

**Kafirin biofilm quality:
Effect of sorghum variety and milling
fractions**

BY

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DECLARATION

I declare that the dissertation herewith submitted for the degree MSc (Agric) Food Science and Technology at the University of Pretoria, has not previously been submitted by me for a degree at any other university or institution of higher education.

Laura da Silva

ABSTRACT

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**By
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Degree: MSc (Agric) Food Science and Technology

Research was done to determine if sorghum bran could be a potential source of kafirin for biofilm preparation. Condensed tannin-free red and white sorghum were decorticated by abrasion until approximately 10% or 25% grain by weight was removed. Kafirin was extracted from the different milling fractions using hot aqueous ethanol with alkali and reducing agent. Kafirin and commercial zein were defatted and free-standing biofilms were prepared by casting in hot aqueous ethanol with added plasticizer.

Compared to whole grain and extraction flours, the brans were darker and considerably higher in protein, fat and polyphenols. This is due to the removal of these components from the grain during abrasive decortication, and their being concentrated in the bran. However, the yield of kafirin from the brans was somewhat lower, due to the fact that kafirin is located solely in the endosperm. The protein extracted from the different dry milling fractions was pure kafirin. However, the purity of the different kafirin preparations was found to differ. Kafirin extracted from the brans was less pure, containing higher levels of fat, non-starch polysaccharides and polyphenols compared to kafirin extracted from whole grain and extraction flours. Higher levels of polyphenols in the kafirin extracted from the brans resulted in the bran kafirin being highly coloured, particularly kafirin from red sorghum bran.

All the kafirin preparations, including those from bran, were able to form biofilms. Kafirin biofilms were stronger, but had poorer elongation compared to zein biofilm. This could be due to the presence of β - and γ -kafirin polypeptides in the kafirin, resulting in high levels of disulphide cross-linking in these films. It is possible that better plasticization could improve the elongation properties of all kafirin biofilms. Kafirin biofilms also had poorer water barrier properties compared to the zein biofilm, possibly related to the fact that the kafirin biofilms were thicker. Higher levels of contaminants in the bran kafirin biofilms resulted in these “multicomponent” films being more highly coloured, less clear and flexible, with stronger odour and rougher surface texture, compared to the other kafirin biofilms. High levels of polyphenols in bran kafirin biofilms could be beneficial by imparting antioxidant activity, possibly prevent oxidative rancidity of the fats in the biofilms and high fat foods such as nuts. Furthermore, the high levels of fats in kafirin prepared from bran could improve the mechanical and water barrier properties of these films, due to the plasticizing and hydrophobic properties of the fats, respectively.

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CHAPTER 1

1 INTRODUCTION

1.1 Statement of the problem

In the SADC (Southern African Development Community) region of Africa the average total production of sorghum grain over a five year period is 400 000 metric tons per annum (Directorate: Statistical Information of the National Department of Agriculture 2001), of which approximately 60 000 metric tons is milled into sorghum flour for food. Generally, before milling the sorghum into flour for food, the grain is decorticated (removal of the outer grain layers). Grain decortication results in considerable quantities of valuable protein-rich co-product (bran) being produced, which is currently sold as animal feed. Applications, such as edible protein coatings, biofilms and biodegradable packaging from high protein cereal co-products like maize gluten (Dickey et al 2001) and rice bran (Gnanasambandam et al 1997) has been reported, resulting in an increase in the potential value of these waste products. Therefore, this sorghum co-product has been identified as a potential source of sorghum protein from which sorghum protein biofilms, like maize protein biofilms, could be manufacture (Enviropak 2002). The sorghum proteins could have the potential to be used as coatings on southern Africa's export fruits and nuts, reducing post-harvest losses. This in turn could reduce the cost of production, trade and distribution of these fresh produce.

Traditionally red sorghum varieties were grown in South Africa, as the red colour is desired by the sorghum beer industry (reviewed by Taylor 1993). But due to greater demand for sorghum flour products, sorghum is now also grown in large quantities for the dry milling industry. However, red sorghum is still the predominate type grown compared to white varieties. This has resulted in large quantities of red sorghum bran co-product, compared to white sorghum, being produced. The characteristic colour of red sorghum is due to anthocyanin and anthocyanidin pigments present in the pericarp (Hahn et al 1984), which are

largely absent in white sorghum varieties. It is therefore important to determine whether sorghum variety i.e. condensed tannin-free red and white sorghums, will have an influence on protein extraction composition, extraction yield and protein quality, and whether this will have an effect on sorghum protein biofilm properties.

Furthermore, due to problems associated with inefficient decortication of sorghum grain or the requirement for whiter sorghum meals (reviewed by Rooney and Waniska 2000), extraction variations in the dry milling industry occurs. Extraction rates range from approximately 95% (high extraction) to 70% (low extraction), with resulting 5% and 30% bran. This variation in extraction rates could have an effect on bran composition, specifically with regard to protein content and composition, as well as other components fat, crude fibre and polyphenols, the latter being concentrated in the bran fractions during decortication. These factors could influence protein extraction yield and protein purity. Differences in protein composition (albumin, globulin, glutelin and prolamin proteins) between the different milling fractions could influence the protein extract composition, which could also have an influence on protein biofilm properties.

1.2 Literature review

The literature review will give a brief overview of sorghum kernel structure and the chemical composition of the different sorghum anatomical parts. Effect of sorghum dry milling on the composition of different milling fractions with particular reference to the sorghum prolamin proteins (kafirins) will also be discussed. Additionally, protein biofilm research, more specifically research done on maize zein biofilms, will be reviewed.

1.2.1 Sorghum: Brief overview

Sorghum (*Sorghum bicolor* (L.) Moench) is an important cereal food staple for millions of the poorest people living in harsh semi-arid areas of the world, especially Africa and Asia (reviewed by Serna-Saldivar and Rooney 1995).

Sorghum (Fig. 1) is unique among tropical cereals, such as maize and rice, in that it is drought tolerant and adaptable to environments where other agricultural crops fail to grow.

Sorghum has become the principle source of energy, protein, vitamins and minerals for these people. Food uses include various kinds of traditional porridges, flatbreads, beer and non-alcoholic beverages (reviewed by Rooney and Waniska 2000). The development of a number of new and improved sorghum and sorghum-wheat composite foods around the world include baked products, tortillas, extruded snacks, noodles and pastas. In most cases, sorghum must undergo milling before it is utilised for food preparation (reviewed by Rooney et al 1986). The term 'milling' usually refers to both decortication and dehulling or pearling of the grain, which involves the removal of the outer pericarp layers, followed by crushing of the decorticated grains into flour or grits.



Fig. 1. Red, condensed tannin-free, hybrid sorghum under cultivation in South Africa (Taylor and Belton 2002)

1.2.2 Kernel structure and chemical composition

Sorghum is classified as a naked caryopsis (kernel losing its hulls or glumes after threshing) (reviewed by Serna-Saldivar and Rooney 1995). In general, the kernel is spherical, ranging in weight from 20 to 30 mg (reviewed by Hosenev 1994). The kernel consists of three distinctive anatomical components whose approximate proportions are similar between different sorghum varieties: pericarp (8%), germ or embryo (10%) and the endosperm (82%) (reviewed by Hosenev 1994). Figure 2 shows a diagrammatic representation of a sorghum kernel. Each anatomical component, and its chemical composition, with specific reference to proteins, will be discussed briefly.

1.2.2.1 Pericarp

The pericarp consists of three major parts, the epicarp, mesocarp and endocarp (Hosenev et al 1974). The epicarp often contains phenolic pigments responsible for pericarp colour and is covered by a thin layer of wax (Earp and Rooney 1982). Pericarp colours include white (colourless), lemon-yellow or red (Rooney and Miller 1982). In sorghum the mesocarp is unique in that some varieties contain starch granules, giving the pericarp a thick, chalky appearance (Rooney and Miller 1982). The endocarp, the innermost layer, consists of cross and tube cells which are responsible for moisture transport throughout the kernel (Rooney and Miller 1982). Directly beneath the cross and tube cell layers, the kernels of some sorghum varieties have a highly pigmented testa layer (Rooney and Miller 1982). This layer develops from the inner integument, and contains large amounts of condensed tannins. Chemically, the pericarp is rich in fibre, and contains minor components including protein (6%), lipids (4.9%), ash (2%) (reviewed by Serna-Saldivar and Rooney 1995) and polyphenolic compounds (Hahn et al 1984). The majority of proteins present in the pericarp are unextractable, possibly due to associations with cell wall materials (Taylor and Schüssler 1986). Amino acid analysis of extracted and residual pericarp proteins shows that these proteins are not prolamins as they are poor in glutamic acid and proline, and relatively rich in lysine compared to the grain protein.

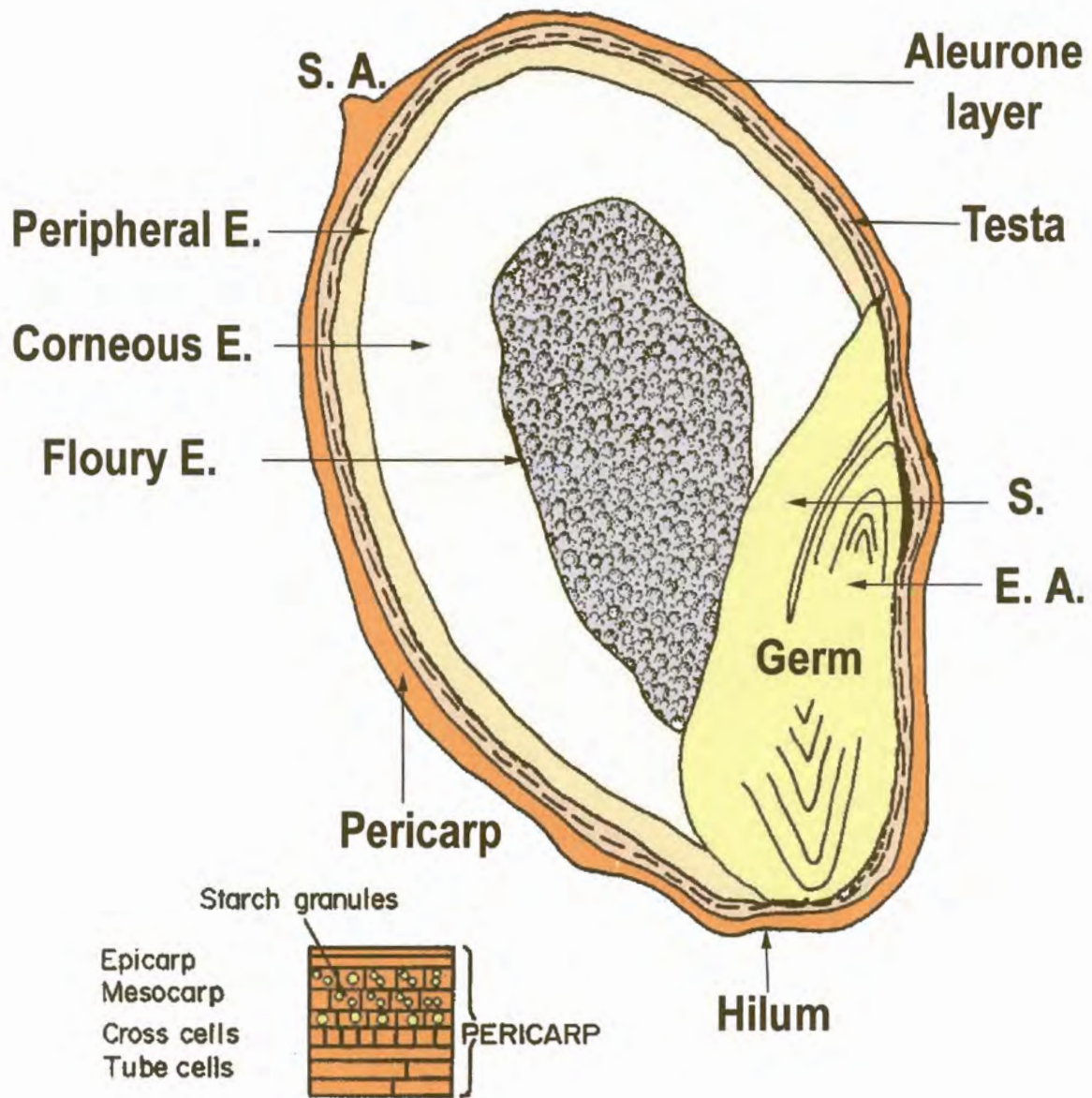


Fig. 2. A sorghum kernel. S.A., stylar area; E., endosperm; S., scutellum; E.A., embryonic axis (adapted from Hosney 1994).

1.2.2.1.1 Phenolic compounds found in sorghum

All sorghums contain phenolic compounds; which are divided into three major categories: phenolic acids, flavonoids and tannins (Hahn et al 1984). They affect the colour, appearance and nutritional quality of sorghum grains and products. Phenolic acids are present in all sorghums, and most contain flavonoids, but only the brown high-tannin (bird-resistant) sorghums contain condensed tannins (Hahn et al 1984). Flavonoids are a diverse class of water-soluble plant polyphenols, based on a C₆-C₃-C₆ flavone skeleton (Fig. 3) (reviewed by Ryan et al 1999). The three-carbon bridge, between the phenyl groups, is commonly cyclised with oxygen. Flavans namely anthocyanin, anthocyanidin, leucoanthocyanin and catechin, are the major flavonoid pigments found in sorghum (Hahn et al 1984). The characteristic colour of red sorghums is primarily due to the presence of anthocyanin and anthocyanidin pigments, although combinations of flavan pigments have been found to be responsible for a variety of pericarp colours.

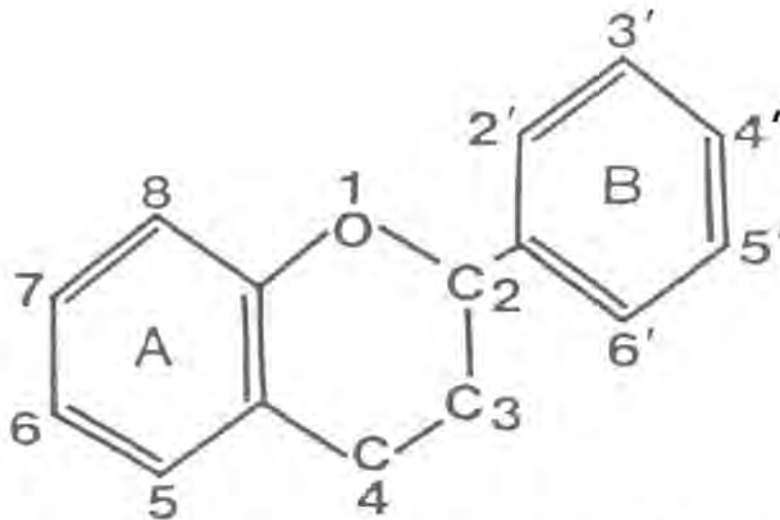


Fig. 3. Basic flavonoid ring structure, flavans have no carbonyl at 4 (Hahn et al 1984).

1.2.2.2 Germ (embryo)

The germ or embryo is the only living part of the mature sorghum kernel, and is composed of the embryonic axis and the scutellum (reviewed by Hosoney 1994). The former contains the new plant and is divided into a radicle and plumule. Upon germination they form the primary roots, as well as the leaves and stems, respectively. The scutellum cells are modified transport cells containing oil globules, protein bodies, minerals and a few starch granules (Rooney and Miller 1982). It serves as a bridge or connection between the endosperm and embryo. Chemically the germ is rich in protein (18.4%), lipids (28.1%) and ash (10.4%) (reviewed by Serna-Saldivar and Rooney 1995). The protein found in the germ occurs mostly as low molecular weight nitrogen (LMWN) (amino acids and peptides), and albumin and globulin proteins (Taylor and Schüssler 1986).

1.2.2.3 Endosperm

The endosperm, the largest anatomical component of the kernel, consists of the aleurone layer, the hard corneous endosperm and soft floury endosperm (Rooney and Miller 1982). Immediately below the aleurone layer is the peripheral endosperm tissue which is two to six cells thick (Rooney and Miller 1982). The peripheral endosperm tissue contains small starch granules embedded in large quantities of protein. The protein forms a dense protein matrix composed mainly of prolamin protein bodies surrounded by a glutelin protein network. The corneous endosperm, also referred to as glassy, horny, or vitreous, is located beneath the peripheral endosperm and is a continuous interface between strongly bound starch and protein bodies embedded in a protein matrix (Hosoney et al 1974). This tight packing arrangement makes the corneous endosperm appear translucent or vitreous. The floury endosperm has loosely packed endosperm cells with small voids occurring between spherical starch granules (Rooney and Miller 1982). The voids permit light to pass through the floury endosperm, giving it an opaque or chalky appearance. Small amounts of protein bodies and little or no protein matrix is found in the floury endosperm. Chemically the endosperm contains large amounts of starch (82.5%), some protein (10.5%) and small amounts of fat (0.6%) and ash (0.4%), plus cell wall

material (reviewed by Serna-Saldivar and Rooney 1995). Starch is the main source of energy utilised during the first stages of germination and plant development. Approximately 80% of the grain protein is found in the endosperm, of which the majority is aqueous alcohol-soluble prolamin proteins (Taylor and Schüssler 1986). Prolamin proteins function as a nitrogen store for the growing plant during germination. The second most important protein fraction in the endosperm is G₃-glutelin, which most probably forms the gluten matrix around the prolamin protein bodies (reviewed by Serna-Saldivar and Rooney 1995).

Table I summarises the mean chemical composition of whole sorghum grain and its anatomical components. These data are useful in estimating the purity of fractionations obtained from various dry milling procedures.

TABLE I
Typical Chemical Composition (%) of Whole Sorghum
and Its Anatomical Parts
(adapted from Serna-Saldivar and Rooney 1995)

Component	Whole grain (%)	Endosperm (%)	Germ (%)	Pericarp (%)
Whole kernel	100	84.2	9.4	6.5
Starch	71.8	82.5	13.4	34.6
Protein	11.3	10.5	18.4	6.0
Fat	3.4	0.6	28.1	4.9
Ash	1.7	0.4	10.4	2.0
Crude Fibre	2.7	-	-	-

(-) no data reported

1.2.3 Sorghum dry milling

The objective of sorghum decortication is the separation of the anatomical components of the grain, that is to separate the endosperm from the pericarp, testa and germ (Hahn 1969, reviewed by Kent and Evers 1994). The endosperm is then reduced into flour or grits, which are then used for food preparation. Decortication processes can be done either by traditional or industrial methods.

1.2.3.1 Traditional sorghum milling

Traditional sorghum milling is a laborious task, usually done by hand by the village women, with wooden mortar and pestle (reviewed by Rooney et al 1986). The cleaned grain is washed, placed in the mortar, and pounded vigorously with the pestle. In general the abrasive action frees the pericarp from the kernel, usually at the mesocarp which is mechanically weak (Shepherd 1981). During pounding, additional water may be added to the sorghum to facilitate pericarp removal. Finally, the decorticated grain is separated from the bran by washing or winnowing, and ground into flour by additional pounding with the mortar or traditional grinding stones (Mukuru 1992).

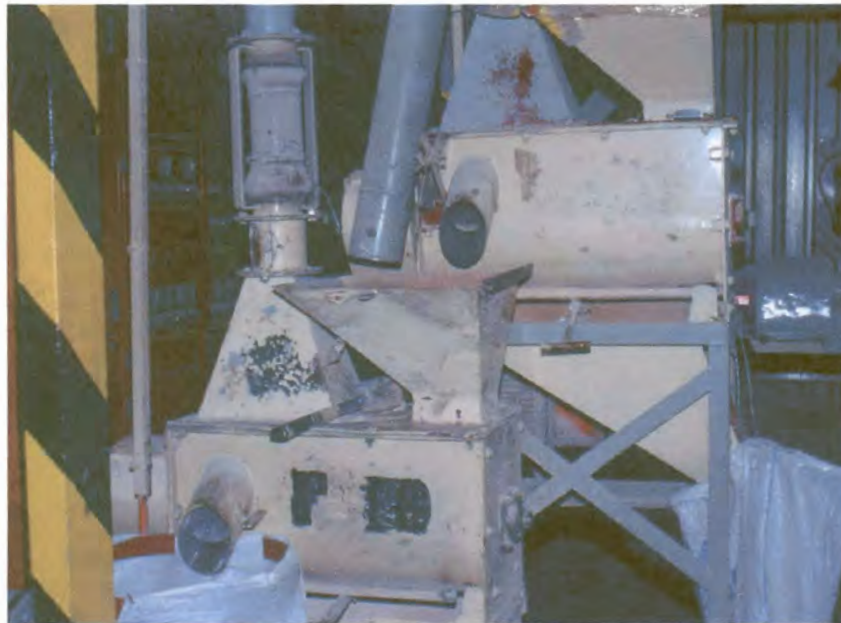
1.2.3.2 Industrial sorghum milling

Industrial milling methods include roller, attrition and abrasive type dehullers used for the milling of cereals such as wheat, maize, rice and barley, many of which have been specially adapted for sorghum (Hahn 1969, Munck et al 1982). Attrition and abrasive types of dehullers have been found to be more effective compared to roller types. In southern Africa, PRL (Prairie Research Laboratory)-dehullers (Fig. 4a) are commonly used for industrial abrasive decortication of sorghum (Taylor and Dewar 2001). The abrasive action is provided by 13 carborundum stones mounted on a horizontal axle (Fig. 4b) (Munck 1995). When the feed gate is opened, grain flows from the hopper into the body of the machine. Bran generated by the action of the abrasive surface is taken off by the fan and bagged at a cyclone. The decorticated (dehulled) grains leave the machine via an overflow outlet. The degree of decortication achieved is adjusted by regulating the feed and adjustable gates, and the speed of the stones.

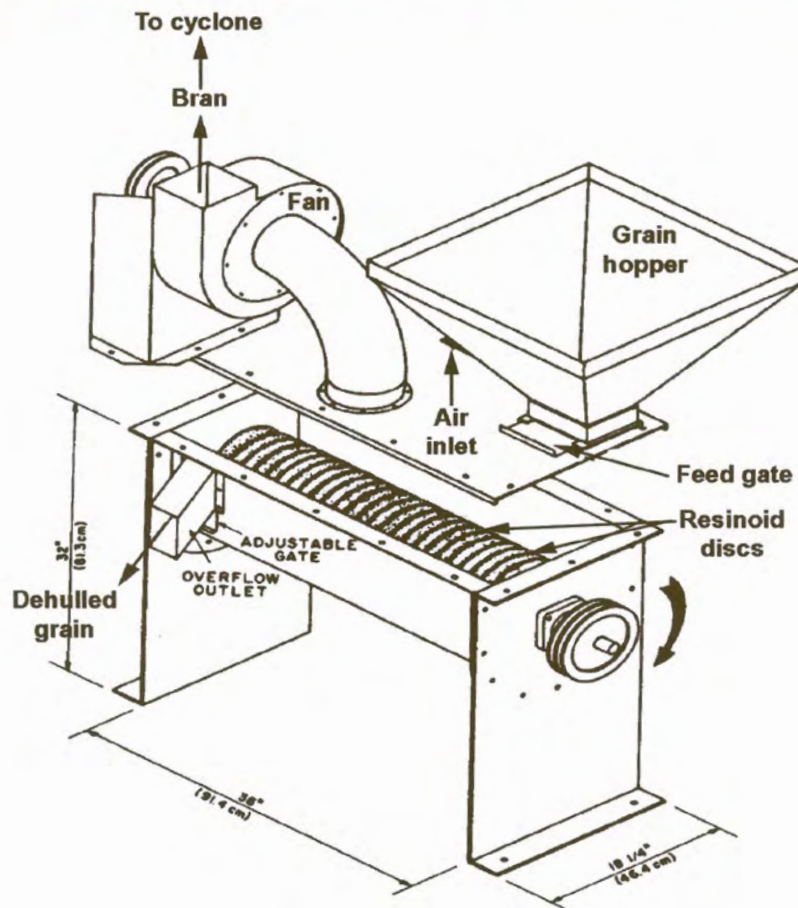
As previously stated, the objective of grain decortication is to abrade off the pericarp and germ with minimal removal of the endosperm. However, due to the inefficiency of industrial operations, as well as variation in kernel morphology (kernel size, shape, pericarp thickness and endosperm hardness), losses ranging from 5 to at least 20% of grain weight can occur (Wills and Ali 1992, reviewed by Rooney and Waniska 2000). Low extraction rate (i.e. 75% endosperm obtained after decortication) can result in large quantities of endosperm being removed from the grain and added to the bran fraction.

1.2.3.2.1 Effect of decortication on sorghum chemical composition

The chemical composition of decorticated sorghum, as well as the abraded bran material, depends on the degree and method of decortication used, as well as kernel characteristics (Munck et al 1982). The composition of decorticated grain at several levels of bran removal is shown in Table II. Hahn (1969) found similar results after serial decortication of whole sorghum grain. According to Hahn (1969) as the amount of bran removed from the kernel increased, the protein, fat, crude fibre and ash content of the decorticated grain decreased and the proximate composition values approached that of hand dissected, pure endosperm. Protein content values may differ due to differences in the initial protein content of the whole grain. Kernels remaining after 39% bran removal were found to be almost completely rounded with no sign of bran or germ, containing only the corneous and floury endosperm (Hahn 1969). The protein content of this material is substantially less than the protein content of the whole grain material. This is due to the loss of 'protein dense' peripheral corneous endosperm, aleurone layer and germ.



a.



b.

Fig. 4a. PRL-Dehuller typically used for the industrial decortication of grain sorghum in southern Africa (photograph courtesy of Prof. J. R. N. Taylor), and 4b. Schematic diagram of the PRL- dehuller (Munck 1995).

Table II
Effect of Decortication on the Composition of Sorghum Grain
(reviewed by Rooney and Waniska 2000)

Percent Removal	Percent Composition				
	Protein	Oil	Crude Fibre	Ash	NFE*
0.0	9.6	3.4	2.2	1.5	83.3
6.0	9.4	3.0	1.3	1.2	85.1
10.0	9.5	2.5	1.0	1.1	85.9
16.1	9.1	2.0	0.8	0.9	87.2
20.2	8.0	1.6	0.7	0.8	88.9

* Nitrogen-Free Extract

The proximate composition of the bran removed by abrasive decortication is shown in Fig. 5. As fibre is concentrated in the outer portions of the kernel, it is removed first reaching a maximum around 3% to 4% (as crude fibre) of the kernel weight removed (Hahn 1969). Maximum values for oil were found to occur at about 12% decortication. The peak content for protein was found to be broader than the other components, indicating removal of the 'protein dense' peripheral corneous endosperm, aleurone layer and germ proteins into the bran fraction.

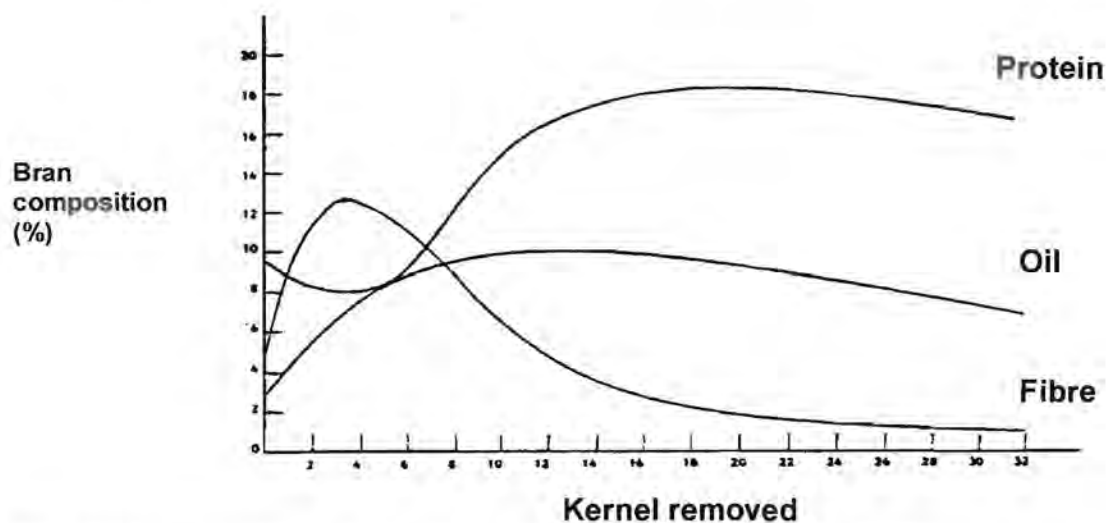


Fig. 5. Composition of bran removed by pearling (Hahn 1969).

1.2.4 Protein-based biofilms

Proteins from numerous agricultural sources, both plant and animal, have been studied for their film-forming abilities. Maize zein, wheat gluten, soy protein, collagen, and milk proteins namely casein and whey, have been studied extensively (reviewed by Gennadios et al 1994a). Other protein sources including fish, egg, cotton seed and cereal proteins such as barley hordein and sorghum kafirin have not received as much attention. Proteins are natural heteropolymers composed of a range of different amino acid units linked by peptide bonds. They are able to form amorphous three dimensional structures such as biofilms, stabilised mainly by non-covalent interactions namely hydrogen bonding and hydrophobic and electrostatic interactions (reviewed by Cuq et al 1998). Functional properties of proteins depend on structural heterogeneity, thermal sensitivity and hydrophilic/hydrophobic behaviour (reviewed by De Graaf 2000), all of which are directly related to protein primary structure; that is the sequence of amino acids and the first level of differentiation between proteins. It is therefore important that the characteristics of different proteins is understood, before biofilms with specific properties can be developed.

Cereal proteins such as wheat gluten (Rayas et al 1997), maize zein (reviewed by Shukla and Cheryan 2001) and sorghum kafirin (Buffo et al 1997) have been identified as potential sources for plant-based protein biofilms. The film-forming properties of zein and wheat gluten have been studied extensively, but according to reviews by Gennadios and Weller (1990) and Krotchta and De Mulder-Johnston (1997), zein is the only one that has been promoted commercially as an edible biofilm or coating. Allergies to wheat gluten is the main reason responsible for the lack of commercialisation of wheat gluten biofilms (reviewed by Gennadios et al 1994). CozeenTM (Park and Chinnan 1995) and VEGE-COAT (Garuda International Inc. 2002) are examples of zein based commercial products.

Cereal protein chemistry started with the separation of wheat flour into starch and gluten by Beccari in the 16th century (Tatham 1995). By the beginning of the 20th century, Osborne (1918) classified all vegetable proteins into four major

classes based primarily on different solubility characteristics: albumins (water-soluble), globulins (salt-soluble), prolamins (aqueous alcohol-soluble), and glutelins (acid- or alkali-soluble). Over the years modifications of Osborne fractionation have been developed to optimise cereal protein extraction and characterisation.

1.2.4.1 Prolamin proteins of maize and sorghum

Zein and kafirin are the aqueous alcohol-soluble prolamin proteins of maize and sorghum, respectively. They are the major storage proteins in these cereals, making up about 50% of the total grain protein (Taylor et al 1984). Extensive homology has been found to exist between the genes coding for zein and kafirin (DeRose et al 1989). Prolamins are characterised as being high in glutamine, and nonpolar amino acids leucine, proline, alanine, valine, isoleucine and methionine (reviewed by Wall and Paulis 1978). The high proportion of non-polar amino acid residues together with the low amount of acidic and basic amino acids, are responsible for their solubility in aqueous alcohol solutions instead of water. Another important amino acid found in prolamin proteins, although in small quantities, is the sulphur-containing cystine (reviewed by Wall and Paulis 1978). Cystine's role in contributing disulphide groups to produce intra- and intermolecular bonds has a significant effect on the solubility and extraction of zein and kafirin proteins. Although kafirin resembles zein in amino acid content in having low levels of lysine and other ionisable amino acids and high content of leucine and proline, kafirin is reported to contain more valine, alanine, and isoleucine than zein as well as more glutamine (reviewed by Wall and Paulis 1978). The solubility of proteins in water and less polar solvents such as ethanol, is related essentially to the distribution of the polar (charged or not) and apolar (hydrophobic) groups of the side chains of the amino acid constituents (reviewed by Cheftel and Cuq 1985). Thus, slight differences in the amino acid composition of kafirin and zein, as discussed above, are believed to result 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 Cheftel and Cuq (1985), free energy of hydration is the transferring of 1 mol of the amino acid from the aqueous solution into the ethanol solution.

Like other prolamins, kafirin is a heterogeneous protein separable into different classes based on differences in solubility, amino acid composition, electrophoretic, chromatographic, and immunological properties (Shull et al 1991). Zeins may be classified into α -zein made up of M_r -21,000-25, 000 polypeptides plus a M_r -10,000 minor polypeptide (also referred to as δ -zein), constituting 75% to 85% of the total zein (Esen 1987). β -Zein includes methionine-rich polypeptides with M_r -17,000-18,000 and constitute 10% to 15% of the total zein. γ -Zein is a one size class polypeptide rich in proline with M_r -27,000 constituting 5% to 10% of the total zein. Shull et al (1991) demonstrated molecular weight, solubility and structural similarities exist between zein and kafirin using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), differential solubility and Western blot analysis. Thus a nomenclature for kafirin based on that of zein described by Esen (1987) was proposed. The kafirins may be classified into α -kafirins with M_r -25,000-23,000, β -kafirins with M_r -20,000, 18,000 and 16,000; and γ -kafirins with M_r -28,000 (Shull et al 1991). According to a review by Shewry (2002) all α -type prolamins are rich in glutamine, proline and non-polar amino acids (leucine, alanine) while β -types are rich in methionine and cystine, and γ -types are rich proline and cystine. A schematic representation of total zein (lane 1) and kafirin (lane 2) polypeptides separated by SDS-PAGE, and the characteristics of the major prolamins groups of sorghum is shown in Fig. 6. However, it should be noted that there is considerable discrepancy in the number of size classes and estimated sizes depending in separating gel concentration and length, and in the calibration standards used by different authors.

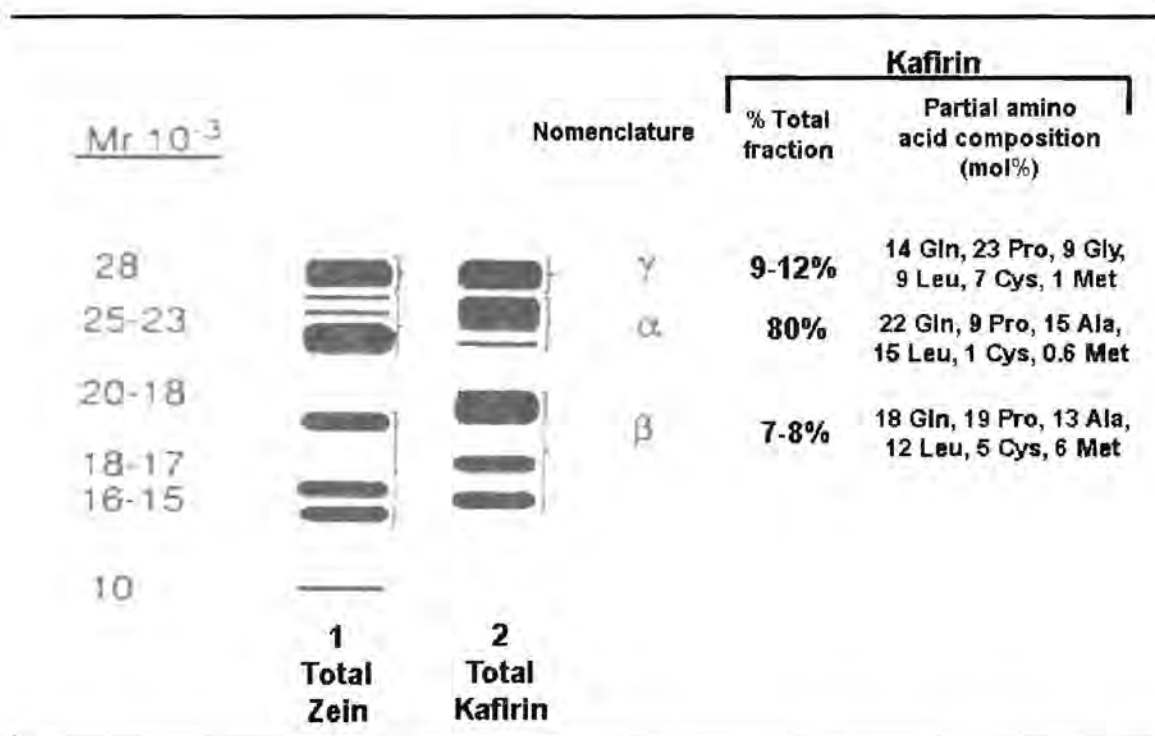


Fig. 6. Schematic representation of total zein (lane 1) and kafirin (lane 2) polypeptides separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) with the characteristics of the major prolamin groups of sorghum (kafirin) (adapted from Shull et al 1991 and Shewry 2002).

Studies conducted by El Nour et al (1998) showed that kafirin extracted and analysed by SDS-PAGE in the unreduced form showed the presence of various protein oligomers (approximately 70% of the total protein) of different *M_r*s, in addition to γ-, α₁- α₂- and β-kafirin monomers. These oligomers were found to comprise γ-, α₁- and α₂-kafirins linked together by disulphide bonds when analysed by SDS-PAGE under reducing conditions. In contrast, β-kafirin was not a component of the oligomers, however, high molecular weight polymers were found to comprise γ- and α₁-kafirins cross-linked with β-kafirin. It was hypothesised by El Nour et al (1998) that β-kafirin can act as a bridge linking together oligomers of γ-, and α₁-kafirins, forming high molecular weight polymers. Therefore, the degree of polymerisation of kafirin protein is assumed to be determined by the competitive linkage through disulphide cross-linking of γ-, and

α_1 -kafirin with α_2 -kafirin (a 'chain terminator') resulting in oligomers (dimers and trimers); or β -kafirin (a 'chain extender') resulting in high molecular polymers.

Commercially prepared zein, used extensively in the study of zein based biofilms, has been reported to differ in electrophoretic (Boundy et al 1967, Parris and Dickey 2001) and chromatographic properties (Parris et al 1997), and amino acid content (Boundy et al 1967) compared to laboratory prepared zein extracted from dry milled maize. In general the absence of β - and γ -zeins and consequently reduced levels of the sulphur containing amino acid, cystine and their resulting cystine-cystine disulphide bonds, has been observed in commercial zein. These differences have been directly related to processing conditions used in wet-milling resulting in the high protein maize gluten co-product used for commercial zein extraction. During commercial wet-milling, whole maize is steeped in a sulphur dioxide solution (active component sulphurous acid) at elevated temperatures (50°C to 60°C) for approximately 40 hr (Boundy et al 1967). This is done to permit separation of germ and fibre from the endosperm as well as to facilitate starch and protein separation. The sulphurous acid acts as a mild reducing agent, cleaving disulphide bonds of intermolecular aggregated zeins in the maize kernel (Parris and Dickey 2001). This, as well as the fact that reduced γ -zein is already soluble in water (Esen 1987), could further increase the solubility of β - and γ -zein and result in their removal from the maize kernel with the steep liquor or made insoluble in the maize (Parris and Dickey 2001).

1.2.4.2 Prolamin protein extraction

In order for prolamin proteins to be used in biofilm processing, high purity protein must be obtained. Processes for the manufacture of zein go back over more than 100 years, with the first known process for zein recovery being reported by Osborne in 1891 (reviewed by Shukla and Cheryan 2001). Extensive reviews by Shukla and Cheryan (2001) and Lawton (2002) on the industrialisation and use of maize zein show the complexity involved in producing a pure, high-grade zein product. To date no methods for the commercial extraction of sorghum kafirin have been published. Due to the increase in non-food applications of zein (and possibly kafirin in the near future), sources of cheap agricultural co-products high

in these proteins are being sort and identified for protein extraction. At the present time zein is commercially extracted, in the U.S.A., from maize grits and corn gluten meal (CGM) (containing a minimum of 60% protein db) obtained from wet milling processes (Dickey et al 2001). However, CGM varies considerably in quality and protein content, and there is evidence that variation in pre-treatment prior to separation of the CGM (that is the steeping and milling conditions) and temperatures used during the final drying step can influence extractability and quality of the zein. Wu et al (1997) reported that the recovery of zein from CGM decreased with an increase in drying temperature of CGM, possibly due to chemical browning resulting in decreased protein solubility and yield.

Solvents that can be used for zein extraction are determined by the solubility characteristics of zein. Due to the high proportion of nonpolar amino acids present in zein, suitable solvents should contain both ionic and non-ionic polar groups as well as nonpolar groups (reviewed by: Shukla and Cheryan 2001, Lawton 2002). According to Shukla and Cheryan (2001) more than 70 solvents for zein, whether pure or in mixtures, have been described in the literature. However, only aqueous ethanol and aqueous isopropanol have been used extensively for commercial extraction of zein. This is possibly due to the availability and easy recovery of ethanol and isopropanol from zein. Furthermore, ethanol and isopropanol are food compatible, and can thus be used for extracting zein intended for food applications. However, according to a feasibility study conducted by Dickey et al (1999), to separate zein as part of a starch to ethanol process; ethanol distillation (or recovery) and product drying are the two major energy-consuming units and cost elements involved in solvent extraction systems. Furthermore, high purity zein with reduced fat content and minimal colour and aroma, necessitates expensive and hazardous non-polar solvent extractions such as hexane or benzene. Hence, a number of studies have since been conducted to develop high quality zein with reduced fat content using less expensive methods (Dickey et al 2001, Dickey et al 2002).

Zein extraction methods most commonly used now are based on the patented process by Carter and Reck (1970), involving a single hot extraction (55 - 65°C)

of CGM with aqueous ethanol or isopropanol, at a ratio of 4:1 aqueous alcohol to gluten, containing 0.25% sodium hydroxide (or pH 12) for 1 hr. The resulting zein is precipitated from the solvent by chilling or dilution with cold water with or without reducing the solution pH with hydrochloric acid. According to Chiue et al (1997) studies have shown that the use of alkali treatment at elevated temperatures readily cleaves amide bonds resulting in deamidation (release of amide groups as ammonia), thus breaking of hydrogen bonds and hydrophobic interactions that stabilise prolamin polypeptides. Deamidation and the partial cleavage of the peptide bonds have been found to increase zein solubility and stability (Boundy et al 1967, Wilson 1988) possibly due to distinct changes in the functional properties of zein (Chiue et al 1997). Characterisation of commercial zein by reverse phase high pressure liquid chromatography (RP-HPLC) showed a broad, poorly resolved peak which was attributed to deamidation reactions resulting from extraction of zein from CGM under alkaline conditions (Parris et al 1997).

As stated, kafirin is similar to zein in molecular weight, solubility, structure, and amino acid composition. Due to the limited amount of information on kafirin extraction procedures, methods used for zein extraction have been the starting point for scientists. However, extracting defatted sorghum gluten (obtained from wet-milling of sorghum) with hot (65°C) 95% ethanol at a ratio of 5:1 alcohol to gluten for 1 hr resulted in low extraction yields of only 5.5% (Buffo et al 1997). This was three times lower compared to zein extracted from CGM under the same conditions. It is possible that the use of almost anhydrous ethanol without reducing agents and alkali reduced kafirin solubility resulting in low extraction yields. As already discussed, the addition of reducing agents and alkali to the solvent system could improve kafirin solubility by cleaving disulphide bonds and deamidating the polypeptides, respectively. Thus investigation into the optimal extraction conditions for kafirin is needed if commercialisation of kafirin is to be made economically viable.

1.2.4.3 Principles involved in protein biofilm formation

The formation of protein biofilms has been described as a three-step process involving denaturation, aggregation and gelation, to form a three dimensional protein matrix (reviewed by: Cuq et al 1998, Debeaufort et al 1998, De Graaf 2000). In general, proteins are solubilised/dispersed in a suitable solvent, for example aqueous alcohol in the case of zein, with additives such as plasticizers by mixing at elevated temperatures. Increased temperature and shear result in protein (or partial) denaturation. According to Wang and Damodaran (1991), protein (partial) denaturation, usually by heat, shear and/or extreme pH, weaken the tertiary and quaternary (three-dimensional) protein structure, exposing previously hidden functional groups such as carbonyl (C=O), amide (N-H) and disulphide (S-S) bonds, as well as side chain amine and hydrophobic groups. These functional groups become available for intermolecular interactions and result in the formation of a three-dimensional protein network, entrapping film components such as plasticizing agents, during protein aggregation and drying, (Gontard et al 1993). The addition of a plasticizing agent to the film is required to overcome film brittleness caused by extensive intermolecular interactions. Plasticizers reduce these forces and increase the mobility of protein chains, thereby improving flexibility and extensibility of the film. Rearrangement of the protein polypeptides, such as in soybean protein biofilms, were reported by Subirade et al (1998) to show structural changes from α -helix into β -sheets. It was hypothesised that hydrogen bonded intermolecular β -sheet structure is essential for protein-protein interactions and network formation in soybean protein and possibly other plant based protein biofilms (Subirade et al 1998).

However, the extent of intermolecular β -sheet hydrogen bonding in zein and kafirin biofilms might not be as high as that found in soybean protein biofilms or other protein biofilms such as wheat. Such assumptions are based on the predicted structural model for zein by Argos et al (1982), using circular dichroism. In this model (Fig. 7) zein is supposedly a collection of nine helical rods packed in a capsular shaped array with the end pieces of the capsules, which consists of a glutamine repeat sequence, being joined together by hydrogen bonding.

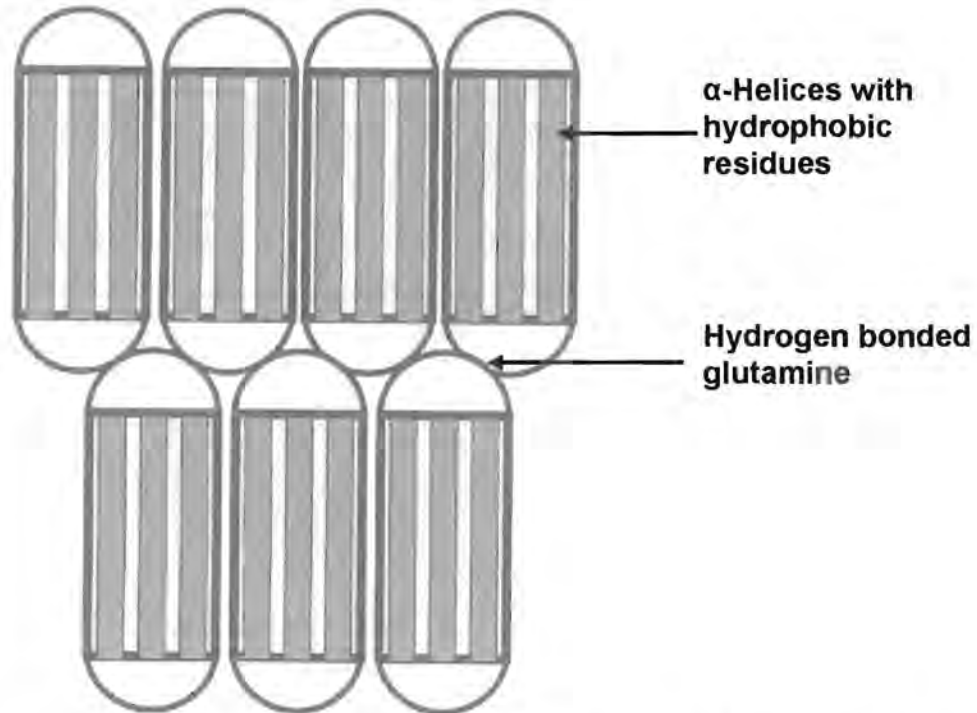


Fig. 7. The structure of zein. Striped regions represent the α -helical region and the curved regions at the end represent hydrogen bonding through glutamine (Taylor and Belton 2002).

Furthermore, Belton et al (according to Taylor and Belton 2002) reported that Fourier transform infrared reflectance (FTIR) studies showed that alkylated kafirin and zein samples, compared to unalkylated samples, only showed a slight increased β -sheet structure, with no other structural changes occurring. In addition, during wet cooking of sorghum and maize Duodu et al (2001) reported that only slight changes in protein secondary structure from α -helical to intermolecular β -sheet conformation of kafirin and zein was observed using FTIR and solid state ^{13}C NMR spectroscopic methods. Thus it can be assumed that only slight (partial) heat denaturation of zein and kafirin will occur during biofilm preparation, and that a high proportion of α -helical conformations (opposed to β -sheets) will occur in these biofilms. Disulphide cross-linking could also occur between polypeptides closely aligned to each other, which could result in a rigid, stabilised biofilm structure. According to a review by Gennadios et al (1994a), hydrophobic and hydrogen bonding are thought to be the primary modes of stabilisation in commercial zein biofilms. However, due to the reduced content of

cystine in commercial zein (as previously discussed) only a limited number of disulphide cross-linking is believed to occur.

1.2.4.4 Biofilm processing

The film forming properties of zein have been studied extensively (Aydt et al 1991, Yamada et al 1995, Lai et al 1997, Lai and Padua 1998, Parris and Coffin 1997, Yoshino et al 2002) compared to the single study on kafirin by Buffo et al (1997). In general, two methods namely the wet and dry process have been used to prepare free-standing zein films.

1.2.4.4.1 Wet process

General zein film-forming procedures involve dissolving the protein at high temperature (70°C for 5 to 10 min) in an appropriate solvent (wet process) with added plasticizers such as glycerol and polyethylene glycol while stirring vigorously, and then casting onto a smooth flat surface (Aydt et al 1991, Yamada et al 1995, Parris et al 1997, Parris and Coffin 1997, Parris and Dickey 2001). The solvent is then allowed to evaporate at ambient temperatures (Aydt et al 1991) or using air oven (Yamada et al 1995, Parris et al 1997) or vacuum oven (Parris and Coffin 1997, Parris and Dickey 2001) drying. Binary solvents comprising a lower aliphatic alcohol (ethanol, isopropanol) and water are known to be good solvents for zein (reviewed by Wilson 1987). However, many kinds of solvents including acetic acid, lactic acid and acetone are known to dissolve zein alone or in the presence of water (reviewed by Lawton 2002). The very low solubility of zein in water alone has been attributed to the high content of hydrophobic interactions between nonpolar amino acids, and the presence of covalent disulphide bonds (reviewed by Wall and Paulis 1978). Solvent systems used to prepare zein film-forming solutions are generally based on aqueous alcohol (Aydt et al 1991, Parris and Dickey 2001) and occasionally aqueous acetone (Yamada et al 1995, Parris and Coffin 1997, Yoshino et al 2002). According to Gontard et al (1992) and Gennadios et al (1993) the functional properties of protein biofilms obtained by the wet process depend on protein

concentration in solution, pH (depending on protein properties), additives, solvent polarity, drying rate, and temperature. Sensitivity of proteins to pH change is usually associated with a high content of ionised polar amino acids (Okamoto 1978). Zein films have been reported to form over a wide pH range because they have low ionised amino acid content and are thus not as sensitive to pH change such as other proteins like wheat gluten and soy.

The effects of different solvents, namely alcohol (ethanol) and acetone (Yamada et al 1995, Parris and Coffin 1997, Yoshino et al 2002) on zein biofilm properties have been studied. According to Yamada et al (1995) there can be many arrangements of zein protein in the film depending on the preparation solvent used. Zein films prepared with aqueous acetone (more hydrophobic solvent) were observed to have good water resistance and barrier properties when compared to zein films prepared from aqueous ethanol (more hydrophilic solvent) (Yamada et al 1995, Parris and Coffin 1997). It has been hypothesised that in more hydrophobic solvents zein aggregates (piles) are formed with more hydrophobic outer surfaces stabilised by the hydrophobic acetone, while the more hydrophilic centre is stabilised by the entrapment of water (Yamada et al 1995). Furthermore, acetone (a smaller, highly volatile hydrophobic molecule compared to water) causes a rapid change in the hydrophilic-lipophilic balance (HLB) at the air side of the zein solution during drying (Yamada et al 1995), making the air side of the zein film relatively hydrophobic (Yoshino et al 2002). Zein deposited at the drying air-surface reduces the successive rapid change in HLB in the remaining solvent, allowing zein to form the film (Yamada et al 1995).

Ethanol on the other hand has both hydrophobic and hydrophilic functional groups, resulting in aqueous ethanol solvents having weaker hydrophobicity than aqueous acetone (Yamada et al 1995, Yoshino et al 2002). When aqueous ethanol is used as a solvent the opposite arrangements is presumed. Zein molecules are thought to aggregate by entrapping small amounts of ethanol in a hydrophobic centre exposing its hydrophilic region where it is stabilised by the remaining more hydrophilic solvent (Yamada et al 1995). After drying the surface hydrophobicity of zein-ethanol films is thought to be less than zein-acetone films

(Yoshino et al 2002). The proposed arrangement of zein would account for observed differences in water vapour permeability between zein-acetone and zein-ethanol films. According to Parris and Coffin (1997) zein films prepared in acetone had lower water vapour permeability values than those prepared in ethanol. In addition, zein films prepared from acetone have been reported to be less uniform and more cloudy in appearance with a rough surface compared to those prepared from ethanol (Yamada et al 1995, Parris and Coffin 1997). Differences in physical properties of different zein films are most likely caused by variation in the internal microstructure of the zein films.

Casting is commonly done on plastic Petri dishes, as they have a smooth non-sticky surface and are easily available. However, size and shape limits the use of such cast films. Aluminium moulds (Aydt et al 1991) and sheets (Pol et al 2002) as well as glass plates (Gennadios et al 1993a) of various sizes and depth have been used. The depth of these casting moulds determines film thickness. Technical difficulties associated with cast-biofilms include uncontrolled film thickness and homogeneity in film properties (Lai and Padua 1997).

1.2.4.4.2 Dry process

Preparation of zein films or sheets by moulding processes (dry process) has also been reported (Lai et al 1997, Lai and Padua 1998). Dry processes involve mixing zein with plasticizers and then kneading the mixture into soft pliable dough after applying heat. The zein-plasticizer dough is then moulded or rolled into films of even thickness. Alternatively, cold water is added to hot zein-plasticizer solutions resulting in the formation of a zein-plasticizer precipitate or zein-resin, that is recovered as a soft solid compound (Santosa and Padua 2000). The resin is subsequently kneaded into a cohesive mouldable resin and rolled into films before drying. Resin-zein biofilms have been reported to have even film thickness and high ductile and tensile strength compared to cast-zein biofilms (Lai et al 1997, Lai and Padua 1998).

Dry processes are based on the thermoplastic (glass transition) properties of proteins under low moisture conditions. According to Hoseney et al (1986), Sperling defines glass transition (T_g) as the onset temperature of coordinated molecular mobility in the polymer main chain, from a meta-stable glassy state (immobile) to an unsteady rubbery (mobile) state. The T_g phenomenon is dependent on macromolecular characteristics such as molecular weight, flexibility or rigidity of the main and side-chains, polarity and presence of intermolecular covalent bonds, as well as the presence and content of plasticizers (Hoseney et al 1986, Cheryan et al 1995). Plasticization by water or polyhydroxy components is critical for the interaction of proteins to form a continuous network from powdered raw materials. According to Lai et al (1997) a number of authors state that zein is unique in terms of thermoplasticity and hydrophobicity, resulting in dough exhibiting viscoelastic behaviour when heated above 60°C with starch.

1.2.4.5 Properties of protein biofilms

The preservation of food products with the use of protein biofilms depends mainly on retarding or slowing down external or internal mechanisms of deterioration. This is achieved through mechanical and barrier properties of protein biofilms. Mechanical and barrier properties are dependent on the type of material (protein) used, its formulation, processing and film thickness, and the presence of bubbles and pinholes (reviewed by Gontard and Guilbert 1994, Park and Chinnan 1995). Physical and barrier transmission properties of most biofilms are influenced by environmental temperature and relative humidity. Therefore these conditions should be standardised prior to and when possible during testing before reliable comparisons can be made between different materials (Aydt et al 1991).

1.2.4.5.1 Mechanical properties

Biofilms must generally be resistant to breakage and abrasion as well as being flexible in order to provide mechanical protection to foods. Tensile strength and percentage elongation at break are the two most commonly measured mechanical properties of biofilms. Tensile strength expresses the maximum stress developed in a film specimen during tensile testing, while the elongation or percentage tensile strain (sample elongation divided by original sample length multiplied by 100) values represents the film's ability to stretch (reviewed by Gennadios et al 1994a, Lai et al 1997). Another important measurement, Young's modulus, is related to the rigidity of the material and is the ratio of tensile stress over tensile strain and measures the force necessary to deform a test specimen (Lai et al 1997).

Structural cohesion between the polymers of protein biofilms has been found to influence mechanical properties (reviewed by Gennadios et al 1994a, Guilbert et al 1996). Cohesion is the ability of polymers to form strong and/or numerous molecular bonds between polymeric chains, thus hindering their separation. This ability depends on the structure of the polymer, especially its molecular length, shape and molecular weight distribution as well as protein concentration and processing conditions (Guilbert et al 1996, Parris et al 1997). Aydt et al (1991) compared the tensile strengths of zein and wheat gluten films. Zein films were found to be brittle compared to wheat gluten films, which were more elastic. Wheat gluten polymers are much larger and more complex in comparison to zein polymers, resulting in numerous associative and covalent bonds, stabilising and strengthening the gluten structure (Bietz and Wall 1980). However, increasing protein concentration can further increase protein-protein bonds necessary to ensure strong cohesion and film formation. According to Parris et al (1997) low purity zein (48.2% protein), isolated from unrinsed dry milled maize flour did not form a film as well as did high purity zein (91.4% protein). This was attributed to the low protein content of the zein isolate and the presence of cellulosic material which could have interfered with protein-protein associations and covalent bonding, reducing strong cohesion necessary to form the film structure.

Although strong cohesion and bond formation is important for film formation, these forces result in protein biofilms being brittle with little flexibility, necessitating the need for plasticizers. Scanning Electron Microscopy (SEM) showed unplasticized zein films to be amorphous spongy-like structures with cracks and voids (Lai et al 1997). Polymer fracturing is usually associated with the formation of cracks at regions where there is localised stress concentration, resulting in low tensile strength values of unplasticized zein films. Plasticizers are defined as low molecular weight substances that dissolve in the polymer, increasing the free volume and chain mobility, resulting in the rearrangement of the three-dimensional protein matrix (Lai et al 1997). Commonly used food-grade plasticizers include water and polyols such as glycerol, sorbitol, mannitol, and polypropylene and polyethylene glycol, and sugars such as sucrose (reviewed by Kester and Fennema 1986). According to Lawton (2002), substituted aryl sulphonamides, lactic acid, dibutyl tartrate and acetanilide are some of the more useful plasticizers used for zein. Considering zein hydrophobicity, hydrophobic plasticizers such as fatty acids (stearic, palmitic and oleic acids) have been found to be effective plasticizers for zein (Lai et al 1997, Lai and Padua 1998, Santosa and Padua 2000).

Mechanical modifications of plasticized films include increased extensibility and flexibility, while decreasing cohesion, elasticity, mechanical resistance and rigidity (Wu and McGinity 1999, Irissin-Mangata et al 2001). Furthermore, film properties are dependent on the characteristics and amount of plasticizers used. According to Santosa and Padua (2000), Lai and co-workers proposed a structural model for zein-oleic acid films based on X-ray scattering measurements. The model consisted of staggered zein planes separated by two layers of oleic acid molecules oriented with their long axis perpendicular to the film plane, suggesting that oleic acid binds to the zein surface during plasticization. The use of hydrophilic plasticizers such as glycerol (GLY) has been reported to result in the migration of GLY to the surface of zein films reducing film flexibility (Parris and Coffin 1997). However, the use of plasticizer blends including GLY with more hydrophobic plasticizers such as polypropylene glycol (PPG) was found to improve the HLB in zein films, preventing plasticizer separation in the film. It was

reported by Parris and Coffin (1997) that zein films containing GYL and PPG at a ratio of 1:3 exhibited elongation values almost fifty times greater than when plasticized with GYL alone. This was believed to be due to synergy existing between GLY and PPG, and certain secondary forces between the protein polymers resulting in reduced intermolecular forces and thus facilitating extensibility of the film. However, plasticization of protein biofilms generally results in decreased tensile strength.

According to Buffo et al (1997) laboratory extracted kafirin (89.04% protein, 6.74% crude fat and 0.04% ash) cast into films, plasticized with GLY and polyethylene glycol (PEG) had lower tensile strength and higher elongation compared to commercial zein (94.82% protein, 1.82% crude fat and 0.34% ash) cast films. It was assumed that the lower protein content of kafirin resulted in reduced tensile strength since non-protein constituents were likely to disrupt homogeneity and continuity of the protein film network. However, the higher fat content of the kafirin could have resulted in increased plasticization of the film above that of the GLY and PEG added. Higher levels of fat could have increased film flexibility and elongation, and reduced the tensile strength of kafirin biofilms compared to the zein biofilms. Defatting of the protein samples could result in better mechanical property comparisons by eliminating the effect of differences in crude fat content.

Loss in tensile strength due to the addition of plasticizers can be overcome with the addition of cross-linking agents for example formaldehyde, citric acid (CA) and butanetetracarboxylic acid (BTCA), by covalently linking together polymer chains in the film (Yamada et al 1995, Parris and Coffin 1997, Lens et al 1999). Cross-linking between protein polymers is thought to improve the breaking strength properties of the film by increasing bond formation and film homogeneity, yet film flexibility can be reduced slightly. However, the toxicity of certain protein cross-linking agents, such as most aldehydes, will limit their use to protein biofilms for non-food applications.

1.2.4.5.2 Barrier properties

Biofilm barrier (permeability) properties, specifically those of gaseous (oxygen and carbon dioxide) and water vapour permeability (WVP) are necessary if food product quality is to be maintained. Barrier properties of biofilms can be defined as states that permit the transmission of permeates through the biofilm (reviewed by Gontard and Guilbert 1994). The primary mechanism for gaseous and vapour flow through a film, in the absence of cracks, pinholes, or other flaws, is by activated diffusion (reviewed by Kester and Fennema 1986). Active diffusion is when the permeate (i.e. water vapour) dissolves in the film matrix at the high concentration side, diffuses through the film driven by a concentration gradient, and evaporates from the other surface. According to Kester and Fennema (1986) diffusion is dependent on size, shape, and polarity of the permeating molecule, intermolecular attractive forces namely hydrogen bonding and van der Waals' forces, and the degree of cross-linking and amount of crystallinity within the film matrix. For polymer materials of crystalline or highly oriented structure, absorption and dissolution of permeate into the polymer matrix is difficult due to closely packed polymer chains (Lai and Padua 1998). For the majority of foods, the gain or loss of moisture leads to either physical or biological defects reducing food quality and shelf life. A loss or gain of water could have detrimental effects on physical attributes such as texture (dry cake or soggy chips) (Park et al 1996), while microbial spoilage could be enhanced by moisture gain and increased product water activity (reviewed by Kester and Fennema 1986). Prevention of lipid oxidation in high fat foods can be reduced with good oxygen barrier biofilms (reviewed by Gennadios and Weller 1990), while the control of internal gas composition of fresh products could result in extended product shelf life (Park 1999, Rakotonirainy et al 2001).

Barrier properties of edible films strongly depend on temperature, relative humidity and film hydratability (reviewed by Kester and Fennema 1986), as well as the chemical nature of the proteins and film microstructure (Lai et al 1997, Lai and Padua 1998). Protein biofilms generally hydrate readily in water and are thus poor barriers against moisture migration. This is attributed to the hydrophilic

(number of polar groups present) nature of proteins facilitating water transport through the film. The plasticizing and/or swelling effect of moisture on hydrophilic protein biofilms has been reported to affect barrier properties of protein biofilms, making them more permeable to gases and vapour under high relative humidity conditions (Gennadios et al 1993b, Lai and Padua 1998). Furthermore, increasing temperature is believed to increase plasticizer and polymer chain mobility, and increase gaseous and vapour diffusion rates, resulting in reduced film barrier properties at higher temperatures. However, the addition of waxes, fatty acids, surfactants and sugars to the protein matrix has been reported to reduce water migration and improve barrier properties (reviewed by Kester and Fennema 1986, Lai et al 1997, Lai and Padua 1998). Lai et al (1997) reported that unplasticized zein films became soft with lighter colour and disintegrated easily after 2 hr of soaking in distilled water. The presence of voids and cracks in unplasticized zein films is believed to allow for increased water absorption. The addition of hydrophobic fatty acids has been reported to reduce water absorption (Park et al 1996, Lai et al 1997, Lai and Padua 1998), therefore maintaining film structure in aqueous environments (Lai et al 1997). The addition of fatty acids is thought to make the structure more hydrophobic with more compact film microstructure and thus increase water resistance (Park et al 1996, Lai et al 1997, Lai and Padua 1998).

Processing conditions such as cast versus resin films (Lai and Padua 1998) and solvents used such as ethanol versus acetone (Yamada et al 1995, Yoshino et al 2002) have also been reported to influence film microstructure, affecting water absorption and permeability properties of zein biofilms. Unlike cast-zein films, resin-zein films observed under SEM showed an oriented microstructure, consisting of closely packed ribbon-like structures (Lai and Padua 1998). Microstructure orientation in resin films was suggested as being responsible for reduced water absorption and permeability of resin-zein films compared to cast-zein films. Furthermore, differences in solvent hydrophobicity such as that found between aqueous acetone and aqueous ethanol has been reported to influence polymer hydrophobic/hydrophilic orientation of zein aggregates, influencing water barrier properties, as discussed earlier. According to Yoshino et al (2002)

differences in WVP exist between the different sides (air dried and basal side) of cast-zein films suggesting a correlation between WVP of the zein film and the contact angle (surface properties) and internal microstructure of the zein films. Increasing contact angle (smooth surface as found with the basal side) results in decreased WVP. It was therefore suggested that when zein films are used for food packaging, choosing the side with a high or low WVP must be considered.

Plasticizer characteristics have been reported to influence water absorption and permeability properties of biofilms. The incorporation of hydrophilic plasticizers such as GLY has been found to reduce the water vapour barrier properties of zein films significantly (Parris and Coffin, 1997). This is due to hydrophilic plasticizers aiding water vapour transmission through the biofilm, as well as increasing free volume between protein chains. Lipids or fatty acids have been added to protein film-forming formulations to improve barrier properties (Lai et al 1997). Fatty acid characteristics have been found to influence barrier properties. According to Lai et al (1997) palmitic acid was more effective than stearic acid in decreasing water absorption of zein films, suggesting that water absorption is influenced by differences in zein film microstructure prepared with different plasticizers. SEM indicated that differences in fatty acid characteristics such as melting point (palmitic acid < stearic acid), resulted in zein-palmitic acid films having more uniform distribution of fatty acids than zein-stearic acid films. It was hypothesised that the higher melting point of stearic acid compared to palmitic acid resulted in quick resolidification of stearic acid, preventing its complete mixing with zein, resulting in less effective plasticization. Furthermore, barrier properties of zein films can be improved by coating with heated oils such as flax (Lai et al 1997, Lai and Padua 1998) or tung (Rakotonirainy et al 2001) oil, thereby sealing off surface pores and preventing rapid absorption of water and reducing permeability.

Zein based biofilms have been observed as being sensitive to moisture transfer (Park et al 1996, Lai et al 1997, Lai and Padua 1998). However, compared to other proteins (albumins and globulins), zein is more hydrophobic, and lamination of hydrophilic protein biofilms (soy, whey and casein) with more hydrophobic zein

has been reported to decreased water vapour permeability of hydrophilic biofilms (Pol et al 2002, Cho et al 2002). Compared to zein, kafirin has been reported as having slightly higher hydrophobicity with reduced solubility (reviewed by Wall and Paulis 1978), it can therefore be assumed that kafirin will have similar or better barrier properties compared to zein, and could therefore be a substitute for zein in applications mentioned thus far.

1.2.5 Conclusions

It is evident from the above literature review that sorghum kafirin, based on its similarities to maize zein, has the potential to be used in edible films and biodegradable packaging. High protein co-products (bran) from the sorghum dry milling industry could be a source of kafirin for extraction. However, different sorghum varieties and variation in the level (high or low) of decortication could have an influence on the colour and composition of the bran, respectively. This in turn could have an effect on the colour, and yield and purity of the kafirin extracted from bran. The colour and purity of kafirin extracted from different dry milling fractions, including bran, could in turn have an effect on the physical, mechanical and water barrier properties of kafirin biofilms. Thus the influence of sorghum variety and level of decortication on the physical, mechanical and water barrier properties of kafirin biofilms will be investigated.

Compared to zein, information on the extraction of kafirin has been purely laboratory based. No commercial processes for the extraction of kafirin have been described. Furthermore, little work has been done in the film forming properties of kafirin. Thus methods used for the commercial extraction of zein and zein biofilm research will be used as a guide for the extraction of kafirin and the preparation and testing of kafirin biofilms. Solvent systems most commonly used for the extraction of zein, at both laboratory and commercial level, are hot aqueous ethanol and isopropanol. However, compared to zein, kafirin is less soluble in ethanol, due to it being more hydrophobic and having higher levels of disulphide cross-linking. Thus the addition of alkali and a reducing agent to the extracting solvent should be used to increase kafirin solubility in aqueous ethanol. Hot ethanol, and more recently acetone, are the most common solvents

used for zein film casting. Commonly used plasticizers include GLY, PEG and PPG, and less common, lactic acid. Casting of zein film forming solutions onto Petri dishes and drying at ambient or elevated temperatures (in air ovens) seems to be the most common and simplest method used to prepare laboratory films for testing. Thus casting of kafirin-ethanol film forming solutions with added plasticizers will be the method used for this research, as it is simple and easily managed on a laboratory scale.

1.3 Objectives

To determine if different sorghum varieties namely condensed tannin-free red and white, will have an influence on protein extractability and composition.

To determine if protein preparations from different sorghum varieties will have an influence on protein biofilm properties, namely film colour, and mechanical and water barrier properties.

To determine if different milling extraction rates: whole sorghum grain (100% extraction), 90% endosperm (high extraction) and 75% endosperm (low extraction), and the resulting 10% and 25% bran fractions, will have an influence on protein extraction and composition.

To determine if the different sorghum protein preparations extracted from the different milling fractions (describe above) will have an influence on protein biofilm properties, namely film colour, and mechanical and water barrier properties.

To compare the different sorghum kafirin biofilms with biofilm from commercial zein with regard to film colour, and mechanical and water barrier properties.

1.4 Hypotheses

It is expected that condensed tannin-free red sorghum will have reduced protein extraction yield and protein quality, compared to tannin-free white sorghum, due to higher polyphenol contamination in red sorghum varieties. High levels of polyphenols in red sorghum kafirin preparations are expected to have a negative effect on protein biofilm properties with regard to film colour, and mechanical and water barrier properties, as polyphenols are reported to be extracted with the kafirin.

Different sorghum dry milling fractions are expected to have different chemical composition with regard to protein, fat, fibre, ash, carbohydrate and polyphenols. Increasing levels of decortication is expected to result in extraction flours of increasing purity, having reduced levels of protein, fat, fibre, ash, and polyphenols compared to whole grain flours. The converse is expected for the bran fractions. Protein extraction yield and purity is expected to increase in extraction flours of higher purity compared to the bran. Increased protein yield and purity could be due to the improved purity of extraction flours and reduced polyphenol contamination. Differences in protein purity could influence protein biofilm properties with regard to film colour, and mechanical and water barrier properties.

CHAPTER 2

2 RESEARCH

The following sub-sections are written in the style of scientific papers, as required for the journal *Cereal Chemistry*. A flow diagram of the experimental design used in this research is shown in Fig. 8.

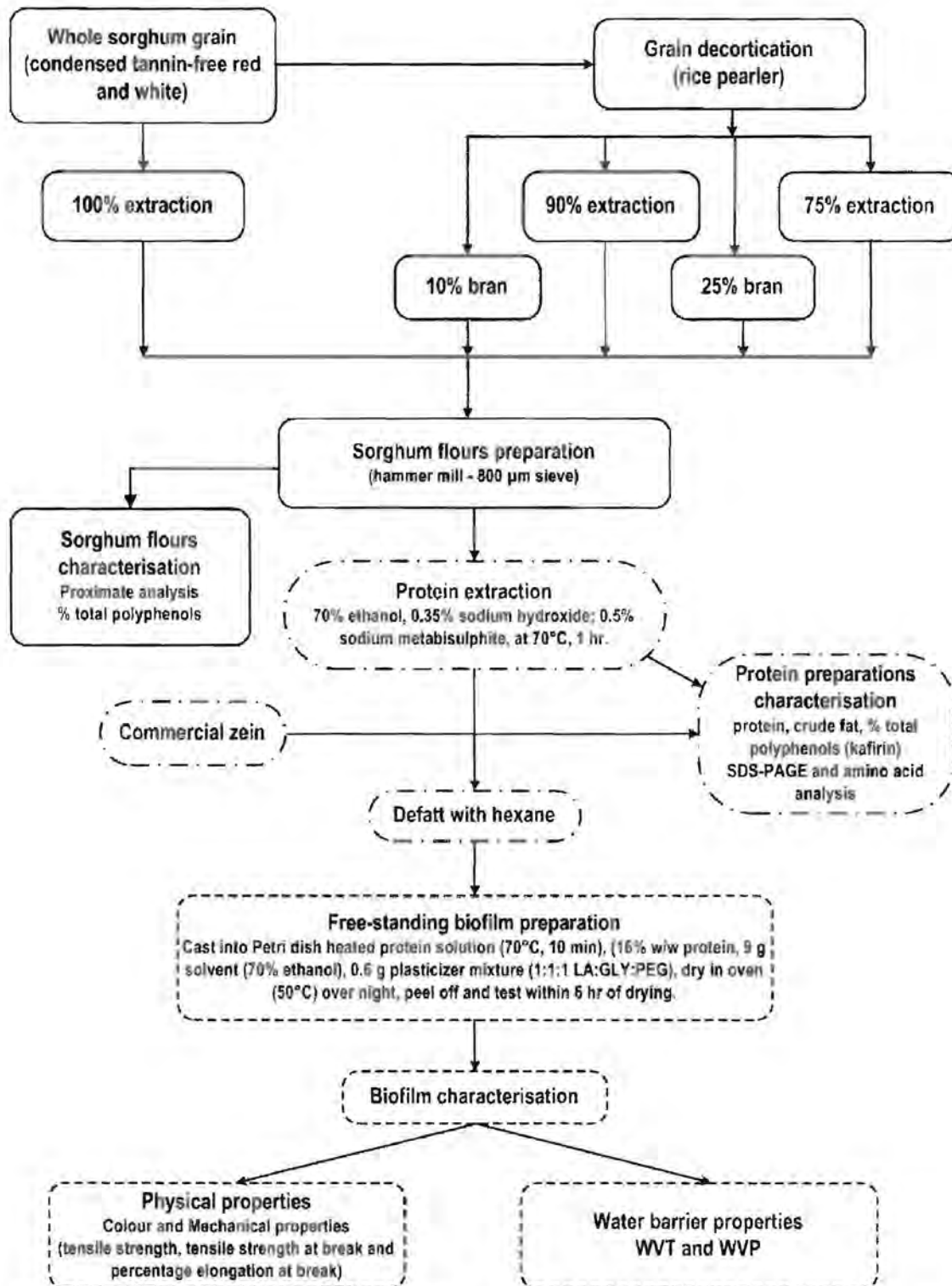


Fig. 8. Experimental design used for the preparation and characterisation of the different sorghum dry milling fractions; extracted protein and free-standing biofilms.

2.1 Effect of decortication on the composition of red and white sorghum milling factions and kafirin extraction

Abstract

Sorghum bran, a co-product of sorghum dry milling, could be a potential source of protein for industrial applications such as biofilms. Condensed tannin-free red and white sorghum samples were decorticated by abrasion until approximately 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 to 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 was somewhat lower than that from whole grain and decorticated grain flours, due to the fact that kafirin is located solely in the endosperm. Also, the kafirin from bran was more contaminated with fat and polyphenols and hence more highly coloured, particularly the kafirin from red sorghum. Thus, sorghum bran from dry milling could be used as a source of kafirin, but further purification steps may be necessary.

2.1.1 Introduction

In the arid parts of Africa and Asia, sorghum (*Sorghum bicolor* (L.) Moench) is an important food staple, making up approximately 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 non-alcoholic 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). This primarily involves the removal of the outer anatomical parts of the grain, namely: the pericarp layers (pericarp and testa) rich in fibre and ash; aleurone layer, rich in ash, protein, and fat; and the germ, rich in protein, fat and ash (Hahn 1969, Rooney and Miller 1982, Taylor and Dewar 2001). Decortication therefore results in large quantities of co-product, usually used for livestock feed. This fraction is commonly referred to 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 co-products such as rice bran (Gnanasambandam et al 1997), and maize gluten meal (Dickey et al 2001), beyond the use of livestock feed has been recognised. Applications for cereal proteins such as edible coatings and biodegradable packaging have been described (reviewed by: Gennadios and Weller 1990, Krotcha and De Mulder-Johnston 1997).

Of the cereal proteins, zein, the aqueous alcohol-soluble prolamin protein of maize, has been used in numerous non-food applications, of which edible coatings is a major one (reviewed by Gennadios and Weller 1990). Kafirin, the aqueous alcohol-soluble prolamin protein of sorghum, is analogous to zein (Shull et al 1991), and has been found to have the potential to be used as a biopolymer for edible and non-edible film and coating applications (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 characterise the kafirin extracted.

2.1.2 Materials and methods

2.1.2.1 Grain samples

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

2.1.2.2 Preparations of sorghum milling fractions

Whole grain flours were prepared by milling clean whole sorghum grain into flour with a hammer mill fitted with a sieve having a mesh opening of 800 μm . For milling fractions, clean whole grain was decorticated by passing the grain through a carborundum cone abrasive-type rice pearler (Maig Braunschweig, Germany) until an approximately 90% or 75% extraction rate was achieved, the approximate range found in the southern African sorghum milling industry. 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 sieve having a mesh opening of 800 μm . All milling preparations were stabilised by vacuum packing and storage at -20°C until further analysis.

2.1.2.3 Characterisation of sorghum milling fractions

Grain hardness was determined visually using the method of Rooney and Miller (1982). Colour of all sorghum flour/bran milling fractions was measured by Tristimulus colorimetry (HunterLab, 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, $103\pm 1^{\circ}\text{C}$ for 3 hr) (method 44-15A), ash (method 08-01), and crude fat (method 30-25). Crude protein was determined using combustion nitrogen analysis (LECO FP528 Protein/Nitrogen Analyser, St Joseph, USA), using 6.25 as the factor to convert nitrogen to protein. Crude fibre

was determined by the Weende Method (Tecator Fibertec system, 1020 Hot Extraction, Höganäs, Sweden). Total polyphenols were determined by the ferric ammonium citrate (FAC) International Standardisation Organisation method (ISO 1988).

2.1.2.4 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 metabisulphite, at a ratio of 1:5 (w/w) flour/bran to extractant with vigorous stirring at $70 \pm 0.1^\circ\text{C}$ for 1 hr. Insoluble matter was removed by centrifugation at $1000 \times g$ 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 precipitate formed. Chilled distilled water (10°C) of approximately equal volume was used to dilute the precipitate. 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 2 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 (Sigma Z3625) was ground to a fine powder with a coffee grinder to ensure similar particle size of all protein samples, the protein repetitions were defatted three times with hexane at ambient temperature at a ratio of 1:10 (w/w) protein to hexane for 1 hr.

2.1.2.5 Characterisation of kafirin preparations

The kafirin and zein preparations were characterised (before defatting) as for sorghum flour/bran preparations, as described above. Amino acid compositions of the defatted protein preparations were determined by RP-HPLC using pre-column derivatisation (PICO-TAG method) (Bidlemeier et al 1984).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), under reduced and non-reduced conditions, of the defatted protein preparations was performed using 140 mm length and 1.5 mm thick gels 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), polymerized with 0.1% (w/v) ammonium persulphate and tetramethyl-ethylenediamine (TEMED). Approximately 33 µg protein was loaded per track. Premixed protein molecular weight makers (Low-range Marker, Cat. No. 1 495 984, Roche Molecular Biochemicals, Indianapolis) were used. Proteins were stained with 1% (w/v) Coomassie Blue R250.

2.1.2.6 Statistical analysis

Multifactor analysis of variance was used to assess the effect of decortication on colour and proximate composition data of the different sorghum milling fractions and extracted kafirin preparations. Means of at least 2 replicate tests were separated with the least significant difference test.

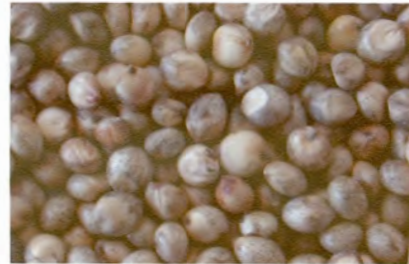
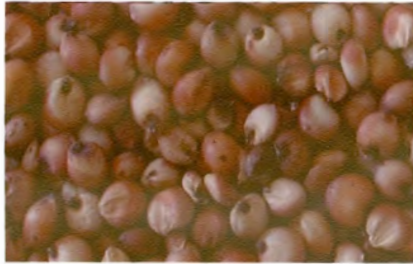
2.1.3 Results and discussion

Visual characterisation of the sorghum grains revealed that NK 283 endosperm texture; the relative proportion of corneous to floury endosperm within the grain, was primarily of intermediate hardness (rating 3) according to the system of Rooney and Miller (1982). The outer translucent corneous endosperm was continuous, making up 50% or less of the total endosperm. Endosperm texture of PANNAR 202-606 was not easy to categorise as just less than 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 due to the different varieties (PANNAR 202 and PANNAR 606) having different endosperm textures.

The whole grains were small, round, and slightly flattened with NK 283 being red compared to PANNAR 202-606 (Fig. 9). At the 90% extraction level, the grains showed signs of some pericarp and germ removal and appeared to be slightly more rounded. The colour of NK 283 appeared less intense, showing patches of white endosperm. This is due to the removal of anthocyanin pigments in the pericarp (Rooney and Murty 1982). At the 75% extraction level, the grains had no pericarp and very little germ remaining. The grains became almost round with some kernels showing signs of fracturing, which was more prominent for PANNAR 202-606. This could be due to differences in endosperm hardness of the different varieties (PANNAR 202 and PANNAR 606). Endosperm hardness affects the processing properties of sorghum grains (Rooney and Miller 1982). In grains with a higher percentage of corneous endosperm, the pericarp (bran) is more readily removed by abrasive decortication from the intact endosperm (Rooney and Miller 1982). Grains with floury endosperm are more difficult to decorticate, and tend to break resulting in losses of fine endosperm particles to the bran fraction (Eggum et al 1982).

NK 283

PANNAR 202-606



a) Whole sorghum grain



b) Decorticated grain, 90% extraction rate



c) Decorticated grain, 75% extraction rate

Fig. 9. Degree of pericarp, germ and endosperm (bran) removal of NK 283 and PANNAR 202-606 sorghums after varying degrees of abrasive decortication.

Table III shows that PANNAR 202-606 whole grain flour (WGF) was lighter, less red and more yellow compared to NK 283 WGF. This is due to the presence of red anthocyanin pigments which are present in the pericarp of red sorghum varieties (Rooney and Miller 1982). Lightness increased with increasing levels of decortication for both NK 283 and PANNAR 202-606 flours. These results were similar to those reported by Awika et al (2002). These authors reported that increasing levels of decortication, using a tangential abrasive dehulling device (TADD) and adjusting yields to standard L values (85 for flour and 67 for grits), resulted in flour and grit being lighter in colour. In addition, as flour and grit colour lightness increased a decrease in decortication yield was observed. Compared to white sorghum, red sorghum was found to have the lowest L values at any given decortication yield.

Bran materials were significantly darker compared to the WGFs and extraction flours (EF). Due to the presence of anthocyanin and anthocyanidin pigments in red sorghum varieties (Rooney and Miller 1982), NK 283 brans were darker and more red and yellow compared to PANNAR 202-606 brans. Ten percent brans were darker and more red and yellow compared to 25% brans for both sorghum varieties. This was presumably due to higher concentrations of pigments in the 10% brans. The relative lightness of 25% brans was probably due to the presence of endosperm material removed during decortication, reducing the polyphenol content.

Table IV shows that the protein and total polyphenol content differed significantly between NK 283 and PANNAR 202-606 WGFs, with NK 283 having 23% more protein and twice the polyphenols. 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 is largely dependent on agronomic conditions including water availability, soil fertility, temperatures and environmental conditions during grain development. In contrast sorghum appearance, namely pericarp colour and thickness, the presence, colour and thickness of the testa and endosperm colour, are all genetically controlled

(Rooney et al 1980). There were no significant differences in fat, crude fibre and ash between the two varieties.

Table III
Sorghum Flour and Bran Colour of NK 283 and PANNAR 202-606, as Measured by Tristimulus Colorimetry.

Milling fraction	Colour ¹				
	L	± a	± b	E ²	
NK 283	Whole flour	68.8±0.1 ^{3e}	+4.5±0.1 ^h	+10.5±0.1 ^{bc}	69.7±0.1 ^e
	90% EF	74.5±0.5 ^g	+3.5±0.1 ^g	+10.3±0.5 ^b	75.0±0.4 ^g
	75% EF	82.1±0.3 ⁱ	+1.2±0.0 ^d	+9.7±0.6 ^a	82.5±0.3 ⁱ
	25% Bran	59.1±0.7 ^c	+8.6±0.2 ^j	+12.8±0.0 ^g	60.7±0.5 ^c
	10% Bran	49.8±0.3 ^a	+10.4±0.1 ^j	+13.5±0.0 ^h	52.4±0.2 ^a
PANNAR 202-606	Whole flour	73.6±0.3 ^f	+0.4±0.0 ^c	+11.0±0.3 ^{cd}	74.3±0.2 ^f
	90% EF	79.9±0.2 ^h	-0.1±0.0 ^b	+11.4±0.0 ^e	80.6±0.1 ^h
	75% EF	84.3±0.4 ^j	-0.6± 0.1 ^a	+11.4±0.7 ^{de}	84.9±0.3 ^j
	25% Bran	66.0±0.2 ^d	+1.4±0.0 ^e	+12.1±0.1 ^f	67.0±0.1 ^d
	10% Bran	56.7±0.2 ^b	+2.3±0.0 ^f	+12.6±0.1 ^g	58.0±0.1 ^b

EF. Extraction flour

1. "L" measures colour intensity with L = 100 for lightness, and L = 0 for darkness, + a = increasing red, - a = increasing green, + b = increasing yellow, - b = increasing blue.

2. $E = \sqrt{L^2 + a^2 + b^2}$

3. Values are means and standard deviations for 2 replicates, means with different letters within a column differ significantly ($p < 0.05$)

Table IV

Chemical Composition (% Dry Basis) and Percentage Distribution of Components of NK 283 and PANNAR 202-606 Whole Flour, 90% and 75% Extraction Flour, and 10% and 25% Brans.

Milling Preparations		Protein (N x 6.25)	Fat	Crude Fibre	Ash	Carbohydrate (by difference)	Total Polyphenols
NK 283	Whole flour	10.7±0.1 ^{1e} [100] ²	3.18±0.49 ^c [100]	2.40±0.16 ^d [100]	1.51±0.0 ^c [100]	82.2±0.7 ^e [100]	0.179±0.025 ^{cd} [100]
	90% EF	10.3±0.2 ^d [86]	2.77±0.35 ^{bc} [66]	1.46±0.05 ^{bc} [56]	1.4±0.0 ^c [86]	84.1±0.6 ^f [92]	0.148±0.011 ^{bc} [90]
	75% EF	9.38±0.1 ^c [65]	1.80±0.42 ^a [36]	0.82±0.12 ^{ab} [25]	1.0±0.0 ^a [53]	87.0±0.4 ^g [79]	0.106±0.021 ^{ab} [38]
	25% Bran	14.4±0.6 ^g [34]	7.45±0.31 ^d [49]	8.88±1.11 ^f [92]	3.0±0.3 ^e [51]	66.4±1.1 ^b [20]	0.653±0.074 ^f [88]
	10% Bran	12.1±0.1 ^f [11]	7.98±0.11 ^d [21]	12.8±0.63 ^h [53]	2.7±0.1 ^{de} [18]	64.4±0.7 ^a [8]	0.902±0.007 ^g [45]
PANNAR 202-606	Whole flour	8.66±0.15 ^b [100]	3.17±0.41 ^c [100]	2.11±0.08 ^{cd} [100]	1.51±0.05 ^c [100]	84.6±0.7 ^f [100]	0.098±0.004 ^{ab} [100]
	90% EF	8.46±0.14 ^b [88]	2.40±0.16 ^{ab} [66]	0.90±0.10 ^{ab} [38]	1.34±0.03 ^{bc} [76]	86.9±0.3 ^g [92]	0.116±0.000 ^{ab} [90]
	75% EF	7.94±0.06 ^a [68]	1.98±0.42 ^a [46]	0.51±0.04 ^a [18]	1.18±0.06 ^{ab} [58]	88.4±0.5 ^h [78]	0.086±0.007 ^a [75]
	25% Bran	10.5±0.17 ^{de} [29]	7.71±0.40 ^d [60]	6.62±0.45 ^e [78]	2.65±0.14 ^d [42]	72.5±0.3 ^d [21]	0.211±0.007 ^{de} [50]
	10% Bran	10.2±0.23 ^d [12]	8.80±0.85 ^e [28]	10.43±0.72 ^g [49]	2.63±0.21 ^d [17]	67.9±1.9 ^c [8]	0.268±0.007 ^e [30]

EF. Extraction flour.

1. Values are the mean and standard deviations of 2 replicates, means with different letters within a column differ significantly ($p < 0.05$)

2. Percentage in whole grain

Decortication altered the chemical composition of the flours. Compared to WGF, increasing decortication resulted in 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 PANNAR 202-606, respectively. Reduction in other components: fat, crude fibre, ash and total polyphenols, also occurred. Loss of these chemical components resulted in the 10% and 25% brans being significantly higher in protein and in fat, crude fibre, ash and total polyphenols compared to WGF and EFs. Compared to WGF, 10% bran was higher in protein by 13% and 17%, fat by 111% and 175%, crude fibre by 433% and 395%, ash by 80% and 73% and total polyphenols by 404% and 173% for NK 283 and PANNAR 202-606, respectively. Compared to WGF, 25% bran was higher in protein by 35% and 21%, fat by 95% and 141%, crude fibre by 267% and 214%, ash by 267% and 214% and total polyphenols by 265% and 115%, respectively. These findings are in general agreement with the results of research on sorghum decortication by Hahn (1969) and Rooney et al (1972).

The colours of kafirin preparations extracted from the milling fractions and commercial zein are shown Table V. For both sorghum varieties lightness of kafirin preparations increased significantly when extracted from flours of lower extraction rate. As lightness increased a decrease in red and yellow values occurred. This is probably related to the lower levels of total polyphenols present for co-extraction in the low extraction flours (75%). As stated, low levels of polyphenols in flours of lower extraction rate are due to the removal of polyphenols, which are concentrated in the grain pericarp, during abrasive decortication of the grain (Rooney and Murty 1982). Commercial zein was darker, more red and yellow compared to kafirin preparations from WGFs and EFs, presumably because it had been extracted from corn gluten prepared from yellow maize (reviewed by Shukla and Cheryan 2001).

Kafirins prepared from the bran fractions were much darker, more red and yellow compared to kafirins prepared from WGFs and EFs. This is no doubt due to higher levels of polyphenols and colour pigments present in the bran fractions, which were extracted with the kafirin. The intense colour of the kafirin preparations extracted from brans is an indication that non-tannin

polyphenols have the ability to bind to kafirin. Kafirin preparations from 10% brans were darker, more red and yellow compared to kafirins prepared from 25% bran, probably due to higher concentration of phenolic and other pigments, in the 10% bran.

Table V
Colour of Defatted Kafirin Preparations from NK 283 and PANNAR 202-606 Milling Fractions and Commercial Zein as Measured by Tristimulus Colorimetry.

		Colour ¹			
		L	± a	± b	E ²
NK 283	Whole flour	81.4±0.9 ^{3cd}	2.7±0.4 ^{fg}	11.4±0.1 ^{bc}	82.3±0.9 ^c
	90% EF	85.3±0.1 ^{ef}	1.3±0.1 ^{de}	11.5±0.1 ^{bc}	86.1±0.1 ^{def}
	75% EF	90.3±0.3 ^g	-0.9±0.1 ^{ab}	8.7±0.6 ^a	90.7±0.2 ^g
	25% Bran	70.9±1.8 ^b	10.1±0.3 ^h	12.9±1.2 ^c	72.7±2.0 ^b
	10% Bran	61.8±2.6 ^a	14.6±1.2 ⁱ	15.7±2.1 ^d	65.4±3.2 ^a
PANNAR 202-606	Whole flour	85.1±2.1 ^{ef}	-0.3±0.5 ^{bc}	13.1±0.0 ^c	86.1±2.0 ^{def}
	90% EF	87.7±0.1 ^{fg}	-0.9±0.0 ^{ab}	11.1±0.2 ^b	88.4±0.1 ^{efg}
	75% EF	88.5±0.2 ^g	-1.4±0.0 ^a	8.8±0.2 ^a	89.0±0.2 ^{fg}
	25% Bran	84.1±0.6 ^{de}	0.8±0.1 ^{cd}	16.1±0.6 ^d	85.6±0.5 ^{de}
	10% Bran	80.0±2.1 ^c	2.1±0.9 ^{ef}	19.0±0.7 ^e	82.3±1.8 ^c
Commercial zein		79.6±0.1 ^c	3.84±0.2 ^g	26.5±0.2 ^f	84.0±0.2 ^{cd}

EF. Extraction flour

1. L- measures colour intensity with L = 100 for lightness, and L = 0 for darkness, + a = increasing red, - a = increasing green, + b = increasing yellow, - b = increasing blue.

2. $E = \sqrt{L^2 + a^2 + b^2}$

3. Values are means and standard deviations for 2 replicates, means with different letters within a column differ significantly ($p < 0.05$)

Table VI shows that whole NK 283 flour yielded 30% more protein compared to PANNAR 202-606, probably due to NK 283 grain having a higher protein content. Protein yields were similar for both sorghum varieties averaging 45-46% of the total grain protein, indicating the relative amount of kafirin was similar for both sorghums. According to Taylor et al (1984), the mean kafirin content of 25 whole sorghum flour varieties was found to be 47.8% when extracted with 60% t-butanol plus reducing agent at ambient temperature. However, values as high as 68% to 73% have been reported by Hamaker et al (1995), when kafirin was obtained by first removing the non-protein nitrogen with albumin and globulin proteins, followed by extraction of protein in a borate buffer with SDS plus reducing agent and precipitation of non-prolamins with 60% t-butanol.

Compared to WGFs, decortication increased the amount of protein extracted from flours of lower extraction rate. Flours of lower extraction rate yielded 16% and 15% more kafirin for 90% EF, and 9% and 15% more kafirin for 75% EF, for NK 283 and PANNAR 202-606, respectively (Table VI). This is despite the fact that flours of lower extraction rate contained less total protein compared to WGFs. Furthermore, protein yields for lower extraction rate flours were higher compared to WGFs by 22% and 16% for 90% EFs and 26% and 24% for 75% EFs, respectively. This tendency indicates that the relative amount of kafirin, compared to other proteins, was higher in flours of lower extraction rate compared to WGFs. This is mainly because kafirin is located solely in the endosperm. Protein yields for NK 283 90% EF and 75% EF, as well as PANNAR 202-606 75% EF were not significantly different, indicating the proportion of prolamins to non-prolamins was similar in these sorghum milling fractions.

Significantly less kafirin was extracted from the bran fractions compared to WGFs and EFs, although the bran fractions contained proportionally more protein (Table VI). The low level of kafirin extracted from the brans is probably due to the bran materials being much richer in non-prolamin proteins compared 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), which is removed from the grain during abrasive decortication (Hahn, 1969). However, increasing decortication resulted in the 25% bran fractions yielding more kafirin compared to brans from the 10% level of decortication. This is due to increasing decortication removing more prolamin-rich corneous endosperm from the grain, and added to the bran materials (Hahn 1969). Of the bran fractions studied, NK 283 25% bran yielded the most kafirin, 42% more kafirin compared to PANNAR 202-606 25% bran, and approximately double the kafirin obtained from the 10% brans. This is presumably due to NK 283 25% bran having considerably more endosperm, and therefore more kafirin for extraction, compared to the other bran fractions. However, protein yields for the 25% brans were not significantly different; indicating the proportion of prolamins to non-prolamins was similar for these bran fractions.

Protein purity of the different kafirin preparations and commercial zein varied (Table VI). Kafirin preparations prepared from flours of lower extraction rate had higher protein contents, ranging from 82% for PANNAR 202-606 90% EF to 90% for NK 283 75% EF. This is because these preparations were lower in fat, polyphenols and other components (possibly ash and non-starch polysaccharides), compared to kafirins prepared from the bran fractions. This is presumably due to the higher purity of lower extraction rate flours, i.e. very low in fat and polyphenols. 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. Defatting proteins with non-polar solvents such as hexane can improve protein purity.

Table VI
Protein Extracted, Protein Yield and Chemical Composition (% Dry Basis) of Kafirin Preparations from NK 283 and PANNAR 202-606 Milling Fractions and Commercial Zein.

		Protein extracted (g protein/ 100 g flour/bran)	Protein yield (% of total protein in flour/bran)	Chemical composition			Protein content of defatted preparation s (N x 6.25)	
				Protein (N x 6.25)	Fat	Total polyphenols		Other (by difference)
NK 283	Whole flour	4.38±0.16 ^{1e}	46.1±1.7 ^d	83.1±2.0 ^{2ef}	12.8±0.8 ^c	0.461±0.106 ^b	3.70±1.40 ^{bc}	88.7±1.1 ^c
	90% EF	5.12±0.07 ^g	56.3±0.8 ^f	87.0±0.1 ^{ef}	10.4±0.3 ^b	0.305±0.013 ^a	2.33±0.44 ^{ab}	90.8±0.9 ^{cd}
	75% EF	4.81±0.10 ^f	57.9±1.2 ^f	90.2±0.1 ^f	9.30±0.8 ^b	0.190±0.035 ^a	0.33±0.96 ^a	93.9±3.8 ^d
	25% Bran	3.43±0.21 ^c	26.7±1.6 ^c	54.1±0.6 ^c	30.7±2.2 ^e	1.423±0.005 ^d	13.84±2.24 ^e	69.4±1.6 ^b
	10% Bran	1.70±0.05 ^a	15.9±0.5 ^a	40.0±4.0 ^{ab}	40.8±0.3 ^f	2.211±0.009 ^e	17.01±4.20 ^f	61.6±6.9 ^a
PANNAR 202-606	Whole flour	3.44±0.12 ^c	45.0±1.5 ^d	77.9±0.6 ^{de}	16.7±0.3 ^d	0.322±0.106 ^{ab}	5.14±0.74 ^c	88.3±0.3 ^c
	90% EF	3.93±0.01 ^d	52.4±0.2 ^e	82.3±0.2 ^{ef}	14.4±1.5 ^c	0.303±0.016 ^a	3.04±1.60 ^{abc}	91.4±0.1 ^{cd}
	75% EF	3.93±0.05 ^d	56.0±0.8 ^f	87.1±0.4 ^{ef}	10.1±0.3 ^b	0.250±0.035 ^a	2.62±0.22 ^{abc}	93.7±0.2 ^d
	25% Bran	2.43±0.06 ^b	26.1±0.6 ^c	46.4±0.5 ^{bc}	44.7±2.5 ^g	0.718±0.122 ^c	8.19±2.55 ^d	72.7±1.6 ^b
	10% Bran	1.84±0.02 ^a	20.4±0.2 ^b	31.8±1.6 ^a	53.9±1.5 ^h	0.754±0.083 ^c	13.60±0.45 ^e	62.0±2.0 ^a
Commercial zein	NA	NA	91.7±0.5 ^g	7.2±0.6 ^a	-	1.15±0.49 ^{ab}	92.5±0.1 ^d	

EF. Extraction flour

1. Values are means and standard deviations of 2 replicates, means with different letters within a column differ significantly (p<0.05)

NA. Not applicable.

- Value not determined

Compared to kafirins prepared from WGFs, bran prepared kafirins were less pure, 52% and 60% less for 10% brans, and 35% and 40% for 25% brans for NK 283 and PANNAR 202-606, respectively (Table VI). This is because they contained significant amounts of fats, total polyphenols and other components. As shown in Table IV, fats, phenolic pigments and non-starch polysaccharides are found in high quantities in sorghum bran. The possible presence of non-starch polysaccharides (namely soluble dietary fibre including hemicellulose, pectins and gums) in the kafirin extracted from the brans could be due to the addition of sodium hydroxide (alkali) and the resulting high pH of the extracting solvent. According to Aoe et al (1993), defatted starch-free rice bran showed sodium hydroxide (2%, pH 14) to be an effective extracting solvent for soluble fibre, yielding 8 g soluble fibre per 100 g rice bran. It is possible some soluble fibre was extracted from the sorghum bran.

There were no significant differences in the final protein content of kafirins prepared from WGFs and 90% EFs, and 90% EFs, 75% EFs and commercial zein, after defatting with hexane (Table VI). Defatting with hexane also increased the purity of bran kafirin preparations by 54% and 95% for 10% bran preparations, and 29% and 57% for 25% bran preparations for NK 283 and PANNAR 202-606, respectively. After defatting, sorghum variety did not seem to influence the final protein content of kafirin preparations extracted from equivalent milling fractions.

Table VII shows that all the kafirin preparations had virtually identical amino acid composition, indicating that sorghum variety and milling fraction did not have an effect on kafirin composition. The amino acid profiles were characteristic of kafirin, 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%). These data are in agreement with Taylor and Schüssler (1986), except for glutamic acid and leucine, which were somewhat higher in this study.

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

		Glu	Leu	Ala	Pro	Phe	Asp	Tyr	Ser	Val	Ile	Thr	Gly	His	Arg	Met	Lys
NK 283	Whole flour	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	Whole flour	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 ¹		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

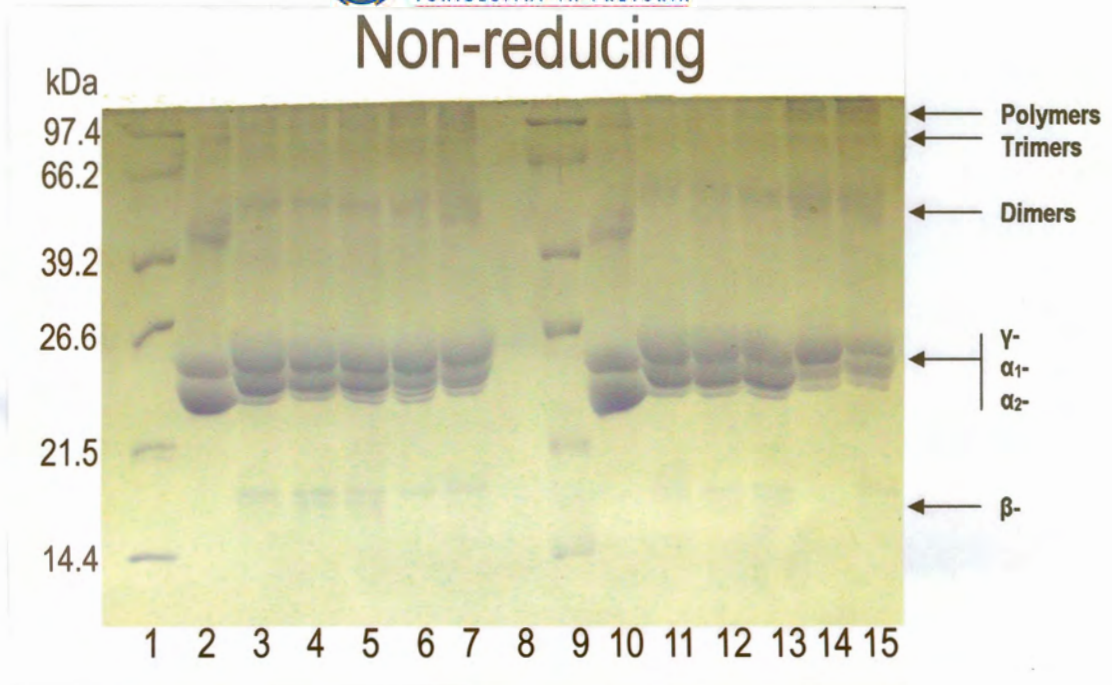
EF. Extraction flour

1. Taylor and Schussler (1986). Prolamins extracted from the endosperm of tannin-free red sorghum, Barnard Red, (Cys/2 = 0.5, NH₃ = 2.4)

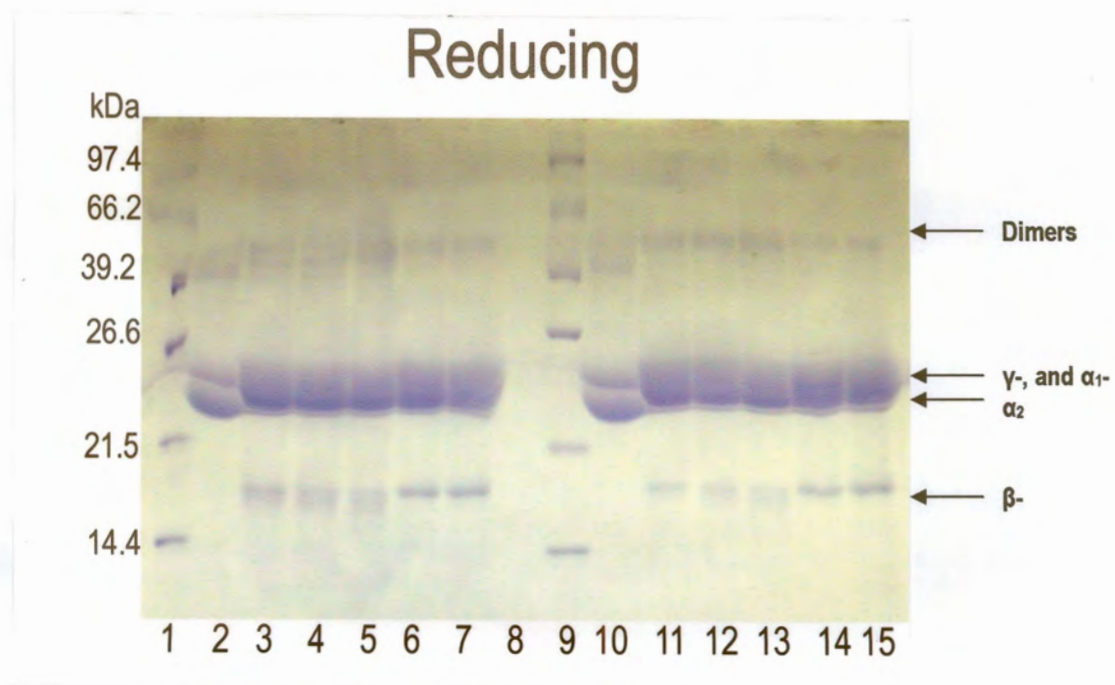
Compared to the commercial zein, kafirin preparations were higher in glutamic acid, glutamine, alanine, valine, glycine and histidine, but lower in leucine, phenylalanine, and serine (Table VII). These differences are similar to those reported for kafirin and zein, resulting in kafirin being more hydrophobic than zein (reviewed by Wall and Paulis 1978).

SDS-PAGE under non-reducing conditions (Fig. 10a) showed that all kafirin preparations, tracks 3 to 7 PANNAR 202-606, and tracks 11 to 15 NK 283, gave essentially identical bands. Bands with M_r of approximately 45,000 to 50,000, and 66,200 and 97,000 are dimers, trimers and oligomers 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 of approximately 26,000; 24,000; 22,000 and 18,000, are the monomeric kafirin polypeptides, γ -, α 1-, α 2-, and β -kafirins, respectively (El Nour et al. 1998). The intensity of the oligomeric proteins appeared to be higher in kafirins from bran (tracks 6, 7, 14 and 15) compared to kafirins extracted from WGFs (tracks 2 and 10) and EFs (tracks 3, 4, 5, 11, 12, and 13), possibly due to the higher levels of polyphenols increasing stain density.

Under reducing conditions (Fig. 10b) all kafirin preparations, tracks 3 to 7 PANNAR 202-606 and 11 to 12 NK 283, were essentially identical, with bands in the 45,000 and 50,000 region, designated reduction-resistant dimers (Duodu et al 2002), and the monomers γ - (M_r 26,000), α 1- (24,000), α 2- (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.



a)



b)

Fig. 10. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of defatted commercial zein and kafirin preparations from NK 283 and PANNAR 202-606 milling fractions under a) non-reducing and b) reducing conditions. Tracks 1 and 9, molecular weight standard; tracks 2 and 10, commercial zein; Tracks 3 to 7 PANNAR 202-606 kafirin preparations (Whole flour; 90% and 75% EF, 25% and 10% bran, respectively); Tracks 11 to 15 NK 283 kafirin preparations (whole flour, 90% and 75% EF, 25% and 10% bran, respectively).

Compared to non-reducing conditions (Fig. 10a), under reducing conditions (Fig. 10b) the monomeric bands of γ - and α 1-kafirins overlapped, forming an intense continuous band. This was also found by El Nour et al (1998). It is presumed that reduction of the oligomers, observed under non-reducing conditions, increased the concentration of these monomeric proteins resulting in band overlapping and increased band intensity.

Additionally, under reducing conditions (Fig. 10b), the β -kafirins in the bran extracted kafirins (tracks 6, 7, 14 and 15) appeared to be darker and more defined compared to β -kafirins in kafirin extracted from WGFs and EFs. Since the level of polyphenols was higher in the kafirins extracted from the brans, it would appear that the polyphenols had a higher affinity towards the β -kafirins. 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 stabilised by hydrogen bonding (Murray et al 1994). This phenomenon was not observed under non-reducing conditions (Fig. 10a, tracks 6, 7, 14 and 15), possibly due to too low concentration of monomeric β -kafirins, due to the formation of cross-linked polymers.

Electrophoretic mobility of commercial zein under both non-reducing (Fig. 10a) and reducing conditions (Fig. 10b) (tracks 2 and 10) showed two major bands of M_r 24,000 and 23,000 to 22,000, corresponding to α -1 and α -2 zein, as well as high molecular weight dimers, of M_r 40,000 to 50,000. Dimer bands appeared more intense under non-reducing conditions, and there were also polymer bands in the 97,000 region. Bands for β -, δ - and γ -zein were not observed. The absence of β -, δ - and γ -zeins in commercial zein agrees with previous findings by Parris et al (1997) using RP-HPLC, and Parris and Dickey (2001) using SDS-PAGE, showing commercial zein to consist almost exclusively of α -zein. Commercial zein is usually extracted from corn gluten meal, a co-product of maize wet milling (reviewed by Shukla and Cheryan 2001). The maize is first steeped with sulphur dioxide, to help soften the kernels and facilitate removal of starch. Sulphur dioxide is thought to weaken the maize matrix structure by breaking disulphide cross-links found in β -, δ -

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). The procedures for zein extraction then include the use of aqueous-alcohol solvents with added alkali, normally sodium hydroxide (reviewed by Shukla and Cheryan 2001). The addition of alkali to the zein solution during extraction is thought to result in the destruction of cystine (Boundy et al 1967), presumably due to the reduction of disulphide bonds, thus improving zein stability in solution.

2.1.4 Conclusions

Sorghum bran, a co-product of the sorghum dry milling industry appears to be a rich source of protein, containing from 11% to 34% of the total grain protein. However, kafirin yields from bran fractions are lower than yields obtained from WGFs and flours of lower extraction rate, because the bran contains proportionally less prolamin protein, as prolamin is located solely in the endosperm. High levels of decortication (25%) of high protein sorghum varieties would increase the prolamin content of bran and result in more kafirin being extracted compared to bran of lower levels of decortication (10%). There are high levels of fat and total polyphenol contaminants in kafirin preparations from bran. Also as the level of polyphenols are much higher in the bran from red sorghum than from white, this results in highly coloured kafirin preparations. Bran kafirin preparations of low purity may require further purification steps if this kafirin is to be used for biofilms and other applications.

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2.2 Physical, mechanical and barrier properties of kafirin protein biofilms from red and white sorghum milling fractions

Abstract

To determine the properties of biofilms prepared from kafirin extracted from different dry milling fractions, including bran. Free-standing biofilms were prepared from defatted kafirin preparations and commercial zein by casting. All the kafirin preparations including those from bran were able to form biofilms. However, differences in film thickness, clarity, flexibility, surface texture, odour and colour were observed between the different kafirin biofilms. Bran kafirin biofilms were highly coloured, less flexible with a less smooth surface texture compared to biofilms from the other sorghum milling fractions, probably due to higher levels of contaminants in bran kafirin. The strong colour of the bran biofilms could limit their use in certain coating applications. Generally, kafirin biofilms had poorer water barrier properties compared to the zein biofilm, possibly due to kafirin biofilms being thicker. Poor flexibility of the bran biofilms could have resulted in microcracks, increasing water vapour transmission through these films. Kafirin biofilms had higher tensile and breaking strength, but poorer elongation compared to the zein biofilm. This is presumably due to the presence of β - and γ -kafirin polypeptides, and high levels of disulphide cross-linking in these films.

2.2.1 Introduction

Biofilms (edible polymer films) can be defined as a thin layer of edible material formed on a food as a coating or placed pre-formed, on or between food components (Krochta and De Mulder-Johnston 1997). Generally coatings are applied and formed directly onto the food product, whereas films are structures, which are applied after being formed separately (Gontard and Guilbert 1994). The main purpose of biofilms is to improve the overall quality of food by extending the shelf life, and improving the mechanical integrity or handling characteristics of the food (Krochta and De Mulder-Johnston 1997) and possibly improve the economic efficiency of synthetic packaging materials (Kester and Fennema 1986). Extending shelf life can be achieved by the inhibition of the migration of moisture, gases or other food components such as lipids and aromas into or out of the food.

A variety of natural polymers, namely polysaccharides, proteins and lipids from various agricultural raw materials have been used, either alone or in combination, to produce composite biofilms (reviewed by Krochta and De Mulder-Johnston 1997). Zein, the aqueous-alcohol soluble prolamin protein of maize has been studied extensively as a source of protein for the formation of edible films and biodegradable packaging (Lai et al 1997, Parris and Coffin 1997, Lai and Padua, 1998). Kafirin, the aqueous-alcohol soluble prolamin protein of sorghum, is similar to zein in molecular weight, solubility, structure, and amino acid composition (Shull et al 1991). However, only one research paper has been published on the film forming properties of kafirin, by Buffo et al (1997). They found that the film forming properties of kafirin appear to be similar to those of zein.

In a previous paper (2.1), sorghum bran, a co-product of the sorghum dry milling industry, was identified as being a source of kafirin. The extracted kafirin could have the potential to be used as an edible coating, to improve the quality and shelf life of southern Africa's export fruit and nuts, or to be used in the formation of biodegradable packaging (Enviropak 2002).

The objectives of this work were to determine what effect kafirin extracted from different sorghum varieties, namely condensed tannin-free red and white, and different dry milling fractions, including bran, will have on kafirin biofilm quality in terms of physical, mechanical and barrier properties.

2.2.2 Materials and methods

2.2.2.1 Kafirin preparations

Kafirin was extracted from NK 283 (red) and PANNAR 202-606 (white) sorghum dry milling fractions: whole grain flour (WGF), 90% and 75% extraction flours (EF) and 10% and 25% brans, using a hot aqueous ethanol solvent system as described (2.1.2.4). Commercial zein was obtained from Sigma (Z3625). All protein samples were defatted before biofilm preparation, as described (2.1.2.4).

2.2.2.2 Biofilm formation

Protein solutions (16% (w/w) protein) were prepared by dissolving defatted protein samples in 9 g solvent (or 20 g for kafirin extracted from the bran) (70% (w/w) absolute ethanol made up with distilled water) and then adding 0.6 g plasticizer mixture (42% of protein content). The plasticizer mixture comprised 1:1:1 (w/w/w) polyethylene glycol 400 (PEG), glycerol (GLY) and 90% lactic acid (LA). The protein preparations, solvent and plasticizer were weighed into a 100 ml Erlenmeyer flask containing a magnetic stirrer bar. The total weight of the flask and contents was recorded. The flask was closed with foil and placed on a stirrer hotplate (70°C). The stirring motion was turned slowly to full and a frozen cooler block was placed on top of the flask to reduce ethanol evaporation. The flask was held for 10 min with rapid stirring. After this time, the flask and contents were reweighed and absolute ethanol added to the original weight of the contents. After replacing lost ethanol due to evaporation, the solution was stirred for a further 20 sec. Aliquots (4 g or 8 g for kafirin extracted from the bran) were weighed into plastic Petri dishes (14 cm diameter) and gently swirled to coat the bottom of the dish. Petri dishes (without lids) were placed on a level surface in a 50°C oven for approximately

18 hr. The oven fan was disconnected to reduce draft. Biofilms were analysed within 6 hr of drying.

2.2.2.3 Biofilm characterisation

2.2.2.3.1 Colour

Biofilm colour was determined using Tristimulus colorimetry (HunterLab, ColorQuest, Hunter Associate Laboratories, Reston, VA) using the L, a, b scale. Biofilms were carefully peeled from the Petri dishes using the sharp point of a divider and broad forceps. The intact film was placed over the window of the HunterLab instrument and the white standard plate was placed over the film to aid light reflectance.

2.2.2.3.2 Sensory evaluation

The physical properties, namely clarity, flexibility, surface texture and odour, of the different kafirin biofilms and the commercial zein biofilm (peeled from the Petri dishes as described) were rated using a trained sensory panel, using a scale from 1 to 5. A clear, flexible, odourless polyethylene bag with a smooth surface texture was used to represent 1 on the scale for all attributes.

2.2.2.3.3 Water barrier properties

A modified ASTM E96-95 (ASTM, 1995) standard test method was used to measure water vapour transmission (WVT) (g/hrm^2) and water vapour permeability (WVP) ($\text{gmm/m}^2\text{hrkPa}$) through the biofilms. A circular template (4 cm diam) was used to cut two discs from each biofilm. Thickness measurements were taken by micrometer (graduated to 1 micron) at the centre and four points around the edge of each cut disc. Thickness was measured as the mean of these five points.

Schott bottles (100 mL) were modified for use 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. The bottles were filled with 90 mL deionised water and biofilms were placed with the surface that was in contact with the Petri dish to the inside of the bottle. After the biofilms were mounted in position, a fibre tap washer (internal diam 3.1 cm) was placed on top of the film and used to create a water-tight seal. The whole assembly was weighed and placed in an incubator (25°C) with circulating air. The relative humidity (RH) inside the incubator was recorded daily (Kane-May KM 8006, Relative Humidity Meter, Welwyn Garden City, UK) and the average RH (approximately RH2 20%) and the RH inside the test bottle (assumed RH1 100%) was used to calculate the RH gradient. Temperature inside the incubator was also recorded daily using an alcohol based thermometer placed in a beaker containing distilled water. The weight loss of each bottle was recorded daily for ten days. Flux, the slope of the mathematical least squares regression of mass over time (g/hr), was calculated and used to calculate WVT and WVP. Water vapour transmission rate was calculated by dividing the slope by the effective surface area of the biofilm. WVP was calculated from flux, thickness and RH gradient according the equation:

$$WVP = \text{flux} \cdot x / A P_0 (RH1 - RH2)$$

were x is biofilm thickness (mm), A is the area of the biofilm surface exposed to permeate (m^2), P_0 is the vapour pressure of pure water (kPa) (at 25°C, P_0 is 3.17 kPa) and $(RH1 (100\%) - RH2 (20\%))$ is the relative gradient used in the experiment.

2.2.2.3.4 Mechanical properties

A modified ASTM D882-97 method (ASTM 1997) was used to determine the mechanical properties of the biofilms. A TA-XT2 Texture Analyser (Stable Microsystems, Goldalming, UK), fitted with tensile rig grips (code ATG), with pieces of sandpaper stuck onto the grips, was used. The initial distance between the grips and the crosshead speed were set at 40 mm and 0.4 mm/s, respectively. From each film, 6 strips (60 mm x 6 mm) were cut using a sharp scalpel and metal ruler. The minimum thickness of each strip was measured

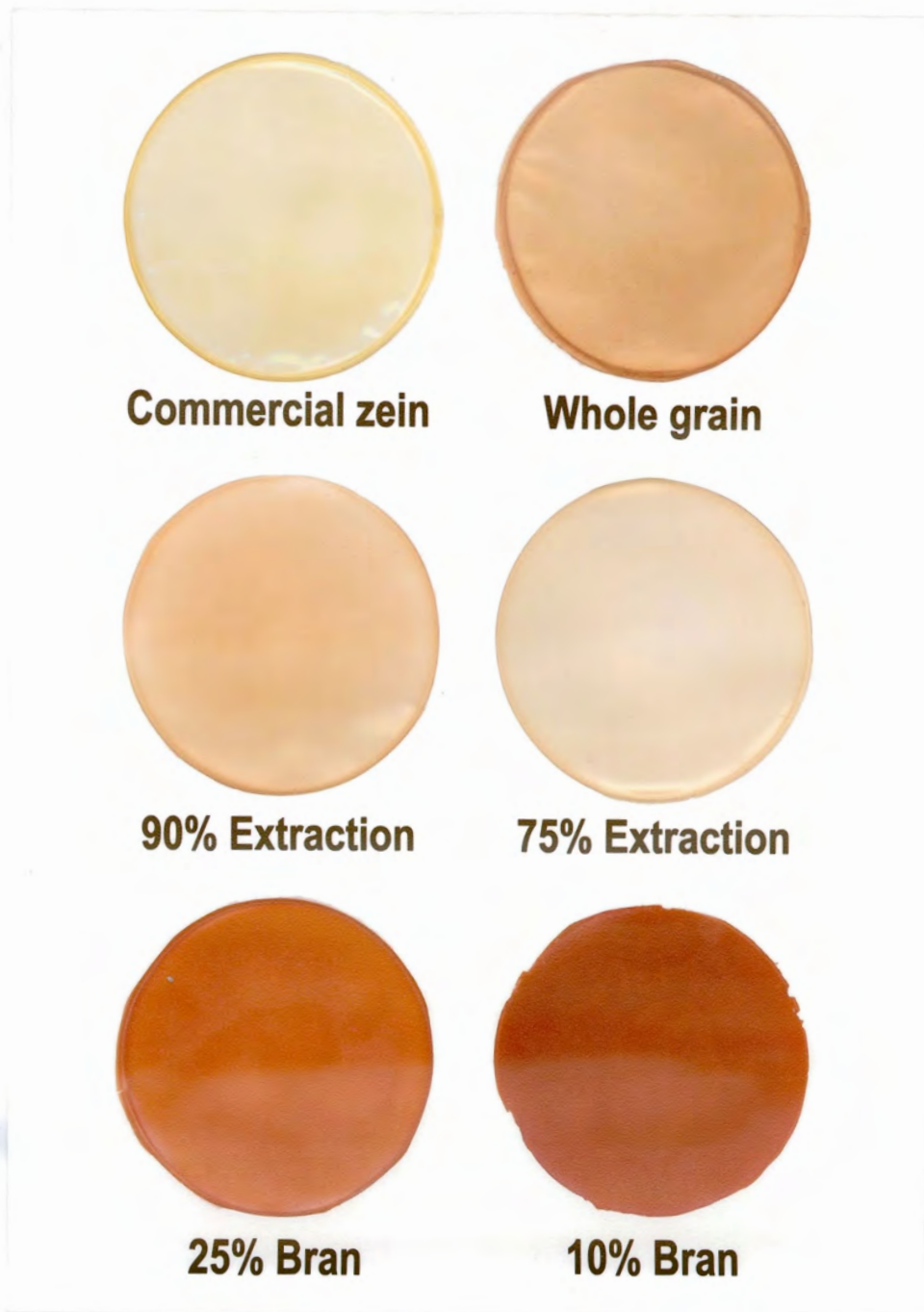
using a micrometer. The films were tested at ambient temperature and RH. The film strips were deformed until breakage occurred. Force and elongation were recorded during extension. Tensile strength (maximum load divided by the minimum cross sectional area of the film strip), tensile strength at break (load at break divided by the original minimum cross-sectional area of the film strip), and percentage elongation at break (extension at the moment of rupture of the film strip divided by the distance between the grips and multiplying by 100) were calculated (ASTM 1997).

2.2.2.4 Statistical analysis

Multifactor analysis of variance was used to assess the colour, mechanical and barrier properties of the kafirin and commercial zein biofilms. Means of at least 2 replicate tests for colour, and 4 replicate tests for mechanical and barrier properties were separated with the least significant difference test.

2.2.3 Results and discussion

Biofilms could be formed from kafirin extracted from the different sorghum dry milling fractions, including the bran. The different kafirin biofilms were found to differ in colour. Their colours ranged from light pink to dark red for NK 283 kafirin biofilms (Fig. 11a) and light tan to dark yellow for PANNAR 202-606 kafirin biofilms (Fig.11b). Furthermore, in Table VIII it can be seen that kafirin biofilms became lighter, less red and yellow when prepared from kafirin extracted from EFs. This is due to these kafirin preparations containing lower levels of polyphenols (2.1.3, Table VI). Similarly, Buffo et al (1997) reported kafirin extracted from sorghum gluten, to result in kafirin biofilms (cast in hot ethanol, with plasticizers) with Lab values of 75.48, 7.27 and 48.10, respectively, except they were more yellow. The high levels of polyphenols in kafirins extracted from the brans resulted in the bran biofilms being highly coloured. Biofilms prepared from kafirin extracted from NK 283 bran were the darkest, and most coloured (dark red). This is due to high levels of anthocyanin polyphenol pigments in red sorghum bran (Rooney and Miller 1982), being extracted with the kafirin.



a)

Fig. 11. Biofilms prepared from defatted commercial zein and kafirin extracted from a) NK 283 and b) PANNAR 202-606, milling fractions (whole grain flour, 90% and 75% extraction flour and 25% and 10% brans).



Commercial zein



Whole grain



90% Extraction



75% Extraction



25% Bran



10% Bran

b)

The zein biofilm was bright yellow compared to the kafirin biofilms (Table VIII), presumably due to xanthophylls and carotenoids being extracted with the zein from the maize or corn gluten meal (CGM) (reviewed by Shukla and Cheryan 2001).

Kafirin biofilms were thicker, less transparent and flexible compared to the zein biofilm (Table VIII). Kafirin biofilms also had less smooth surface texture and were generally lower in odour than the zein biofilm. Biofilms prepared from kafirin extracted from EFs were thinner, with improved clarity, flexibility, surface texture smoothness and had less odour, compared to biofilms prepared from kafirin extracted from the brans. Improved physical attributes of kafirin biofilms prepared from EFs, is presumably due to the higher purity of these kafirin preparations, which ranged from 90.8 to 93.9% protein after defatting with hexane (2.1.3, Table VI). In addition, lower levels of volatiles most probably resulted in these films having less odour than the bran kafirin biofilm preparations.

During film casting it was observed that the film forming solutions of kafirin were more viscous and gelled quickly upon cooling, compared to the commercial zein. Kafirins extracted from the bran gelled and formed a lumpy mass, which necessitated the need to double the amount of solvent used. Doubling the amount of solvent used in the film formulation improved kafirin solubility and resulted in a pourable film forming solution. However, it was still very viscous. The high viscosity was probably simply due to the fact that there was more material as the bran kafirin was only 61-73% pure after defatting with hexane, where as WGFs and EFs were 88% and more than 90% pure, respectively (2.1.3, Table VI). Additionally, the non-starch polysaccharides in the bran could have been hydrated by the solvent. The bran biofilms were thicker, presumably mainly because they contained more material, and probably also because they were less smooth.

Gelling of the kafirin film forming solutions made casting difficult as a smooth even layer was not always achieved. This resulted in kafirin biofilms having a more rough texture, compared to the zein biofilm (Table VIII). Possible

reasons for the greater gelling of kafirin compared to commercial zein, could be due to the presence of β - and γ -kafirin, (2.1.3, Fig. 10a and b). These prolamin polypeptides are rich in sulphur containing amino acids and are reported to be involved in the formation of kafirin oligomers by disulphide cross-linking (El Nour et al 1998). Furthermore, high temperatures, as occurred during kafirin extraction and film casting, have been reported to increase the presence of these kafirin oligomers, and cause slight secondary structural changes in the kafirin polypeptides (Duodu et al 2002). Slight secondary structural changes in the kafirin polypeptides could result in increased intermolecular bonding occurring between the polypeptides and thus result in kafirin being more prone to gelling than commercial zein.

Table VIII
Physical Properties and Colour of Kafirin Biofilms from NK 283 and PANNAR 202-606 Milling Fractions and Zein Biofilm.

	Thickness (μm)	Clarity ¹	Flexibility ²	Surface texture ³	Odour ⁴	Colour ⁵				
						L	$\pm a$	$\pm b$	E ⁶	
NK 283	Whole flour	98 \pm 5 ^{7bc}	3	2	2	3	59.1 \pm 2.38 ^c	21.6 \pm 2.4 ^e	30.7 \pm 0.6 ^e	70.0 \pm 1.0 ^c
	90% EF	115 \pm 7 ^d	2	2	2	2	71.1 \pm 0.4 ^e	8.7 \pm 0.1 ^c	23.0 \pm 0.3 ^d	75.3 \pm 0.5 ^e
	75% EF	107 \pm 10 ^{cd}	2	1	2	1.5	78.0 \pm 0.6 ^f	2.9 \pm 0.5 ^b	16.5 \pm 1.3 ^b	79.8 \pm 0.3 ^f
	25% Bran	132 \pm 2 ^{ef}	4	3	3	4	37.2 \pm 1.4 ^b	40.7 \pm 0.1 ^f	22.8 \pm 0.7 ^d	59.6 \pm 1.1 ^b
	10% Bran	148 \pm 6 ^g	4	3	3	4.5	31.1 \pm 0.4 ^a	41.5 \pm 0.2 ^f	18.9 \pm 0.3 ^c	55.2 \pm 0.5 ^a
PANNAR 202-606	Whole flour	102 \pm 11 ^{bc}	3	2	2	3	75.6 \pm 0.2 ^f	0.5 \pm 0.0 ^a	21.9 \pm 1.0 ^d	78.7 \pm 0.1 ^f
	90% EF	92 \pm 12 ^b	2	2	2	2	78.0 \pm 0.8 ^f	-0.7 \pm 0.1 ^a	18.0 \pm 0.6 ^{bc}	80.0 \pm 0.7 ^f
	75% EF	92 \pm 12 ^b	2	1	2	1.5	76.9 \pm 0.1 ^f	-1.1 \pm 0.1 ^a	22.1 \pm 0.4 ^d	80.0 \pm 0.2 ^f
	25% Bran	130 \pm 4 ^e	4	3	3	4	65.6 \pm 1.9 ^d	6.9 \pm 0.7 ^c	31.7 \pm 0.6 ^{ef}	73.2 \pm 0.2 ^d
	10% Bran	142 \pm 9 ^{fg}	4	3	3	4.5	61.0 \pm 1.9 ^c	12.0 \pm 2.0 ^d	32.3 \pm 0.3 ^f	70.1 \pm 1.2 ^c
Commercial Zein	74 \pm 6 ^a	1	1	1	4	83.8 \pm 0.4 ^g	-1.3 \pm 0.0 ^a	10.6 \pm 0.3 ^a	84.4 \pm 0.5 ^g	

EF. Extraction flour

1. Clarity (1 clear to 3 transparent to 5 translucent); 2. Flexibility (1 flexible to 5 brittle); 3. Surface texture (1 smooth to 5 rough); 4. Odour (1 odourless to 5 strong odour).

5. L- measures colour intensity with L = 100 for lightness, and L = 0 for darkness. + a = increasing red, - a = increasing green, + b = increasing yellow, - b = increasing blue.

 6. $E = \sqrt{L^2 + a^2 + b^2}$.

 7. Values are means and standard deviations for 2 replicates, means with different letters within a column differ significantly ($p < 0.05$)

Figure 12 suggests that the bran kafirin biofilms had better water barrier properties (mean 11.3-12.2 g/hrm²) as they reduced WVT by between 16% and 29% compared to biofilms prepared from kafirin extracted from WGFs and EFs (13.1-15.9 g/hrm²) and commercial zein (14.9 g/hrm²). However, WVT calculations do not take biofilm thickness into consideration. As stated, bran biofilms were much thicker than biofilms prepared from kafirin extracted from WGFs, EFs and commercial zein (Table VIII). The increased thickness of bran biofilms could have reduced WVT through the films.

Zein biofilm had significantly lower WVP than all the kafirins biofilms (Fig. 13). The bran kafirin biofilms, especially those prepared from kafirin extracted from 10% brans and PANNAR 202-606 25% bran had in general the highest WVP. No trend in WVP was observed for biofilms prepared from kafirin extracted from WGFs and EFs from the two sorghum varieties. WVP calculations do take biofilm thickness into consideration, thus eliminating differences in film thickness. However, according to Park and Chinnan (1995), WVP through a biofilm can increase as biofilm thickness increases. Reasons for increased WVP include changes in the film structure due to increased film thickness, as well as possible swelling of hydrophobic films which could further alter the film structure increasing WVP. Furthermore, the poorer flexibility (Table VIII) of the bran biofilms made them more difficult to handle, possibly resulting in microcracks in the film which could also increase WVP. Although the primary mechanism for vapour flow through a biofilm is by active diffusion; cracks, pinholes, or other flaws have been reported to decrease water vapour barrier properties of biofilms (reviewed by Gontard and Guilbert 1994). These results differ from Buffo et al (1997), who reported the WVP of kafirin biofilm to be slightly lower (5.5 g mm/m²hrkPa) than commercial zein biofilm (5.7 g mm/m²hrkPa). However, the kafirin preparations contained far more crude free fat compared to the commercial zein, possibly increasing kafirin biofilm hydrophobicity and thus reducing WVP.

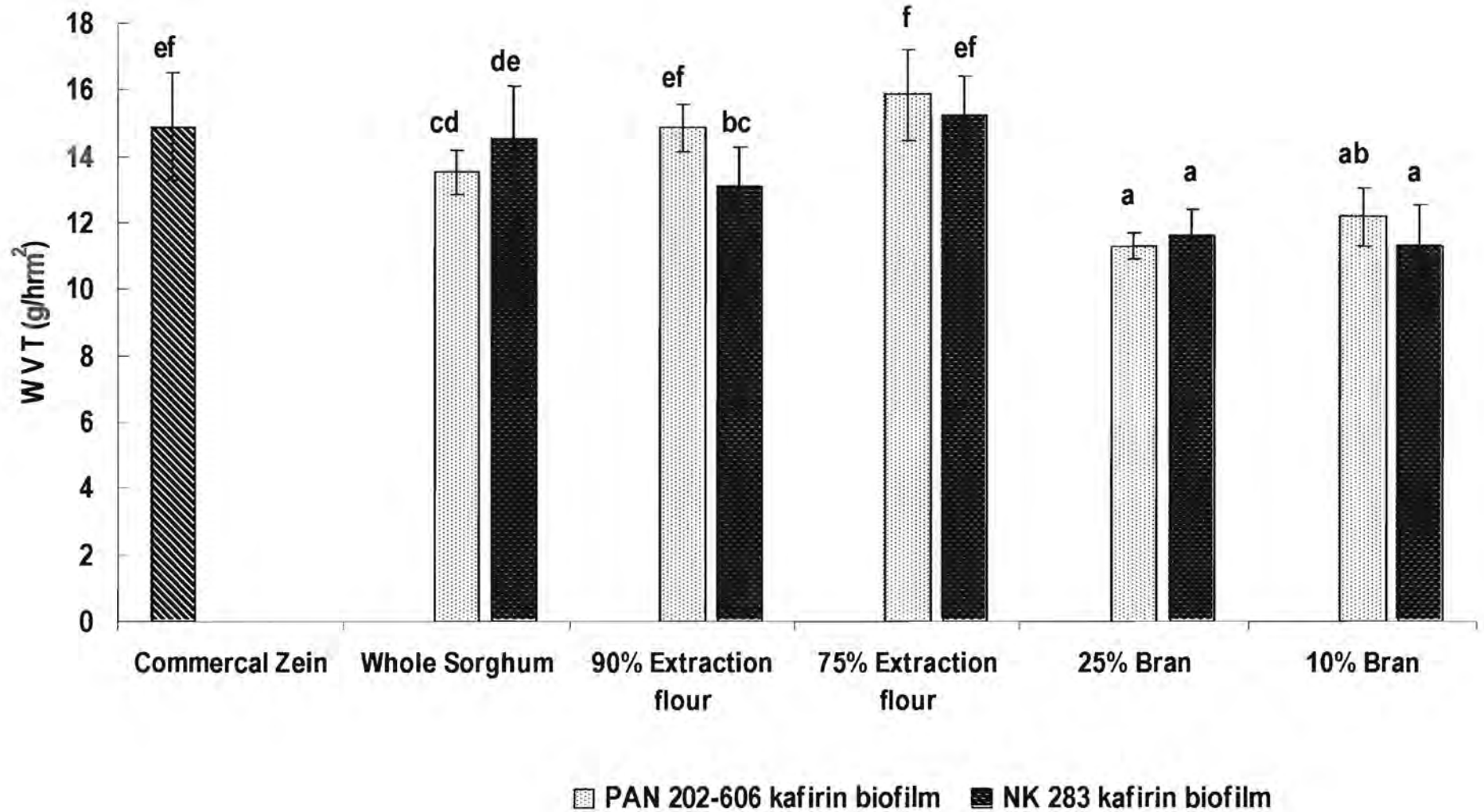


Fig. 12. Water vapour transmission (WVT) of biofilms prepared from defatted kafirin preparations from NK 283 and PANNAR 202-606 milling fractions and commercial zein. Bars with different letter differ significantly ($p < 0.05$).

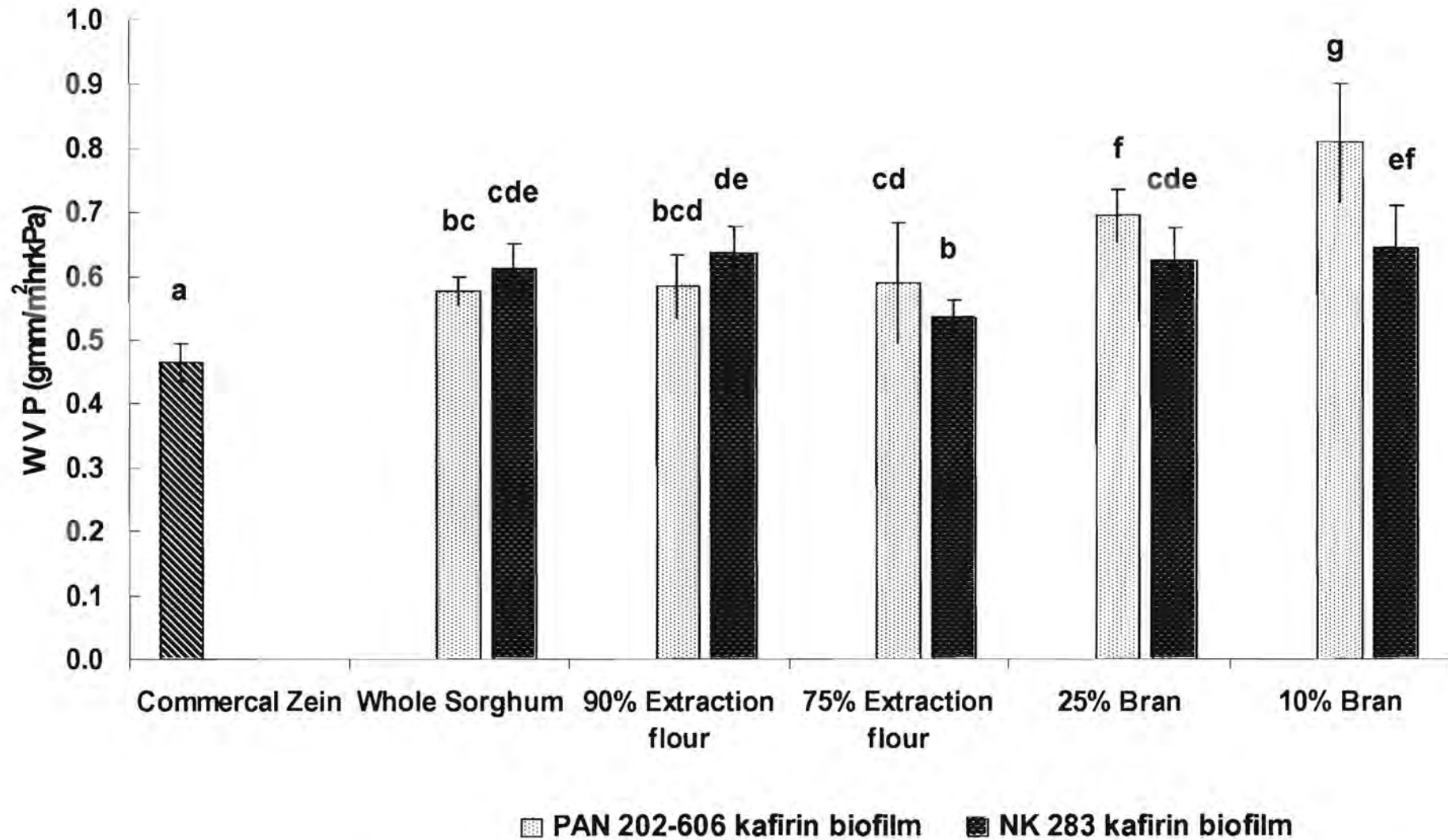


Fig. 13. Water vapour permeability (WVP) of biofilms prepared from defatted kafirin preparations from NK 283 and PANNAR 202-606 sorghum milling fractions and commercial zein. Bars with different letters differ significantly ($p < 0.05$).

Figure 14 shows that all the kafirin biofilms had higher tensile strength (40 to 70% stronger) than the zein biofilm. In Figure 15 it can also be seen that the tensile strength at break of the kafirin biofilms was also higher. The higher tensile and breaking strength of the kafirin biofilms, compared to the zein biofilm, is perhaps due to a higher level of disulphide cross-linking occurring between the kafirin polypeptides. As stated, the extracted kafirin was found to contain far higher levels of cystine rich β - and γ -kafirin polypeptides (2.1.3, Fig. 10a and b), than the commercial zein. Cross-linking (such as disulphide cross-linking) is believed to increase the tensile and breaking strength properties of biofilms by increasing bond formation, while reducing film flexibility (Parris and Coffin 1997, Lens et al 1999). In general, no difference in tensile or breaking strength was observed between biofilms prepared from kafirin extracted from the different sorghum varieties or dry milling fractions. However, it would appear that kafirin of increasing purity could result in biofilms with increasing tensile and breaking strength.

From Figure 16, it can be seen that the zein biofilm had significantly much higher elongation than the kafirin biofilms. Possibly due to lower levels of β - and γ -zein polypeptides in commercial zein compared to the kafirin preparations, as well as more effective plasticization of the zein. Of the kafirin biofilms, the bran biofilms had in general the lowest elongation. Again, this is presumably due to the high levels of β - and γ -kafirin polypeptides in these kafirin preparations. Also, higher levels of contaminants in the kafirin extracted from the bran, presumably resulted in poor protein-protein intermolecular bonding, as well as reducing the effect of the plasticizers. It is probable that the mechanical properties of the bran biofilms could be improved by purifying the kafirin and better plasticization could improve the elongation properties of all kafirin biofilms.

The results obtained in this study differ from those reported by Buffo et al (1997). According to Buffo et al (1997), kafirin biofilms had tensile strength of 2.1 N/mm², (much lower, Fig 14) and percentage strain of 106.1% (much higher, Fig 16) compared to that found in this study. Apart from variations in film preparation, plasticizer composition and testing conditions, it is possible that higher levels of crude free fat (6.74%) in the kafirin prepared by Buffo et al (1997), had a significant plasticizing effect on these films, influencing their mechanical properties.

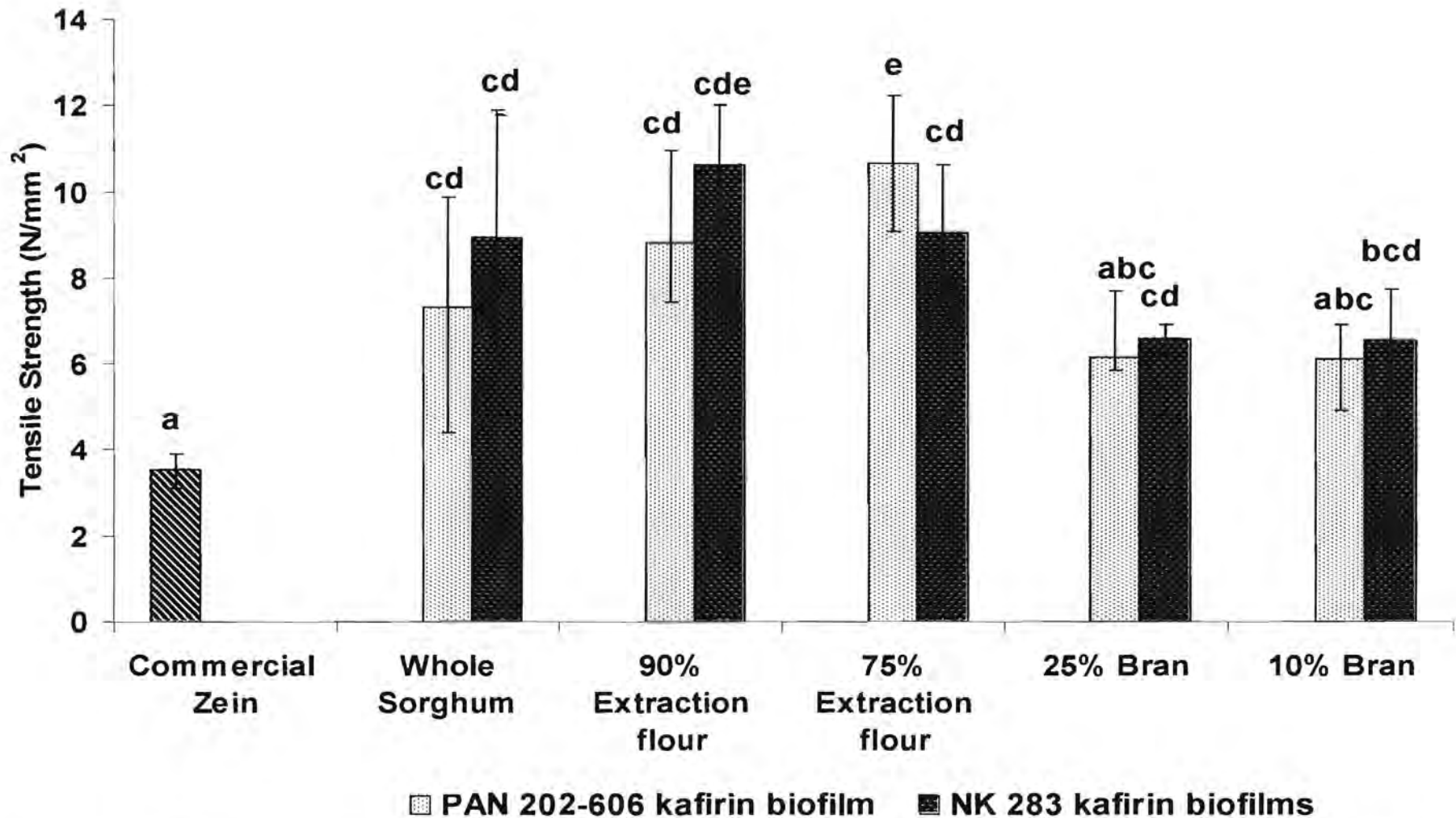


Fig. 14. Tensile strength, calculated with the mean minimum cross-sectional area, for biofilms prepared from defatted kafirin preparations from NK 283 and PANNAR 202-606 sorghum milling fractions and commercial zein. T - Standard error of 4 replica plates, each having 6 strips tested, bars with different letters differ significantly ($p < 0.05$).

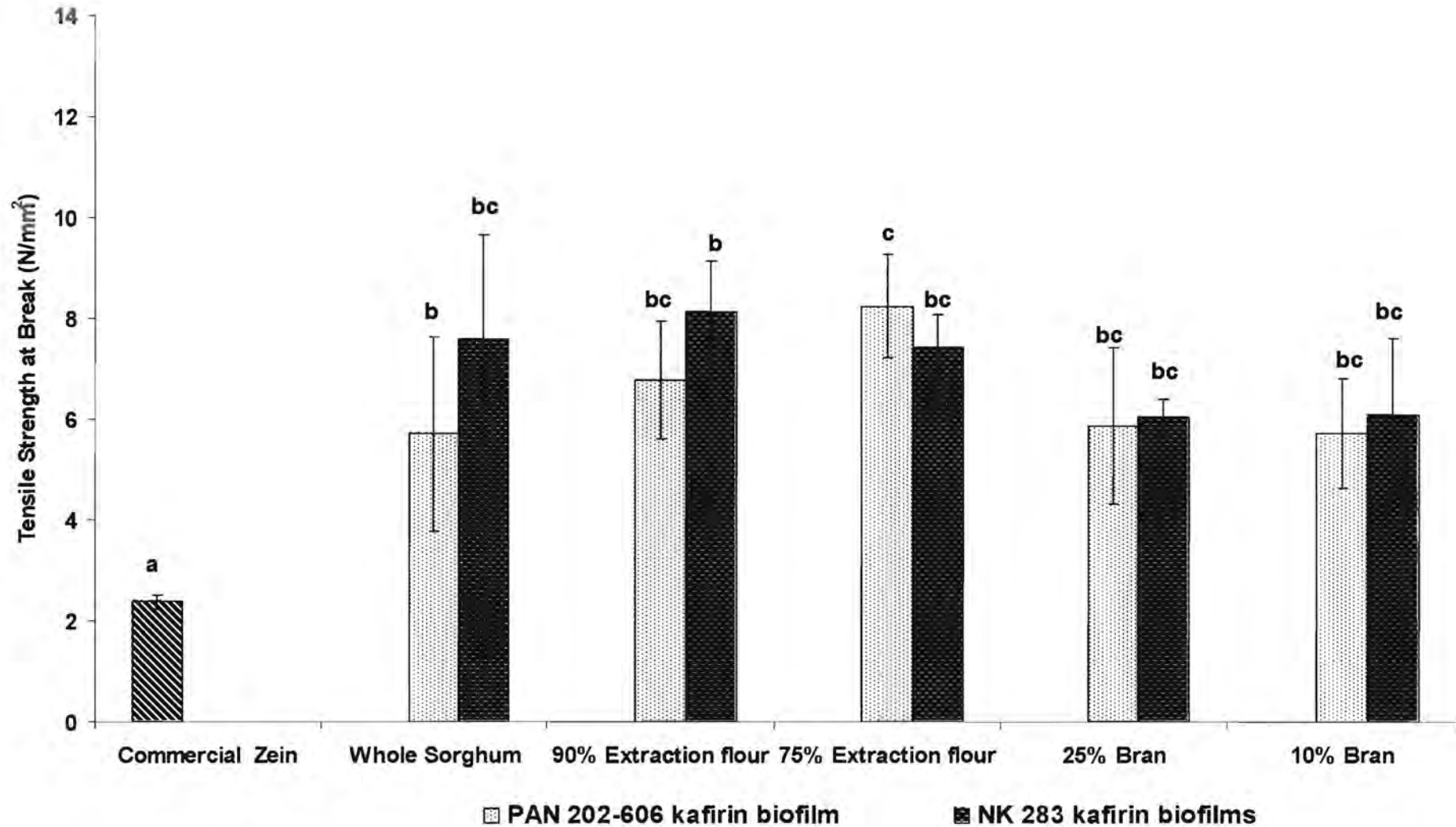


Fig. 15. Tensile strength at break, calculated with the mean minimum cross-sectional area, for biofilms prepared from defatted kafirin preparations from NK 283 and PANNAR 202-606 sorghum milling fractions and commercial zein. T - Standard error of 4 replica plates, each having 6 strips tested, bars with different letters differ significantly ($p < 0.05$).

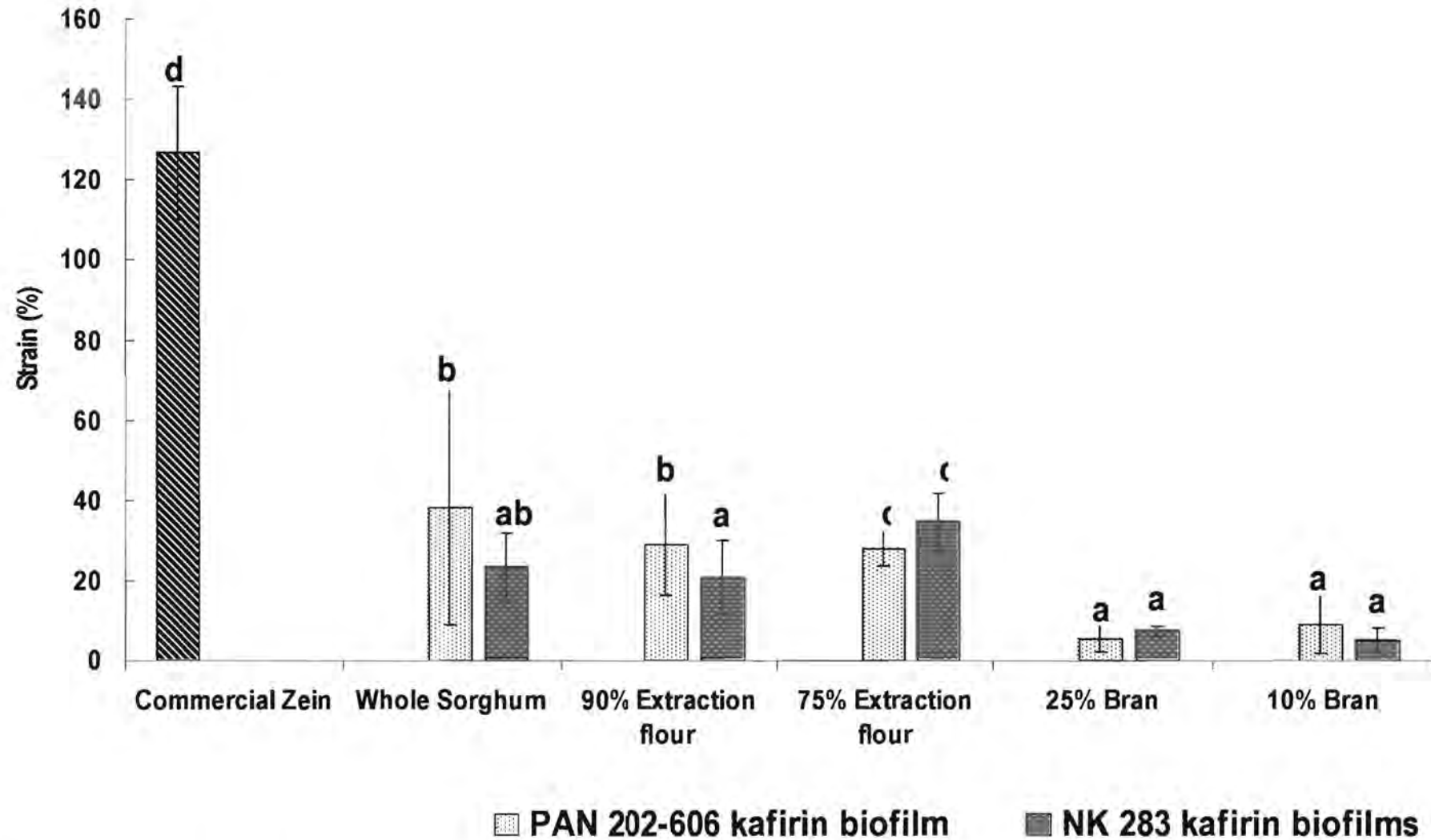


Fig. 16. Percentage strain (percentage elongation at break) of biofilms prepared from defatted kafirin preparations from NK 283 and PANNAR 202-606 sorghum milling fractions and commercial zein. T - Standard error of 4 replica plates, each having 6 strips tested, bars with different letters differ significantly ($p < 0.05$).

2.2.4 Conclusions

Kafirin preparations from different sorghum dry milling fractions including bran are able to form biofilms. However, differences in physical and water barrier properties are observed between the different kafirin preparations, seemingly due to differences in kafirin purity. Generally, kafirin biofilms had lower water barrier properties, presumably due to these films being thicker than the zein biofilm. Lower water barrier properties of the bran kafirin biofilms could also be due to the presence of microcracks in these films. Kafirin biofilms were stronger, with higher breaking strength than the zein biofilm, possibly due to higher levels of disulphide cross-linking between the kafirin polypeptides. However, kafirin biofilms had much lower elongation than the zein biofilm. It is probable that the mechanical properties of kafirin biofilms from bran could be improved by purifying the kafirin and better plasticization could improve the elongation properties of all kafirin biofilms. Alternatively, by keeping the crude free fat in the kafirin preparation, the water barrier and mechanical properties of these films could be improved, due to the fats plasticizing and hydrobic properties, respectively.

The colour of the different bran biofilms could limit their use in certain coating applications. For instance red kafirin would be better, even desirable for coating red fruits such as litchis and plums, while yellow kafirin would be more suitable for coating nuts and deciduous fruits, such as pears.

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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 prolamins 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 (α -, β - and γ -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 possibly 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. Notwithstanding 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) through 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

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.

CHAPTER 4

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 polypeptides, 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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