JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Properties of Heat-Treated Sorghum and Maize Meal and Their Prolamin Proteins

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The digestibility of sorghum protein is reduced when wet cooked. Size exclusion chromatography (SEC) together with other protein analytical techniques was applied to further elucidate the effects of cooking on the sorghum and maize and their prolamin proteins. Sorghum and maize meal and their respective tertiary butanol extracted kafirin and zein were wet heat treated by boiling or pressure cooking. As expected, the in vitro pepsin protein digestibility of sorghum meal and kafirin reduced with boiling and pressure cooking, whereas the decrease in maize meal and zein protein digestibility was much less. SDS-PAGE showed that the boiled and pressure-cooked kafirin was more polymerized than the corresponding zein preparations. SEC of kafirin also revealed a substantially increased high molecular weight peak with boiling and pressure cooking. In contrast, the high molecular weight peak was very small for control and wet heated treated zein. The highly polymerized kafirin occurs as a result of extensive disulfide bonding of kafirin monomers during cooking. Cooking sorghum meal and kafirin also resulted in a relative change in secondary structure from α-helical to β-sheet, as determined by Fourier transform infrared spectroscopy. The pepsin indigestible kafirin residues were mainly in β-sheet conformation. In contrast, the conformational changes were very small for cooked maize meal and zein. Disulfide bonds formed during heating cause polymeric kafirin formation and also promote realignment of kafirin into β-sheet structures. These conformational changes apparently cause the lower proteolysis susceptibility of kafirin.

KEYWORDS: Sorghum; maize; kafirin; zein; cooking; digestibility; size exclusion chromatography; electrophoresis; Fourier transform infrared; protein structure

INTRODUCTION

Wet-cooked non-tannin sorghum grain flour has lower protein digestibility compared to other major cereals, for example, maize (*1*), which is anatomically and chemically very similar. The lower protein digestibility of cooked sorghum has negative implications with regard to its use as a staple food and monogastric animal feed and in industrial uses such as brewing and bioethanol. Hence, there has been extensive research into the cause of cooked sorghum's lower protein digestibility. The most widely accepted theory is that it is as a result of polymerization of kafirins, the prolamin storage proteins of sorghum, through disulfide bonding (*2*), particularly involving the cysteine-rich β- and γ-kafirins (*3*). The main evidence in support of this theory is of two types. First, treatment of sorghum flour with reducing agents that break disulfide bonds, such as sodium bisulfite, L-cysteine, dithiotheitol, and 2-mercaptoethanol, improves the cooked protein digestibility of sorghum (*4*). Second, there is direct evidence that cooking sorghum and also kafirin itself causes an increase in the proportion of kafirin dimers and oligomers (*5*−*7*) and even high molecular weight polymers (*6*), as seen by SDS-PAGE. According to Duodu et al. (*5*) kafirin polymerization on cooking is more extensive than occurs with zein, the maize prolamin. This did not seem to be the case in the work of Nunes et al. (*6*), although these workers observed the formation of a M_r 66K prolamin oligomer on cooking sorghum but not maize. Furthermore, it has been proposed (*8*) that prolamin polymers of M_r >100K are formed in sorghum and maize on cooking. This was based on the fact that with SDS-PAGE there was a large increase in prolamin oligomers and monomers when flours were cooked in the presence of 2-mercaptoethanol and an increase when samples were cooked under nonreducing conditions and then subsequently treated with 2-mercaptoethanol. However, as there was no analysis of uncooked samples, it was not possible to clearly ascertain whether there were differences in the degree of prolamin polymerization between sorghum and maize.

Thus, an important issue with regard to the correctness of the theory appears to be whether the kafirin does in fact polymerize more by disulfide bonding on cooking than zein. In the work reported here we have employed size exclusion chromatography (SEC) together with other protein analytical techniques to further elucidate the effects of cooking on the sorghum and maize prolamin structure.

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10.1021/jf802672e CCC: $40.75
Published on Web 01/14/2009

EXPERIMENTAL PROCEDURES

Materials. Grain of a mixture of two white non-tannin, tan plant sorghum cultivars PANNAR PEX 202 and 606 was decorticated with a rice pearler (Miag, Braunschweig, Germany) so that essentially all of the pericarp germ had been removed. The decorticated grain was then milled with a laboratory hammer mill to pass through an 800 μm opening screen. Commercial white maize (cultivar unknown) was purchased decorticated and degermed and was hammer milled as above.

Kafirin and zein prolamins were extracted from the sorghum and maize meals, respectively. The meals were first defatted three times using *n*-hexane at room temperature. Albumins and globulins and non-protein-nitrogen-containing compounds were then removed by extraction with 1.25 M NaCl (*9*). The residues were then washed with distilled water. This was followed by extraction of the kafirin and zein using aqueous tertiary butanol (60% w/w) for 1 h at room temperature (1 part of flour to 5 parts of solvent on a weight basis). The extraction was repeated three times, and the extracts were recovered by centrifugation. Part of the solvent was evaporated off at room temperature overnight in a fume hood. The pH was then adjusted to 4.5 with 1 M HCl to precipitate the prolamins. The slurry was then filtered and freeze-dried. The protein contents (N × 6.25) of the kafirin and zein preparations were 89 and 87% (as is basis), respectively.

The extraction of prolamin was done specifically under nonreducing conditions to extract the protein in its native state from the endosperm. If a reducing agent had been included in the extract, the kafirin would have been broken down into its component polypeptides. The yield of extracted prolamin without a reducing agent was about 35% for both extracted zein and kafirin. Thus, the kafirin and zein samples represented a fraction of the total endosperm prolamin protein.

Other chemicals and reagents used in this work were of analytical grade.

Cooking Treatments. One gram samples of sorghum and maize meals, kafirin, and zein were suspended in 5 mL of distilled water. The samples were then placed in a boiling water bath (96 °C) for 30 min or pressure cooked (110 °C) for 10 min. The controls were not cooked. After cooking, the samples were freeze-dried.

Analyses. *In Vitro Protein Digestibility.* In vitro protein digestibility was assayed according to the pepsin method (*10*) as modified by Duodu et al. (*5*). Two hundred milligram samples of the sorghum and maize meals and 50 mg samples of the prolamins were digested with porcine pepsin (Sigma P-7000). The undigested residue was collected by centrifugation (5000*g*) for 10 min, dried, and weighed. Nitrogen was determined by combustion analysis. Protein digestibility was expressed as the difference between the nitrogen content of the sample and the residual nitrogen content after pepsin digestion, divided by the nitrogen content of the sample. The indigestible residue from digestion of kafirin was also collected for further analyses.

Size Exclusion Chromatography. SEC was performed on kafirin, zein, and indigestible kafirin residue. One hundred milligram samples were dissolved in 1.5 mL of solvent. The solvent consisted of urea and potassium phosphate buffer (pH 3) dissolved into a liquid mixture of ethanol, water, and lactic acid at a concentration of 5:2:3 (w/w), respectively. The final concentration of urea was 1 M, and phosphate buffer was 0.02 M. To aid solvation, the samples were sonicated with a probe sonicator (Microson, Misonix Inc., New York) in an ice bath for 2 min using a power of 4 W. The combination of ethanol, water, and lactic acid has been used to dissolve kafirin protein at 70 °C for film production (*11*). Several solvents were experimented with to dissolve kafirin. This solvent system resulted in a negligible amount of pellet after centrifugation, showing that virtually all of the kafirin was solubilized. The other solvent systems tried did not have lactic acid or had lower lactic acid content and no urea. These solvents were not so effective as they left a large pellet after centrifugation, indicating that little kafirin was solubilized. The samples were then loaded on a SEC column (1 cm internal diameter × 65 cm long) containing Superose 6B (Pharmacia Fine Chemicals, Uppsala, Sweden). The samples were eluted with the same solvent at a rate of 2 mL/h at room temperature (23 °C). The eluent containing the fractionated proteins was collected in 2 mL aliquot fractions. The fractions were then analyzed at 280 nm to obtain a chromatographic profile. The wavelength 280 nm was chosen on the basis of a wavelength scan done on the kafirin and zein samples.

Table 1. Effects of Different Heat Treatments on the Digestibility of Sorghum and Maize Meal

treatment[a]	digestibility[b] (%)	mean reduction in digestibility (%)
	Sorghum	
control	82.6 (1.52) b	
boiled	59.9 (5.36) a	27
pressure cooked	56.7 (6.89) a	33
	Maize	
control	89.1 (0.41) c	
boiled	79.9 (2.97) b	10
pressure cooked	69.8 (2.69) a	21

[a] Boiled means heat treated in a boiling water bath for 30 min and pressure cooked for 10 min. [b] Average values with standard deviation in parentheses. Different letters show significant differences at $P < 0.05$ for sorghum and maize meals separately.

The peak fractions were diluted to 10 mL with distilled water and adjusted to pH 4.5 with 1 M HCl, and then the solvent was evaporated at room temperature overnight in a fume hood to concentrate the protein. This was then suspended in distilled water (4 °C) and then centrifuged at 7000*g* at 4 °C. The pellet was then freeze-dried and analyzed by SDS-PAGE.

SDS PAGE. SDS-PAGE under nonreducing and reducing conditions was performed on kafirin, zein, and the collected peak fractions from SEC. A Nupage Bis-Tris mini 4−12% gradient gel (separation range of 1.5−300K) (Invitrogen Life Technologies, Carlsbad, CA) was used. Loading was approximately 10 μg of protein. Protein molecular markers 14.4−97.2 $M_r \times 10^3$ (catalog no. 1 495 984, Roche Molecular Biochemicals, Indianapolis, IN) was used. Electrophoresis was carried out at 220 V with a current of 80 mA. The gels were stained with Coomassie Blue R-250, destained, and scanned.

Fourier Transform Infrared Spectroscopy (FTIR). All samples were stored in a desiccator containing silica gel for at least 48 h to ensure minimal moisture before spectroscopic analysis. Cooked and control sorghum and maize meals, cooked and control kafirin and zein, and indigestible kafirin residue after pepsin digestion were analyzed. FTIR was performed using a Perkin-Elmer Spectrum GX FTIR System (Waltham, MS) equipped with attenuated total reflectance (ATR) crystal. The sample was placed on the ATR crystal (zinc selenide), covering the crystal surface. The sample was gently squeezed by a screw for contact with the crystal. The spectra (64 scans at 8 cm^{-1} resolution with an interval of 1 cm^{-1}) were collected at a frequency range 4000−600 cm^{-1}. The empty crystal was used as background. The FTIR spectra were Fourier deconvoluted using a resolution enhancement factor of 1.5 and a full-height bandwidth of 15 cm^{-1}.

Statistical Analysis. Unless otherwise stated, mean values were obtained from three independent experiments. One-way analysis of variance (ANOVA) was performed on the data (dependent variables) to determine the effect of different cooking treatments (as independent variables). The least significance difference test was performed at $P < 0.05$.

RESULTS AND DISCUSSION

Pepsin Digestibility of Endosperm Meals and Extracted Kafirin and Zein. As has been extensively reported (*2*), wet cooking sorghum endosperm flour resulted in a greater reduction in in vitro protein digestibility than wet cooking maize flour (**Table 1**). Interestingly, there was no significant difference ($P \geq 0.05$) in the protein digestibility of boiled and pressure-cooked sorghum flour, whereas the pressure-cooked maize flour gave lower protein digestibility. This can be attributed to the greater disruption of the maize endosperm protein matrix compared to the sorghum protein matrix that occurs on pressure cooking (*12*). Increased disruption results in greater digestibility.

In the case of the aqueous *tert*-butanol-isolated prolamins (without a reducing agent), the digestibility of the isolated

Table 2. Effects of Different Heat Treatments on the Digestibility-Extracted[a] Kafirin and Zein

treatment[b]	digestibility[c] (%)	mean reduction in digestibility (%)
	Kafirin	
control	76.0 (4.8) c	
boiled	57.6 (1.6) a	24
pressure cooked	64.3 (1.6) b	15
	Zein	
control	98.7 (0.22) b	
boiled	93.9 (3.65) a	5
pressure cooked	95.4 (1.98) ab	3

[a] Kafirin and zein were extracted with aqueous tertiary butanol at room temperature. [b] Boiled means heat treated in a boiling water bath for 30 min and pressure cooked for 10 min. [c] Average values with standard deviations in parentheses. Different letters show significant differences at $P < 0.05$ for kafirin and zein separately.

untreated kafirin was very much lower than that of the corresponding zein (also extracted with *tert*-butanol without a reducing agent) (**Table 2**). This finding is consistent with the disulfide bonding theory, as it has been shown that kafirins isolated in the absence of reducing agent, as in this work, are polymerized by disulfide bonding to a considerable extent (*13*). The table also shows that the protein digestibility of the isolated kafirin decreased with boiling to a much greater extent than the zein. Surprisingly, in view of the amount of research into sorghum protein digestibility, this finding seems to be novel. Up until now, workers have cooked the flours and then isolated the kafirin and zein, for example, Nunes et al. (*6*), or analyzed endosperm protein concentrates (*8*) or isolated protein body preparations (*5*), which contain other proteins in addition to the prolamins. It is also important to note that the results (**Table 2**) are for kafirin and zein extracted with tertiary butanol without a reducing agent and not for the whole endosperm prolamin. Notwithstanding this, El Nour et al. showed that this solvent extracts monomeric and disulfide linked kafirin polymers of M_r 18 to >100K.

SDS-PAGE of Isolated Cooked Kafirin and Zein. SDS-PAGE under nonreducing conditions (**Figure 1a**) of the untreated and cooked kafirin (lanes 1−3 show that the predominant bands were in the region of 42−48 M_r. These have been generally identified variously as kafirin dimers (*5*, *13*). There were also some bands of <26 M_r, which are the kafirin monomers, and faint bands of M_r around 66 and 98K and higher, which are probably kafirin trimers and tetramers. In contrast, with zein (lanes 4−6), the concentrations of the protein bands was more even, but also appearing in groups: $M_r \leq 24$ (zein monomers) (*14*), M_r 39−45 (probably zein dimers), M_r 66−70 (probably trimers), $M_r \geq 97$ k zein (probably tetramers and larger oligomers). There were different effects on the kafirin and zein proteins with cooking. Boiling the kafirin (lane 2) resulted in a significant general reduction in band intensity, and pressure cooking (lane 3) resulted in a dramatic reduction in band intensity. In contrast, neither boiling (lane 5) nor pressure cooking (lane 6) had a substantial effect on zein band intensity.

SDS-PAGE under reducing conditions (**Figure 1b**) of the kafirin and zein samples gave a dramatically different band pattern from nonreducing conditions. With both the kafirin and zein samples, the predominant bands were the monomers. This shows clearly that the bands of higher M_r seen under nonreducing conditions were essentially all disulfide bond cross-linked kafirin and zein oligomers, as was found by El Nour et al. (*13*) working with kafirin. **Figure 1b** also shows that there was no difference in kafirin band intensity between the untreated, boiled, and pressure-cooked kafirin (lanes 1−3), in contrast to the situation under nonreducing conditions (**Figure 1a**, lanes 1−3). This finding indicates that with cooking, a proportion of the kafirin monomers was polymerized by disulfide bonding into polymers of M_r >200K, such that they were too large to enter the polyacryamide gel (**Figure 1a**, lanes 2−3). This did not seem to be the case with zein, as there was no difference in band intensity between the cooked and uncooked samples (**Figure 1a**, lanes 4−6). The results further indicate that the degree of the kafirin polymerization was related to cooking energy, with pressure cooking resulting in greater polymerization than boiling.

In addition to the monomeric prolamins observed with SDS-PAGE under reducing conditions (**Figure 1b**) there were also very faint bands of higher M_r. There existence suggests that these oligomers were resistant to reduction, possibly because of nonaccessibility of the disulfide bonds or other modes of polymerization (*5*).

SEC of Cooked Zein and Kafirin. SEC was performed only on isolated kafirin and zein that had been subjected to the various treatments and not on the meals. Extracts of the meals were too impure to give good chromatograms. SEC of untreated kafirin showed three peaks, peak 1 at fractions 8−11, peak 2 at 15−20, and peak 3 at 25−30, and a shoulder at fractions 12−14 (**Figure 2a**). With SEC, molecular weight decreases with elution time (fraction number). The peak area ratio of peak 1 and shoulder to peak 2 was approximately 25:75 for control kafirin. This ratio changed to 45:55 and 41:59 for peak 1/peak 2 for boiled and pressure cooking, respectively. Thus, although boiled and pressure-cooked kafirin showed the same peaks as the control, peak 1 (high molecular weight material) increased dramatically in area and peak 2 (lower molecular weight material) decreased dramatically in area and peak 3 changed to a much lesser extent. This indicates that with boiling and pressure cooking there was extensive polymerization of the kafirin. With untreated zein the high molecular fraction (indicated by shoulder) was very low, only resolving into a shoulder (**Figure 2b**). The estimated area ratio under the shoulder to peak 2 for untreated zein was about 2:98. For boiling and pressure cooking, the area under the shoulder/or peak 1 to peak 2 was 6:94 and 10:90, respectively. These values first indicate that the extracted and untreated zein was much less polymerized than the kafirin. Second, when zein was heat treated in boiling water and pressure cooked, the degree of polymerization was very much less than with kafirin.

Similar SEC results have been reported to occur for extracted protein from rice that had been flaked (*15*). It was found that there was an increase in the first elution peak as detected at 280 nm and a decrease in the second elution peak for extracted protein from flaked rice in comparison to raw rice, which was attributed to polymerized prolamin protein through aggregation by disulfide bonding. These polymerized proteins were resistant to proteolysis by trypsin.

SDS-PAGE of Kafirin and Zein SEC Fractions. Peak 1, the shoulder between the peaks (where sufficient material was available) and peak 2 were recovered by precipitation. For reasons unknown, peak 3 did not precipitate at pH 4.5 and thus could not be recovered for further analysis. With SDS-PAGE under nonreducing conditions (**Figure 3a**), all of the kafirin peaks from the untreated, cooked, and pressure-cooked treatments gave essentially the same band pattern, although the intensity of the protein bands in peak 1 was considerably lower (lanes 2, 5, and 7). This suggests that this high molecular weight protein peak comprised mostly extensively polymerized kafirin proteins, with a very low proportion of oligomers and mono-

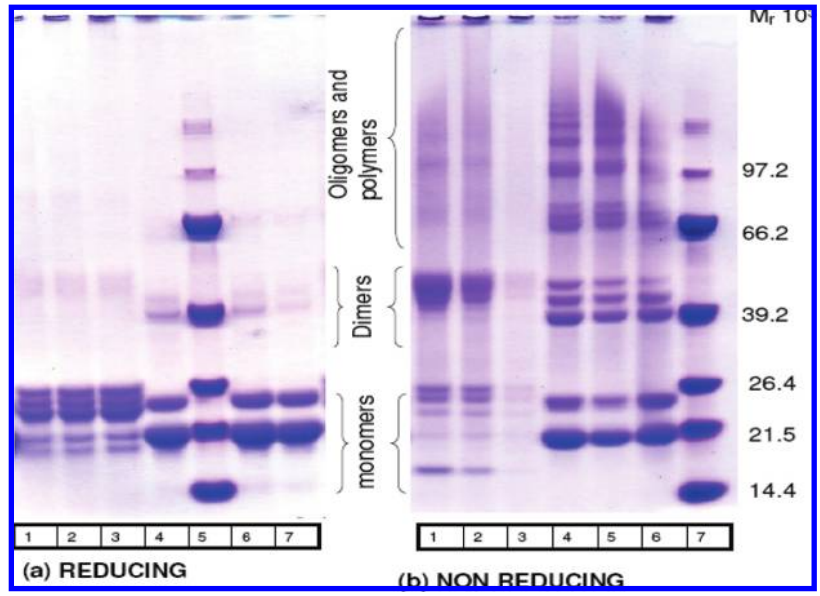

Figure 1. SDS-PAGE of heat-treated kafirin and zein under reducing and nonreducing conditions. Under reducing condition lanes 1−7 are, respectively, kafirin control, kafirin boiled, kafirin pressure cooked, zein control, molecular weight markers, zein boiled, and zein pressure cooked. Under nonreducing condition lanes 1−7 are, respectively, kafirin control, kafirin boiled, kafirin pressure cooked, zein control, zein boiled, zein pressure cooked, and molecular weight markers.

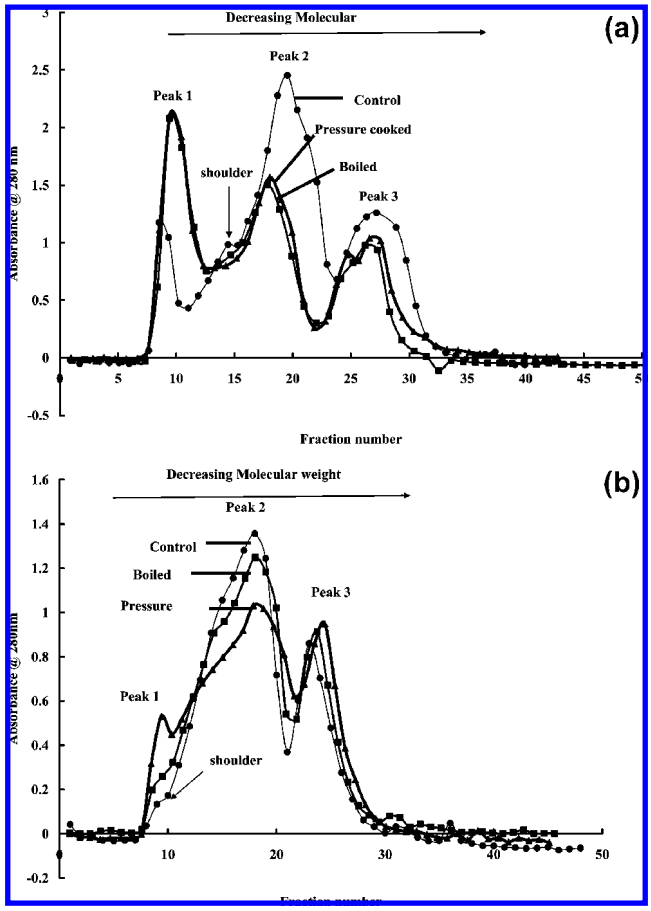

Figure 2. Size exclusion chromatogram of heat-treated kafirin (**a**) and zein (**b**) protein (thin line, control; medium weight line, boiled; heavy line, pressure cooked).

mers. The situation appeared to be slightly different with the zein SEC fractions. Peak 1 from all of the treatments (bands 10, 12, and 14) showed a relatively higher intensity of poorly resolved bands of high molecular weight (M_r > 97K < 200K). This suggests that the zein proteins eluted at peak 1 were somewhat less polymerized than the corresponding kafirin proteins.

Under reducing conditions (**Figure 3b**) virtually all of the kafirin and zein proteins from the SEC peaks were resolved as monomers and dimers, confirming that the proteins were originally polymerized by disulfide bonding.

FTIR of Sorghum, Maize Meals, and Their Extracted Prolamin Proteins. Deconvoluted FTIR spectra of sorghum meal from the frequency range of 1750−1450 cm^{-1} showed two main regions (**Figure 4**). The frequency range from about 1700−1600 cm^{-1} has been identified as the amide I region and that from 1575−1475 cm^{-1} as amide II for kafirin (*7*, *12*, *16*, *17*) and zein (*16*). Sorghum and maize meals showed a single peak in the amide I region at 1650 cm^{-1} and a peak at about 1540 cm^{-1} (**Figure 4a**). The peaks at 1650 and 1545 cm^{-1} have been characterized as indicating α-helical structures (*7*, *12*, *16*, *17*). When sorghum flour was wet cooked by boiling and under pressure, an additional peak at 1625 cm^{-1} was observed (Figure **4a**). This peak has been characterized as β-sheet structures (*7*, *12*, *16*, *17*). There was also a shift in the peak from 1545 to 1525 cm^{-1} in the amide II region with wet cooking in boiling water and cooking under pressure. This also indicates the formation of β-sheet structures (*16*). There were no major changes in the deconvoluted spectra when maize meal was wet cooked in boiling water and cooked under pressure (**Figure 4b**). However, a small shoulder at 1625 cm^{-1}, indicative of some β-sheet structure, occurred as a result of wet cooking (both boiling and pressure cooking).

The control kafirin had one main peak at 1650 cm^{-1} with a shoulder at 1625 cm^{-1} in the amide I region (**Figure 4c**). In the amide II region, untreated kafirin showed a peak at 1545 cm^{-1} and a shoulder at about 1520 cm^{-1}. When the kafirin was wet cooked in boiling water, a peak at 1625 cm^{-1} indicative of β-sheet structures was formed. Pressure cooking also resulted in a peak at 1625 cm^{-1}, but the intensity of the peak was lower than when boiled. The peaks of wet cooked in boiling water and pressure-cooked kafirin only showed peak broadening in the amide II region. There were no significant changes in heat-

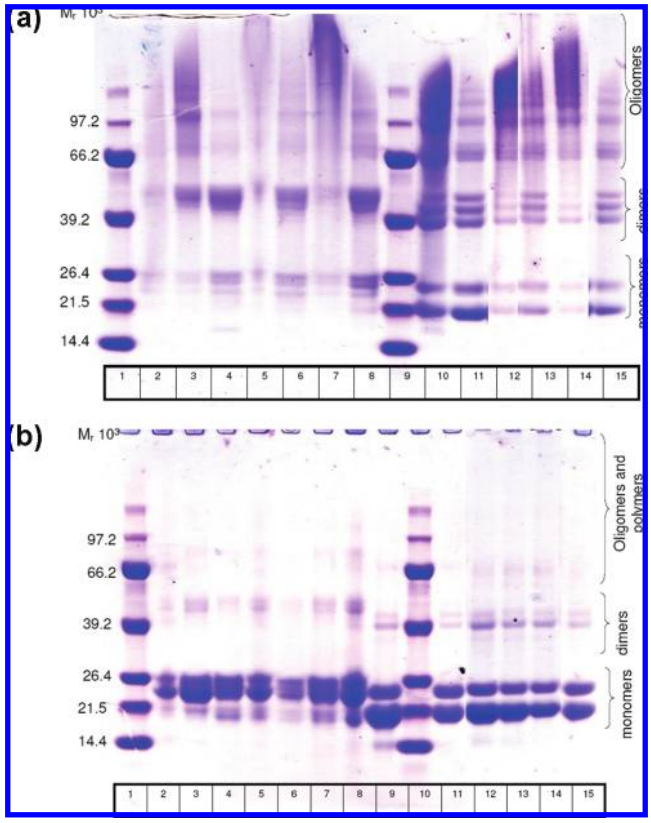

Figure 3. SDS-PAGE of the peaks from SEC of heat-treated kafirin and zein proteins. Under nonreducing condition lanes 1−15 are, respectively, molecular weight marker, kafirin control peak 1, kafirin control shoulder (before peak 2), kafirin control peak 2, kafirin boiled peak 1, kafirin boiled peak 2, kafirin pressure cooked peak 1, kafirin pressure cooked peak 2, zein control peak, molecular weight marker, zein control shoulder (at position of peak 1), zein boiled peak 1, zein boiled peak 2, zein pressure cooked peak 1, and zein pressure cooked peak 2. Under reducing condition lanes 1−15 are, respectively, molecular weight marker, kafirin control peak 1, kafirin control shoulder (before peak 2), kafirin control peak 2, kafirin boiled peak 1, kafirin boiled peak 2, kafirin pressure cooked peak 1, kafirin pressure cooked peak 2, zein control peak 2, molecular weight marker, zein control shoulder (at position of peak 1), zein boiled peak 1, zein boiled peak 2, zein pressure cooked peak 1, and zein pressure cooked peak 2.

treated zein protein in comparison to untreated in the amide I region (**Figure 4d**). In the amide II region, pressure-cooked zein showed a shoulder broadening at 1525 cm^{-1}, possibly indicative of some β-sheet structures, in comparison to untreated and boiled kafirin.

The formation of β-sheet structures of kafirin and sorghum meal proteins as a result of wet cooking is in agreement with the literature (*7, 12, 16, 17*). The formation of β-sheet structures has been suggested to be one of the causes of reduced sorghum protein digestibility during wet cooking (*16*). This is because, in zein, far less or even insignificant β-sheet structures are formed during wet cooking. The change in secondary structure from α-helical to β-sheet structures in kafirin may have been a cause of the reduced susceptibility to proteolysis by pepsin. The peak intensity at 1625 cm^{-1} indicative of β-sheet structures was less for pressure cooking in comparison to boiled kafirin (**Figure 4c**). The digestibility of pressure-cooked kafirin was also significantly ($P < 0.05$) higher than that of boiled kafirin (64.3 compared to 57.6%, **Table 2**). This seems to indicate an inverse relationship between kafirin digestibility and β-sheet formation.

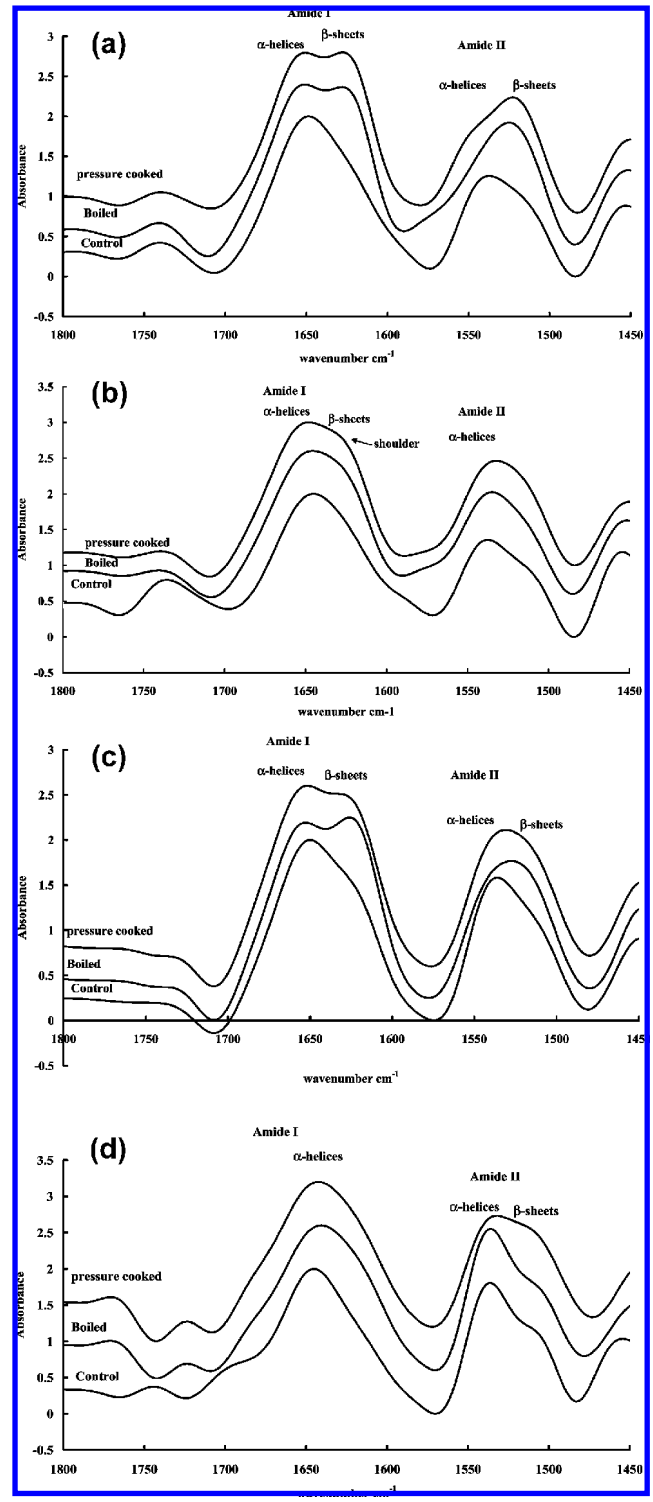

Figure 4. Deconvoluted FTIR spectra of heat-treated sorghum (**a**) and maize (**b**) meal and their respective extracted heat treated kafirin (**c**) and zein (**d**).

Thus, kafirin with a higher ratio of β-sheet to α-helical structure will probably have lower digestibility than that with less β-sheet structure.

FTIR of Residual Kafirin after Pepsin Digestion. FTIR was not done on the zein indigestible residue as negligible residues were collected after zein digestion on account of its high digestibility. FTIR of indigestible residue of untreated, boiled, and pressure-cooked kafirin showed amide I (1700−1600 cm^{-1}) and amide II (1575−1475 cm^{-1}) bands (**Figure 5**). The amide I indigestible residues of the wet heat treated by boiling

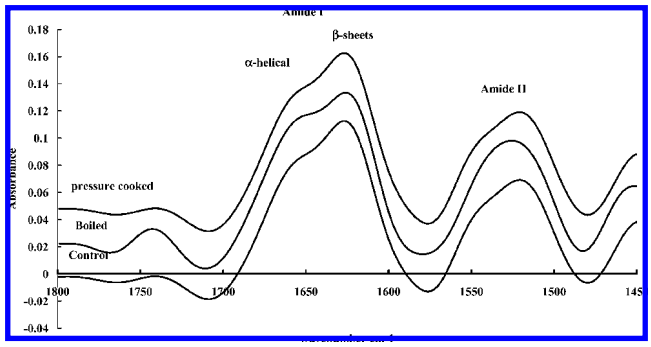

Figure 5. Deconvoluted FTIR of heat-treated indigestible kafirin residues.

and pressure cooking and untreated showed predominantly β-sheet instead of α-helices. The peak ratio between β-sheet and α-helices also seems to be similar between untreated and wet heat treated (boiling and pressure cooking) kafirin indigestible residues. This appears to confirm that β-sheet structures are less susceptible to proteolysis by pepsin than α-helices. During wet cooking, it has been reported that kafirin can denature to form random coils, and subsequent cooling favors the β-sheet structures (*7*).

Conclusions. When sorghum protein is wet cooked, the kafirin protein extracted with aqueous tertiary butanol under nonreducing conditions can polymerize to form high molecular weight polymers through disulfide bonding. There is also a secondary protein conformational change in the kafirin from α-helical to β-sheet structures. These changes occur minimally with tertiary butanol extracted zein. Thus, the changes appear to be the causes of the decrease in the protein digestibility of sorghum when cooked. However, as these observed bahaviors are only for the one sorghum and maize variety, this needs to be confirmed in a wider study. In addition, as the findings are for the prolamin species extracted in the absence of reducing agent, the implications with respect to the unextractable prolamin fractions still need to be determined.

ABBREVIATIONS USED

FTIR, Fourier transform infrared; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; SEC, size exclusion chromatography; ATR, attenuated total reflectance; M_r, relative molecular weight.

ACKNOWLEDGMENT

We acknowledge the Industrial Metals and Minerals Research Institute (IMMRI, University of Pretoria) for allowing us to use the FTIR equipment and the Department of Biochemistry (University of Pretoria) for allowing us to use the SEC equipment.

LITERATURE CITED

(1) Mertz, E. T.; Hassen, M. M.; Cairns-Whittern, Kirkleis, A. W.; Tu, L.; Axtell, J. D. Pepsin digestibility of proteins in sorghum and other major cereals. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1–2.

(2) Duodu, K. G.; Taylor, J. R. N.; Belton, P. S.; Hamaker, B. R. Factors affecting sorghum protein digestibility. *J. Cereal Sci.* **2003**, *38*, 117–131.

(3) Oria, M. P.; Hamaker, B. R.; Schull, J. M. Resistance of sorghum α-, β-, and γ-kafirins to pepsin digestion. *J. Agric. Food Chem.* **1995**, *43*, 2148–2153.

(4) Hamaker, B. R.; Kirkleis, A. W.; Butler, L. G.; Axtell, J. D.; Mertz, E. T. Improving the *in vitro* protein digestibility of sorghum with reducing agents. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 626–628.

(5) Duodu, K. G.; Nunes, A.; Delgadillo, I.; Parker, M. L.; Mills, E. N. C.; Belton, P. S.; Taylor, J. R. N. Effect of grain structure and cooking on sorghum and maize *in vitro* protein digestibility. *J. Cereal Sci.* **2002**, *35*, 161–174.

(6) Nunes, A.; Correia, I.; Barros, A.; Delgadillo, I. Characterisation of kafirin and zein oligomers by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Agric. Food Chem.* **2005**, *53*, 639–643.

(7) Byaruhanga, Y. B.; Emmambux, M. N.; Belton, P. S.; Wellner, N.; Ng, K. G.; Taylor, J. R. N. Alteration of kafirin and kafirin film structure by heating with microwave energy and tannin complexation. *J. Agric. Food Chem.* **2006**, *54*, 4198–4207.

(8) Ezeogu, L. I.; Duodu, K. G.; Taylor, J. R. N. Effects of endosperm texture and cooking conditions on the in vitro starch digestibility of sorghum and maize flours. *J. Cereal Sci.* **2005**, *42*, 33–44.

(9) Taylor, J. R. N.; Schussler, L.; Van Der Walt, W. H. Fractionation of proteins from low tannin sorghum grain. *J. Agric. Food Chem.* **1984**, *32*, 149–154.

(10) Hamaker, B. R.; Kirleis, A. W.; Butler, L. G.; Axtell, J. D.; Mertz, E. T. Effect of cooking on the protein profiles and in vitro digestibility of sorghum. *J. Agric. Food Chem.* **1986**, *34*, 647–649.

(11) Emmambux, M. N.; Stading, M.; Taylor, J. R. N. Kafirin film property modification with tannins. *J. Cereal Sci.* **2004**, *40*, 127–135.

(12) Ezeogu, L. I.; Duodu, K. G.; Emmambux, M. N.; Taylor, J. R. N. Influence of cooking conditions on the protein matrix of sorghum and maize floury and vitreous endosperm flours. *Cereal Chem.* **2008**, *85*, 397–402.

(13) El-Nour, I. N. A.; Peruffo, A. D.; Curioni, A. Characterization of sorghum kafirin in relation to their cross-linking behaviour. *J. Cereal Sci.* **1998**, *28*, 197–207.

(14) Esen, A. A proposed nomenclature for the alcohol-soluble proteins (zeins) of maize (*Zea mays L.*). *J. Cereal Sci.* **1987**, *52*, 218–219.

(15) Mujoo, R.; Chandrashekar, A.; Zakiuddin, S. A. Rice protein aggregation during the flaking process. *J. Cereal Sci.* **1998**, *28*, 187–195.

(16) Duodu, K. G.; Tang, H.; Grant, A.; Wellner, N.; Belton, P. S.; Taylor, J. R. N. FT-IR and solid state ^{13}C NMR spectroscopy of proteins of wet cooked and popped sorghum and maize. *J. Cereal Sci.* **2001**, *33*, 261–269.

(17) Gao, C.; Taylor, J.; Wellner, N.; Byaruhanga, Y. B.; Parker, M. L.; Mills, E. N. C.; Belton, P. S. Effect of preparation conditions on protein secondary structure on biofilm formation of kafirin. *J. Agric. Food Chem.* **2005**, *53*, 306–312.

Received for review August 29, 2008. Revised manuscript received November 21, 2008. Accepted December 4, 2008. We acknowledge University of Pretoria and the National Research Foundation of South Africa for funding the research.

JF802672E