Extraction and Film Properties of Kafirin from Coarse Sorghum and Sorghum DDGS by Percolation

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ABSTRACT

The high cost of kafirin and zein restricts their use for bioplastic and food applications. Effective, simple and rapid kafirin/zein isolation processes are required. Here a percolation-type aqueous ethanol solvent extraction process from coarse meals (grits) and coarse sorghum DDGS for kafirin and zein isolation employing a low ratio of extractant to meal (2.5:1) was investigated, which is potentially applicable in the grain bioethanol industry. Post-extraction filtration times were more than twice as fast using coarse meals compared to fine flours. Washing the meals to prior to extraction to remove starch improved protein preparation purity to 73-85% compared to 68-72% for unwashed meals. Hence, no subsequent filtration or centrifugation step is required to clean up the kafirin/zein solution prior to solvent evaporation. With a single extraction step, kafirin/zein yields were 48% (protein basis) for DDGS and 53-70% for washed sorghum/maize meals. Cast films were used as a model bioplastic system to evaluate extracted kafirin/zein functional properties. DDGS kafirin films had rough surfaces but had the lowest water uptake and in vitro digestibility, due to heat induced disulfide crosslinking during DDGS processing. Extraction by percolation using coarse meal/DDGS has potential to improve kafirin/zein viability.

INTRODUCTION

For the past 30 years there has been an upsurge in research into zein and kafirin for bioplastics and food (gluten-free applications), largely driven by environmental concerns (Taylor et al 2013; Taylor et al 2016). However, there are very few actual commercial products made from zein or kafirin. High cost and inferior functional properties of these materials are the main reasons given (Lawton 2002; Lagrain et al 2010). The availability of very large amounts of protein-rich co-products, from bioethanol production using maize or sorghum, has potential to improve the economics of zein and kafirin extraction (Wang et al 2009; Anderson and Lamsal 2011).

Commercially, zein is generally extracted from corn gluten meal using aqueous ethanol or aqueous isopropanol at high pH and high temperature, 70°C, using a modified method patented by Carter and Reck (Lawton 2002). Kafirin is not produced commercially at any significant level. However, compared to zein, kafirin has potentially better functional properties in terms of film formation, as an encapsulation vehicle and as an emulsifying agent, as it is more hydrophobic and less digestible than zein (Bean and Ioeger, 2015; Xiao et al 2015; Espinosa-Ramírez and Serna-Saldivar 2016). Without a commercial source, kafirin is unlikely to fulfill its potential as a biomaterial. Lately, there has been activity in developing processes to extract zein and kafirin during the grain bioethanol production process (Anderson and Lamsal 2011). Generally, studies have involved extracting the proteins from distillers dried grains and solubles (DDGS) (Cookman and Glatz 2009; Wang et al 2009). However, one process has been described where zein is extracted from the grain prior to the dry grind bioethanol process (Anderson and Lamsal 2011; Shane et al 2015). In fact, there is a more general interest in recovering various co-products ahead of the process for economic and environmental reasons (Purtle and Zullo 2015).

Concerning extraction technologies, raw materials of small particle size, 200-400 μm have been shown to extract high yields of zein in a short time by increasing the rate of diffusion of the solvent into the endosperm particles (Shukla et al 2000). Also, vigorous mixing has been shown to reduce diffusion limitations during zein extraction (Dickey et al 1998). However, in the bioethanol dry grind process, fine grinding that would produce a flour is not economical and not done in practice (Anderson and Lamsal 2011). A coarse meal produced by hammer milling is generally used. An extraction method that also does not involve fine milling of DDGS before kafirin or zein extraction would be advantageous.

Percolation of solvent through particulate plant materials is a method used to extract essential oils and to de-fat flaked soy (Kaufmann and Christen 2002; Becker1971). Thus, the present study investigated a percolation-type process for kafirin extraction using decorticated coarsely ground sorghum and sorghum DDGS, using a low ratio of solvent to meal. Cast free-standing films were used as a model to determine the functionality of the extracted proteins in terms of stability in water and water uptake and break down under simulated digestion. Zein was extracted from coarse maize meal to determine whether the extraction methodology could be more widely applied. Two different additives to the aqueous ethanol solvent systems were investigated. NaOH was used to give high pH as per the method of Carter and Reck (1970) and acetic acid to give low pH, in a similar way to Wang et al. (2009), who used HCl as part of an aqueous ethanol extractant for kafirin from sorghum DDGS.

MATERIALS AND METHODS

Materials

Total kafirin was extracted from commercial decorticated (approx. 90% extraction rate) red non-tannin meal (grits) (Tiger Brands, Bryanston, South Africa) and from commercial sorghum DDGS meal, kindly donated by Dr G. Peterson, Texas A&M University, Lubbock, Texas. For comparison, total zein was extracted from refined (approx. 80% extraction rate) white maize meal (grits) (Pride Milling, Vorsterskroon, South Africa).

The particle sizes of the meals and DDGS measured by sieving 100 g of the material through a stacked set of standard sieves of mesh size 2000, 1000, 500 and 250 µm. Material retained on each sieve was weighed and the percentage particle size distribution recorded. The analysis was carried out in triplicate. Particle size of the meals was >96% between 500 and 2000 µm for sorghum and maize meals and >90% for sorghum DDGS.

The sorghum meal was milled into a fine flour using a laboratory hammer mill (Falling Number 3100, Huddinge, Sweden) fitted with a 250 µm sieve and had a particle size of 96% <250 µm.

Methods

Kafirin and Zein Extraction

Extraction was carried out using the percolation apparatus shown in Figure 1. The apparatus comprised a glass vessel with jacket for circulation of hot water to maintain the extraction temperature and a tap at the bottom to drain the extraction solvent. A 250 µm opening stainless steel mesh screen was formed into a basket to contain the coarse meals within the vessel and closed the bottom of the vessel. A handle was attached to the mesh basket to enable agitation.

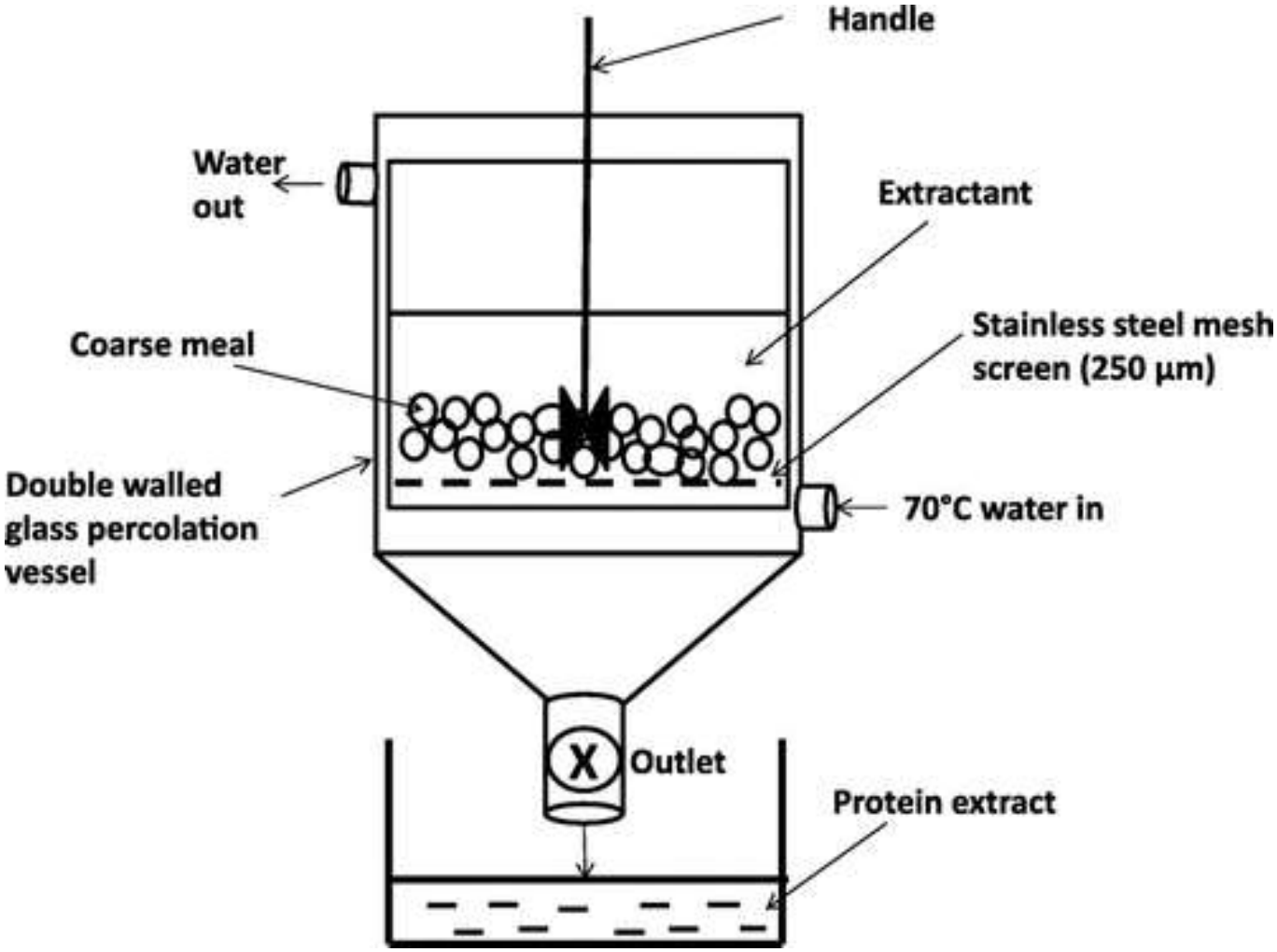

Fig. 1. Percolation vessel.

The meals/flours (100 g) were transferred to the basket in the extraction vessel. In one treatment, the effect of washing the meals to remove adhering starch was investigated. Kafirin and zein are prolamin proteins and so by definition are insoluble in water (Wilson et al 1981; Evans et al 1987). Consequently, these proteins would not be lost during the washing process to remove starch. The meals were immersed in ambient temperature (25^{o}C) tap water and agitated and the water drained away. This was repeated until the wash water was no longer cloudy.

Kafirin/zein extraction was carried out with 70% (w/w) aqueous ethanol containing 1.0% (w/w) sodium metabisulfite plus 0.35% (w/w) glacial acetic acid or 0.35% (w/w) NaOH at 70°C for 1 hour using 100 g flour/meal and 250 mL extractant, i.e. an extractant to meal/flour ratio of 2.5:1. Extraction conditions were based on our previous work (Emmambux and Taylor 2003). The ratio of flour to meal was reduced by half compared to our previous work (5:1) enabling a significant reduction in the amount of solvent used without all the solvent being absorbed into the meal. During this time the mixture was agitated every 5 min of the extraction period and the same solvent was percolated through the flours or meals twice. On completion of extraction, the filtration time was recorded as the time taken for extractant to drain out of the percolation apparatus. No further filtration was carried out.

On draining from the percolation apparatus, the alcoholic solvent was allowed to evaporate from the extract overnight at ambient temperature from shallow open trays placed in a fume hood. After evaporation of the ethanol from the extract, cold distilled water (200 mL) (8-10°C) was added to precipitate the kafirin/zein preparations. When sodium hydroxide was included in the extractant, the pH of the protein slurry was adjusted to approximately pH 5

with 1M HCl before the cold water addition. The precipitated protein concentrates were then recovered by vacuum filtration before air drying at ambient temperature.

Protein Content

Protein contents (N x 6.25) were determined using the Dumas total combustion method, Approved Method 46-30 (AACC, 2000). Sorghum and maize meals and flours used for extraction contained 10.0% and 7.5% protein (dw basis), respectively and sorghum DDGS had 28.2% protein. Protein yields for the extractions were calculated as weight of total recovered protein divided by the grain protein content × 100.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

The protein preparations were characterized by SDS-PAGE under reducing and non-reducing conditions as described by Anyango et al. (2013) on pre-prepared 4−12% Bis-Tris (BT) gradient gels (Invitrogen Life Technologies, Carlsbad, CA) using an X Cell SureLock Mini-Cell electrophoresis unit (Invitrogen Life Technologies). The protein loading was ≈10 μg. Invitrogen Mark12 Unstained Standard was used. Protein bands were stained with Coomassie Brilliant Blue R-250 and photographed using a flat-bed scanner.

Kafirin and Zein Film Preparation

Films were cast from the kafirin and zein preparations as described by Taylor et al. (2005b) without the inclusion of a plasticizer. The protein preparations, equivalent to 1.2 g, 100% protein were dissolved in 70% (w/w) aqueous ethanol at elevated temperature (70°C). Protein solutions (1.5 g) were weighed into rectangular silicone baking trays (28 mm x 69 mm) and gently swirled around to distribute the contents evenly on the bottom of the

trays. Films were dried on level shelves in an oven (not forced draft) at 50°C overnight. Films were weighed before being photographed by scanning.

Film Water Uptake

Films (28 x 69 mm) immersed in 0.2 M sodium phosphate buffer (pH 6.8) were incubated at 39°C for 12 hours. On cooling, film integrity was recorded by scanning whilst the films were still in the sodium phosphate buffer. Films were then removed from the buffer and the surface water carefully blotted with a paper towel before weighing. Percentage water uptake of the films was calculated as follows:

$$\% \text{ water uptake} = \frac{\text{Mass of films after immersion in buffer (mg) - initial dry mass (mg)}}{\text{Initial dry mass (mg)}} \times 100$$

Scanning Electron Microscopy

Films, both before and after the water uptake test were mounted on a stub with double-sided tape and sputter coated with gold before being viewed using a Joel JSM-5800 LV scanning electron microscope (Tokyo, Japan).

In vitro Protein Digestibility of Kafirin and Zein Films

Approximately 250 mg of each film sample was accurately weighed into centrifuge tubes. Pre-heated (39°C) pepsin (1.0 g/L, Sigma P-7000) in 0.1 M HCl (pH 1.9) (35 mL) was added and samples were incubated for 1h at 39°C with constant rotation. On completion of the digestion period, the residual material was separated from the enzyme solution by centrifugation and the supernatant carefully removed. The pellet was washed 3 times with distilled water (10 mL) before the pellet was dried at 105ºC for 24 hours, cooled and weighed. A further set of samples were exposed to pepsin digestion and then after washing

with distilled water were incubated, with shaking at 39°C for 12 h with 35 mL 0.5 M, KH_2PO_4, phosphate buffer, (pH 7.75), containing 3 g/L pancreatin (Sigma P-7545). Again, on completion of the digestion period, the residual material was separated from the enzyme solution by centrifugation and the supernatant carefully removed. The pellet was washed 3 times with distilled water (10 mL) before the pellet was dried at 105ºC for 24 hours, cooled and weighed. The percentage pepsin digestion and pepsin digestion combined with pancreatin digestion were determined using the weight of the material both pre- and post-digestion . Pancreatin digestion alone was determined by difference between the combined pepsin and pancreatin digestion and pepsin digestion.

Statistical Analysis

Experiments were repeated at least twice. One-way analysis of variance (ANOVA) was performed using SPSS software (IBM Software, Johannesburg, South Africa). Significant differences among the means were determined by Fisher's Least Significant Difference Test (LSD) at a 5% significance level.

RESULTS AND DISCUSSION

Kafirin and Zein Extraction Yield, Protein Purity and Composition

Using the percolation method, filtration of the kafirin containing solvent was far more rapid with the coarse meals than the fine flour (Table 1). Filtration of the fine flour took more than twice the time (570-572 s) than unwashed coarse meals (254-260 s) or washed coarse meals (254-259 s). Since fine flours rapidly block filters, conventional laboratory extraction methods for kafirin and zein use centrifugation to separate the fine flour from the extract (Emmambux and Taylor 2003). Washing of coarse meals did not affect filtration times. The faster filtration time of the meals was probably due to the larger inter-particle spaces between

TABLE I

Effects of Raw Materials, Extractant, and Prewashing on Filtration Time, Extraction Yield (g of protein extracted/total protein in 100 g of raw material, db), and Protein Purity (g of protein/100 g of protein preparation, db) of Flour and Meals Extracted Using a Modified Percolation Procedure[a]

Sample	Extract Filtration Time (s)		Extraction Yield		Protein Preparation Purity	
	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
Sorghum flour						
70% Aq. EtOH + 1% $Na_2S_2O_5$ + 0.35% AA	570 ± 2C	NA	50.0 ± 0.6C	NA	67.7 ± 1.5A	NA
70% Aq. EtOH + 1% $Na_2S_2O_5$ + 0.35% NaOH	572 ± 2C	NA	54.3 ± 3.0C	NA	67.2 ± 0.9B	NA
Meals						
Sorghum meal						
70% Aq. EtOH + 1% $Na_2S_2O_5$ + 0.35% AA	259 ± 3Ba	255 ± 0Aa	59.9 ± 0.4Bb	52.8 ± 0.4Ba	68.1 ± 0.2Ca	76.5 ± 0.1Bb
70% Aq. EtOH + 1% $Na_2S_2O_5$ + 0.35% NaOH	255 ± 2Aa	259 ± 2Ca	61.3 ± 0.2Bb	57.8 ± 1.5Ca	68.9 ± 0.4Ca	85.1 ± 0.1Eb
Sorghum DDGS meal						
70% Aq. EtOH + 1% $Na_2S_2O_5$ + 0.35% AA	254 ± 1Aa	254 ± 0Ca	53.5 ± 0.5Ab	48.4 ± 1.5Aa	77.3 ± 0.8Ea	78.5 ± 0.1Ca
Maize meal						
70% Aq. EtOH + 1% $Na_2S_2O_5$ + 0.35% AA	260 ± 2Ba	259 ± 1Ca	68.3 ± 0.9Cb	55.6 ± 2.4BCc	68.3 ± 1.4Ca	73.0 ± 0.3Ab
70% Aq. EtOH + 1% $Na_2S_2O_5$ + 0.35% NaOH	260 ± 1Bb	257 ± 1Ba	71.7 ± 2.0Da	70.1 ± 1.3Da	71.8 ± 0.1Da	80.3 ± 0.9Db

[a] Mean values with different uppercase letters in a column differ significantly from each other ($P < 0.05$). Mean values for each flour or meal type within a dependent variable (for example, filtration time) with a different lowercase letter in a row differ significantly from each other ($P < 0.05$). NA indicates not applicable, not able to wash finely milled flour; $n = 2$. Aq. EtOH = aqueous ethanol; $Na_2S_2O_5$ = sodium metabisulfite; AA = acetic acid; and DDGS = distillers dried grains and solubles.

large particles making solvent passage easier and faster than though the small spaces between fine flour particles (Palmer 1999). With the coarse meals, no further filtration or centrifugation appeared to be necessary prior to prolamin recovery from the solvent as the extracts were clear. However, extracts from the flour were cloudy.

Protein extraction yields of sorghum and maize using the percolation methodwere 50-72% (Table 1). These values were comparable to the 54.3% obtained when fine sorghum flour was extracted with the same solvent, (inclusive of NaOH), with vigorous stirring, using a solvent to flour ratio of 5:1 at 70°C (Taylor et al 2005a).

Furthermore, the protein extraction yields were higher with unwashed coarse sorghum meal (60 and 61%) compared to those from sorghum flour (50 and 54%) using the percolation method. This may have been due to more extractant being retained by the flour than the meal, reducing the volume of extractant recovered and consequently reducing the protein yield from the flour. In practice, more complete kafirin extraction would be obtained from either flour or meal by running additional extractant through the spent grain, enabling the recovery of more solubilized protein. This extractant could then be re-used as the extraction solvent with fresh grain. The inclusion of acetic acid instead of NaOH in the extraction mixture resulted in a reduction in kafirin extraction yield (up to 10%) (Table 1). The sacrifice of extraction yield with the inclusion of acetic acid was offset by not needing to increase the pH of the protein solution prior to protein precipitation, as is required with NaOH.

Protein yields from washed meals were lower than from unwashed meals, for all three meal substrates: sorghum, maize and sorghum DDGS (Table 1). This was probably simply because of residual wash water remaining in the meal and consequent dilution of the solvent to below 70% (w/w) ethanol and hence reducing its kafirin/zein solvation capacity. This

could be corrected in future by adding less water when preparing the solvent. Notwithstanding this, the lower yield obtained with washing the sorghum and maize meals was compensated by the substantially increased kafirin and zein preparation purity, from 77% and 85% for kafirin and from 72% and 80% for zein, when acetic acid or NaOH was included in the extractant, respectively (Table 1). This was due to the removal of starch granules and fine starch-rich particles. This will be discussed in detail later.

Protein yield from DDGS was only ~90% of that achieved using sorghum meal. Yield was also lower than previously published methods using DDGS (Wang et al 2009). This was probably due to the poor solubility of the DDGS kafirin as a result of disulfide crosslinking caused by exposure to high temperatures during its processing (Wolf and Lawton 1997). SDS-PAGE of the kafirin extracted from DDGS showed a high proportion of disulfide linked polymers (Figure 2) and there may have been higher molecular weight polymers present in the DDGS that were not easily solubilized by the percolation process used. Importantly, the purity of the kafirin preparation from the sorghum DDGS was considerably higher than that from the unwashed sorghum meal, 77% versus 68%, and comparable to that from the washed sorghum meal (77%) (Table 1), but slightly lower than the 82% quoted in literature (Lau et al 2015). This confirms that it was the presence of starch in the protein preparations from the unwashed meals that reduced their purity, as there is very little starch in DDGS. Starch is almost completely hydrolyzed and fermented during the bioethanol production process resulting in little residual starch in DDGS (Corredor et al 2006).

Concerning extraction of zein, percolation process resulted in a somewhat higher yield than with kafirin (approx.13% of unwashed meal and 6% for washed meal with inclusion of acetic

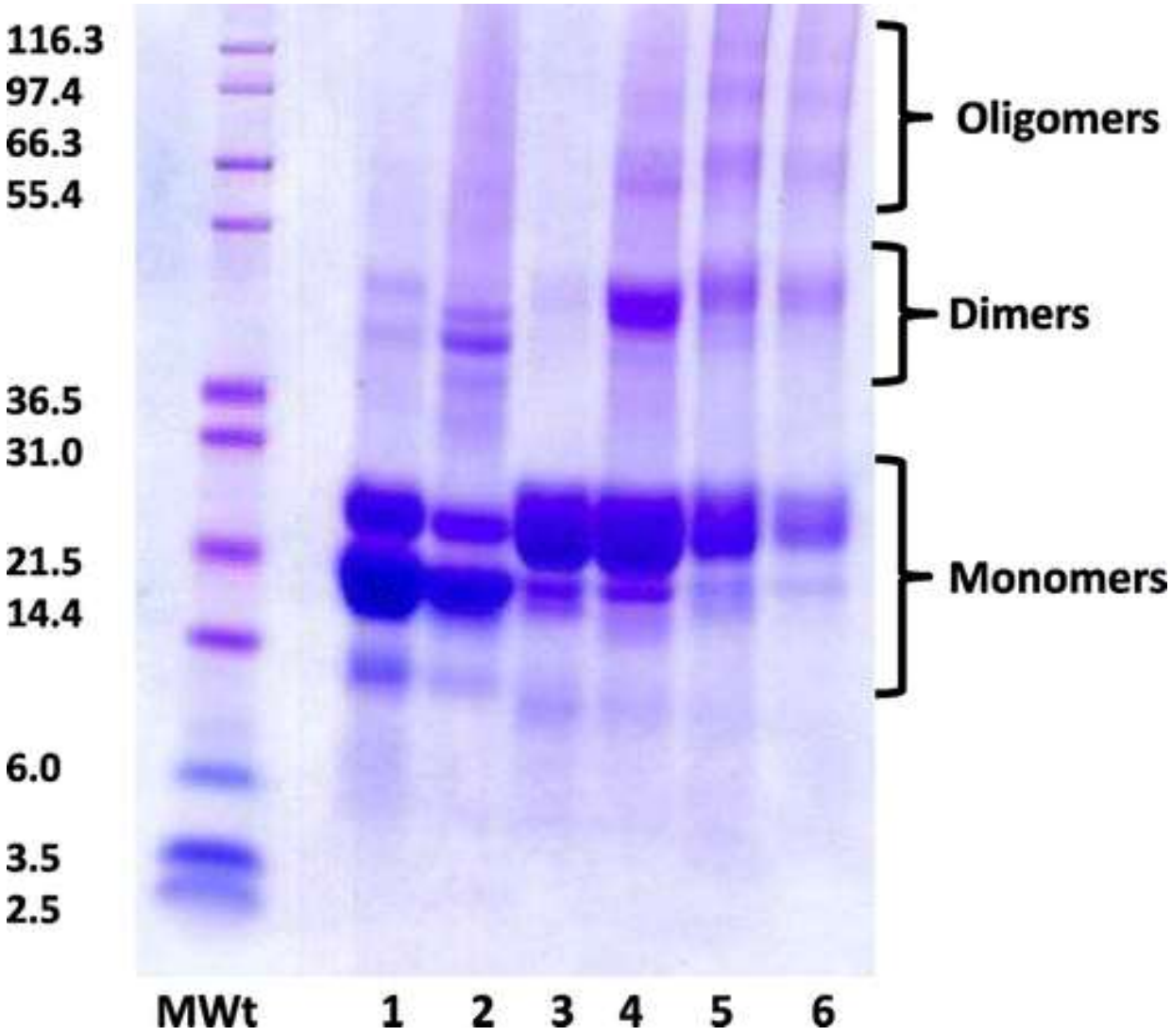

Fig. 2.
SDS-PAGE of kafirin and zein preparations extracted from washed sorghum, maize, and washed sorghum DDGS meal under reducing and nonreducing conditions. MWt = molecular weight standards; track 1 = kafirin, reducing conditions; track 2 = kafirin, nonreducing conditions; track 3 = DDGS kafirin, reducing conditions; track 4 = DDGS kafirin, nonreducing conditions; track 5 = zein, reducing conditions; and track 6 = zein, nonreducing conditions.

acid) and similar purity (Table 1). The higher extraction is probably because zein is less hydrophobic than kafirin (Duodu et al 2003) and hence more soluble in aqueous ethanol.

SDS-PAGE of kafirin and zein preparations under reducing and non-reducing conditions confirmed the presence of all the prolamin sub-classes (Figure 2). Bands with molecular weights of approximately 13 kDa-28 kDa (monomers) were observed which correspond to the α_1- α_2-,γ- and β-kafirin or zein sub-classes (El Nour et al 1998; Shewry, 2002). Under non-reducing conditions, the bands with molecular weights of approximately 13 kDa were fainter in all the tracks. Bands of approximately 40 kDa-50 kDa (dimers) occurred in all kafirin and zein preparations under non-reducing conditions (Figure 2, tracks 2, 4, 6) and became fainter on reduction. The relative concentration of the dimers was greater in DDGS kafirin preparations (track 4) than kafirin extracted from sorghum meal (track 2) or zein (track 6), indicating a higher degree of disulfide linked polymerization caused by exposure to high temperatures during processing as described above. Some of these polymers would have been too large to enter the gel (Emmambux and Taylor, 2009), resulting in the fainter bands of track 6.

Film Functionality

Film Visual Appearance

Having confirmed the composition of the extracted kafirin and zein preparations, free-standing films were used as a model to determine film or coating functionality in terms of stability in aqueous solutions and degradation under simulated digestion. No plasticizer was added to the films, so as expected all the films were brittle. The kafirin films were yellow to orange in color, whereas the zein films were pale yellow (Figure 3). None of the films were

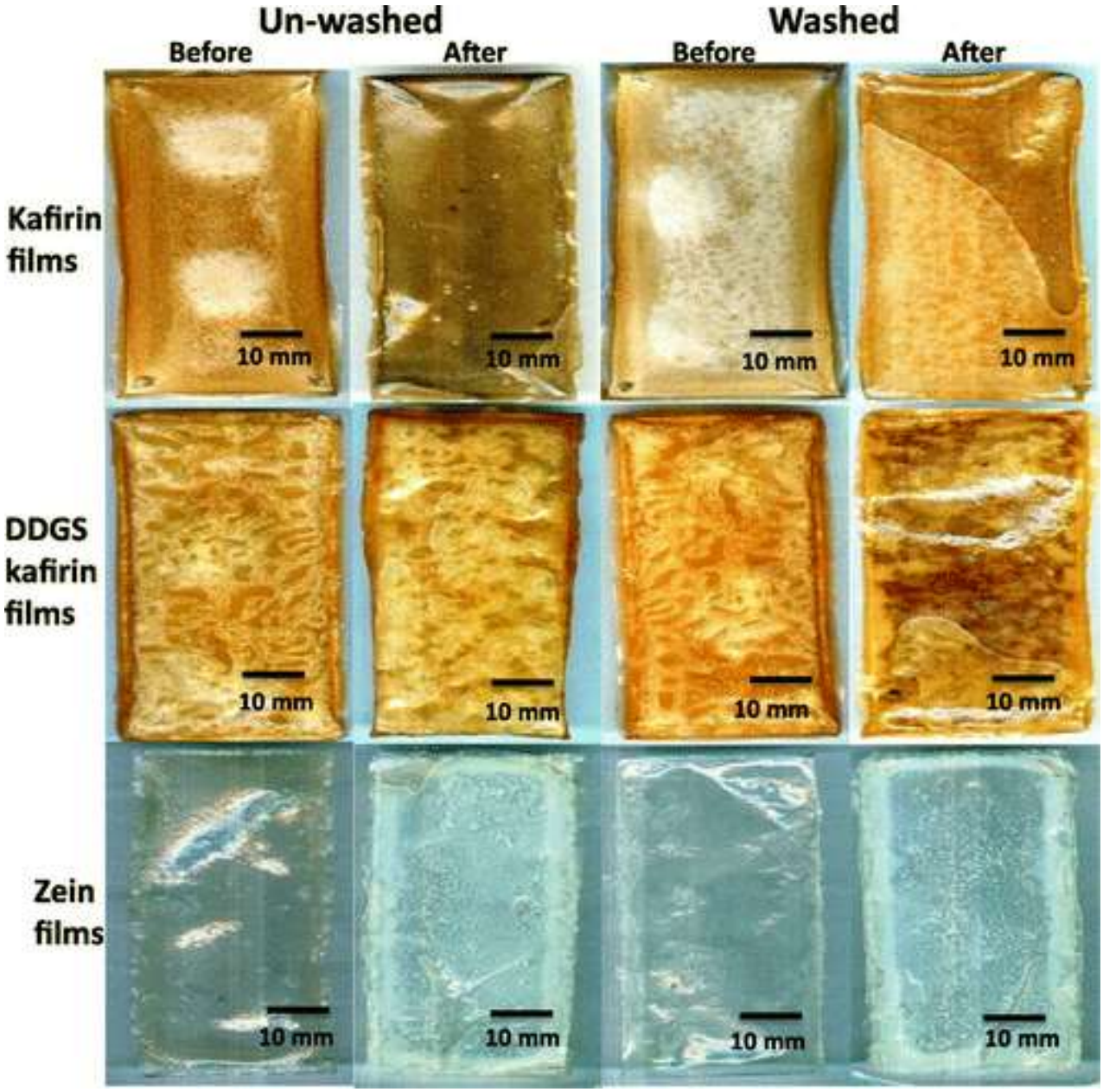

Fig. 3.
Effects of washing the meals prior to kafirin or zein extraction on film phosphate buffer (0.2M, pH 6.8) absorption before and after immersion for 12 h at 39°C.

transparent unlike similar films containing a plasticizer including lactic acid which aids the solubilization of the prolamins (Taylor et al 2005b). The surface of the DDGS kafirin films were rougher than any of the other films, probably due to decreased solubility of the kafirin as a result of heat induced polymerization (Figure 2) (Hamaker et al 1986). Microwave heat treatment of kafirin prior to film formation has been shown to result in films with rough surfaces and was attributed to un-dissolved kafirin (Byaruhanga et al 2005). There was no apparent visual effect of washing the meals prior to protein extraction on film formation (Figure 3).

Film Water Uptake

After incubation of the films at 39°C for 12 hours in 0.2 M phosphate buffer (pH 6.8), all films absorbed water but remained whole (Figure 3). The swelling of zein films was more obvious than that of the kafirin films. Particularly noticeable on soaking of the zein films was the formation of a white surface layer. It was probable a similar surface layer occurred on the kafirin film surfaces but was less noticeable due to the darker colour of the kafirin films. A similar white powdery deposit was reported to occur when a kafirin coating was applied to litchis but no explanation for this occurrence was given (Taylor et al 2006).

SEM of kafirin and zein films prepared from preparations extracted from non-washed meals (Figure 4) showed the presence of starch granules with some pores (indicated by arrows) before soaking. DDGS kafirin films showed no starch granules but had small pores distributed throughout the films. After soaking all the films made from kafirin and zein extracted from non-washed meals developed pores and defects. On soaking, water was absorbed by the starch granules present as impurities in the protein preparation, increasing

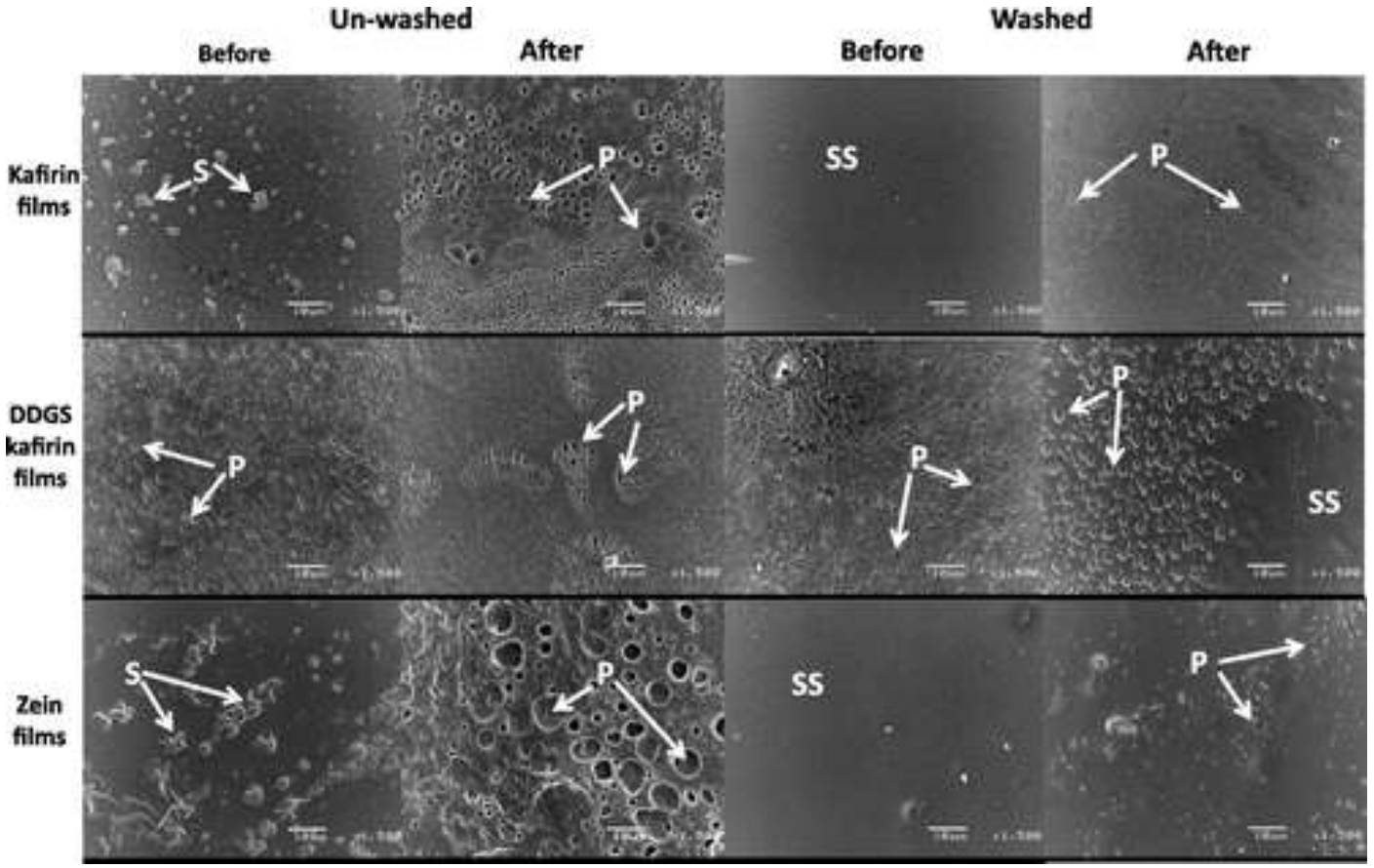

Fig. 4.
Scanning electron microscopy images showing the effects of washing the meals prior to extraction on film surface morphology before and after immersion in 0.2M phosphate buffer (pH 6.8) for 12 h at 39°C. SS = smooth surface; P = pores; and S = starch granule.

their size. Extended soaking times would cause some of the starch granules to become dislodged from the film matrix, resulting in the observed holes.

The presence of little or no starch in the DDGS kafirin resulted in fewer, smaller holes in the kafirin DDGS films on soaking (Figure 4). SEM of films made from preparations extracted from washed sorghum and maize meals (Figure 4) had smooth surfaces with very few defects. After soaking, these films showed only a few small pores which were evenly distributed throughout the films. After soaking, DDGS kafirin films (Figure 4) developed numerous pores. The reason for this is unclear but may be related to the inferior film quality caused by the poorer solubility of DDGS kafirin in the film casting solution due to heat induced polymerization, as described.

Measured water uptake showed clear differences between the different kafirin and zein preparations (Table 2). Kafirin and zein extracted from non-washed meal had water uptakes of 65.4% and 66.7% respectively, which is similar to the 70.7% reported by Emmambux et al (2004) for kafirin films. Removing starch by pre-washing the meals before kafirin and zein extraction decreased the water uptake of the kafirin films by approx. 15% and zein films by approx. 11%. Overall DDGS kafirin films had the lowest water uptake of 37% without pre-washing and 35% with pre-washing. This low water uptake was in spite of the films poor visual quality and was again probably due to the highly cross-linked nature of the DDGS kafirin. Similarly, it has been found that films made from microwave heat treated kafirin had lower water vapor transmission than that of non-heat treated kafirin (Byaruhanga et al 2005). This was also attributed to heat induced disulfide crosslinking. As expected, since the DDGS contained little starch, pre-washing DDGS grain prior to kafirin extraction resulted in little difference in water uptake on soaking these films (Table 2). Clearly, the removal of starch

TABLE II
Sodium Phosphate Buffer (0.2M, pH 6.8) Uptake of Kafirin and Zein Films[z]

Preparation and Source	Extraction Pretreatment	Water Uptake (%)
Kafirin, washed sorghum meal	Unwashed	65.4 ± 0.2e
	Washed	55.1 ± 0.5c
Kafirin, washed sorghum DDGS	Unwashed	37.4 ± 0.1b
	Washed	35.0 ± 0.1a
Zein, washed maize meal	Unwashed	66.7 ± 0.1f
	Washed	58.9 ± 0.5d

[z] Mean values with different lowercase letters in a column differ significantly from each other ($P < 0.05$); $n = 2$. DDGS = distillers dried grains and solubles.

TABLE III
Effects of Kafirin and Zein Preparations from Different Sources on the In Vitro Digestibility of Kafirin and Zein Films[z]

Preparation and Source	Pepsin Digestion (%)	Pancreatin Digestion (%)	Combined Pepsin and Pancreatin Digestion (%)
Kafirin, washed sorghum meal	38.1 ± 2.2a	21.9 ± 1.7b	60.0 ± 1.7b
Kafirin, washed sorghum DDGS	40.6 ± 3.6a	14.8 ± 1.5a	55.4 ± 1.5a
Zein, washed maize meal	61.0 ± 3.9b	12.2 ± 1.7a	73.2 ± 1.7c

[z] Mean values with different lowercase letters in a column differ significantly from each other ($P < 0.05$); $n = 2$. DDGS = distillers dried grains and solubles.

and heat induced crosslinking in the case of DDGS kafirin resulted in films with much better resistance to water uptake. The ability of a film or coating to remain functional for the lifespan of its use is critical to the success of a particular application.

In vitro Simulated Digestion of Kafirin and Zein Films

Knowledge of the degradation of a film in a simulated digestive environment gives an indication of the efficacy of the material if used as an encapsulating agent for an ingested active ingredient. In vitro pepsin digestion of zein films was much higher (61%) than either kafirin film types (38-41%) (Table 3). Similar pepsin digestion of kafirin (41.5%) and zein films (54.4%) have been reported (Taylor et al. 2007). Zein films had similar pancreatin digestibility to DDGS kafirin (12% vs 15%), both of which were lower than that of films made from sorghum meal (22%). Overall, DDGS kafirin films (55%) had the lowest digestibility, followed by kafirin films (60%), with zein films being the most readily digested (73%). Again, it appears that the heat induced polymerization that the DDGS had undergone during processing was responsible for its films lower digestibility, as was found by Byaruhanga et al (2005) when using microwave heat-treated kafirin. The ranking of the films, in terms of digestibility closely matched that of film water uptake (Table 2) and was probably related to the relative hydrophobicity of the proteins (Belton et al 2006) and in the case of the DDGS, heat induced disulfide polymerization.

CONCLUSIONS

This work has shown that good yields of kafirin and zein that have good film functional properties can be obtained by extraction of the proteins from coarse meals using a percolation-type extraction procedure. The percolation process uses coarse meals as available in grain bioethanol manufacture. Furthermore, less solvent is required, and filtration times are

faster than previously described extraction technologies. Moreover, no further filtration or centrifugation prior to protein recovery is required, all factors making the process potentially more economically viable. The use of DDGS as a raw material could improve the economic viability of the process still further. The high protein content of DDGS results in acceptable yields of high purity kafirin, which when made into films have better functional properties in terms of lower water uptake and lower digestibility than kafirin or zein extracted from coarse meals, due to heat induced disulfide crosslinking that occurred during DDGS processing.

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