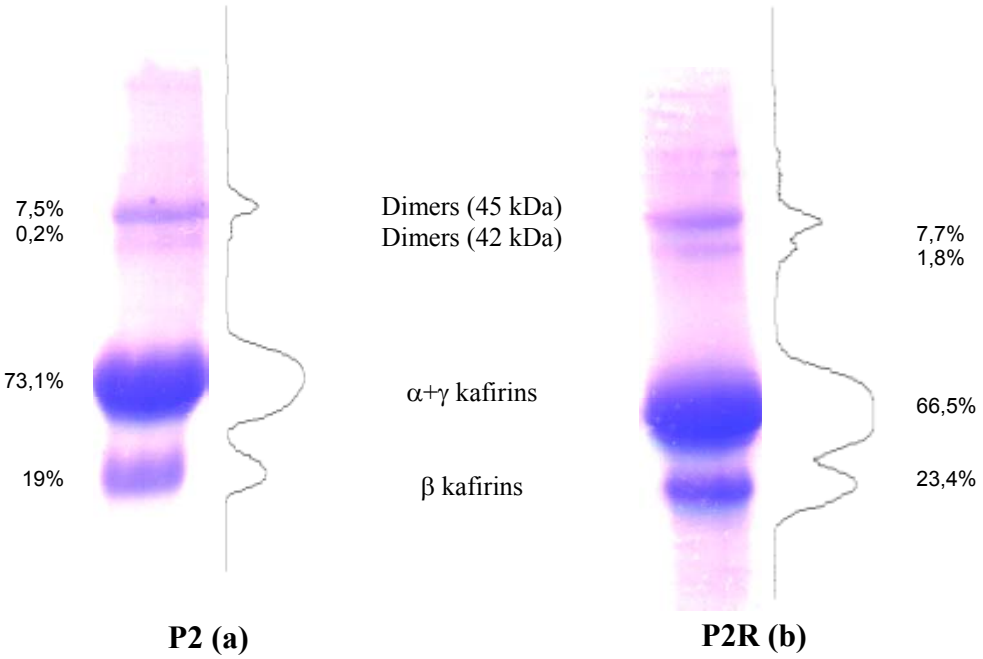
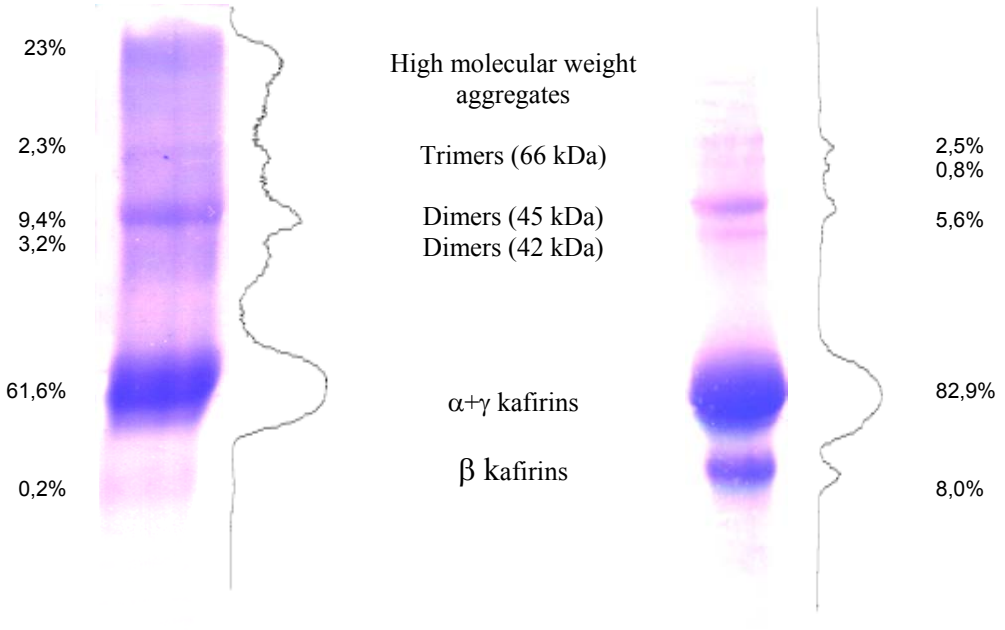


Figure 2C Electrophoretic profile of prolamin 2 (P2) fraction extracted from uncooked NK 283 sorghum. (a) Non reduced and (b) Reduced.



P2C (a) **P2CR (b)**

Figure 2D Electrophoretic profile of prolamin 2 (P2) fraction extracted from cooked NK 283 sorghum. (a) Non reduced and (b) Reduced.

Kafirin and zein hydrophobicity and primary structure

Relatively higher hydrophobicity of kafirins compared to zeins has been suggested to be a possible factor affecting sorghum protein digestibility. Hydrophobic proteins would be expected to have lower enzyme accessibility since enzymes function in an aqueous environment. Though *alpha*-prolamins of sorghum and maize have virtually the same degree of hydrophobicity, *gamma*-kafirin appears to be more hydrophobic than *gamma*-zein and this may be a contributing factor to the observed lower digestibility of cooked sorghum compared to cooked maize[5]. Though kafirins and zeins share extensive homology, there are slight differences in primary structure of the *gamma*-kafirins and zeins and it has been suggested that this may have a bearing on the differences observed in sorghum and maize protein digestibility[5]. *Gamma*-zein has eight tandem repeats (occurring in succession) of the sequence PPPVHL from residues 31 to 78 with a variant, PPPVHV at residues 67 to 72. In contrast, *gamma*-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 to 57. Secondly, *gamma*-zein has two tandem repeats (occurring in succession) of the sequence QPHPCPCQ from residues 97 to 112. A variant QPHPSPCQ occurs at residues 105 to 112. In contrast, *gamma*-kafirin does not have either of the repeat sequences QPHPCPCQ or QPHPSPCQ (see Figure 3).

Change in protein secondary structure

Spectroscopic studies have shown that on cooking, sorghum and maize proteins undergo a change in secondary structure from an *alpha*-helical to antiparallel, intermolecular *beta*-sheet conformation[35,36]. Even though the changes in sorghum appear to occur to a slightly greater extent compared with maize, 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.

Gamma-zein

10	20	30	40	50	60
MRVLLVALAL	LALAASATST	HTSGGCGCQP	**PPPVHLPPPV**	**HLPPPVHLPP**	**PVHLPPPVHL**
70	80	90	100	110	120
PPPVHLPPPV	**HVPPPVHL**PP	PPCHYPTQPP	RPQPHP**QPHP**	***CPCQQPHPSP***	**CQ**LQGTCGVG
130	140	150	160	170	180
STPILGQCVE	FLRHQCSPTA	TPYCSPQCQS	LRQQCCQQLR	QVEPQHRYQA	IFGLVLQSIL
190	200	210	220		
QQQPQSGQVA	GLLAAQIAQQ	LTAMCGLQQP	TPCPYAAAGG	VPH	

Gamma-kafirin

10	20	30	40	50	60
MKVLLVALAL	LALASAASTL	TTGGCGCQTP	HLP**PPPVHLP**	**PPVHLPPPVH**	**LPPPVHV**PPP
70	80	90	100	110	120
PPQCHPHPTL	PPHPHPCATY	PPHPSPCHPG	HPGSCGVGGG	PVTPPILGQC	IEFLRHQCSP
130	140	150	160	170	180
AATPYCSPQC	QALRQQCCQQ	LRQVEPLHRY	QAIFGVVLQS	IQQQQPQGQS	SPLPALMAAQ
190	200	210			
IAQQLTAMCG	LGVGQPSPCA	SCSPFAGGVH	Y		

Figure 3. Comparison of the primary structures of *gamma*-zein and *gamma*-kafirin showing relative differences in number of tandem repeats.

Needs for further research

The protein digestibility of sorghum is influenced by a number of factors. Some factors are more important than others depending on whether one is dealing with uncooked or cooked grain or the nature of the grain, that is, whole grain, endosperm, protein bodies or the extracted proteins. However, the exact reason why cooked maize has better protein digestibility compared to cooked sorghum is still not clear. This is more puzzling given the extensive similarities in protein body structure and prolamin primary structure between the two cereals. There are still some needs for further research into the problem of reduced cooked sorghum protein digestibility and why cooked maize appears to behave differently. Some of these are outlined below.

- There is general agreement that phytate is able to form less digestible complexes with sorghum proteins and this could reduce digestibility. It has been suggested that the degree of phytate-protein binding is influenced by the structural and chemical properties of both phytate and the protein rather than the total phytate concentration. A study of the mechanisms by which different forms of phytate complex sorghum proteins and how this influences digestibility may be important.

- Sorghum proteins are able to bind cell wall components and this may affect digestibility. The mechanisms by which this occurs are not clear and it has been hypothesised that this could be either by the direct attachment of proteins to non-starch polysaccharide components in the cell wall and/or by ferulic acid-mediated crosslinking. There is a need for these proposed mechanisms to be investigated. It is also important to find out how cooking affects sorghum protein-cell wall adhesion. This may have an effect on milling processes.

- In high tannin sorghums, the effect of tannins on protein digestibility is well known. However, the influence if any, of smaller molecular weight phenolic substances such as flavonoids and phenolic acids on digestibility is not very clear. Undertaking a study designed to test the hypothesis that these smaller phenolic substances may polymerise under oxidising conditions to form larger compounds that can complex protein could provide some useful information.

- There is a need for establishment of an efficient protocol for preparation of pure sorghum and maize protein bodies in high yield for further studies. Sucrose gradient centrifugation could be investigated. Further studies of protein body ultrastructure could be conducted using electron microscopy or atomic force microscopy.

- Protein crosslinking mainly through disulphide bonding appears to be perhaps the most important factor affecting cooked sorghum protein digestibility. However, it does not explain the poorer digestibility of sorghum proteins compared to maize though disulphide crosslinks are formed in both cereals on cooking. It has been suggested that the extent of crosslinking in sorghum may be higher than in maize. Quantification of disulphide crosslinks formed in both cereals during cooking could shed some light on this. The possibility of forming non-disulphide crosslinks such as dityrosine bridges has been suggested and this could be investigated in sorghum and maize. The exact nature of pepsin-indigestible, reduction-resistant oligomeric protein species (M_r 45 000 – 50 000) in cooked sorghum and the nature of the crosslinks is not well understood and this could be investigated.

- Kafirin and zein hydrophobicity appear to be important. The *gamma*-kafirins seem to be more hydrophobic than the *gamma*-zeins. It is not known how the *beta*-kafirins compare with the *beta*-zeins with regard to hydrophobicity and this could be investigated. Studies on the relative hydrophobicities of the isolated kafirins and zeins could be extended to different sorghum and maize varieties in order to establish whether there are any specific trends. The *beta*-kafirin primary structure needs to be determined.

- Spectroscopic studies could be conducted on uncooked and cooked forms of the isolated proteins in both sorghum and maize namely, *alpha*- *beta*- and *gamma*-prolamins. This will provide information about whether changes in protein secondary structure differ between the two cereals and possible relation to digestibility.

- There is a need to find an expression system for kafirins. Also a study could be conducted in which more digestible proteins such as the zeins or coixins are expressed in sorghum in order to determine how they affect digestibility.

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