

Sorghum Proteins: The Concentration, Isolation, Modification, and Food Applications of Kafirins

Normell Jhoe de Mesa-Stonestreet, Sajid Alavi, and Scott R. Bean

Abstract: Celiac disease is a serious condition affecting millions of individuals. Those afflicted with this illness are resigned to a lifelong avoidance of products containing the storage prolamin proteins found in cereal grains wheat, rye, and barley. Since many food products are based on these cereals, especially wheat, celiac patients have very limited food choices, and those that are available to them are generally poor in quality, often nutritionally deficient, and expensive. Furthermore, this condition also indirectly affects their families and friends with whom they share meals. Thus, a burgeoning need exists to develop nutritious, palatable, and affordable foods, especially staples like bread and pasta, for these individuals and their families and friends who are accustomed to wheat based products. Grain sorghum and its proteins are safe for celiac patients and individuals with varying levels of gluten intolerances. However, the main sorghum proteins, kafirins, are resistant to digestion. They are also difficult to extract and modify in an industrial-scale process and with food-compatible chemicals, thus limiting their use in foods. This review describes studies on kafirin extraction and methods for modifying sorghum proteins for improved nutrition and functionality, as well as food applications. Armed with this knowledge, scientists and technologists will be in a better position to identify opportunities that will further enhance the nutritional and functional value of sorghum proteins.

Keywords: celiac disease, gluten-free, kafirin, protein isolation, sorghum protein

Introduction

Gluten intolerance is a serious and prevalent issue

Prolamin proteins in the cereal grains wheat (gluten), rye (secalin), and barley (hordein), are known to bring about an allergic response or a detrimental autoimmune reaction in certain individuals. The latter, a condition called celiac disease (CD), afflicts 1 in 133 Americans (Fasano and others 2003). CD (also referred to as celiac sprue, nontropical sprue, and gluten-sensitive enteropathy) is a chronic, genetic disease characterized by the formation of autoantibodies and the destruction of the mucosal lining of the small intestine, which results in nutrient malabsorption. Typical symptoms associated with CD are abdominal pain, diarrhea, and constipation. Long-term complications of this disease include anemia, osteoporosis, miscarriage, liver diseases, cancers of the intestine, and depression or anxiety (NIDDK 2009). In many individuals, the disease becomes evident only during adulthood, and is sometimes triggered after surgery, pregnancy, childbirth, viral infection, or severe emotional stress. For this reason, even though 1% of the U.S. population is thought to be afflicted with CD, about 97% of these cases are undiagnosed (Mintel 2007). A life-long avoidance of products containing gluten, secalin, and hordein is the

only treatment. Additionally, because gluten is found not only in foods but also in medicines, vitamins, beauty products, stamps, and envelope adhesives, celiacs have to be exceedingly judicious.

Market research shows a heightened demand for gluten-free products

Increased awareness and diagnosis of CD and gluten sensitivity have spurred the demand for gluten-free products. Mintel (2007) reported that the gluten-free foods and beverages market in 2006 was \$700 million and is projected to grow annually at a rate of 15% to 25% to \$1.3 billion by 2010. The largest numbers of new introductions were in bakery goods and snacks, which were 22.7% and 17.5%, respectively, of the 1339 gluten-free products introduced in 2006.

Creating gluten-free foods is challenging

Unfortunately, the quality of gluten-free products has not kept up with the rising demand. Gluten replacement in food presents several challenges. First, because gluten is a unique structure-building protein, its removal from baked products (especially bread) and pasta, results in very poor sensory qualities and product shelf life (Gallagher and others 2004). Second, gluten-free products are made primarily from isolated starches, thus are poor in fiber, protein, vitamins, and minerals (Berti and others 2004; Engleson and Atwell 2008). Third, gluten-free products are more expensive than comparable conventional products because of the added burden of ensuring the absence of cross-contamination and because production is at a smaller scale (Mintel 2007). Generally, gluten-free products are 240% more expensive than their gluten-free counterparts (Lee and others 2007). Fourth, there is limited availability of gluten-free selections in dining establishments (Mintel 2007), regular grocery stores (Lee and others 2007), and schools (Swientek 2008). Although studies have been conducted

MS 20091282 Submitted 12/23/2009, Accepted 2/19/2010. Authors de Mesa-Stonestreet and Alavi are with Dept. of Grain Science and Industry, Kansas State Univ., Manhattan, KS 66506, U.S.A. Author Bean is with USDA-ARS GMPRC, Manhattan, KS 66502, U.S.A. Direct inquiries to author de Mesa-Stonestreet (E-mail: jhoe@ksu.edu).

U.S. Dept. of Agriculture, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination.

Mention of firm names or trade products does not constitute endorsement by the U.S. Dept. of Agriculture over others not mentioned.

to address some of these technological and nutritional issues, there is still a need to develop gluten-free ingredients and processes that are safe and economical for celiacs and those with gluten sensitivities.

Sorghum is a safe ingredient for gluten-free products

Sorghum is a cereal grain that is safe for celiac patients (Ciacci and others 2007). Sorghum flour is an attractive alternative to wheat flour for the celiac market because of its neutral flavor and the use of hybrids with a white pericarp. These white grained sorghum lines produce a flour similar to wheat flour in appearance and do not impart an unusual color to the flour. Furthermore, sorghum utilization helps address food security issues because it is a drought resistant crop that easily withstands harsh cultivating conditions in impoverished regions of Asia and Africa. The United States is the world's top sorghum producer, followed by India and Nigeria (U.S. Grains Council 2008). In 2007, the United States produced 12.83 million metric tons, or 20% of the world's sorghum supply. Sorghum is the 3rd most widely produced crop in the United States and 5th in the world. Although mostly supplied to the feed industry in the United States, sorghum is an important staple in parts of Asia and Africa (Dendy 1995). Thus, sorghum is important economically and it ensures food security in a number of countries worldwide.

Cooking sorghum reduces its nutritional value

In Asia and Africa, sorghum is traditionally prepared in a number of ways including porridges, flat breads, alcoholic beverages, and snacks (Murty and Kumar 1995). However, cooking sorghum, especially wet cooking, reduces its digestibility (Hamaker and others 1986; Zhang and Hamaker 1998; Duodu and others 2002; Duodu and others 2003; Nunes and others 2004; Ezeogu and others 2008; Emmambux and Taylor 2009), making it less available for the body to use. For celiac patients who typically suffer from malnutrition due to poor nutrient absorption, it is even more important for nutrients to be more readily available. Thus, a challenge exists to make sorghum proteins more digestible. Additionally, to make sorghum protein a commercially viable ingredient, it has to be concentrated and/or isolated at an industrial scale using processes and/or chemicals that are compatible with food grade applications.

In this review, we describe methods used to concentrate, isolate, and modify sorghum proteins and also identify new developments in uses of sorghum and sorghum proteins in gluten-free foods, specifically in staples like bread and pasta.

Overview of Sorghum Proteins

As extensive literature is available on characterizing sorghum proteins, only an overview is given to serve as basis for discussion. For a recent review on sorghum protein chemistry and structure, see Belton and others (2006).

Sorghum proteins

Sorghum (*Sorghum bicolor* L. Moench) grain has protein content varying from 6% to 18%, with an average of 11% (Lasztity 1996). Sorghum proteins can be broadly classified into prolamins and non-prolamin proteins. Kafirins, the major storage proteins, are classified as prolamins, and as such, they contain high levels of proline and glutamine and are soluble in nonpolar solvents such as aqueous alcohols (Shewry and Tatham 1990). Kafirins account for 77% to 82% of the protein in the endosperm, whereas non-prolamin proteins (namely, albumins, globulins, and glutelins)

make up about 30% of the proteins (Belton and others 2006). Since maize and sorghum are closely related genetically (both belong to the same tribe of the grasses [Andropogoneae]), the large volume of research on maize prolamins, called zein, has served as a framework for studying kafirins. Shull and others (1992) even utilized procedures developed for maize to characterize the proteins of sorghum based on solubility, molecular weight, and structure. This review primarily focuses on kafirin proteins.

Kafirin classification and microstructure

Kafirins are classified as either α , β , γ , or δ based on molecular weight and solubility. Depending on whether it is floury or vitreous, sorghum endosperm contains about 66% to 84% α -kafirin, 8% to 13% β -kafirin, and 9% to 21% γ -kafirin and low levels of a poorly characterized δ -kafirins (Lasztity 1996; Belton and others 2006). The α -kafirins are divided into 2 groups of polypeptides with molecular weights (M_w) of 23 and 25 kDa. These proteins are rich in nonpolar amino acids and are found primarily as monomers and oligomers. These proteins do not crosslink extensively and form mainly intramolecular disulfide bonds. The β -kafirins have a M_w of approximately 18 kDa, are rich in the sulfur-containing amino acids methionine and cysteine, and are found in monomeric and polymeric forms. The γ -kafirins have a M_w of approximately 20 kDa and are rich in the amino acids proline, cysteine, and histidine. These subunits are found as oligomers and polymers. Both β - and γ -kafirins form intermolecular and intramolecular disulfide bonds and are highly crosslinked. The δ -kafirins have a M_w of about 13 kDa and are rich in methionine (Belton and others 2006). Overall, sorghum prolamins are rich in glutamic acid and nonpolar amino acids (proline, leucine, and alanine), but almost absent with the essential amino acid lysine.

The microstructure of kafirins in relation to the glutelin protein matrix and starch granules, as well as its reaction to chemicals and processing, have been studied using scanning and transmission electron microscopy (Seckinger and Wolf 1973; Hoseny and others 1981; Taylor and others 1984a; Rooney and Pflugfelder 1986; Shull and others 1992; Oria and others 1995a; Oria and others 2000; Duodu and others 2002; Elkhalfi and others 2006), as well as confocal laser scanning microscopy (Schober and others 2007; Wu and others 2007; Choi and others 2008). The previously mentioned studies have shown that kafirins are located primarily in spherical protein bodies, which are embedded in a glutelin protein matrix, and are surrounded by starch granules. A schematic representation of this relationship is shown in Figure 1 and 2. The protein bodies are 0.4 to 2 μ m in diameter (Taylor and others 1984a), with an outer "shell" composed mainly of crosslinked β - and γ -kafirins, and an interior comprised predominantly of α -kafirin (Shull and others 1992; Duodu and others 2003).

While kafirins in most sorghum cultivars are tightly bound in spherical protein bodies, sorghum protein bodies in the highly digestible mutant sorghum cultivar P851171 grown at the Purdue Univ. Agronomy Research Center were irregularly shaped with numerous invaginations (Oria and others 2000). These researchers attributed the ease of digestibility to this unique microstructure. The invaginations provided a greater surface area for enzymatic digestion; the highly digestible α -kafirins were more homogeneously dispersed throughout the interior of the protein body rather than simply localized in the central portion; and, the poorly digestible γ -kafirins were concentrated at the base of the invaginations of the protein body rather than at the protein body periphery encapsulating α -kafirins like in normal sorghum cultivars.

Sorghum protein digestibility

Duodu and others (2003) extensively reviewed the factors affecting sorghum protein digestibility, which were broadly categorized as exogenous and endogenous factors. The former involves interactions of proteins with non-protein components (for example, polyphenols, phytates, lipids, starch, and cell wall components), while the latter entails only protein-protein interactions.

Polyphenols, lipids and cell wall components form complexes with kafirins that are resistant to digestion. Cooking enhances the interaction of kafirins with these compounds, further reducing protein digestibility (Duodu and others 2003). A 50% reduction was observed as a result of the complexation of total kafirins with sorghum condensed tannins (Taylor and others 2007). Taylor and others (2007) also found that sorghum tannins bound preferentially to γ -kafirins than to either α - and β -kafirins because of the high proline content of γ -kafirins. However, not all sorghum lines contain tannins, thus this would only be an issue in sorghum lines containing tannin. Likewise, phytic acid also complexes with kafirins. Unlike the previously mentioned components, however, phytic acid content is reduced upon cooking, reducing its role in protein digestibility. Starch affects sorghum protein digestibility differently. Although the bulk of the literature suggests that sorghum proteins inhibit starch gelatinization and its digestion (Duodu and others 2002; Ezeogu and others 2008), the presence of starch mutually reduces sorghum protein digestibility (Duodu and others 2003; Wong and others 2009). *In vitro* protein digestibility, however, can be improved with the addition of α -amylase to either raw (Wong and others 2009) or cooked (Duodu and others 2002) sorghum flour. Likewise, the presence of the glutelin matrix that binds kafirin protein bodies and starch granules reduces protein digestibility (Wong and others 2009).

In general, kafirins tend to be more hydrophobic than other cereal prolamins (Belton and others 2006). While these proteins have some hydrophilic tendencies in the raw state, cooking at high moisture emphasizes their hydrophobicity. This reversal in water absorption upon cooking may be due to extensive disulfide bonding, which results in the polymerization of kafirin monomers and realignment of kafirin into β -sheets. This structural change then prevents swelling, imbibition of water, and reduces protein's susceptibility to proteolysis (Belton and others 2006; Emmambux and Taylor 2009). Cooked sorghum showed a decrease in the amount of albumin, globulin and kafirins and a concomitant rise in the percentages of cross-linked glutelin and nonextractable proteins (Hamaker and others 1986). Additionally, cooking sorghum in high moisture resulted in an increase in the amount of pepsin-indigestible proteins from 19.3% (raw) to 35.2% (cooked). These results presented by Hamaker and others (1986) indicated that the reduction in protein digestibility during cooking at high mois-

ture was brought about by protein polymerization. On the other hand, protein digestibility of sorghum cooked in limited water, such as popping or extrusion, was either greater than (MacLean and others 1983; Mertz and others 1984; Fapojuwo and others 1987; Hamaker and others 1994), or the same as that of uncooked sorghum (Dahlin and Lorenz 1993; Parker and others 1999). This phenomenon was attributed by Parker and others (1999) to the explosive disruption of the cell walls and expansion of starch, leading to immediate accessibility of enzymes to proteins.

Kafirin functionality in food systems

Protein functionality is related to protein size, molecular structure and conformation, charge distribution, and molecular interactions. The functional roles of proteins in food systems include

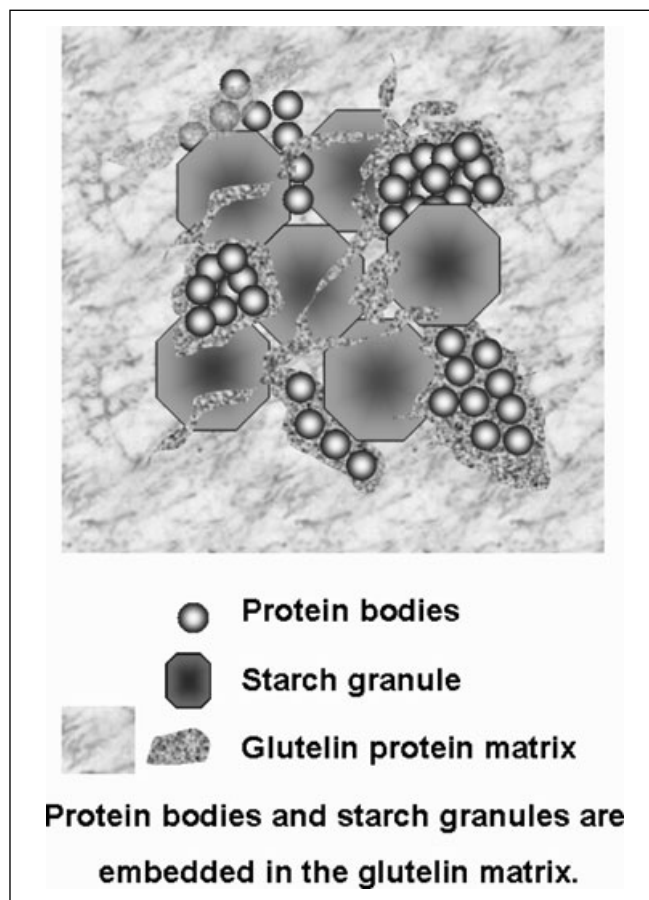


Figure 2—Sorghum protein bodies in relation to starch and the glutelin matrix.

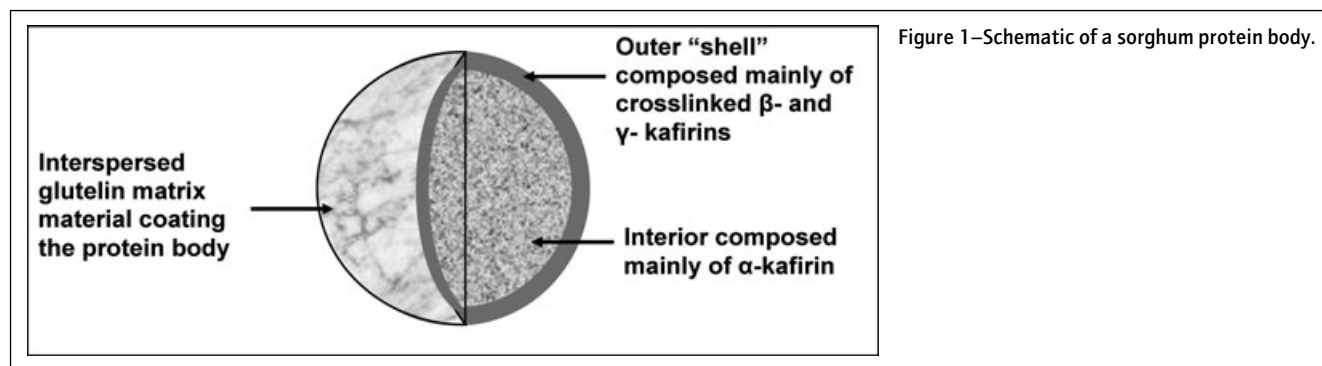


Figure 1—Schematic of a sorghum protein body.

solubility, viscosity, water binding, gelation, elasticity, emulsification, foaming, gas holding capacity, and fat and flavor binding, and these properties define their applications in foods. Extensive literature on these properties exists and a discussion of which is beyond the scope of this review (see for example Nakai 1983; Kinsella and Soucie 1989; Damodaran and Paraf 1997; Zayas 1997; Sikorski 2001). This section focuses on the functionality of kafirins.

Oom and others (2008) studied the rheological properties of kafirins in a viscoelastic dough system. Their study showed that although the extensional viscosity of isolated kafirin dough immediately after mixing was similar to those found in gluten-based dough, it became rapidly stiff over time. Oom and others (2008) speculated that this was due to disulfide crosslinking of kafirin monomers. When kafirin was mixed with starch and water, however, no dough could be formed. The researchers inferred that kafirin's inability to form composite viscoelastic doughs could be a result of its extremely hydrophobic nature (that is, its exclusion of water prevented its hydration and plasticization).

Kulamara and others (2004) found that sorghum flour has poor viscoelastic properties and that the observed rheological properties result primarily from starch gelatinization. These researchers studied the effects of water level (70%, 80%, 90%, and 100% flour weight basis) and temperature of mixing (22 and 100 °C) on the rheological properties of sorghum flour using the Instron Universal Testing Machine. Extensibility was greater at higher water levels. Dough mixed with boiling water had increased extensibility, reduced hardness, increased cohesiveness, and higher gumminess values, which were most likely due to starch gelatinization. In the same study, they conducted parallel plate oscillatory tests with a dynamic rheometer. Dough samples were subjected to a constant stress of 6 Pa with frequency ranging from 0.1 to 100 Hz at 25 °C. The storage modulus was reduced with increasing water levels because of dilution. And due to starch gelatinization, it was also lower in dough mixed with boiling water than with cold water.

Schober and others (2007) studied the rheological properties of sorghum dough subjected to sourdough fermentation and investigated the effect of adding hydroxypropyl methyl cellulose (HPMC) to the dough. Dynamic oscillatory testing at 1 Hz in the linear viscoelastic region was conducted by using a serrated plate measuring system with the following temperature profile to simulate the baking and cooling processes: (1) a linear temperature ramp from 25 to 95 °C in 47 min; (2) 10 min at 95 °C; and, (3) a linear gradient down from 95 to 25 °C in 47 min. The target strain was 5×10^{-4} . Compared with maize and potato starch doughs, sorghum flour dough had a higher $|G^*|$ (absolute value of the complex dynamic shear modulus) over the temperature range tested and lower phase angles, indicating it was firmer or more resistant to deformation and more elastic, respectively. Additionally, unlike in starch doughs, these parameters changed over a broader range for sorghum flour doughs due to its broader particle size distribution and because of delayed starch gelatinization resulting from sorghum starch particles being embedded in the surrounding protein. Sorghum dough became thinner as a result of sourdough fermentation. There was no notable difference in $|G^*|$ seen between sorghum flour dough with and without sourdough treatment. After gelatinization, however, $|G^*|$ was significantly higher for sorghum flour dough with sourdough treatment. Hence, sourdough treatment resulted in a stronger starch gel upon subsequent heating.

In another study, Schober and others (2005) utilized a texture analyzer equipped with a forward extrusion cell having a 10 mm nozzle to analyze bread batters using flours from different

sorghum hybrids. The maximum extrusion force (at 8 to 18 mm distance) indicated batter firmness/consistency. Batter consistency varied amongst the samples, with extrusion forces ranging from 3.5 to 10.1 N. This test was also used to adjust the amount of water added to obtain constant consistency of 4.9 N for bread baking as a method to standardize the amount of water added to the bread formula. Bread made from commercial flour with this consistency was of good quality, hence was taken as the standard. The researchers found that low consistency batters (5% more water than the standard water content of 105%, flour weight basis) had improved specific volume and crumb texture.

The effect of fermentation on the functional properties of sorghum flour was also described by Elkhalfa and others (2005). These researchers found that the protein solubility of sorghum flour increased in the acidic range (pH 2–4); oil-binding capacity, emulsifying capacity, and emulsifying stability increased; water-binding capacity decreased; and, no foaming capacity was observed in either fermented or unfermented flours. However, since this study considered sorghum flour in its totality and did not focus on either sorghum proteins or kafirins, it is possible that other factors, aside from protein modification resulting from fermentation, could have possibly played a role in the observed changes in functionality.

Food Uses of Sorghum-Based Ingredients

Gluten-free bread and pasta that have the same quality of wheat-based counterparts are the most highly desired foods by celiacs, and yet are the most difficult to formulate. A plethora of reviews and articles on gluten-free foods, including sorghum breads and pasta, exist. Recent publications on these include those by Gallagher and others (2004), Schober and others (2006), and Taylor and others (2006) and books edited by Arendt and Dal Bello (2008) and by Gallagher (2009), to which the reader is referred. This review describes recent developments in the uses of kafirins in bread and pasta.

Hamaker and others (2008) patented the production of leavened products made from non-wheat cereal proteins. The inventors claim that the composition comprises of non-wheat starch, flour, or a mixture of non-wheat cereal storage proteins from either maize, sorghum, millet, rice or oat, and a co-protein such as casein, elastin, γ -zein, or γ -kafirin. The inventors explained that co-proteins, when mixed with cereal prolamins (especially zein and kafirin), will stabilize the β -sheet formation in the non-wheat prolamins and facilitate the formation of dough that retains its viscoelasticity for an extended period of time under room temperature. Stabilization of the β -sheet conformation is believed to be brought about by binding of the prolamins and co-protein. An example of the method for making bread is: (a) conditioning a mixture of the prolamins and co-protein between 35 and 50 °C with 5% to 25% (w/w) moisture content for 1 to 36 h, preferably between 12 and 24 h; (b) preparing a leavened dough with the conditioned protein mixture, starch, water, sugar, salt, ammonia, and dry yeast in a mixer at 35 °C; (c) proofing the dough for 35 min at 35 °C; and, (d) baking at 220 °C for 20 min. Conditioning the protein mixture is thought to meld the prolamins and co-protein to form a network comparable to wheat gluten. With the exception of zein, which needs to have a moisture content during conditioning between 10% and 25% (w/w), the moisture content of the prolamins/storage protein or co-protein is sufficient to hydrate the proteins and convert it from the glassy to flowable state. It is worth noting that mixing the leavened dough is done at a higher temperature than most traditional processes,

which are usually done at ambient temperature. This is probably because proteins like zein require higher temperatures to exhibit extensibility.

A patent by Engleson and others (2009) describes a system for gluten replacement of food products, including yeast-leavened dough, involving the use of a combination of a gluten-free gas-retaining agent and setting agent. The cited gas-retaining ingredients include polymers like chewing gum base, butyl rubber, paraffin, and petroleum wax, to name a few; and, setting agents include kafirin, zein, egg, whey, and soy proteins, caroubin, casein, shellac, and hydrocolloids, to name a few. The gas-retaining agents enable the dough to hold carbon dioxide generated by the leavening agent within the gas cells, while the setting agents bring about strain hardening upon increase in temperature and evaporation of water. The patent describes preparation of bread dough at room temperature using a kitchen bowl mixer, proofed at 46 °C with a relative humidity of 85%, and then baked for 30 min at 221 °C. Leavened pan breads were reported to have specific volumes of 3.8 to 6 mL/g, similar to those of wheat bread. The patent claims that this gluten replacement system can be used to make pasta, crackers, pizza crust, and leavened bread.

Suhendro and others (2000) studied the effects of the modes of cooking and drying on the qualities of noodles made from decorticated sorghum flour. These cooked a mixture of 100 g sorghum flour, 90 mL water, and 1% salt using either a hot-plate or a microwave oven. The preheated mixtures were passed through a forming extruder to produce the noodles. Three methods of drying were evaluated (slow air drying, 1-stage hot air drying, and 2-stage drying with high and low humidity). Noodles preheated in the microwave yielded better qualities (that is, firmer, less chewy, less sticky, and low dry matter losses) because less starch gelatinization occurred when the noodles were heated in the microwave rather than when they were cooked on a hot plate. The 2-stage drying method yielded the best noodles because hot, moist drying enhanced amylose mobility and reassociation. Starch retrogradation hinders water absorption, thereby reducing the amount of starch leaching into the cooking water. Rapid hot air drying shortened the period for starch retrogradation, and the temperature for slow air drying was too low to promote amylose mobility; thus, both methods resulted in inferior noodle quality. Timing of amylose solubilization and dispersion, noodle formation and amylose retrogradation were critical in obtaining noodles of good quality. These researchers also reported that finer flour yielded better noodles.

In studying noodles made using 4 sorghum grain varieties, Liu (2009) found that sorghum noodle quality was also highly dependent on starch properties. Liu (2009) prepared noodles with a formulation containing sorghum flour, corn starch, dried egg whites, whole eggs, xanthan gum, salt, and water. The ingredients were blended in a batch mixer, kneaded by hand, sheeted and cut using a noodle machine, and then cooked in boiling water. Textural properties were evaluated with a texture analyzer and starch pasting properties were analyzed with a rapid visco-analyzer (RVA). Sorghum flours with lower starch pasting peak viscosity, shorter peak development time, and lower peak temperature produced more desirably firm noodles. While shorter peak development time and low gelatinization temperature (indicators of rapid starch swelling and gelatinization) are also desired in making wheat flour noodles, contrary to sorghum flour noodle, high starch pasting peak viscosity (a measure of starch swelling power) is more desirable. However, Liu (2009) did not find starch pasting properties to be significantly related to cooking loss. Similar to the findings

reported by Suhendro and others (2000), Liu (2009) reported a positive correlation between amylose content and cooked noodle firmness. Unlike in wheat noodles where high protein content of the flours results in better noodles, Liu (2009) found that sorghum protein content was not related to cooked noodle firmness or tensile strength.

Pre-cooked pasta based on sorghum flour has also been prepared by extrusion cooking and forming (Cheng and others 2007). This pasta had similar cooking quality (water absorption and cooking loss) as commercial wheat-based pasta. Although non-wheat noodles, including those made from sorghum flour, were reported to rely primarily on starch for their quality (Suhendro and others 2000; Liu 2009), the reasonably good quality of pre-cooked sorghum pasta observed by Cheng and others (2007) can also possibly be attributed to modification of sorghum proteins during extrusion. Similar to the disruption of maize protein bodies and dispersion of α -zein during extrusion (Batterman-Azcona and others 1999), the relatively high mechanical energy input in the pre-cooked pasta extrusion process may have led to the disruption of kafirin protein bodies and its formation of a protein structural network that reduced dry matter losses. This was, however, not confirmed experimentally, and more research is needed in this area. In wheat flour pasta, proteins are responsible for ensuring strength and quality of the products, and improving the functionality of sorghum proteins could lead to improved sorghum pasta quality.

To summarize this section, sorghum is safe for celiacs and individuals with gluten sensitivities, and improving its protein digestibility is of exceeding importance. Better protein digestibility not only increases sorghum utilization in foods and offers gluten-intolerant individuals an alternative nutritional source but also helps populations in developing nations, for which sorghum is a diet staple, to maximize protein intake. And, although protein digestibility is the foremost concern, improved protein functionality further expands its application in foods. Developing a concentrated source of sorghum proteins with enhanced nutritional and functional characteristics can open more doors for utilization of sorghum in foods. Consequently, this review next presents methods for extraction and concentration of sorghum proteins and describes strategies for protein modification.

Isolation of Sorghum Proteins

Wet-milling of sorghum

Wet-milling is a physico-chemical separation of the components of grain, namely, germ, bran, fiber, starch, and protein. Corn is the grain traditionally used for wet-milling but its shortage during World War II led to the utilization of sorghum grain as the starting raw material in the commercial production of starch and dextrose (Zipf and others 1950). For 22 y, sorghum was used in a commercial wet-milling facility in Corpus Christi, Tex., U.S.A. (Rooney and Serna-Saldivar 2000), but its use was later discontinued because of incomplete starch recovery, low oil yield, and high wax content in the grain (Yang and Seib 1995). Additionally, the economic competitiveness of using sorghum over corn was gone because the price of grain sorghum rose and almost paralleled that of corn. To date, there are no known commercial sorghum wet-milling operations in the United States. Munck (1995) describes an elaborate wet-milling process with 10 possible products (germ, crude oil, refined oil, fiber, protein, protein meal [referred to as sorghum gluten meal], wet starch, dry starch, dextrin, and glucose) while Rooney and Serna-Saldivar (2000) illustrate a commercial

wet-milling process for grain sorghum. A schematic diagram of a simplified wet-milling process is shown in Figure 3.

Given the intricate attachment of sorghum proteins and starch, it is not surprising that poor starch recovery and residual protein content in starch are of great concern in sorghum wet-milling. Using the same wet-milling process, starch recovery and residual protein content in starch from yellow maize is about 90% and 0.12%, respectively, while that from regular sorghum is only about 86% and 0.20%, respectively (Perez-Carrillo and Serna-Saldivar 2006). Of interest to this review on sorghum proteins is the amount of protein recovered and the use of chemicals and enzymes in wet-milling that facilitate the separation of sorghum grain components. Sorghum protein fraction (also referred to as gluten fraction) yields (that is, the dry weight of the protein fraction obtained from wet-milling divided by the initial dry total solids weight in the kernel multiplied by 100) ranging from 8.23% to 25.60% have been reported (Moheno-Perez and others 1997; Buffo and others 1998; Wang and others 2000; Xie and Seib 2000). Protein contents of the sorghum protein (gluten) fraction range from 44.31% to 58.20% (Buffo and others 1998; Xie and Seib 2000).

Critical to the wet-milling process and the subject of most sorghum wet-milling studies is steeping of sorghum. Sorghum grain is steeped in water to toughen the bran and soften the endosperm for easy separation. Chemicals and enzymes can be added to the steeping water to facilitate the separation of grain components and increase starch recovery. Sulfur dioxide (SO₂), sodium metabisulfite, sodium bisulfite or sodium hydrogen sulfite, with an effective concentration of 0.05% to 0.30% SO₂, are typically added to solubilize the protein matrix enveloping the starch granules in the endosperm (Zipf and others 1950; Yang and Seib 1995, 1996; Moheno-Perez and others 1997; Buffo and others

1998; Xie and Seib 2000, 2002; Wang and others 2000; Serna-Saldivar and Mezo-Villanueva 2003; Perez-Carrillo and Serna-Saldivar 2006). Sometimes, lactic acid (0.40% to 1.4% [w/w]) is also added to facilitate protein solubilization. Cell-wall-degrading enzymes and proteases have also been used in wet-milling of sorghum in attempt to increase starch yield and reduce protein content in the starch (Moheno-Perez and others 1997; Wang and others 2000; Serna-Saldivar and Mezo-Villanueva 2003; Perez-Carrillo and Serna-Saldivar 2006). While the addition of protease significantly increased starch recovery (Mezo-Villanueva and Serna-Saldivar 2004; Perez-Carrillo and Serna-Saldivar 2006), the use of cell-wall-degrading enzymes alone did not have a significant benefit (Moheno-Perez and others 1997; Wang and others 2000; Perez-Carrillo and Serna-Saldivar 2006). These enzymatic studies are additional evidences showing the complexity of starch-protein binding in sorghum.

Steeping experiments have been done on temperature and holding time, and the optimum conditions lie within 48 to 55 °C for 24 to 48 h. Grain to steep water ratio in laboratory wet-milling is usually 1 : 2 (Moheno-Perez and others 1997; Wang and others 2000; Xie and Seib 2000, 2002; Serna-Saldivar and Mezo-Villanueva 2003; Perez-Carrillo and Serna-Saldivar 2006) whereas that in commercial wet-milling is 1 : 5 (Rooney and Serna-Saldivar 2000). Whole grain sorghum is the typical starting material. Using decorticated grain as the starting raw material is not beneficial due to high starch losses (Zipf and others 1950; Yang and Seib 1996), lack of improvement in starch brightness and insignificant reduction in protein contamination of starch (Yang and Seib 1996). The use of sorghum grits as the starting raw material, instead of whole grain sorghum, yielded starch with less protein contamination and improved brightness (Higiro and others 2003).

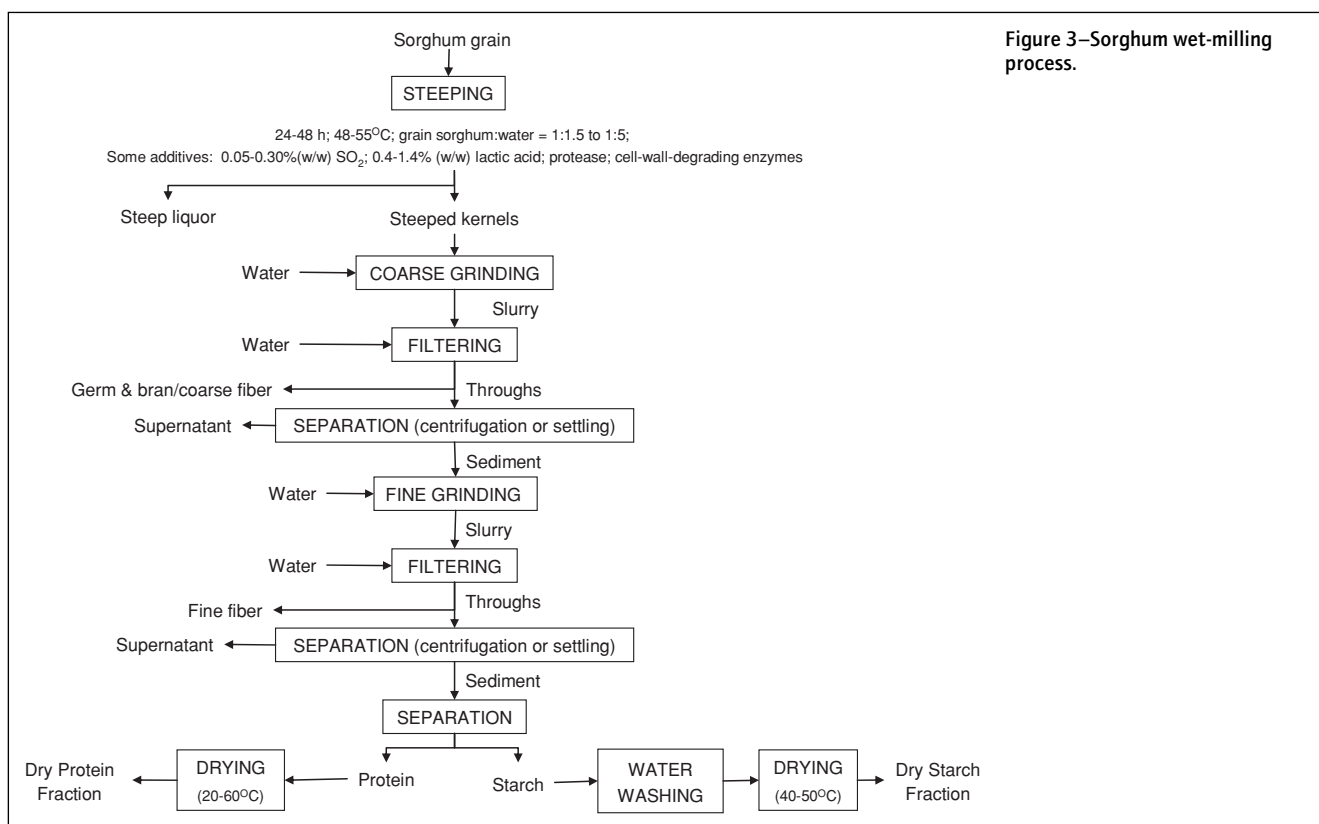


Figure 3—Sorghum wet-milling process.

Traditional methods of protein extraction

Sorghum proteins traditionally have been extracted and classified based on the Osborne procedure (Osborne 1907). This classification method divides proteins into water soluble albumins, salt soluble globulins, alcohol soluble prolamins, and acid or base soluble glutelins (Virupaksha and Sastry 1968; Taylor and others 1984b; Hamaker and others 1995; Wrigley and Bekes 2001). This method, however, does not cleanly separate protein fractions and generally results in significant overlap among the fractions. Many variants of this method have been used to extract sorghum proteins. The Landry–Moureaux method later further divided prolamins into those extractable in aqueous alcohol alone and those extractable in aqueous alcohol plus a reducing agent (reviewed by Hamaker and others 1995). In this procedure, sequential extraction results in the following protein fractions: albumins and globulins extracted with NaCl solution (fraction I); kafirin-1 extracted with 60% *t*-butanol (fraction II); kafirin-2 (also referred to as crosslinked kafirin) extracted with 60% *t*-butanol with 2-mercaptoethanol (2-ME) (fraction III); glutelin-like proteins extracted with alkali borate buffer with 2-ME (fraction IV); true glutelins extracted with alkali borate and sodium dodecyl sulfate (SDS) (fraction V); and, nonextractable proteins determined by protein content analysis of the residue. Although the Landry–Moureaux method fine-tuned the Osborne procedure, it still does not provide much information about the functionality of sorghum proteins.

Alkaline extraction

Wu (1978) obtained sorghum proteins from whole ground sorghum by using an alkaline extraction process. Extraction was carried out by preparing a slurry with 150 g of ground sorghum and 900 mL 0.1 to 0.15 N sodium hydroxide solution, pH 11.8 to 11.9. The slurry was centrifuged and the supernatant was collected and adjusted to pH 4.8 to precipitate the proteins. The protein concentrate in the form of the precipitate was freeze-dried. The concentrates had protein contents ranging from 48% to 60%, depending on grain variety. Solubilities of the concentrates were 90% at a pH range of 8.7 to 10.8; 15% to 22% at pH 2.1; and, were insoluble between pH 3.5 to 5.8. The authors of this study did not identify the types of protein extracted. However, judging from the amount of protein extracted, some kafirin may have been solubilized by this process. Assuming the bulk of the protein was albumin/globulin and glutelins, some 5% to 15% of the extracted protein may have been kafirin. In addition, it was not noted if any modification occurred to the proteins due to the extreme pH used in the extraction process.

Separation of non-prolamins and prolamins

Hamaker and others (1995) used a procedure first applied to extract maize proteins that differentiated non-prolamins (or non-kafirins) from prolamins (or kafirins). In this method, flour samples were first extracted with sodium chloride to remove the albumins, globulins, and nonprotein nitrogen contained in the supernatant. The resulting pellets were then extracted with sodium borate, a detergent (SDS), and 2-ME at pH 10, with a flour-solvent ratio of 1 : 10. After a 1-h extraction, the suspension was centrifuged, and then 60% *t*-butanol was added to the supernatant to precipitate the detergent-extractable nonkafirins. After standing for 2 h with occasional stirring, the mixture was centrifuged, and then the supernatant, containing kafirins, was separated from the pellet. This procedure allowed kafirins to be obtained as one group and facilitated further identification of the different types of kafirins.

However, because these proteins were extracted primarily for characterization purposes, and not for food use, selecting food-grade chemical reagents was of little concern.

Following the previously mentioned procedure by Hamaker and others (1995), Park and Bean (2003) investigated the factors affecting sorghum protein extraction and then optimized these conditions to reduce extraction time. Their studies revealed that pH, detergent type, reducing agent type and sample-to-solvent ratio significantly affected protein extraction. From pH 2.5 to 10, the amount of protein extracted by SDS increased with increasing pH. SDS, an anionic detergent, was exceedingly superior to the cationic detergent dodecylammonium bromide and zwitterionic detergent SB 3–12, regardless of concentration. SDS concentration of 2% extracted the most amount of protein, with no further increases in extraction at higher concentrations. β -ME at 2% extracted more proteins than either dithiothreitol or tris(2-carboxyethyl) phosphine hydrochloride. In contrast to the 1 : 10 flour-solvent ratio used by Hamaker and others (1995), Park and Bean (2003) found that the optimum ratio was 1 : 20. Furthermore, by pooling the protein extracts from three 5-min extractions, Park and Bean (2003) shortened total extraction time from 1 h to 21 min, while obtaining the same amount of protein. They also determined that the same non-kafirins were precipitated by 60% *t*-butanol, 60% 1-propanol, and 70% ethanol. Thus, these solvents can be interchanged. Additionally, acetone can be used to precipitate kafirins.

Use of sonication

Sonication has been utilized to improve extraction of sorghum proteins and to rapidly separate sorghum protein and starch (as in the case of sorghum starch isolation). Bean and others (2006) investigated the effects of various extraction and precipitation conditions, including the use of ultrasound, on recovery and purity of kafirins. These researchers extracted protein from whole ground sorghum flour with 70% ethanol at 50 °C for 1 h, with and without reducing agents (sodium metabisulfite, glutathione, and cysteine), and with 4 min sonication. Lipid was first removed from the extract by diluting ethanol to 60% and centrifuging. The supernatant was then collected for protein precipitation. Protein sedimentation was done by further diluting the ethanol solution from 50% to 30% with water, with or without sodium chloride and with or without lowering the pH to 2.5. After continual mixing and centrifugation, the precipitates were collected and air-dried overnight at room temperature, and then analyzed for protein content and characterized. The researchers found that extracting with ethanol alone resulted in poor protein purity (31% to 52% protein content), and that the addition of a sonication step increased protein content by 15% to 26%. The use of ethanol with either glutathione or sodium metabisulfite (without sonication), on the other hand, yielded a larger percentage of extracted protein (about 70% to 80%). These reducing agents were preferred over β -ME due to their suitability for foods. Lowering pH enhanced protein precipitation because kafirins have low levels of the positively charged amino acids arginine, lysine, and histidine, which are responsible for the solubility of proteins at low pH. While the addition of NaCl increased the amount of protein precipitated in some conditions, overall, its addition did not show a significant improvement in the amount of protein precipitated over the other methods (that is, either lowering ethanol concentration or reducing pH). Precipitating sorghum proteins by dilution to 50% ethanol, with or without NaCl and lowering pH to 2.5, yielded the highest protein content (purest precipitate) under most extraction conditions.

Zhao and others (2008) also used sonication to extract proteins from sorghum and characterized these using size exclusion and reversed-phase high-performance liquid chromatography (HPLC). A sorghum meal slurry containing sodium borate (pH 10) and SDS was sonicated at 10W for 30 s. Sonication is believed to reduce the molecular weight of large proteins by breaking covalent bonds through shear degradation. While sonication extracted more polymeric proteins than SDS borate buffer alone, the amount of proteins this method extracted was fewer than that extracted by a 24-h extraction with SDS borate buffer.

Park and others (2006) also used sonication to disrupt sorghum protein structures to isolate sorghum starch. Sorghum flour was mixed with various protein extraction buffers containing sodium borate buffer, SDS and different reducing agents (β -ME, dithiothreitol, and sodium metabisulfite), and then sonicated. The researchers concluded that 2-min sonication of sorghum flour with 12.5 mM sodium borate buffer, pH 10, with 0.5% SDS (w/v) and 0.5% sodium metabisulfite (w/v) were the optimum conditions for producing sorghum starches with protein contents below 0.06%.

Extraction of sorghum polymeric proteins

Differential solubility is often used in studying the molecular weight distribution of wheat proteins. In wheat, this differentiation is useful in determining the strength of the dough and, therefore, is a predictor of functionality. Differentiating sorghum proteins on the basis of solubility provides insight into the extent of crosslinking. Several researchers have utilized this technique to study sorghum proteins (for example, Oria and others 1995a, b; El Nour and others 1998; Nunes and others 2005; Ioerger and others 2007). These studies have shown that high molecular weight polymeric proteins were linked by disulfide (SS) bonds and that these polymers were primarily made up of γ -kafirins. The α - and β -kafirins were found as monomers and also participated in the formation of oligomers. Vitreous endosperm had higher amounts of crosslinked proteins than did flourey endosperm. Formation of high molecular weight aggregates was promoted by cooking. While most of these researchers utilized the previously described procedures for extracting kafirins, Ioerger and others (2007) used a different method for extracting sorghum proteins based on solubility. In their study, a multistep extraction procedure divided sorghum proteins into soluble proteins (SP), insoluble proteins (IP) and residue proteins (RP). SP were those proteins extracted from sorghum flour with sodium borate, pH 10, buffer with 2% SDS. After continuous shaking and centrifugation of this mixture, IP were extracted from the pellet with sodium borate, pH 10, buffer using sonication (30 s at 10 W). Then, after centrifugation, RP were extracted from the remaining pellet with sodium borate, pH 10, buffer with 2% SDS and 2% β -ME. After centrifugation, the protein content remaining in the pellet was analyzed. Aliquots of the extracts were analyzed by size exclusion-HPLC (SEC) and the percentages of each extract were determined. Flourey endosperm had a higher SP percentage (47.2%) than vitreous endosperm (36.7%), while the RP portions did not differ significantly. A more notable difference was seen in the IP portion wherein vitreous endosperm had a greater proportion of IP (45.3%) than flourey endosperm (35.9%). Furthermore, the IP fraction of the vitreous endosperm had more polymeric proteins than the IP fraction of the flourey endosperm. These factors indicated that proteins in vitreous endosperm were more extensively crosslinked and had higher molecular weights than proteins in flourey endosperm. Ioerger and others (2007) postulated that the SP and IP extracts were analogous to the kafirin-1

(or fraction II) and kafirin-2 (or fraction III) fractions, respectively, of the Landry-Moureaux procedure; and, that the RP fraction was most likely made up of non-prolamin proteins.

Glacial acetic acid extraction

Taylor and others (2005) developed a kafirin extraction method using glacial acetic acid because existing procedures pose problems for the food industry. For instance, they noted that *t*-butanol is toxic and that aqueous ethanol is not acceptable to certain religions. The researchers hypothesized that the low dielectric constant of glacial acetic acid (6.1) enables it to dissolve highly hydrophobic proteins such as kafirin. Dielectric constant of a solvent is inversely proportional to the extent of interaction occurring between 2 charged particles in solution. As glacial acetic acid has a low dielectric constant, proteins tend to unfold and hydrophobic groups interact with the solvent just as easily as these would with each other. In this study, aqueous alcohol extractants (70% ethanol at 70 °C and 55% isopropanol at 40 °C), each containing sodium metabisulfite and sodium hydroxide, were compared against extractants containing glacial acetic acid with and without sodium metabisulfite at 25 °C. Additionally, the researchers tested the effect of presoaking sorghum flour in sodium metabisulfite prior to extraction with glacial acetic acid at 25 °C. After extraction, kafirin preparations were defatted with hexane. The results of their experiments showed that after defatting, the purity of the kafirins extracted with either aqueous alcohol extracts were not significantly different from the purity of the kafirins obtained by glacial acetic acid extraction with presoaking in 0.5% sodium metabisulfite for 16 h. Pretreatment with sodium metabisulfite was necessary in obtaining the desired purity as extraction with glacial acetic acid alone had poor kafirin yield and purity.

Wang and others (2009) compared the properties of kafirins isolated from sorghum dried distiller's grain with solubles (DDGS) using the acetic acid method developed by Taylor and others (2005), an acidic-ethanol method originally used for maize, and the alkaline-ethanol method modified for sorghum by Emmambux and Taylor (2003). In the acetic acid method, sorghum was presoaked in sodium metabisulfite for 16 h prior to extraction with glacial acetic acid. The resulting protein was defatted with petroleum ether. In the acidic-ethanol method, defatted sorghum DDGS was mixed with 70% ethanol, the pH was adjusted to 2 using HCl, and then sodium sulfite was added. After continual stirring for 2 h at 78 °C, the mixture was centrifuged and the supernatant was collected and dehydrated by a rotary evaporator. A 2nd defatting procedure was performed. In the alkaline-ethanol method, sorghum DDGS was mixed with 70% ethanol, 0.35% NaOH and 0.5% sodium metabisulfite, and then stirred for 1 h at 70 °C. Next, the mixture was centrifuged, then the supernatant was diluted with distilled water to 40% ethanol. This suspension was held at -20 °C overnight to promote precipitation, and then centrifuged. The pellet was rinsed with distilled water, dried at 49 °C overnight, and then defatted. Analysis of protein content showed that acetic acid and alkaline-ethanol extraction procedures gave higher yields and purity than the acid-ethanol method. The extraction rates and protein contents obtained were 44.1% and 98.94%, respectively, for acetic acid extraction; 24.2% and 42.32%, respectively, for acidic-ethanol extraction; and, 56.8% and 94.88%, respectively, for alkaline-ethanol extraction. Wang and others (2009) surmised that acidic-ethanol was not strong enough to dissolve denatured proteins and that the extent of disulfide bond disruption is diminished at low pH. Furthermore, presoaking with a reducing agent in the glacial acetic acid procedure led

to a higher extraction percentage. The Fourier transform infrared spectroscopic analysis of the samples revealed that kafirin extracted by acetic acid and alkaline-ethanol had a greater distribution of α -helices and random coils than kafirin extracted by acidic-ethanol. Additionally, only kafirin extracted with acidic-ethanol had β -sheet conformations. Wang and others (2009) inferred that the presence of β -sheets was due to the higher extraction temperature used in the acidic-ethanol method. Differential scanning calorimetry showed a glass transition peak at around 230 °C for all powdered protein extracts. Size exclusion chromatography revealed that acetic acid and acidic-ethanol extraction methods extracted more of high molecular weight polymeric proteins (approximately 20 to 30 kDa) than the alkaline-ethanol method. Additionally, γ -kafirins were observed only in the alkaline-ethanol extracts. Reversed phase high-performance liquid chromatograms of all the extracts from sorghum DDGS were not as sharp as those seen in kafirins extracted directly from sorghum endosperm, indicating the possibility of protein degradation or modification due to the extreme conditions of processing and extraction. A summary of all the protein extraction procedures discussed previously is shown in Table 1.

Concentration of Sorghum Proteins

Kafirins, without any modification, exhibit limited functionality. At present, available literature focuses mainly on kafirin extraction and its application in films. Scientists find it challenging to develop economical, food compatible and non-toxic extraction procedures that can be scaled up to a commercial process because of the propensity of these proteins to form extensively aggregated networks and tightly bound structures. Without first developing a suitable kafirin concentrate, modifying its properties will be even more challenging. However, most cereal protein concentration procedures that have been described in literature are either based on cereals other than sorghum, or are secondary processes with the main goal being separation of starch for downstream applications such as ethanol production.

A protein concentrate can be developed by treating flours with α -amylase to breakdown starch, a process called liquefaction, and then washing out the degraded material. Liquefaction of flour yields concentrates containing protein, fiber, and lipids. This process does not use harsh and toxic chemicals, making the end product safe for food use. Typically, liquefaction is used to produce maltodextrins and sugars for ethanol production from starch. However, some researchers have used this process to produce protein concentrates. Paredes-Lopez and others (1990) made protein concentrates from amaranth flour with 26% to 28% protein by treating flour with either heat-stable α -amylase or glucoamylase. Shih and Daigle (1997) treated rice flour with a heat-stable α -amylase and obtained a concentrate with 65% protein. When they further treated the concentrate with cellulase and hemicellulase, protein content was raised to 76%. Paraman and others (2006) also used enzymatic treatment of rice with a heat stable α -amylase and a cellulase to isolate proteins up to 86% concentration. Barrows and others (2009) applied for a patent describing the production of protein concentrate from starch containing grain or oil seed using enzymes that hydrolyze starch, maltodextrins and β -glucans.

Extrusion liquefaction

Conventional liquefaction is a batch process wherein a 30% to 40% w/w solids starch slurry adjusted to pH 6 to 6.5 is jet-cooked together with a thermostable α -amylase at 103 to 105 °C for 5 min., or at 95 °C for 1 to 2 h (Bigelis 1993). When flours

(for example, rice, corn, and sorghum flours) are used as starting materials, this step can be followed by treatment with other carbohydrate-hydrolyzing enzymes such as glucoamylase, cellulose, and hemicellulase as discussed previously. To speed up the process, liquefaction can be combined with extrusion. Extruding starches and flours degrades and gelatinizes starch thermomechanically, making the substrate more amenable to enzymatic attack. Meagher and Grafelman (1999) published a patent describing the liquefaction of cereal grain starch using an extruder. The inventors described how wet or dry milled corn was extruded in a single-screw extruder and then passed through a static mixer (attached to the extruder) where thermostable α -amylase was added. Liquefaction ensued in the postextrusion reactor, a barrel attached to the end of the static mixer, and in a receiving tank where the extrudate was held at 90 °C for 15 min. Vasanthan and others (2001) also used extrusion-enzyme liquefaction for starch dextrinization in barley flours. These researchers extruded barley flours in a twin-screw extruder and found the optimum processing temperature that maximized α -amylase activity while minimizing its inactivation was 100 °C. Materials extruded with 50% moisture (flour dry weight basis) had the highest dextrose equivalent due to increased starch gelatinization and enzyme hydrolysis. They also found that degree of hydrolysis at the same moisture and temperature at 4% α -amylase was twice that at 2% α -amylase.

As for other cereal flours and starches, liquefaction of sorghum flour is also typically carried out to produce sugars for ethanol production (Corredor and others 2006; Wu and others 2007; Perez-Carrillo and others 2008). Supercritical fluid extrusion, a modification of the conventional extrusion process, has also been used for cooking whole sorghum flour prior to liquefaction for ethanol production (Zhan and others 2006). These researchers found that extrusion with supercritical carbon dioxide effectively disrupted the protein matrix surrounding starch, making the material easier to liquefy. The above studies point towards the utility of the extrusion process for concentration of sorghum proteins, and a method for concentrating insoluble sorghum proteins was recently developed using decorticated sorghum flour by extrusion-enzyme liquefaction (de Mesa and others 2008; and de Mesa-Stonestreet and others 2009) (Figure 4). Decorticated sorghum flour was liquefied with a thermostable α -amylase in either, or both, the extruder or batch mixer. Then, the liquefied material was boiled to inactivate the enzyme, washed and centrifuged. The protein concentrate in form of the precipitate was then collected and freeze-dried. It was thought that the extrusion process disrupted the sorghum protein bodies and glutelin matrix and simultaneously brought about starch gelatinization, which in turn facilitated liquefaction by a thermostable α -amylase. This was a relatively speedier process that yielded concentrates with higher protein contents (up to 80%), and made sorghum proteins more digestible. Further research is needed to optimize the extrusion and enzyme liquefaction processes and characterize and modify the isolated proteins for use in foods. Elkhalfa and others (2009) also recently reported a preparation of sorghum protein-enriched flour that involved digesting boiled whole grain sorghum flour with α -amylase overnight at ambient temperature.

Modification of Sorghum Proteins

Sorghum use in food is limited by its poor digestibility and lack of functionality, which are exacerbated during wet-cooking. Protein modification studies have been undertaken in attempt to overcome these problems, and these can be classified

Table 1—Methods of protein extraction and classification.

Method	Reagent	Function/interactions broken	Proteins obtained
Osborne Procedure (Osborne 1907; Virupaksha and Sastry 1968; Taylor and others 1984b; Hamaker and others 1995; Wrigley and Bekes 2001)	distilled water, 1% NaCl solution	breaks noncovalent electrostatic interactions	albumins globulins
	aqueous alcohol (for example, 70% v/v ethanol, 60% t-butanol, 70% isopropanol)	weakens hydrophobic interactions and acts as a solvent	prolamins
	Acidic or basic solution (for example, 0.4% NaOH, alkali borate buffer, pH 10)	noncovalent electrostatic interactions	glutelins
Landry–Moureaux sequential extraction procedure (Hamaker and others 1995)	0.5 M NaCl solution	breaks hydrophilic interactions	albumins and globulins
	60% t-butanol	weakens hydrophobic interactions and acts as a solvent	prolamins (kafirin-1)
	60% t-butanol + 0.5% 2-mercaptoethanol (2-ME)	2-ME is a reducing agent that breaks covalent disulfide bonds	prolamins (kafirin-2/crosslinked kafirins)
	12.5 mM alkali-borate buffer + 0.5% 2-ME	breaks noncovalent electrostatic interactions and disulfide bonds	glutelin-like proteins
	12.5 mM alkali-borate buffer + 2% sodium dodecyl sulfate (SDS)	SDS is an anionic detergent that breaks hydrogen bonds and hydrophobic interactions	true glutelins (supernatant); nonextractable proteins (precipitate; determined by nitrogen combustion)
Alkaline extraction (Wu 1978)	NaOH solution, pH 11.9	break noncovalent hydrophilic interactions and acts as a solvent	possibly glutelin
	HCl solution, pH 4.8	precipitate proteins	
Non-kafirin/kafirin sequential extraction (Hamaker and others 1995; Park and Bean 2003)	12.5 mM sodium borate buffer, pH 10 + 1% or 2% SDS + 2% 2-ME ^a	breaks noncovalent electrostatic interactions, hydrogen bonds, and disulfide bonds	total proteins ^b
	60% t-butanol	nonkafirin precipitation	kafirins and nonprotein nitrogen (supernatant); detergent-extractable non-kafirins (precipitate)
	acetone (8 : 1 ratio, acetone to sample)	kafirin precipitation	kafirins (precipitate)
Sonication with ethanol (Bean and others 2006)	70% ethanol (+ sonication) ^c	ethanol breaks hydrophobic interactions and solubilizes prolamins; sonication breaks crosslinks that hold large protein aggregates	kafirin monomers, crosslinked kafirins, lipids
	dilution of ethanol to 60% with water	precipitate lipids	kafirin monomers and crosslinked kafirins (supernatant)
	further dilution of ethanol (ranging from 50% to 30%) ^d	precipitates prolamins	kafirin monomers and crosslinked kafirins
Sonication with sodium borate and SDS (Zhao and others 2008; Park and others 2006)	12.5 mM sodium borate buffer, pH 10 + 2% SDS (+ sonication; 10W, 30 s)	buffer breaks noncovalent electrostatic interactions; SDS breaks hydrogen bonds and hydrophobic interactions; sonication breaks large protein aggregates	detergent-extractable proteins, crosslinked proteins
	12.5 mM sodium borate buffer, pH 10 + 2% SDS + 2% β -ME	buffer and SDS function as mentioned previously; β -ME breaks disulfide bonds	detergent-extractable proteins, crosslinked proteins
Polymeric protein sequential extraction using differential solubility and sonication (Ioerger and others 2007)	12.5 mM sodium borate buffer, pH 10 + 2% SDS	breaks noncovalent electrostatic interactions, hydrogen bonds and hydrophobic interactions	soluble proteins (supernatant) ^e
	12.5 mM sodium borate buffer, pH 10 + 2% SDS + sonication (10W, 30 s)	sonication breaks large protein aggregates; buffer functions as above	insoluble proteins (supernatant) ^e ; heavily crosslinked residue protein (precipitate) ^e
Glacial acetic acid extraction of kafirins (Taylor and others 2005)	pre-soak for 16 h in 0.5% Sodium metabisulfite (SMS) at 25 °C	breaks disulfide bonds	
	glacial acetic acid NaOH for adjusting pH to 5	breaks hydrophobic interactions kafirin precipitation	kafirins
Acidic-ethanol method (Wang and others 2009)	70% ethanol, pH 2 + SMS	weakens hydrophobic interactions, breaks disulfide bonds and acts as a solvent	kafirins
Alkaline-ethanol method (Emmambux and Taylor 2003; Wang and others 2009)	70% ethanol + 0.35% NaOH + 0.5% SMS	weakens hydrophobic interactions, breaks electrostatic and disulfide bonds, and acts as a solvent	kafirins
	dilution of ethanol to 40%, -20 °C	kafirin precipitation	

^aAlternative reducing agents include dithiothreitol (DTT), tris(2-carboxyethyl) phosphine hydrochloride, glutathione, and sodium metabisulfite (SMS).

^bAlternatively, albumins and globulins can be removed with 1% NaCl solution prior to extraction of the remaining proteins (kafirins and glutelins). This also allows isolation of the glutelin fraction after kafirins have been extracted.

^cIn some experiments, a reducing agent (SMS or glutathione or cysteine) was added. SMS and glutathione extracted the most protein. The addition of reducing agents to ethanol was more effective in extracting proteins than sonication.

^dNaCl may be added and pH may be lowered to 2.5.

^eSoluble, insoluble, and residue proteins are analogous to the kafirin-1, kafirin-2, and glutelin fractions of the Landry–Moureaux procedure.

into 3 broad categories—biochemical/chemical, enzymatic, and thermo-mechanical.

Biochemical/chemical modification: fermentation

Fermentation of sorghum porridge improved protein digestibility (El Khalifa and El Tinay 1995; Yousif and El Tinay 2001; Taylor and Taylor 2002; Elkhalfia and others 2006). Yousif and El Tinay (2001) found a marked increase in sorghum *in vitro* protein digestibility (from 51.8% to 75.6%) after 24 h of fermentation. After 24 h, the albumin and globulin fractions decreased, the kafirin content increased, but there was no clear trend in the changes occurring in the crosslinked kafirin, glutelin-like, true glutelin, and nonextractable protein fractions. Hence, while these researchers purport that enhanced *in vitro* protein digestibility after fermentation was due to the partial degradation of complex storage proteins into simpler and more soluble products, the changes in the protein fractions do not clearly indicate how this came about.

Taylor and Taylor (2002) also observed a decrease in water soluble proteins in sorghum flour accompanied by higher *in vitro* protein digestibility as a result of a fermentation. As such, these researchers inferred that rather than being broken down into smaller subunits, prolamins, and glutelins underwent structural changes during fermentation that made them more accessible to pepsin digestion. Furthermore, this structural change was attributed to the reduction in pH from about 6 to 3.4 and to the increase in titrable acidity due to lactic acid fermentation. Most of these changes took place during the 1st day and only slight variations were observed over the next 5 d of the study. While fermented sorghum flours had higher *in vitro* protein digestibility than raw sorghum flour, cooking the fermented flours still resulted in lower *in vitro* protein digestibility (11.5%) in comparison to raw sorghum flour. However, the fermented and cooked sorghum flour had greater protein digestibility than cooked sorghum flour that was not fermented (2% digestibility). While Taylor and Taylor (2002) asserted that changes occurred in both prolamins and glutelins, Elkhalfia and others (2006) reported that proteolysis of the glutelin fraction occurred and that the kafirin protein bodies remained intact (as observed with scanning electron microscopy). Furthermore, these researchers also reported that insoluble protein aggregates still formed in fermented sorghum flour after cooking in boiling water.

Fermentation affected not only protein digestibility but also its functional properties (Elkhalfia and others 2005). For example, it was found that fermentation shifted the solubility of sorghum proteins by 2 pH units, with unfermented sorghum flour having a minimum solubility at pH 4 and fermented samples having a minimum solubility at pH 6 (Elkhalfia and others 2005), which suggests modifications of the proteins during fermentation. Fermentation increased the ability of sorghum flour to act as a gelling or firming agent, which is useful in foods like puddings. Although water-binding capacity of sorghum flour decreased, its oil-binding capacity increased by 7% as a result of fermentation. A reduced water-binding capacity makes it desirable for making thinner gruels, while a higher oil-binding capacity makes it useful in foods requiring oil retention. The emulsifying capacity of sorghum flour peaked to 52.83% (an increase of 7%) and emulsifying stability was 52.11% (9% increase) after 16 h of fermentation. This functional property makes it applicable in mayonnaise, salad dressings and frozen desserts. Both fermented and unfermented sorghum flour showed no foaming capacity.

The effects of fermentation on sorghum protein modification were also seen in bread. Schober and others (2007) studied the changes taking place in sorghum proteins in gluten-free sorghum bread undergoing sourdough fermentation. The researchers reported that sourdough fermentation brought about a more stable crumb structure in bread based on the observation that gluten-free sorghum bread that underwent sourdough fermentation had no hole in the crumb and had a higher loaf height than breads without sourdough fermentation. Additionally, its hardness values in texture profile analysis (TPA) showed that it had a softer crumb and that it staled at a slightly slower rate. Their experiments ruled out that chemical acidification brought about these beneficial changes. Instead, evidence pointed toward proteolysis as the primary mechanism. Confocal scanning laser microscopy revealed degradation of protein aggregates in sourdough-fermented bread. In contrast, bread with the same formula but without sourdough fermentation had some protein aggregation, and bread that was chemically acidified to the same pH as sourdough-fermented bread (5.2) had even more heavily aggregated proteins. Schober and others (2007) inferred from the SEC data that many proteins were degraded into fragments small enough that crosslinking upon baking was no longer possible.

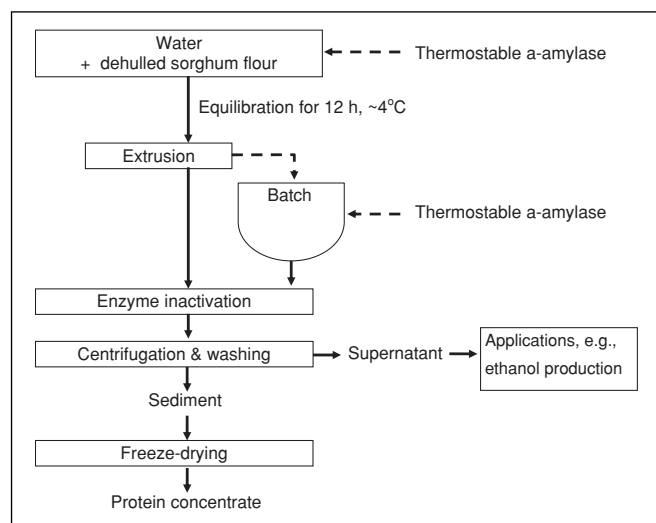


Figure 4—Extrusion-liquefaction process for producing sorghum protein concentrate.

Biochemical/chemical modification: protein-polysaccharide conjugation

Babiker and Kato (1998) conjugated sorghum protein with dextran or galactomannan to improve its functional properties. The researchers first extracted sorghum proteins in an aqueous alkaline (pH 8) medium containing 2-ME. Conjugation was carried out by first preparing a 10% mixture of sorghum protein with either dextran or galactomannan at a ratio of 1 : 5, which was then freeze-dried. The powdered mixtures were heated to 60 °C at 70% relative humidity in a desiccator containing saturated potassium bromide solution for 7 d. Both sorghum protein-dextran and sorghum protein-galactomannan conjugates were 90% to 95% soluble at all pH levels, even when heated to 90 °C. Emulsifying capacity of the conjugates was almost twice that of sorghum protein alone, and the dextran conjugate was superior to the galactomannan conjugate. Stabilities of the emulsions with the dextran and galactomannan conjugates were 10 and 7 times better, respectively, than sorghum protein alone. It is important to note, though, that because an aqueous alkaline medium was used to extract the proteins, albumins, globulins, and possibly some glutelins, rather than

kafirins, may be the proteins primarily extracted. Since albumins and globulins are initially soluble in water or saline solutions, this modification may not be effective for kafirins, and due to the low levels of albumins and globulins in sorghum, this procedure would likely yield only relatively low levels of modified proteins from a given batch of sorghum flour.

Biochemical/chemical modification: reducing agents

Reducing agents have been used to modify both the *in vitro* digestibility (Hamaker and others 1987; Rom and others 1992; Arbab and El Tinay 1997; Zhang and Hamaker 1998; Elkhalfa and others 1999; Choi and others 2008) and extractability (Park and Bean 2003; Bean and others 2006) of sorghum proteins. Of the reducing agents tested, sodium metabisulphite, glutathione, and L-cysteine are suitable for some food use. These reagents work by breaking disulfide linkages in kafirins and the protein matrix. Using scanning electron microscopy, Rom and others (1992) showed that boiling a sorghum flour suspension with sodium bisulfite for 20 min resulted in a breakdown of the protein matrix and pitting of the protein bodies. Using confocal laser scanning microscopy, Choi and others (2008) depicted breakdown of the protein matrix and increased starch digestion resulting from cooking a sorghum flour suspension with sodium bisulfite. Ezeogu and others (2008) also used confocal laser scanning microscopy to show the effect of cooking with and without 2-ME on the formation of protein matrices in vitreous and floury sorghum endosperm flour. These researchers found that cooking without a reducing agent resulted in discontinuities and an expansion of the protein matrix in floury sorghum endosperm flour but not in the vitreous fraction, indicating a greater degree of protein crosslinking in the latter. On the other hand, cooking with 2-ME reduced the density of the web-like protein network in both floury and vitreous endosperm flours due to the breakage of disulfide bonds.

Biochemical/chemical modification: enzymatic hydrolysis

Enzymes have advantages over chemical methods of protein modification. First, reaction rates are fast and highly specific and conditions are generally mild, which limits damage to the nutritional quality of proteins and reduces production of toxic substances. Second, and probably most important, enzymatic methods are generally safer than corresponding chemical based methods. On the flip side, enzymes are not as cost effective as chemical processes. Additionally, while specificity of enzymatic reactions is a commonly cited desirable attribute, it can also be a deterrent to its adoption because several enzymes may be needed to accomplish the job of a single chemical process.

Proteases are utilized to modify protein nutritional and sensory qualities (for example, digestibility, allergenicity, bitterness, and so on) and protein functionality (for example, solubility, dispersibility, foaming, water or oil binding, dough elasticity, and so on) (Adler-Nissen 1986; Nielsen 1997; Kunst 2003). In sorghum, proteolysis has been primarily used to isolate sorghum starch, to improve starch digestion for animal feeds and to improve starch hydrolysis for ethanol production, but there is limited research on enzymatic hydrolysis of kafirins. Yang and Seib (1995) used a type II protease from *Aspergillus oryzae* to aid in removing sorghum proteins during sorghum starch isolation. The researchers were able to reduce the protein content from 0.7% to 1.1% (dry weight basis, db) to 0.5% to 0.6% (dry weight basis) by treating sorghum starch with the protease. When isolating sorghum starch, Xu (2008) treated sorghum flour with pepsin, and achieved an almost complete hydrolysis of the proteins after 4 h. The isolated starch had 0.5% protein content.

Xu (2008) also found that pepsin pretreatment improved sorghum starch digestibility. Similarly, Mezo-Villanueva and Serna-Saldivar (2004) were able to achieve greater starch recovery after steeping sorghum flour for 24 and 48 h using NeutraseTM, a neutral metalloprotease requiring Zn²⁺ and Ca²⁺ for its activity. Zhang and Hamaker (1998) found an increase in starch digestibility of cooked sorghum flours by 7% to 14% when sorghum flours were pretreated with pepsin. Benmoussa and others (2006) also found that sorghum starch digestion profile over a 12 h period was significantly improved by pepsin pretreatment of raw sorghum flour for feed use.

Sorghum flour has also been treated with protease to facilitate starch liquefaction. Perez-Carillo and Serna-Saldivar (2007) treated decorticated and whole sorghum flour slurry with NeutraseTM. The slurry containing NeutraseTM was heated to 60 ± 1 °C for 30 min prior to the addition of a thermostable α-amylase. Their experiments showed that protease pretreatment resulted in a 44.7% reduction in liquefaction time. Using the same protease pretreatment procedure described above, Perez-Carrillo and others (2008) found that decorticated sorghum flour treated with protease had approximately 50% more reducing sugars than its untreated counterpart and that fermentation time was reduced from 60 to 22 h. However, none of the previously mentioned studies on sorghum starch isolation and liquefaction investigated the impact of proteases on structure, digestibility, or functionality of the residual proteins.

Ng'andwe and others (2008) treated raw and wet-cooked sorghum flour with a combination of an aminopeptidase (FlavourzymeTM) and potassium metabisulfite at 40 °C for 7 h. An aminopeptidase rather than a sulphhydryl protease was specifically used because the latter enzyme is inactivated by potassium metabisulfite. Confocal laser scanning micrographs and transmission electron micrographs revealed that the exogenous protease alone digested the glutelin protein matrix surrounding the starch granules in both raw and cooked sorghum flours, and that the presence of both enzyme and potassium metabisulfite in the mixture brought about the reduction of not only the glutelin matrix, but also the exterior parts of the protein bodies. SDS-polyacrylamide gel electrophoretic patterns also showed that potassium metabisulfite was effective in reducing kafirin polymers and oligomers into monomers.

Kamath and others (2007) hydrolyzed isolated α-kafirin in Tris-HCl buffer (pH 7.6) containing SDS with chymotrypsin. Hydrolysis was carried out at 37 °C for 4 h with 40 μg chymotrypsin/ mg α-kafirin. The researchers obtained a hydrolysate rich in peptides that inhibited (*in vitro*) angiotensin I converting enzyme (ACE). Inhibiting ACE is useful in treating high blood pressure (reviewed by Kamath and others 2007).

Biochemical/chemical modification: deamidation

Deamidation is known to improve solubility, emulsification activity and stability, and foaming of plant storage proteins (Haard 2001). This reaction is characterized by the conversion of the amide groups of asparagine and glutamine to carboxyl groups, which can be accomplished chemically (under acidic or basic conditions) or enzymatically. Acidic deamidation with 0.05N HCl for 15 to 30 min at 95 °C was successful in improving the solubility of zein (Casella and Whitaker 1990). On the other hand, alkali deamidation at pH 11 and 25 °C was conducted on rice protein isolates by Paraman and others (2007). A procedure for the enzymatic deamidation of food proteins, primarily soy, corn, rice, egg, and milk proteins, was described in the patent by Hamada

and Marshall (1992). This patent described the optimization of deamidation by first heat denaturing or enzymatically hydrolyzing proteins prior to deamidation with peptidoglutaminase. The denaturation and/or hydrolysis steps were necessary in opening the protein structures to provide more sites for peptidoglutaminase to act on. While deamidation has not been used in sorghum, its success in zein and corn gluten indicates that it can also be used in modifying kafirins. However, pretreatment of kafirins by thermo-mechanical and/or chemical means may be necessary because of the nature of sorghum protein bodies.

Thermo-mechanical modification: irradiation

Fombang and others (2005) used γ -irradiation to modify sorghum proteins. Sorghum porridge cooked with sorghum flour irradiated at 10 kGy showed a significantly higher *in vitro* protein digestibility than that cooked with untreated sorghum flour. The researchers hypothesized that irradiation cleaved kafirin disulfide bonds and fragmented proteins, leading to reduced disulfide crosslinking during cooking. This more open structure would have been more susceptible to proteolytic digestion. Digestibility of porridge made from flour irradiated at 50 kGy, however, was lower than that of porridge made from 10 kGy irradiated flour but higher than that of porridge made with untreated flour. Fombang and others (2005) attributed the reduced digestibility to crosslinking and aggregation at higher doses and to production of Maillard products that inhibit proteolytic activity.

Thermo-mechanical modification: extrusion

Extrusion of sorghum flour improves its protein digestibility. MacLean and others (1983) found that decortication and low moisture extrusion improved digestibility of sorghum when prepared into porridge and fed to preschool children. Mertz and others (1984) established that after boiling with water, dried and ground sample of decorticated sorghum flour extruded at low moisture and 350 °C had 22% higher *in vitro* protein digestibility than decorticated sorghum flour. In a study by Fapojuwo and others (1987), extrusion also raised *in vitro* protein digestibility of sorghum by about 30%. These researchers also saw that there were no significant differences in digestibilities between 2 moisture levels (15% and 25%), but that increasing screw speed from 50 to 125 rpm, as well as raising temperature (50, 125, and 200 °C), significantly increased protein digestibility. Fapojuwo and others (1987) also reported that pretreatment of sorghum grain with 4% calcium hydroxide (pH 11) further increased the protein digestibility of extruded sorghum grain. Dahlin and Lorenz (1993) processed whole sorghum flour in a single-screw extruder and evaluated the effect of feed moisture (15%, 25%), processing temperature (100 and 150 °C) and screw speed (100 and 150 rpm) on *in vitro* protein digestibility. Their results revealed that extruding with 15% feed moisture at 150 °C and 100 rpm were the optimum conditions for yielding extrudates with high *in vitro* protein digestibility. While they found that extruding at low moisture and high temperature raised protein digestibility, they saw that the effect of raising screw speed was less obvious. Dahlin and Lorenz (1993) explained that extruding at 15% moisture gave better digestibility values probably by reducing the reaction rate of degradative processes. These degradative processes, however, were not specified. Additionally, these researchers said that extruding at 150 °C instead of 100 °C favored digestibility due to greater denaturation of protein and inactivation of enzyme inhibitors.

Hamaker and others (1994) studied the *in vitro* protein digestibility and protein distribution of cooked flour porridges of decorti-

cated only and decorticated and extruded sorghum flours. Their study showed that extrusion of decorticated sorghum flours raised protein digestibility by 18%. They also found that the percentage of prolamins extractable by 60% t-butanol increased by 12% and that the percentage of prolamins extractable by t-butanol with a reducing agent decreased by 17%. This indicated a shift in protein distribution towards the more digestible fraction, which could explain the improvement in protein digestibility. However, this was also accompanied by a shift in the glutelin fraction to the nonextractable fraction. The latter, though, did not appear to affect protein digestibility.

Batterman-Azcona and others (1999) studied the relationship between extrusion and maize protein body degradation. They found that α -zeins remained intact under mild processing conditions and were released only at a specific mechanical energy (SME) of about 100 kJ/kg. At 165 kJ/kg, the protein bodies were completely disrupted, α -zein was dispersed and it was inferred that these formed protein fibrils. Even though kafirins bear a high degree of homology to zein, the extensive crosslinking that occurs in kafirins during cooking may require a higher specific mechanical energy to open the protein bodies (Hamaker and others 1986; Ezeogo and others 2008).

Conclusions

Sorghum is an attractive raw material and a good source of protein for wheat-free products due to the neutral flavor and color of specific varieties, low allergenicity and its ability to grow in drought-like conditions. Although sorghum has been mainly used for animal feed in the United States, it has a huge potential for food use, including as a source of concentrated proteins for incorporation in gluten-free foods. However, the acceptability of sorghum and its proteins as food ingredients depends not only on their nutritional characteristics, but also on sensory and technological properties. At present, a huge gap exists between the present and desired nutritional and functional characteristics of sorghum and its proteins, limiting their use in foods. Sorghum proteins have low digestibility, which is further reduced during cooking with high moisture. Furthermore, unlike wheat proteins, sorghum proteins are not highly functional. Concentration and/or modification of sorghum proteins could be one way to address this challenge. However, research to date has focused on sorghum protein extraction with non-food compatible and unsafe chemicals, and incorporation of kafirins in highly demanded staples like bread and pasta is scant.

Thus, areas for future research include development of economical, food-compatible and safe methods for concentrating and/or extracting sorghum proteins, especially kafirins, that can be scaled up to a commercial level and modification of the functional properties of kafirins to increase the scope of their applications in foods.

Acknowledgment

The authors would like to acknowledge the Great Plains Center for Sorghum Improvement for partial funding of ongoing research on sorghum proteins at Kansas State Univ. This is contribution number 10-185-J from the Kansas Agricultural Experiment Station, Manhattan, Kans., U.S.A.

References

- Adler-Nissen J. 1986. Enzymic hydrolysis of food proteins. New York: Elsevier Science Publishing Co. Inc. 427 p.

- Arbab ME, El Tinay AH. 1997. Effect of cooking and treatment with sodium bisulphite or ascorbic acid on the *in vitro* protein digestibility of two sorghum cultivars. *Food Chem* 59(3):339–43.
- Arendt EK, Dal Bello F. 2008. *Gluten-free cereal products and beverages*. Oxford, UK: Elsevier Inc. 464 p.
- Babiker EE, Kato A. 1998. Improvement of the functional properties of sorghum protein by protein-polysaccharide and protein-protein complexes. *Nahrung* 42(5):286–9.
- Barrows FT, Bradley CA, Kearns RD, Wasicek BD, Hardy RW, inventors; The United States of America, as represented by the Secretary of Agriculture, Washington, DC, US, Montana Microbial Products, assignees. 2009 Oct 15. Protein concentrate from starch containing grains: composition, method of making, and uses thereof. U.S. patent application 20090259018.
- Batterman-Azcona SJ, Lawton JW, Hamaker BR. 1999. Effect of specific mechanical energy on protein bodies and α -zeins in corn flour extrudates. *Cereal Chem* 76(2):316–20.
- Bean SR, Ioerger, BP, Park, SH, Singh H. 2006. Interaction between sorghum protein extraction and precipitation conditions on yield, purity, and composition of purified protein fractions. *Cereal Chem* 83(1):99–107.
- Belton PS, Delgado I, Halford NG, Shewry PR. 2006. Kafirin structure and functionality. *J Cereal Sci* 44(3):272–86.
- Benmoussa M, Suhendra B, Aboubacar A, Hamaker BR. 2006. Distinctive sorghum starch granule morphologies appear to improve raw starch digestibility. *Starch* 58(2):92–9.
- Berti C, Riso P, Monti LD, Porrini M. 2004. *In vitro* starch digestibility and *in vivo* glucose response of gluten-free foods and their gluten counterparts. *Eur J Nutr* 43(4):198–204.
- Bigelis R. 1993. *Carbohydrases*. In: Nagodawithana T, Reed G, editors. *Enzymes in food processing*. 3rd ed. San Diego, Calif.: Academic Press Inc. p 121–58.
- Buffo RA, Weller CL, Parkhurst AM. 1998. Wet-milling factors of sorghum and relationship to grain quality. *J Cereal Sci* 27(3):327–34.
- Casella MLA, Whitaker JR. 1990. Enzymatically and chemically modified zein for improvement of functional properties. *J Food Biochem* 14(6):453–475.
- Cheng EM, Alavi S, Bean S. 2007. Sorghum-based pre-cooked pasta utilizing extrusion processing. 25rd Biennial Sorghum Research and Utilization Conference, Jan. 14–16, Santa Ana Pueblo, New Mexico. Book of Abstracts.
- Choi SJ, Woo HD, Ko SH, Moon TW. 2008. Confocal laser scanning microscopy to investigate the effect of cooking and sodium bisulfite on *in vitro* digestibility of waxy sorghum flour. *Cereal Chem* 85(1):65–9.
- Ciacci C, Maiuri L, Caporaso N, Bucci C, Del Giudice L, Massardo DR, Pontieri P, Di Fonzo N, Bean SR, Ioerger B, Londei M. 2007. Celiac disease: *In vitro* and *in vivo* safety and palatability of wheat-free sorghum food products. *Clin Nutr* 26(6):799–805.
- Corredor D, Bean SR, Schober TJ, Wang D. 2006. Effect of decortivating sorghum on ethanol production and composition of DDGS. *Cereal Chem* 83(1):17–21.
- Dahlin K, Lorenz K. 1993. Protein digestibility of extruded cereal grains. *Food Chem* 48(1):13–8.
- Damodaran S, Paraf A. 1997. *Food proteins and their applications*. New York: Marcel Dekker, Inc. 681 p.
- De Mesa NE, Alavi S, Bean SR, Schober TJ. 2008. Modification of sorghum proteins for enhanced functionality. AACC Annual Meeting, Sept. 21–24, Honolulu, Hawaii. Supplement of Abstracts. *Cereal Foods World*, 53: A56.
- De Mesa-Stonestreet NJE, Alavi S, Bean SR, Schober TJ, Gwirtz J. 2009. Sorghum protein concentration and modification for improved nutrition and functionality. Institute of Food Technologists Annual Meeting and Expo, June 6–9, Anaheim, Calif. Book of Abstracts.
- Dendy DAV. 1995. Sorghum and millets: production and importance. In: Dendy DAV, editor. *Sorghum and millets: chemistry and technology*. St. Paul, Minn.: American Assoc. of Cereal Chemists. p 11–26.
- Duodu KG, Nunes A, Delgado I, Parker ML, Mills ENC, Belton PS, Taylor JRN. 2002. Effect of grain structure and cooking on sorghum and maize *in vitro* protein digestibility. *J Cereal Sci* 35(2):161–74.
- Duodu KG, Taylor JRN, Belton PS, Hamaker BR. 2003. Factors affecting sorghum protein digestibility. *J Cereal Sci* 38(2):117–31.
- El Khalifa O, El Tinay AH. 1995. Effect of fermentation and germination on the *in vitro* protein digestibility of low and high tannin cultivars of sorghum. *Food Chem* 54(2):147–50.
- El Nour INA, Peruffo ADB, Curioni A. 1998. Characterization of sorghum kafirins in relation to their cross-linking behavior. *J Cereal Sci* 28(2):197–207.
- Elkhalifa AEO, Chandrashekar A, Mohamed BE, El Tinay AH. 1999. Effect of reducing agents on the *in vitro* protein and starch digestibilities of cooked sorghum. *Food Chem* 66(3):323–6.
- Elkhalifa AEO, Schiffler B, Bernhardt R. 2005. Effect of fermentation on the functional properties of sorghum flour. *Food Chem* 92(1):1–5.
- Elkhalifa AEO, Bernhardt R, Bonomi F, Iametti S, Pagani MA, Zardi M. 2006. Fermentation modifies protein/protein and protein/starch interactions in sorghum dough. *Eur Food Res Technol* 222(5–6):559–64.
- Elkhalifa AEO, Georget DMR, Barker SA, Belton PS. 2009. Study of the physical properties of kafirin during the fabrication of tablets for pharmaceutical applications. *J Cereal Sci* 50(2):159–65.
- Emmambux MN, Taylor JRN. 2003. Sorghum kafirin interaction with various phenolic compounds. *J Sci Food Agric* 83(5):402–7.
- Emmambux MN, Taylor JRN. 2009. Properties of heat-treated sorghum and maize meal and their prolamin proteins. *J Agric Food Chem* 57(3):1045–50.
- Engleson J, Atwell B. 2008. Gluten-free product development. *Cereal Foods World* 53(4):180–4.
- Engleson JA, Lendon CA, Hope J, Casper JL, inventors; Cargill Inc., assignee. 2009 Apr 16. System for gluten replacement in food products. U.S. patent application 20090098270.
- Ezeogu LI, Duodu KG, Emmambux MN, Taylor JRN. 2008. Influence of cooking conditions on the protein matrix of sorghum and maize endosperm flours. *Cereal Chem* 85(3):397–402.
- Fapojuwu OO, Maga JA, Jansen GR. 1987. Effect of extrusion cooking on *in vitro* protein digestibility of sorghum. *J Food Sci* 52(1):218–9.
- Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, Elitsur Y, Greetz PHR, Guandalini S, Hill ID, Pietzak M, Ventura A, Thorpe M, Kryszak Db, Fornaroli F, Wasserman, SS, Murray, JA, Horvath K. 2003. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States. *Arch Int Med* 163(3):286–92.
- Fombang EN, Taylor JRN, Mboufung CMF, Minnaar A. 2005. Use of γ -irradiation to alleviate the poor protein digestibility of sorghum porridge. *Food Chem* 91(4):695–703.
- Gallagher E. 2009. *Gluten-free food science and technology*. Ames, Iowa: Blackwell Publishing Ltd. p 256.
- Gallagher E, Gormley TR, Arendt EK. 2004. Recent advances in the formulation of gluten-free cereal-based products. *Trends Food Sci Technol* 15(3–4): 143–52.
- Haard NE. 2001. Enzymic modification of proteins in food systems. In: Sikorski ZE, editor. *Chemical and functional properties of food proteins*. Lancaster, Pa.: Technomic Publishing Co. Inc. p 155–90.
- Hamada JS, Marshall E, inventors; United States of America as represented by the Secretary of Agriculture, assignee. 1992 Jan 21. Enzymatic deamidation of food proteins for improved food use. U.S. patent 5,082,672.
- Hamaker BR, Kirleis AW, Mertz ET, Axtell JD. 1986. Effect of cooking on the protein profiles and *in vitro* digestibility of sorghum and maize. *J Agric Food Chem* 34(4):647–9.
- Hamaker BR, Kirleis AW, Butler BJD, Axtell JD, Mertz ET. 1987. Improving the *in vitro* protein digestibility of sorghum with reducing agents. *Proc Natl Acad Sci* 84(3):626–28.
- Hamaker BR, Mertz ET, Axtell JD. 1994. Effect of extrusion on sorghum kafirin solubility. *Cereal Chem* 71(5):515–7.
- Hamaker BR, Mohamed AA, Habben JE, Huang CP, Larkins BA. 1995. Efficient procedure for extracting maize and sorghum kernel proteins reveals higher prolamin contents than the conventional method. *Cereal Chem* 72(6):583–8.
- Hamaker BR, Mejia CD, Mauer LJ, Campanella OH, inventors; Purdue Research Foundation, assignee. 2008 Mar 27. Leavened products made from non-wheat cereal proteins. International patent application WO 2008/036646 A1.
- Higiro J, Flores RA, Seib PA. 2003. Starch production from sorghum grits. *J Cereal Sci* 37(1):101–9.
- Hoseney RC, Varriano-Marston E, Dendy DA. 1981. Sorghum and millets. *Adv Cereal Sci Technol* 4: 71–144.
- Ioerger B, Bean SR, Tuinstra MR, Pedersen JF, Erpelding J, Lee KM, Herrman TJ. 2007. Characterization of polymeric proteins from vitreous and floury sorghum endosperm. *J Agric Food Chem* 55(25):10232–9.
- Kamath V, Niketh S, Chandrashekar A, Rajini PS. 2007. Chymotryptic hydrolysates of α -kafirin, the storage protein of sorghum (*Sorghum bicolor*) exhibited angiotensin converting enzyme inhibitory activity. *Food Chem* 100(1):306–11.
- Kinsella JE, Soucie WG. 1989. *Food proteins*. Champaign, Ill.: The American Oil Chemists' Society. 431 p.
- Kulammarva AG, Garlepy Y, Sosle VR, Ngadi M, Raghavan V. 2004. Rheological properties of sorghum dough. ASAE/CSAE Meeting Paper Nr: 046036. St. Joseph, Mich.: ASAE.
- Kunst T. 2003. Protein modification to optimize functionality, protein hydrolysates. In: Whitaker JR, Vorag AGJ, Wong DWS. New York: Marcel Dekker Inc. p 221–36.
- Lasztyry R. 1996. Sorghum proteins. In: Lasztyry R, editor. *The chemistry of cereal proteins*. 2nd ed. Boca Raton, Fla.: CRC Press. p 227–48.
- Lee AR, Ng DL, Zivin J, Green PHR. 2007. Economic burden of a gluten-free diet. *J Hum Nutr Diet* 20(5):423–30.
- Liu L. 2009. Evaluation of four sorghum hybrids in a gluten-free noodle system. [MSc thesis]. Manhattan, Kans.: Kansas State Univ. 103 p. Available from: <http://krex.k-state.edu>.
- MacLean WC Jr, de Romana GL, Ganatady A, Graham GG. 1983. The effect of decortication and extrusion on the digestibility of sorghum by preschool children. *J Nutr* 113(10):2071–7.
- Meagher MM, Grafelman DD, inventors; Board of Regents, assignee. 1999 Nov 9. Method for liquefaction of cereal grain starch substrate and apparatus thereof. U.S. patent 5,981,237.
- Mertz ET, Hassen MM, Cairns-Whittern C, Kirleis AW, Tu L, Axtell JD. 1984. Pepsin digestibility of proteins in sorghum and other major cereals. *Proceedings of the National Academy of Science U.S.A.-Biological Sciences* 81(1):1–2.
- Mezo-Villanueva M, Serna-Saldivar SO. 2004. Effect of protease addition on starch recovery from steeped sorghum and maize. *Starch* 56(8):371–8.
- Mintel: food allergies and intolerance-US-April 2007 [Internet]. Chicago, Ill.: Mintel Intl. Group Ltd.; c2009. Available from: <http://academic.mintel.com.er.lib.k-state.edu>. Accessed 2009 October 13.
- Moheno-Perez JA, Almeida-Dominguez HC, Serna-Saldivar SO. 1997. Effect of fiber degrading enzymes on wet milling and starch properties of different types of sorghums an maize. *Starch-Stärke* 49(12):480–4.
- Munck L. 1995. New milling technologies and products: whole plan utilization by milling and separation of botanical and chemical components. In: Dendy DAV, editor. *Sorghum and millets: Chemistry and technology*. St. Paul, Minn.: American Assoc. of Cereal Chemists. p 223–81.
- Murty DS, Kumar KA. 1995. Traditional uses of sorghum and millets. In: Dendy DAV, editor. *Sorghum and millets: chemistry and technology*. St. Paul, Minn.: American Assoc. of Cereal Chemists. p 185–221.
- Nakai S. 1983. Structure-function relationship of food proteins with an emphasis on the importance of protein hydrophobicity. *J Agric Food Chem* 31(4): 676–83.
- Ng'andwe CC, Hall AN, Taylor JRN. 2008. Proteolysis of sorghum endosperm proteins when mashing with raw grain plus exogenous protease and potassium metabisulphite. *J Inst Brew* 114(4): 343–8.
- [NIDDK] Natl. Inst. of Diabetes and Digestive and Kidney Diseases. 2009. Celiac disease. Bethesda, Md.: NIDDK Clearing Houses Publications Catalog; c2009. Available from: www.digestive.niddk.nih.gov. Accessed 2009 February.
- Nielsen PM. 1997. Functionality of protein hydrolysates. In: Damodaran S, Paraf A, editors. *Food proteins and their applications*. New York: Marcel Dekker Inc. p 443–72.
- Nunes A, Correia I, Barros A, Delgado I. 2004. Sequential *in vitro* pepsin digestion of uncooked and cooked sorghum and maize samples. *J Agric Food Chem* 52(7):2052–8.
- Nunes A, Correia I, Barros A, Delgado I. 2005. Characterization of kafirin and zein oligomers by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *J Agric Food Chem* 53(3):639–43.
- Oom A, Pettersson A, Taylor JRN, Stading M. 2008. Rheological properties of kafirin and zein prolamins. *J Cereal Sci* 47(1):109–16.
- Oria MP, Hamaker BR, Shull JM. 1995a. Resistance of sorghum α -, β -, γ -kafirins to pepsin digestion. *J Agric Food Chem* 43(8):2148–53.
- Oria MP, Hamaker BR, Shull JM. 1995b. *In vitro* protein digestibility of developing and mature sorghum grain in relation to α -, β -, and γ -kafirin disulfide crosslinking. *J Cereal Sci* 22(1):85–93.
- Oria MP, Hamaker BR, Axtell JD, Huang CP. 2000. A highly digestible sorghum mutant cultivar exhibits a unique folded structure of endosperm protein bodies. *Proc Natl Acad Sci U.S.A.* 97(10):5065–70.

- Osborne TB. 1907. The proteins of the wheat kernel. Washington, D.C.: Carnegie Inst. of Washington.
- Paraman I, Hettiarachchy NS, Schaefer C, Beck MI. 2006. Physicochemical properties of rice endosperm proteins extracted by chemical and enzymatic methods. *Cereal Chem* 83(6):663–7.
- Paraman I, Hettiarachchy NS, Schaefer C. 2007. Glycosylation and deamidation of rice endosperm protein for improved solubility and emulsifying properties. *Cereal Chem* 84(6):593–9.
- Paredes-Lopez O, Barba de la Rosa AP, Carabez-Trejo A. 1990. Enzymatic production of high-protein amaranth flour and carbohydrate rich fraction. *J Food Sci* 55(4):1157–61.
- Park SH, Bean SR. 2003. Investigation and optimization of the factors influencing sorghum protein extraction. *J Agric Food Chem* 51(24):7050–4.
- Park SH, Bean SR, Wilson JD, Schober TJ. 2006. Rapid isolation of sorghum and other cereal starches using sonication. *Cereal Chem* 83(6): 611–6.
- Parker ML, Grant A, Rigby NM, Belton PS, Taylor JRN. 1999. Effect of popping on the endosperm cell walls of sorghum and maize. *J Cereal Sci* 30(3):209–16.
- Perez-Carrillo E, Serna-Saldivar SO. 2006. Cell wall degrading enzymes and proteases improve starch yields of sorghum and maize. *Starch-Starke* 58(7):338–44.
- Perez-Carrillo E, Serna-Saldivar SO. 2007. Effect of protease treatment before hydrolysis with α -amylase on the rate of starch and protein hydrolysis of maize, whole sorghum and decorticated sorghum. *Cereal Chem* 84(6):607–13.
- Perez-Carrillo E, Serna-Saldivar SO, Alvarez, MM, Cortes-Callejas ML. 2008. Effect of sorghum decortication and use of protease before liquefaction with thermoresistant α -amylase on efficiency of bioethanol production. *Cereal Chem* 85(6):792–8.
- Rom DL, Schull JM, Chandrashekar A, Kirleis AW. 1992. Effects of cooking and treatment with sodium bisulfite on *in vitro* protein digestibility and microstructure of sorghum flour. *Cereal Chem* 69(2):178–81.
- Rooney LW, Pflugfelder RL. 1986. Factors affecting starch digestibility with special emphasis on sorghum and corn. *J Animal Sci* 63(5):1607–23.
- Rooney LW, Serna-Saldivar SO. 2000. Sorghum. In: Kulp K, Ponte JG Jr, editors. *Handbook of cereal science and technology*. 2nd ed. New York: Marcel Dekker Inc. p 149–75.
- Schober TJ, Messerschmidt M, Bean SR, Park SH, Arendt EK. 2005. Gluten-free bread from sorghum: quality differences among hybrids. *Cereal Chem* 82(4): 394–404.
- Schober TJ, Bean S, Arendt E, Fenster C. 2006. Use of sorghum flour in bakery products. *AIB technical bulletin XXVIII*(3):1–6.
- Schober TJ, Bean SR, Boyle DL. 2007. Gluten-free sorghum bread improved by sourdough fermentation: biochemical, rheological and microstructural background. *J Agric Food Chem* 55(13):5137–46.
- Seckinger HL, Wolf MJ. 1973. Sorghum protein ultrastructure as it relates to composition. *Cereal Chem* 50(4):455–65.
- Serna-Saldivar SO, Mezo-Villanueva M. 2003. Effect of a cell-wall-degrading enzyme complex on starch recovery and steeping requirements of sorghum and maize. *Cereal Chem* 80(2):148–53.
- Shewry PR, Tatham AS. 1990. The prolamin storage proteins of cereal seeds: structure and evolution. *Biochem J* 267(1):1–12.
- Shih FF, Daigle K. 1997. Use of enzymes for the separation of protein from rice flour. *Cereal Chem* 74(4):437–41.
- Shull JM, Watterson JJ, Kirleis AW. 1992. Purification and immunocytochemical localization of kafirins in *Sorghum bicolor* (L. Moench) endosperm. *Protoplasma* 171(1–2):64–74.
- Sikorski ZE. 2001. Chemical and functional properties of food proteins. Lancaster, Pa.: Technomic Publishing Co. Inc. 473 p.
- Suhendro EL, Kunez CF, McDonough CM, Rooney LW, Waniska RD. 2000. Cooking characteristics and quality of noodles from sorghum. *Cereal Chem* 77(2): 96–100.
- Swientek B. 2008. Eating by subtraction. *Food Technol* 62(10): 44–52.
- Taylor J, Taylor JRN, Dutton MF, de Kock S. 2005. Glacial acetic acid—a novel food-compatible solvent for kafirin extraction. *Cereal Chem* 82(5):485–7.
- Taylor J, Bean SR, Ioerger BP, Taylor JRN. 2007. Preferential binding of sorghum tannins with gamma-kafirin and the influence of tannin binding on kafirin digestibility and biodegradation. *J Cereal Sci* 46(1):22–31.
- Taylor JRN, Taylor JRN. 2002. Alleviation of the adverse effect of cooking on sorghum protein digestibility through fermentation in traditional African porridges. *Int J Food Sci Technol* 37(2):129–37.
- Taylor JRN, Novellie L, Liebenberg NVDW. 1984a. Sorghum protein body composition and ultrastructure. *Cereal Chem* 61(1):69–73.
- Taylor JRN, Schussler L, Van Der Walt WH. 1984b. Fractionation of proteins from low-tannin sorghum grain. *J Agric Food Chem* 32(1):149–54.
- Taylor JRN, Schober TJ, Bean SR. 2006. Novel food and non-food uses for sorghum and millets. *J Cereal Sci* 44(3):252–71.
- U.S. Grains Council: World sorghum production and trade [Internet]. Washington, D.C.: U.S. Grains Council; c2008. Available from: <http://www.grains.org/sorghum>. Accessed 2009 October 13.
- Vasanthan T, Yeung J, Hoover R. 2001. Dextrinization of starch in barley flours with thermostable alpha-amylase with extrusion cooking. *Starch* 53(12):616–22.
- Virupaksha TK, Sastry LVS. 1968. Studies on the protein content and amino acid composition of some varieties of grain sorghum. *J Agric Food Chem* 16(2): 199–203.
- Wang FC, Chung DS, Seib PA, Kim YS. 2000. Optimum steeping process for wet milling of sorghum. *Cereal Chem* 77(4):478–83.
- Wang Y, Tilley M, Bean S, Sun XS, Wang D. 2009. Comparison of methods for extracting kafirin proteins from sorghum distillers dried grains with solubles. *J Agric Food Chem* 57(18):8366–72.
- Wong JH, Lau T, Cai N, Singh J, Pedersen JF, Vensel WH, Hurkman WJ, Wilson JD, Lemaux PG, Buchanan BB. 2009. Digestibility of protein and starch in Sorghum (*Sorghum bicolor*) is linked to biochemical and structural features of grain endosperm. *J Cereal Sci*. 49(1):73–82.
- Wrigley CW, Bekes F. 2001. Cereal-grain proteins. In: Sikorski ZE, editor. *Chemical and functional properties of food proteins*. Lancaster, Pa.: Technomic Publishing Co. Inc. p 373–406.
- Wu YV. 1978. Protein concentrate from normal and high-lysine sorghums: preparation, composition and properties. *J Agric Food Chem* 26(2): 305–9.
- Wu X, Zhao R, Bean SR, Seib PA, McLaren JS, Madl RL, Tuinstra M, Lenz MC, Wang D. 2007. Factors impacting ethanol production from grain sorghum in the dry-grind process. *Cereal Chem* 84(2):130–6.
- Xie XJ, Seib PA. 2000. Laboratory procedure to wet-mill 100 g of grain sorghum into six fractions. *Cereal Chem* 77(3):392–5.
- Xie XJ, Seib PA. 2002. Laboratory wet-milling of grain sorghum with abbreviated steeping to give two products. *Starch-Starke* 54(5):169–78.
- Xu X. 2008. *In vitro* digestibility of starch in sorghum differing in endosperm hardness and flour particle size. [MSc thesis]. Manhattan, Kans.: Kansas State Univ. 61 p. Available from: <http://krex.k-state.edu>.
- Yang P, Seib PA. 1995. Low-input wet-milling of grain sorghum for readily accessible starch and animal feed. *Cereal Chem* 72(5):498–503.
- Yang P, Seib PA. 1996. Wet milling of grain sorghum using a short steeping period. *Cereal Chem* 73(6):751–5.
- Yousif NE, El Tinay AH. 2001. Effect of fermentation on sorghum protein fractions and *in vitro* protein digestibility. *Plant Foods Hum Nutr* 56(2):175–82.
- Zayas JF. 1997. *Functionality of proteins in food*. New York: Springer-Verlag. 373 p.
- Zhan X, Wang D, Bean SR, Mo X, Sun XS, Boyle D. 2006. Ethanol production from supercritical-fluid-extrusion cooked sorghum. *Ind Crop Prod* 23(3):304–10.
- Zhang GY, Hamaker BR. 1998. Low alpha-amylase starch digestibility of cooked sorghum flours and the effect of protein. *Cereal Chem* 75(5):710–3.
- Zhao R, Bean SR, Ioerger BP, Wang D, Boyle DL. 2008. Impact of mashing on sorghum proteins and its relationship to ethanol fermentation. *J Agric Food Chem* 56(3):946–53.
- Zipf RL, Anderson RA, Slotter RL. 1950. Wet milling of grain sorghum. *Cereal Chem* 27(6):463–76.