Making kafirin, the sorghum prolamin into a viable alternative protein source

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Abstract
Kafirin, the sorghum prolamin is the most hydrophobic of the prolamins, and forms disulfide cross-links due to its high cysteine content. It is noted for its slow digestibility and it does not trigger an adverse response when consumed by celiacs. These properties make kafirin potentially valuable in both food and non-food applications, especially as a bioplastic and encapsulating agent. Despite these valuable properties, to date there is no commercial production of kafirin and consequently no commercial products. Extraction technologies that could be up-scaled for industrial use are described. Also, genetic and physico-chemical techniques that have been applied to improve the functional and nutritional properties of kafirin as a functional food ingredient and as a bioplastic are reviewed. It is proposed that kafirin extraction and bioplastic manufacture should be located at the site of grain bioethanol production. This would enable the use of a consistent supply of inexpensive, high protein feedstock from sorghum DDGS, as well as a ready supply of inexpensive ethanol for extraction. In addition, equipment would be available for solvent recovery and transportation costs could be minimized. These factors would contribute to making kafirin economically viable as an alternative protein source.

Keywords:
Sorghum, prolamin, kafirin, bioethanol, distillers dried grains with solubles (DDGS), bioplastic, nutrition, functional properties, food ingredient, brewery spent grain
1. Introduction: Sorghum as a sustainable crop

A dream of a ‘green economy’ has been around for some considerable time, where all the world’s needs in terms of fuel and polymers are met from renewable biomass (1). Up until now this has remained a dream, since in reality the world continues to be dependent on the petrochemical industry. Can sorghum play a role in realising, at least in part, this dream of a green economy? In terms of production, sorghum is the fifth most important cereal crop, with a global production of approx. 69 million tonnes (2). Currently, sorghum only accounts for <3% of world cereal production, dwarfed by maize (40%), rice (29%) and wheat (28%). So at the moment there is just not enough sorghum grown for it to play a major role in a green economy. Importantly, however, sorghum is probably the most hardy major cereal crop and is better adapted to cultivation in hot and arid regions than the major cereals, with a generally lower water requirement. For example, it has been shown that sorghum will out yield maize under conditions of moderate and severe water deficit (3,4). With climate change causing higher temperatures, more erratic rainfall and more frequent droughts in many regions, there is a strong case for increased sorghum cultivation (5).

As a generalization, in countries such as the USA, Mexico, Argentina, Brazil and Australia and the European Mediterranean countries, sorghum has been cultivated almost exclusively as a grain for animal feed, cattle, pig and poultry feeding. In contrast, in Africa, India and China, sorghum grain has traditionally been used as a human food and for making alcoholic and non-alcoholic beverages. Today, sorghum grain is also being used in large quantities in the USA for bioethanol production and in Africa for the industrial brewing of opaque beer and increasingly for lager and stout beers and non-alcoholic malt-based beverages. Additionally, both across Africa and in Western countries, flour from industrially-milled sorghum is currently being processed into porridge and gluten-free types of foods. These sorghum processing industries are generating very large quantities of protein-rich co-products. These co-products are currently used as animal feed but have the potential to serve as feedstock for the production of sorghum protein.
As with most cereals, the major protein in sorghum grain is a prolamin-type protein. The sorghum...
prolamin was first isolated 100 years ago and given the name of kafirin (6). Kafirin has some
interesting properties that make it potentially valuable in both food and non-food applications. It is
probably the most hydrophobic prolamin and is only slowly digestible (7). Kafirin has been widely
investigated as a bioplastic and encapsulating agent (8) (Figure 1). As its amino acid sequences are
rather dissimilar to the prolamins of wheat, barley and rye, it does not trigger an adverse response
when consumed by celiacs (9). Despite these valuable properties, to date there is no commercial
production of kafirin.

Since there have been several good reviews recently dealing mainly with the chemistry and
functional properties of kafirin, these will be briefly outlined. See for example de Mesa-Stonestreet
et al. (10), Taylor et al, (8), Bean and Ioerger, (11), Taylor et al., (12), Taylor and Taylor, (13) and
Xiao et al (14). However, reviews concerning kafirin have not focussed on the critical issue as to
what is required to turn a dream, beginning with a good idea, into reality. In an attempt to meet this
need, this review examines potential industrial sources of kafirin, technologies, which could be used
to improve the viability of industrial kafirin extraction, the genetic improvement of kafirin’s
nutritional and functional properties, and physical and chemical modifications of kafirin to improve
its functionality in food and non-food applications (Figure 1).

2. Drivers of kafirin availability: grain bioethanol, brewing, milling waste

In many less developed countries, sorghum is a staple food for many of the most impoverished, food
insecure people in the world and so until now has not been considered as a source of protein as a
food ingredient or for industrial applications. However, as indicated, there are developing sorghum
industries which produce currently underutilised protein-rich co-products. e. Commercial brewing of
traditional opaque beer from sorghum has a long history and is still a major industry in southern
Africa (15). Further, linked with increased urbanization in sub-Saharan Africa, the development of
lager-type beer brewing using sorghum has spread across Africa (16). Worldwide, the need for
gluten-free foods and beverages has driven the development of gluten-free lager-type beer brewing, again using sorghum. In the USA, sorghum grain is widely used in the production of bioethanol (17), and its use is being adopted by other countries. The technology of brewing and that of bioethanol production using sorghum grain are almost identical (Figure 2). Both processes involve size reduction of the sorghum grain by milling, followed by cooking to gelatinize the starch. The starch is then converted to simple sugars using amylase enzymes. This is followed by yeast fermentation. At this point currently the processes differ. Bioethanol production is continued with a distillation step producing ethanol and silage (Figure 2a). The silage is centrifuged and the wet grains dried to produce distillers dried grains with solubles (DDGS). After fermentation, the brewing process involves wort separation, clarification of the wort and then bottling and pasteurization of the beer (Figure 2b). The solid residual material after wort separation is known as brewer’s spent grain, which is then dried.

Figure 2. Flowchart of (a) grain bioethanol production and (b) sorghum lager/stout beer brewing process

In 2016, bioethanol production from sorghum in the US was approximately 3% of a total production
of 57 billion litres, that is 1.7 billion liters, with some 1.3 million tonnes of sorghum DDGS (18). Beer production in 2012, using sorghum was just short of 2 billion litres in Nigeria alone (19), the world’s largest sorghum brewing country, resulting in 5500 tonnes of sorghum brewers spent grain. However, in Nigeria, the use of sorghum in beer production is not consistent, mainly due to frequent changes in government policy concerning the import of barley malt and sorghum and the changing of import levies and excise duties on barley malt and beer (19). Nevertheless, it can be seen that both industries have potential to produce huge quantities of sorghum co-products, brewers spent grain and DDGS. These co-products are similar in composition due to the fact that hydrolysis of starch into fermentable sugars during the production processes increases the protein concentration by up to four times that in the grain, to some 40% for brewers spent grain (dry basis) (20) and 30-45% for DDGS (dry basis) (21). The kafirin content of DDGS is approximately 40% (13). Increased lysine content of the co-product protein may also occur due to the inclusion of spent yeast. This has been reported at 3.4 g/100 g from spent grains from 77% sorghum malt: 23% maize grits brew (22) and 2.1 g/100g for DDGS (including spent yeast), (23). Potentially these protein-rich co-products could improve the sustainability and economic viability of the brewing and bioethanol processes, by providing a valuable protein source for human food or for use in high value non-food applications, particularly as bioplastic materials (Figure 1).

When sorghum is milled to remove bran layers to improve the palatability of sorghum-based food products, another potential raw material for kafirin extraction is generated. Bran from the dry milling of sorghum has been shown to have higher protein content than the grain it was derived from (24). This was a result of the bran containing some of the grain outer endosperm, which is rich in protein. Kafirin could be extracted from bran but with lower yields and more impurities than that extracted from whole or decorticated grain. Nevertheless, it has been shown that the kafirin extracted from bran could be made into bioplastic films (25) and potentially used for other industrial applications.
3. Chemistry of kafirin

The majority of sorghum proteins are the kafirins, which are endosperm specific (26), and comprise approximately 80% of the total endosperm nitrogen (27). They are found within discrete protein bodies of the seed storage tissue, acting as a store for nitrogen, carbon and sulfur (28), where they remain inert until required during germination (29).

The kafirins are rich in glutamine, proline, alanine and leucine but contain little lysine (29). Based on their solubility, electrophoretic mobility, amino acid composition and sequences, molecular masses and immunochemical cross-reactions, and DNA sequences, they have been classified into four classes, α-, β-, γ-, and δ-kafirin (7, 30, 31), equivalent to the α-, β-, γ- and δ-zeins of maize, with which they share a considerable degree of homology (32). Each class can be further separated into several subclasses (7, 30). All classes are small (≤ 28 k) polypeptides and differ mostly in the amounts of methionine (high in β-kafirins and β- and γ-zeins) and cysteine (high in γ-zeins and β- and γ-kafirins) (28). As a result of the high levels of cysteine, β- and γ-kafirins exhibit extensive cross-linking by disulphide bonds (7). The γ-prolamins are stabilised by inter-chain disulfide bonds and are readily soluble in water in their monomeric form (33). All the other kafirin classes are insoluble in water but soluble in aqueous ethanol. Kafirins are considered the most hydrophobic of the cereal prolamin proteins. However, they do have some hydrophilic characteristics (7). The high degree of hydrophobicity of kafirin and the ability to form extensive disulfide cross-links are usually considered to impact negatively on the functional properties of isolated kafirin, particularly its protein digestibility (34). The challenge is to take advantage of these apparently negative attributes and use them in a positive manner to make functional food ingredients and bioplastic materials with superior properties to other plant-based proteins.

The secondary structure of kafirin, containing all the kafirin classes is 40-60% α-helical, (7). Kafirin with the γ-class removed was shown to have a similar structure to that of kafirin with all the classes
present, whereas isolated γ-kafirin was found to be predominately random coil with some β-sheet conformation when analysed by FTIR (35). The method of kafirin extraction, drying conditions, as well as cross-linking methods employed, influence the secondary structure of kafirin by generally increasing the amount of β-sheet present, with a concurrent decrease in the α-helical content (36-39).

In spite of the availability of protein sequences for kafirin (40), to date there are no structural models for kafirin or any of its different classes, but it is thought to be similar to those proposed for α-zein. The classic ‘Argos’ model for α-zein is based on a group of nine anti-parallel α-helices arranged in a cylinder, with the polar amino acids are on the surface and the hydrophobic amino acids hidden within the helices of the cylinder (41). The polar amino acids are available to form intra- and intermolecular hydrogen bonds in the same plane. At the ends of the cylinder of helices are glutamine-rich turns, which allow bonding between molecules in different planes. The ‘Argos’ model was modified and extended to include all α-prolamins (42). Other models have subsequently been proposed based on extended helical hairpin, rod or ribbon-like structures, 17 nm in length, 4.5 nm wide and 1.2 nm thick, with clearly defined hydrophilic and hydrophobic domains (43-46).

Structural analysis of kafirin using small-angle X-ray scattering (SAXS) showed an ellipsoid of slightly smaller size than that for zein, with dimensions of 11.8 (L) x 1.5 (W) x 1.5 (D) nm in 60% tert-butanol and 10.0 (L) x 1.1 (W) x 1.1(D) nm in 65% isopropanol (47).

4. Enhanced kafirin extraction technologies

Zein, the prolamin protein of maize, which is in many ways homologous to kafirin (32), is commercially extracted from corn gluten meal at elevated temperature, high pH and using either aqueous ethanol or aqueous isopropanol as solvents. The process used is modified from a patented method of Carter and Reck (48). Kafirin is not produced commercially at any significant level and subsequently there are no commercial products made from isolated kafirin. High cost and inferior
functional properties of prolamin bioplastics are the main reasons given for the lack of commercial products (8, 48, 49). However, without a reliable, economical supply of kafirin, which has consistent and suitable functional properties, there will continue to be no products made from kafirin in the market place. This is in spite of a large volume of published research into kafirin, which indicates that it has considerable potential in terms of film formation, as an encapsulation vehicle and as an emulsifying agent (8, 11, 14, 50). As indicated in Section 3, kafirin’s hydrophobic nature and slow digestibility are advantageous when kafirin is used as a bioplastic material. Recently, there has also been research indicating that kafirin can be modified and used to produce a cohesive viscoelastic material (51), which may have applications in gluten-free bakery products.

A comprehensive review of methodologies used for sorghum protein extraction was published by De Messa-Stonestreet et al (10). Many of these methods for kafirin extraction are based on the classic Osborne protein fractionation (52) or modifications thereof, such as that of Landry-Moureaux (53), where a sequential extraction is carried out. Other protein extraction methods are not specific for kafirin or use reagents which are not food-compatible. The latter issue is important when the kafirin is destined for use as a food ingredient or as a bioplastic material to be used with food. Cost and availability of solvent should also be considered. Ethanol is a food-compatible solvent that has been used for kafirin extraction. By its nature, a bioethanol plant has a consistent, inexpensive supply of ethanol, which could be used for kafirin extraction. Also distillation equipment on the bioethanol site could be used for solvent recovery after the kafirin extraction. If kafirin extraction was carried out on the same site as bioethanol production then solvent and transport costs could be minimised, improving the financial viability of the kafirin extraction. Simple, one-stage extraction methodologies using ethanol as solvent, that are specific for kafirin, are required for potential industrial extraction of kafirin.
4.1 Kafirin extraction methods based on an aqueous ethanol solvent

One such method, based on a patent by Carter and Reck (54) has been widely used as a laboratory method for extraction of all the kafirin classes (55, 56). This method uses a 1 h extraction at elevated temperature (70°C) with 70% aqueous ethanol containing, 0.35% sodium hydroxide and 0.5% sodium metabisulfite. The food-compatible reducing agent, sodium metabisulfite, is used to break disulfide bonds present within and between kafirin molecules, increasing the solubility of the kafirin in the aqueous ethanol and consequently increasing the amount of extractable protein. The increased kafirin yield resulting from the reduction of disulfide bonds on addition of a reducing agent to an extraction solvent has been clearly demonstrated (57-59). Kafirin extracted using this method has been shown to contain all the kafirin classes and has been used to make a range of kafirin bioplastics (25, 35, 36, 55, 38, 39, 60-65).

The inclusion of sodium hydroxide in the aqueous ethanol extractant has also been shown to improve kafirin yield from whole grain sorghum (37). The effect was to improve the efficiency of the reducing agent due to the reduction of disulfide bonds followed by the formation of cysteric acid and dehydroalanine (66), thus reducing the amount of cysteine available for disulfide bond reformation (37). In addition, since kafirin is rich in glutamine (29), the combined effect of high temperature (70°C) and sodium hydroxide on kafirin was to deamidate the kafirin glutamine residues, reducing glutamine/glutamine interactions and introducing electrostatic repulsion via charged glutamine residues and consequently further increasing kafirin solubility (37). A disadvantage of using sodium hydroxide as a constituent of kafirin extractant was that more non-protein contaminants were extracted, but these were removed by a defatting step. All the kafirin classes were extracted, with or without the inclusion of sodium hydroxide and no additional proteins were co-extracted. However, the re-solubilization of the kafirin prior to film formation was affected by the extraction solvent composition and the method of drying the kafirin after isolation. Freeze-dried kafirin extracted in the absence of sodium hydroxide was more difficult to solubilize than when
sodium hydroxide had been present during the extraction. When both kafirin preparations were
dried at 40°C, both were more difficult to solubilize than the freeze-dried preparations. Again, the
kafirin extracted in the presence of sodium hydroxide was more readily soluble than when sodium
hydroxide was absent. Drying the protein in the presence of mild heat probably enabled disulfide
cross-links to reform thus reducing the kafirin solubility.

Recovery of kafirin, when sodium hydroxide is used as part of the extractant, necessitates the
reduction in pH before protein precipitation (55). To avoid this, the sodium hydroxide was replaced
with acetic acid as part of the extraction solvent (67). Acetic acid was chosen since it is a good
solvent for kafirin (60) and so could possibly assist kafirin solvation during the extraction process.
The kafirin composition was not affected, as shown by SDS-PAGE. However, a reduction in kafirin
yield of approximately 10% was found, but this sacrifice was offset by removing the pH adjustment
step prior to kafirin recovery. This would be advantageous in a commercial process. Other workers,
however, have found that the yield reduction was far larger, in excess of 50%, when kafirin was
extracted from DDGS, using aqueous ethanol acidified with hydrochloric acid and containing
sodium metabisulfite compared to the same solvent with sodium hydroxide (56). Lower extraction
yields were probably a result of more intensive cross-linking caused by excessive exposure to damp
heat during the DDGS processing.

Aqueous ethanol at elevated temperature is highly flammable and its use is not acceptable for
certain religious groups. A search for alternative food-compatible solvents for kafirin, applicable for
industrial extraction of kafirin was undertaken (60). The most comprehensive study on zein solubility
was carried out by Evans and Manley in the early 1940s and produced a list of some 70 different
solvents and solvent combinations (68-70). No equivalent study has been carried out on kafirin
solubility. Whilst it is accepted that kafirin is more hydrophobic than zein (7) and - more difficult to
solubilize than zein, food-compatible solvents for kafirin have been selected based on the work of
Manley and Evans. Taylor et al (60) identified lactic acid (25°C) and glacial acetic acid (25°C) (primary solvents) and 55% (w/w) isopropanol (25°C), and 70% (w/w) aqueous ethanol (40°C), (secondary solvents), as the solvents most readily able to dissolve kafirin (13 g/100 g) at the given temperatures.

4.2 Kafirin extraction based on acetic acid

Solubility is not the same as extractability. Solubility refers to how much of a given substance dissolves in a given solvent at a specific temperature, whereas extraction involves the solubilization, separation and isolation of a specific substance (e.g., a protein) from other cellular constituents with which it is intimately connected chemically or physically. The efficacy of the three kafirin solvents identified by Taylor et al (60) as kafirin extractants is shown in Table 1. SDS-PAGE of the kafirin extracted using each of the three solvents was essentially the same and showed that all the kafirin classes had been extracted. Glacial acetic acid with no reducing agent or with a reducing agent mixed with the glacial acetic was found to be a very poor kafirin extractant. However, when a sodium metabisulfite soak was applied before extraction with glacial acetic acid, the kafirin yield was higher than that of either aqueous ethanol or aqueous isopropanol with sodium hydroxide and a reducing agent (71). The sodium metabisulfite pre-soak reduced the disulfide bonds of the kafirin polypeptides and was thought to disrupt the sorghum matrix proteins, allowing the kafirin to be solubilized by the glacial acetic acid. Although the extraction procedure was carried out at ambient temperature, temperature control during protein recovery and purification was necessary to avoid excessive kafirin polymerization. The efficacy of glacial acetic as a kafirin extractant was probably due to its low dielectric constant, enabling the solubilization of the hydrophobic kafirin. In addition, the protonation of kafirin and partial unfolding of the kafirin molecules in glacial acetic acid, as reported for zein (72), may have facilitated better kafirin solvation and consequently higher extraction yields.
Table 1: Effect of different extractants on yield, recovery and protein purity of kafirin.

<table>
<thead>
<tr>
<th>Extractant</th>
<th>Yield (% of Total Grain Protein)</th>
<th>Recovery of Kafirin (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Purity (% dwb)</th>
<th>Purity after defatting (% dwb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70% ethanol + 0.5% sodium metabisulfite + 0.35% NaOH at 70°C</td>
<td>54.3b (3.0)</td>
<td>74.4-79.8</td>
<td>76.6a (2.5)</td>
<td>89.3a (2.8)</td>
</tr>
<tr>
<td>55% isopropanol + 0.5% sodium metabisulfite + 0.35% NaOH at 40°C</td>
<td>55.3b (0.2)</td>
<td>75.8-81.3</td>
<td>73.1a (1.9)</td>
<td>91.2a (1.9)</td>
</tr>
<tr>
<td>Glacial acetic acid at 25°C</td>
<td>25.0a (1.0)</td>
<td>34.2-36.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glacial acetic acid + 0.5% sodium metabisulfite at 25°C</td>
<td>25.0a (1.4)</td>
<td>34.2-36.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-soak (16 hr) 1.0% sodium metabisulfite, glacial acetic acid at 25°C</td>
<td>61.0c (0.3)</td>
<td>83.6-89.7</td>
<td>68.0a</td>
<td>92.9a (0.5)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values calculated according to estimates of the kafirin content of sorghum grain (68-73%) made by Hamaker et al (27)

Values in the same column but with different letters are significantly different at the 95% level

Figures in parentheses indicate standard deviations

However effective the extraction solvent, the yield of kafirin is limited by the kafirin content of the source material. Feedstock for laboratory extraction of kafirin is generally whole grain sorghum or decorticated grain, both of which have relatively low protein contents of approximately 11% (50). The much higher protein content of DDGS, of approximately 40%, is potentially a more economical feedstock for kafirin extraction. Three different solvent systems were investigated, under reducing conditions, for kafirin extraction from sorghum DDGS (56). Kafirin yields were 44.2% with glacial acetic acid, 24.2% with acidified ethanol and 56.8% with sodium hydroxide/ethanol. Protein purity (98.9%) was highest when the acetic acid method of Taylor et al (71) was used. All the protein preparations were mainly α-helical in structure and composed of α1-, α2- and β-kafirins.

Using a modified percolation-type process for extraction of kafirin from coarsely milled, commercially decorticated sorghum meal and DDGS, Muuhiwa et al (67) achieved extraction yields of 53% and 48%, respectively. Protein purities of 76.5% (sorghum meal) and 78.5% (DDGS) were obtained when aqueous ethanol containing sodium metabisulfite and acetic acid was used as extraction solvent. The sorghum meal required a pre-washing with water to remove starch-rich fines. This step was not needed when DDGS was used as there was little residual starch after the
bioethanol process. The lower yield from the DDGS was attributed to disulfide cross-linking caused by high temperature processing of the DDGS as was found with zein (73). Kafirin bioplastic films made from the DDGS extracted kafirin had better functional properties in terms of lower water uptake and lower digestibility than kafirin extracted from coarsely milled meal. The advantages of the percolation-type process is that coarse meals, as available from grain bioethanol production can be used without further milling. Furthermore, less solvent is required, filtration times are faster than other extraction technologies and no further filtration or centrifugation prior to protein recovery is required. All these factors point to a technology that could easily and economically be adapted for industrial extraction of kafirin. A further idea, which had been suggested to enable the extraction of prolamins with potentially better functional properties, is to extract the prolamins from the grain ahead of bioethanol production. This has been described for zein (74-75) but not kafirin.

The purpose of laboratory kafirin extraction process is to study the components of kafirin in a form as close as possible to their true in vivo nature (11). In contrast, an industrial extraction process has to economically extract the maximum amount of protein, with properties most appropriate for the intended end use of that protein. It is known that the conditions used for extraction and purification of prolamins influences their composition (58) and the functionality of those prolamins (76). Some reducing agents are more effective than others at reducing kafirin polymers during the extraction process. Sodium metabisulfite enabled the precipitation of greater amounts of higher-purity kafirin than glutathione with an aqueous ethanol extraction solvent (58). Also, in the absence of a reducing agent but with sonication, the kafirins extracted exhibited a far wider molecular weight range than when a reducing agent was used (58). Concerning the composition of prolamins extracted with different solvent combinations, Schober et al (76) found that for zein, the presence of NaOH or sodium metabisulfite as part of the extractant resulted in non-functional zeins with respect to viscoelastic mass formation. Functional zeins were characterized by high levels of α-zeins and low levels of β+γ-zeins. Zein's film-forming ability was found to be less sensitive to the amount of β+γ-
zeins present. Isolated kafirin is even more difficult to functionalize than zein (76), probably due to its more hydrophobic nature and its greater propensity to form disulfide cross-links. The majority of published research on kafirin bioplastics has been carried out on laboratory extracted kafirin, containing all the kafirin classes. However, it is probable that bioplastics with different functional properties could be obtained if kafirins with different compositions were used.

5. Genetic modification of kafirin to improve nutritional quality and functionality

When processed by wet cooking, sorghum generally has a lower protein nutritional quality than other cereals. The Protein Digestibility Corrected Amino Acid Score (PDCAAS) of wet-cooked sorghum is in the range 0.10-0.22, compared with that of cereals such as maize, rice and wheat at approximately 0.47, 0.53-0.66 and 0.44, respectively (77). The low PDCAAS of wet-cooked sorghum is due to the combination of its poor protein digestibility and low content of the indispensable (essential) amino acid lysine. The latter is common to most cereals, but the poor protein digestibility is apparently unique to sorghum. It is believed to be caused by polymerization of kafirins and crosslinking of kafirins with endosperm proteins through disulfide cross-linking involving the cysteine-rich γ- and β-kafirin classes (34, 59, 78).

Kafirin and zein, its maize homolog, are also very inert proteins even among the cereal prolamin proteins, with kafirin being rather more inert than zein (76). For example, only commercial zein (a zein preparation with a low level of γ-zein subclass) will form a viscoelastic wheat gluten-like mass when kneaded with water (79). Zein preparations with all the subclasses and kafirin require pre-treatments before they will form viscoelastic masses. See section 6.1.1.

Research to improve the nutritional quality and functionality of the protein in sorghum grain by genetic means has been ongoing since the 1970s. The work is summarized in Table 2. Basically, three approaches have been used: chemical mutagenesis, selection of naturally occurring genotypes
with modified kafirin expression and recombinant DNA technology (genetic engineering).

**Table 2 Genetic improvements in the nutritional and functional quality of kafirin**

<table>
<thead>
<tr>
<th>Mode of genetic improvement</th>
<th>Research</th>
<th>Findings and benefits</th>
<th>Problems</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical mutagenesis</td>
<td>Back crossing P-721 opaque high lysine mutant and effects on protein digestibility and protein expression</td>
<td>Improved raw and wet-cooked protein digestibility</td>
<td>Reduced quantity of kafirin as improvement is due to overexpression of non-kafirin proteins. Floury (soft) grain endosperm texture</td>
<td>Weaver et al. (118) Da Silva et al. (78) Benmoussa et al. (119)</td>
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<td></td>
<td>Dough and bread making performance of high lysine, high protein digestibility (HLHD) line</td>
<td>Improved dough and bread quality of wheat-HLHD sorghum composite flour Viscoelastic mass formed with HLHD composite flour and wheat gluten</td>
<td></td>
<td>Goodall et al. (82)</td>
</tr>
<tr>
<td>Recombinant DNA technology</td>
<td>RNAi suppression of expression of specific kafirin subclasses, especially γ-kafirin and effects of grain lysine content, protein digestibility, Osborne protein factions, protein body and endosperm structure</td>
<td>Improved kafirin digestibility, with compensatory synthesis of other kafirins Co-suppression of several kafirin subclasses associated with floury grain endosperm texture</td>
<td></td>
<td>Grootboom (120) Da Silva et al. (59, 78) Grootboom et al. (86)</td>
</tr>
<tr>
<td></td>
<td>Brewing and bioethanol performance of sorghum lines with RNAi suppressed expression specific kafirin subclasses</td>
<td>Improved hot water extract (starch solubilisation) and free amino nitrogen</td>
<td></td>
<td>Kruger et al. (84)</td>
</tr>
<tr>
<td></td>
<td>Transgenic sorghum lines with down regulation of γ-kafirin kafirin expression and coding for wheat high molecular weight glutenin (HMWG) protein</td>
<td>No effect of suppression of γ-kafirin expression alone protein body structure. However, no effect on protein digestibility Expression of wheat HMWG protein</td>
<td>No improvement in kafirin digestibility Sorghum expressing HMWG would not be gluten-free</td>
<td>Kumar et al. (85)</td>
</tr>
<tr>
<td>Chemical mutagenesis and recombinant DNA technology</td>
<td>Structure and rheological properties of viscoelastic masses formed from kafirins isolated from high protein digestibility P-721 opaque lines and transgenic lines with</td>
<td>Reduced expression of β- and γ-kafirin subclasses resulted in viscoelastic masses with broad, ribbon-like fibrils, like gluten, but all kafirins exhibited similar</td>
<td></td>
<td>Eihassan et al. (51)</td>
</tr>
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<thead>
<tr>
<th>Selection of naturally occurring genotypes</th>
<th>modified kafirin expression</th>
<th>viscoelastic behavior</th>
<th>Cremer et al. (87)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum lines with allelic variation at the kafirin loci and characterization of endosperm proteins</td>
<td>β-kafirin null mutation associated with thioredoxin expression</td>
<td>Line with novel γ-kafirin allele had low starch</td>
<td>Cremer et al. (89)</td>
</tr>
</tbody>
</table>

Through chemical mutagenesis, researchers at Purdue University developed a high lysine sorghum line P-721 opaque with up to 60% higher lysine (80). However, the mutation causes suppression of synthesis of several types of kafirins: γ-kafirin, 25 kDa α-kafirin and some β-kafirin classes. This results in a far lower quantity of kafirin in this high lysine-type, sorghum, approximately. 50% lower compared to its normal parent (81) and approximately lower by 30% in a line which also exhibited the high protein digestibility trait (78). With regard to kafirin functionality, composite flour of high lysine-protein digestibility (HLHD) sorghum with wheat had improved dough and bread-making quality compared to a composite flour of normal sorghum with wheat (82). The authors attributed this improvement to the kafirin protein in the HLHD sorghum being freed during kneading from the protein bodies, where it is normally entrapped, which resulted in improved dough viscoelasticity. Freeing the kafirin from the protein bodies would presumably be facilitated in these high protein digestibility sorghum lines as their kafirin protein bodies are folded in shape resulting in a high surface area, as opposed to being essentially spherical in shape in normal sorghum (83).

The application of RNAi (RNA interference) technology to suppress the synthesis of specific combinations of α-, γ- and δ-kafirins together with reduced expression of Lysine Ketoglutarate Reductase (LKR), a lysine degrading enzyme, substantially improved both wet-cooked protein digestibility and lysine content in sorghum grain without reducing the proportion of kafirin (59, 78).
SDS-PAGE revealed additional kafirin bands in the transgenic line, indicating compensatory up-regulation of synthesis of certain kafirin classes (78). This transgenic high protein digestibility, high lysine-type sorghum also would appear to have superior grain bioethanol and brewing performance, as it yielded higher levels of high water extract (starch solubilization) and considerably increased free amino nitrogen compared to its null controls (84).

Kumar et al. (85) produced transgenic sorghum events with down regulation of γ-kafirin expression. These authors and Grootboom et al. (86) showed that this down regulation alone was not sufficient to improve sorghum protein digestibility. Additionally, however, Kumar et al. (85) found that reduction in accumulation of a predicted 20 kDa α-kafirin improved sorghum protein digestibility. They also expressed a wheat high molecular weight glutenin protein in sorghum. Unfortunately, they did not investigate the impact of this expression on sorghum flour dough functionality. A problem with expressing wheat proteins is that the sorghum would no longer be “gluten-free” and suitable for consumption by celiacs.

Concerning kafirin viscoelastic properties, Elhassan et al. (51) investigated the structure and rheological properties of kafirins isolated from both P-721 opaque type and the RNAi HLHD transgenic sorghum line. It appeared that reduced expression of the β- and γ-kafirin classes resulted in the dough-like viscoelastic masses that were formed by kneading, exhibiting broad, ribbon-like fibrils, similar to those observed in wheat gluten viscoelastic masses. However, there was no substantial difference in the viscoelastic properties between these lines and their controls.

Regarding selection of naturally occurring genotypes, proteomic analysis of 28 inbred sorghum lines, focusing on lines with null expression of β-kafirin showed that such lines had reduced levels of kafirins (87). Also, there was an association with the expression of thioredoxin, a protein that can reduce disulfide bonds and the expression of which has been implicated in improved sorghum
protein digestibility (88). With regard to the influence of kafirin expression on grain bioethanol performance, Cremer et al. (89) studied 10 sorghum lines with allelic variation in β-, γ- and δ-kafirins. They found an association between β-kafirin and grain digestibility and free amino nitrogen, but starch content was the major determinant of ethanol yield. Also, a novel γ-kafirin allele was found to be associated with high fermentation efficiency.

6. Physical and chemical modification of kafirin to improve functionality as a food and non-food ingredient

Table 3 summarizes published research on kafirin extracted using different methodologies, the resultant kafirin functional properties and modifications applied to improve those properties, with some suggestions of potential applications. For convenience, treatments that have been or could be applied to kafirin to modify its properties are discussed under the headings of physical and chemical. However, such a division is somewhat arbitrary as, for example, the physical treatments of heat and shear cause chemical reactions.

Table 3. Summary of extraction method, protein modification, functional properties and potential applications of kafirin bioplastics

<table>
<thead>
<tr>
<th>Kafirin bioplastic materials</th>
<th>Sorghum source and extraction method used</th>
<th>Modification</th>
<th>Properties and potential applications</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Films cast from aqueous ethanol</td>
<td>Sorghum gluten after wet milling-95% aqueous ethanol for 1 h at 65°C. No reducing agent, defatted prior to extraction</td>
<td>Plasticiser PEG 400 plus glycerol</td>
<td>Similar to commercial zein films. Biopolymer for edible or non-edible applications</td>
<td>Buffo et al (99)</td>
</tr>
<tr>
<td>Whole grain flour-70% aqueous ethanol +0.5% sodium metabisulphite for 1 h at 70°C with and without 0.35% sodium hydroxide or sequential extraction, kafirin extracted with 60% tert-butanol + 0.05% DTT,</td>
<td>Freeze dried or dried at 40°C</td>
<td>Plasticiser 1:1 PEG/glycerol/lactic acid</td>
<td>Tert-butanol extracted kafirin was most readily soluble, formed uniform films, consistent thickness. Stronger and less extensible with lower WVP than commercial zein films. Addition of sodium hydroxide</td>
<td>Gao et al (37)</td>
</tr>
<tr>
<td>Kafirin bioplastic materials</td>
<td>Sorghum source and extraction method used</td>
<td>Modification</td>
<td>Properties and potential applications</td>
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<tr>
<td>kafirin defatted</td>
<td>to aqueous ethanol improved kafirin yield and resulted in stronger, less extensible films with similar WVT properties to commercial zein films. Kafirin dried with the application of heat and extracted with aqueous ethanol caused protein aggregation resulting in difficulty in re-solubilisation.</td>
<td>Freeze dried Plasticiser 1:1 PEG/glycerol/lactic acid</td>
<td>Stronger and less extensible with lower WVP than commercial zein films. Bran films highly colored due to co-extracted phenolics. Fruit and nut coatings</td>
<td>Da Silva et al (25)</td>
</tr>
<tr>
<td>Whole grain flour + milling fractions including bran 70% aqueous ethanol + 0.35% sodium hydroxide +0.5% sodium metabisulphite for 1 h at 70°C, kafirin defatted</td>
<td>Plasticiser 1:1 PEG/glycerol/lactic acid</td>
<td>Increasing levels of plasticizer reduced Tg Extensibility increased and WVT decreased with increased plasticiser Packaging and wrapping if properties were improved</td>
<td>Gillgren and Stading (105)</td>
<td></td>
</tr>
<tr>
<td>Decorticated sorghum flour Aqueous ethanol plus sodium metabisulphite, kafirin defatted</td>
<td>Air dried Plasticiser 1:1 PEG/glycerol/lactic acid</td>
<td>Tannin cross-linked films: stronger and less extensible, no change WVP, lower oxygen transfer, less digestible and slow biodegradation than untreated kafirin films</td>
<td>Emmambux et al (36), Byaruhanga et al (39), Taylor et al (110)</td>
<td></td>
</tr>
<tr>
<td>Decorticated sorghum flour 70% aqueous ethanol + 0.35% sodium hydroxide +0.5% sodium metabisulphite for 1 h at 70°C, kafirin defatted</td>
<td>Freeze dried Plasticiser 1:1 PEG/glycerol/lactic acid Tannic acid or sorghum condensed tannins (SCT)</td>
<td>DDGS kafirin films were rougher but had lower water uptake and lower in vitro digestibility than films made from kafirin extracted from coarse sorghum meal</td>
<td>Muhiwa et al (67)</td>
<td></td>
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<tr>
<td>Decorticated coarse sorghum meal or sorghum DDGS 70% aqueous ethanol + 0.35% sodium hydroxide or 0.35% acetic acid +0.5% sodium metabisulphite for 1 h at 70°C, using modified</td>
<td>Air dried No plasticiser</td>
<td></td>
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<table>
<thead>
<tr>
<th>Kafirin bioplastic materials</th>
<th>Sorghum source and extraction method used</th>
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<th>Properties and potential applications</th>
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<tbody>
<tr>
<td>percolation method</td>
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<tr>
<td><strong>Films cast from glacial acetic acid</strong></td>
<td>Decorticated sorghum flour 70% aqueous ethanol + 0.35% sodium hydroxide +0.5% sodium metabisulphite for 1 h at 70°C or 60% tert-butanol + 0.05% DTT, kafirin defatted</td>
<td>Freeze dried Plasticiser 1:1 PEG/glycerol/lactic acid Microwave heat, wet and dry</td>
<td>Kafirin extracted with tert-butanol was closer to native state. Microwave heat treatment of kafirin films was more effective at increasing tensile strength than wet heating kafirin prior to film formation. Also decreased film protein digestibility and slowed biodegradation. Both treatments resulted in intermolecular disulphide crosslinking of kafirin monomers and increased β-sheet structure</td>
<td>Byaruhanga et al (38, 39, 96)</td>
</tr>
<tr>
<td><strong>Films cast from aqueous ethanol with inclusion of antimicrobial agents</strong></td>
<td>Whole grain flour-70% aqueous ethanol +0.5% sodium metabisulphite for 1 h at 70°C with and without 0.35% sodium hydroxide, kafirin defatted</td>
<td>Freeze dried Plasticiser 1:1 PEG/glycerol/lactic acid Citral or quercetin as antimicrobial agents</td>
<td>Inclusion of citral decreased tensile strength but increased film elongation. Quercetin addition did not change film tensile properties. Both lowered oxygen permeability of films but did not affect WVP. Citral showed a wide range of antibacterial activity Bioactive packaging to improve food safety and quality</td>
<td>Giteru et al (101)</td>
</tr>
<tr>
<td><strong>Coatings solution kafirin in aqueous ethanol, applied by dipping</strong></td>
<td>Decorticated sorghum flour 70% aqueous ethanol + 0.35% sodium hydroxide +0.5% sodium metabisulphite for 1 h at 70°C, kafirin defatted</td>
<td>Air dried Plasticiser 1,2 propanediol +glucono-δ-lactone (GDL)</td>
<td>Good gas barrier, poor moisture barrier, decreased fruit respiration rate and reduced ethylene production resulting in retarded senescence and increased shelf-life. GDL thought to improve kafirin solvation and improve coating barrier properties Extension of shelf life of climacteric fruits including pears and avocado</td>
<td>Buchner et al (102) Taylor et al (103)</td>
</tr>
<tr>
<td><strong>Kafirin films cast from aqueous ethanol plus sodium</strong></td>
<td>Wet milling Plasticiser polyethylene glycol</td>
<td>High pH improved kafirin solubility, increasing levels of plasticizer increased WVT, decreased mechanical strength and increased film</td>
<td>Lal et al (100)</td>
<td></td>
</tr>
<tr>
<td>Kafirin bioplastic materials</td>
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<tr>
<td>hydroxide, Gelatin capsule dip coated with same solution</td>
<td>Whole grain flour-70% aqueous ethanol +0.5% sodium metabisulphite + 0.35% sodium hydroxide for 1 h at 70°C, kafirin defatted</td>
<td>Kafirin+ calcium hydrogen orthophosphate + magnesium stearate</td>
<td>Tablets had acceptable hardness and friability. Drug release was greater at acid pH (pH 1.3) than at pH 6.8. Tablet excipient, sustained release tablet matrix</td>
<td>Elkhalifa et al (121)</td>
</tr>
<tr>
<td>Compression molded tablets</td>
<td>Whole grain flour-70% aqueous ethanol +0.5% sodium metabisulphite + 0.35% sodium hydroxide for 1 h at 70°C, kafirin defatted</td>
<td>Acetic acid/dichloromethane (4:1) +polycaprolactone (PCL)</td>
<td>Cylindrical fibres, 300-500 nm with hydrophilic surface, swelling reduced and flexibility increased with increased PLC, Controlled release, wound healing, tissue engineering</td>
<td>Xiao et al (97)</td>
</tr>
<tr>
<td>Electro spinning</td>
<td>Whole grain sorghum flour, defatted. Sequential extraction with sodium chloride, followed by tert-butanol and ultra sonication</td>
<td>Freeze dried Plasticiser 1:1:1 PEG/glycerol/lactic acid Shear</td>
<td>Spherical, porous structures 10–20 µm diameter With shear during formation an open porous matrix was formed Not suitable as biomaterial due to chronic inflammatory response when implanted in mouse model</td>
<td>Taylor et al (61)</td>
</tr>
<tr>
<td>Microparticles Coacervation from a solution of kafirin in glacial acetic acid</td>
<td>Decorticated sorghum flour 70% aqueous ethanol + 0.35% sodium hydroxide +0.5% sodium metabisulphite for 1 h at 70°C, kafirin defatted</td>
<td>Freeze dried Plasticiser 1:1:1 PEG/glycerol/lactic acid Catechin or sorghum condensed tannins (SCT)</td>
<td>Encapsulation of catechin or SCT did not increase the size of microparticles, SCT reduced digestibility more than catechin. Antioxidant release was 70% with catechin, 50% with SCT over 4 h period Encapsulation for controlled release</td>
<td>Taylor et al (63)</td>
</tr>
<tr>
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<td>Wet heat increased microparticle size and changed microparticle shape to oval and increased the size of microparticle vacuoles,</td>
<td>Anyango et al (65)</td>
</tr>
<tr>
<td>Kafirin bioplastic materials</td>
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<td></td>
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<td>glutaraldehyde</td>
<td>Glutaraldehyde increased microparticle size but not vacuole size, shape elongated. Both enabled more bioactive to be bound than collagen Binding bone morphogenetic protein-2 (BMP-2) for bioactive scaffolds</td>
<td>Lau et al (123)</td>
</tr>
<tr>
<td>Coacervation from a solution of kafirin in aqueous ethanol, sodium chloride used for phase separation</td>
<td>Sorghum DDGS, washed with hot water to remove solubles, dried at 50°C and defatted 70% aqueous ethanol + 0.35% sodium hydroxide +0.5% sodium metabisulphite for 1 h at 70°C</td>
<td>Freeze dried</td>
<td>Kafirin primarily α-class and high molecular weight polymers. Microparticles were various sized, spherical particles, with crinkled surface. In vitro protein digestibility of drug loaded microparticles was higher than empty microparticles. Oral nutraceutical and drug delivery system</td>
<td>Lau et al (124)</td>
</tr>
<tr>
<td></td>
<td>Whole grain sorghum flour, glacial acetic acid after sodium metabisulphite pre-soak</td>
<td>Freeze dried</td>
<td>Various sized, spherical particles, with crinkled surface. In vitro protein digestibility’s of drug loaded microparticles was higher than empty microparticles. The majority of the encapsulated material was retained by the microparticles under simulated gastric and intestinal conditions</td>
<td>Lau et al (124)</td>
</tr>
<tr>
<td>Microparticle films Cast from colloidal suspension of kafirin microparticles in dilute acetic acid</td>
<td>Decorticated sorghum flour 70% aqueous ethanol + 0.35% sodium hydroxide +0.5% sodium metabisulphite for 1 h at 70°C, kafirin defatted</td>
<td>Plasticiser 1:1:1 PEG/glycerol/lactic acid</td>
<td>Microparticle films 20 µm thick; water stable; slowly biodegradable</td>
<td>Taylor et al (62)</td>
</tr>
<tr>
<td></td>
<td>Freeze dried</td>
<td></td>
<td>Treatment of microparticles with heat and transglutaminase ahead of making films resulted in thicker films, with reduced tensile strength, whereas, films made from microparticles treated with glutaraldehyde had increased tensile strength and were</td>
<td>Anyango et al (64)</td>
</tr>
<tr>
<td>Kafirin bioplastic materials</td>
<td>Sorghum source and extraction method used</td>
<td>Modification</td>
<td>Properties and potential applications</td>
<td>Reference</td>
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<td>stable in water in spite of loss of plasticizer by dissolution.</td>
<td>Taylor et al (122)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No abnormal inflammatory response when implanted in rodent model. Bioactive biomaterial scaffolds</td>
<td></td>
</tr>
<tr>
<td>Nanoparticles Coacervation from a solution of kafirin in aqueous ethanol</td>
<td>Whole grain sorghum flour, defatted. Sequential extraction with sodium chloride, followed by tert butanol and ultra sonication</td>
<td>Encapsulated curcumin Kafirin/curcumin Kafirin/caboxymethyl chitosan(CMC)/curcumin</td>
<td>Spherical particles, approximate mean particle size 200 nm for kafirin/curcumin, 236 nm kafirin/CMC curcumin. Slow release with simulated GI conditions up to 6 h. Delivery system for bioactive compounds</td>
<td>Xiao et al 2015a</td>
</tr>
<tr>
<td>Nanoparticle stabilized Pickering emulsions Coacervation from a solution of kafirin in glacial acetic acid, then emulsified with an oil</td>
<td>Whole grain sorghum flour, defatted. Sequential extraction with sodium chloride, followed by tert butanol and ultra sonication</td>
<td>Pickering emulsions or double emulsions and stabilised with hydrogel matrix</td>
<td>Spherical nanoparticles used as interfacial stabilizer for Pickering emulsions. Protect curcumin against UV light, retard lipid oxidation but not resistant to high pH and temperature or pepsin digestion. Improvements with stabilisation in hydrogel but reduced curcumin bioavailability Oral administration of Pickering emulsions as bioactive encapsulation agents</td>
<td>Xio et al (125-129)</td>
</tr>
<tr>
<td>Thermoplastic molding</td>
<td>Decorticated sorghum flour Aqueous ethanol plus sodium metabisulphite, kafirin defatted</td>
<td>Freeze dried Plasticisers lactic acid or PEG 400 or lauric acid</td>
<td>Plasticisation with lactic acid resulted in the lowest strength and highest strain, whereas lauric acid showed the highest strength and lowest strain. Values for PEG 400 were slightly lower than for lauric acid. Compression molded slabs had similar tensile properties to cast kafirin films using the same plasticisers but at lower plasticizer level</td>
<td>Di Maio et al (107)</td>
</tr>
<tr>
<td>Thermoplastic</td>
<td>Sorghum DDG used directly</td>
<td>Grafting with</td>
<td>Films had good strength and wet stability. Thermoplastic</td>
<td>Reddy et al</td>
</tr>
</tbody>
</table>
### 6.1 Physical treatments

#### 6.1.1 Shear

As mentioned in Section 5, the physical shear imparted by dough kneading is thought to free the kafirin from the protein bodies in HLHD sorghum mutants, enabling formation of more viscoelastic sorghum-wheat flour composite doughs. With maize, it has been shown that high mechanical energy (specific mechanical energy of ≥100 kJ/kg) applied during extrusion cooking (90, 91) and roller flaking (92) can disrupt the zein protein bodies. This seems to facilitate formation of wheat gluten dough-like zein fibrils (90). The formation of zein and kafirin proteins into fibrils (also referred to as strands or fibers) seems to be an essential step in the process of forming a functional viscoelastic dough (51, 93). With sorghum, extrusion cooking in combination with α-amylase treatment (extrusion-liquefaction process) can produce a protein concentrate of up to 82% protein with relatively high in vitro digestibility (66%) (10). However, the specific effect of extrusion cooking on the kafirin protein bodies does not seem to have been investigated.

Protein microparticles have potential as encapsulating agents for delayed or controlled release of pharmaceuticals (61). The effect of shear on kafirin microparticle formation has been investigated (61). When kafirin microparticles were made by simple coacervation with water from a solution of kafirin in aqueous ethanol, the application of high shear resulted in reduction of microparticle size, with the formation of some continuous matrix material. When glacial acetic acid was used as the original kafirin solvent, and shear was applied, microparticles were no longer observed. A continuous open matrix was formed resembling a sponge. This was attributed to the fragile nature of the kafirin microparticles.

<table>
<thead>
<tr>
<th>Kafirin bioplastic materials</th>
<th>Sorghum source and extraction method used</th>
<th>Modification</th>
<th>Properties and potential applications</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>molding films</td>
<td>methacrylates</td>
<td>materials</td>
<td></td>
<td>(130)</td>
</tr>
</tbody>
</table>
6.1.2 Thermal treatment

Regarding thermal effects, as explained in Section 5, wet cooking (often referred to as hydrothermal treatment) reduces the digestibility of sorghum proteins through disulfide bond formation involving the cysteine-rich β- and γ-kafirins. This has been shown to take place not just in the sorghum flour but also at the protein body level and with isolated kafirin (94, 95). Although heat-induced cross-linking of kafirin has negative implications with regard to the protein quality of sorghum foods, it improves kafirin functionality as a bioplastic material. This has been demonstrated when heat treatments were applied to kafirin films (38, 39, 96), kafirin microparticles (65) and kafirin microparticle films (64).

Film tensile strength was increased but extensibility and water vapor permeability (WVP) of the films were decreased, when kafirin was wet-heat treated using microwaves prior to film formation (38). Superior film functional properties were found when kafirin was dried in the presence of heat after protein recovery (37). These improvements were associated with increased levels of β-sheet structure both in the kafirin and in the films (37, 39). Microwave heating after film formation was found to be more effective at increasing kafirin film tensile strength (96). Although this treatment had no effect on film WVP, film biodegradation was slowed.

Although very thin, kafirin microparticle films have better properties in terms of WVP, lower protein digestibility, (62) and water stability, (64), than the previously described cast kafirin films (60), further attempts were made to improve their functional properties using cross-linking by heat treatment (64). Unfortunately these treatments were unsuccessful and incomplete films were formed. The films had a rough surface and the microparticles were incompletely fused. This was attributed to reduction in solubility of the kafirin microparticles in the aqueous acetic acid film casting solution due to disulfide cross-linking of the kafirin proteins.
In order to increase the amount of bioactive material that could be bound to kafirin microparticles, Anyango et al (65) applied heat-induced polymerization in an attempt to make larger structures. Cross-linking pre-formed microparticles with wet heat up to 75°C resulted in larger kafirin microparticles, with larger vacuoles. The large microparticles appeared to be formed from coalesced, round nanostructures. The advantage of these larger microparticles was that they could be loaded with more bioactive ingredient than smaller, non-heat- treated microparticles.

6.2 Chemical treatments

6.2.1 Different solvents

As explained in Section 4, the nature of the solvent used to extract kafirin influences the composition of kafirin extracted in terms of classes and its degree of solvation. Concerning dough functionality, Schober et al. (76) found that kafirin extracted with 83% isopropanol (seemingly comprising predominantly α-kafirin) formed a cohesive mass when mixed in water at 55°C. However, while the kafirin mass was initially somewhat extensible, it hardened very quickly. The addition of the disulfide bond-breaking reducing agent 2-mercaptoethanol to the water enabled the mass to remain slightly extensible for a few minutes. The fact that the kafirin extracted in 83% isopropanol was probably low in β- and γ-kafirins and the presence of mercaptoethanol had a positive effect on dough formation, suggested that extensive disulfide bonding was not desirable for kafirin dough functionality. This is supported by the finding that isolated total zein containing α-, β-, γ- and δ-zein did not form a viscoelastic mass when mixed with water, unlike with commercial zein (predominantly α-zein (79)). The authors found that by first completely solubilizing the total zein in glacial acetic acid and then drying the zein into a film, a viscoelastic mass could be formed when it was mixed with water. This treatment did not work with isolated kafirin (Nijla, Taylor and Taylor, unpublished data). However, Elhassan et al. (51) showed that solubilization of kafirin in glacial acetic acid, followed by rapid addition of cool water under low shear resulted in the formation of a network of kafirin fibrils. These fibrils could readily be kneaded into a viscoelastic mass, which
maintained its functionality over prolonged storage (several days) at 10°C. As stated in Section 5, viscoelastic masses could be formed from a range of kafirin preparations with considerably differing levels of the β- and γ-kafirin classes. Surprisingly, the levels of these kafirin classes appeared to have little influence on the relative viscous flow and elastic properties of the kafirin masses, with the materials being predominantly elastic in all cases.

Solvation of kafirin is required prior to the application of the various technologies used for kafirin film and microparticle formation. The solvent used for kafirin solvation affects the viscosity and sometimes the secondary structure of the protein, resulting in changes to the functional properties of the bioplastic material (8). Like kafirin extraction, the most common solvents for kafirin solvation are aqueous ethanol and glacial acetic acid. Glacial acetic acid is considered a better solvent for kafirin than aqueous ethanol (60) and this has also been shown for zein (72). Zein behaves as a polymer in aqueous ethanol but fully dissolves in glacial acetic acid, becoming protonated and more unfolded, thus exposing a greater surface area for hydration and solvation (72). The same may be true for kafirin. Improved kafirin solubility was given as the reason for improved kafirin film functional properties when films cast from glacial acetic acid were compared to those of kafirin films cast from aqueous ethanol (60). With kafirin microparticle preparation, microparticles of different size and morphology were formed when different solvents were used (61). Microparticles made by simple coacervation from aqueous ethanol were small (1-3 µm), smooth spheres with few internal holes, whereas those made by the same method but using glacial acetic acid as the initial solvent, were larger (1-10 µm) spheres, with a rough surface and many internal holes.

Again using acetic acid as a kafirin solvent, this time mixed in a 4:1 solution with dichloromethane, kafirin and polycaprolactone were used to electro-spin fibre mats, as a potential biomaterial suitable for controlled release, wound healing or tissue engineering (97). Carnosic acid, a natural anti-inflammatory agent with antioxidant and antitumor properties was co-dissolved with the
kafirin/ polycaprolactone in the spinning solution and encapsulated during the spinning process. A 1:2 ratio of kafirin to polycaprolactone gave the most desirable functional properties for the intended applications and a ratio of 1:1 kafirin to polycaprolactone released the greatest amount of carnosic acid (58.1%) within 5 h.

6.2.2 Plasticization

Most bioplastics are brittle and plasticizers are added to improve mechanical properties including flexibility. Plasticizers are small, non-volatile compounds, which reduce protein-protein interactions, increase free-volume and decrease glass transition temperatures (T_g) (98). The most common plasticizer used in kafirin bioplastic films is glycerol, generally in combination with the following: polyethylene glycol 400 (99); polyethylene glycol 300 (100); lactic acid and polyethylene glycol 400 (25, 36, 39, 101), and glucono-delta-lactone (102, 103). Film extensibility was increased with plasticization but film strength (104) and water barrier properties were decreased (105). Water has a major plasticization effect on prolamins and so when glycerol and other hygroscopic plasticizers are used, the T_g of the protein is further lowered (48) and films become even more extensible. When glycerol was used at high levels, with time, the kafirin films became brittle as the glycerol leached out, resulting in less extensible films with greater water permeability (64). Very low levels of glycerol adsorbs onto or into the kafirin structure and acts as an internal plasticizer by forming glycerol-protein interactions. Due to these chemical interactions the plasticiser does not leach out but remains functional (104, 106).

The inclusion of plasticizers enables kafirin to be formed into a viscoelastic melt (107). Compression molded slabs of kafirin had comparable tensile properties to those of cast films with the same plasticizers but at lower plasticizer content.
6.2.3. Chemical cross-linking

Very little work has been carried out on the improvement of the properties of kafirin bioplastics using chemical cross-linking agents. The main reason for this is that many of the commonly used chemicals, such as aldehydes, for example, glutaradehyde are not food-compatible. However, glutaradehyde treatment has been applied to kafirin microparticle films (64). The resultant films were stable and maintained their integrity and flexibility in ambient temperature water, despite loss of plasticizer by solubilization. A further study on glutaraldehyde treatment of kafirin microparticles resulted in the formation of larger microparticles, as found with heat cross-linking (65). Disulfide cross-linking was not involved with glutaraldehyde treatment. Atomic force microscopy revealed that spindle shaped nanostructures were formed when glutaraldehyde cross-linking was applied.

Natural, food-compatible cross-linking agents are advantageous when the intended final uses are in food systems or for biomedical applications. Tannins are plant polyphenols, which cross-link prolamin proteins strongly through hydrogen bonding with proline-rich regions of the prolamins (55). This property impacts negatively on the nutritional value of sorghum, as it reduces the protein digestibility of sorghum foods (34). More positively, sorghum polyphenols exhibit considerable antioxidant activity (108) and so have potential health benefits depending on their degree of absorption and metabolism (109).

Kafirin films modified with tannic acid and sorghum condensed tannins (SCT) were found to have better tensile properties at higher relative humidity than uncross-linked films (36). These properties were attributed to reduced molecular mobility and less free volume. As would be expected, SCT cross-linked kafirin and kafirin films had lower protein digestibility and the films were more slowly biodegradable under high moisture conditions than untreated films (110). The kafirin used for this study contained all the kafirin classes. It was found that the proline rich γ-kafirin class, with its high proline content (23 mole%) preferentially binds to condensed tannins (110).
Exploiting the large surface area of kafirin microparticles made by simple coacervation from a solution of kafirin in acetic acid, kafirin polyphenol cross-linking and subsequent slow digestion, encapsulation of catechin and sorghum condensed tannins (SCT) was investigated as a potential delivery vehicle for controlled release of dietary antioxidants (63). Under simulated digestive conditions, little or no digestion occurred after 4 h, and antioxidant release was approximately 70% and 50% for catechin and SCT respectively. Since bound SCT have been found to retain half of their antioxidant activity (111), it was suggested that even more antioxidant activity would be available to act as a free radical sink in the gastrointestinal tract (63).

6.2.4 Enzymic crosslinking

It has been noted that enzymes which promote intra- or intermolecular protein cross-linking can be used to improve prolamin bioplastic properties (112). No such improvements were found when kafirin microparticle films were treated with transglutaminase (64). Transglutaminase catalyzes an acyl transfer reaction in which cabxyamide groups of peptide-bound, glutamine residues are acyl donors and primary amines act as acyl acceptors resulting in ε-(γ-glutamyl)-lysine bridges (113). Although rich in glutamine, kafirin has low lysine content, which would explain these poor results (64).

6.2.5 Reducing agents

It has been shown clearly by many researchers that the addition of reducing agents, which break disulfide bonds in proteins, can substantially reduce or even reverse the adverse effect of disulfide bond mediated cross-linking on sorghum protein digestibility. A number of different reducing agents have been shown to be effective, including L-cysteine, sodium bisulfite, 2-mercaptoethanol and dithiotheitol (114) and ascorbic acid (115). Through the use of enzyme-linked immunosorbant (ELISA) assay to quantity the specific kafirin classes, Oria et al. (116) showed that with wet cooking, the proportion of undigested cysteine-rich β- and γ-kafirin classes increased greatly (2-2.5-fold), in
contrast to the smaller increase in α-kafirin (approx. 50%). In contrast, when wet cooking was carried out in the presence of sodium bisulfite, the digestibility of all three major kafirin classes, α-, β- and γ- was substantially decreased. This work confirmed the central role of β- and γ-kafirin in inhibiting sorghum protein digestibility through disulfide crosslinking and the effectiveness of disulfide-bond breaking reducing agents in largely preventing this.

With regard to the effects of reducing agents on kafirin functionality, the work by Schober et al. (76) on kafirin dough functionality, found that the addition of the disulfide-bond-breaking reducing agent 2-mercaptoethanol to the water enabled the isolated kafirin mass to remain extensible for a few minutes.

6.2.6 Oxidizing agents

It has been suggested that oxidation of the polyphenols in sorghum by molecular oxygen leading to the formation of quinones, and in turn peroxides, which are oxidizing agents, may inhibit sorghum protein digestibility (presumably through disulfide bond mediated cross-linking (34)). However, no direct evidence has been presented. With regard to direct effects of oxidizing agents on commercial zein (which largely lacks γ-zein), it has been found that “dough” preparation in the presence of hydrogen peroxide greatly enhances the resulting extensibility of zein viscoelastic masses (117). This finding was attributed to hydroxylation of aliphatic amino acid side chains in the zein through oxidation by the hydrogen peroxide. Hydrogen peroxide treatment also increased the extensibility of viscoelastic masses prepared from total zein (i.e. zein with all the classes) (Njila, Taylor and Taylor, unpublished data).

Perhaps surprisingly, in view of the well-described effects of crosslinking on kafirin film properties (Sections 6.1.2, 6.2.3, 6.2.4), there is a dearth of research into the effects of oxidants on the functional properties of kafirin and zein bioplastics. With cast films made from commercial zein,
Taylor et al. (117) showed that treatment of the zein with hydrogen peroxide did not cause evident disulphide or other covalent bond-mediated polymerization of the zein. However, film water uptake was enhanced. This effect was also attributed to hydroxylation of zein aliphatic amino acid side chain residues. Enhancement of cast kafirin film water uptake has also been found when kafirin was treated with hydrogen peroxide (Njila, Taylor and Taylor, unpublished data).

7. Conclusions

For sorghum to play a role in the dream of a ‘green economy’, even for niche protein-based biopolymer products, kafirin bioplastics have to compete functionally and economically with synthetic plastics. The best hope for this is to link their manufacture with bioethanol production and to produce high value products, potentially for biomedical or pharmaceutical applications, which are not as sensitive to high costs as food applications. This means that kafirin extraction and bioplastic manufacture would have to occur on the same site as sorghum bioethanol production, where there is a constant supply of high protein feedstock from DDGS, a cheap and plentiful supply of ethanol and a means of recovering that ethanol after the bioplastics have been produced. This process would have the added advantage of considerably reducing transportation costs. Currently, sorghum is grown in insufficient quantities to supply food, brewery and bioethanol production needs. Predicted climate changes of increased temperatures and reduced and erratic rainfall may drive increased sorghum production, but sorghum yields still lag behind those of maize and other cereals. Yield improvements are needed but are slow in coming.

Perhaps the most realistic approach would be to take existing cereal protein rich co-products from the brewing and bioethanol production, where commonly blends of grains are used as feedstocks, such as maize, barley, wheat plus sorghum. Research is required into the functional properties of bioplastics made from such mixed prolamins.
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