Sorghum Bran as a Potential Source of Kafirin

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ABSTRACT

Sorghum bran, a coproduct of sorghum dry milling, could be a source of protein for industrial applications. Condensed tannin-free red and white sorghum samples were decorticated by abrasion until ≈10 or 25% grain by weight was removed. Kafirin was then extracted from the milling fractions using an aqueous ethanol based solvent system. The brans were darker and considerably higher in protein and fat compared with the whole grain flours and decorticated grain flours, with the 25% bran having higher protein than the 10% bran. This is due to increased contamination of the bran with protein-dense, corneous endosperm. The protein extracted from all the milling fractions, including the brans, was pure kafirin. However, the yield of kafirin from the brans (15.9–26.7% of total protein present) was somewhat lower than that from whole grain and decorticated grain flours (45.0–57.9% of total protein present), due to the fact that kafirin is located solely in the endosperm. Also, the kafirin from bran was more contaminated with fat, polyphenols, and other substances, and more highly colored, particularly the kafirin from red sorghum. Thus, sorghum bran could be used as a source of kafirin but further purification steps may be necessary.

In the arid parts of Africa and Asia, sorghum (*Sorghum bicolor* L. Moench) is an important food staple, contributing ≈70% of protein and energy in the daily diet of these people (reviewed by Serna-Saldivar and Rooney 1995). Food uses include various traditional porridges, flatbreads, beers, and other nonalcoholic beverages (reviewed by Rooney and Waniska 2000). Before being used as food, sorghum grain generally undergoes abrasive decortication, also known as dehulling or pearling (Taylor and Dewar 2001). Decortication involves the removal of the outer anatomical parts of the grain: the pericarp layers (pericarp and testa) rich in fiber and ash; aleurone layer rich in ash, protein, and fat; and germ rich in protein, fat, and ash (Hahn 1969; Rooney and Miller 1982). Decortication, therefore, results in large quantities of coproduct, usually used for livestock feed. This fraction is commonly referred to as bran in milling terminology (Rooney and Murty 1982). However, due to the inefficiency of industrial milling processes, a large amount of protein-dense peripheral corneous (horny) endosperm may be removed with the bran (Rooney et al 1972).

The potential value of high protein cereal coproducts such as rice bran (Gnanasambandam et al 1997) and maize gluten meal (Dickey et al 2001) beyond the use of livestock feed has been recognized. Applications for cereal proteins such as edible and nonedible coatings, biodegradable packaging, fibers, binders, and thermoplastics have been described (reviewed by Gennadios and Weller 1990; Krotcha and De Mulder-Johnston 1997; Lawton 2002).

Of the cereal proteins, zein, the aqueous alcohol-soluble prolamin protein of maize, has been used most widely (Lawton 2002). Kafirin, the aqueous alcohol-soluble prolamin protein of sorghum, is analogous to zein (Shull et al 1991) and has potential for edible and nonedible films and coatings (Buffo et al 1997).

The objectives of this work were to determine whether kafirin can be extracted from red and white sorghum milling fractions, including bran, and to characterize the kafirin extracted.

MATERIALS AND METHODS

Sorghum Samples

Two condensed tannin-free sorghum cultivars, NK 283, a red hybrid (ex. Nola, Randfontein, South Africa, 1998) and PANNAR 202 and 606, a mixed white tan plant hybrid (ex. B. Koekemoer, Lichtenburg, South Africa, 2001) were used.

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Preparation of Sorghum Milling Fractions

Whole grain flours were prepared by milling clean whole sorghum grain into flour with a hammer mill fitted with an 800-µm sieve. For milling fractions, clean whole grain was decorticated by passing the grain through a carborundum cone abrasive rice pearler (Maig, Braunschweig, Germany) until an extraction rate of 90 or 75% (the typical range in southern African sorghum milling) was achieved. The brans, 10 and 25% extraction were retained. The decorticated grain and bran fractions were each milled into flour with a hammer mill fitted with an 800-µm sieve. All milling preparations were stabilized by vacuum packing and storage at –20°C until further analysis.

Characterization of Sorghum Milling Fractions

Grain hardness was determined visually using the method of Rooney and Miller (1982). Color of all sorghum flour/bran milling fractions was measured by Tristimulus colorimetry (ColorQuest, Hunter Associate Laboratories, Reston, VA), using the L, a, b scale. Approved Methods of the American Association of Cereal Chemists (AACC 2000) were used to determine moisture (one-stage air oven drying, 103 ± 1°C for 3 hr) (Approved Method 44-15A), ash (Approved Method 08-01), and crude fat (Approved Method 30-25). Crude protein was determined using combustion nitrogen analysis (FP528 protein/nitrogen analyzer, Leco, St Joseph, MI), using 6.25 as the factor to convert nitrogen to protein. Crude fiber was determined by the Weende method (Fibertec system, 1020 hot extraction, Tecator, Höganäs, Sweden). Total polyphenols were determined by the ferric ammonium citrate (FAC) International Organization for Standardization method (ISO 1988).

Kafirin Extraction

Kafirin was prepared by batch extraction of the milled flour and bran fractions, using a modification of the method of Carter and Reck (1970) using 70% (w/w) absolute ethanol in distilled water, 0.35% (w/w) sodium hydroxide, and 0.5% (w/w) sodium metabisulfite at a ratio of 1:5 (w/w) flour-bran to extractant with vigorous stirring at 70 ± 0.1°C for 1 hr. Insoluble matter was removed by centrifugation at 1,000 × *g* for 5 min at ambient temperature. The clear supernatant was decanted into a stainless steel tray, and the ethanol was removed from the extract by evaporation in a fume cabinet at ambient temperature until a viscous liquid sediment formed. Chilled distilled water (10°C) of approximately equal volume was used to dilute the sediment. The pH was then reduced to pH 5.0 with 1*M* HCl to liberate all the protein from suspension. The precipitated protein was then recovered by vacuum filtration through a Buchner funnel using two layers of Whatman No. 4 filter paper. The resulting wet kafirin was frozen at –20°C and then freeze dried. The freeze-dried kafirin preparations were weighed, milled into a fine powder with a coffee grinder, and stored in an airtight bottle at 10°C until further analysis. Commercial zein (Cat. Z3625, Sigma, Johannesburg, South Africa,) was ground to a fine powder with a coffee grinder to ensure similar particle size of all protein samples. The protein preparations were defatted three times with hexane at ambient temperature at a ratio of 1:10 (w/w) protein to hexane for 1 hr.

Characterization of Kafirin Preparations

The kafirin and zein preparations were characterized (before defatting) as for sorghum flour/bran preparations. Amino acid compositions of the defatted protein preparations were determined by RP-HPLC using precolumn derivatization (PICO-TAG method) (Bidlingmeyer et al 1984).

SDS-PAGE under reducing and nonreducing conditions of the defatted protein preparations was performed using gels 140 mm long and 1.5 mm thick on a Bio-Rad vertical electrophoresis system (Protean II xi Cell, Bio-Rad Laboratories, Hercules, CA) according to Gallagher (1999). Separating and stacking gel concentrations were 12 and 3.9% (v/v) acrylamide, respectively, prepared with 40% acrylamide-bis (19:1) ready-to-use solution (Merck, Halfway House, South Africa). Protein (≈33 μg) was loaded per track. Premixed protein molecular weight makers (low-range marker, Cat. 1495984, Roche Molecular Biochemicals, Indianapolis, IN) were used. Proteins were stained with Coomassie Blue R250.

Statistical Analysis

Multifactor analysis of variance was used to assess the effect of decortication on color and composition data of the different sorghum milling fractions and extracted kafirin preparations.

Means of at least two replicate tests were separated with the least significant difference test.

RESULTS AND DISCUSION

Visual characterization of the sorghum grains revealed that NK 283 endosperm texture was primarily of intermediate hardness (rating 3) according to the system of Rooney and Miller (1982). Endosperm texture of PAN 202-606 was not easy to categorize as just <50% of the grains viewed showed floury endosperm (rating 5), while the rest showed endosperm of intermediate hardness (rating 3) (Rooney and Miller 1982). This is probably because different cultivars (PAN 202 and PAN 606) have different endosperm textures.

The whole grains were small, round, and slightly flattened; NK 283 was red compared with PAN 202-606. At the 90% extraction level, the grains showed signs of some pericarp and germ removal and appeared to be slightly more rounded. The color of NK 283 appeared less intense, showing patches of white endosperm. This is due to the removal of anthocyanin and anthocyanidin polyphenolic pigments in the pericarp (Rooney and Murty 1982). At the 75% extraction level, the grains had no pericarp and very little germ remaining. Some of the NK 283 grains showed signs of endosperm staining with anthocyanin/anthocyanidin pigments, while PAN 202-606 grain appeared almost completely white. The grains became almost round, with some kernels showing signs of fracturing, which was more prominent for PAN 202-606. This could be due to differences in endosperm hardness of the two cultivars (PAN 202 and PAN 606).

TABLE I
Color of Milling Fractions from NK 283 and PANNAR 202-606 Measured by Tristimulus Colorimetery[a,b]

Milling Fractions[c]	Color Values L	±a	±b
NK 283			
WGF	68.8 ± 0.1e	+4.5 ± 0.1h	+10.5 ± 0.1bc
90% EF	74.5 ± 0.5g	+3.5 ± 0.1g	+10.3 ± 0.5b
75% EF	82.1 ± 0.3i	+1.2 ± 0.0d	+9.7 ± 0.6a
25% Bran	59.1 ± 0.7c	+8.6 ± 0.2i	+12.8 ± 0.0g
10% Bran	49.8 ± 0.3a	+10.4 ± 0.1j	+13.5 ± 0.0h
PANNAR 202-606			
WGF	73.6 ± 0.3f	+0.4 ± 0.0c	+11.0 ± 0.3cd
90% EF	79.9 ± 0.2h	–0.1 ± 0.0b	+11.4 ± 0.0e
75% EF	84.3 ± 0.4j	–0.6 ± 0.1a	+11.4 ± 0.7de
25% Bran	66.0 ± 0.2d	+1.4 ± 0.0e	+12.1 ± 0.1f
10% Bran	56.7 ± 0.2b	+2.3 ± 0.0f	+12.6 ± 0.1g

[a] L = color intensity with L = 100 for lightness, and L = 0 for darkness; +a = increasing red, –a = increasing green; +b = increasing yellow, –b = increasing blue.
[b] Values are means and standard deviations for two replicates, means with different letters within a column differ significantly ($P < 0.05$).
[c] WGF, whole grain flour; EF, extraction flour.

TABLE II
Chemical Composition (g/ 100 g, db) and % Distribution of Components of NK 283 and PANNAR 202-606 Milling Fractions[a]

Milling Fractions[b]	Protein (N × 6.25)	Fat	Crude Fiber	Ash	Carbohydrate (by difference)	Total Polyphenols
NK 283						
WGF	10.7 ± 0.1e (100)	3.18 ± 0.49c (100)	2.40 ± 0.16d (100)	1.51 ± 0.0c (100)	82.2 ± 0.7e (100)	0.179 ± 0.025cd (100)
90% EF	10.3 ± 0.2d (86)	2.77 ± 0.35bc (66)	1.46 ± 0.05bc (56)	1.4 ± 0.0c (86)	84.1 ± 0.6f (92)	0.148 ± 0.011bc (90)
75% EF	9.38 ± 0.1c (65)	1.80 ± 0.42a (36)	0.82 ± 0.12ab (25)	1.0 ± 0.0a (53)	87.0 ± 0.4g (79)	0.106 ± 0.021ab (38)
25% Bran	14.4 ± 0.6g (34)	7.45 ± 0.31d (49)	8.88 ± 1.11f (92)	3.0 ± 0.3e (51)	66.4 ± 1.1b (20)	0.653 ± 0.074f (88)
10% Bran	12.1 ± 0.1f (11)	7.98 ± 0.11d (21)	12.8 ± 0.63h (53)	2.7 ± 0.1de (18)	64.4 ± 0.7a (8)	0.902 ± 0.007g (45)
PANNAR 202-606						
WGF	8.66 ± 0.15b (100)	3.17 ± 0.41c (100)	2.11 ± 0.08cd (100)	1.51 ± 0.05c (100)	84.6 ± 0.7f (100)	0.098 ± 0.004ab (100)
90% EF	8.46 ± 0.14b (88)	2.40 ± 0.16ab (66)	0.90 ± 0.10ab (38)	1.34 ± 0.03bc (76)	86.9 ± 0.3g (92)	0.116 ± 0.000ab (90)
75% EF	7.94 ± 0.06a (68)	1.98 ± 0.42a (46)	0.51 ± 0.04a (18)	1.18 ± 0.06ab (58)	88.4 ± 0.5h (78)	0.086 ± 0.007a (75)
25% Bran	10.5 ± 0.17de (29)	7.71 ± 0.40d (60)	6.62 ± 0.45e (78)	2.65 ± 0.14d (42)	72.5 ± 0.3d (21)	0.211 ± 0.007de (50)
10% Bran	10.2 ± 0.23d (12)	8.80 ± 0.85e (28)	10.43 ± 0.72g (49)	2.63 ± 0.21d (17)	67.9 ± 1.9c (8)	0.268 ± 0.007e (30)

[a] Values are the mean and standard deviations of two replicates, means with different letters within a column differ significantly ($P < 0.05$). Values in parentheses are % in whole grain.
[b] WGF, whole grain flour; EF, extraction flour.

After milling, NK 283 whole grain flour (WGF) was darker, more red, and less yellow compared with PAN 202-606 WGF (Table I) because of the red pigments from the pericarp. Lightness (L) increased with increasing levels of decortication for both NK 283 and PAN 202-606 flours, but NK 283 flours had the lower L values at both decortication levels. These results are similar to those reported by Awika et al (2002) working with red and white sorghum. These authors reported that increasing levels of decortication using a tangential abrasive dehulling device (TADD) resulted in flour and grits being lighter in color with the red sorghum giving the lowest L values at any given decortication yield.

The bran materials were significantly darker compared with the WGF and extraction flours (EF) (Table I) due to the presence of anthocyanin/anthocyanidin pigments. NK 283 brans were darker, more red, and more yellow than PAN 202-606 brans. The 10% extraction brans were darker, more red, and more yellow compared with 25% brans for both cultivars. This was presumably due to higher concentrations of pigments in the pericarp-rich 10% brans. The relative lightness of 25% brans was probably due to the presence of endosperm material removed during decortication, reducing the pigment content.

Table II shows that the protein and total polyphenol contents differed significantly between NK 283 and PAN 202-606 WGF, with NK 283 having 23% more protein and twice the polyphenols (accounting for its darker and far more red color). These differences are presumably due to genetic variation as well as differences in agronomic and environmental growing conditions (reviewed by Serna-Saldivar and Rooney 1995). Protein content is usually the most variable component and it is largely dependent on agronomic conditions, including water availability, soil fertility, temperatures, and environmental conditions during grain development. In contrast, sorghum appearance (pericarp color and thickness), the presence, color and thickness of the testa, and endosperm color are all genetically controlled (Rooney et al 1980). There were no significant differences in fat, crude fiber, and ash between the two cultivars.

Decortication altered the chemical composition of the flours. Compared with WGF, increasing decortication resulted in a reduction in the mean protein by 4 and 2% in the 90% EF, and 12 and 9% in the 75% EF for NK 283 and PAN 202-606, respectively. Reduction in other components (fat, crude fiber, ash, and total polyphenols) also occurred.

Loss of these chemical components resulted in the 10 and 25% extraction brans being significantly higher in protein and in fat, crude fiber, ash, and total polyphenols compared with WGF and EF. Compared with WGF, 10% bran was higher in protein by 13 and 17%, fat by 111 and 175%, crude fiber by 433 and 395%, ash by 80 and 73%, and total polyphenols by 404 and 173% for NK 283 and PAN 202-606, respectively. Compared with WGF, 25% bran was higher in protein by 35 and 21%, fat by 95 and 141%,

TABLE III
Color of Defatted Kafirin Preparations from NK 283 and PANNAR 202-606 Milling Fractions and Commercial Zein as Measured by Tristimulus Colorimetery[a,b]

Milling Fractions[c]	L	±a	±b
NK 283			
WGF	81.4 ± 0.9cd	2.7 ± 0.4fg	11.4 ± 0.1bc
90% EF	85.3 ± 0.1ef	1.3 ± 0.1de	11.5 ± 0.1bc
75% EF	90.3 ± 0.3g	–0.9 ± 0.1ab	8.7 ± 0.6a
25% Bran	70.9 ± 1.8b	10.1 ± 0.3h	12.9 ± 1.2c
10% Bran	61.8 ± 2.6a	14.6 ± 1.2i	15.7 ± 2.1d
PANNAR 202-606			
WGF	85.1 ± 2.1ef	–0.3 ± 0.5bc	13.1 ± 0.0c
90% EF	87.7 ± 0.1fg	–0.9 ± 0.0ab	11.1 ± 0.2b
75% EF	88.5 ± 0.2g	–1.4 ± 0.0a	8.8 ± 0.2a
25% Bran	84.1 ± 0.6de	0.8 ± 0.1cd	16.1 ± 0.6d
10% Bran	80.0 ± 2.1c	2.1 ± 0.9ef	19.0 ± 0.7e
Commercial zein	79.6 ± 0.1c	3.84 ± 0.2g	26.5 ± 0.2f

[a] L = color intensity with L = 100 for lightness, and L = 0 for darkness; +a = increasing red, –a = increasing green; +b = increasing yellow, –b = increasing blue.
[b] Values are means and standard deviations for two replicates, means with different letters within a column differ significantly ($P < 0.05$).
[c] WGF, whole grain flour; EF, extraction flour.

TABLE IV
Protein Extracted, Protein Yield, and Chemical Composition (g/100 g, db) of Kafirin Preparations from NK 283 and PANNAR 202-606 Milling Fractions and Commercial Zein[a]

Milling Fractions[b]	Protein Extracted (g of protein/100 g of flour/bran)	Protein Yield (% of total protein in flour/bran)	Chemical Composition				Protein Content of Defatted Preparations (N × 6.25)
			Protein (N × 6.25)	Fat	Total Polyphenols	Other (by difference)	
NK 283							
WGF	4.38 ± 0.16e	46.1 ± 1.7d	83.1 ± 2.0ef	12.8 ± 0.8c	0.461 ± 0.106b	3.70 ± 1.40bc	88.7 ± 1.1c
90% EF	5.12 ± 0.07g	56.3 ± 0.8f	87.0 ± 0.1ef	10.4 ± 0.3b	0.305 ± 0.013a	2.33 ± 0.44ab	90.8 ± 0.9cd
75% EF	4.81 ± 0.10f	57.9 ± 1.2f	90.2 ± 0.1f	9.30 ± 0.8b	0.190 ± 0.035a	0.33 ± 0.96a	93.9 ± 3.8d
25% Bran	3.43 ± 0.21c	26.7 ± 1.6c	54.1 ± 0.6c	30.7 ± 2.2e	1.423 ± 0.005d	13.84 ± 2.24e	69.4 ± 1.6b
10% Bran	1.70 ± 0.05a	15.9 ± 0.5a	40.0 ± 4.0ab	40.8 ± 0.3f	2.211 ± 0.009e	17.01 ± 4.20f	61.6 ± 6.9a
PANNAR202-606							
WGF	3.44 ± 0.12c	45.0 ± 1.5d	77.9 ± 0.6de	16.7 ± 0.3d	0.322 ± 0.106ab	5.14 ± 0.74c	88.3 ± 0.3c
90% EF	3.93 ± 0.01d	52.4 ± 0.2e	82.3 ± 0.2ef	14.4 ± 1.5c	0.303 ± 0.016a	3.04 ± 1.60abc	91.4 ± 0.1cd
75% EF	3.93 ± 0.05d	56.0 ± 0.8f	87.1 ± 0.4ef	10.1 ± 0.3b	0.250 ± 0.035a	2.62 ± 0.22abc	93.7 ± 0.2d
25% Bran	2.43 ± 0.06b	26.1 ± 0.6c	46.4 ± 0.5bc	44.7 ± 2.5g	0.718 ± 0.122c	8.19 ± 2.55d	72.7 ± 1.6b
10% Bran	1.84 ± 0.02a	20.4 ± 0.2b	31.8 ± 1.6a	53.9 ± 1.5h	0.754 ± 0.083c	13.60 ± 0.45e	62.0 ± 2.0a
Commercial zein	na	na	91.7 ± 0.5g	7.2 ± 0.6a	nd	1.15 ± 0.49ab	92.5 ± 0.1d

[a] Values are means and standard deviations of two replicates, means with different letters within a column differ significantly ($P < 0.05$); na, not applicable; nd, value not determined.
[b] WGF, whole grain flour; EF, extraction flour.

crude fiber by 267 and 214%, ash by 267 and 214%, and total polyphenols by 265 and 115%, respectively. The much higher concentration of polyphenols in the bran accounts for the much darker color compared with the WGF and EF. These findings on the effect of decortication on sorghum grain composition are in general agreement with the results of research on sorghum decortication by Hahn (1969) and Rooney et al (1972).

With regard to the color of the kafirin preparations extracted from the different milling fractions, Table III shows that for both sorghum cultivars, kafirin lightness (higher L values) increased significantly when kafirin was extracted from flours of lower extraction rate. As lightness increased, there was a decrease in red and yellow values. This is probably related to the lower levels of pigments in the EF, as indicated by the lower levels of total polyphenols present (Table II). Commercial zein was darker, more red, and more yellow compared with kafirin preparations from WGF and EF, presumably because it had been extracted from corn gluten from yellow maize (reviewed by Shukla and Cheryan 2001).

Kafirins prepared from the bran fractions were darker, more red (especially from NK 283), and more yellow (especially from PAN 202-606) compared with kafirins prepared from WGF and EF (Table III). This is, no doubt, due to higher levels of pigments present in the bran fractions, which were extracted with the kafirin. The higher color of the kafirin preparations extracted from brans is an indication that nontannin polyphenols have the ability to bind to kafirin. Kafirin preparations from 10% brans were darker, more red, and more yellow compared with kafirins prepared from 25% bran, probably due to the higher concentration of pigments in the pericarp-rich 10% bran.

Table IV shows that whole NK 283 flour yielded 30% more protein on extraction with the aqueous ethanol based solvent compared with PAN 202-606, presumably due to the higher protein content of NK 283 grain. Protein yields were similar for both sorghum cultivars, averaging 45–46% of the total grain protein, indicating that the relative amount of kafirin was similar for both sorghums. This compares to a mean kafirin content of 47.8% for 25 sorghum cultivars reported by Taylor et al (1984) using 60% *t*-butanol plus reducing agent as an extractant. However, higher values (68–73%) have been reported by Hamaker et al (1995) when kafirin was obtained by first removing the nonprotein nitrogen with albumin and globulin proteins, followed by extraction of protein in a borate buffer with SDS plus reducing agent and precipitation of nonprolamins with 60% *t*-butanol.

Decortication increased the amount of protein extracted. The 90% EF yielded 16 and 15% more protein; 75% EF yielded 9 and 15% more, compared with WGF for NK 283 and PAN 202-606, respectively. This despite the fact that flours of lower extraction rate contained less total protein. This tendency indicates that the relative amount of kafirin, compared with other proteins, was higher in the flours of lower extraction rate. This is mainly because kafirin is located solely in the endosperm (Taylor and Schüssler 1986).

Significantly less protein was extracted from the bran fractions compared with WGF and EF (Table IV), although the bran fractions contained proportionally more protein (Table II). The low level of protein extracted from the brans is probably due to the bran materials being much richer in nonprolamin proteins relative to prolamins. According to Jones and Beckwith (1970), amino acid analysis of bran material showed it to be rich in albumin and globulin proteins. Albumins and globulins are concentrated in the grain germ (Taylor and Schüssler 1986) that is removed from the grain during abrasive decortication (Hahn 1969). However, increasing decortication resulted in the 25% bran fractions yielding more protein compared with from the 10% bran. This is presumably due to increased decortication removing more prolamin-rich corneous endosperm from the grain, which is added to the bran material (Hahn 1969). Of the bran fractions, most protein was extracted from NK 283 bran, 42% more protein compared with PAN 202-606 25% bran, and approximately double the protein obtained from the 10% brans. This is presumably because the NK 283 25% bran contained considerably more protein (Table II) and more endosperm and therefore more kafirin for extraction compared with the other bran fractions.

Protein purity of the different kafirin preparations and commercial zein varied (Table IV). Kafirin preparations prepared from EF had the highest protein contents, ranging from 82% for PAN 202-606 90% EF to 90% for NK 283 75% EF before defatting. This is because these preparations were lower in fat, polyphenols and other components (possibly ash and nonstarch polysaccharides) compared with kafirins prepared from the bran fractions. Commercial zein had the highest purity (92%) with the lowest level of fat, presumably because it was prepared from corn gluten, which is high in protein (minimum of 60% protein db) (reviewed by Shukla and Cheryan 2001). According to Parris and Dickey (2001), the principal lipid components found in zein extracted from dry-milled maize are free fatty acids (80%), as most of the triacylglycerols are removed with the insoluble matter during centrifugation of the extract.

Kafirin preparations from the brans were the least pure. The 10% brans contained only 69 and 71% of the protein, and 25% brans contained only 78 and 82% of the protein for NK 283 and PAN 202-606, respectively, compared with the WGF preparations (Table IV). This is because they contained considerable amounts of fat, total polyphenols, and other components. As shown in Table II, fat, phenolic pigments, and nonstarch polysaccharides are found

TABLE V
Amino Acid Composition (g/100 g of protein) of Defatted Kafirin Preparations from NK 283 and PANNAR 202-606 Milling Fractions and Commercial Zein

Milling Fractions[a]	Glu	Leu	Ala	Pro	Phe	Asp	Tyr	Ser	Val	Ile	Thr	Gly	His	Arg	Met	Lys
NK 283																
WGF	28.5	16.7	11.1	10.0	5.8	5.7	5.0	4.8	4.4	3.8	2.9	1.7	1.7	1.7	1.4	0.2
90% EF	27.3	16.5	10.7	9.7	5.8	5.5	5.0	4.7	4.3	3.9	2.8	1.7	1.6	1.7	1.5	0.3
75% EF	28.3	17.3	10.9	9.8	6.0	5.8	5.2	4.9	4.5	4.1	2.9	1.7	1.7	1.8	1.7	0.3
25% Bran	28.2	16.9	11.0	12.0	6.1	5.5	5.1	5.0	4.4	4.0	3.0	1.7	1.6	1.6	1.5	0.1
10% Bran	27.5	16.2	10.8	9.6	5.7	5.4	4.8	5.1	4.3	4.0	2.9	1.8	1.5	1.7	1.5	0.2
PANNAR 202-606																
WGF	27.2	16.2	10.7	9.8	5.7	5.5	5.0	4.8	4.3	3.9	2.9	1.9	1.7	1.8	1.6	0.4
90% EF	26.9	16.0	10.6	9.7	5.6	5.4	5.0	4.8	4.3	3.8	2.9	1.9	1.7	1.9	1.6	0.4
75% EF	27.2	16.4	10.8	9.8	5.8	5.4	5.0	4.8	4.2	3.8	2.9	1.9	1.6	1.9	1.6	0.4
25% Bran	26.8	16.2	10.5	10.0	5.7	5.2	4.9	4.8	4.3	3.8	2.9	1.8	1.6	1.7	1.5	0.2
10% Bran	26.5	16.0	10.6	9.6	5.5	5.4	4.7	4.9	4.3	3.8	3.0	1.9	1.6	1.9	1.5	0.3
Commercial zein	24.4	20.6	9.7	10.2	7.0	5.4	5.3	6.2	3.7	4.1	2.9	1.3	1.4	1.6	1.8	0.1
Kafirin[b]	22.0	14.7	10.6	9.8	5.8	5.2	4.9	3.9	4.8	4.3	2.8	1.7	2.2	1.8	2.4	0.2

[a] WGF, whole grain flour; EF, extraction flour.

[b] Taylor and Schüssler (1986). Prolamins extracted from the endosperm of tannin-free red sorghum, Barnard Red, (Cys/2 = 0.5, NH_3 = 2.4).

in high quantities in sorghum bran. Nonstarch polysaccharides (soluble dietary fiber including hemicelluloses, pectins and gums) probably accounted for much of the "other" component in the bran kafirin preparation due to the inclusion of sodium hydroxide (alkali) and the resulting high pH of the extraction solvent. Aoe et al (1993), working with defatted starch-free rice bran, found that sodium hydroxide (2%, pH 14) was an effective extraction solvent for soluble fiber, yielding 8 g of soluble fiber/100 g of rice bran. Defatting increased the protein contents of all the kafirin preparations, especially those from the brans, although the protein content was still some 20–30% lower than the kafirin preparations from the WGF and EF (Table IV). After defatting, there was no effect of cultivar on the protein content of the kafirin preparations.

Table V shows that the amino acid profiles of all the kafirin preparations were characteristic of kafirin (Taylor and Schüssler 1986), as they were rich in glutamic acid (27–28%), leucine (16–17%), alanine (11%), and proline (10–12%), and poor in lysine (0.1–0.4%). Also, there were no clear differences in amino acid profiles between the kafirin preparations from the different milling fractions or between the two cultivars. However, compared with the commercial zein, the kafirin preparations were higher in glutamic acid, alanine, valine, glycine, and histidine, but lower in leucine, phenylalanine, and serine. These differences are similar to those previously reported (reviewed by Wall and Paulis 1978).

SDS-PAGE under nonreducing conditions (Fig. 1A) showed that all kafirin preparations (lanes 3–7 [PAN 202-606] and lanes 11–15 [NK 283]) gave essentially identical bands. Bands with M_r ≈45,000 to 50,000 and 66,200, and 97,000 are dimers, trimers,

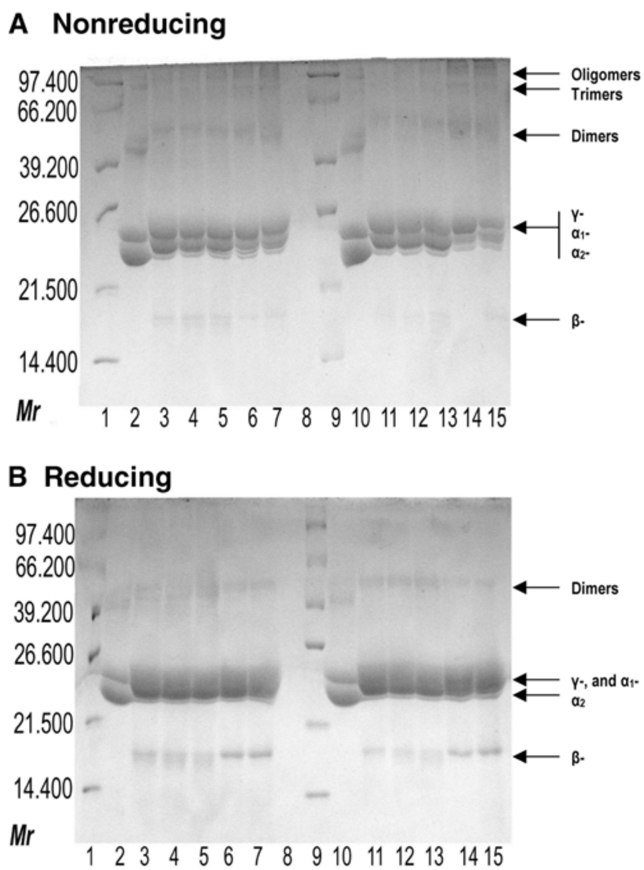

Fig. 1. SDS-PAGE of defatted kafirin preparations from NK 283 and PANNAR 202-606 milling fractions and commercial zein under nonreducing (**A**) and reducing (**B**) conditions. Lanes 1 and 9: molecular weight standards; 2 and 10: commercial zein; 3 to 7: PAN 202-606 kafirin preparations (WGF, 90% EF, 75% EF, 25% bran, 10% bran, respectively); 11 to 15: NK 283 kafirin preparations (WGF, 90% EF, 75% EF, 25% bran,10% bran, respectively).

and oligomers, respectively, of the kafirin polypeptides (El Nour et al 1998). They are believed to be due to disulphide cross-linking of the monomeric units α-, β-, and γ-kafirins. The bands with M_r ≈26,000 (γ-kafirins), 24,000 (α1-kafirins), 22,000 (α2-kafirins), and 18,000 (β-kafirins) are the monomeric kafirin polypeptides, respectively (El Nour et al 1998). The SDS-PAGE and amino acid analysis data thus show that the protein extracted from WGF, EF, and brans was essentially pure kafirin. A small difference noted was that the staining intensity of the oligomeric kafirins from the brands appeared to be higher (lanes 6, 7, 14, and 15) compared with kafirins extracted from WGF (lanes 3 and 11) and EF (lanes 4, 5, 12, and 13), possibly due to bound polyphenols increasing stain density.

Under reducing conditions (Fig. 1B), all kafirin preparations (lanes 3–7 PAN 202-606 and lanes 11–15 NK 283) were, again, essentially identical, with bands in the 45,000 and 50,000 region designated reduction-resistant dimers (Duodu et al 2002). The monomers were γ- (M_r 26,000), α1- (24,000), α2- (22,000), and β-kafirins (18,000). Dimers are not normally present under reducing conditions (El Nour et al 1998). However, Duodu et al (2002) observed reduction-resistant dimers in cooked sorghum proteins. It was postulated that cooking leads to the formation of disulphide-bonded oligomeric proteins, resistant to reducing agents. It is possible that hot aqueous-alcohol extraction used in this study increased the presence of these reduction-resistant dimers.

In contrast to the kafirin preparations, bands for β- and γ-zein were not observed (Fig. 1A and B, lanes 2 and 10). Their absence in commercial zein agrees with findings by Parris et al (1997) using RP-HPLC, and Parris and Dickey (2001) using SDS-PAGE, which showed commercial zein to consist almost exclusively of α-zein. Commercial zein is usually extracted from corn gluten meal, a coproduct of maize wet milling (reviewed by Shukla and Cheryan 2001). The maize is first steeped with sulfur dioxide to help soften the kernels and facilitate removal of starch. Sulfur dioxide is thought to weaken the maize matrix structure by breaking disulfide cross-links involving β- and γ-zeins. Once reduced, these proteins are apparently water-soluble and are possibly eliminated with the steep water (reviewed by Shukla and Cheryan 2001).

Under reducing conditions (Fig. 1B), the β-kafirins in the kafirins extracted from brans (lanes 6, 7, 14, and 15) appeared to be darker and more defined compared with β-kafirins in the kafirin extracted from the WGF and EF. Because the level of polyphenols was higher in the kafirins extracted from the brans, it would appear that the bound polyphenols had a higher affinity toward the β-kafirins. The β-kafirins have been reported to be very high in the amino acid proline (reviewed by Shewry 2002). Proline-rich proteins have a high affinity for plant polyphenols, primarily driven by hydrophobic association, which may then be stabilized by hydrogen bonding (Murray et al 1994). This phenomenon was not observed under nonreducing conditions (Fig. 1A, lanes 6, 7, 14, and 15), possibly because concentration of monomeric β-kafirins was too low due to the formation of cross-linked oligomers.

CONCLUSIONS

Bran, a coproduct of the sorghum dry-milling industry is a rich source of protein. However, kafirin yields from bran fractions are lower than yields obtained from WGF and EF. This is because the bran contains proportionally less prolamin protein because prolamin is located solely in the endosperm. High levels of decortication of high-protein sorghum can increase the prolamin content of bran and result in more kafirin being extracted. There are, however, high levels of fat, polyphenol, and probably nonstarch polysaccharide contamination in kafirin preparations from bran. Also, kafirin from red sorghum bran is highly colored due to the high levels of polyphenols. Thus, bran could be a useful source of kafirin for industrial purposes but further purification steps may be required.

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