# CHAPTER 3

## 3 DISCUSSION

In this chapter the following will be discussed: Methodological considerations of the experimental methods used in this research, namely sorghum variety and decortication, kafirin extraction and characterisation (SDS-PAGE and amino acid analysis), biofilm preparation, and mechanical and water barrier property testing; the extraction of kafirin from sorghum bran, and the major characteristics and components of bran kafirin biofilms.

Condensed tannin-free sorghum varieties, NK 283 (red hybrid) and PANNAR 202-606 (mixed white hybrid) were used to prepare the different sorghum milling fractions, including the bran, from which kafirin was extracted and used for the preparation of kafirin biofilms. The chemical compositions of the different sorghums used in this study were typical of sorghum according to a review by Serna-Saldivar and Rooney (1995). However, NK 283 contained more protein and had twice the level of total polyphenols, compared to PANNAR 202-606. The higher protein content of NK 283, compared to PANNAR 202-606, is assumed to be due to differences in the agronomic and environmental growing conditions of these sorghums (reviewed by Serna-Saldivar and Rooney 1995), as they were grown in different locations in South Africa during different years (near Randfontein, Gauteng Province, 1998 and Lichtenburg, North West Province, 2001, respectively).

Differences in total polyphenol content, and consequently the colour of the different sorghum varieties used, are due mainly to genetic variation (Rooney et al 1980). The colour of the red sorghum is due to the presence of anthocyanin and anthocyanidin phenolic pigments in the grain's pericarp, which are largely absent in the white sorghum (Hahn et al 1984). Compared to white sorghum, red sorghum is the predominant type cultivated in South Africa (reviewed by Taylor and Belton 2002). This is in part due to the development of the sorghum (opaque) beer industry during the 20th century, which requires red sorghum for its production (reviewed by Taylor 1993). The presence of anthocyanin and anthocyanidin pigments in red sorghum malt gives the beer its characteristic pink-brown colour.

Although the production of sorghum beer is somewhat less today, red sorghum is still the most common variety grown. Thus, the surplus red sorghum produced is now used to meet the growing demand for the production of sorghum flour, used in the preparation of foods including porridges, snack foods, energy drinks and breakfast cereals (Sooliman 1993). As a result, far more red sorghum bran is produced compared to white. Thus the effect of sorghum variety (red versus white) on the extraction and purity of kafirin, and their effect on kafirin biofilm properties were investigated. This was done to determine if red sorghum bran could be an acceptable material for kafirin extraction, compared to white, thereby determining if the large amounts of industrial red sorghum bran could meet the possible demand needed for the commercial extraction of kafirin. Secondly, information on the properties of the different bran kafirin preparations and their biofilm properties (such as colour) will assist in identifying possible markets for the use of different kafirin preparations. This will help ensure the full utilisation of available industrial bran (red and white) for kafirin extraction.

The reason why bran of low (10%) and high (25%) levels of abrasive decortication were produced was to mimic industrial sorghum dry milling operations and obtain bran of different levels of decortication that are most likely to occur in industrial operations. In South Africa, industrial abrasive decortication is commonly done using PRL-dehullers, followed generally by hammer milling of the sorghum endosperm material into meal or flour (Taylor and Dewar 2001). However, such methods are still inefficient and can result in losses ranging from 5 to at least 20% of the grain weight, depending on the level of decortication desired, e.g. high decortication level for red sorghum to make whitish meal. Increasing levels of grain decortication result in the complete removal of the grain's pericarp and germ; but increasing removal of endosperm from the grain into the bran (Hahn 1969). This could have an influence on the protein content and composition of different bran sources, as well as the extraction and purity of different kafirin preparations. Thus, the effect of decortication level on the extraction and purity of kafirin preparations, and their biofilm properties was investigated. These data could give an indication as to possible yields and purity of kafirin extracted from different sources of industrial bran, and what biofilm properties can be expected. However, it is possible that abrasive decortication using a pilot scale rice pearler, as used in this research, was a more 'gentle' and controlled process compared to industrial

decortication, and reduced levels of grain cracking could have occurred. Thus less kafirin rich corneous endosperm might have been in laboratory prepared bran compared to industrial bran.

Kafirin was extracted from the different sorghum dry milling fractions by batch extraction using hot aqueous ethanol containing alkali (sodium hydroxide) and a food grade reducing agent (sodium metabisulphite). This was done by a modification of the patented method of Carter and Reck (1970) for the commercial extraction of zein from corn gluten meal. However, compared to zein, kafirin is less soluble in aqueous ethanol, primarily due to slight differences in their amino acid composition (kafirin contains more hydrophobic amino acids valine, alanine and isoleucine than zein) (Wall and Paulis 1978). Differences in amino acid composition results in kafirin having a lower free energy of hydration than zein, thereby making kafirin more hydrophobic than zein (reviewed by Duodu et al 2003). According to Taylor et al (1984), kafirin is best extracted with 60% tertiary butanol with 0.05% dithiothreitol, a reducing agent used to break inter- and intra-molecular disulphide bonds, improving kafirin solubility. Alternatively, Hamaker et al (1995) suggested the use of an alkaline buffer containing sodium dodecyl sulphate (SDS) and a reducing agent followed by the precipitation of the non-prolamin proteins of sorghum with 60% tertiary butanol. However, these solvent systems are not food compatible and are therefore not suitable for the extraction of kafirin intended for food coating applications.

To improve the solubility and extraction of kafirin in aqueous ethanol reducing agent and alkali were added to the extracting solvent, and the extraction was carried out at high temperature (70°C). Sodium metabisulphite (a food compatible reducing agent) was used to ensure food compatibility of the extracted kafirin as it is intended for coating fruits and nuts. The use of alkali treatment at elevated temperatures (75°C) is believed to improve the solubility of prolamins by cleaving off amide groups, resulting in deamidation of amide containing amino acids (glutamine, asparagine and proline) (Boundy et al 1967, Chiue et al 1997, Wilson 1988). Protein deamidation is thought to cause unfolding of the proteins, break hydrogen bonds and disrupt hydrophobic interactions, thus increasing protein solubility. However, extraction of kafirin at temperatures higher than ambient temperature could result in protein denaturation, and the formation of high

molecular weight oligomers (Duodu et al 2002). This could reduce the solubility of kafirin in aqueous ethanol. In addition, the used of alkali treatment for the extraction of zein is thought to extract other proteins besides zein, resulting in increased protein extraction yield (reviewed by Lawton 2002). Hence, the purity of the different kafirin preparations was determined using SDS-PAGE and amino acid analysis.

SDS-PAGE is the electrophoretic system most commonly used to resolve and identify the heterogeneous polypeptides of prolamins by estimating their molecular weight against standards of known molecular weight. The polypeptide separation is performed using size-based separation in an electric field, as the presence of SDS gives all the polypeptides the same negative charge (reviewed by Reubsaet et al 1998). The polyacrylamide gel acts as a porous media resulting in a molecular sieving effect and the gel pore size is dependent on the separating gel concentration (reviewed by: Hames 1990, Michalski and Shiell 1999). Common gel concentrations used for the analysis of kafirin range from 12.4% (Taylor and Schüssler 1986) to 15% (El Nour et al 1998, Duodu et al 2002). However, it should be noted that SDS-PAGE is an empirical technique and large errors in the estimated molecular weights may arise if the prolamin polypeptides differ in amino acid composition from the proteins used as molecular weight standards (Noelken et al 1981).

SDS-PAGE of kafirin is commonly performed under reducing conditions to separate the monomeric polymers ($\alpha$-, $\beta$- and $\gamma$-kafirin). According to El Nour et al (1998) and Duodu et al (2002), SDS-PAGE under non-reducing conditions results in different band patterns and shows the presence of high molecular weight oligomers not previously observed under reducing conditions. However, kafirin is less soluble under non-reducing conditions, resulting in insoluble material not able to penetrate the gel. To improve kafirin solubility under non-reducing conditions (as well as reducing conditions), 10 mg protein was dissolved in 3 mL buffer (opposed to 10 mg/1 mL buffer previously used) and the samples were mixed vigorously in a test tube with added glass beads to break up insoluble kafirin particles. The boiling time was also increased from 5 min (commonly used) to a total of 15 min, and the samples were mixed periodically during the boiling step to

ensure complete kafirin solubilisation. The steps used resulted in a clear protein-buffer solution.

Kafirin purity can also be characterised by its amino acid composition. The amino acid composition of kafirin has been reported from several sources, and generally good agreement exists. Kafirin is high in glutamic acid (glutamine), and non-polar amino acids (leucine, alanine, valine and proline) and low in lysine and cystine (reviewed by Wall and Paulis 1978, Taylor and Schüssler 1986). Although amino acid analysis is considered one of the best quantitative methods used to identify proteins, problems such as variability in the inherent liability of the different amino acids to hydrolysis exist (West et al 1996). Additionally, differences in instrument performance, hydrolysis/derivatisation conditions, sample contamination and data reduction methods do occur. These influence amino acid analysis results reported by different laboratories and care should be taken when comparing values reported in the literature.

Variability in the susceptibility of different amino acids to hydrolysis includes the destruction of cystine and tryptophan, and the deamidation of glutamine and asparagine to their corresponding acids, glutamic acid and aspartic acid, respectively (West et al 1996). Deamidation of glutamine and asparagine results in the content of the latter four amino acids being reported as glutamic acid plus glutamine and aspartic acid plus asparagine. Information on the levels of cystine and deamidation reaction products between the different extracted kafirin preparations and commercial zein could give further evidence as to why kafirin was more prone to gelling during biofilm preparation, compared to commercial zein. Higher levels of cystine in kafirin could result in increased levels of polypeptide disulphide cross-linking (reviewed by Wall and Paulis 1978), possibly resulting in protein aggregation and gelling. Higher levels of deamidation reaction products in commercial zein could reduce protein gelling (reviewed by Lawton 2002), possibly improving protein solubility in aqueous ethanol.

Free-standing kafirin and zein biofilms were prepared by casting heated aqueous ethanol protein solutions (16% protein (w/w)) with added plasticizer mixture, into Petri dishes and drying at 50°C overnight. Preliminary work showed that using a controlled drying temperature reduced differences in solvent evaporation rate, as

found on different days due to variation in environmental (temperature and relative humidity) conditions, occurring in our laboratory. Drying of the films at 50°C resulted in films that were clear and glossy, compared to those dried at ambient temperature. However, the effect of drying at different elevated temperatures, which could have affected the film forming properties of these films, was not studied.

Since the different protein preparations contained different levels of crude fat, all protein samples were defatted before biofilm preparation, to eliminate the possible effect of fat on mechanical and water barrier properties of the different biofilms. Free fatty acids, including palmitic, stearic (Lai et al 1997) and oleic acid (Lai and Padua 1998) have been reported to be good plasticizing agents for zein. They can improve the water barrier properties of zein biofilms due to their highly hydrophobic nature. During the preparation of the film casting solutions, it was observed that all the kafirin preparations had poor solubility in 70% ethanol, even at high temperatures, compared to commercial zein. This is because kafirin is by nature less soluble in aqueous ethanol due to its slightly different amino acid composition (reviewed by Wall and Paulis 1978), and possibly the high temperature (70°C) used for kafirin extraction could have resulted in the formation of insoluble high molecular weight oligomers (Duodu et al 2002). To improve the solubility of kafirin in aqueous ethanol, preliminary research showed lactic acid (LA) to be an important plasticizing component, in combination with glycerol (GYL) and polyethylene glycol 400 (PEG). The improved solubility of kafirin in aqueous ethanol, in the presence of LA, could possible be due to the addition of hydrogen ions, which could have increased the positive charge on the kafirin.

In addition, it was found that high shear (vigorous stirring) was required to ensure kafirin solubilisation and the formation of a homogenous solution. Not withstanding this, kafirin film casting solutions were still very viscous and gelled quickly upon cooling, compared to commercial zein. The high viscosity of the kafirin film casting solutions made pouring difficult and probably caused the uneven film surface and film thickness. It is possible that the high viscosity and quick gelling of kafirin film casting solutions could also render glass plate spreading methods (with a thin-layer chromatography spreader bar) (Aydt, et al 1991) difficult. It is possible that the high viscosity and quick gelling of the kafirin will cause the the solution to fold and

wrinkle during spreading, rather than forming a smooth even surface, using this technique. The use of masking tape strips bordering the glass plate would be necessary to hold the casting solution, preventing it from running off the edges of the glass plate.

Due to differences in the purity of the different protein preparations, film casting solutions were prepared according to constant protein (16% protein (w/w)), and cast by constant weight of casting solution (4 g solution/Petri dish). This procedure was probably the major cause of the different films having different thicknesses (biofilm thickness increased with decreasing protein purity). This is undoubtedly due to low purity kafirin preparations resulting in films with higher levels of total solids, compared to films prepared from high purity kafirin preparations. Due to the low purity of the bran kafirin preparations (50 to 60% pure) the amount of solvent was not sufficient to dissolve the kafirin preparations and resulted in thick, lumpy solutions that were not possible to pour. Therefore, twice the amount of solvent was used, films being cast using 8 g solution/Petri dish. It is possible that using a constant material to solvent ratio could have resulted in film forming solutions with similar viscosity, and variation in film thickness between different kafirin preparations could have been reduced. However, this would result in bran kafirin biofilms having proportionally less protein per film compared to the more pure kafirin preparations. Less protein per film could have an influence on the tensile and WVT properties of the bran kafirin films.

The water barrier properties of the kafirin and zein biofilms were determined by measuring the water vapour transmission (WVT) and water vapour permeability (WVP) though the biofilms using a modification of the ASTM E96-95 (ASTM 1995) "cup method". Water barrier properties of protein biofilms are an important property, indicating the films ability to control water vapour transport between a food system and its environment (Gennadios et al 1994b). Schott bottles were modified by accurately drilling a hole (3.3 cm diam) in the centre of the plastic screw top, removing the centre up to and including the inner ridge of the top, and then used for testing the water barrier properties of the different biofilms. The modified Schott bottle with the aid of a fibre tap washer was effective in creating a water tight seal. However, the shape (contours of the bottle) could have an influence on the WVT rate of this system. In addition, the stagnant air layer

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existing between the underside of the film and the surface of the water contained in the 'cup', is believed to resist water vapour transport through the film, resulting in an underestimation of water permeability, ranging between 5 and 46% (Gennadios et al 1994b). Since the kafirin film is intended for coating fruits and nuts, it can be assumed that a stagnant air layer existing between the coating and the fruit/nut would be negligible, compared to that using the cup method. This would most possibly affect the water permeability of the coating when used on fruit or nuts, compared to the cup method used. In view of these factors, only the trends observed between the different biofilm preparations for WVT and WVP were considered.

Furthermore, due to the high weight of this assembly (Schott Bottle filled with water), small changes in weight during testing could not be determined using an analytical balance. Therefore, determination of the weight change over a period of 10 days was necessary to obtain enough data to calculate WVT and WVP. It was found that flux (weight loss (g) over time (hr)) for all samples was linear ($R^2$ ranged from 0.991 to 0.999). Using a longer test period opposed to the short test period (approximately 24 hr) (Park and Chinnan 1994, Parris et al 1997) commonly used for determining water barrier properties of films; could be more reliable considering that coatings on fruits or nuts are expected to function over an extended time period (several weeks) during transport and storage.

Mechanical properties, namely tensile strength, tensile strength at break and percentage elongation at break, of the kafirin and zein biofilms were determined using a Stable Microsystems TA-XT2 Texture Analyser. Tensile strength expresses the maximum stress developed in the film during testing, while the elongation value represents the film's ability to stretch (reviewed by Gennadios et al 1994a). The mechanical properties of biofilms are largely influenced by the temperature and relative humidity of the surrounding environment during testing. Therefore, films should be conditioned at a known temperature and relative humidity before and during the tensile test to ensure comparative results. Commonly used methods include controlled relative humidity environmental chambers (25°C at 50% RH) wherein the films are conditioned and tested (Cho et al 2002) or the use of sorbostats or desiccators in which saturated salt solutions are used to obtain a certain relative humidity at 25°C (Lai and Padua 1998). But

testing is usually done under ambient laboratory conditions. Preliminary studies showed that uncontrolled laboratory conditions (approximately 20°C and 16 RH) gave very large variations in the tensile test of conditioned strips (conditioned at 25°C, 50% RH saturated calcium chloride solution in a desiccator, for 48 hr) prepared from the same biofilm. This is presumably due to the relative humidity equilibrium in the desiccator being altered once it was opened (hence relative humidity and temperature was quickly reduced). The reduced relative humidity in the desiccator could have resulted in the films losing moisture over the duration of the testing period (approximately 5 hr). Therefore to minimise variation between replicate tests, due to moisture loss, all films were tested within 6 hrs of drying at ambient temperature and relative humidity (which remained relatively constant throughout the day). However, variations in ambient temperature and relative humidity on different test days undoubtedly influenced repeatability between tests. Thus only the trends observed between the tensile strength data of the different biofilm preparations were considered.

As hypothesised, sorghum kafirin can form free-standing biofilms, like maize zein. However, kafirin biofilms had higher tensile and breaking strength compared to the zein biofilm. But the zein biofilm had higher elongation and better water barrier properties. Sorghum variety and different dry milling fractions had an influence on the colour and the purity of the different kafirin preparations, respectively; which influenced the colour and physical properties of their kafirin biofilms. Kafirin colour is dependent on the type and level of polyphenols present in different sorghum varieties and their dry milling fractions, which are extracted with the kafirin (Jones and Beckwith 1970). While kafirin purity is dependent on the chemical composition of the different dry milling fractions and the extraction of components, namely fats, non-starch polysaccharides, ash and polyphenols with the kafirin. From this research, it is clear that kafirin extracted from industrial bran (high in protein, fat, ash, crude fibre and polyphenols) will have low purity, ranging from approximately 30 to 50% protein, containing high levels of fats, polyphenols and other components, possibly non-starch polysaccharides and ash. Furthermore, red and white industrial bran will result in red and yellow kafirin and kafirin biofilms, respectively.

The low purity of kafirin extracted from bran, due to the high level of fats, can easily be improved by removing the fats with non-polar solvents such as hexane. Fat extraction of the bran prior to kafirin extraction could reduce the level of fat extracted with the kafirin, also improving kafirin purity. However, the non-polar solvents used for fat extraction are expensive and hazardous due to high flammability. Alternatively, the extracted fats could be considered as a useful functional component of bran kafirin biofilms. Bran kafirin biofilms (prepared from defatted kafirin) were found to be brittle and difficult to handle, with very low elongation compared to biofilms prepared from kafirin extracted from WGFs and EFs. The extracted fats (presumably a combination of free fatty acids and triacylglycerides) could improve the mechanical and handling properties of bran kafirin biofilms due to its plasticizing effect. According to Lai and co-workers (1997, 1998) fats, in the form of free fatty acids, are becoming important plasticizing agents in the preparation of zein biofilms.

Methods that have been employed to improve the colour of kafirin preparations, could be used on kafirin extracted from bran, include washing the kafirin with absolute ethanol to remove the phenolic pigments (Jones and Beckwith 1970), and using non-polar solvents such as hexane to remove hydrophobic carotenoid pigments (reviewed by Shukla and Cheryan 2001). Again, such methods use large volumes of expensive, hazardous organic solvents. Alternatively, these contaminants could be considered natural colourants, and it could be advantageous to match the colour of the different kafirin preparations with applications where film colour would improve the overall appearance of the product. For example, red kafirin could be used to coat red fruits such as litchis and plums, while yellow kafirin could be used for nuts and deciduous fruits.

Furthermore, the activity of plant phenols as potent antioxidants, free radical scavengers and metal chelators in preventing lipid peroxidation is well known (reviewed by Cook and Samman 1996). This has resulted in efforts to extract these compounds from a number of food industrial wastes including grape marc from the red winemaking industry, to use them as food lipid antioxidants (Bonilla et al 1999). Thus, the high levels of phenolic compounds in bran kafirin biofilms could impart lipid antioxidant activity. This could prevent oxidative rancidity of the high levels of fat in bran kafirin biofilms, as well as, give added antioxidant protection to

high fat foods such as nuts. Furthermore, this could eliminate the need for synthetic antioxidants such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) currently used in zein based edible coating on nuts to prevent lipid rancidity (reviewed by Gennadios et al 1994a).

When considering the complex composition of bran kafirin biofilms, it might be appropriate to consider these films as composite or multicomponent films. Multicomponent films are defined as being heterogeneous in nature, consisting of a blend of polysaccharides, proteins and/or lipids (reviewed by Wu et al 2002). These films utilise the distinct functional characteristics of each class of film component, with the result biofilms with specific functional properties required by certain applications can be designed. The presence of non-starch polysaccharides in the bran kafirin biofilms is probably responsible for reducing the flexibility and elongation of these biofilms. The reduced film flexibility and poor elongation of bran kafirin films could be due to formation of numerous hydrogen bonds between aligned polysaccharide polymer chains (reviewed by: Kester and Fennema 1986, Krotchta and De Mulder-Johnston 1997). In addition, the incompatibility of different biopolymers (proteins, polysaccharides and fats) in aqueous media is well documented (Tolstoguzov 1993), and could result in poor interaction between the proteins and polysaccharides in the bran kafirin biofilms. The incompatibility of the different biopolymers in bran kafirin preparations could render common casting methods inadequate in the formation of biofilms with desired functional properties, possibly requiring chemical modification (such as plasticization and cross-linking agents) of the biopolymers. Bran kafirin film flexibility and elongation could be improved by better plasticization. The interaction between the proteins and polysaccharides could be enhanced with the use of cross-linking agents such as polypropyleneglycol alginate (PGA) (Shih 1996, reviewed by Wu et al 2002). Under alkaline conditions PGA is known to cross-link with amino acid groups in proteins, as well as hydroxyl groups in polysaccharides.

Furthermore, by not removing the residual fat in the kafirin preparations, especially the bran kafirin preparations, it is possible that the water barrier properties of these films could be improved. This is due to the high hydrophobicity of fats, which have been reported to reduce moisture transfer through zein biofilms (Lai et al 1997, Lai and Padua 1998). In addition, as already discussed, the fats could also improve

the mechanical properties of these films due to their plasticizing effect. However, the incompatibility of high levels of fats with proteins (reviewed by McHugh 2000) and polysaccharides (reviewed by Wu et al 2002), in aqueous solutions is well known. Retaining the high levels of fats in bran kafirin preparations could further reduce biopolymer interactions, resulting in poor tensile properties, phase separation and the migration of the fats to the surface of the bran kafirin biofilms. According to reviews by McHugh (2000) and Wu et al (2002), protein-lipid, and polysaccharide-lipid interactions in edible films and coatings can be improved with emulsifying agents or surfactants (such as the phospholipid lecithin). Emulsifying agents lower the interfacial tension that exists between the dispersed (fat droplets) and the continuous (protein/polysaccharide and solvent) phases. Thus, reducing phase separation and the coalescence of the fat droplets, and their migration to the surface of the film. Mechanical work, such as homogenisation of the film casting solution, can also be used to reduce the size of the fat droplets, improving emulsification and homogeneity of the casting solution (reviewed by McHugh 2000).

Considering the multicomponent nature of bran kafirin preparations, physical (thermoplastic extrusion) modification, as well as chemical modification of the biopolymers, could also be considered as an alternative method of producing bran kafirin biofilms. According to Tolstoguzov (1993), thermoplastic extruded products can be regarded as heterophase polymer melts of incompatible water-plasticized biopolymers. In the process of thermoplastic extrusion, proteins, polysaccharides and/or fats are melted simultaneously at high pressure (up to 100 atm) and temperature (140-200°C) in a shear field, converted into a plastic mass, forced through a shaping die and rapidly hardened by cooling. However, the final biofilm preparation would have to be used in biodegradable packaging applications (bags, wraps) and not for the direct coating of fruits and nuts.

In the light of the above, it would appear that kafirin extracted from bran (both red and white industrial bran) could be used for the coating of fruits and nuts. However, this research shows that the full potential of this rich protein source is not being fully utilised, as less than half the proteins present in the bran were extracted. If the full potential of this protein source is to be met, methods to

improve the extraction of more or all of the sorghum proteins in bran should be investigated.

The different solubility properties of the different cereal proteins, namely albumins (water-soluble), globulins (salt-soluble), glutelins (acid- or alkali-soluble) and prolamins (aqueous alcohol-soluble) (Osborne 1918), will make it difficult to find a common solvent system able to extract all the sorghum proteins simultaneously. A serial extraction system, that is using different solvents for the extraction of the different proteins in a step-wise process, might be effective, but not practical on an industrial scale. According to reviews by Shukla and Cheryan (2001) and Lawton (2002), a number of solvent systems apart from aqueous alcohol alone, have been found to extract the prolamin proteins of maize. For example, hot aqueous alcohol solvents containing an alkali have been reported to improve the extraction of zein (reviewed by Lawton 2002). It is believed that the improved extraction yield could be due to the extraction of other proteins as well as zein. However, SDS-PAGE and amino acid analysis showed that this was not the case for kafirin in this research, as the extracted kafirin was pure kafirin. It is possible the concentration of alkali used in this study was not sufficient to extract the alkali-soluble proteins. Pre-soaking treatments, using strong alkali/acid and reducing agents, of the bran could possibly improve the solubility and extractability of the different proteins. Also, variations in the composition of the extracting solvent (alkali, reducing agents and ethanol, or other solvents) and extraction conditions (temperature and time) used should be considered in improving the extraction of sorghum proteins from bran. The extraction of film forming components other than protein, namely polysaccharides and fats, should also be considered as they may contribute to the overall functional properties of these multicomponent films. According to Rojas et al (2002) dry milled sorghum flour, containing starch and protein, can be used to prepared cast edible films.

In the event that the extraction of sorghum proteins from bran can be improved, it should be noted that the composition of these protein preparations will most probably influence their film forming ability in different solvents and the physical and water barrier properties of the films. Wheat gluten is an example of a heterogeneous protein, consisting of prolamin (gliadin) and glutelin (glutenin) type proteins, as well as, small amounts of albumins and globulins, and lipids and

carbohydrates (reviewed by Gennadios et al 1994a). Like zein, aqueous ethanol is the most common solvent used for wheat gluten film-casting solutions, however, alkaline or acidic conditions are required to form homogenous solutions (Gontard et al 1992, Gennadios et al 1993a).

Furthermore, each type of protein could impart certain functional properties that could be beneficial or detrimental to the overall functional properties of these multicomponent films. Albumin and globulin proteins such as those found in rice bran (Gnanasambandam et al 1997) and soy protein (Pol et al 2002) have been reported to have good film forming properties. In addition, globulin proteins have cohesive and adhesive properties, and have good emulsification properties (reviewed by Gennadios et al 1994a). Such properties could be beneficial in multicomponent films (reviewed by: McHugh 2000, Wu et al 2002), by improving the incorporation of fats and hence their plasticization effect in the film, and possibly ensuring good adhesion of the coating onto fruits and nuts. However, films made from albumin and globulin proteins are reported to have poor moisture barrier properties due to their hydrophilic nature (Gnanasmbandam et al 1997, Pol et al 2002). In this respect, lamination of hydrophilic protein biofilms such as soy protein (Pol at al 2002) and whey protein (Cho et al 2002) with the more hydrophobic zein protein, with or without the addition of fatty acids, has been found to improve the water vapour barrier properties of hydrophilic protein films. According a review by Duodu et al (2003) slight differences in the amino acid composition of kafirin and zein are believed to result in kafirin being more hydrophobic than zein. Thus it can be assumed that the lamination of hydrophilic protein biofilms with the more hydrophobic kafirin could also improve the water vapour barrier properties of hydrophilic protein biofilms.

# 4 CONCLUSIONS AND RECOMMENDATIONS

Kafirin preparations from different sorghum dry milling fractions including bran are able to form biofilms. The colour and purity of the different kafirin preparations (and thus their biofilms) is affected by sorghum variety (red or white) and the composition of the different dry milling fractions used for kafirin extraction. Compared to kafirin from EFs, kafirin from bran contained high levels of fat and were highly coloured due to high levels of polyphenols, particularly kafirin from red sorghum bran. The colour of the bran kafirin biofilms could limit their use to certain coating applications. However, these natural pigments could improve the overall appearance of red fruits such as litchis in the case of red kafirin, and nuts and pears in the case of yellow kafirin. Furthermore, the high levels of phenolic compounds in bran kafirin biofilms could prevent oxidative rancidity of fats in these biofilms, and give added protection to high fat products such as nuts.

Compared to the zein biofilm, kafirin biofilms had higher tensile and breaking strength, but less elongation. This is most likely due to the presence of β- and γ-kafirin polypetides, resulting in high levels of disulphide cross-linking in kafirin films. In addition, kafirin biofilms, in particular the bran biofilms, appeared to have poorer water barrier properties, presumably due to these films being thicker than the zein biofilm. Furthermore, poorer flexibility and handling properties of the bran biofilms could have resulted in microcracks in these films, decreasing water barrier properties. Extracted fats (formerly considered contaminants in the kafirin) could improve the mechanical and water barrier properties of bran kafirin biofilms, due to their plasticizing properties and high hydrophobicity, respectively.

Bran, a protein rich co-product from the sorghum dry-milling industry, contains approximately 11% to 34% of the total grain protein. However, hot aqueous ethanol, with alkali and a reducing agent only extracted the kafirin proteins, less than half the protein in the bran. This is due to non-prolamin proteins being insoluble in aqueous ethanol. Methods to extract and utilise more proteins in bran should be found so that the full potential of this resource, for biofilm production, can be met.

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