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Effect of Preparation Conditions on Protein Secondary Structure and Biofilm Formation of Kafirin

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Various extraction and drying conditions for the isolation of kafirin from dry-milled, whole grain sorghum have been investigated, with a view to optimizing extraction of the protein for commercial food coatings and packaging films. The addition of sodium hydroxide to an aqueous ethanol extractant increased the yield and solubility of kafirin. Subsequent heat drying at 40 °C was shown to cause the kafirin to aggregate as indicated by an increase in intermolecular β-sheets. Extraction of the flour using ethanol (70%, w/w) with 0.5% (w/w) sodium metabisulfite and 0.35% (w/w) sodium hydroxide at 70 °C followed by freeze-drying of the protein was found to produce a yield of 54% kafirin with good film-forming properties. The kafirin films were assessed for their sensory properties, tensile strength, strain, and water vapor permeability. Fourier transform infrared spectroscopy was used to study the secondary structure of the extracted kafirins. The best films were made with kafirin containing a large proportion of nativelike α-helical structures with little intermolecular β-sheet content as indicated by the Fourier transform infrared reflectance peak intensity ratios associated with these secondary structures. The principal factor affecting the secondary structure of the protein appeared to be the temperature at which the protein was dried. Heat drying resulted in a greater proportion of intermolecular β-sheets. Any industrial-scale extraction must therefore minimize protein aggregation and maximize native α-helical structures to achieve optimal film quality.

KEYWORDS: Kafirin; biofilm; sorghum; protein secondary structure; FT-IR

INTRODUCTION

Interest and research activity in the development of edible and biodegradable protein films from renewable natural resources have increased in recent years due to growing environmental concerns and waste disposal costs (*1*−*3*). In general, protein films and coatings have lower oxygen permeabilities than polyethylene films, which make them good barriers for oxygen sensitive products; they tend to have lower tensile strengths and elongation values than synthetic films, but these are generally sufficient to allow usage for wraps, pouches, and coatings (*4*). Protein films normally have higher water vapor permeabilities (WVP) than edible wax coatings and low-density polyethylene packaging films.

Zein, the prolamin protein from maize, is an unusual protein because it is soluble only in aqueous alcohols. Biofilms made from zein are not water soluble but relatively transparent and grease resistant; they have the lowest WVP values observed for protein films, comparable to those of cellulose derivatives. Zein films have been comprehensively studied and also used commercially for food coating and packaging (*1*, *4*−*7*). The sorghum prolamin protein, kafirin, is similar to zein (*8*). On the basis of sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) and solubility differences, kafirin polypeptides can be classified into α- ($M_r = 23$ and 25×10^3), β- ($M_r = 16$, 18, and 20×10^3), and γ-kafirin ($M_r = 28 \times 10^3$) (*9*). These are equivalent to the zein polypetides, α- ($M_r = 18$ and $21-25 \times 10^3$), β- ($M_r = 17$ and 18×10^3), and γ-zein ($M_r = 27 \times 10^3$) as described by Esen (*10*). Kafirin is more difficult to solubilize than zein (*11*). Zein is readily extracted with 55% (v/v) 2-propanol with or without a reducing agent (*12*). Whereas a less polar solvent, 60% (v/v) tertiary butanol with a reducing agent is required to extract kafirin (*13*). These solubility differences are due to kafirin being more hydrophobic (*14*) and more disulfide cross-linked (*15*, *16*) than zein. Fourier transform infrared reflectance (FT-IR) studies have shown that both kafirin and zein contain high levels of α-helices and possibly some unordered structures as well as some β-sheet structures (*17*). Kafirin has the potential to form films with superior gas and water vapor barrier characteristics. Like zein, kafirin is plant

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based, which is important for religious considerations, and it is not known to be allergenic, unlike wheat and soy protein (*18*). The specific advantage for Southern Africa is that a kafirin-based material could be a value-added product manufactured from waste from the sorghum milling and brewing industry. Kafirin could be a substitute for zein in biofilm production, but only one laboratory-scale study on kafirin films has been published (*19*).

Our objective was to find a method for extracting kafirin suitable for industrial use from dry-milled sorghum. It has been reported that aqueous *tert*-butyl alcohol with dithiothreitol (DTT) is the most efficient extractant for extracting kafirin (*13*). However, both *tert*-butyl alcohol and DTT are expensive chemicals and not food compatible, making them unsuitable for commercial production of kafirin where the intended end use is for food coatings. The effectiveness of extractants consisting of aqueous ethanol containing the food compatible reducing reagent, sodium metabisulfite, with or without sodium hydroxide was investigated and compared with *tert*-butyl alcohol extraction. FT-IR spectroscopy was used to investigate changes in kafirin structure during extraction and drying. The kafirin films were assessed for their sensory properties, tensile strength, strain, and WVP. The relationship between the protein secondary structures and their film-forming properties was explored.

MATERIALS AND METHODS

Materials. A mixture of two condensed tannin-free white sorghum cultivars (PANNAR 202 and 606, ex. B. Koekemoer, Lichtenburg, South Africa, 2001) was used. Clean whole grain was milled using a laboratory hammer mill (Falling Number, Huddinge, Sweden) fitted with an 800 μm opening screen. Commercial zein (Z3625), tertiary butanol, ethanol (absolute), sodium hydroxide, DTT, sodium metabisulfite, glycerol, lactic acid, and poly(ethylene glycol) (PEG-400) were of analytical reagent grade and obtained from Sigma Aldrich (United Kingdom or South Africa).

Preparation of Kafirin by Ethanol Extraction with or without Sodium Hydroxide. Kafirin was extracted from sorghum flour using a modification (*20*) of the method of Carter and Reck (*21*). Sorghum flour was extracted for 1 h with 70% (w/w) aqueous ethanol containing 0.5% (w/w) sodium metabisulfite, either with or without 0.35% (w/w) sodium hydroxide at 70 °C under constant stirring. The extract was separated by centrifugation at 3000 rpm (1000*g*) for 5 min, and the solvent was allowed to evaporate from the supernatant overnight at ambient temperature from shallow open trays placed in a fume cupboard. The protein was then washed with a minimal amount of cold (<10 °C) distilled water and adjusted to approximately pH 5 to precipitate out the protein. The protein was recovered by filtration before freeze drying or oven drying at 40 °C. The dried kafirin and commercial zein were defatted with hexane at ambient temperature at a protein-to-solvent ratio of 1:10 (w/w). All of the defatted protein preparations were characterized by amino acid analysis (data not shown) and by SDS−PAGE and were found to have an amino acid composition (*22*) and SDS−PAGE band pattern consistent with that of total kafirin (*15*).

Preparation of Kafirin by *tert*-Butyl Alcohol Extraction. Albumins and globulins and nonprotein nitrogen-containing compounds were extracted first with sodium chloride (1.25 M), and then, the flour was washed with distilled water to remove the excess salt (*13*). A single extraction using *tert*-butyl alcohol (60%, w/w) containing 0.05% DTT (w/w) was then carried out on the flour for 1 h at ambient temperature (25 °C). The resulting extract was separated by centrifugation at 3000 rpm (1000*g*) for 5 min. The solvent was then allowed to evaporate, and the protein was recovered as described above. The extracted kafirin was freeze-dried or dried at 40 °C.

Determination of Protein. Protein was determined as total nitrogen using the Dumas total combustion method and converted to total protein using a conversion factor of 6.25. Percentage protein yield values for the extractions were calculated as weight of total recovered protein divided by the total amount of protein present in the grain multiplied by 100.

Characterization of Kafirin Proteins by SDS−PAGE. Protein preparations were subjected to SDS−PAGE under both reducing and nonreducing conditions. A Protean II (Bio-Rad, Hercules, CA) vertical electrophoresis system using gels of 140 mm length and 1.5 mm thick was used according to Gallager (*23*). Separating gel and stacking gel concentrations were 12 and 3.9% acrylamide, respectively, prepared from a stock of 40% (w/v) acrylamide/bis (19:1) (Merck, Halfway House, South Africa). The different protein preparations were loaded to constant protein (≈10 μg). Molecular weight markers (low-range protein marker, Roche Molecular Biochemicals, Indianapolis, IN) were used. Proteins were stained with Coomassie Brilliant Blue R250.

Dissolution Test. The dissolution of the extracted kafirin was measured in 70% (w/w) aqueous ethanol, and the solvent was used to prepare kafirin films. Ethanol (20 mL, 70%) was added to 0.5 g of kafirin powder, and the mixture was stirred and heated at 70 °C for 10 min and then filtered with Whatman filter paper under vacuum. The insoluble residues were dried to a constant weight in a ventilated oven at 100 °C. The calculation of solubility was based on the subtraction of the dried insoluble residues from the total weight of protein. The parameter measured is not the thermodynamic solubility but is an indication of how much material may be readily contained in the solvent system and thus is a useful practical guide.

Film Casting. Kafirin protein powder (1.2 g, protein basis) was weighed into a 100 mL Erlenmeyer flask. Ethanol (7.5 g, 70%, w/w), premixed with 0.5 g of plasticizers [similar to those used by Buffo et al. (*19*)], was added along with a magnetic stirrer bar. The total weight of the flask plus contents was recorded. The samples were heated to 70 °C and held for 10 min with rapid stirring, under condensation. After this time, the flask and contents were reweighed and absolute ethanol was added until the original weight was obtained (to replace the ethanol, which had evaporated). Aliquots (4 g) were weighed into 9 cm diameter plastic Petri dishes and gently swirled to coat the bottom of the dish. The Petri dishes were placed without lids on a level surface (checked with a spirit level) in an oven at 50 °C (not forced draught) overnight to evaporate the solvent.

FT-IR Spectroscopy. Spectra of kafirins (dry powder and films) were obtained on a FTS 175 Spectrometer (Bio-Rad, United Kingdom) using a Golden Gate Diamond Horizontal Attenuated Total Reflectance sampling device (Specac, United Kingdom). Spectra (128 scans at 2 cm^{-1} resolution) were collected within the frequency range of 4000−800 cm^{-1}. The empty crystal was used as the background. Fourier self-deconvolution was carried out with the spectrometer software with a *K* factor of 1.5 and a half width of 15 cm^{-1}. The ratios of peak intensity at 1650 and 1620 cm^{-1} were calculated to indicate different contents of α-helices and intermolecular β-sheets, respectively. The samples were dried over silica gel in a desiccator for 1 week before analysis.

Light Microscopy of Kafirin Powders. The powders were mixed with one drop of distilled water to make a paste. After 30 min, the paste was mixed with a small amount of molten low melting point agarose (TypeVII, A-4018, Sigma Aldrich), which was then solidified over ice and chopped into small pieces. These pieces were postfixed in 2% (w/v) aqueous osmium tetroxide for 2 h and then dehydrated through an ethanol series. After three washes in absolute ethanol, the pieces were transferred to acetone and then infiltrated and embedded in Spurr resin (Agar Scientific, Stansted, United Kingdom). Semi-thin sections, 1 μm thick, were cut with a glass knife and stained with 1% (w/v) toluidine blue in 1% (w/v) Borax, examined with an Olympus BX60 light microscope, and photographed.

Sensory Evaluation of Kafirin Films. Shortly after drying, the film properties, clarity, color, flexibility, surface texture, and odor were compared subjectively with those of films of commercial zein cast under the same conditions by a trained sensory panel.

Tensile Properties of Kafirin Films. A modified method based on ASTM D882-97 (*24*) was used. Films were not conditioned prior to analysis. Films were cut into strips, 60 mm × 6 mm, and the thickness was measured in five places along the length using a micrometer. Texture analysis was performed using a TA-XT2 Texture Analyzer (Stable Micro Systems, Goldalming, United Kingdom) using tensile grips coated with abrasive paper. The settings were as follows: force

Table 1. Characterization of Kafirins Prepared Using Different Extraction Processes

extractant[a]	extraction temp	drying conditions	yield[b] (%)	protein purity (db)	solubility[b] (%)	FT-IR (powder) α-helix:intermolecular β-sheet (1650 cm^{-1}:1620 cm^{-1} peak intensity ratio)
tB/DTT	RT[c]	freeze-dried	32 (3.7) a	82.4 (1.63) a	100 (0.1) d	1.39 (0.02) e
tB/DTT	RT[c]	40 °C			95 (1.6) c	0.90 (0.03) a
Et/$Na_2S_2O_5$/NaOH	70 °C	freeze-dried	54 (3.0) c	84.2 (2.43) a	95 (1.1) c	1.10 (0.01) d
Et/$Na_2S_2O_5$/NaOH	70 °C	40 °C			93 (0.2) c	0.96 (0.02) b
Et/$Na_2S_2O_5$	70 °C	freeze-dried	38 (0.8) b	83.4 (0.49) a	90 (1.2) b	1.00 (0.01) c
Et/$Na_2S_2O_5$	70 °C	40 °C			80 (2.0) a	0.90 (0.01) a

[a] tB/DTT = 60% *tert*-butyl alcohol + 0.05% DTT. Et/$Na_2S_2O_5$/NaOH = 70% ethanol + 0.5% sodium metabisulfite with 0.35% sodium hydroxide. Et/$Na_2S_2O_5$ = 70% ethanol + 0.5% sodium metabisulfite without 0.35% sodium hydroxide. [b] Figures in parentheses indicate standard deviations. [c] Room temperature, approximately 25 °C. [d] Dry weight basis. Values in the same column but with different letters are significantly different at the 95% level.

measured in tension, pretest speed, 1.0 mm/s; test speed, 0.4 mm/s; post-test speed, 8 mm/s; distance set according to the expected elongation. At least six strips from each Petri dish film were analyzed, and at least three films from each treatment were used for each experiment. Tensile testing was completed within 6 h of removal of films from the drying oven. The maximum force and distance at break was recorded, and the stress and strain were calculated.

Water Vapor Transmission (WVT) and WVP. The ASTM method E96-97, Standard Test Method (*24*), was modified for use as follows: Schott bottles (100 mL) were modified by accurately drilling a hole (33 mm) in the center of the plastic screw top and removing the inner ridge of the top. A circular template (40 mm diameter) was used to cut circles from the cast films to ensure that circles of a constant size were used for the test. Distilled water (90 mL) was placed in each Schott bottle. The circle of film was placed over the mouth of the bottle followed by a fiber tap washer (external diameter 39 mm), which covered the glass rim of the bottle. The lid of the bottle was then screwed in place. The assembly was water tight. The assembled bottles were weighed on a top pan balance to two decimal places before placing in a fume cupboard with the fan switched on. A control bottle containing water but no film was used to monitor the water loss with no barrier present. Weight loss was recorded daily for up to 14 days. At least three replicates were used for each treatment. Temperature and relative humidity have a significant effect on the functional properties of protein films (*2*). In the experiments described here, no attempt was made to control these factors but experiments were carried out on films at the same time thus ensuring that they all experienced the same conditions. The values given for WVT and WVP are thus not absolute values but do allow comparison of film properties.

Statistical Analysis. Analysis of variance using the least squares procedure was applied to the yield and purity of the protein, the solubility of kafirin in different solvents, FT-IR (powder), tensile, WVT, and WVP tests to determine whether significant differences existed ($p < 0.05$) between the means of the different treatments in each case.

RESULTS AND DISCUSSION

Kafirin Preparation and Characterization. The inclusion of sodium hydroxide in the aqueous ethanol extractant with a reducing agent significantly improved the extraction yield of kafirin, from 38 to 54% of flour protein (**Table 1**), better than the yield obtained with 60% (w/w) *tert*-butyl alcohol containing DTT (32%). This is in contrast to the findings of Taylor et al. (*13*) who reported that aqueous *tert*-butyl alcohol containing DTT was the most efficient extractant for kafirin. This apparent anomaly is probably due to the use of a single extraction in the present study rather than the multiple extraction procedure used by Taylor et al. (*13*). In addition, the aqueous ethanol extraction was at 70 °C whereas the aqueous *tert*-butanol extraction was carried out at room temperature.

When sodium hydroxide was used as part of the kafirin extractant, more nonprotein contaminants (kafirin purity 67%) were extracted than if sodium hydroxide was not used (74%). However, after the samples were defatted, there was no significant difference in the purity of the kafirin regardless of its method of extraction. This indicates that extraction of kafirin in the presence of sodium hydroxide coextracts more fat than if sodium hydroxide is not used. This in agreement with Landry and Moureaux (*25*) who found a decrease in the purity of zein when extracted in the presence of sodium hydroxide due to the coextraction of lipoproteins and nonprotein nitrogen.

In terms of amino acid composition, the different protein preparations were all rich in glutamine (assayed as glutamic acid), leucine, alanine, and proline and low in lysine, essentially the same as that shown for kafirin by Taylor and Schüssler (*22*). Wall and Paulis (*11*) reviewed early methods used for the extraction of glutelin proteins from sorghum and maize and found that dilute sodium hydroxide was commonly used for this purpose. Thus, it was considered a possibility that some glutelin proteins would be coextracted when sodium hydroxide was used as part of the extractant. This was not found to be so. The amino acid composition of the extracted proteins was not changed when sodium hydroxide was included in the extractant, and no additional proteins were coextracted. Thus, the aqueous ethanol solvent was specific for kafirin prolamin proteins even with the inclusion of some alkali. Of the freeze-dried samples, the *tert*-butyl alcohol-extracted kafirin was the most soluble. It was completely soluble in the film-casting solvent (70% ethanol, w/w). The kafirin extracted with ethanol and NaOH was significantly less soluble than the *tert*-butyl alcohol-extracted kafirin, which had been freeze-dried. It had the same solubility as kafirin extracted with *tert*-butyl alcohol and dried at 40 °C and better solubility than that extracted without NaOH. Kafirin extracted without sodium hydroxide and oven dried had the worst solubility.

Under reducing conditions, SDS−PAGE of the kafirins (**Figure 1a**) showed the presence of γ-, α_1-, α_2-, and β-kafirin in all samples, with α_1-kafirin (M_r = 24−25 kDa) predominating. There was no significant difference among the various preparations. The band patterns were in agreement with the kafirin study by El Nour et al. (*15*). When the SDS−PAGE analysis was carried out under nonreducing conditions (**Figure 1b**), a few faint bands of high molecular weight were present when kafirin was extracted without NaOH but not in samples extracted with NaOH.

The observation that the addition of sodium hydroxide to the aqueous ethanol extractant improved the yield of kafirin extracted from whole grain sorghum is consistent with commercial zein extraction practice where sodium hydroxide is used to increase yield (*26*). The combined effect of high temperature (70 °C) and sodium hydroxide on proteins is to deamidate glutamine and asparagine residues to form glutamic and aspartic

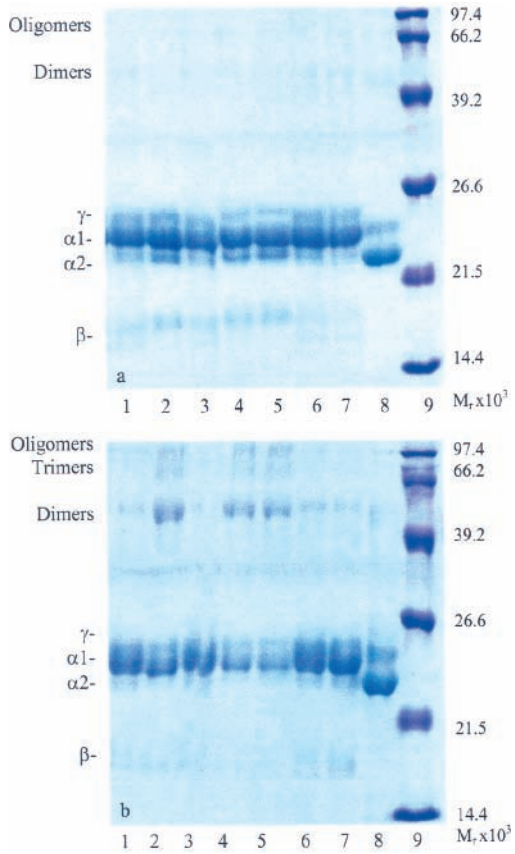

Figure 1. SDS–PAGE of sorghum protein preparations extracted from whole grain sorghum flour (unless otherwise stated) with and without NaOH in the extractant (protein loaded at constant weight). (**a**) Under reducing conditions and (**b**) under nonreducing conditions. Lanes: 1, 70% ethanol + 0.5% sodium metabisulfite with NaOH, freeze-dried; 2, 70% ethanol + 0.5% sodium metabisulfite without NaOH, freeze-dried; 3, 70% ethanol + 0.5% sodium metabisulfite with NaOH, dried at 40 °C; 4, 70% ethanol + 0.5% sodium metabisulfite without NaOH, dried at 40 °C; 5, 70% ethanol + 0.5% sodium metabisulfite without NaOH, dried at 40 °C; 6, 70% ethanol + 0.5% sodium metabisulfite with NaOH, freeze-dried; 7, sorghum endosperm, 70% ethanol + 0.5% sodium metabisulfite with NaOH, freeze-dried; 8, commercial zein; and 9, molecular weight (MW) standards.

acid (*27*). Kafirin is rich in glutamine (*28*); thus, the addition of sodium hydroxide probably improved kafirin solubility by deamidating glutamines, thereby reducing glutamine/glutamine interactions and introducing electrostatic repulsion via charged glutamate residues. In addition, the solubility of the kafirins may also have been increased because of the enhanced efficiency of the reducing reagent in the presence of sodium hydroxide (**Figure 1**), causing the clevage of intra- or/and intermolecular disulfide bonds resulting in a higher extraction yield. Cleavage of disulfide bonds would occur first followed by the formation of cysteic acid and dehydroalanine (*27*), which may reduce the amount of cysteine available for disulfide-bonding reformation after the extraction.

FT-IR was used to investigate the secondary structure of the extracted kafirins. Fourier self-deconvoluted spectra revealed that the amide I band has two main peaks around 1650 and 1620 cm^{-1} (**Figures 2** and **3**). The peak around 1650 cm^{-1} can be assigned to α-helical (*17*) conformations; random coil absorbance is believed to be in the region of 1645−1637 cm^{-1} and thus overlaps the peak assigned to α-helix (*29*), while the peak around 1620 cm^{-1} can be attributed to intermolecular β-sheets (*28*, *29*), a secondary structure associated with protein

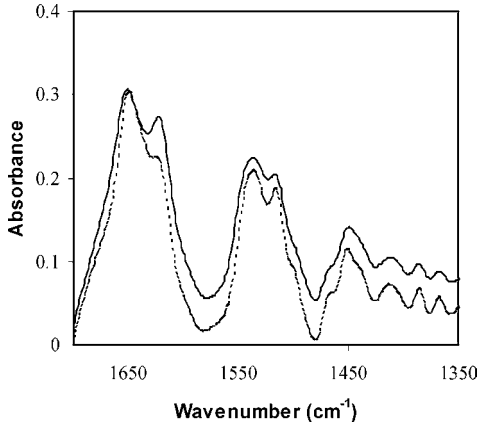

Figure 2. Deconvoluted FT-IR spectra of kafirins from different solvent extractions (samples freeze-dried). Key: –, 70% ethanol with $Na_2S_2O_5$ and NaOH; - - -, 60% *tert*-butyl alcohol with DTT.

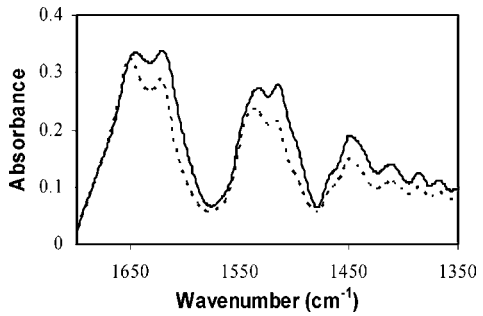

Figure 3. Deconvoluted FT-IR spectra of kafirin extracted with 70% ethanol, with (- - -) or without (–) NaOH (samples freeze-dried).

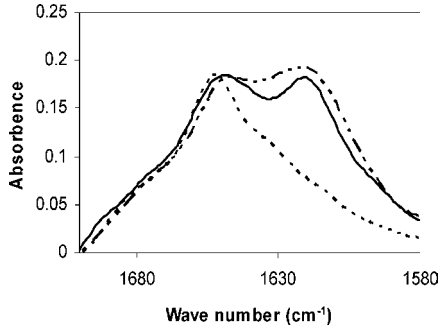

Figure 4. Deconvoluted FT-IR spectra of the soluble and insoluble fractions (all measured in the dry state) of the kafirin (extracted with 70% ethanol with and without NaOH, dried at 40 °C). Key: –, kafirin extracted without NaOH; - - -, aqueous ethanol soluble fraction; and - – -, aqueous ethanol insoluble fraction.

aggregation. However, because the native kafirin is predominantly an α-helical protein (*17*), the change of the ratio of the intensities at 1650 to 1620 cm^{-1} may be taken as an indication of the degree of structural transition from native to aggregated protein, which takes place during the extraction and drying processes. The intermolecular β-sheet peak intensity of the ethanol-extracted freeze-dried kafirin is higher than that of *tert*-butyl alcohol-extracted freeze-dried kafirin (**Table 1** and **Figure 2**). The FT-IR spectra also show that the freeze-dried kafirin extracted with ethanol containing NaOH has a lower intermolecular β-sheet content than kafirin extracted without NaOH (**Table 1** and **Figure 3**). Similar observations were made when comparing the kafirin dried at 40 °C.

When the soluble and insoluble fractions from the kafirin dissolution test (both fractions were air-dried) were measured separately, the FT-IR spectra revealed that the soluble kafirin

Table 2. Effect of Extractant and Protein Drying Conditions on the Thickness and Tensile Properties of Kafirin Films[a]

protein	extractant	drying conditions	thickness mean (μm)	stress at break (N/mm^2)	strain at break (%)
kafirin	tB/DTT	freeze-dried	113 (30) b	2.0 (0.3) ab	141.6 (20.8) b
kafirin	Et/$Na_2S_2O_5$	40 °C	270 (13) c	1.6 (0.4) a	13.5 (4.0) a
kafirin	Et/$Na_2S_2O_5$/NaOH	40 °C	110 (17) b	4.1 (1.5) bc	15.3 (1.9) a
kafirin	Et/$Na_2S_2O_5$	freeze-dried	120 (9) b	5.5 (0.6) c	17.4 (5.6) a
kafirin	Et/$Na_2S_2O_5$/NaOH	freeze-dried	101 (17) b	5.9 (2.5) c	21.5 (9.8) a
commercial zein	not known	not known	59 (7) a	1.8 (0.3) a	203 (39.6) c

[a] tB/DTT = 60% *tert*-butyl alcohol + 0.05% DTT. Et/$Na_2S_2O_5$/NaOH = 70% ethanol + 0.5% sodium metabisulfite with 0.35% sodium hydroxide. Et/$Na_2S_2O_5$ = 70% ethanol + 0.5% sodium metabisulfite without 0.35% sodium hydroxide. Figures in parentheses indicate standard deviations. Values in the same column but with different letters are significantly different at the 95% level.

Table 3. Effect of Inclusion of NaOH in the Extractant and the Protein-Drying Conditions on the Thickness and Water Vapor Barrier Properties of Kafirin Films[a]

protein	extractant	drying conditions	thickness mean (μm)	WVT (g/h/m^2)	WVP (g mm/m^{2}hkPa)
kafirin	tB/DTT	freeze-dried	106 (10) b	8.7 (0.3) a	0.22 (0.01) a
kafirin	Et/$Na_2S_2O_5$	40 °C	203 (23) c	15.0 (1.2) c	1.28 (0.17) c
kafirin	Et/$Na_2S_2O_5$/NaOH	40 °C	126 (22) b	10.9 (0.6) b	0.57 (0.09) b
kafirin	Et/$Na_2S_2O_5$	freeze-dried	110 (5) b	10.9 (1.4) b	0.50 (0.04) b
kafirin	Et/$Na_2S_2O_5$/NaOH	freeze-dried	131 (22) b	9.8 (1.7) ab	0.54 (0.15) b
commercial zein	not known	not known	57 (10) a	10.6 (1.5) ab	0.24 (0.03) a

[a] tB/DTT = 60% *tert*-butyl alcohol + 0.05% DTT. Et/$Na_2S_2O_5$/NaOH = 70% ethanol + 0.5% sodium metabisulfite with 0.35% sodium hydroxide. Et/$Na_2S_2O_5$ = 70% ethanol + 0.5% sodium metabisulfite without 0.35% sodium hydroxide. Figures in parentheses indicate standard deviations. Values in the same column but with different letters are significantly different at the 95% level.

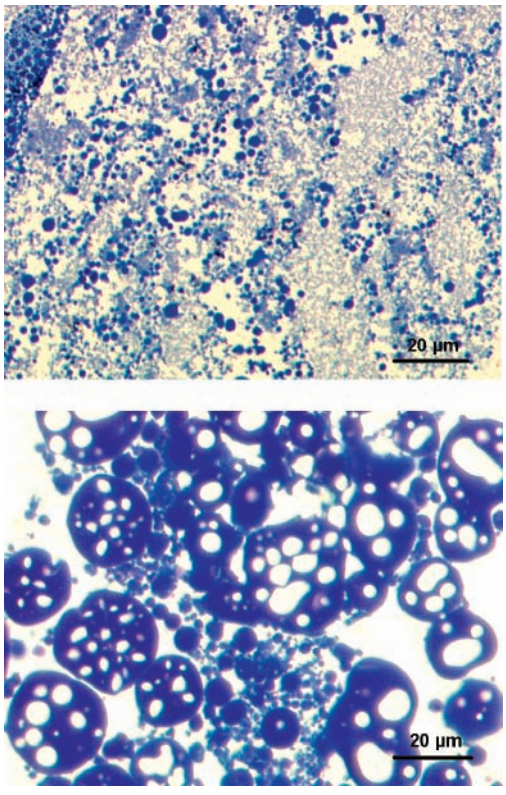

Figure 5. Light microscopy of kafirins extracted with 70% ethanol, sodium metabisulfite, and sodium hydroxide at 70 °C; freeze-dried (top) and dried at 40 °C (bottom).

contained more of the α-helical form of the protein while the insoluble residue had a higher intermolecular β-sheet content (**Figure 4**). Such results might be explained, since an intermolecular β-sheet is indicative of protein aggregation (*30*); the aggregated molecules are more difficult to disperse in the solvent as shown (**Table 1**).

The structural properties of the various kafirins were influenced by the method of drying. Generally, the freeze-dried kafirins were easier to dissolve in aqueous ethanol than kafirin dried at 40 °C. Similarly 1650 cm^{-1}:1620 cm^{-1} peak intensity ratios in the FT-IR spectra of the freeze-dried samples were higher than those of samples dried at 40 °C (**Table 1**). These results suggest that protein aggregation occurs during the heat-drying process. Light microscopy (**Figure 5**) supports this view, showing the fine particles of the freeze-dried sample in contrast to the large aggregated particles in the heat-dried material.

Properties of Films Cast from Kafirin and Commercial Zein. The solubility of the different kafirin preparations in the film casting solution influenced the thickness and also the mechanical properties of the films. This is in agreement with Banker (*31*) who reported that good solvation and polymer chain extensibility are needed for production of strong, cohesive films. Kafirin, predominately in the native α-helical form (extracted with *tert*-butyl alcohol and freeze-dried), dissolved readily and formed even, uniform films of consistent thickness. Conversely, kafirin with a higher intermolecular β-sheet content (kafirin extracted without sodium hydroxide and dried at 40 °C), dissolved poorly and was prone to gelation resulting in uneven films of more variable thickness.

The results of sensory assessment, measurement of tensile (**Table 2**), and water vapor barrier properties (**Table 3**) showed that those cast from *tert*-butyl alcohol-extracted kafirin (freeze-dried) had superior sensory and mechanical properties. Under the same protein-drying conditions, films formed from kafirin extracted with NaOH had better sensory properties (better clarity, color, flexibility, and surface texture) (data not shown) than films formed from kafirin extracted without NaOH. It was also found that when kafirin was extracted with NaOH, the protein-drying conditions had less effect on the film properties than with kafirin extracted without NaOH. Films cast from freeze-dried kafirin extracted with NaOH had better sensory properties (better clarity, color, flexibility, and

surface texture) (data not shown) than films cast from kafirin dried at 40 °C, but there were no difference in film thickness, tensile properties, or water vapor barrier properties. Films formed from kafirin extracted without NaOH and dried at 40 °C had poorer sensory properties (much less clear, much darker, with a much rougher surface texture) (data not shown) were thicker with lower tensile strength, similar extensibilty, and higher WVP than those formed from freeze-dried kafirin extracted without NaOH.

Freeze-dried kafirin extracted with NaOH formed films of very similar sensory quality to that of commercial zein, being only slightly inferior in clarity and slightly darker than *tert*-butyl alcohol-extracted kafirin films. All of the kafirin films were stronger (higher stress at break) than those made from commercial zein but were less extensible (showing a lower strain at break) (**Table 2**). This is in contrast to the findings of Buffo et al.(*19*), who reported that kafirin films had lower tensile strength and higher elongation at break than commercial zein films. The authors attributed their observations to the lower protein content of the kafirin as compared to that of the commercial zein. Comparison with this work is difficult as the kafirin extraction was not comparable to that used in the present study. The WVT of the kafirin and zein films was similar, but as the zein films were thinner, the WVP of zein films was lower than that of the kafirin films (**Table 3**). This may have been due to the zein dissolving more readily than the kafirin in the film-casting solvent resulting in more even, thinner films.

In conclusion, the addition of sodium hydroxide to the aqueous ethanol extractant improved the yield and solubility of kafirin extracted from dry-milled whole grain sorghum and also appeared to increase the efficiency of the reducing reagent. Freeze drying the kafirin prevented aggregation of the protein, which was a problem in heat-dried protein. It was found that the higher the proportion of nativelike α-helical structure of the protein remaining after extraction and drying (tB/DTT kafirin and freeze-dried Et/$Na_2S_2O_5$/NaOH kafirin), the more desirable were the film properties (good sensory properties, higher tensile strength, higher strain, and low WVP). This is possibly due to the kafirin, which has a greater proportion of α-helical structure being more readily dispersed in the film-casting solution, the films formed from this protein consequently being more homogeneous. Such folded proteins may also pack together more effectively than denatured, aggregated proteins to form the film structure. Therefore, any industrial-scale extraction must aim to minimize protein aggregation by maximizing the retention of the nativelike α-helical structure of the kafirin. Taking into consideration yield levels, film-forming properties, and food compatibility, an extraction procedure of 70% (w/w) ethanol with 0.5% (w/w) sodium metabisulfite and 0.35% (w/w) sodium hydroxide at 70 °C was shown to be an appropriate method for the commercial extraction of kafirin. Films formed from kafirin prepared by this method are somewhat stronger but less extensible than those formed from commercial zein, although they have similar WVT properties. This indicates that in some circumstances they may be used as an alternative to zein-based materials and may have uses complementary to those of zein in other circumstances.

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