NON-STARCH POLYSACCHARIDES, PROTEIN AND STARCH: FORM FUNCTION AND FEED - HIGHLIGHT ON SORGHUM

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Summary

In terms of structure and chemical composition, sorghum grain is remarkably similar to maize. The digestibility of sorghum starch is lower than that of maize starch and the major factor responsible for this appears to be a subtle difference in the endosperm protein bodies and surrounding protein matrix, which envelop the starch granules. The sorghum prolamin proteins (kafrins) in the protein bodies seem to be more cross-linked than their maize zein counterparts and cross-link more when the grain is subjected to wet-cooking. The cross-linking is primarily due to disulphide bonding. These cross-linked proteins seem to form a barrier to the egress of hydrolytic enzymes and probably also limit starch granule expansion during gelatinisation. Chemical treatments such as addition of sulphites and dry physical treatments such as fine milling, extrusion cooking or popping to disrupt the protein matrix seem to improve starch digestibility and may be of practical value. Selection of floury endosperm type sorghum may also be useful. Mutants with high protein digestibility that are also richer lysine have been identified and it is possible that these traits can be incorporated into sorghum lines with good agronomic properties.

I. INTRODUCTION

The chemistry and structure of sorghum grain is unique (Taylor and Dewar, 2001), as is the case with all other cereal grains. Knowledge of and understanding of sorghum’s uniqueness is the first step to optimising its exploitation. This paper will examine the unique features of the structure and chemistry of the sorghum grain with respect to nutritional negatives compared to other cereal grains. Possible strategies for alleviating these negatives will be discussed.

II. POLYPHENOLS

The grain of most sorghum cultivars contains higher levels of polyphenols than the grain of other cereal species. The red non-tannin sorghums are highly pigmented with polyphenolic anthocyanins and anthocyanidins. These appear to help protect the grain on the plant from insect and fungal attack. It is possible that they are slightly antinutritional. They bind strongly to the grain starch (Beta, 1999) and protein, but do not significantly affect digestibility (Duodu et al., 2002). However, the antioxidant properties of these compounds probably more than outweigh any antinutritional properties.

III. CELL WALLS

The endosperm cell walls of sorghum grain (Verbruggen, 1996) and maize grain (Huisman et al. 2000) are rich in water inextractable (insoluble) glucuronarabinoxylan type pentosans. Unlike for example barley, but like maize, the sorghum endosperm walls are not broken down during germination. Access by endogenous hydrolytic enzymes to the contents of the endosperm cells is via portals in the cell walls (Glennie, 1984). It is therefore probable

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that the cell walls themselves constitute something of a barrier to digestion. The cell walls are also intimately bound to the endosperm matrix protein. The mechanism of attachment is not known, but it is possible that the phenolic acid ferulic acid plays a role (Parker et al., 1999) (Figure 1).

Figure 1. Autofluorescence micrograph of popped sorghum (left) and popcorn (right) fragmented cell walls (from Parker et al., 1999)

IV. PROTEINS

a) Protein matrix and protein bodies

As in maize, the sorghum endosperm proteins are of two general types: a matrix and the protein bodies. The matrix envelops the starch granules and the protein bodies (Fig. 2). The matrix protein is probably simply remnants of the endosperm cytoplasmic protein. It is soluble in aqueous alkali and therefore in terms of the Osborne protein classification it can be considered as glutelin-type protein (Taylor et al., 1984). However, it is fundamentally different from the wheat glutenins, the glutenins, which are storage proteins. The protein bodies, which are essentially spherical and 1-2 microns across (Fig. 2), are comprised of prolamin proteins (Taylor et al., 1984). The prolamin of sorghum is called kafrin. Unlike in wheat and barley, but like in maize, the bodies persist in the mature grain, but are not membrane-bound. Kafirin, like the maize prolamin, zein, comprises three major protein species, designated alpha, beta and gamma (Shull et al., 1991). These are small proteins of molecular weight ranging from approx. 16 to 28 k. Alpha, which makes up about 80% of the total kafirin/zein fraction, is concentrated in the core of protein body (the cork of the cricket ball). Beta- and gamma-kafirin/zein are concentration at the surface of the protein body (the leather cover) (Oria et al., 1995). Beta- and gamma-kafirin/zein are rich in cysteine and are linked together with themselves (El Nour et al. 1998; Duodu et al., 2002) and with alpha-kafirin/zein, and probably with the matrix proteins, into oligomers of polypeptides by disulphide bonds. When wet-cooked the degree of linking increases (Duodu et al., 2002). We think that large polymers of molecular weight at least 100 k are formed. For reasons not known the degree of cross-linking on wet-cooking with kafirin seems to be greater than with zein. It also appears that some of the cross-links, particularly in kafirin, may not be disulphide bonds, since not all the oligomers can be broken down into monomers with reducing agents that are used for this purpose (Duodu, et al., 2002).
Figure 2. TEM of sorghum protein bodies (PB) and matrix (M)

b) Protein digestibility

The formation of these cross-linked proteins is believed to be the major, but probably not the only, cause of the well-described reduction in the protein digestibility of sorghum on wet-cooking (Table 1) (Duodu et al., 2002; 2003). It has been proposed that the cross-linked gamma- and beta-kafrins at the surface of the protein bodies are poorly digestible and that they form a barrier to protease enzymes reaching the readily digestible alpha-kafrin at the core (Oria et al., 1995). Strong evidence in support of this theory comes from the research of Prof B R Hamaker and co-workers of Purdue University. They have discovered sorghum mutant lines with high protein digestibility, even when cooked (Oria et al., 2000). The protein bodies of these mutants are not generally spherical, but are highly invaginated, in section like the petals of a flower (Fig. 3). Unlike in the protein bodies of normal sorghum, the beta- and gamma-kafrins are concentrated at the base of the invaginations. Hence, they would not constitute a barrier to digestion of the alpha-kafrin. Indirect support for this theory comes from the fact that kafrin polypeptide cross-linking occurs as normal in these mutants (Duodu, 2000).

Table 1. Effect of wet-cooking on the in vitro pepsin protein digestibility (g/kg) of whole grain, endosperm and protein body enriched sorghum and maize flours (adapted from Duodu et al., 2002).

<table>
<thead>
<tr>
<th>Grain</th>
<th>Treatment</th>
<th>Whole grain</th>
<th>Endosperm</th>
<th>Protein body enriched</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red med</td>
<td>Raw</td>
<td>591</td>
<td>657</td>
<td>728</td>
</tr>
<tr>
<td>sorghum</td>
<td>Wet cooked</td>
<td>305</td>
<td>359</td>
<td>442</td>
</tr>
<tr>
<td>White hard</td>
<td>Raw</td>
<td>558</td>
<td>674</td>
<td>743</td>
</tr>
<tr>
<td>sorghum</td>
<td>Wet cooked</td>
<td>366</td>
<td>394</td>
<td>635</td>
</tr>
<tr>
<td>White hard</td>
<td>Raw</td>
<td>666</td>
<td>674</td>
<td>688</td>
</tr>
<tr>
<td>maize</td>
<td>Wet cooked</td>
<td>620</td>
<td>636</td>
<td>674</td>
</tr>
</tbody>
</table>
Figure 3. TEM of protein bodies from normal (left) and a highly digestible mutant (right) (From Oria et al., 2000)

V. STARCH

Characteristics

With regard to starch, the starch granules of sorghum like those of maize, but unlike those of wheat and barley, are only of one type, large essentially spherical granules of 10-16 microns across (Taylor and Belton, 2002). A minor difference between sorghum and maize is that sorghum contains starch granules in the mesocarp layer of the pericarp (bran) in addition to the normal starch endosperm granules. However, in quantity these are insignificant compared with the number of granules in the endosperm.

It is well known that sorghum starch has amongst the highest gelatinisation temperatures of all starches. The gelatinisation temperature range of 68-78°C is some five degrees higher than that of maize (Taylor and Belton, 2002). It is speculated that the long amylopectin “a” chains which entangle with each other, may be longer in sorghum than in maize. Even if so, this may simply be a consequence of the sorghum kernels being borne on the top of the plant and exposed to direct sun and higher temperature, rather than being in the shade and slightly cooler temperature, as in the case of maize. The high temperature in the sorghum kernels could lead to greater “a” chain synthesis.

a) Starch digestibility

The impact of sorghum’s higher starch gelatinisation temperature on starch digestibility depends on the situation. If fully gelatinised, isolated sorghum starch is no doubt just as digestible as maize starch. However, in food and feed systems it is generally flour with much of the cell contents structure intact, and not pure starch, that is consumed. In this situation, sorghum has lower cooked starch digestibility than maize (Fig. 4) (Ezeogu, et al., in press). The cause of this appears to be the same as the reduced protein digestibility on wet-cooking. Addition of reducing agents that break protein disulphide bonds increases starch digestibility (Fig. 4) (Zhang and Hamaker, 1998; Ezeogu et al., in press). We have also observed that the protein matrix around the starch granules in sorghum appears to remain more intact than that of maize, after the starch has been gelatinized. Both these pieces of evidence indicate that kafirin cross-linking is involved in the lower starch digestibility of sorghum. It can be speculated that the cross-linked proteins in the matrix enveloping the starch granules inhibit the access of amylase enzymes to the granules. Further, the cross-linking of the proteins may inhibit expansion and disruption of the starch granules during gelatinisation, hence, slightly limiting egress of the amylases between the starch molecules.
Figure 4. *In vitro* starch digestibility of sorghum and maize floury (squares) and vitreous (circles) endosperm treated without (open symbols) and with a reducing agent (closed symbols) (from Ezeogu *et al.*, in press)

The adverse effects of protein cross-linking on sorghum protein and starch digestibility seem to be predominantly a consequence of protein cross-linking occurring as result of wet-cooking. In poultry feeding, grain is often consumed in the raw (uncooked) state. Notwithstanding this, sorghum has lower starch digestibility and metabolisable energy than maize. The latter cannot be fully accounted for by the slightly lower oil content of sorghum grain, compared to maize. It has long been speculated (Rooney and Pflugfelder, 1986) that the sorghum protein matrix plays a role. There is evidence that there is greater cross-linking of the proteins in the matrix of raw sorghum compared to maize. However, at present the evidence is really only circumstantial. A common method to extract the prolamin proteins from maize or sorghum is to first extract with aqueous alcohol and then to extract with aqueous alcohol plus reducing agent. The theory is that the reducing agent breaks disulphide bonds between the prolamins, extracting the so-called cross-linked prolamins. Analysis of the literature reveals that the relative proportion of prolamin extracted with reducing agent is much higher in sorghum than in maize (Table 2) (Duodu *et al.*, 2003), thus indicating that in raw grain the kafirins in sorghum are more highly cross-linked than the zein in maize. Hence, it could well be that these cross-linked kafirins present a barrier to digestion, accounting for the lower starch digestibility and metabolisable energy of sorghum. The problem is to prove this, as the process of analysis necessitates destruction of the native state of the proteins.

VI. STRATEGIES FOR ALLEVIATION OF NUTRITIONAL NEGATIVES

a) Chemical and physical treatments

The idea of using exogenous pentosanases to break down sorghum endosperm cell walls seems at first sight to be attractive. This would remove a potential barrier to digestion of the cell contents. However, it has been found that sorghum cell walls are only hydrolysed to a limited extent by endoxylanase (Verbruggen, 1996). This appears to be because of the high degree of arabinose substitution and the presence of considerable amounts of glucuronic acid in the sorghum arabinoxylans. The use of accessory enzymes to remove the arabinose improves degradation of the arabinoxylan somewhat.
Table 2. Prolamin 1 and prolamin 2 protein fractions (g/kg protein) in raw sorghum and maize (adapted from Duodu et al., 2003)

<table>
<thead>
<tr>
<th>Sorghum</th>
<th>Maize</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kafirin 1</td>
<td>173</td>
</tr>
<tr>
<td>199</td>
<td>351</td>
</tr>
<tr>
<td>99</td>
<td>153</td>
</tr>
<tr>
<td>200</td>
<td>440</td>
</tr>
<tr>
<td>200</td>
<td>330</td>
</tr>
<tr>
<td>Mean</td>
<td>174</td>
</tr>
</tbody>
</table>

Since the protein matrix and bodies both are somewhat poorly digestible themselves and seem to be the major factor reducing starch digestibility, they would seem to be the most important focus area. With regard to digesting them with exogenous protease enzymes, their hydrolysis by proteases is rather slow (Taylor and Boyd, 1986). This is presumably related to their apparently high degree of cross-linking and that they are insoluble in aqueous solution. In fact, the mode of digestion of the matrix and protein bodies by proteases is a process of erosion, primarily at the outer surfaces (Taylor and Evans, 1989).

In in vitro studies good success in improving both sorghum protein and starch digestibility has been achieved through the addition of the food grade reducing agent bisulphite/metabisulphite (Zhang and Hamaker, 1998). This is presumed to be due to it breaking protein disulphide bonds. Whether its addition in poultry feed would be effective remains to be seen, since sulphites can have adverse physiological effects, in particular they are allergens.

The simple expedient of soaking the sorghum grain to hydrate it may be of value. Hale (1973) reported that “reconstitution”, i.e. storing sorghum grain at 30-35% moisture under oxygen limiting conditions for some 3 weeks, resulted in it having markedly higher protein digestibility, similar to that obtained with steam flaking. We have found that hydration of sorghum grain can be accelerated by soaking in very dilute alkali (Dewar et al., 1997). We attribute this to the alkali hydrating the cell wall arabinoxylans.

In ruminant animal feeding, the process of tempering followed by steam flaking is routinely used with sorghum grain feeding as it improves starch and protein digestibility (Rooney, 1992). Steam flaking disrupts the grain subcellular structure, especially the vitreous endosperm, allowing more surface area contact between the digestive enzymes and the protein and starch. The improvement in starch digestibility is also presumably due to the disruption process enabling more expansion of the starch granules during gelatinisation.

According to Hancock (2000) in poultry feeding grain particle size is much more critical in sorghum than in maize. Sorghum needs to be more finely milled than maize. For both soft and hard sorghums, reducing the grain particle size to a maximum of 500 microns resulted in the growth performance of broiler chicks approaching that with maize. This effect is again presumably mainly as a result of disrupting the sorghum grain subcellular structure and also of increasing the grain surface area for enzymic attack. In the poultry industry, the process of pelleting is widely used. The ground grain is conditioned with steam and then passed though a die to create a pellet that reduces dust, improves palatability and feed handling (Rooney, 1992). The steam partially gelatinises the starch so that it acts as binder. It is doubtful whether the protein matrix is disrupted in the process. However, the possible negative impact of what is in effect a wet-cooking process on protein cross-linking does not seem to have been assessed. In this respect, extrusion cooking and popping which are essentially cell disruption dry cooking processes may have advantages. It has been found that
neither extrusion cooking (Hamaker et al., 1994) nor popping (Parker et al., 1999) negatively affect sorghum protein digestibility.

**b) Selection and breeding of cultivars**

As shown in Figure 3, we have recently found that in wet-cooked sorghum the starch digestibility of the vitreous endosperm is lower than that of the floury endosperm even when the endosperm particles are of similar size. Since in the floury endosperm the protein matrix is much thinner, this suggests that sorghum grain with a high proportion of floury endosperm would have better starch digestibility than grain with a high proportion of vitreous endosperm. There is considerable literature on feeding ruminant animals with maize of different endosperm texture that supports this contention (Philippeau et al., 1999; Correa et al., 2002).

The improved digestibility of the sorghum mutant lines from Purdue University with the invaginated protein bodies has already been described. These mutants also have additional potential benefits for poultry feed. They are high lysine types (Weaver et al., 1998). Some also have high starch digestibility, apparently caused by morphological differences in their starch granules (Benmoussa et al., 2004).

**VII. CONCLUSIONS**

We are making slow but steady progress in understanding why the nutritional value of sorghum, especially with respect to its protein and starch digestibility, is slightly lower than that of other cereal grains. Direct comparison with maize has proved to be useful because of the close similarity in chemical composition and subcellular structure. The evidence points clearly to disulphide bond cross-linking of the beta- and gamma-kafirin proteins being a major factor in reducing protein and starch digestibility. However, what we do not understand is why in view of the great similarity between the prolamin proteins of sorghum and maize, cross-linking should occur to a greater degree in sorghum.

With regard to strategies for alleviating sorghum’s nutritional negatives, physical treatments to disrupt the subcellular structure and selection of less vitreous endosperm types appear to be the best options at present. Development of high digestibility, high lysine sorghum lines which have good agronomic characteristics seems to be a good idea but may just be a pipedream. The very beneficial characteristics of sorghum that enable it be cultivated in the semi-arid tropics are probably closely related to its slight nutritional negatives.

**REFERENCES**


