OVERVIEW: SORGHUM PROTEINS AND FOOD QUALITY

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INTRODUCTION

When considering sorghum proteins and their contribution to functionality and food quality of sorghum-based foods, a fairly small amount of research has been done on sorghum compared to most of the other major cereal grains. Potential functional properties of sorghum proteins can perhaps best be broken down into two general areas: 1) grain proteins and their interaction to form structures that impact food properties, and 2) the influence of proteins on other grain constituents, principally starch. Overall, sorghum flour proteins, as is the case with maize and millet, are not considered to have a large role in creating textures in foods. One reason is that the majority of sorghum grain proteins, the kafirin storage proteins, are found encapsulated in protein bodies that remain intact during low shear cooking and, thus, are not normally available to form functional structures in foods. However, recent evidence on viscoelastic behavior of maize storage protein, as well as extended sorghum protein structures that appear to form during cooking, implies a possible significant role for sorghum proteins in quality characteristics of sorghum foods. A few studies have shown how sorghum proteins affects other grain constituents, including its influence starch gelatinization behavior and starch digestion rate. It is the purpose of this paper to present an overview of work done on sorghum proteins as they affect sorghum food quality, and to speculate how sorghum proteins can be manipulated through processing or genetics to create foods with desirable, and perhaps new, attributes. This review will focus principally on the potential of sorghum proteins to form structures that impact function properties and quality of sorghum-based foods.

It is first useful to review briefly sorghum grain protein composition and chemistry to understand how proteins might behaves in processing of sorghum foods. Using the solubility-based classification scheme of Landry and Moureaux[1] that was modified for sorghum by Jamunathan *et al.*[2], sorghum proteins were divided into albumins (water-soluble), globulins (salt-soluble), kafirins (prolamins, aqueous alcohol-soluble), cross-linked kafirins (aqueous alcohol + reducing agent-soluble), and glutelins (detergent + reducing agent + alkaline pH-soluble). Sorghum was different compared to maize in that it contained a higher proportion cross-linked kafirins to kafirins than was found in maize. This suggested a higher propensity towards intermolecular disulfide-crosslinking among kafirins than occurs in maize, despite a high degree of sequence homology between the storage proteins of the two cereals. In regards to relative proportions of protein classes, Hamaker *et al.*[3] showed that kafirins, taken as a whole, comprise about 70% of whole grain flour protein and about 80% of decorticated flour protein. They went on to suggest that a reasonable, and simpler, classification of sorghum proteins would be kafirins and non-kafirins to reflect the homogeneous nature and different origin of the kafirin storage prolamins opposed to the heterogeneous group of non-kafirin proteins involved in cellular functions.

Kafirin proteins are synthesized and translocated into the lumen of the endoplasmic reticulum where they accumulate and form protein bodies. They have been further classified as alpha-, beta-, and gamma-kafirins based on differences in their molecular weight, extractability, and structure, and cross-reactivity with analogous maize zeins[4]. Alpha-kafirin makes up about 80% of the total kafirin and is considered the principal storage protein of sorghum, beta-kafirin comprises about 5% of total kafirin, and gamma-kafirin about 15%. Two important considerations regarding kafirins as they act in food systems are their cysteine contents and their placement within the protein body.

While alpha-kafirin has a low level of cysteine, beta- and gamma-kafirins have high levels of 5 and 7 mol% cysteine, respectively. Internal protein body structure is such that gamma-kafirin, and beta-kafirin to a lesser degree, encapsulate alpha-kafirin in a disulfide-bound polymeric network[5]. In the sorghum endosperm, non-kafirins form a coating around the protein bodies that effectively "glues" them into a matrix that surrounds the starch granules of the vitreous endosperm portion (Fig. 1).

Sorghum proteins must form structures with themselves or with other constituents during processing and/or cooking to directly impact functional properties and quality of sorghum-based foods. The following sections will consider recent studies in our laboratory and by others that provide some evidence that sorghum proteins may either act or have the potential to act to impart quality changes or even new functionalities for sorghum flours.

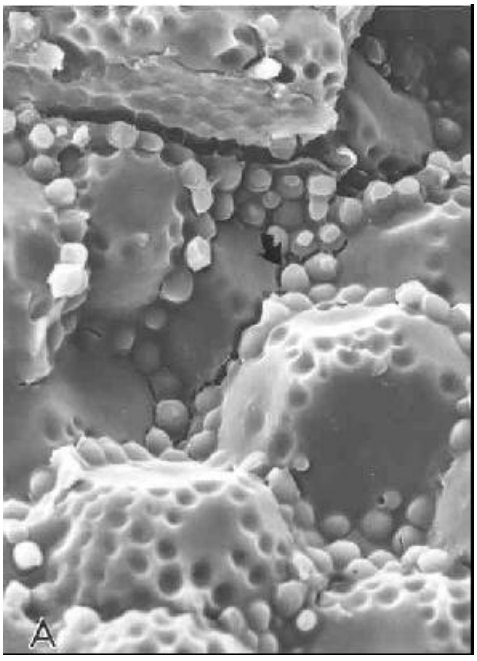


Figure 1. Scanning electron micrograph of vitreous portion of sorghum endosperm. Protein bodies are indicated by the arrow.

SORGHUM PROTEIN-PROTEIN INTERACTIONS

Viscoelasticity studies

Sorghum kafirins are generally not thought of as functional proteins. First, their encapsulation in rigid protein bodies that persist through most food preparations effectively prevents them from forming polymeric structures that would affect

functionality. Second, even if released from the confines of protein bodies, obvious molecular differences between kafirins and the highly viscoelastic wheat proteins would appear to preclude similar viscoelastic properties. Additionally, kafirin and zein proteins do not contain an analogous protein class to the HMW glutenin proteins of wheat that is principally responsible for gluten elasticity. Yet, despite these dissimilarities, recent studies[6,7] have shown that isolated zein protein, analogous in many ways to kafirins, can be made viscoelastic with resulting affects on bread dough strength and loaf volume. In the study of Bugusu *et al.*[7], functionality of zein was dependent on conditioning the moistened protein overnight at 35 degrees C, a temperature above its glass transition temperature (Tg) of around 27 degrees C. A fundamental difference between wheat and maize prolamins is their Tg when hydrated. At the moisture levels of a wheat dough system, the Tg of the gluten protein is below room temperature, while Tg of zein protein is above room temperature (common in the US). Lawton[6] showed that isolated zein became viscoelastic above its Tg.

Bugusu *et al.*[7] tested this principle in a dough and bread system using a 20% sorghum/80% wheat flour composite. Commercial isolated zein was added at 1, 5, and 10% levels of the sorghum portion of the composite mix. Following conditioning at 35 degrees C overnight, doughs were made and rheological properties assessed using a mixograph (Fig. 2).

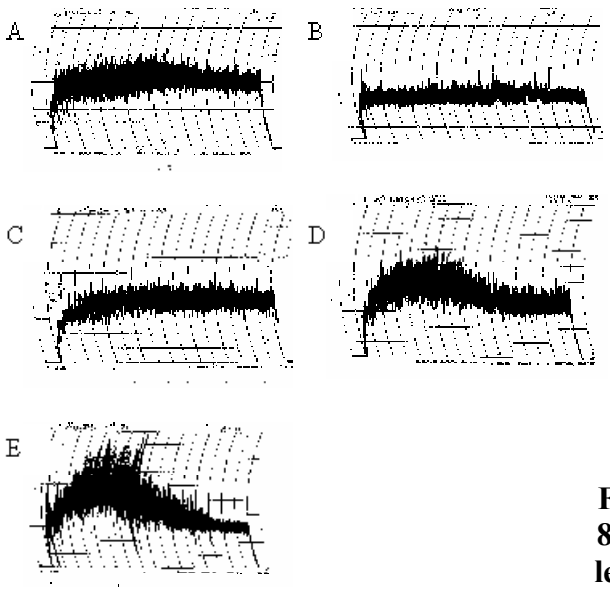


Fig. 2. Mixograms of 20% sorghum-80% wheat dough with added zein at levels of 0%, 1%, 5%, and 10%, B-E, respectively. A is the control 100% wheat.

The addition of zein protein clearly increased dough strength, though did not result in ideal dough mixograph profiles (Fig 2c-e). Dough extensibility also increased as demonstrated by a stress elongation test. Bread loaf volume (cubic cm) was likewise affected increasing from 715.8 to 739.2 and 809.2 with addition of 5 and 10% zein, respectively. Thus, formation of zein fibrils during dough mixing not only increased

dough strength and extensibility, but also aided in the capture of carbon dioxide gas to increase loaf volume. Confocal laser scanning microscopy of the gluten and alpha-zein proteins in bread[8], visualized by autofluorescence of the former and antibody-fluorescent tagged in the latter, showed the supporting linkages that were made by the zein protein (Fig. 3).

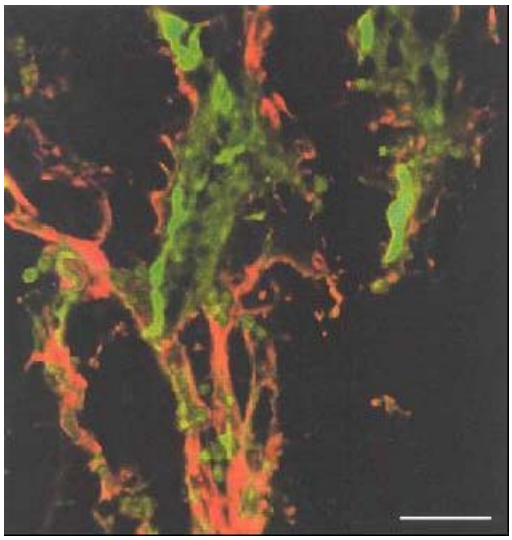


Fig. 3. Confocal laser scanning micrograph of wheat gluten (green) and maize zein (red) in bread. Maize zein appears to connect some gluten fibrils providing better ability of the dough to trap carbon dioxide and leading to the observed increase in loaf volume in a sorghum-wheat composite bread when zein was added.

While not giving a direct route to use sorghum kafirin protein in composite breads, these studies do show the potential that kafirin can be a functional component in baked products. Moreover, it suggests that the functional properties of kafirin protein, particularly alpha-kafirin, could be improved further, perhaps through molecular approaches such as protein engineering. Obviously a real constraint in utilizing kafirins in a positive and functional way is their encapsulation in protein bodies. However, solutions may exist there also either by disruption of bodies through processing, as discussed in the following section, or through modification of the protein body structure itself. In this regard, the high protein digestibility sorghum mutant with altered protein body morphology (Fig. 4) shows a structure where alpha-kafirin is perhaps positioned for greater protein-protein interaction during processing[9]. In mature seeds (not shown), there is some melding of protein bodies in the mutant sorghum, while wild-type protein bodies remain distinct and separate. A future goal perhaps would be to have sorghum/wheat composite flours where kafirin protein is a contributor to development of the gluten network, resulting in higher proportions of sorghum flour that could be used.

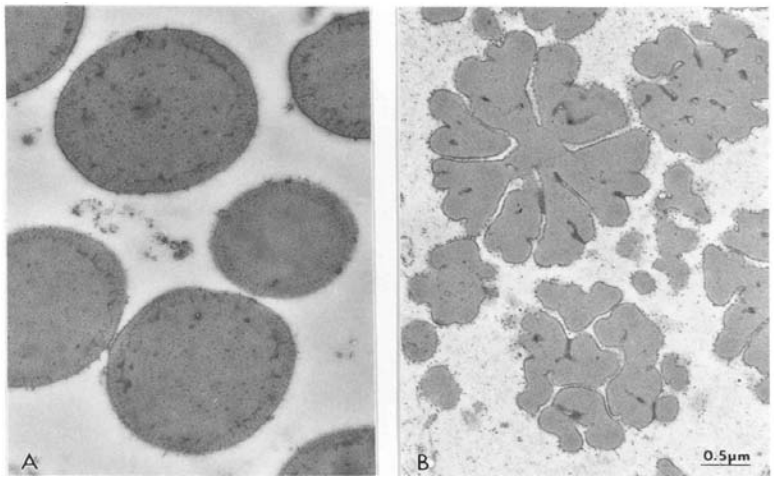


Fig. 4. Transmission electron micrographs of protein bodies from normal (left) and high protein digestibility mutant (right) sorghum genotypes.

DISRUPTION OF PROTEIN BODIES DURING PROCESSING

With approximately 80% of sorghum endosperm protein contained within the structure of protein bodies, it is necessary to understand the forces required to disrupt protein bodies and release kafirins to realize their potential as functional component to a food system. In this regard, we conducted a study on disruption of maize protein bodies that should well reflect what happens to sorghum protein bodies processed under the same conditions. Normal maize or sorghum protein bodies are presumably made rigid by the disulfide-linked polymeric nature of the gamma- and beta-prolamin proteins found at the body periphery. The structural role of gamma-prolamin is highlighted in the high protein digestibility sorghum mutant mentioned above where change in location of the protein from the body periphery to the base of the folded structure results in altered structure. To disrupt normal protein body structure, shear forces must be applied in the food preparation process. In the following described experiments, high pressure, high temperature extrusion processing was used to determine conditions necessary to not only disrupt, but also to disperse zein[10].

Transmission electron microscopy was used to assess microstructural and chemical changes that occur in zeins in corn flour extruded over a range of shear forces, as measured by specific mechanical energy [SME = (2π x Torque x RPM)/feed rate]. The SME required to physically disrupt the protein bodies and disperse the zein contained within was determined. A single-screw extruder was used with three screw types (30/1 L/D; 1:1, 3:1, and dispersive) to attain a wide range of SME for corn flour extrudates. Extrudates, as well as corn flour, were ground and fixed for microscopic sectioning using a standard method. Dried samples were infiltrated for one day each in 20, 40, 60, and 80% LR White resin in ethanol and then placed for two weeks in 100% resin prior to polymerization by heating. Immunochemistry was also performed using alpha-zein specific rabbit polyclonal antiserum to visualize zein-containing protein structures.

Uncooked and cooked protein bodies appeared the same showing that thermal treatment alone does not disrupt body structure. Protein bodies retained their structural integrity up to a SME of about 100 kJ/kg where there was an elongation and some melding of protein bodies (Fig. 5). At a SME of 165 kJ/kg, individual protein bodies were no longer visible and alpha-zein appeared dispersed, while at a high SME of 387 kJ/kg alpha-zein was highly disrupted with no remnants of protein bodies (Figs. 6-7). Disulfide-mediated polymerization of alpha-zein was found in these extrudates. In a subsequent study (Tandjung and Hamaker, *unpublished*), zein in extrudates was shown to impart brittleness (similar to crispness) in extrudates, particularly when extrudates were brought to an intermediate moisture content of about 14%. This was related to the relatively high Tg of zein and to speculated fibril formation in the extrudate.

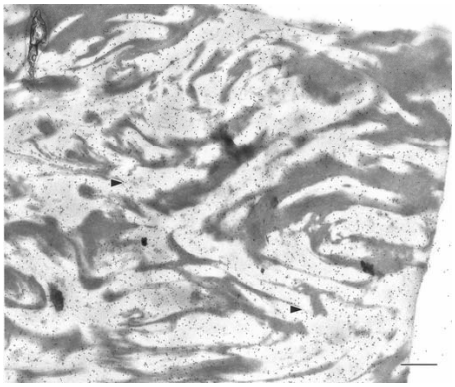


Fig. 5. Maize protein bodies (light staining structures) in extrudate processed with medium shear of about 100 kJ/kg specific mechanical energy. Note elongation and some melding of protein bodies

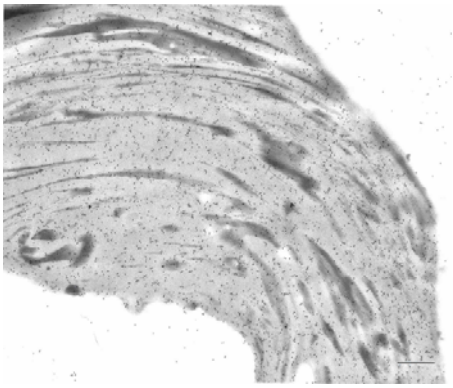


Fig. 6. Maize protein bodies (light staining structures) in extrudate processed with medium-high shear of 165 kJ/kg specific mechanical energy. Note dispersal of zein and no noticeable protein body structures

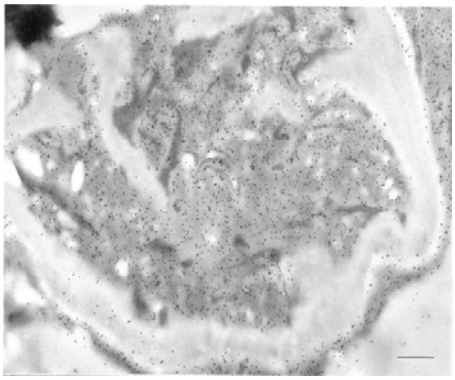


Fig. 7. Maize protein bodies (dark staining structure in center of light staining semicircular structure) in extrudate processed with high shear of close to 400 kJ/kg specific mechanical energy.

The relevance of this study to sorghum flour utilization rests in the finding that cooking alone does not disrupt protein body structure. A SME of about 100 kJ/kg is needed to begin to disfigure protein bodies to the extent that the prolamin may interact in a functional way. However, still higher SME's were required to disperse zein. Zeins were not dispersed per se throughout the cooked starch, but associated with like proteins to form what may amount to fibrils with some functional characteristics. In a practical sense, these shear forces are not very high, however, would rarely be reached in normal processing of sorghum-based foods. Thus, outside of extrusion processing it is not clear what other common food processes would produce sufficient shear to break apart normal protein bodies and release kafirin to achieve possible functional behavior. Perhaps a genetic change resulting in alteration of protein body structure, such as that described above, may be the best way to realize an effect of sorghum proteins in food systems.

CHANGES IN SORGHUM PROTEINS DURING COOKING

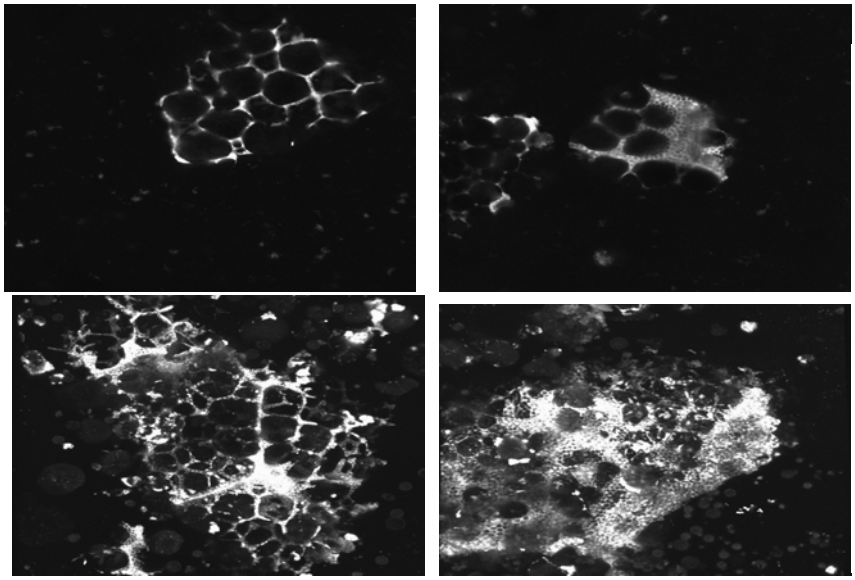
Changes have been documented that occur to kafirin proteins during the cooking process that affect protein digestibility, and recent evidence indicates that similar changes among non-kafirin proteins may also impact functional properties of sorghum-based foods. Cooking reduces digestibility of sorghum kafirins through disulfide-mediated polymerization principally among the gamma- and beta-kafirin proteins found at the periphery of the protein bodies[11]. Thus, the encapsulated major storage protein, alpha-kafirin, becomes more difficult to access by digestive proteases. Certainly the high proportion of cysteine residues in gamma-kafirin, 14 as deduced by cDNA sequencing[12], and less so in beta-kafirin suggest that these proteins would be particularly prone to forming disulfide-linked polymers. El Nour *et al.*[13] reported interesting data suggesting that beta-kafirin, in particular, is a component determining degree of polymerization. It was present in non-reducing solvent unextractable protein, and though itself is not present in dimeric or trimeric forms, beta-kafirin may act as a bridge linking oligomers of alpha$_1$- and gamma-kafirin. Alpha$_1$-kafirin has been designated the higher MW major storage polypeptide of M_r 24-29 and alpha$_2$-kafirin the minor lower MW polypeptide of M_r 22. They further went on to suggest that beta-kafirin is a 'chain extender' and alpha$_2$-kafirin a 'chain terminator'. Another important piece of the puzzle was reported recently by Duodu *et al.*[14]. They showed, using FTIR and solid state ^{13}C NMR spectroscopy, that cooking causes more intermolecular beta-sheet structure that seemed to be more pronounced in sorghum

than in maize proteins, and suggested that change from alpha-helical to beta-sheet conformation could facilitate formation of disulfide-linked polymers.

These studies form an interesting base to better understanding disulfide-mediated polymerization at the molecular level and further studies of this type and at the sequence and molecular structural level could delineate why sorghum kafirins behave as they do during cooking. Yet, one of the perplexing problems still confronting sorghum protein researchers is why sorghum kafirins behave as they do in relation to digestibility when they have such high similarity, e.g. sequence homology and location, with their analogous maize zein counterparts. Surely it is possible that protein structural differences alone may account for this odd phenomenon of substantial decrease in digestibility of sorghum proteins on cooking, but other possibilities also exist – such as presence of non-protein promoters of polymerization or protein conformational changes.

In other recent and unpublished studies in our laboratory, we have developed possibly yet another view of how sorghum proteins behave during cooking that is different from other cereal proteins. In an attempt to understand how sorghum proteins impact functionality in thick pastes, as well as how they may retard digestion of starch, confocal laser scanning microscopy was used to visualize protein structures. The protein dye, 3-(4-carboxybenzoyl) quinoline-2-carboxaldehyde (CBQCA), fluoresces only after reaction with primary amines in proteins which makes it particularly useful in this application as it does not have to be washed out prior to analysis. The dye covalently binds to proteins or amino acids and forms highly fluorescent compounds as shown in the reaction (Product Information, Molecular Probes, Inc., Eugene, OR).

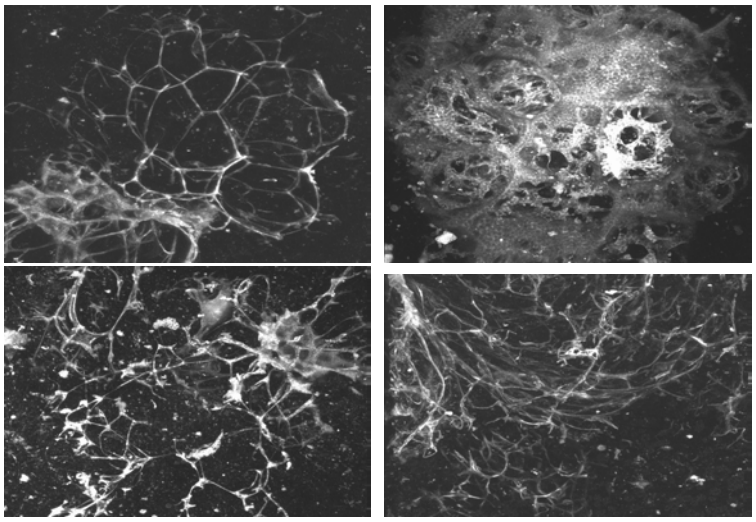
Micrographs shown in Figs. 8-12 show 3-D images (except where noted) comprising greater than 20 laser-generated optical planes that are compressed into 2-D pictures. Protein in flour and cooked sorghum pastes are shown in Fig. 8 and 9. As is typical of vitreous portions of sorghum endosperm, protein in raw flour is shown in white surrounding dark areas where starch granules exist. Protein bodies are seen as small circular bodies embedded in a larger protein matrix (Fig. 8c,d). We speculate that the CBQCA dye did not fully penetrate the protein bodies, thus highlighting their spherical structure. After cooking, sorghum proteins appear to have formed extended, web-like structures (Fig. 9a,c,d) or sheet-like structures with starch embedded within (Fig. 9b). Similar structures were seen in the high protein digestibility mutant sorghum (Fig. 10). When a reducing agent, sodium bisulfite, was added to the cooking broth, formation of these structures was considerably reduced (not shown) indicating that a disulfide-mediated polymerization process was in play. Maize proteins, when cooked, showed far less of these highly extended structures (Fig. 11) and rice proteins after cooking appeared mostly to aggregate (Fig. 12).



A B

C D

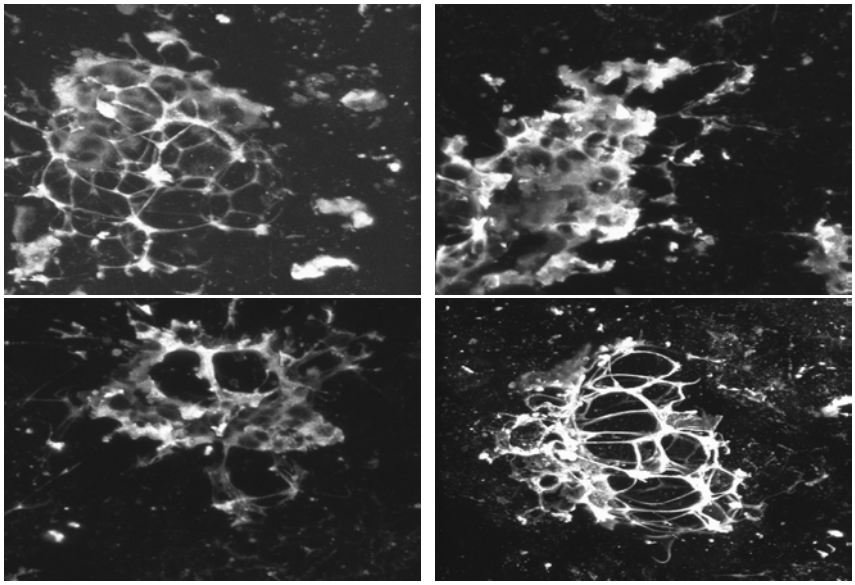
Fig. 8. Confocal micrographs of raw normal sorghum (P721N), protein (bright areas) stained with CBCQA, A and B single optical plane, (A-starch granules linked by protein, B-protein bodies embedded in matrix), C and D reconstructed 3D images (in 2D picture).



A B

Fig. 9. Confocal micrographs of cooked normal sorghum (P721N), protein (bright areas) stained with CBCQA (reconstructed 3D images), showing the web-like (A, C, D) and sheet-like (B) protein structures.

C D

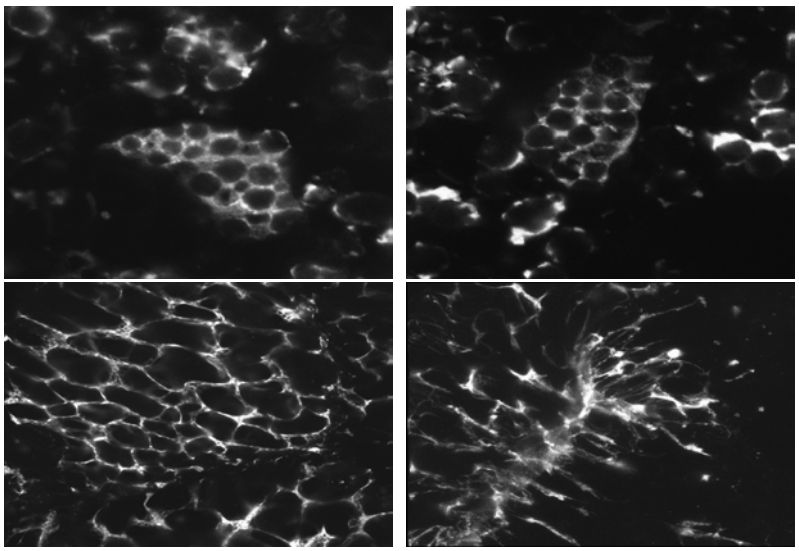


A B

C D

Fig. 10. Confocal micrographs of cooked high protein digestibility sorghum, protein (bright areas) stained with CBCQA (reconstructed 3D images), showing the web-like (A, C, D) and sheet-like (B) protein structures

A B



C D

Fig. 11. Confocal micrographs of raw (A and B, single optical planes) and cooked (C and D, reconstructed 3D images) maize, protein (bright areas) stained with CBCQA. Microstructure in raw is similar to sorghum. Cooked sample shows the less well developed web-like protein structures but no sheet-like structures observed.

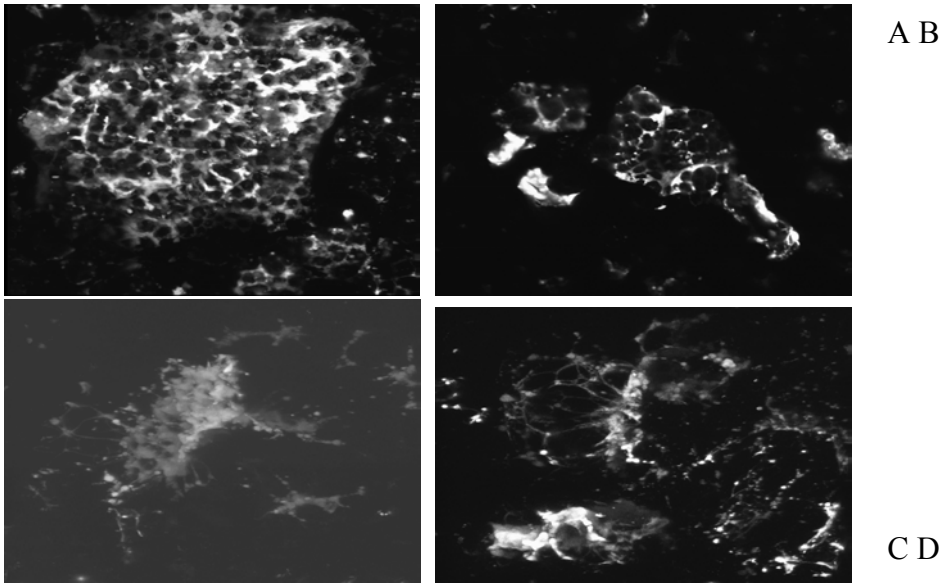


Fig. 12. Confocal micrographs of raw (A and B, single optical planes) and cooked (C and D, reconstructed 3D images) rice, protein (bright areas) stained with CBCQA. In raw samples, the starch granules are embedded in the protein matrix while in cooked the proteins seems to aggregate together

Formation of these large extended web-like protein structures during cooking of sorghum flour paste appears to fit well with a general hypothesis that sorghum proteins undergo changes during cooking that include disulfide-mediated polymerization. Interestingly in this work it is apparent that non-kafirin proteins also participate in this process, as these extended structures must principally be formed from this protein group. This suggests to us that a non-protein component of sorghum flour, such as a weak oxidant (e.g., a quinone), is promoting polymerization. Alternatively, from the work of Duodu *et al.*[14] is it possible that a factor is facilitating greater changes in protein conformation in sorghum pastes? We are currently further studying the former possibility.

So how could these protein structures affect sorghum food quality? One possibility is that such structures could impede full starch gelatinization, thereby influencing paste viscosity and gel strength of the thin and thick porridges. Chandrashekar and Kirleis[15] showed that addition of reducing agent prior to cooking increased degree of gelatinization in sorghum pastes. Related to this we are also currently investigating the effect of sorghum protein on starch digestion rate of cooked sorghum foods. Other functional outcomes in sorghum foods may also be possible.

CONCLUSIONS

Sorghum proteins likely provide a greater role in the functionality and quality of sorghum foods than has previously been thought. This overview has shown that kafirin proteins have a potential to contribute to the viscoelasticity of developed gluten networks, but only if released from the confines of the rather rigid protein body structures into which they are packaged. Also the comparably high Tg of maize zeins

and sorghum kafirins conceivably could confer the desirable property of crispness of products. Finally, proteins in general appear to behave differently in sorghum than in maize or rice by forming extensive extended web-like or sheet-like structures during cooking. This could impact functional properties and quality of sorghum products, as well as could affect nutritional properties. Further knowledge of how to manipulate proteins so that they can be more functional is needed, as well as better understanding the diverse nature of sorghum germplasm as it relates to protein functionality and product quality.

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