

Modification of kafirin film properties by microwave heating

By

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DECLARATION

I declare that the thesis which I hereby submit at the University of Pretoria for the award of the degree of PhD (Food Science) is my work and has not been submitted by me for a degree to any other University or institution of higher education.

Yusuf Byenkya Byaruhanga

July 2005

DEDICATION

Dedicated to my family.

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ABSTRACT

Modification of kafirin film properties by microwave heating

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Kafirin, the prolamin of sorghum, is hydrophobic and produces biodegradable and/or edible films of good tensile and barrier properties. However, the diverse packaging requirements necessitate modification of the functional properties of kafirin films. Microwave heating is known to cross-link proteins and has been used to modify the functional properties of whey protein films. Here, microwave heating was applied to kafirin before film casting and to cast kafirin films. The effects of microwaving on the tensile and barrier properties, digestibility, biodegradability, microstructure and protein conformation and cross-linking of kafirin films were investigated.

Microwaving of kafirin powder before film casting had no effect on the film functional properties. Microwave heating of wetted kafirin to 96°C for 2 min and microwaving of cast films at 80 W for 2 min increased tensile strength, decreased strain and slowed the biodegradability of the films. Microwaving before film casting decreased film water vapour permeability (WVP), while microwaving after film casting had no effect. The microstructure of the films indicated a general decrease in the number and size of pores with microwaving. Microwaving decreased the digestibility of kafirin, films made from microwaved kafirin and non-plasticised films cast in aqueous ethanol (AE) but had no effect on the digestibility of plasticised films cast from AE and films cast from glacial acetic acid (GAA). The GAA cast films had a higher digestibility than AE cast films, suggesting GAA mediated deamidation.

FTIR, SDS-PAGE and SS-bond assay were used to study the changes in the conformation and cross-linking of kafirin after microwave heating. Microwave heating increased the β -sheet conformation of the kafirin in films microwaved before and after casting. However, the kafirin in the films microwaved before casting was predominantly β -sheet while that of the films that were microwaved cast was predominantly α -helical in conformation. It is postulated that exposure of kafirin to moisture (in microwave heating before film casting) and to GAA (in microwave heating after film casting) altered the kafirin conformation before the application of the microwaves. SDS-PAGE indicated microwave-induced intermolecular SS and/or non-SS covalent cross-linked kafirin oligomers. The oligomers seem to be responsible for the reduced digestibility of kafirin and kafirin films after microwave heating. SS bond assay confirmed that microwaving induced SS cross-linking. The increase in glass transition temperature of non-plasticised films after microwaving further suggests microwave induced cross-linking.

It is proposed that the microwave heating induced SS cross-links created a dense protein network in the kafirin films, which resulted in the increased tensile strength, reduced strain and digestibility and slowed biodegradability of kafirin films. The conformational changes following microwaving seem to have facilitated the cross-linking of the kafirin.

Although microwave heating of kafirin can modify kafirin film properties, the kafirin must be microwaved wet. Microwave heating after film casting was more effective than microwaving before film casting. The increased tensile strength and decreased WVP of microwaved kafirin films suggests that they could provide increased protection of e.g. coated fruits against mechanical damage and moisture loss.

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1. INTRODUCTION

1.1. Problem statement

The food industry is one of the major end-users of synthetic plastic packaging materials like plastics, many of which are non-biodegradable (Demicheli 1996). Increased use of synthetic packaging materials has led to serious environmental problems due to their non-biodegradability. This is manifested in the overloading of municipal solid waste disposal systems (reviewed by Selke 1996).

Consequently, environmental awareness and concern has led to a paradigm shift towards the use of biodegradable materials, particularly from agricultural produce and food processing co-products to produce packaging films that are compatible with the environment and some that are edible (reviewed by Tharanathan 2003). At the same time, there is increased interest to diversify the utilization of agricultural produce to a wider range food and non-food applications, in order to increase the value derived from crops (Salmoral et al 2000b).

These pressures have led to increased research and development into the use of natural polymers to produce biodegradable and/or edible coating and films for use as packaging materials in both food and non-food applications. It has been shown that commercially available biopolymers such as cellulosic materials (Park and Chinnan 1995), starch (Stading et al 2001) and proteins (reviewed by Krochta 2002) can be used to produce biodegradable films. Plant proteins such as from rice (Cho et al 1998), wheat (gluten) (Aydt et al 1991; Gontard and Ring 1996), soy (Salmoral et al 2000b), beans (Salmoral et al 2000a) and maize (zein) (Aydt et al 1991; Lai and Padua 1998) have been used to produce films at laboratory scale. Zein is one of the very few proteins that is produced and utilised at industrial scale for the production of films, coatings and fibres (Shukla and Cheryan 2001; Lawton 2002).

Another protein that has shown potential for film production is kafirin. Kafirin is the prolamins of sorghum, and comprises most of the protein in the grain (reviewed by Taylor and Belton 2002). Sorghum (*Sorghum bicolor* (L) Moench) is one of the world's major cereals. It is a drought-tolerant crop and thus mainly grown in regions characterised by low rainfall (ICRISAT/FAO 1996; FAO 2004). In developing countries,

sorghum is used for food, animal feed and for brewing. Sorghum plays a crucial role in food security in developing countries as a source of energy and protein and its production is a major economic activity (reviewed by Taylor and Belton 2002). Since kafirin is similar to zein (Shull et al 1991), highly hydrophobic compared to most other proteins (Duodu et al 2003), has reduced digestibility when cooked (Duodu et al 2002) and is not known to be allergenic (Skerritt 1988), it offers advantages over other proteins used in making films. Kafirin has been successfully used to produce films by Buffo et al (1997). However, the kafirin films had lower tensile strength and higher strain compared to zein films. Subsequently, a number of researchers have investigated different aspects of kafirin films to improve their functional properties. Taylor et al (2005a) investigated a number of food compatible solvents to identify new kafirin solvents for film casting and found that glacial acetic acid could be used for film casting at lower temperatures as compared to aqueous ethanol. Da Silva and Taylor (2005) studied the effect of sorghum variety and milling fractions on kafirin film quality and found that kafirin extracted from different sorghum varieties and milling fractions made films with higher tensile strength and shorter elongation as compared to those of commercial zein. Emmambux et al (2004) used tannins to cross-link kafirin, resulting in increased film tensile strength and reduced oxygen permeability.

Successful use of proteins for biodegradable and/or edible films lies in achieving stability and functionality during storage and intended use without adversely affecting their biodegradability (reviewed by Krochta and De Mulder-Johnston 1997). Although protein films have tensile and barrier properties that allow them to be used in coatings and packaging of some foods (Park et al 1994a; Park et al 1994b; Rakotonirainy et al 2001), the diverse range of food packaging and coating requirements and applications necessitate modification of their properties (reviewed by Krochta 2002).

As mentioned, Emmambux et al (2004) showed that chemical cross-linking with tannins can modify kafirin. Modification of proteins can also be achieved by inducing cross-linking using physical means e.g. thermal and electromagnetic energy (Damodaran 1996), which could improve the functional properties of protein films. Thermal treatment has been reported to increase the tensile strength of protein-based films (Roy et al 1999; Perez-Gago et al 1999; Perez-Gago and Krochta 2001). Use of electromagnetic waves such as ultraviolet light (Gennadios et al 1998; Rhim et al

1999), γ -irradiation (Vachon et al 2000) and microwaving (Kaya and Kaya 2000) has also been found to improve the tensile properties of protein films. Applying microwave energy in the production of kafirin films could therefore improve their functional properties.

1.1.2. Hypotheses

Microwave heating will promote cross-linking reactions in kafirin because conventional heat (Shimada and Cheftel, 1988b; Duodu et al 2001; Duodu et al., 2002) and microwave-generated heat (Valentinova et al 2000; Farag et al 2001) modify the structure of the proteins through exposing different internal and external functional groups, which promote inter- and intra-molecular interactions. In turn, these interactions will affect kafirin film functional properties.

Since in the use of microwaves to heat products, heat generation and transfer take place by molecular polarisation (Ohlsson and Bengtsson 2001) instead of molecular collision as in convection/conduction heating, the effects of microwave heating on kafirin will be different from those of conventional heating.

Protein film properties are related to the protein conformation (Subirade et al 1998). Hence, the effects on film properties of microwaving the kafirin before film casting will be different from those of microwaving the film after casting due to the differences in the structural organisation of kafirin before and after film casting.

1.1.3. Objectives

To determine the effect of microwave heating of kafirin on the functional properties of kafirin films

To determine the effect of microwave heating of cast films on their functional properties

To determine the mechanisms of the effects of microwave heating on the functional properties of kafirin films.

2. LITERATURE REVIEW

2.1. Proteins as film forming material

The formulation of films and coatings requires the use of at least one component capable of forming a structural matrix with sufficient cohesiveness (reviewed by Debeaufort et al 1998). Biopolymeric materials, which provide such a cohesive matrix include: cellulose, starch, chitosans and proteins. These biopolymers can produce a film or coating that is both biodegradable and edible. Proteins are one of the natural biopolymer materials that have been most successfully used in films and coatings (reviewed by Cuq et al 1998).

Proteins are polymeric compounds that provide structure and biological activity in plants and animals. The monomers of proteins are amino acids, which in addition to the amino ($-NH_2$) and a carboxyl ($-COOH$) groups, also have different side groups attached to the central carbon. The side groups lend unique character to individual amino acids and may be hydrophobic, hydrophilic, positively or negatively charged (reviewed by Cheftel et al 1985; Damodaran 1996). The individual amino acids are linked together by peptide bonds in a sequential order specific to each protein species. This forms a polypeptide chain that is also called the primary structure of a protein. Depending on the sequential order of the amino acids in the polypeptide chain, the protein will assume different structures. The secondary structure is the spatial structure that the polypeptide chain assumes along its axis. At normal pH and temperature, the polypeptide chain assumes a native structural conformation that is thermodynamically stable. The native conformation is related to the polarity, hydrophobicity and steric hindrance of the amino acid side chains. The main secondary structures found in native protein include α -helices, β -sheets, β -turns and random coils. The secondary structure may then be organised into tertiary structures, which are three-dimensional organisations with regions of α -helices, β -turns and β -sheets. The interactions and linkages involved in the formation and stabilising the secondary and tertiary protein structures include steric interactions, van der Waals interactions, hydrogen bonding, electrostatic interactions, hydrophobic interactions, and disulphide cross-links. It is these interactions and linkages, which stabilise the native protein that are taken advantage of in the formation and stabilisation of the protein network in a protein-based film.

The successful use of proteins in film and coating production is due to their polymeric structure arising from the different amino acid monomers (Cuq et al 1998). The mechanical properties of films depend on the type of film forming material especially on its structural cohesion, which in turn depends on the structure of the polymer and especially its molecular length, geometry and the type of and position of its lateral functional groups (Banker 1966). Regularly branched polymer molecules like proteins tend to have greater cohesive strength than the more uniform non-branched polymers like some polysaccharides (Cuq et al 1998). Also, because proteins are made up of different amino acid monomers, unlike polysaccharides, which have the same monomer, their complex structure offers more functional properties (reviewed by Damodaran 1996; Krochta 2002). Thus, proteins have potential for forming intermolecular bonding and interactions at numerous positions (Gontard et al 1993; Cuq et al 1998). This could be instrumental in the modification of the functional properties of protein films.

In the making of films, the native protein structure is disrupted either by dissolution in an appropriate solvent or by heating the protein to temperatures above its glass transition temperature (T_g). Proteins are formed into a film by either casting the protein solution onto a flat surface followed by evaporating off the solvent or by thermoforming e.g. extrusion, compression or injection moulding (reviewed by Cuq et al 1998; De Graaf 2000). Figure 2.1 is a schematic presentation of the two film making procedures. As the film is formed, the protein interactions and linkages that stabilise the native protein are re-formed. New protein interactions resulting from process treatments may be formed to stabilise the film network and confer its properties (reviewed by Krochta 2002).

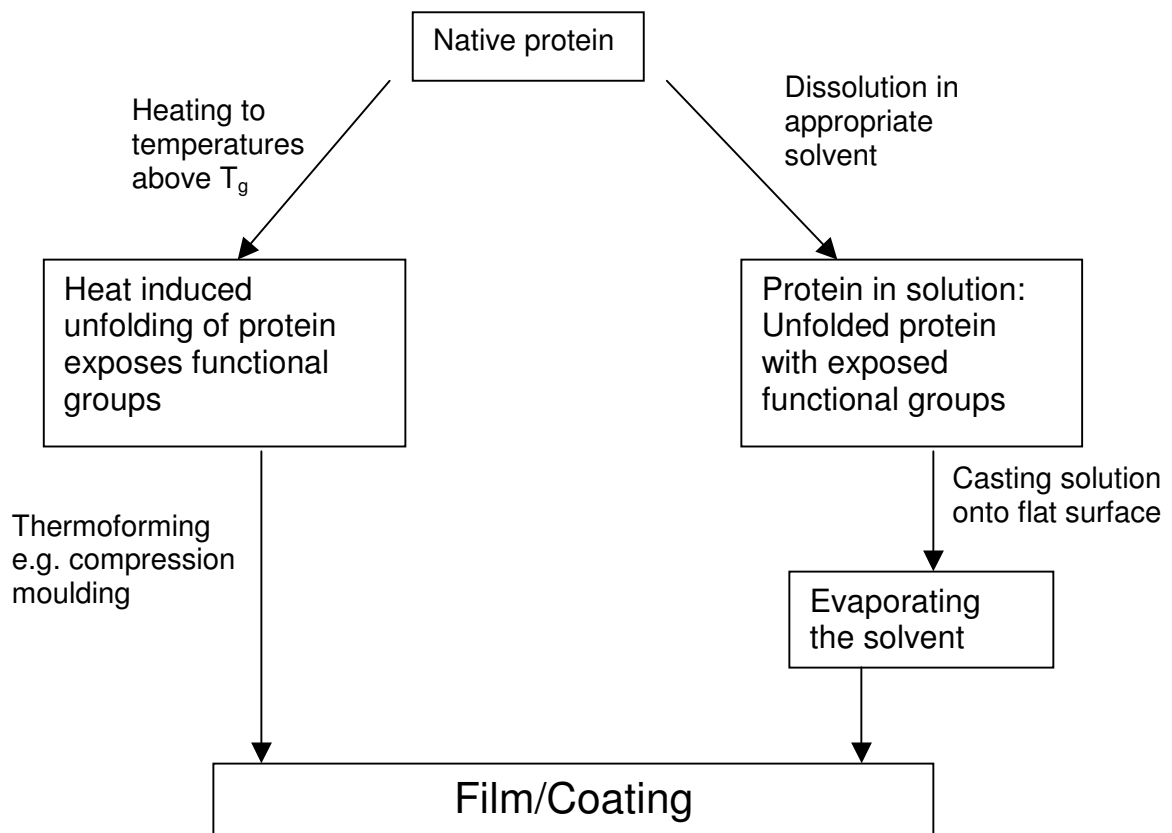


Fig. 2.1. Protein film/coating forming procedures

2.2. Sorghum Kafirins

2.2.1. Kafirin structure and composition

Cereal grains contain on average 10-12% protein (Shewry and Halford 2002). According to Taylor et al (1984b), sorghum protein content can vary from 7-13%. Storage proteins account for about 50% of the total protein in mature cereal grains (Shewry and Halford 2002). Based on the Osborne type of protein fractionation, sorghum proteins can be categorised into albumins (soluble in water), globulins (soluble in saline solution), glutelins (soluble in acid or base solutions) and prolamins (soluble in aqueous alcohol solution). The major storage protein of sorghum is the prolamins kafirin that constitutes some 68-73% of the whole grain flour protein and 77-87% of the total endosperm protein (Hamaker et al 1995).

Kafirins are a complex mixture of different protein species. Based on their molecular weight, structure, solubility, immunological properties and similarity to the zein proteins, Shull et al (1991) categorised kafirins into: α -kafirins, β -kafirins and γ -kafirins. Alpha-kafirins have apparent molecular weight (M_r) of 23,000 to 25,000 and are soluble in 40-90% tert-butanol. The β -kafirins have M_r 16,000-20,000 and are soluble in 10-60% tert-butanol and the γ -kafirins have M_r 28,000 and is soluble in 10-80% tert-butanol. The α -kafirins compose most of the sorghum kafirin fraction and the β -kafirins the least. Table 2.1 summarises some characteristics of these kafirin species.

TABLE 2.1 Characteristics of the Different Kafirins Species

Fraction	% of Total Kafirins^a	Fraction M_r^b	Polymer or Monomers^c
α -kafirins	80	23,000-25,000	Monomers
β -kafirins	7-9	16,000-20,000	Polymers
γ -kafirins	9-12	28,000	Polymers

a. Shull et al (1991)

b. Watterson et al (1993)

c. Shewry (2002)

Research by El Nour et al (1998) revealed extensive disulphide cross-linking in kafirin proteins with subsequent formation of oligomers of high molecular weight. The disulphide cross-links pre-existed in the kafirins and were not a result of oxidation of SH groups during extraction. Seventy percent of the total tert-butanol extracted kafirins existed as S-S linked oligomers. Such a degree of polymerisation could play a role in kafirin film formation and film properties.

Kafirins are located in organelles called protein bodies of the grain starchy endosperm. Protein bodies are circular in section of 0.4-2.0 μ m diameter (Taylor et al 1984a). The protein bodies are mostly located in the outer vitreous endosperm of the sorghum grain (Watterson et al 1993) and may be irregularly or spheroid shaped depending on their location in the endosperm (Shull et al 1992). Transmission electron microscopy of

immunocytochemically stained protein bodies showed that most of the α -kafirin is located inside the protein body while the γ - and β -kafirin are mostly found at the periphery of the protein body (Shull et al 1992). Figure 2.2 shows the location of different zein protein species in the protein bodies of maize endosperm, as proposed by Lending and Larkins (1989), which is similar to that reported by Shull et al (1992) for the different kafirin species in sorghum.

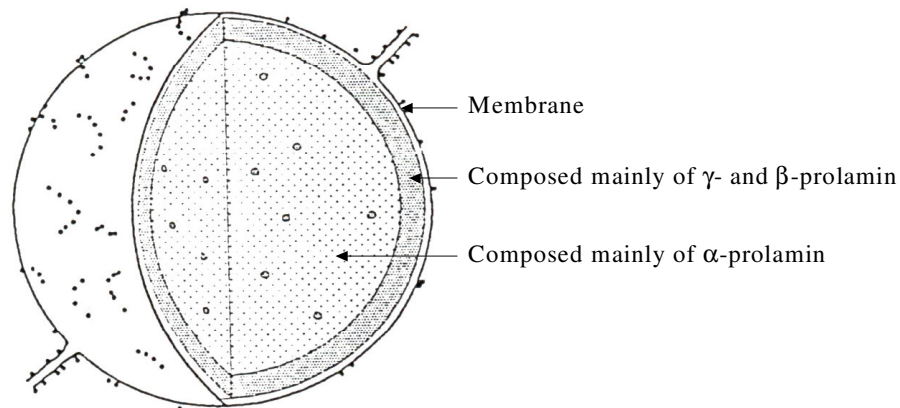


Fig. 2.2. Location of the different protein species in the protein body of maize endosperm proposed by Lending and Larkins (1989)

2.2.1.1. Molecular conformation of kafirins

Protein conformational structures help in understanding the functionality of proteins and how such functionality can be taken advantage of in different food and non-food applications. Using Fourier Transform-Infrared (FTIR) spectroscopy, the secondary structure of native kafirin has been found to be mainly α -helical (59%) and some β -sheets (Duodu et al 2001). Unfortunately, the molecular conformation of kafirin polypeptides is not known. Nonetheless, the extensive homology between zein and kafirin, as demonstrated by various researchers (DeRose et al 1989; Coleman et al 1990; Shull et al 1991), suggests that kafirin and zein should have similar molecular conformation. Using the knowledge of amino acid sequences and circular dichroism,

Argos et al (1982) proposed a structural model for α -zein (Fig. 2.3). The proposed model consists of nine topologically anti-parallel adjacent helices clustered within a cylinder of oval cross-section with the helical and cylindrical axes aligned. The top and bottom of the helical cylinder are proposed to consist of polar glutamine residues, which comprise the turn spans between helices. Polar and hydrophobic residues appropriately distributed along the helical surfaces allow intra- and inter-molecular hydrogen bonds and Van der Waals interactions among neighbouring helices in the rod shaped zein molecules. A cluster of α -helices corresponding to the zein homologous repeat units and contained within the cylindrical surface capped by polar glutamine residues allows hydrogen bonding and hydrophobic interactions to maintain zein structural integrity as well as intermolecular packing. The similarity of kafirin to zein (DeRose et al 1989; Coleman et al 1990; Shull et al 1991) suggests that α -kafirin could well have the same conformation as α -zein.

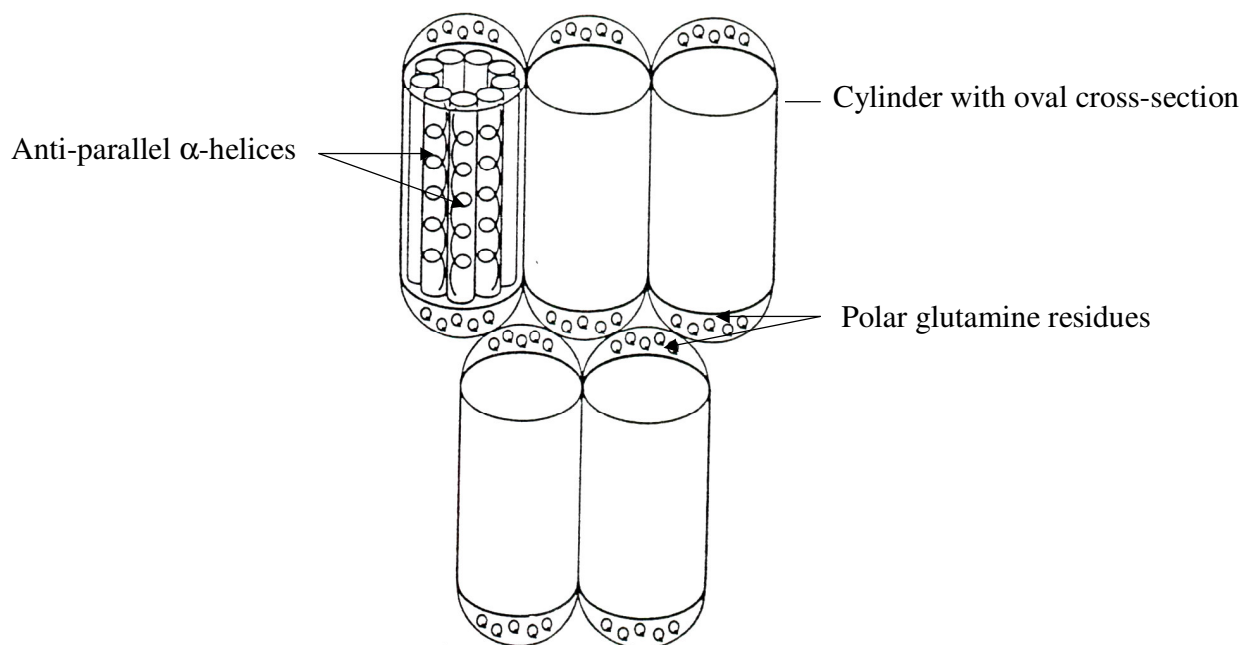


Fig. 2.3. Proposed structural model of α -zein proteins (Argos et al 1982).

2.2.2. Properties of kafirins

2.2.2.1. Solubility

As stated, kafirin is aqueous alcohol-soluble and thus is extracted in aqueous alcoholic solvents. Aqueous tert-butanol (60% v/v), isopropanol (70% v/v) (Taylor et al 1984b) and hot ethanol (70% w/w) (Emmambux and Taylor 2003) have been used to extract kafirin. Reducing agents like mercaptoethanol, dithiothreitol (Taylor et al 1984b; El Nour et al 1998) and sodium metabisulphite (Emmambux and Taylor 2003) are generally added to the aqueous alcoholic solvents to break disulphide bonds so as to solubilise the cross-linked kafirins.

2.2.2.2. Hydrophobicity

Kafirins and zeins are known to be hydrophobic proteins (Wall and Paulis 1978; Shewry 1995). It has also been suggested that kafirins are more hydrophobic than zein thus less soluble (Duodu et al 2003). The amino acid composition of kafirins reported by various workers is shown in Table 2.2. All the three kafirin species are low in lysine. The β - and γ -kafirins are relatively high in cysteine (half cystine) which can form disulphide cross-links. Also, β -kafirin is high in methionine (Watterson et al 1990; Shull et al 1992). It can also be seen that the hydrophobic amino acids proline, alanine, valine methionine, isoleucine, leucine and phenylalanine comprise up to 53% of the total amino acid residues. Evans et al (1987) estimated a total hydrophobic amino acid content of 58.3 mol% in kafirin. Duodu et al (2003) determined the hydrophobicity of kafirins and zeins based on their calculated values of hydration free energy. The authors found that α -kafirins and α -zeins have the same level of hydrophobicity. However, the γ -kafirin was found to be slightly more hydrophobic than γ -zein. This suggests that due to the hydrophobic nature of the kafirins, they could make films with good water vapour barrier properties.

TABLE 2.2 Amino Acid Composition of Prolamins in the Sorghum Grain

Amino acid	Amount (mole%)				
	Whole Kafirin ¹	Whole Kafirin ²	α -Kafirin ³	β -Kafirin ³	γ -Kafirin ³
Lysine	0	0.1	0.4	0.5	0.4
Histidine	1.0	0.9	1.2	0.9	6.9
Arginine	2.3	1.1	0.8	2.7	2.0
Asparagine	4.8	6.2	5.6	3.3	0.6
Threonine	2.1	1.9	3.2	4.6	4.2
Serine	3.1	3.4	5.8	4.6	5.0
Glutamine	25.0	24.8	21.8	17.7	13.8
Half-cystine	1.9	0.4	1.1	4.9	6.9
Glycine	1.1	1.2	4.1	6.8	8.6
Tyrosine	3.7	4.2	2.3	3.0	2.1
Proline	7.2	8.5	8.9	9.7	22.6
Alanine	12.3	10.0	15.2	13.4	6.3
Valine	5.8	4.0	4.8	5.2	5.8
Methionine	1.2	0.6	0.6	5.7	1.0
Phenylalanine	5.1	5.9	4.0	1.9	1.8
Isoleucine	5.2	4.1	4.4	2.3	2.6
Leucine	15.9	17.0	15.1	12.0	9.0

1. Taylor et al (1984a).

2. Watterson et al (1990)

3. Shull et al (1992)

2.2.2.3. Digestibility

Digestibility is a measure of the susceptibility of a protein to proteolytic enzyme action. Digestibility may therefore, be used as an indicator of protein availability, an aspect of the nutritional value of a protein. In films, however, digestibility is important because it can act as an indicator of how biodegradable the film is, whether the film can be applied on surfaces with proteolytic enzyme activity and to what extent the film will contribute to the dietary protein if consumed as part of a product.

The digestibility of kafirin has been a subject of some *in-vivo* and many *in-vitro* studies. It is generally agreed that wet cooking reduces the *in-vitro* digestibility of sorghum protein (Hamaker et al 1986; Oria et al 1995a; Oria et al 1995b; Duodu et al 2002; Nunes et al 2004). *In-vitro* protein digestibilities of cooked and uncooked sorghum reported by various workers are shown in Table 2.3. It is apparent in Table 2.3 that depending on the cultivar, cooking of sorghum results in approximately 20 to 75% decrease in *in-vitro* digestibility of kafirin.

TABLE 2.3 *In-Vitro* Protein Digestibility of Cooked and Uncooked Sorghum Reported by Various Workers

Grain Sample	<i>In-vitro</i> Protein Digestibility (%)		Reference
	Uncooked	Cooked	
Sorghum cultivar P721N (whole grain flour)	79	58	Rom et al (1992)
Sorghum cultivar P721N (whole grain flour)	73.2	55.2	Oria et al (1995b)
Sorghum cultivar P721N (decorticated grain flour)	69.2	43.6	Oria et al (1995a)
Sorghum cultivar M-35-1 (whole grain flour)	38.8	10.7	Elkhalifa et al (1999)
Sorghum cultivar P721N (whole grain flour)	80.7	64.8	Hamaker et al (1986)
Red sorghum NK 283 (whole grain flour)	59.1	30.5	Duodu et al (2002)
White sorghum KAT 369 (whole grain flour)	55.8	36.6	Duodu et al (2002)
Red sorghum NK 283 (whole grain flour)	68.6	32.7	Nunes et al (2004)
Sorghum Kenyan Local White (whole grain flour)	75.6	18.0	Nunes et al (2004)
White sorghum KAT 369 (whole grain flour)	66.8	14.5	Nunes et al (2004)

Most studies on protein digestibility of sorghum have involved whole or decorticated sorghum flour. Thus the models developed to explain the effect of heat on sorghum protein digestibility have mainly focused on the kafirin protein body. It is suggested that cooking changes the protein so that they cannot be digested as well as they are before

cooking, thus resulting in reduced digestibility (Rom et al 1992). Using TEM, researchers (Taylor and Evans 1989; Oria et al 1995a) found that protein body digestion was initiated from the surface, where the γ - and β -kafirin are located. Alpha-kafirin was found to be the last of the kafirins to be digested (Oria et al 1995a). However, when the reducing agent sodium bisulphite was applied to the flour (Rom et al 1992; Oria et al 1995a), the protein digestibility of the cooked sorghum flour was increased and formation of pits at the periphery of the protein body was observed. It has been suggested (Hamaker et al 1987) that the formation of disulphide cross-links during cooking results in “toughening” at the periphery of the protein body where the cysteine rich γ - and α -kafirins are mostly found.

Duodu (2000) extracted kafirin using aqueous tert-butanol with dithiothreitol and subjected it to cooking. *In-vitro* protein digestibility was reduced on cooking regardless of whether the protein was alkylated or un-alkylated. However, alkylated kafirin samples (uncooked and cooked) were more digestible than the un-alkylated. This seems to suggest that other factors in addition to disulphide cross-links were involved in the decreased digestibility of kafirin on cooking. However, Duodu et al (2003) suggested that although other factors may be involved, intermolecular disulphide bonding plays the biggest role in the observed decrease in kafirin digestibility on cooking. Since wet heating decreases the *in vitro* kafirin digestibility and since heat is applied in the film forming procedure, it can be hypothesised that film forming procedure will decrease the digestibility of kafirin films compared to that of kafirin.

Spectroscopic studies by Duodu et al (2001) showed that when sorghum is cooked, the kafirin secondary structure assumes more β -sheet conformation at the expense of α -helical structure. Whether this change in the kafirin secondary structure directly contributes or is a preamble to the changes that lead to the decrease in protein digestibility is not clear. It is also not known whether such heat-induced changes in the secondary structure of kafirin would affect the properties of kafirin films.

2.2.3. Kafirin suitability for film making

Kafirin has been shown to form films of properties similar to those of zein films (Buffo et al 1997), probably because the two proteins are closely similar (DeRose et al 1989).

However, due to some small differences, kafirin could potentially form films of better functional properties as compared to those of zein and water-soluble proteins. For example, Duodu et al (2003) found that γ -kafirin is more hydrophobic than γ -zein, which suggests that kafirin could form films of lower water vapour permeability than zein and water-soluble proteins. Furthermore, research by Duodu et al (2001, 2002) suggests that on heating, kafirin undergoes more cross-linking than zein. This suggests that kafirin can form films of increased tensile strength and perhaps lower barrier properties due to the increased cross-linking upon heating. In fact research by Da Silva and Taylor (2005) showed that kafirin films had a higher tensile strength than films made from commercial zein, attributable to high level of disulphide cross-linking due to the presence of β - and γ -kafirins. In addition, kafirin can potentially be extracted from cheap and readily available materials such as spent sorghum grain from breweries. Besides, kafirin is not known to be allergenic (Skerritt 1989), which gives it an advantage over proteins to which consumers are allergic.

2.3. Protein-based films

An edible film is defined as a thin layer of edible material formed on a food as a coating or placed (as a pre-formed stand alone film) on/or between food components (reviewed by Krochta and De Mulder-Johnston 1997; Krochta 2002). Stand-alone films may be used as covers, wraps, and separation layers. Films and coatings function as a barrier to inhibit migration of moisture, oxygen, carbon dioxide, aromas and lipids, carry food ingredients and/or improve mechanical integrity or handling characteristics of the food. Other functions of films and coatings may include providing gloss, colour and a non-sticky surface (reviewed by Krochta 2002).

Protein-based films are considered biodegradable and/or edible depending on the formulation, formation method and modification treatments. Films are regarded edible if food-grade protein and additives are used and protein changes resulting from acceptable processes like heat and salt addition are involved in their formation (reviewed by Krochta and De Mulder-Johnston 1997).

Films should, therefore, meet certain desired functional properties in order for them to be used for the various purposes. Some of the important properties of edible films include tensile strength, colour, gas and water vapour permeability.

2.3.1. Properties of protein-based films

2.3.1.1. Mechanical and barrier properties

To ensure their integrity, protein films should have adequate tensile strength and be free of minor defects. Interactions between the protein and other film components contribute to the mechanical behaviour of films (reviewed by Krochta and De Mulder-Johnston 1997). The mechanical properties commonly used to characterise films are the tensile strength (TS), elongation (E) and the elastic or Young's modulus (EM). According to ASTM (1998), TS is the pulling force per film cross sectional area. E is the elongation of the film expressed as a percent of the original test film length. It measures the degree to which the film can stretch before breaking. EM also called Young's modulus is determined by the ratio of the pulling force/area to the degree of film stretch. EM measures the stiffness of a film. Generally protein films have lower TS and E than synthetic polymer films as shown in Table 2.4.

TABLE 2.4 Tensile Properties of Some Protein Based and Synthetic Films

Film	Tensile Strength (MPa)	Elongation (%)	Reference
Kafirin: PEG ¹ :LA ² :glycerol (2.3:1:1:1)	4.1	63.5	Taylor et al (2005)
Kafirin: PEG ¹ :LA ² :glycerol (2.3:1:1:1)	7.0	35.0	Da Silva and Taylor (2005)
Kafirin: PEG ¹ :LA ² :glycerol (2.3:1:1:1)	2.4	109	Emmambux et al (2004)
Kafirin:glycerol:PEG (2.2:1:1)	2.1	106.1	Buffo et al (1997)
Zein glycerol:PEG (2.2:1:1)	2.6	84.4	Buffo et al (1997)
Zein:glycerol	13.5	Np ³	Gennadios et al (1993a)
Gluten:glycerol:ammonium hydroxide	3.8	Np	Gennadios et al (1993a)
Gluten:glycerol (2.5:1)	2.6	276	Gennadios et al (1993b)
Gluten:glycerol (2.5:1)	1.8	25	Aydt et al (1991)
Zein:glycerol	0.4	Np	Aydt et al (1991)
Zein: oleic acid (1.4:1)	8.7	11.9	Lai and Padua (1998)
Zein	7-30	Np	Yoshino et al. (2002)
Polyvinylidene chloride	48.4-138	20-40	Briston (1988)
Oriented polystyrene	72	20	Briston (1988)
Low density polyethylene	13	500	Briston (1988)
High density polyethylene	26	300	Briston (1988)

1. PEG, polyethylene glycol
2. LA, lactic acid
3. Np, data not provided

The mechanical and barrier properties of protein films are influenced by factors such as the structure and cross-linking of the protein and the presence of plasticisers including moisture. The protein structure and cross-linking has been suggested to affect the tensile properties of soy films (Subirade et al 1998). Regarding kafirin, Gao et al (2005) showed that the protein with higher proportions of β -sheet conformation produced films with different mechanical properties as compared to those to those made from less cross-linked kafirin that had lower proportion of β -sheet conformation.

Concerning plasticisers, they are added to protein films to decrease film stiffness (EM) and increase flexibility. Normally, as the plasticiser amount increases permeability and E increase, while TS and EM decrease (Parris and Coffin 1997). In some protein films, plasticiser addition allows achievement of the desired mechanical properties with no substantial effect on barrier properties (reviewed by Krochta 2002). However, the use of plasticisers to modify protein film mechanical properties affects the film barrier properties. Mate and Krochta (1996) reported exponential increases in water vapour permeability of whey protein and lactoglobulin films with increasing amounts of glycerol plasticiser. Increased plasticisation also increased the oxygen permeability of the films.

The influence of various factors on the functional properties of protein films can be explained through molecular mobility. The glass transition temperature (T_g) of a polymeric material governs the mobility of the polymer molecules. At T_g , proteins undergo a dramatic change in their dynamic properties similar to those exhibited by synthetic amorphous polymers (Ringe and Petsko 2003). Thus, at low temperatures (below T_g), a protein is hard and brittle or glassy and becomes leathery and changes to a rubbery structure at higher temperatures (above T_g). The temperature at which the protein changes from a glassy to leathery behaviour is designated as the T_g (Van de Velde and Kiekens 2002). With increasing temperature above T_g , the mobility of polypeptides increases drastically and their reactivity is enhanced provided the decomposition temperature of the protein is not reached (Toufeili et al 2002). Using differential scanning calorimetry and pressure rheometry, Madeka and Kokini (1996) found that the increased mobility of zein molecules above the T_g enabled them to cross-link, possibly through disulphide exchange reactions. Similar increased molecular mobility and cross-linking above T_g has been reported in gluten (Toufeili et al 2002). T_g of protein films largely depends on their chemical composition, molecular size, presence of plasticisers and structural and conformation factors such as chain branching, cross-linking and crystallinity (Cherian et al 1995; Padua and Wang 2002). T_g therefore, separates two domains according to clear structural differences and the protein polymer properties are significantly modified when the temperature rises above the T_g . Thus, the change of film functional properties can be attributed to the change from a glassy to the rubbery state (Gontard and Ring 1996).

Water is an ubiquitous and uncontrollable plasticiser in protein films (reviewed by Krochta 2002). The water content of films is influenced by the water activity (a_w) of the product on which the film is used, the relative humidity of the environment in which the film is held and/or the nature of the film material (reviewed by Krochta 2002). Research by Lawton (2004) showed that relative humidity affected plasticiser action in zein films. The type of plasticiser used determined the extent of the changes seen in the tensile properties of films stored at different relative humidity values. Depending on the plasticiser used, there were large differences in the amount of water absorbed by the zein films. Films containing hygroscopic plasticisers absorbed too much water at high relative humidity and became weak, while films containing more hydrophobic plasticisers had good mechanical properties at high relative humidity values. The influence of water content on film functional properties may be explained in terms of a_w or disruptive water-polymer hydrogen bonding in a polymer hydrogen-bonded network (Gontard and Ring 1996). However, the water content and temperature dependence of protein film properties can be better explained in terms of T_g (Ringe and Petsko 2003). Studies have shown that increasing plasticisation resulting from increased water content of gluten films decreases their T_g (Gontard and Ring 1996; Toufeili et al 2002). Madeka and Kokini (1996) reported a similar decrease in T_g of zein from 139°C (for dry zein) to 47°C (for zein with 6.6% moisture content). This decrease in T_g is consistent with the plasticising effect of water on proteins (Matveev et al 2000). Lawton (2004) reported that both hydrophilic and hydrophobic plasticisers reduce the T_g of zein, with the more hydrophilic plasticisers being most effective. It appears that plasticisers, including water, affect the functional properties of protein films through their effect on molecular mobility that may be measured as changes in T_g .

Protein films have high WVP compared to synthetic polymer films, for example polyethylene and ethylene vinyl alcohol (Table 2.5). The high WVP of protein films is attributed to the fact that proteins are more hydrophilic as compared to synthetic polymers (reviewed by Krochta 2002). Hence moisture can readily diffuse through the protein film network. It is also apparent from Table 2.5 that the WVP of protein films can vary depending on film composition and testing conditions, which suggests that plasticisers and RH affect film permeability.

TABLE 2.5 WVP of Some Protein Based and Synthetic Films

Film	Test Conditions	WVP (g mm m ⁻² d kPa)	Reference
Kafirin: PEG ¹ :LA ² :glycerol (2.3:1:1:1)	25°C, 30/85% RH ³	0.53-0.81	Da Silva and Taylor (2005)
Kafirin: PEG ¹ :LA ² :glycerol (2.3:1:1:1)	25°C, 30/85% RH	0.41-0.44	Taylor et al (2005a)
Kafirin: PEG ¹ :LA ² :glycerol (2.3:1:1:1)	25°C, 30/85% RH	0.98	Emmambux et al (2004)
Kafirin:glycerol:PEG (2.2:1:1)	25°C, 50/100% RH	132.0	Buffo et al (1997)
Zein: PEG ¹ :LA ² :glycerol (2.3:1:1:1)	25°C, 30/85% RH	0.47	Da Silva and Taylor (2005)
Zein: PEG ¹ :LA ² :glycerol (2.3:1:1:1)	25°C, 30/85% RH	0.24	Taylor et al (2005a)
Zein glycerol:PEG (2.2:1:1)	25°C, 50/100% RH	136.8	Buffo et al (1997)
Zein:glycerol (4.9:1)	21°C, 85/0% RH	10.0	Park and Chinnan (1995)
Gluten:glycerol (3.1:1)	21°C, 85/0% RH	53.2	Park and Chinnan (1995)
Gluten:glycerol (2.5:1)	26°C, 50/100% RH	108.4	Aydt et al (1991)
Zein:glycerol	26°C, 50/100% RH	35.1	Aydt et al (1991)
Zein: oleic acid (2:1)	25°C, 53/0% RH	1.58	Lai and Padua (1998)
Zein	40°C, 90/0% RH	0.02-0.22	Yoshino et al (2002)
Zein: oleic acid (1.4:1)	25°C, 51/0% RH	1.92	Wang and Padua (2004)
Zein: oleic acid (1.4:1)	25°C, 100/0% RH	3.70	Wang and Padua (2004)
Cellophane	38°C, 90/0% RH	7.27	Taylor (1986)
Low density polyethylene	38°C, 90/0% RH	0.08	Smith (1986)
High density polyethylene	38°C, 90/0% RH	0.02	Smith (1986)
Ethylene vinyl alcohol (68% Vinyl alcohol)	38°C, 90/0% RH	0.25	Foster (1986)

1. PEG, polyethylene glycol

2. LA, lactic acid

3. RH, Relative humidity

Oxygen permeability (OP) of protein films is important because oxygen influences reactions that may lead to food deterioration e.g. oxidation and respiration (Miller and Krochta 1997). In contrast to WVP, protein films tend to have lower OP compared to synthetic polymer films (Table 2.6). This can be attributed to the polar nature of proteins, which leads to a high cohesive energy density in the protein, thus does not readily allow the non-polar oxygen molecule to diffuse through the film network (Miller and Krochta 1997).

According to Pascat (1986), molecular orientation affects permeability of films. Polymer orientation is commonly used in the synthetic film industry to reduce permeability values by up to 50%. Arvanitoyannis et al (1994) studied the gas permeability of undrawn and uniaxially drawn potato and rice starch films. Uniaxial drawing of the films induced polymer orientational and conformational changes that lowered gas permeability. Lai and Padua (1998) compared the properties of cast zein films to resin zein films of the same formulation. They found that the WVP of resin films was considerably lower than the cast films. The resin films also maintained better tensile properties compared to the cast films. The better properties exhibited by resin zein films was attributed to molecular structural changes and orientation in the film matrix.

TABLE 2.6 Oxygen Permeability (OP) of Some Protein and Synthetic Polymer Films

Film	Test Conditions	OP ($\text{cm}^3 \mu\text{m m}^{-2} \text{d kPa}$)	Reference
Kafirin: PEG ¹ :LA ² :glycerol (2.3:1:1:1)	23°C, 0% RH	135	Emmambux et al (2004)
Zein:glycerol (4.9:1)	30°C, 0% RH ³	13.0-44.9	Park and Chinnan (1995)
Gluten:glycerol (3.1:1)	30°C, 0% RH	9.6-24.2	Park and Chinnan (1995)
Zein	24°C	1.03×10^{-2}	Yoshino et al (2002)
Cellophane	23°C, 50% RH	16	Taylor (1986)
Low density polyethylene	23°C, 50% RH	1870	Salame (1986)
High density polyethylene	23°C, 50% RH	427	Salame (1986)
Ethylene vinyl alcohol (70% vinyl alcohol)	23°C, 95% RH	12	Salame (1986)
Ethylene vinyl alcohol (70% vinyl alcohol)	23°C, 0% RH	0.1	Salame (1986)

1. PEG, polyethylene glycol
2. LA, lactic acid
3. RH, Relative humidity

The barrier properties of protein films are influenced by the protein's molecular conformation, polymer chain interaction and molecular mobility (Kester and Fennema 1986). Since T_g is a reflection of molecular mobility and interaction, the gas and water vapour permeability of films tend to be low below T_g and high above T_g . Below the T_g , proteins exist mostly in a glassy state in which polypeptide chain mobility is limited. Thus they exhibit low barrier properties and a high tensile stress (reviewed by Krochta 2002). However, at temperatures above T_g , the protein polymers exist in a rubbery state where polypeptide chain mobility is increased and this can lead to an increase in gas and WVP, and low tensile stress (Cherian et al 1995).

Environmental conditions like RH and temperature affect the WVP and gas permeability of films. Lai and Padua (1998) reported that the WVP and E of zein films increased while TS decreased with increasing relative humidity. It appears high RH leads to water absorption by biopolymers resulting in swelling and conformational

changes. The absorbed water plasticises the film matrix and increases its permeability. Increasing temperatures lead to higher coefficients of diffusion due to increased molecular mobility of the plasticisers and polymer chains (reviewed by Krochta 2002).

2.3.1.2. Biodegradability

Degradation is a process by which a material is reduced to a much less complex form (ASTM, 1996). This may be achieved through bio-, photo- process or solubilisation in water (Selke, 1996). A biodegradable material is one that can be broken down by microbial action within a reasonable period of time to carbon dioxide, methane, water and humus-type materials. Biodegradation involves a total loss of the chemical identity of the starting material (Selke 1996). Table 2.7 shows the degradability of some common packaging materials. Biopolymers and their derivatives, for example wood, cellophane and paper are generally biodegradable, while the synthetic polymers are not.

TABLE 2.7 Degradability of Some Packaging Materials Under Different Degradation Processes (Selke 1996)

Material	Biodegradable	Water Soluble	Oxidizable	Photodegradable
Aluminium	No	No	Very slow	No
Cellophane	Yes	Disruptable	At high temperature	Very slow
Paper	Yes	Disruptable	At high temperature	Very slow
Wood	Yes	No	At high temperature	Very slow
Polyester*	No	No	At high temperature	Very slow
Polystyrene*	No	No	At high temperature	Very slow
Polyethylene*	No	No	At high temperature	Very slow
Glass	No	No	No	No

* Synthetic material

Little work has been reported on the biodegradability of protein films. This is presumably because researchers assume that as the proteins are readily digestible,

the films will be biodegradable. Using film digestibility as an indicator of biodegradability, Yamada et al (1995) reported that cross-linking of zein films with 1,2-epoxy-3-chloropropane decreased their digestibility. Mezgheni et al (1998) investigated the effects of γ -irradiation induced cross-links on the biodegradability of caseinate films using *Pseudomonas aeruginosa*. They found that degradation was delayed for eight days in films that contained the highest content of cross-links. Ou et al (2004) studied the effect of different pHs and the addition of ferulic acid, tannin and hydrogen peroxide to the film forming solutions on the digestibility of soy protein films. High pH, ferulic acid, tannin and hydrogen peroxide were found to decrease the digestibility of the films. Yamauchi et al (2001) reported that disulphide cross-linked collagen films were insoluble in water and more resistant to degradation by collagenase than the non-cross-linked collagen films. Slowed biodegradability of films presumably due to increased cross-linking has also been reported in heat treated whey protein films (Le Tien et al 2000) and γ -irradiated whey, soy and casein protein films (Lacroix et al 2002).

2.4. Modification of protein films

The functional properties of a film are related to the polymer structure and chemistry, the nature of the solvent system, the presence of additives and conditions during film formation. As polymer chain length increases, so does tensile strength (reviewed by Krochta 2002). Uniform distribution of polar groups along the polymer chain increases tensile strength and improves the barrier properties due to the increased likelihood of inter-chain cross-linking (reviewed by Gennadios et al 1994b). In spite of their ability to form numerous inter- and intra-molecular cross-links, proteins form films of inferior functional properties as compared to synthetic polymer films (reviewed by Kester and Fennema 1986; Krochta and De Mulder-Johnston 1997). The relatively inferior functional properties coupled with the varied packaging requirements in the food industry, necessitates modification of the protein film properties.

Modification of proteins involves the use of chemical and/or physical agents to cause changes in the protein functionality (reviewed by Cheftel et al 1985; Damodaran 1996). Modification processes may involve, deamidation, cross-linking and hydrolysis of the protein to a certain extent. Modification is important in the use of proteins for various applications in the food industry. In applications such as films and coatings, a certain

degree of protein cross-linking must occur in order to form a film of given mechanical and barrier properties (De Graaf 2000). This is achieved by adding chemical cross-linking agents, enzymic treatments, manipulation of processing parameters like time and temperature and application of electromagnetic waves or a combination of these.

2.4.1. Approaches to modification of protein films

The literature reveals that there are essentially three general approaches to the modification of protein films:

1. Applying a modifying agent before film formation. This involves modification of the protein before it is formed into a film. For example, Larre et al (2000) made films from native and 20% deamidated gluten and found that deamidation decreased film tensile strength and strain. Modification of the protein can also be used in the thermoforming method of film formation and can be combined with approach 3.
2. Applying a modifying agent during film formation. Here the protein is first solubilised into a film forming solution where the modifying agents, chemical or physical, are applied. This is the most common approach used. For example Perez-Gago and Krochta (2001) heated whey protein film forming solution at increasing temperature and found that film solubility decreased with increasing heating time and temperature. The whey films became stronger and more stretchable as the time and temperature of the film-forming solution increased. Gamma-irradiation has also been applied to casein film forming solutions, which resulted in increased tensile strength (Brault et al 1997; Vachon et al 2000).
3. Applying a modifying agent after film formation. In this approach, the film is first made then the modifying agent applied. It therefore tends to be limited to physical modifying agents. Ultraviolet light applied to soy protein films increased their tensile strength (Gennadios et al 1998). γ -irradiation applied on cast zein films increased their tensile strength (Wang and Padua 2001) and microwaving increased the tensile strength of whey protein films (Kaya and Kaya 2000). Heat treatment was used to increase the tensile strength of cast whey films (Miller et al 1997), wheat gluten films (Ali et al 1997) and soy protein films (Kim et al 2002).

These different approaches can have varied effects on the functional properties of the modified protein films and may have implications in the choice and application of the different modification technologies or agents.

2.4.2. Chemical agents

Emmambux (2004) comprehensively reviewed chemical modifying agents. Briefly, however, chemical agents for modification include acids, organic solvents, enzymes, polyphenols and commercial cross-linking agents. Some acids are known to deamidate and/or partially hydrolyse proteins (Mimouni et al 1994; Chiue et al 1997). In cross-linking of proteins, some chemicals react with the carboxyl groups of aspartic and glutamic acids and the C-terminal amino acids, the imidazolyl group of histidine and the thioester group of methionine. Examples of such chemical cross-linkers include carbodiimides, which couple carboxyl and amino groups of different protein molecules (Wong and Wong 1992). However, chemicals such as carbodiimides are not safe for food use though they are very effective cross-linkers. Research by Emmambux et al (2004) showed that natural agents like plant tannins can cross-link kafirin resulting in increased tensile strength and decreased oxygen permeability.

Other natural potent chemical cross-linkers are some products of the Maillard reactions (Yen et al 1989; Hattori et al 2000). Degradation products of reducing sugars and ascorbic acid e.g. glyoxal and methylglyoxal cross-link proteins (Wells-Knecht et al 1995; Fayle et al 2000). A combination of chemical and enzyme treatments can be used to induce thiol-disulphide interchange and thiol-oxidation reactions, thereby forming new inter- and intra-molecular disulphide cross-links (Krochta and McHugh 1996).

Enzymes have been used to modify proteins through partial hydrolysis (Mimouni et al 1994), deamidation (Lakkis and Villota 1992) and cross-linking (Larre et al 2000). Transglutaminase is one of the enzymes that have been widely investigated in cross-linking of proteins and improving film properties. Transglutaminase catalyses an acyl transfer reaction between the γ -carboxamide group of peptide-bound glutamine residues (acyl donors) and a variety of primary amines (acyl acceptors) including the ϵ -amino group of lysine residues (Dickinson 1997). It can also modify proteins by means of amine incorporation, cross-linking and deamidation (Motoki and Seguro 1998).

Transglutaminase was used to cross-link gelatine (Lim et al 1999), whey and soybean globulin (Yildirim and Hettiarachchy 1998), deamidated gluten (Larre et al 2000) and gluten (Lai and Chiang 2003) films. Use of transglutaminase resulted in increased tensile strength and better barrier properties. The changes in properties of the films were attributed to transglutaminase catalysed cross-linking of the protein as demonstrated by SDS-PAGE data (Larre et al 2000). Since the subject of this thesis is the effect of microwaves on kafirin film properties, research into the effects of physical agents on protein film will be reviewed in detail.

2.4.3. Physical agents

2.4.3.1. Heat

Heat is the most common physical agent used for modifying proteins. The protein-protein interactions that stabilise the secondary and tertiary protein structures can be destabilised with mild heat. Thus a 10°C increase in temperature results in a 600-fold increase in the rate of denaturation of proteins (Cheftel et al 1985). Regarding modification of protein films, heat treatment has been used to increase the tensile strength of whey protein films (Perez-Gago et al 1999) and gluten films (Roy et al 1999).

The susceptibility of proteins to heat modification depends on many factors, including the nature of the protein, protein concentration, water activity, pH, and ionic strength. Water plays a major role in the heat modification of proteins (Cheftel et al 1985), possibly through its action as a heat conductor and its reduction of the protein T_g , thus increasing the polypeptide mobility (Toufeili et al 2002). Madeka and Kokini (1996) reported that increasing moisture content decreased the temperature at which cross-linking reactions are initiated in zein. Thus, the effects of wet heat on proteins are somewhat different from those of dry heat. Therefore, the rate of modification of proteins depends on moisture content as well as the temperature and time of exposure.

When heated in water, hydrophobic proteins like kafirin tend to expel water and become less soluble as temperature increases, while hydrophilic proteins tend to absorb more water (Belton et al 1994). The expulsion of water is possibly due to the exposure of hydrophobic groups as the protein is denatured. Thermal effects on

proteins may also involve aggregation of the unfolded molecules, splitting of disulphide cross-links, chemical alteration of amino acid residues and/or formation of new covalent cross-links (Cheftel et al 1985; Damodaran 1996).

According to Finley (1985) mild heat alters the tertiary structure of proteins. As the heating becomes severe, the side chains of the amino acids lysine, cysteine and arginine react with free acid groups of glutamic and aspartic acid or their amides to yield isopeptide cross-links. This induces changes in the protein functionality. Excessively high temperatures (180–300°C) lead to racemization of amino acid residues in the protein.

One of the amino acid residues in proteins most easily affected by heat is cystine. The reactivity of cystine makes it a precursor of a number of new cross-links (Otterburn 1989). Under wet or dry heating conditions, cystine is readily degraded to form a dehydroalanyl residue and a S-thiocysteiny residue as shown in Figure 2.4. Once the dehydroalanyl residue is formed, a number of possible reactions can occur leading to lanthionine, lysinoalanine, ornithinoalanine and histidinoalanine intra- and inter-molecular cross-links.

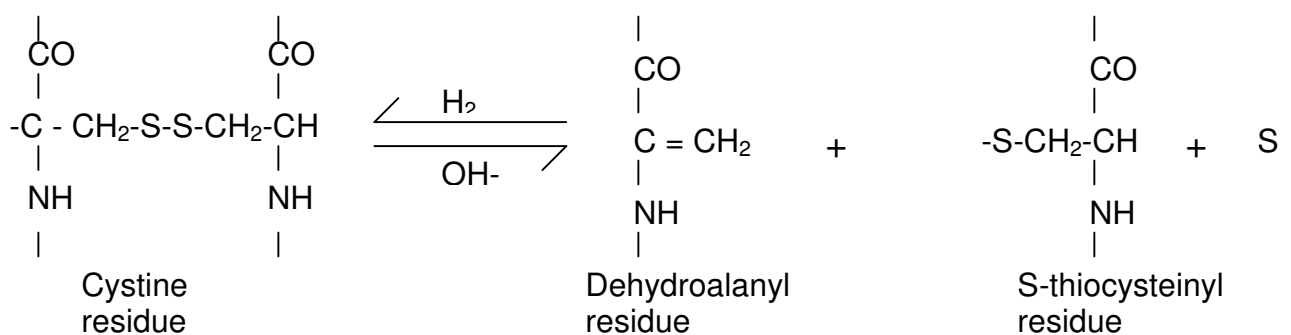


Fig. 2.4. Degradation of cystine residue (Otterburn 1989)

Other isopeptide cross-links e.g. the ε-N(γ-glutamyl) lysylamide (Fig. 2.5) between free amino groups of lysine and carboxylic groups of glutamic acids may be produced as a result of heating protein (Sikorski 1997).

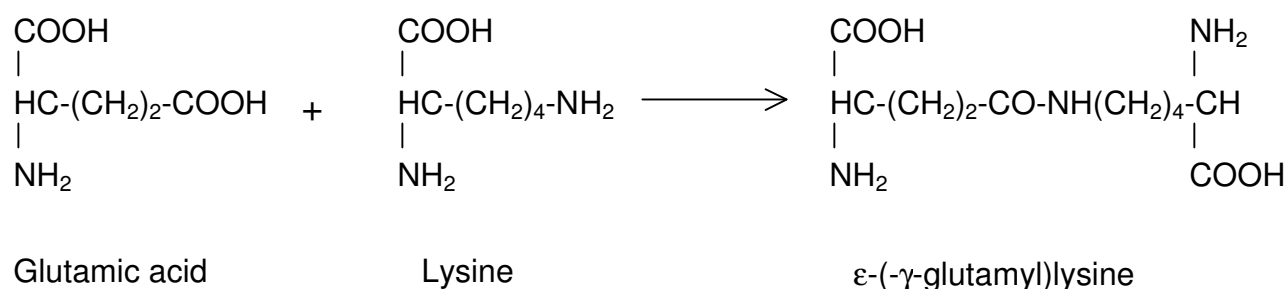


Fig. 2.5. Formation of the ϵ -N(γ -glutamyl) lysylamide isopeptide cross-link under severe dry heat (Sikorski 1997).

The disulphide bond is one of the covalent cross-links that plays an important role in the stabilisation of protein secondary structure (Feeney and Whitaker 1988). Disulphide cross-links may be inter- or intra-molecular (Cheftel et al 1985; Feeney and Whitaker 1988). Disulphide cross-links play an important role in the structure and functionality of gluten (Andrews et al 1995) and kafirin (El Nour et al 1998). The importance of disulphide cross-links in forming and stabilising the gluten structure and its influence on wheat dough properties have been extensively reviewed by Shewry and Tatham (1997) and Lindsay and Skerritt (1999). Heat has been reported to alter the number of disulphide bonds in whey protein gels or interchange them resulting in changes in properties such as protein solubility and gel elasticity and firmness (Shimada and Cheftel 1988a; Shimada and Cheftel 1988b). Yamauchi et al (2001) reported that films made from disulphide cross-linked collagen had higher tensile strength than those made from uncross-linked collagen. In kafirin, heat induced intermolecular disulphide bonds and their role in reducing the digestibility of the protein have been reported by various workers (Hamaker et al 1986; Hamaker et al 1987; El Nour et al 1998; Duodu, et al 2001; Duodu et al 2002; reviewed by Duodu et al 2003; Nunes et al 2004).

A considerable proportion of native kafirin exists as oligomers stabilised by disulphide links among others (El Nour et al 1998). During the extraction of kafirin, reducing agents, like sodium metabisulphite have been used to dis-aggregate the polymerised kafirins by breaking disulphide bonds, thus increasing extractability (Oria et al 1995b). It is possible that the reducing agents could expose more cysteine residues for the formation of SS cross-links in the film network. The structure of the protein in a film depends on the type of protein used and the treatment it is subjected to before, during

and after casting of the film. Such treatment will determine the molecular nature and weight of the polypeptide chains as well as the degree of cross-linking in the film protein network thus its functionality (reviewed by Krochta 2002).

2.4.3.2. Electromagnetic waves

These are defined as coupled electric and magnetic fields that move at the speed of light and exhibit typical wave behaviour (Beiser 1987). As they travel from one point to another, these waves carry electromagnetic energy in the propagating electric and magnetic fields. Electromagnetic energy is used for different applications in the food industry. It is used to heat food, decontaminate packaging materials and to increase food safety and shelf life by destroying microorganisms. In general, there are three common electromagnetic waves that have been used to modify protein films, namely γ -radiation (10^8 - 10^{16} GHz), ultraviolet light (10^6 - 10^7 GHz) and microwaves (300-300,000 MHz). Ultrasound (168-860 KHz) has also been used in some food applications (Banerjee et al 1996). According to Cheftel et al (1985), electromagnetic waves of varying frequencies and wavelength induce cross-links and/or cleavages in protein polypeptide chains, resulting in modified functional properties.

2.4.3.2.1. Ultrasound

The effects of ultrasound frequency and exposure time on the functional properties of whey protein and sodium caseinate films were investigated by Barnajee et al (1996). They found that the average tensile strength of the treated films was higher than that of the control and that increased exposure time resulted in stronger films, possibly as a result of ultrasound induced protein linkages.

2.4.3.2.2. Ultraviolet light

Rhim et al (1999) investigated the effects of ultraviolet light on physical properties of cast zein and gluten films. Treatment with ultraviolet light resulted in an increase of film tensile strength. An increase in tensile strength in soy protein films treated with ultraviolet light has also been attributed to ultraviolet light induced cross-links (Gennadios et al 1998).

2.4.3.2.3. Gamma-irradiation

Gamma-irradiation has been used to improve the mechanical strength of casein-based films (Brault et al 1997; Mezgheni et al 1998; Vachon et al 2000). Wang and Padua (2001) used γ -irradiation on zein films and reported an increase in tensile strength to a peak at 20 kGy with elongation showing a reverse trend and Young's modulus unaffected. The authors attributed the increase in tensile properties of the films to intermolecular cross-links induced in the protein by the applied γ -irradiation.

2.4.3.2.4. Microwaves

Microwaves are part of the electromagnetic spectrum that falls between the frequencies of 300-300,000MHz and wavelength of 1 m - 1 mm in air (Ohlsson and Bengtsson 2001). The International Radio Regulations provide two frequencies i.e. 915 ± 25 and 2450 ± 50 MHz for commercial, industrial and domestic use (Lambert 1980). The energy in the microwave part of the spectrum varies from 10^{-7} - 10^{-3} electron volts. According to Copson (1975), the use of microwaves for heating food was first reported in 1945 in Percy Spencer's patent titled *Treating Foodstuffs*. Today, microwave energy is used in many operations in the food industry including heating, dehydrating, thawing, blanching, pasteurising and baking.

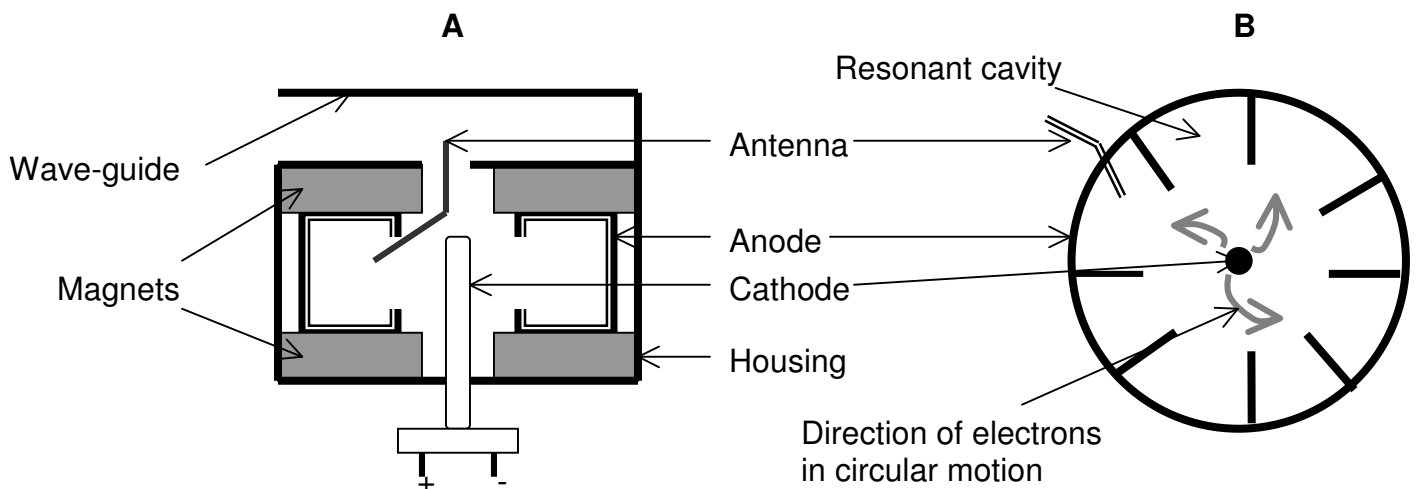


Fig. 2.6. Major components of the magnetron; A) longitudinal section, B) cross-section showing electron motion and resonant cavities. Adapted from Gallawa (2005).

Microwaves are generated by a magnetron. A magnetron is a high voltage system whose basic components include the cathode, the anode constructed with resonant cavities, the antenna and magnets (Fig. 2.6). The basic structure and operation of the magnetron has been detailed by Gallawa (2005). In brief, however, the process of generating microwaves starts by supplying a high direct current voltage to the cathode, which then releases electrons by the thermionic emission process. With the magnetic force supplied by the magnets, the emitted electrons are forced to move in a circular motion around the circular area between the anode and cathode. In so doing, the electrons cause currents around the resonant cavities, which in turn radiate the microwaves. The microwaves are then transmitted to the wave-guide by the antenna.

Based on their interaction with microwaves, materials can be classified into reflecting, absorbing and transparent (Ohlsson and Bengtsson 2001). Reflecting materials are mostly metals e.g. aluminium where a small amount of the microwaves penetrate only a few microns and the rest are reflected. Such reflecting materials are used in the making of wave-guides in microwave ovens. Transparent materials do not absorb microwave energy to any significant extent and just let it go through them. Glass, some plastics and ceramics fall in this category and are thus used as microwaving containers. Materials that absorb microwave energy contain polar constituents e.g. water, plasticisers and polar amino acid residues. Food materials of varying composition fall in this category.

In conventional heating methods, the heat is applied outside the food material and it is transferred to the centre of the material by conduction and/or convection. Heat transfer by conduction/convection is essentially by molecular collision and is dependant on the thermal conductivity of the material. The thermal conductivity of food materials is generally low (Szczesniak 1983). Thus, it takes long for the interior of the material to reach the desired temperature. In contrast, energy transfer in microwave heating is by molecular polarisation, which is dependant on the dipole character of molecules (Fig. 2.6). Thus microwave energy rapidly generates heat within the food material (Harrison 1980; Mudgett 1982). It is for this reason that microwave energy is gaining increasing use as a rapid and effective heating method for industrial use.

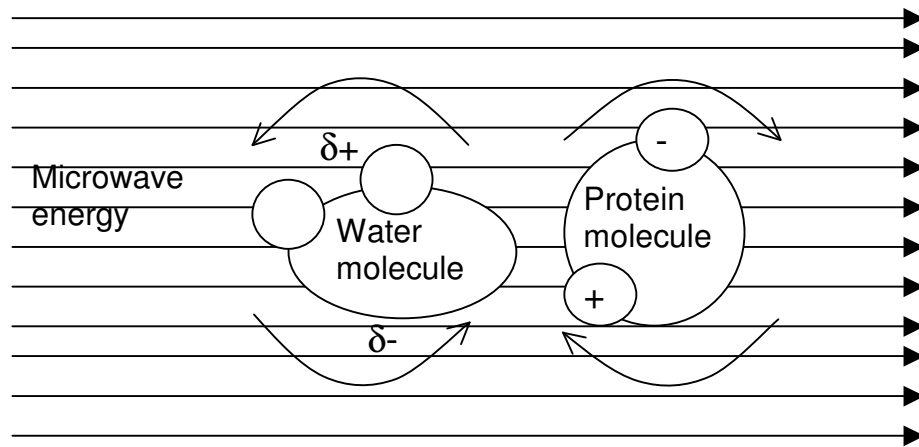


Fig. 2.7. An illustration of microwave heating by molecular polarisation (Adapted from Ohlsson and Bengtsson 2001)

In heating foods using microwaves, the heat is internally generated by dipole rotation, as in the case of water and ionic polarization of molecules, as in the case of proteins, induced by microwave energy as illustrated in Figure. 2.6. With films, water, protein and plasticiser molecules would be involved in such processes. When a dipolar molecule interacts with microwaves, it continuously rotates to align itself with the applied electromagnetic field (Decareau 1985). The continuous rotation at a high frequency of the dipolar molecule against its surroundings results in the generation of heat due to friction, which is called the molecular friction effect (reviewed by Curnutte 1980; Ohlsson and Bengtsson 2001).

The heating efficiency of microwaves is determined by the dielectric properties of the heated material (Mudgett 1982). The dielectric properties of interest in microwave processing are the dielectric constant (k') and the dielectric loss factor (k''). The dielectric constant reflects the ability of a material to store microwave energy, while the dielectric loss factor indicates the ability of the material to dissipate microwave energy as heat. The rate of microwave power absorption and extent of penetration are a function of both the frequency of the microwaves and dielectric properties of the material. The penetration depth is the depth at which about 37% of the microwave energy at the surface remains (Ohlsson and Bengtsson 2001).

The dielectric properties of food materials are generally related to their chemical composition and physical structure. Molecular polarity is the major determinant of the

dielectric constant of a material. Strong dipolar character of a molecule is associated with high dielectric constant values (Curnutte 1980). A high dielectric constant coupled with a high loss factor results in a high and rapid heating effect when a material is subjected to microwave energy. The dielectric properties are also known to vary significantly with microwave frequency and temperature of the material (Mudgett 1982). According to Ohlsson and Bengtsson (2001) the dielectric constant of a material increases almost linearly with the water content, while the dielectric loss factor only shows a moderate increase, except at high salt content. Moisture content influences the dielectric constant of food because of the strong dipolar character of water molecules.

The effect of composition on a material's dielectric properties seems to be complex. As stated, the dielectric constant increases with moisture content of most food materials (Ohlsson and Bengtsson 2001). However, the dielectric loss factor shows an inconsistent trend with increasing moisture content (Padua 1993; Bircan and Barringer 1998).

Mohsenin (1984) reviewed the dielectric properties of foods and agricultural products. He reported that for the same moisture content, the dielectric constant and loss factor of grain foods vary with different microwave frequency. Nelson and Stetson (1975) showed frequency dependence of dielectric properties of sorghum and other grains for a range of 250 Hz to 12 GHz at 24°C. The dielectric properties of materials therefore, seem to be related to their chemical composition, microwave frequency and temperature. Free water and dissociated salts have high dielectric activity while bound water, associated salts and food components e.g. proteins have low activity. The dielectric properties of a material and thus the heating effect will change as it is microwaved due to changes in temperature and composition as the microwave heating progresses.

Effects of microwave energy on proteins

Since electromagnetic energy causes chemical changes in proteins, microwaves have been used to modify functional properties of proteins (Cheftel et al 1985). It seems the effect of electromagnetic waves on proteins is characterised by disorganising the tertiary and secondary structures and breaking of stabilising bonds, thus leading to the

exposure of the various functional groups (Joly 1965; Cheftel, et al 1985). Work done by Villamiel et al (1996) showed that microwave heating of milk at 600 W caused more protein denaturation and Maillard reactions as compared to conventional heating at 70°C for 30 min. Using SDS-PAGE, Farag et al (2001) found that microwave heating of beef liver caused an increase in the levels of high molecular weight protein bands with a concomitant decrease in low molecular weight protein bands. This demonstrates the polymerisation of low molecular weight proteins under the influence of microwaves. The changes in protein molecular weight are dependent on the time of exposure and the power setting of the microwave equipment. Such changes in the molecular weight of proteins are attributed to the inter- and/or intra-molecular protein interaction, which may include the formation of disulphide bonds between pairs of cysteine residues (Cheftel et al 1985). As mentioned, Kaya and Kaya (2000) used microwave energy to dry whey protein films. They found that microwaving increased tensile strength of the films though it did not affect their WVP. Although the increase in tensile strength was not attributed to anything, it is possible that microwave induced cross-linking of the whey protein films was involved.

2.5. Conclusions

Physical methods, for example microwave heating, have been shown to modify functional properties of protein-based films. Such methods provide an opportunity to avoid addition of chemical agents of modification to protein films. Since kafirin properties, for example solubility, digestibility and hydrophobicity, are affected by heat, this suggests the potential of microwave heating in modifying kafirin film properties. However, no work has been reported on microwave heating and its effects on the properties of kafirin and kafirin films and virtually none on any type of protein films. Since the mode of heat generation and transfer in microwave heating is by molecular polarisation unlike collision in conventional convection/conduction heating, it can be hypothesised that the effects of microwave heating on kafirin will be different and more useful than those of conventional heating.

3. RESEARCH

3.1. EFFECT OF MICROWAVE HEATING OF KAFIRIN ON THE FUNCTIONAL PROPERTIES OF KAFIRIN FILMS*

Abstract

To improve the functional properties of cast kafirin films, dry kafirin, extracted with an aqueous ethanol based solvent at 70°C, was microwave heated. No effect on film tensile properties was found. Two strategies were employed to improve the effect of microwaves: extraction of kafirin using an aqueous tert-butanol based solvent at ambient temperature to minimize temperature-induced denaturation and wetting the kafirin to increase its dielectric properties. Microwave heating this kafirin to 90 or 96°C and holding at these temperatures for 1-2 min more than doubled maximum tensile strength and Young's modulus and decreased strain by about one-third compared to films made from non-microwaved kafirin. Film water vapour permeability was reduced by at least one-third. Digestibility of the microwaved kafirin and films was also substantially decreased and film biodegradability was slowed slightly. Microwave heating gave a film microstructure with fewer and smaller size pores. SDS-PAGE showed microwave-induced intermolecular cross-linking of the kafirin monomers, which was possibly responsible for the modification of film properties. Microwave heating of kafirin can be used to modify kafirin film properties but the kafirin must be microwaved wet and be as close as possible to its native state.

* This Chapter has been published in part in Cereal Chemistry 82 (5): 565-573.

3.1.1. Introduction

Kafirin is the prolamin protein of sorghum. Its potential to form cast films similar to those of maize prolamin, zein was first shown by Buffo et al (1997). Subsequently, various researchers have investigated different aspects of kafirin films. Da Silva and Taylor (2005) found that kafirin extracted from milling fractions of red and white sorghum was able to form films with higher tensile strength than those from commercial zein. Gao et al (2005) found extraction of kafirin at elevated temperature adversely affected film quality. Taylor et al (2005a) studied the functional properties of kafirin films cast with different solvents and found that films cast with glacial acetic acid at 25°C were of most consistent quality.

Protein films such as those from zein and kafirin generally have tensile and barrier properties that allow them to be used in some food coating applications (Park et al 1994a; Park et al 1994b; Rakotonirainy et al 2001). Modification of protein film properties is necessary to render the film functional over a diverse range of packaging requirements (Krochta 2002). Technologies that have been used to improve the tensile properties of protein films can be categorized into chemical and physical methods. Chemical methods involve the use of chemical cross-linking agents such as tannins (Ou et al 2004; Emmambux et al 2004), dialdehydes (De Graaf 2000) and/or enzymes like transglutaminase (Yildirim and Hettiarachchy 1998). Emmambux et al (2004) cross-linked kafirin films with tannins and found a significant increase in film tensile strength and a decrease in oxygen permeability.

Regarding physical methods, thermal treatment has been reported to increase the tensile strength of whey films (Perez-Gago et al 1999; Perez-Gago and Krochta 2001) and gluten films (Roy et al 1999). Other physical methods used to change tensile properties of protein films include electromagnetic energy of various frequencies. Ultraviolet light (Gennadios et al 1998; Rhim et al 1999), ultrasonic waves (Banerjee et al 1996), γ -radiation (Vachon et al 2000) and microwaves (Kaya and Kaya 2000) have been reported to improve the tensile strength of protein films.

Microwaves are non-ionising electromagnetic waves in the frequency range 300 to 300,000 MHz, corresponding to wavelengths from 1 m to 1 mm (Ohlsson and

Bengtsson 2001). In heating foods using microwaves, the heat is internally generated by dipole rotation and ionic polarization of molecules induced by microwave energy, which create friction with subsequent transfer of heat to the surrounding (reviewed by Curnutte 1980). Water and protein molecules would be involved in such a process. Conversely, in conventional convection/conduction heating, the heat energy is supplied from outside the food and transferred by molecular collision. Thus, microwave energy offers advantages over conventional heating methods because of its rapid internal heat generation (Mudgett 1982). In the work reported here, microwave energy was used to thermally modify kafirin structure before casting films. The effect of exposure to microwave heating on the functional properties of kafirin films was examined.

3.1.2. Materials and methods

3.1.2.1. Extraction of kafirin

Kafirin was extracted from milled decorticated sorghum grain (a mixture of two white, condensed tannin-free hybrids, PANNAR 202 and 206). Two extraction procedures were used: extraction with an aqueous ethanol based solvent as described by Emmambux and Taylor (2003) and extraction with aqueous tert-butanol as described by Taylor et al (1984b). The defatted aqueous ethanol extracted kafirin had a protein content of 95.6% and that of the defatted aqueous tert-butanol extracted kafirin was 81.2% (db, N x 6.25). The lower protein content of the latter could be due to greater co-extraction of grain components such as non-starch polysaccharides, which may contaminate kafirin preparations (Da Silva and Taylor 2004).

3.1.2.2. Dielectric properties of kafirin

The dielectric constant, dielectric loss factor and power penetration depth of the kafirin powder were determined using the perturbation resonant cavity method described by Sharma and Prasad (2002). Measurements were made at both frequency bands allocated for industrial microwave heating, i.e. 2.45 GHz and 915 MHz.

3.1.2.3. Microwave heating of kafirin

A 2 kW Sairem GMP 20KSM microwave generator (Sairem, Neyron, France) fitted with a power regulator was used for heating. A wave-guide was used to concentrate the microwaves onto a sample. Kafirin powder (containing 1.44 g protein) was

weighed into a glass test tube. Figure 3.1.1 illustrates the configuration of the microwaving apparatus used. Using a burette clamp, the test tube containing the sample was held vertically to expose the sample in the centre of the wave-guide (Fig 3.1.1). The sample was then microwave heated as described below.

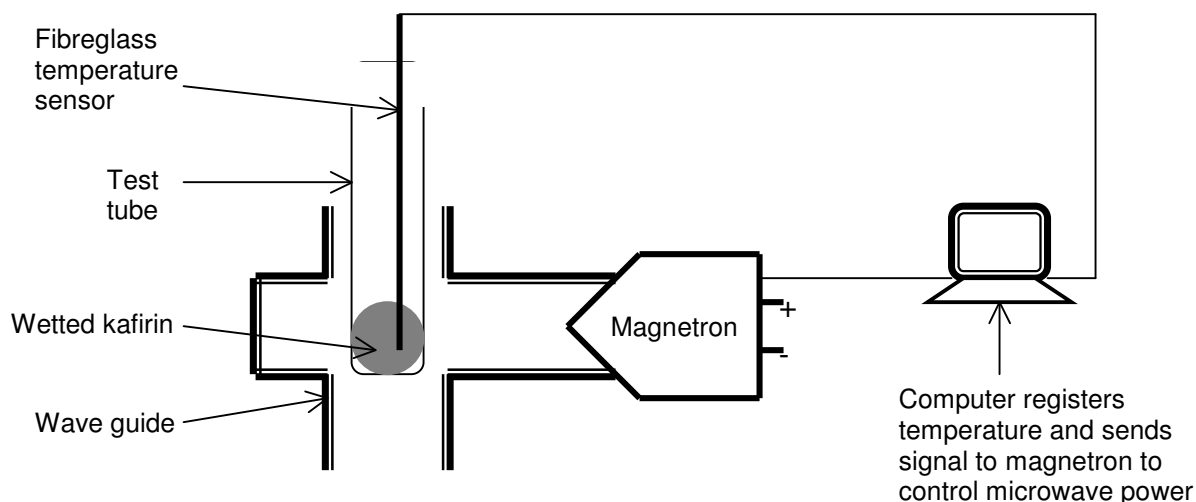


Fig. 3.1.1. Apparatus for microwaving kafirin before film casting

Aqueous ethanol extracted kafirin powder (moisture content, 7% wb) in the test tube was covered with a plastic lid containing a hole for inserting the temperature sensor probe. The kafirin was then microwave heated at rates of 100, 200 and 300 W for 1 and 2 min. The maximum temperature attained in the kafirin was recorded using a FISO FTI-10 fibre optic temperature sensor and recording system (FISO Technologies, Quebec City, Canada). Aqueous ethanol extracted kafirin powder that received no microwave heating was used as the control.

Aqueous tert-butanol extracted kafirin powder (containing 1.44 g protein) was thoroughly mixed (vortexed) with 6.0 g of distilled water. The test tube was then covered with a lid containing a hole for the temperature sensor probe and then held at 4°C overnight to equilibrate. Three microwave heating procedures were used: (1) wet kafirin was microwave heated at rates of 50, 60, 70, 80, and 90 W for 1 min, (2) wet

kafirin was microwave heated at 80 W to set temperature targets of 70, 80 and 90°C and held at these temperatures for 1 min, (3) wet kafirin was microwave heated to 90 and 96°C and held at these temperatures, in the microwave unit, for periods of up to 10 min. The microwave heated wet kafirin was centrifuged at 7,200 *g* at ambient temperature for 5 min. Excess water was decanted off and then the protein was freeze-dried. Untreated wetted aqueous tert-butanol extracted kafirin powder was used as the control.

3.1.2.3.1. Estimation of energy absorbed by sample

The estimated absorbed energy (EAE) was calculated using the equation $Q = MC\theta$, where Q is the absorbed energy expressed as Joules per gram of sample, M is the mass of sample (g), C is the specific heat capacity of the sample (J/g.°C), θ is the change in sample temperature (°C) (Singh and Heldman 1993). The specific heat capacity of the samples was calculated using the equation described by Singh and Heldman (1993). The limitations of using this approach to calculate the EAE are discussed in section 4.1.

3.1.2.4. Film casting

The kafirin was mixed with 9 g glacial acetic acid (98%) and 0.6 g plasticiser (lactic acid: polyethylene glycol 400: glycerol in 1:1:1 weight ratio) in an Erlenmeyer flask at ambient temperature and films were cast following the procedure of Taylor et al (2005a). In brief, 4 g of the film forming solution was weighed into 9 cm diameter Petri dishes and gently swirled to coat the bottom of the dish. The open Petri dishes were placed in an oven at 50°C without forced draft and dried overnight.

3.1.2.5. Film functional properties

The procedure described by Taylor et al (2005a) was used to determine the maximum tensile strength, tensile strength at break, strain and Young's modulus, except that the film strips (60 x 6 mm) were first conditioned. Conditioning of the filmstrips was done at 50% RH for 48 hr as described by Emmambux et al (2004). The films had an average thickness of approx. 100 μ m. The filmstrips were then mounted between the tensile grips (40 mm apart) and the tension was applied with a crosshead speed of 0.4 mm/s.

The Water Vapour Permeability (WVP) of the films was determined using the procedure described by Taylor et al (2005a) with modification. Schott bottles (100 mL) and glass vials (4 mL screw neck, 45 x 14.7 mm) were used. The two different vessels were used because the WVP of films made from microwaved kafirin was to be compared to that of kafirin films that were microwaved after casting. The latter could only be microwaved in film pieces of 19 x 70 mm, the maximum size that could be accommodated in the microwaving apparatus used. Therefore, smaller vessels of diameter less than 19 mm had to be used in addition to the Schott bottles. Circular discs of 40 and 12 mm diameter (for Schott bottles and vials, respectively) were cut from the film and their thickness measured with a micrometer. The bottles and vials were filled with distilled water to leave a gap of 22 and 7 mm, respectively, between the film and water level. The film discs were then mounted on top of Schott bottles and vials with plastic screw tops drilled accurately to provide circular openings of 33 and 9 mm diameter for Schott bottles and vials, respectively. The bottles and vials were placed in a fume cabinet with the fan on at 25°C. Weight change of the bottles and vials was recorded daily for 14 days. The RH of the fume cabinet was monitored daily and the mean RH was taken for calculation. The apparent WVP was then calculated according to the method of Gennadios et al (1994a), which includes correction for the stagnant air gap resistance between water and film.

The digestibility of the kafirin and films was determined using the pepsin method of Hamaker et al (1987). Sample portions (10 mg protein basis) were digested with 998 units of pepsin P7000 (Sigma, St. Louis, USA) at 37°C for 2 hr. Protein (N x 6.25) was determined by the Dumas combustion method. Digestibility was determined on the basis of the protein contents of the kafirin and the kafirin films after treatment.

Film biodegradability was determined using a procedure based on the ASTM method D5512-96 (ASTM 1996). The procedure involved burying the films in well fermented compost, based on horse manure and sawdust, of moisture content 37 and 60% held in tightly covered plastic buckets. The buckets were held for 28 days in an incubator at average temperature 50°C. Films were cut, mounted onto 35 mm plastic film slide frames and then photographed using a flatbed scanner. The films were then conditioned at 50% RH in a dessicator containing concentrated calcium nitrate as described by Emmambux et al (2004) for 48 hr and buried in the compost. During the

28 days of incubation, the containers were aerated daily by removing the lid for 1 hr. The containers were not mixed to avoid physical damage of the buried films. At intervals, films were removed from the compost, examined and scanned. Low-density polyethylene films were included for comparison.

For scanning electron microscopy (SEM), kafirin films were freeze-fractured in liquid nitrogen at -80°C and mounted on aluminium stubs using double-sided carbon tape, with the fracture surface exposed at the top. The mounted films were then sputter coated with gold and viewed by SEM at 5 kV.

3.1.2.6. SDS-PAGE

Gradient sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted using a discontinuous Tris HCl/Glycine buffer system. An acrylamide: bisacrylamide ratio of 19:1 was used to make gels (12.5 cm x 16 cm and 1.5 mm thickness) of concentration gradient 3.9-12% for the kafirin powder extracts and 3.9-15% for the films and the digestibility residues. Samples (10 mg protein) were mixed in 1 mL sample buffer consisting 0.125 M Tris-HCl pH 6.8, 20mL glycerol, 40 g/L SDS and 0.05 g/L bromophenol blue in Eppendorf tubes containing glass beads. For reducing and non-reducing conditions the sample buffer also contained 10 mL mercaptoethanol or distilled water, respectively. For the digestibility residues, the 10 mg of sample were obtained from multiple digestions. The sample preparations were then heated in a boiling water bath for 15 min with periodic vortex mixing. Approx. 60 μg protein was loaded per well. Protein molecular weight markers (Boehringer, Mannheim, Germany) were used. Electrophoresis was done on a Protean II xi vertical cell with a 1000 Powerpac (Bio-Rad Laboratories, Hercules, USA) for 12 hr at constant current of 50 mA. The gels were stained using 0.1% (w/v) Coomassie Brilliant Blue R250, destained and scanned.

3.1.2.7. Statistical analysis

Unless otherwise stated, mean values were obtained from three independent microwaving experiments. One-way analysis of variance (ANOVA) was performed on the data to determine the effect of all the different microwave treatments on the individual kafirin film properties. The least significance difference test was performed at $P < 0.05$ on treatment effects for which significant differences were found.

3.1.3. Results and discussion

3.1.3.1. Dielectric properties of kafirin

Table 3.1.1 shows the dielectric properties of kafirin powder. The powder had a dielectric constant lower than those of insulators such as rubber, paraffin wax, shellac and porcelain (Uvarov et al 1971). The dielectric constant of kafirin powder was close to that of air. Thus kafirin powder can be regarded as an insulator, possibly because of its low charge density, due to its high content of the non-polar amino acids leucine and alanine (Shewry 2002).

TABLE 3.1.1 Dielectric Properties of Aqueous Ethanol Extracted Kafirin Powder Measured at 915 and 2450 MHz^a

Frequency (MHz)	915	2450
Dielectric Constant	1.58	1.55
Dielectric Loss Factor	0.016	0.021
Power Penetration		
Depth (m)	3.99	1.13

a. Values are means of triplicate determinations

3.1.3.2. Microwave heating of dry, elevated temperature aqueous ethanol extracted kafirin powder

The temperature of the kafirin increased with increasing microwave power and time of exposure (Table 3.1.2). The kafirin powder changed in colour and granularity with increasing microwave power and time of exposure. The samples exposed to 300 W in which about 109-160 J/g were absorbed, contained brown to black lumps that did not dissolve completely during the film casting process. These lumps remained in the films giving them a rough surface. It seems that increasing microwave power induced points of combustion within the kafirin powder. Dry heating of proteins in the presence of oxygen leads to thermal degradation of certain amino acid residues e.g. tryptophan, tyrosine, methionine and cysteine (Damodaran 1996). This leads to development of burnt flavours, browning accompanied by formation of new intra- and inter-molecular cross-links and isopeptide interactions that may form insoluble protein polymers

(Otterburn 1989). In addition, it is possible that the impurities in the kafirin extract, presumably non-starch carbohydrates, could have been involved in Maillard browning reactions with the protein.

TABLE 3.1.2 Characteristics of Microwaved Aqueous Ethanol Extracted Kafirin Powder and Characteristics and Tensile Properties of Films Made from the Kafirin

Microwave power (W)	0 (Control)	100		200		300	
Microwaving Time (min)		1	2	1	2	1	2
Maximum Temperature of Kafirin (°C)	23	46	59	73	105	115	158
Estimated Absorbed Energy (J/g)	0	27.4	36.7	59.5	97.6	109.5	160.5
Maximum Tensile Strength (MPa)	2.93a ^a (0.13) ^b	3.95a (1.02)	4.02a (1.05)	3.90a (0.33)	3.78a (0.47)	3.46a (0.21)	3.00a (0.50)
Tensile Strength at Break (MPa)	2.88a (0.15)	3.89a (0.99)	3.95a (0.97)	3.83a (0.30)	3.72a (0.44)	3.39a (0.22)	2.92a (0.50)
Strain (%)	73.6a (19.8)	85.4a (14.0)	78.1a (17.7)	72.4a (15.1)	68.7a (19.9)	65.5a (17.5)	78.4a (8.7)
Appearance of Kafirin Powders	Cream in colour and free flowing powder (fluffy)	No change in colour and fluffiness		Yellow tinge and not as fluffy, contained small lumps		Slightly brown, contained small dark lumps. Burnt odour.	
Appearance of Kafirin Films	Smooth and flexible, clear and transparent.	Smooth and flexible, clear and transparent		Flexible but not as smooth, clear and transparent. Yellow tinge in colour.		Rough surface. Yellow to brown in colour. Dark lumps.	

a. Values in the same row with similar letters are not significantly different ($p>0.05$).

b. Values in parentheses are standard deviations of the means.

c. Appearance of kafirin powder after 2 min microwaving and appearance of films made from kafirin powder microwaved for 2 min.

However, microwave heating of kafirin powder did not have a significant effect on the tensile properties of the films (Table 3.1.2). The standard deviation of the parameters measured was quite high in some cases, possibly blurring the effects of microwave heating. This was possibly because microwave heating of kafirin powder did not affect the kafirin in a controlled manner. Perhaps the most likely reason for this was the low dielectric properties of the kafirin powder (Table 3.1.1), which could not allow for useful modification of the protein using microwave energy. The dielectric properties of foods, i.e. the dielectric constant and loss factor, are mostly determined by the moisture content (Spahioglu and Barringer 2003). Thus, dry food components e.g. proteins have low dielectric properties and in some cases are regarded as being dielectrically inert (Miller et al 1991).

Additionally, the extraction at 70°C, which was necessary because kafirin is only soluble in aqueous ethanol at elevated temperature (Taylor et al 1984b), could have resulted in heat denaturation of the kafirin. SDS-PAGE under reducing and non-reducing conditions of kafirin extracted using aqueous ethanol at 70°C and aqueous tert-butanol at 25°C (Fig. 3.1.2) showed band patterns that were typical of kafirin and consistent with the α -, β - and γ -kafirin (Shull et al 1991). Bands of approx. M_r 50,000 could also be seen. Similar bands have been described as dimers resulting from disulfide cross-linking of the monomeric kafirins (El Nour et al 1998; Duodu et al 2002). Under non-reducing conditions, the M_r 50,000 band was less intense in the aqueous tert-butanol extracted kafirin (track 1) as compared to the aqueous ethanol extracted kafirin (track 2). Under reducing conditions, the M_r 50,000 bands in the aqueous tert-butanol extracted kafirin (track 1) decreased more in intensity as compared to those in the aqueous ethanol extracted kafirin (track 2). This suggests that the kafirin extracted with aqueous ethanol at 70°C contained more reduction resistant dimers as compared to the kafirin extracted with aqueous tert-butanol at 25°C. Gao et al (2005) also found that drying of extracted kafirin at elevated temperature (40°C) resulted in an increase in the proportion of β -sheet content relative to native α -helical structure, which appeared to result in kafirin films of high tensile strength.

From the foregoing, it was realized that to effectively modify the functional properties of kafirin films, the kafirin not only had to be extracted using a method that did not expose it to heat damage, but the protein's dielectric properties had to be increased.

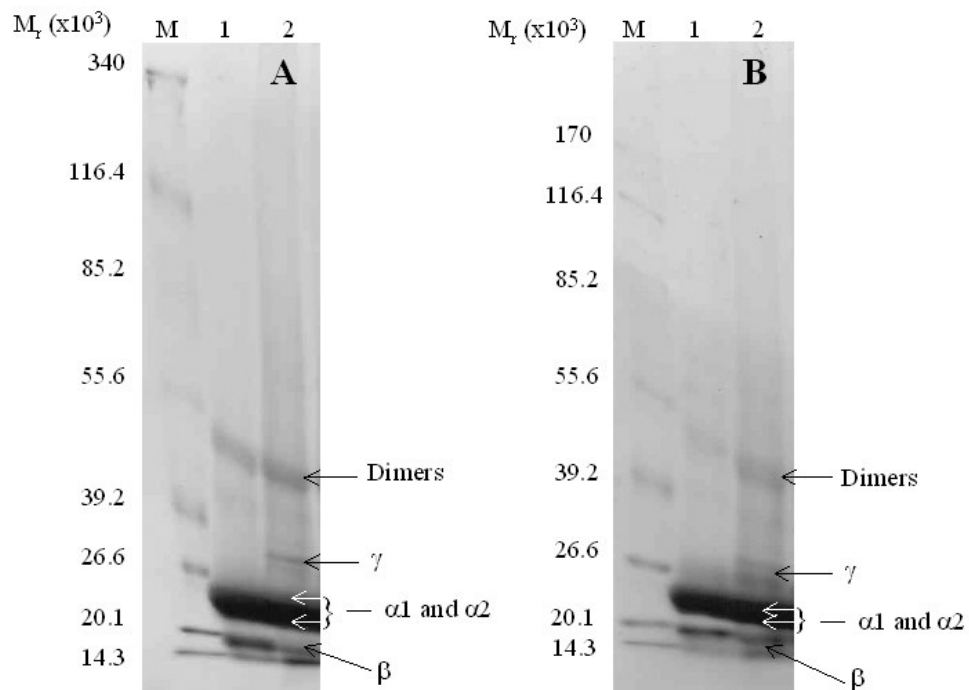


Fig. 3.1.2. Gradient SDS-PAGE of kafirin extracted using different methods. (A) Non-reducing, (B) reducing conditions. Tracks M = molecular weight markers; 1 = aqueous tert-butanol extracted kafirin; 2 = aqueous ethanol extracted kafirin. MW = Molecular Weight.

3.1.3.3. Microwave heating of wet, ambient temperature aqueous tert-butanol extracted kafirin

SDS-PAGE of films made from microwave-heated kafirin (96°C, 3 min) and their residues of pepsin digestion (RPD) (Fig. 3.1.3) showed bands consistent with the α -,

β - and γ -kafirin bands (Shull et al 1991). In addition, bands of approx. M_r 40,000 and 50,000 were present with varying intensity among the different samples. These bands are kafirin oligomers (El Nour et al 1998; Duodu et al 2002). Under non-reducing conditions (Fig. 3.1.3A) there was an increase in the intensity of the oligomer bands in the microwave-heated kafirin (track 2) and film that was made from it (track 3) as compared to the untreated kafirin (control) (track 1). There was also, a decrease in the intensity of the β -kafirin band in the film (track 3) as compared to the control (track 1) and the microwave heated kafirin (track 2). Taylor et al (2005b) also found a low intensity of β -kafirin bands and a higher intensity of high molecular weight proteins in kafirin that was extracted using a glacial acetic acid based solvent as compared to aqueous ethanol or isopropanol extracted kafirin. The decrease in the intensity of β -kafirin bands in the kafirin film coinciding with the increase in the intensity of oligomer bands suggests the involvement of the β -kafirin in the formation of oligomers, since it has been reported that β -kafirin acts as a chain extender in the polymerisation of γ - and α -kafirin through disulfide cross-links (El Nour et al 1998).

The intensity of the α -, β - and γ -kafirin bands in all the RPDs (tracks 4-6) decreased as compared to before digestion, under both non-reducing (Fig. 3.1.3A) and reducing conditions (Fig. 3.1.3B). For the RPDs of the film, these bands were the least intense (track 6). The decrease in the intensity of these protein bands in the RPDs was possibly because of their involvement in the formation of oligomers and higher molecular weight fractions that may not have entered the gel. This effect is most pronounced in track 6, the RPDs of film made from microwaved kafirin, where the oligomer bands are most intense. The fact that there were monomeric kafirin species remaining in the RPDs of the films, which were 86-96% digestible, suggests that the monomers were only slightly more digestible than oligomers.

With SDS-PAGE under reducing conditions (Fig. 3.1.3B), there was a slight decrease in the intensity of oligomer bands in all samples as compared to the non-reducing conditions (Fig. 3.1.3A). This suggests the involvement of disulfide bonds in the cross-linking of the monomeric kafirins to form the oligomers. However, the non-cleavage of all the oligomer bands indicates the possible involvement of inaccessible disulfide and/or non-disulfide cross-links, as suggested by Duodu et al (2002). These results

support the suggestion that the observed changes in the functional properties of kafirin films, i.e. increased tensile strength and decreased strain, WVP and digestibility were a result of microwave induced intermolecular disulfide and/or non-disulfide cross-links.

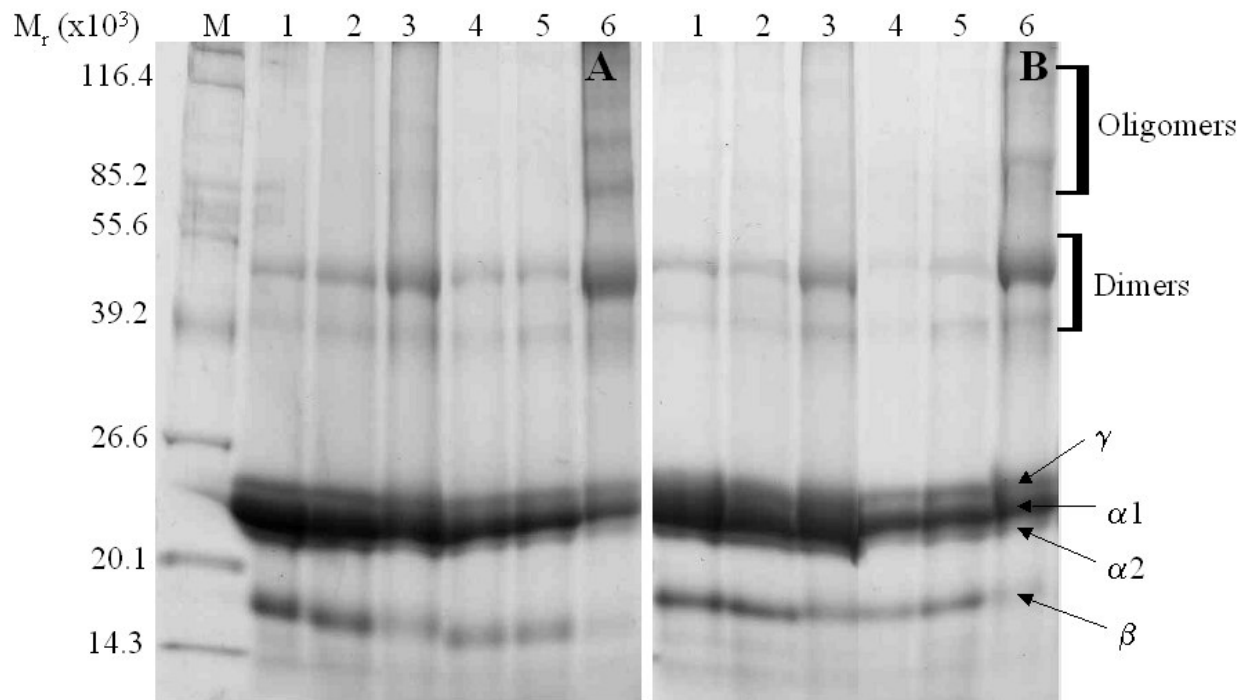


Fig. 3.1.3. Gradient SDS-PAGE of microwave heated wet kafirin, the kafirin films and their residues of pepsin digestion (RPD). A) Non-reducing, B) reducing conditions. Track M = molecular weight markers, 1 = untreated kafirin (control), 2 = microwave heated kafirin (96°C, 3 min), 3 = film made from microwave heated kafirin (96°C, 3 min), 4 = RPD of untreated kafirin, 5 = RPD of microwave heated kafirin (96°C, 3 min), 6 = RPD of film made from microwave heated kafirin (96°C, 3 min). MW = Molecular weight.

The mean maximum tensile strength, tensile strength at break and strain of the control films made from the aqueous tert-butanol extracted kafirin were 1.14 MPa, 1.10 MPa and 136%, respectively (Table 3.1.3) compared to films made from aqueous ethanol extracted kafirin which had maximum tensile strength, tensile strength at break and strain of 2.93 MPa, 2.88 MPa and 73.6%, respectively (Table 3.1.2). This shows that the films made from ambient temperature aqueous tert-butanol extracted kafirin were less strong but more extensible than those made from elevated temperature aqueous ethanol extracted kafirin. The high tensile strength of the films made from ethanol extracted kafirin is in agreement with the report by Gao et al (2005) who found that high temperatures used to dry kafirin increased film tensile strength.

TABLE 3.1.3 Characteristics of Aqueous Butanol Extracted Kafirin that was Microwaved Wet to Different Temperatures at 80 W for 1 min and Appearance and Tensile Properties of Films Made from the Kafirin

Microwave Power (W)	0 (Control)	80	80	80
Maximum Temperature of Kafirin (°C)	24	77	84	92
Time Taken to Reach Maximum Temperature (sec)	n.a. ^a	40	44	49
Estimated Absorbed Energy (J/g)	0	189.8	214.8	243.5
Maximum Tensile Strength (MPa)	1.14a ^b (0.17) ^c	1.17a (0.11)	1.28ab (0.10)	1.58b (0.19)
Tensile Strength at Break (MPa)	1.10a (0.18)	1.13a (0.11)	1.23ab (0.08)	1.50b (0.20)
Strain (%)	136b (26)	131b (16)	93a (8)	86a (25)
Appearance of Kafirin Powders	Cream in colour and free flowing (fluffy)	No change in colour and fluffiness	No change in colour and fluffiness	Yellow tinge, not as fluffy contained small lumps
Appearance of Kafirin Films	Smooth, flexible, clear and transparent	Smooth, flexible, clear and transparent.	Smooth, flexible, clear and transparent	Smooth, flexible, clear and transparent Yellow tinge in colour.

a. n.a., not applicable

b. Means with different letters, in the same row, are significantly different ($p < 0.05$)

c. Values in parentheses are standard deviations of the means

Concerning the difference between microwave heating of kafirin powder and microwaving wet kafirin, Table 3.1.3 shows that during microwave heating of wet kafirin, the time taken to reach e.g. 77°C was 40 sec at 80 W. In contrast, it took 2 min

for a higher microwave power (100 W) to achieve just 59°C during microwaving of kafirin powder (Table 3.1.2). The EAE also shows that much more energy (about 189.8 J/g) was absorbed using 80 W on the wet sample as opposed to about only 36.7 J/g that were absorbed when 100 W were applied on the dry kafirin sample. In addition, there appeared to be less adverse effect on the quality of the kafirin as indicated by its colour and granularity. This shows that microwave heating of wet kafirin was more effective at bringing about desirable changes in film properties than microwaving kafirin powder. This is presumably as a result of the addition of moisture, which increased the dielectric properties of the kafirin powder. It is also apparent (Table 3.1.3) that there was a 2-7°C variation from the set target temperature. The possible reasons for this are discussed in section 4.1.

Microwave heating of wet kafirin at constant power (80 W) to 70, 80 and 90°C and holding it at these temperatures for 1 min resulted in a progressive increase in the EAE, the maximum tensile strength and tensile strength at break and a decrease in strain of the films (Table 3.1.3). The maximum tensile strength increased from about 1.14 MPa to up to 1.58 MPa (approx. 39% higher), tensile strength at break increased from about 1.10 MPa to up to 1.50 MPa and strain decreased from about 136% to as low as 86% (approx. 37% lower) in the films made from microwave heated kafirin. The increase in tensile strength was positively related to the increase in EAE. Also, there were less adverse effects on the quality of the films, as indicated by colour, transparency and surface texture, compared to the films that had been prepared from dry, elevated temperature aqueous ethanol extracted kafirin.

Similarly, microwaving the wet, aqueous tert-butanol extracted kafirin at increasing microwave power (50-90 W) for 1 min resulted in increased film maximum tensile strength from about 1.14 MPa to up to 1.82 MPa, tensile strength at break from about 1.10 MPa to up to 1.77 MPa, and decreased strain from about 136% to as low as 85% (Table 3.1.4). Again, the increase in tensile strength was positively related to the increase in EAE. Microwaving at 70 W increased the kafirin's maximum temperature to 93°C (about 247 J/g of EAE) and showed the most effect on increasing tensile strength and decreasing strain of the films. Higher microwave power, which heated the kafirin to slightly higher temperature, did not give any further change in film tensile properties. Microwave heating of the kafirin to 90°C (about 236 J/g of EAE) for increasing time

periods increased the maximum tensile strength from about 1.37 MPa to up to 2.79 MPa (approx. 104% higher), the tensile strength at break from about 1.36 MPa to up to 2.61 MPa, and the Young's modulus from about 21 MPa to up to 56 MPa (approx. 167% higher), and decreased the strain from about 142% to as low as 90% (approx. 37% lower) in the films made from microwaved kafirin (Table 3.1.5). The shortest microwaving time that showed the most effect on film tensile properties was 2 min. Microwaving times longer than 2 min did not give any further significant change in film tensile properties. Microwave heating of the wet kafirin to a higher temperature (96°C, about 257 J/g of EAE) for increasing time periods similarly affected the tensile properties of the films. The increased kafirin temperature did not give additional changes in film tensile properties but the shortest microwaving time required to modify film tensile properties was only 1 min.

TABLE 3.1.4 Tensile Properties of Films Made from Wet Aqueous Tert-Butanol Extracted Kafirin that was Microwave Heated at Different Microwave Power and Held at These Powers for 1 min

Microwave Power (W)	0 (Control)	50	60	70	80	90
Maximum Temperature of Kafirin (°C)	24	60	64	93	95	95
Estimated Absorbed Energy (J/g)	0	129.0	143.2	247.1	254.3	254.3
Maximum Tensile Strength (MPa)	1.14a ^a (0.17) ^b	1.28a (0.09)	1.60b (0.15)	1.69b (0.01)	1.82b (0.21)	1.71b (0.05)
Tensile Strength at Break (MPa)	1.10a (0.18)	1.26a (0.10)	1.57b (0.13)	1.63b (0.02)	1.77b (0.25)	1.66b (0.04)
Strain (%)	136b (26)	131b (19)	127b (13)	96a (10)	86a (9)	85a (11)

a. Means with different letters, in the same row, are significantly different ($p < 0.05$)

b. Values in parentheses are standard deviations of the means

TABLE 3.1.5 Tensile Properties of Films Made from Wet Kafirin that was Microwave Heated to 90 and 96°C and Held at These Temperatures for Different Time Periods

Microwaving Time (min)	0 (Control)	1		2		3		4		10	
Temperature (°C)		90	96	90	96	90	96	90	96	90	96
Estimated Absorbed Energy (J/g)	0	236.3	257.8	236.3	257.8	236.3	257.8	236.3	257.8	236.3	257.8
Maximum Tensile Strength (MPa)	1.37a ^a (0.17) ^b	2.43b (0.36)	2.65b (0.49)	2.38b (0.19)	3.04b (0.38)	2.79b (0.45)	3.13b (0.73)	2.38b (0.40)	2.74b (0.76)	2.16b (0.41)	2.57b (0.35)
Tensile Strength at Break (MPa)	1.36a (0.30)	2.20ab (0.57)	2.48b (0.53)	2.08ab (0.41)	2.55b (0.42)	2.61b (0.25)	2.75b (0.72)	2.34b (0.39)	2.64b (0.87)	2.03ab (0.49)	2.45b (0.37)
Strain (%)	142b (20.8)	131b (12)	105a (9)	112ab (11)	83a (16)	108a (10)	102a (17)	90a (9)	99a (20)	90a (12)	108a (10)
Young's Modulus (MPa)	21a (5.7)	46b (10)	75b (14)	56b (8)	101b (15)	54b (11)	104b (18)	37b (10)	92b (11)	44b (10)	75b (12)

a. Means in the same row with different letters are significantly different ($p < 0.05$)

b. Values in parentheses are standard deviations of the means

Emmambux et al (2004) obtained a 108% increase in the tensile strength of kafirin films by cross-linking with tannins. The results reported here are also consistent with earlier research that reported that heat treatment of film-forming solutions resulted in increased tensile strength of soy films (Stuchell and Krochta 1994), whey protein films (Perez-Gago et al 1999; Perez-Gago and Krochta 2001) and pea-protein films (Choi and Han 2001). Microwaving of whey protein films was also found to increase their tensile strength (Kaya and Kaya 2000). The increase in tensile strength and Young's modulus of the protein films resulting from heat treatment has been attributed to the formation of intermolecular cross-links (Roy et al 1999; Perez-Gago and Krochta 2001; Choi and Han 2001). Heat induced cross-links, including disulfide and non-disulfide bonds, have been reported in kafirin (Oria et al 1995a; Duodu et al 2002; Duodu et al 2003; Nunes et al 2004). It is probable that microwave heating of kafirin induced similar intermolecular cross-links that resulted in the observed changes in the film tensile properties.

As with the results reported here, it was found that with whey protein films made from film forming solutions that had been heated at 70, 80, 90 and 100°C for time periods for up to 20 min there was no effect of holding time on film strength (Perez-Gago and Krochta 2001). According to Wedemeyer et al (2000) this can be attributed to the fact that all the functional groups with the potential to form cross-links reacted so that further heating could not necessarily produce more cross-linking. It is possible that a similar scenario occurred in the case of microwave-heated kafirin. Of the three conditions used to treat wet kafirin, microwave heating to 96°C (about 257 J/g) was the most effective in terms of changing film tensile properties, while microwaving at 80 W (about 189-243 J/g) to different temperatures for 1 min was the least effective. This was possibly because microwave heating at 96°C was high enough to cause substantial cross-linking of the kafirin, as reflected in the EAE.

The WVP of films made from the kafirin that had been microwave heated to 96°C decreased from about 2.50 to as low as 1.33 g mm m⁻² h⁻¹ kPa⁻¹ (approx. 47% lower) and from about 0.84 to as low as 0.55 g mm m⁻² h⁻¹ kPa⁻¹ (approx. 35% lower), as determined using vials and Schott bottles, respectively (Table 3.1.6). The shortest microwaving time required to decrease the WVP was 2 min. Increasing microwave

time did not give further significant decreases in film WVP. A heat induced decrease in film WVP has also been reported for soy protein films (Stuchell and Krochta 1994) and whey protein films (Le Tien et al 2000). The decrease in WVP was attributed to heat induced protein cross-linking. Increased polymer chain interaction and a high degree of cross-linking tend to reduce the permeability of vapours across bioplastic films (Kester and Fennema 1986). In contrast to the effect of microwave heating on kafirin film WVP, modification of kafirin films with tannins did not affect their WVP (Emmambux et al 2004). This is possibly because the interaction between tannins and kafirin molecules involves mainly hydrogen bonding that is weaker as compared to the covalent linkages such as the disulfide bond which seem to be involved in the microwave heated kafirin. Additionally, tannins cross-link kafirin by forming bridges between its polypeptides, which can act as spacers between kafirin molecules, thus allowing for easy vapour diffusion (Emmambux et al 2004). It is also apparent that the WVP determined in the vials was higher than that determined in the Schott bottles. In the method used to determine WVP, the relative humidity inside the bottle is taken to be 100% (Gennadios et al 1994a). The difference in the volume of vials and Schott bottles could have affected the internal vapour pressure such that the vials had a higher vapour pressure leading to higher WVP readings compared to the Schott bottles. Nonetheless, the WVP readings obtained from both the vials and Schott bottles showed the same trend.

TABLE 3.1.6 Water Vapour Permeability of Films Made from Kafirin that was Microwave Heated to 96°C and Held at 96°C for Different Time Periods

Microwaving Time (min)	0	1	2	3	4	10
	(Control)					
WVP (g mm m ⁻² h ⁻¹ kPa ⁻¹):						
Determined in Vials	2.50a ^a	1.60ab	1.63b	1.33b	1.35b	1.38b
	(0.4) ^b	(0.6)	(0.3)	(0.5)	(0.6)	(0.7)
Determined in Schott	0.84a	0.62b	0.67b	0.55b	0.55b	0.57b
Bottles	(0.02)	(0.14)	(0.13)	(0.13)	(0.14)	(0.24)

a. Means in the same row with different letters are significantly different (p<0.05)

b. Values in parentheses are standard deviations of the means

Microwaving the wet kafirin to 96°C resulted in a progressive decrease in its digestibility (Table 3.1.7). The digestibility of the kafirin decreased from about 58% in the control to 45% for kafirin that was microwave heated to 96°C for 10 min, approx. 22% lower. This is in agreement with the well-described phenomenon, that wet heating reduces the digestibility of kafirin (Hamaker et al 1986; Duodu et al 2002; Nunes et al 2004). Similarly, microwave heating the kafirin reduced the digestibility of the films, from about 96% in the control to as low as 87% for film made from kafirin microwave heated to 96°C for 10 min, approx. 10% lower. The reduction in digestibility of kafirin on heating is probably a result of heat-induced disulfide and/or non-disulfide cross-links reducing enzyme access (reviewed by Duodu et al 2003).

The digestibility of the kafirin films was much higher (86.6-96.3%) than that of the kafirin (45.0-58.1%) from which they were made, showing that film casting had an effect on film digestibility. This was possibly due to the effect of glacial acetic acid, which was used as the solvent for film casting. Chiue et al (1997) reported a pronounced change in the functional properties of zein after heating it in 0.05 M HCl. This change in zein functional properties was attributed to deamidation that caused breakage of the hydrogen bonds and hydrophobic interactions. It is possible that the glacial acetic acid used to cast the films caused deamidation of kafirin thus making the films more digestible.

TABLE 3.1.7 *In Vitro* Protein Digestibility (%) of Wet Kafirin Microwave Heated to 96°C and Held at 96°C for Different Time Periods and Films Made from It

Microwaving Time (min)	0 (Control)	1	2	3	4	10
Kafirin	58.1a ^a (1.7) ^b	52.5b (1.4)	50.4b (2.0)	46.8c (1.1)	49.3bc (2.5)	45.0bc (3.0)
Films	96.3a (1.3)	89.7b (3.0)	94.9ab (2.1)	93.5ab (2.9)	92.1bc (1.4)	86.6c (1.6)

a. Means in the same row with different letters are significantly different ($p < 0.05$)

b. Values in parentheses are standard deviations of means

Fig. 3.1.4. illustrates the progressive degradation of kafirin films buried in low (A) and high (B) moisture content compost. Degradation of the films progressed through shrinking, wrinkling, development of holes and fragmentation, before total degradation. In terms of colour, the films turned milky and opaque in the early days of incubation and became brown to black in the later days. Films buried in the low and high moisture compost were totally degraded after 20 and 14 days of incubation, respectively. However, after 28 days, fragments of films made from microwave-heated kafirin were found in the low moisture content compost. The fragments were black, wrinkled, brittle and had holes. This was an anomaly possibly arising from variations in the film and/or compost. In comparison, the low-density polyethylene films remained whole and clear with only slight wrinkling during the duration of the test.

Films buried in the high moisture compost degraded generally faster (approx. 6 days earlier) than those in low moisture compost, possibly because the higher moisture content promoted faster disintegration of the films and greater microbial growth and metabolism. At low moisture content, less water is available for microbial metabolism hence less microbial degradation. In both the low and high moisture composts, the films made from microwaved kafirin degraded slightly more slowly as compared to the control films. The slower degradation of the films was possibly due to microwave induced intermolecular cross-links (Fig 3.1.3), which would slow enzymatic digestion of the protein. Slowed biodegradation of protein films has been reported in heat treated whey protein films (Le Tien et al 2000) and γ -irradiated whey, casein and soy protein films (Lacroix et al 2002). This was attributed to heat and γ -irradiation induced intermolecular cross-links.

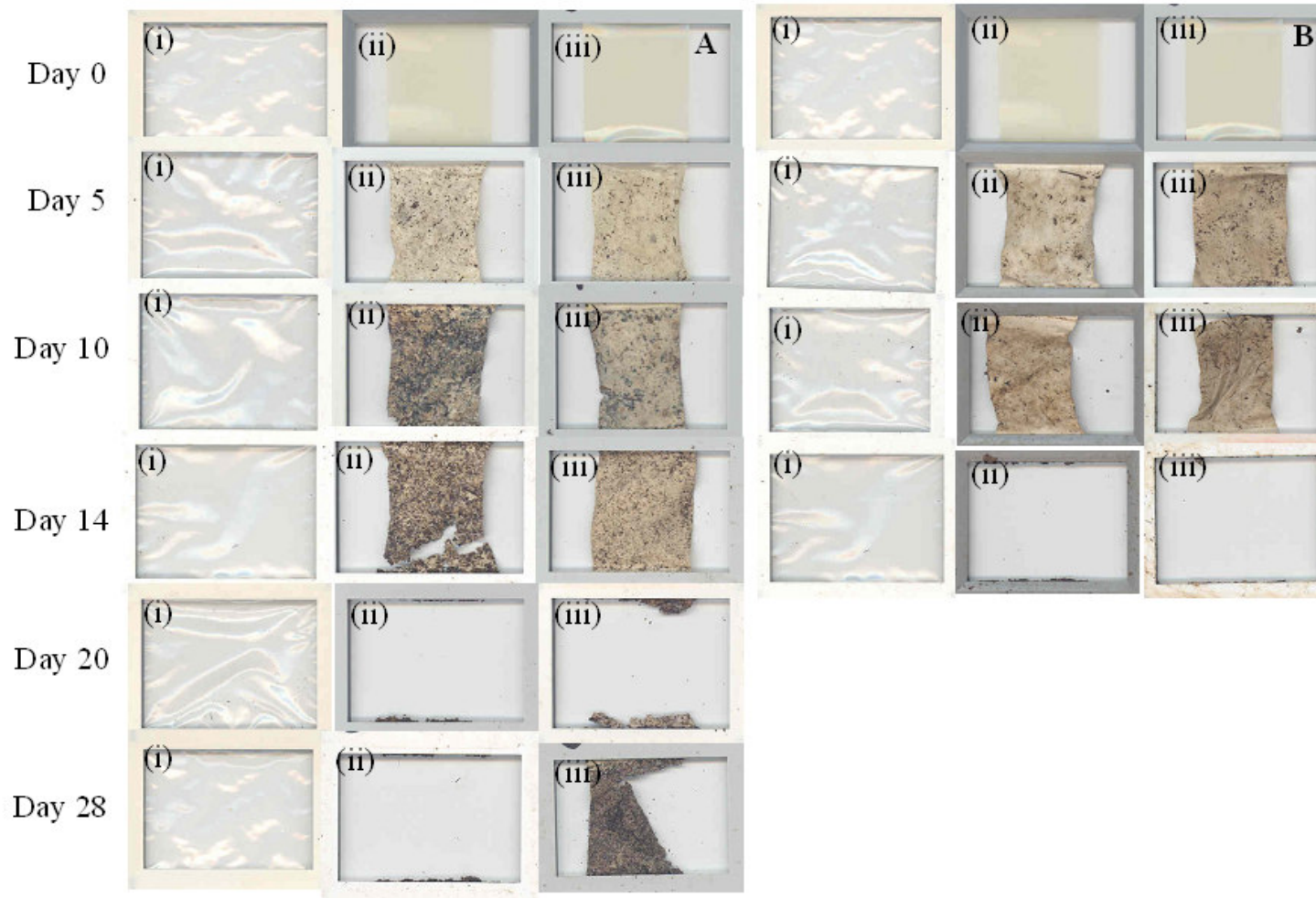


Fig. 3.1.4. Degradation of films made from microwave-heated wet kafirin (96°C for 10 min) buried in compost for 28 days at 50°C. (A) Low moisture compost (37%) (B) high moisture compost (60%). (i) = low density polyethylene film, (ii) = control, film made from untreated kafirin, (iii) = film made from microwave-heated kafirin.

Fig. 3.1.5 compares the microstructure of the fracture surfaces of films made from kafirin that was microwave heated to 96°C for 10 min with that of film made from kafirin that had not be microwave heated. Pores could be seen that appeared to be evenly distributed throughout the film (as they were across the film fracture surface). The pores were possibly due to the presence of air bubbles in the films. Microwaving of kafirin affected the microstructure of films. Microwave heating the kafirin seemed to make the film pores smaller in size and fewer in number (Fig. 3.1.5b). This was probably because microwave heating and subsequent cross-linking of the kafirin caused the bursting of the big bubbles leaving fewer small ones. Emmambux et al (2004) also reported changes in film pore size and density when kafirin films were modified with tannins. In addition, they reported that the microstructure of the tannin modified kafirin films was less uniform, showing differences in striations between the bottom and top part of the fracture surface. The microstructure through the film (across the film fracture surface) in this present work was generally more uniform (Fig. 3.1.5). This difference can be attributed to the different solvents used to cast the films and/or the method of modification used. The reduction in pore size and number resulting from microwave heating of kafirin could have contributed to the observed changes in the functional properties of the films, increased tensile strength, decreased strain, WVP and digestibility, as well as the slightly slower biodegradability.

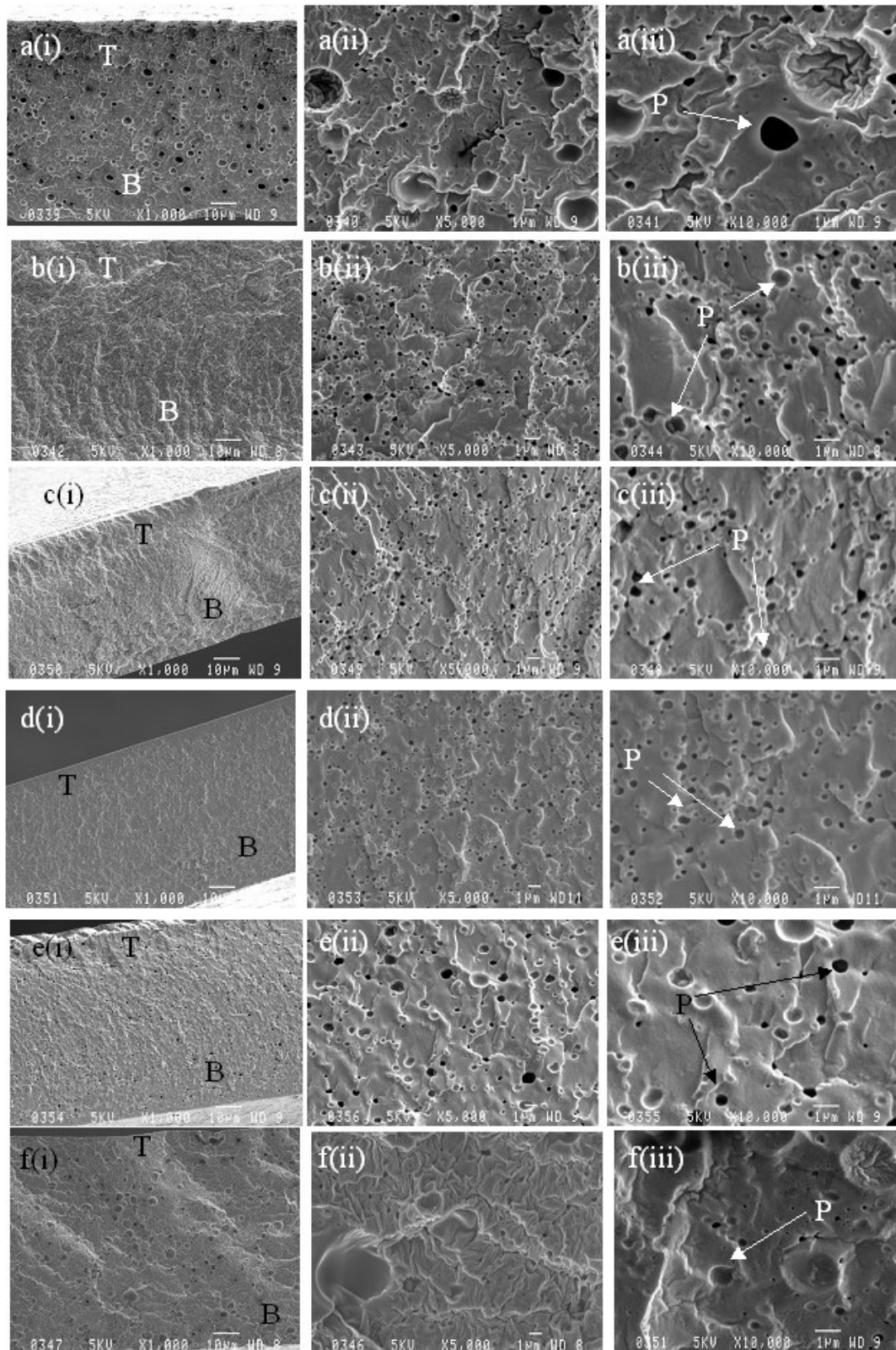


Fig. 3.1.5. Scanning electron micrographs of freeze fracture surfaces of films made from kaffirin that was microwave heated at 96°C for different time periods. a) = control, b) = 1 min, c) = 2 min, d) = 3 min, e) = 4 min, f) = 10 min, T = top part of fracture surface, B = bottom part of fracture surface, P = pore like structure.

3.1.4. Conclusions

Microwave heating of kafirin can be used to modify the functional properties of kafirin films such as tensile and barrier properties as well as digestibility and biodegradability. However, for it to be effective, the kafirin must be wetted to increase its dielectric properties and the kafirin used must be as close as possible to its native or undenatured state. It appears the formation of intermolecular disulfide and possibly non-disulfide crosslinks, as interpreted from SDS-PAGE, are at least in part responsible for the changes in the functional properties of the films.

3.1.5. Literature cited

- ASTM. 1996. American Society for Testing and Materials. Standard practice for exposing plastics to a simulated compost environment using an externally heated reactor; Designation D5512-96. Annual Book of the American Society for Testing and Materials Standards: West Conshohocken, USA.
- ASTM. 1998. American Society for Testing and Materials. Standard method for tensile properties of thin plastic sheeting, Designation D882-97. Annual Book of the American Society for Testing and Materials Standards: West Conshohocken, USA.
- Banerjee, R., Chen, H., and Wu, J. 1996. Milk protein-based edible film mechanical strength changes due to ultrasound process. *J. Food Sci.* 61:824–828.
- Buffo, R. A., Weller, C. L., and Gennadios, A. 1997. Films from laboratory-extracted sorghum kafirin. *Cereal Chem.* 74:473-475.
- Chiue, H., Kusano, T., and Iwami, K. 1997. Deamidation-induced fragmentation of maize zein, and its linked reduction in fatty acid binding capacity as well as antioxidative effect. *Food Chem.* 58:111-117.
- Choi, W.-S., and Han, J. H. 2001. Physical and mechanical properties of pea-protein-based edible films. *J. Food Sci.* 66:319-322.
- Curnutte, B. 1980. Principles of microwave radiation. *J. Food Prot.* 43:618–624.
- Damodaran, S. 1996. Amino acids, peptides and proteins. Pages 321-429, in: *Food Chemistry 3rd Ed.* O. R. Fennema, ed. Marcel Dekker: New York.
- Da Silva, L. S., and Taylor, J. R. N. 2004. Sorghum bran as a potential source of kafirin. *Cereal Chem.* 81:322-327.

- Da Silva, L. S., and Taylor, J. R. N. 2005. Physical mechanical and barrier properties of kafirin films from red and white sorghum milling fractions. *Cereal Chem.* 82:9-14.
- De Graaf, L. A. 2000. Denaturation of proteins from a non-food perspective. *J. Biotechnol.* 79:299-306.
- Duodu, K. G., Taylor, J. R. N., Belton, P. S., and Hamaker, B. R. 2003. Factors affecting sorghum protein digestibility. *J. Cereal Sci.* 38:117–131.
- Duodu, K. G., Nunes, A., Delgadillo, I., Parker, M. L., Mills, E. N. C., Belton, P. S., and Taylor, J. R. N. 2002. Effect of grain structure and cooking on sorghum and maize in vitro protein digestibility. *J. Cereal Sci.* 35:161–174.
- El Nour, I. N. A., Peruffo, A. D. B., and Curioni, A. 1998. Characterisation of sorghum kafirins in relation to their cross-linking behaviour. *J. Cereal Sci.* 28:197-207.
- Emmambux, N. M., and Taylor J. R. N. 2003. Sorghum kafirin interaction with various phenolic compounds. *J. Sci. Food Agric.* 83:402-407.
- Emmambux, N. M., Stading, M., and Taylor, J. R. N. 2004. Sorghum kafirin film property modification with hydrolysable and condensed tannins. *J. Cereal Sci.* 40:127-135.
- Gao, C., Taylor, J., Wellner, N., Byaruhanga, Y. B., Parker, M. L., Mills, E. M. C., and Belton, P. S. 2005. Effect of preparation conditions on protein secondary structure and biofilm formation of kafirin. *J. Agric. Food Chem.* 53:306-312.
- Gennadios, A., Weller, C.L., and Gooding, C.H. 1994a. Measurement errors in water vapour permeability of highly permeable, hydrophilic films. *J. Food Eng.* 21:395-409.
- Gennadios, A., Rhim, J. W., Handa, A., Weller, C. L., and Hanna, M. A. 1998. Ultraviolet radiation affects physical and molecular properties of soy protein films. *J. Food Sci.* 63:225–228.
- Hamaker, B. R., Kirleis, A. W., Mertz, E. T., and Axtell, J. D. 1986. Effect of cooking on the protein profiles and in vitro protein digestibility of sorghum and maize. *J. Agric. Food Chem.* 34:647–649.
- Hamaker, B. R., Kirleis, A. W., Butler, L. G., Axtell, J. D., and Mertz, E. T. 1987. Improving the in-vitro protein digestibility of sorghum with reducing agents. *Proc. Natl. Acad. Sci. USA.* 84:626–628.
- Kaya, S., and Kaya, A. 2000. Microwave drying effects on properties of whey protein isolate edible films. *J. Food Eng.* 43:91-96.

- Kester, J. J., and Fennema, O. R. 1986. Edible films and coatings: A review. *Food Technol.* 40 (12):47-59.
- Krochta, J. M. 2002. Proteins as raw materials for films and coatings: definitions, current status and opportunities. Pages 1–41, in: *Protein-based Films and Coatings*. A. Gennadios, ed. CRC Press: Boca Raton, USA.
- Lacroix, M., Le, T. C., Ouattara, B., Yu, H., Letendre, M., Sabato, S. F., Mateescu, M A., and Patterson, G., 2002. Use of γ -irradiation to produce films from whey, casein and soya proteins: structure and functional characteristics. *Radiation Physics Chem.* 63:827-832.
- Le Tien, C., Letendre, M., Ispas-Szabo, P., Mateescu, M. A., Delmas-Patterson, G., Yu, H. L., and Lacroix, M. 2000. Development of biodegradable films from whey proteins by cross-linking and entrapment in cellulose. *J. Agric. Food Chem.* 48:5566-5575.
- Miller, L. A., Gordon, J., and Davis E. A. 1991. Dielectric and thermal transition properties of chemically modified starches during heating. *Cereal Chem.* 68:441-448.
- Mudgett, R. E. 1982. Electrical properties of foods in microwave processing. *Food Technol.* 36(2):109-115.
- Nunes, A., Correia, I., Barros, A., and Delgadillo, I. 2004. Sequential *in vitro* pepsin digestion of uncooked and cooked sorghum and maize samples. *J. Agric. Food Chem.* 52:2052-2058.
- Ohlsson, T., and Bengtsson, N. 2001. Microwave technology and foods. Pages 65-140, in: S. L. Taylor, ed. *Advances in Food and Nutrition Research Volume 34*. Academic Press: San Diego, USA.
- Oria, M. P., Hamaker, B. R., and Shull, J. M. 1995a. Resistance of sorghum α -, β -, and γ - kafirins to pepsin digestion. *J. Agric. Food Chem.* 43:2148-2153.
- Otterburn, M. S., 1989. Protein cross-linking. Pages 247-261, in: *Protein Quality and Effects of Processing*. R. D. Phillips and J. W. Finley, eds. Marcel Dekker: New York.
- Ou, S., Kwok, K. C., and Kang, Y. 2004. Changes in *in-vitro* digestibility and available lysine of soy protein isolate after formation of film. *J. Food Eng.* 64:301-305.
- Park, H. J., Chinnan, M. S., and Shewfelt, R. L. 1994a. Edible corn-zein film coatings to extend storage life of tomatoes. *J. Food Processing Preservation* 18:317-331.

- Park, H. J., Chinnan, M. S., and Shewfelt, R. L. 1994b. Edible coating effects on the storage life and quality of tomatoes. *J. Food Sci.* 59:568–570.
- Perez-Gago, M. B., and Krochta, J. M., 2001. Denaturation time and temperature effects on solubility, tensile properties and oxygen permeability of whey protein edible films. *J. Food Sci.* 66:705-710.
- Perez-Gago, M. B., Nadaud, P., and Krochta, J. M. 1999. Water vapor permeability, solubility and tensile properties of heat denatured versus native whey protein films. *J. Food Sci.* 64:1034-1037.
- Rakotonirainy, A. M., Wang, Q., and Padua, G. W. 2001. Evaluation of zein films as modified atmosphere packaging for fresh broccoli. *J. Food Sci.* 66:1108–1111.
- Rhim, J. W., Gennadios, A., Fu, D., Weller, C. L., and Hanna, M. A. 1999. Properties of ultraviolet irradiated protein films. *Lebens. Wissen. Technol.* 32:129-133.
- Roy, S., Weller, C. L., Gennadios, A., Zeece, M. G., and Testin, R. F. 1999. Physical and molecular properties of wheat gluten films cast from heated film-forming solutions. *J. Food Sci.* 64:57–60.
- Sharma, G. P., and Prasad, S. 2002. Dielectric properties of garlic (*Allium sativum* L) at 2450 MHz as a function of temperature and moisture content. *J. Food Eng.* 52:343-348.
- Shewry, P. R., 2002. The major seed storage proteins of spelt wheat, sorghum, millets and pseudocereals. Pages 1-24, in: *Pseudocereals and Less Common Cereals*. P. S. Belton and J. R. N. Taylor, eds. Springer, Berlin.
- Shull, J. M., Watterson, S. J., and Kirleis, A. W. 1991. Proposed nomenclature for the alcohol-soluble proteins (kafirins) of *Sorghum bicolor* (L. Moench) based on molecular weight, solubility and structure. *J. Agric. Food Chem.* 39:83-87.
- Singh, P., and Heldman, D. R. 1993. *Introduction to Food Engineering*, 2nd Edition. Pages 1-499. Academic Press, London.
- Spahioglu, O., and Barringer, S. A. 2003. Dielectric properties of vegetables and fruits as a function of temperature, ash and moisture content. *J. Food Sci.* 68:234-239.
- Stuchell, Y. M., and Krochta, J. M. 1994. Enzymatic treatments and thermal effects on edible soy protein films. *J. Food Sci.* 59:1332–1337.
- Taylor, J., Taylor, J. R. N., Dutton, M. F., and De Kock, S. 2005a. Identification of kafirin film casting solvents. *Food Chem.* 90:401-408.
- Taylor, J., Taylor, J. R. N., Dutton, M. F., and De Kock, S. 2005b. Glacial acetic acid-a novel food-compatible solvent for kafirin extraction. *Cereal Chem.* In press.

- Taylor, J. R. N., Schüssler, L., and Van der Walt, W. H. 1984b. Fractionation of proteins from low tannin sorghum grain. *J. Agric. Food Chem.* 32:149-154.
- Uvarov, E. B., Chapman, D. R., and Isaacs, A. 1971. *A Dictionary of Science* 4th ed. Pages 109-110. Penguin: Harmondsworth, UK.
- Vachon, C., Yu, H. L., Yefsah, R., Alain, R., St-Gelais, D., and Lacroix, M. 2000. Mechanical and structural properties of milk protein edible films cross-linked by heating and γ -irradiation. *J. Agric. Food Chem.* 48:3202-3209.
- Wedemeyer, W. J., Welker, E., Narayan, M., and Scheraga, H. A., 2000. Disulfide bonds and protein folding. *Biochem.* 39:4207-4216.
- Yildirim, M., and Hettiarachchy, N. S. 1998. Properties of films produced by cross-linking whey proteins and 11S globulin using transglutaminase. *J. Food Sci.* 63:248-252.

3.2. EFFECT OF MICROWAVE HEATING OF CAST KAFIRIN FILMS ON THEIR FUNCTIONAL PROPERTIES*

Abstract

Edible films can be produced from kafirin but the diverse food product requirements necessitate modification of the films. To modify their functional properties, kafirin films were cast, with and without plasticiser, from glacial acetic acid (GAA) and aqueous ethanol (AE) then microwaved. Microwave heating at 80 W for 2 min caused an about two and four fold increase in the tensile strength of GAA cast non-plasticised and plasticised films, respectively. Film glass transition temperature increased by about 15%. Film biodegradability was slowed. Digestibility of non-plasticised films cast from AE decreased by about 25%, while that of films cast from GAA and the plasticised films cast from AE was not affected. GAA cast films had higher digestibility than AE cast ones, possibly as a result of acid mediated deamidation of kafirin. SDS-PAGE indicated microwave induced kafirin oligomers. Scanning electron microscopy of fracture surfaces of microwave heated plasticised films showed more wrinkled structures compared to non-microwaved films, whereas the non-plasticised films appeared more brittle with microwaving. The results indicate that microwave heating induced intermolecular disulphide cross-linking was involved in modifying the properties of kafirin films.

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3.2.1. Introduction

Physical treatments such as ultraviolet light and γ -irradiation have been used to modify functional properties of cast synthetic polymer films (Chapiro 1988). Similarly, properties of cast protein films can also be modified using the same technique. For example, thermal treatment has been used to increase the tensile strength of cast whey protein films (Miller et al 1997), wheat gluten films (Ali et al 1997) and soy protein films (Kim et al 2002). Electromagnetic waves such as ultraviolet light (Gennadios et al 1998; Rhim et al 1999) and γ -radiation (Vachon et al 2000) have been used to modify the functional properties of protein films. Kaya and Kaya (2000) used microwaving to increase the tensile strength of whey protein films. Such modifications in film properties have been attributed to intermolecular cross-linking of the protein induced by the physical agent used (Vachon et al 2000; Quinn et al 2003).

In Chapter 3.1 it has been shown that microwaving of kafirin before casting of films increases the tensile strength but decreases the strain, water vapour permeability and digestibility of the films. However, to achieve this, the kafirin powder had to be wetted first. To avoid this additional step of having to wet the kafirin powder first, casting the films then treating them with microwave energy was investigated as a process for modifying kafirin film properties.

3.2.2. Materials and methods

3.2.2.1. Extraction of kafirin

Kafirin was extracted from milled decorticated sorghum grain (a mixture of two white, condensed tannin-free hybrids, PANNAR 202 and 206), as described in Chapter 3.1. The extracted kafirin had a protein content of 81.2% (db, N x 6.25).

3.2.2.2. Film casting

Films were cast in glacial acetic acid with and without plasticiser, as described in Chapter 3.1. For the digestibility study, films were also cast in aqueous ethanol with and without plasticiser following the procedure of Taylor et al (2005a). Before microwaving, films were cut into sample pieces of 18 x 66 mm and conditioned for 48 hr at 50% relative humidity (RH) in a dessicator containing saturated calcium nitrate.

3.2.2.3. Microwave heating of films

This was done using a 2 kW Sairem GMP 20KSM microwave oven (Sairem, Neyron, France) fitted with a power regulator. A wave-guide was used to concentrate the microwaves onto the film sample. Film samples were held between two Teflon frames (19 x 70 mm) to avoid buckling. The frames were fastened together with screws. The samples were then microwave heated in a cylindrical quartz tube (20 mm, internal diameter). Figure 3.2.1 illustrates the set up of the microwaving apparatus. The quartz tube could be slid in and out of the wave-guide with the microwave switched off. Films were exposed to various microwaving conditions, namely 60, 80, 100 and 120 W for 1, 2, 3 and 4 min. Timing of exposure to microwave power started with switching on and ended with switching off the microwave. The quartz tube was manually turned through 360° every 30 seconds to ensure even heating of the sample. The temperature of the films after microwaving was measured using a Thermalert MID infrared thermometer (Raytek, Santa Cruz, USA). The controls were films cast with and without plasticiser but not microwaved. The estimated absorbed energy (EAE) (J/g) was calculated as described in Chapter 3.1.

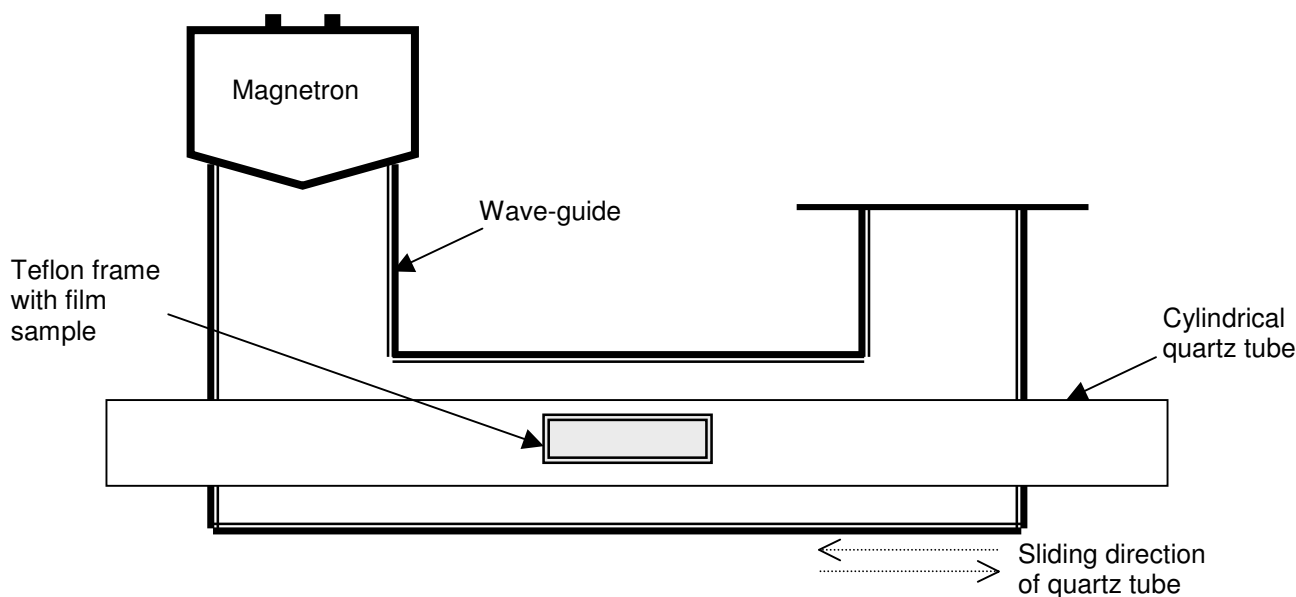


Fig. 3.2.1. Apparatus used for microwave heating of cast films.

3.2.2.4. Film functional properties

3.2.2.4.1. Film tensile property measurement

Films had a mean thickness of 63 and 94 μm for the non-plasticised and plasticised films, respectively. The maximum tensile strength, tensile strength at break, elongation and Young's modulus were determined using a TA-XT2 texture analyser (Stable Microsystems, Godalming, United Kingdom) fitted with tensile grips (code A/TG) as described in Chapter 3.1. Film treatments that gave the most increase in tensile strength were used for determination of other functional properties.

3.2.2.4.2. Barrier properties

The water vapour permeability (WVP) of the films was determined as described in Chapter 3.1. Oxygen permeability of non-plasticised films was measured following the procedure described by Emmambux et al (2004) using a Mocon Oxtran 2/90 (Modern Controls, Minneapolis, USA). A square portion of film (25 cm^2) was masked between two aluminium sheets with an opening of 5 cm^2 . The thickness of the film was measured at 5 points over the circular surface and placed in the instrument. Oxygen permeability was read from the instrument and expressed as $\text{cm}^3\text{ }\mu\text{m m}^{-2}\text{ d}^{-1}\text{ kPa}^{-1}$.

3.2.2.4.3. In vitro protein digestibility and biodegradability

In vitro digestibility was determined as described in Chapter 3.1. Non-plasticised and plasticised films cast from glacial acetic acid and aqueous ethanol were analysed for digestibility. Film biodegradability was determined as described in Chapter 3.1.

3.2.2.5. Moisture content determination

Microwave heated films were conditioned at 50% RH for 48 hr. Samples of 300-400 mg were weighed into aluminium dishes and dried in a circulating air oven at 105°C for 24 hr.

3.2.2.6. SDS-PAGE

Gradient sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted under non-reducing conditions using 3.9-15% acrylamide gradient gels, as described in Chapter 3.1.

3.2.2.7. Determination of glass transition temperature (T_g)

The T_g of non-plasticised films was determined by dynamic mechanical analysis (DMA). DMA was performed under tension using a Rheometrics RSA-II (Rheometric Scientific, Piscataway, USA), as described by Stading et al (2001). The temperature scans were performed from -10°C to 60°C at $5^{\circ}\text{C min}^{-1}$. The temperature at which the glassy modulus changed to transition modulus was used for the calculation of the T_g of the films. Linear regression was applied to fit lines to the glassy modulus and transition modulus. T_g was determined by the intersection of the two linear lines.

3.2.2.8. Scanning electron microscopy (SEM)

This was done following the procedure described in Chapter 3.1.

3.2.2.9. Statistical analysis

Unless otherwise stated, mean values were obtained from three independent microwaving experiments. One-way analysis of variance (ANOVA) was performed on the data to determine the effect of different microwave treatments on the individual kafirin film properties. The least significant difference test was performed at $p < 0.05$ on treatment effects for which significant differences were found.

3.2.3. Results and discussion

The mean moisture content of microwaved plasticised and non-plasticised films was 23 and 8% (wb), respectively. The moisture content of both microwaved plasticised and non-plasticised films was not different from that of the respective control films. Gennadios et al (1996) reported a decrease in moisture content of soy protein films heated at 80 and 95°C for up to 24 hr. The results reported here suggest that the films reabsorbed moisture to equilibrium after microwaving. However, the moisture content of plasticised films was almost 3 times higher than that of non-plasticised films. This was probably because the hydrophilic nature of glycerol and polyethylene glycol used as plasticisers (Di Gioia et al 1998). Such hydrophilic plasticisers cause more absorption of moisture into films (Lawton 2004).

There was an increase in the maximum tensile strength, tensile strength at break and Young's modulus ($p < 0.05$) with microwave heating of both plasticised and non-plasticised kafirin films (Table 3.2.1). In the non-plasticised films, the maximum tensile

strength increased from a mean of 12.8 MPa up to 22.1 MPa (about two times higher), Young's modulus also increased from 943 MPa up to 1609 MPa (also about two times higher) but strain, which was in this case very low, was not affected. In the plasticised films, the maximum tensile strength increased from 1.9 MPa to 6.9 MPa (nearly four times higher), Young's modulus increased from 51 MPa to 296 MPa (nearly six times higher) and in contrast to the non-plasticised films, strain decreased from 132% to 23% (nearly six times lower). This indicates that the films became stronger and less extensible with microwaving. In both the non-plasticised and plasticised films, the tensile strength at break showed a trend similar to that of maximum tensile strength. The thickness of the non-plasticised and plasticised films was not affected by microwaving. As noted, the non-plasticised films were generally not extensible, with strain of only about 1.5%, far lower than that of the plasticised films. Plasticisers make protein films more extensible by forming a lubricating layer between protein molecules (Kester and Fennema 1986; Sothornvit et al 2002), which tends to decrease the tensile strength, while increasing strain of the films (Lens et al 2003; Lawton 2004).

As stated, the mean increase in the tensile strength in the plasticised films was several times higher than that in the non-plasticised films, although the non-plasticised films were of higher tensile strength (Table 3.2.1). Also, the increase in film temperature after microwave heating was nearly twice as great in the plasticised films (from 22 to 45°C) as in the non-plasticised films (from 22 to 35°C). The estimated absorbed energy (EAE) was several times higher in the plasticised films than in the non-plasticised ones. This suggests that microwave heating of plasticised films was more effective than microwave heating the non-plasticised ones. Microwave heating of a food material depends on its dielectric properties, i.e. dielectric constant and loss factor, which are largely determined by the amount of polar molecules (Sharma and Prasad 2002). It appears that plasticisation increased the dielectric properties of kafirin films. Perhaps the most likely mode was through the relatively high dielectric constant of the plasticisers. The dielectric constants of glycerol and lactic acid are 46.5 and 22.0, respectively (Wohlfarth 1974), while that of polyethylene glycol 400 is 12.4 (Shrewsbury 2004). As mentioned, hydrophilic plasticisers increase amount of moisture absorbed in a protein film (Lawton 2004). Since water has a high dielectric constant of 80 (Wohlfarth 1974), foods containing substantial amounts of moisture tend to have high dielectric properties and thus are heated more effectively by

microwaves than those that are dry (Sharma and Prasad 2002). It is shown in the Chapter 3.1 that kafirin powder had to be wetted so as to effectively microwave heat it. Microwave heating of wetted kafirin produced plasticised films with tensile strength of about two times higher than that of films produced from non-microwave heated kafirin. Thus, compared to microwave heating of kafirin before film casting (Chapter 3.1), microwave heating of cast kafirin films was more effective at increasing the tensile strength of plasticised kafirin films.

With both the plasticised and non-plasticised films, film temperature was directly related to microwave power (Table 3.2.1). However, the highest increase in maximum tensile strength for both the plasticised and non-plasticised films was achieved at 80 W. Microwave heating at 80 W resulted in EAE of about 7 and 47-56 J/g for the non-plasticised and plasticised films, respectively. Conversely, microwave heating at 100 W resulted in EAE about 7-9 and 59-61 J/g for the non-plasticised and plasticised films, respectively. Microwave heating at 100 W yielded films of reduced maximum tensile strength, tensile strength at break and Young's modulus, although these were still higher than in the control. This suggests that microwave heating the films at 80 W was probably the most effective. Further, films microwave heated at 60 and 80 W did not show a change in colour as compared to the non-microwaved films, whereas films microwave heated at 100 W showed a slight brown colour development, while those subjected to microwave power of 120 W were burnt and too brittle for tensile testing. Such colour changes are indicative of the beginning of negative effects of thermal treatment on the kafirin films. As shown in Table 3.2.1, the EAE in both non-plasticised and plasticised films increased with increasing microwaving power, suggesting increased heat exposure with microwaving. Excessive heating of proteins causes destruction of protein polymer chains (Cheftel et al 1985; Damodaran 1996), which could weaken the protein network in the films.

TABLE 3.2.1 Tensile Properties of Plasticised (P) and Non-plasticised (NP) Kafirin Films Microwave Heated at Different Powers and Held there for Different Time Periods

Microwave Power (W)	Time (min)	Film Temperature After Microwaving (°C)		Estimated Absorbed Energy (J/g)		Maximum Tensile Strength (MPa)		Tensile Strength at Break (MPa)		Strain (%)		Young's Modulus (MPa)	
		NP	P	NP	P	NP	P	NP	P	NP	P	NP	P
0 (Control)	0	22	22	0	0	12.8a ^a (2.7) ^b	1.9a (0.5)	12.3a (2.4)	1.6a (0.5)	1.5a (0.59)	132.0e (32.5)	943a (112)	51a (26)
	60	30	39	4.2	38.5	18.1b (2.0)	3.4bc (0.6)	17.8b (2.3)	2.8ab (0.6)	1.7a (0.4)	94.0cde (15.4)	1334b (205)	114b (19)
	2	30	40	4.2	40.9	18.3b (1.7)	3.0ab (0.9)	17.2b (1.6)	2.3ab (0.7)	1.7a (0.1)	98.5cde (37.4)	1329b (116)	99ab (43)
	3	32	43	5.3	47.2	18.6b (1.5)	2.9ab (0.7)	17.2b (1.3)	2.3ab (0.5)	1.7a (0.1)	118.1de (10.3)	1406b (103)	97ab (22)
	4	33	43	5.9	47.2	17.9b (1.0)	3.4bc (0.4)	17.2b (1.5)	2.6b (0.3)	1.6a (0.0)	93.5cde (22.8)	1359b (62)	119bc (19)
80	1	35	43	7.1	47.2	20.8cd (0.1)	5.6de (1.2)	20.2c (0.3)	3.9cd (1.1)	1.7a (0.0)	54.0abe (47.3)	1494b (16)	232de (62)
	2	35	45	7.1	52.0	19.9c (1.0)	6.9e (1.4)	19.6bc (1.0)	4.5d (0.7)	1.7a (0.2)	22.8a (12.6)	1405b (56)	287e (37)
	3	35	45	7.1	52.0	19.5c (1.4)	6.7e (1.9)	18.5bc (0.3)	4.7d (1.2)	1.7a (0.4)	24.2a (9.5)	1461b (80)	296e (91)
	4	36	47	7.5	56.8	19.5c (1.7)	5.8de (0.6)	19.4bc (1.7)	4.5d (0.5)	1.3a (0.2)	25.5a (11.7)	1609b (52)	269e (41)
	100	36	48	7.5	59.2	19.8c (1.4)	3.8bc (0.7)	19.5bc (1.6)	2.6b (0.4)	1.7a (0.4)	88.0bcde (26.7)	1448b (145)	147bc (39)
100	2	37	48	9.4	59.2	22.1d (0.8)	4.8cd (0.6)	21.9c (0.9)	3.0bc (0.3)	1.9a (0.5)	80.1b (13.0)	1387b (234)	187cd (24.2)
	3	37	49	9.4	61.1	17.8abc (3.5)	3.9bc (0.7)	17.6bc (3.2)	2.5b (0.4)	1.7a (0.4)	70.7b (10.4)	1249ab (372)	152bc (36)
	4	37	49	9.4	61.1	16.8ab (0.8)	3.2b (0.4)	16.6b (0.6)	2.3ab (0.2)	1.4a (0.3)	69.8b (12.4)	1286b (30)	117b (4)
	120	45	56	12.4	77.0	ND ^c	ND	ND	ND	ND	ND	ND	ND

a. Mean values in the same column with different letters are significantly different ($p < 0.05$)

b. Values in parentheses are standard deviation of means

c. ND = Not determined because films were too brittle for testing.

Table 3.2.2 shows that the mean T_g of non-plasticised kafirin films increased significantly ($p<0.05$) with microwave heating, from 68.6°C in the control up to 78.9°C in the microwave-heated films. This is consistent with the results of Emmambux et al (2004) who reported increasing T_g of kafirin films with increasing levels of tannin cross-linking. T_g is a reflection of the molecular mobility in a biopolymer (Ringe and Petsko 2003). The T_g of biopolymers increases with chain rigidity and the intensity of intermolecular interactions, including hindrance to internal rotation along the macromolecular chain (Matveev et al 2000). Thus, increase in the T_g of a protein has been interpreted as a result of increased intermolecular cross-linking that decreases the mobility of protein polymer chains (Madeka and Kokini 1996; Sothornvit et al 2002).

TABLE 3.2.2 T_g of Non-Plasticised Kafirin Films Cast from Glacial Acetic Acid Microwave Heated at 80 W for Different Time Periods

Microwaving Time (min)	T_g (°C)
0 (Control)	68.6ab ^a (3.2) ^b
1	69.5abc (3.7)
2	65.7a (1.9)
3	76.2cd (8.6)
4	78.9d (8.6)

a. Mean values in the same column with different letters are significantly different ($p<0.05$)

b. Values in parentheses are standard deviation of means.

Table 3.2.3 shows that microwave heating decreased the pepsin digestibility of non-plasticised films cast from aqueous ethanol by about 25% but the digestibility of plasticised films cast from aqueous ethanol and the non-plasticised and plasticised

films cast from glacial acetic acid was not affected. The lack of effect of microwave heating on the digestibility of plasticised films cast from aqueous ethanol was probably due to the presence of plasticisers. As a plasticiser forms a lubricating layer between protein molecules (Sothornvit et al 2002), it presumably increased the free volume between the kafirin molecules making them more susceptible to enzymic attack. Heat-induced reduction in kafirin digestibility has been widely reported and attributed to the formation of disulfide and/or non-disulfide cross-links which make some peptide bonds inaccessible to pepsin (Hamaker et al 1987; Oria et al 1995b; Duodu et al 2002; Nunes et al 2004). Thus, the decrease in digestibility of microwaved non-plasticised kafirin films cast from aqueous ethanol was probably due to microwave-induced intermolecular cross-links.

The digestibility of films cast from glacial acetic acid was several times higher than that of films cast from aqueous ethanol (Table 3.2.3). The higher digestibility of acetic acid cast films is probably primarily related to the fact that the glacial acetic acid films were cast at room temperature and the aqueous ethanol films were cast at 70°C. Such high temperatures have been reported to cause kafirin cross-linking (Gao et al 2005), which was probably responsible for the low digestibility aqueous ethanol films. The higher digestibility of the glacial acetic acid cast films is probably also related to the fact that acids cause deamidation of glutamine and asparagine residues in proteins (Damodaran 1996). Deamidation is known to increase enzyme susceptibility of whey proteins (Lakkis and Villota 1992) and wheat gluten (Mimouni et al 1994). Deamidation also reduces protein-protein interactions like hydrogen bonding (Sastry and Virupaksha 1969; Mimouni et al 1994; Chiue et al 1997). Argos et al (1982) proposed a α -zein model that shows the involvement of glutamine residues in intra- and inter-molecular hydrogen bonding, which plays an important role in the structural stability of zein. Since kafirin and zein are closely similar (DeRose et al 1989), its probable that deamidation of kafirin reduced hydrogen bonding and adversely affected its structural stability.

TABLE 3.2.3 Protein Digestibility of Plasticised and Non-Plasticised Kafirins Films Cast from Aqueous Ethanol (AE) and Glacial Acetic Acid (GAA) and Microwave Heated at 80 W

Microwaving Time (min)	Protein Digestibility (%)			
	Films Cast from GAA		Films Cast from AE	
	Plasticised	Non-plasticised	Plasticised	Non-plasticised
0 (Control)	87.3a ^a (3.8) ^b	87.9a (2.9)	24.9a (4.1)	28.7bc (2.9)
1	85.1a (0.3)	89.9a (2.1)	24.8a (4.3)	24.0abc (3.3)
2	92.9a (4.0)	87.5a (1.7)	25.9a (1.1)	26.1abc (4.5)
3	88.4a (3.7)	82.3a (4.3)	20.4a (4.5)	23.2ab (4.6)
4	84.7a (3.3)	88.6a (0.4)	29.6a (4.6)	21.1a (2.1)

a. Mean values in the same column with different letters are significantly different ($p < 0.05$)

b. Values in parentheses are standard deviation of means.

SDS-PAGE of microwave-heated kafirin films and their residues after pepsin digestion (RPD) (Fig 3.2.2) shows bands consistent with α -, β - and γ -kafirins (Shull et al 1991), except for the RPD of films cast from glacial acetic acid in which the β -kafirins bands were almost non-existent (tracks 5 and 6). Bands of M_r approximately 40k and 50k also occurred in all samples. Bands of similar M_r have been described as cross-linked dimers of kafirin (El Nour et al 1998). The relative concentration of these dimers was much higher in the RPDs (tracks 5-8) than in the films (tracks 1-4), because of their relatively lower digestibility. In the RPDs (tracks 5-8), there were also higher molecular weight oligomers. In contrast, the relative concentration of the monomeric kafirins was much lower in the RPDs (tracks 5-8) than in the films (tracks 1-4). This shows that the

monomeric kafirins were much more pepsin digestible as compared to the dimers and oligomers. The almost complete absence of β -kafirin monomers in the glacial acetic acid cast kafirin film RPDs (tracks 5-6) is presumably related to the much higher digestibility of the films cast in glacial acetic acid as compared to those cast in aqueous ethanol (Table 3.2.3).

From Fig 3.2.2, it appears that oligomer formation due to microwave induced cross-linking is actually involved in the reduction of protein digestibility following microwave heating of kafirin (Table 3.2.3). Cross-linking would cause structural changes that would not allow complete accessibility of pepsin to the peptide bonds in the kafirin polymers. Such cross-linking was probably responsible for the increase in tensile strength accompanied by a decrease in strain after microwave treatment of the kafirin and films (Table 3.2.1). Formation of cross-links and subsequent protein polymerisation is well known to occur on heating kafirin (Duodu et al 2002; Nunes et al 2004). Cross-linking has also been proposed as the cause of increased tensile strength and reduced strain in soy protein films (Gennadios et al 1996; Kim et al 2002) and whey protein films (Miller et al 1997) that were thermally treated.

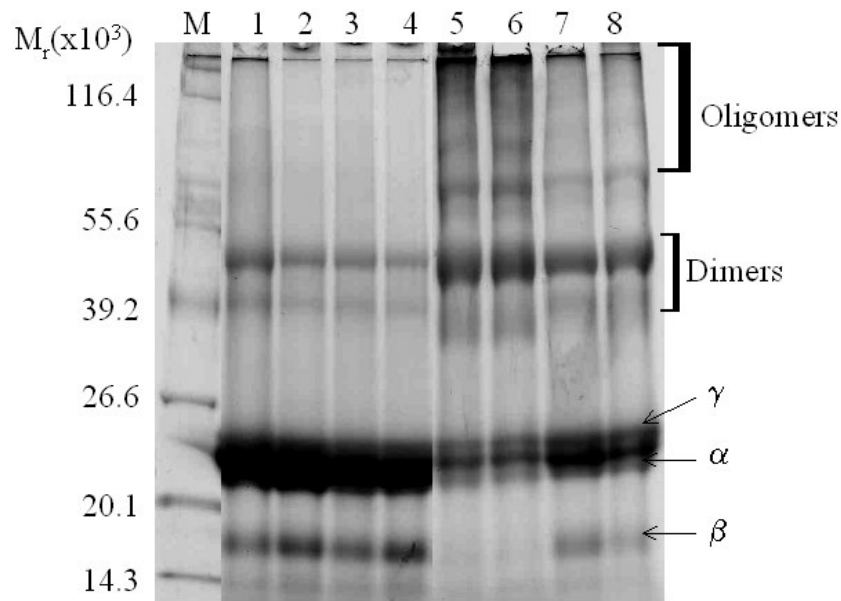


Fig. 3.2.2. SDS-PAGE under non-reducing conditions of microwave heated (80W for 3 min) plasticised and non-plasticised kafirin films and their residues of pepsin digestion (RPD). Tracks M = molecular markers, 1 = plasticised film cast from glacial acetic acid, 2 = non-plasticised film cast from glacial acetic acid, 3 = plasticised film cast from aqueous ethanol, 4 = non-plasticised film cast from aqueous ethanol, 5 = RPD of plasticised film cast from glacial acetic acid, 6 = RPD of non-plasticised film cast from glacial acetic acid, 7 = RPD of plasticised film cast from aqueous ethanol, 8 = RPD of non-plasticised film cast from aqueous ethanol

Microwave heating had no effect on the WVP of the plasticised or non-plasticised kafirin films (Table 3.2.4). This is consistent with the results of Emmambux et al (2004) who cross-linked kafirin films with tannins and found no difference between the WVP of modified and non-modified films. The authors postulated that at RHs higher than 60% kafirin changed from a glassy to a more rubbery state because of the absorbed moisture, which enhanced water diffusion so that tannin cross-linking did not have a significant effect on the WVP of kafirin films. Moisture absorption at high RH was also

reported to cause extensive swelling of the gluten network, which increased water diffusion through gluten films (Gontard et al 1993). In this work, WVP was determined at a RH gradient of 85-38%. Under such conditions, it is probable that swelling of the kafirin network occurred due to the absorbed moisture leading to increased moisture diffusion through the kafirin films.

These results, however, contrast those of films made from microwave heated kafirin where the WVP decreased by about 45% (Chapter 3.1). The mean WVP of films cast from microwave heated kafirin was $1.5 \text{ g mm m}^{-2}\text{h}^{-1}\text{kPa}^{-1}$ compared to $2.6 \text{ g mm m}^{-2}\text{h}^{-1}\text{kPa}^{-1}$ for the films that were microwaved cast. Yoshino et al (2002) found that the gas permeability of zein films cast from acetone depended on the relative humidity during film drying. They suggested that the drying rate of zein-acetone solution in low relative humidity was faster than in high relative humidity, thus the zein molecules associated rapidly to form a larger volume of associated zein body in the film. At high humidities, because of the plasticiser effect of moisture, the zein molecules easily moved during drying leading to a smaller volume of associated zein in the film. Thus the free volume between zein molecules was higher in the high humidity dried zein films than in the low humidity dried films resulting in a higher permeability. It seems a similar scenario happened when kafirin was first cross-linked by microwave heating, leading to a higher volume of associated kafirin body in the film that is cast. This could leave less free volume between kafirin molecules across which moisture could diffuse, resulting in a low WVP. In addition, under such conditions, it is possible that plasticiser molecules migrated and coalesced outside the film as described by Van Eck (2004). Conversely, in the films that were microwaved cast, the dissolution and casting process evenly distributes the plasticiser in the uncross-linked kafirin leading to a smaller volume of associated kafirin body in the film. This would leave more free volume across which moisture could diffuse, leading to a high WVP even after microwave heating of the cast film.

The WVP of plasticised films was higher ($p < 0.05$) than that of the non-plasticised films (Table 3.2.4). Such an increase in WVP with plasticisation was also reported in wheat gluten films (Park et al 1994c; Irissin-Mangata et al 2001) and zein films (Park et al 1994c; Parris and Coffin 1997; Park and Chinnan 1995). Lawton (2004) studied the effect of plasticisers on water absorption of zein films and found that hygroscopic plasticisers such as glycerol and polyethylene glycol led to more moisture absorption

by the films. Water compatible plasticisers increase WVP of protein films through two modes: their effect on increasing intermolecular free space as a result of increasing polymer chain mobility and their hydrophilic nature. This allows for increased absorption and diffusion of water vapour through the film (Kester and Fennema, 1986).

TABLE 3.2.4 WVP of Plasticised and Non-Plasticised Kafirin Films Cast from Glacial Acetic Acid and Microwave Heated at 80 W for Different Times

Microwaving Time (min)	Water Vapour Permeability ($\text{g mm m}^{-2} \text{h}^{-1} \text{kPa}^{-1}$)		
	Determined in Vials		Determined in Schott Bottles
	Plasticised	Non-plasticised	Non-plasticised
0 (Control)	2.80a ^a (0.20) ^b	0.93a (0.20)	0.39a (0.05)
1	2.50a (0.30)	1.10a (0.30)	0.44a (0.06)
2	2.60a (0.20)	0.98a (0.20)	0.39a (0.03)
3	2.50a (0.30)	0.76a (0.30)	0.39a (0.08)
4	2.80a (0.10)	0.90a (0.30)	0.41a (0.13)

a. Mean values in the same column with different letters are significantly different ($p < 0.05$)

b. Values in parentheses are standard deviation of means.

Only non-plasticised films were tested for oxygen permeability, since they were the only ones microwaved whole, thus could offer an area of 25 cm^2 , the minimum required for the testing instrument. However, an effect of microwaving on the oxygen permeability of the films could not be established, as there was a high variability and failed tests (Table 3.2.5). The variability and failed tests were probably a result of cracks in the films, as they were brittle due to non-plasticisation.

TABLE 3.2.5 Oxygen Permeability (OP) of Non-Plasticised Kafirin Films that were Microwaved Cast at 80 W for Different Time Periods

Microwaving Time (min)	OP (cm ³ /μm.m ² /day kPa)
0 (control)	24.8 (3.5) ^a
1	30.7 (6.7)
2	Fail ^b
3	Fail
4	49.5 (29.5)

a. Values in parentheses are standard deviation of means

b. Failed test

Fig. 3.2.3. illustrates the progressive degradation of kafirin films buried in low (A) and high (B) moisture content compost. Degradation of the films progressed through shrinking, wrinkling, development of holes and fragmentation before total degradation. In terms of colour, the films turned milky and opaque initially and became brown to black later. Films buried in the low and high moisture compost were essentially totally degraded after 20 and 14 days of incubation, respectively. After 28 days, fragments of films made from microwave-heated kafirin were found in the low moisture content compost. The fragments were black, wrinkled, brittle and had holes. This was an anomaly, possibly arising from variations in the film and/or compost. In comparison, the low-density polyethylene films remained whole and clear with only slight wrinkling during the test period.

Films buried in the high moisture compost degraded faster (approximately 6 days earlier) than those in low moisture compost probably because the higher moisture content promoted greater hydration of the films and more microbial growth and metabolism. In both the low and high moisture compost, the microwave heated films degraded somewhat slowly as compared to the control films, probably because of microwave induced intermolecular cross-links and consequent structural changes, which made some of the protein less accessible to microbial proteolytic enzymes. Similarly, microwaving of kafirin before film casting also slowed the biodegradability of the films (Chapter 3.1). Slowed biodegradation of films has also been reported in heat-

treated whey protein films (Le Tien et al 2000) and γ -irradiated whey, casein and soy protein films (Lacroix et al 2002). Heat and γ -irradiation induced cross-links were postulated to account for the observed slowed biodegradation of the treated films. Similarly, disulphide cross-linked collagen films were found to be more resistant to degradation by collagenase than the un-cross-linked collagen films, suggesting that SS cross-linking made some of the protein less accessible to the enzyme (Yamauchi et al 2001).

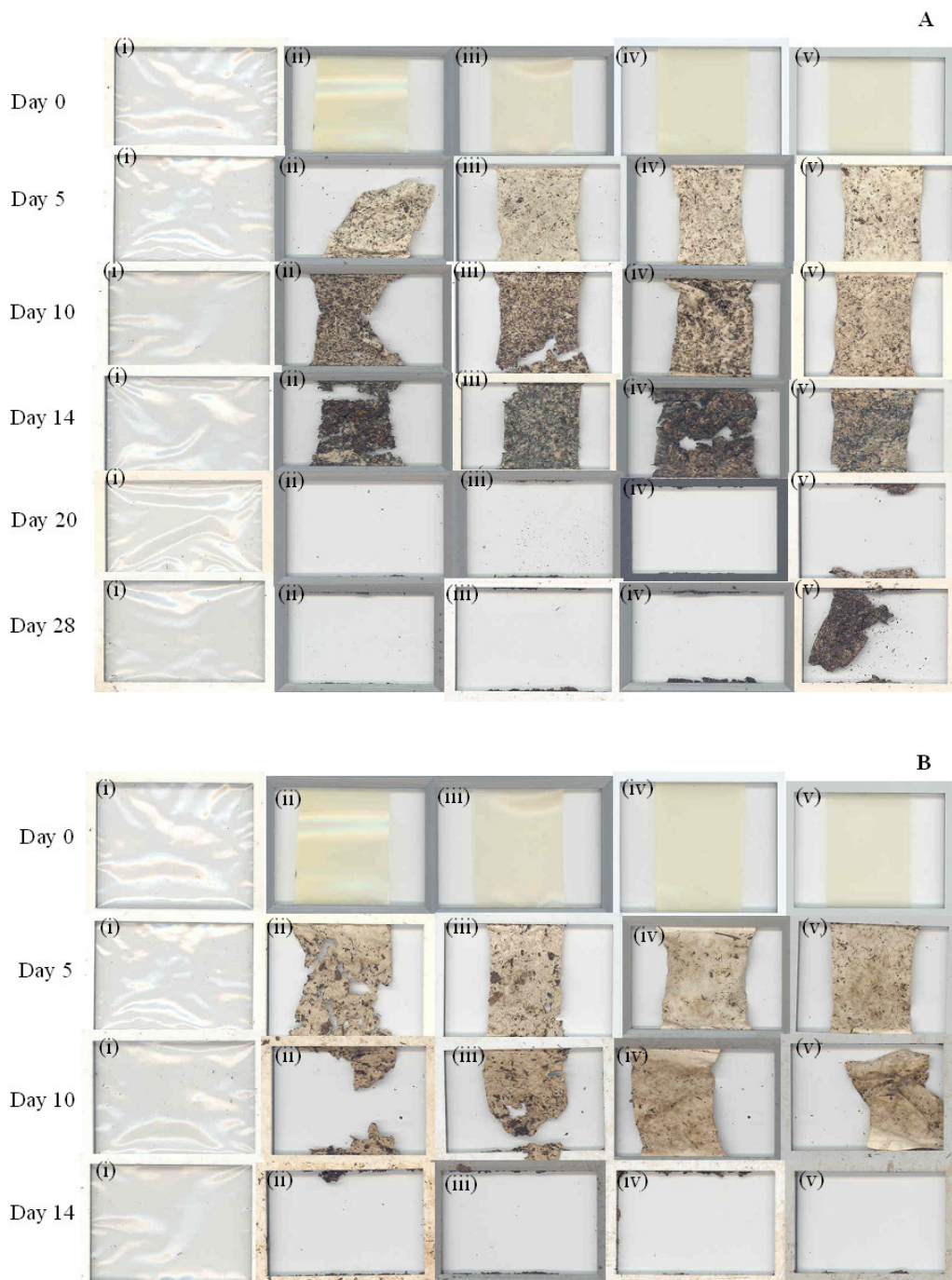


Fig. 3.2.3. Degradation of kafirin films buried in compost for 28 days at 50°C. (A) Low moisture compost (37% moisture content) (B) high moisture compost (60% moisture content). (i) = polyethylene film, (ii) = non-plasticised untreated film, (iii) = plasticised untreated film, (iv) = non-plasticised film microwaved at 80 W for 4 min, (v) = plasticised film microwaved at 80 W for 4 min.

Scanning electron microscopy (SEM) revealed that the microwave heated plasticised films (Fig. 3.2.4A iv-xv) appeared to have more wrinkled or folded structures (F) and smaller pore sizes (P) as compared to the non-microwaved control (Fig. 3.2.4A i-iii). The top and bottom parts of the films were similar in appearance with the pores regularly distributed. A reduction in the number of pores was evident with increasing microwaving time. Compared to the films cast from microwaved kafirin (Chapter 3.1), the structure of the plasticised films that were microwaved cast showed more folded or wrinkled structures. The difference was possibly a result of conformational and cross-linking differences in the kafirin resulting from when the microwaving was applied during making of the film. However, in both cases, the pores appeared to become fewer in number and smaller in size with microwaving. As mentioned (Chapter 3.1), the pores were presumably due to presence of air bubbles. Some of these pores could have burst as a result of microwave heating and subsequent cross-linking of the kafirin in the cast films.

The structure of microwave heated non-plasticised films (Fig. 3.2.4B iv-xv) appeared to be more brittle, in some cases with fractured pieces (FP), as compared to the control (Fig. 3.2.4A i-iii). The brittle appearance could be related to the non-extensible nature of the non-plasticised films (Table 3.2.1). As with the plasticised films, the top and bottom parts of the non-plasticised films appeared similar with the pores regularly distributed. These findings are consistent with earlier SEM studies, which showed that heating affects film microstructure by causing cavities in soy protein films (Kim et al 2002). Also, using SEM, Emmambux et al (2004) found that tannin modified kafirin films were less striated with larger but fewer pores as compared to the control, suggesting that protein cross-linking can affect the microstructure of kafirin films, as seen here with microwave cross-linking of kafirin. Therefore, the decrease in pore size and number was probably caused by microwave induced cross-linking and was, presumably related to the changes in film functional properties, such as biodegradability, tensile strength and strain.

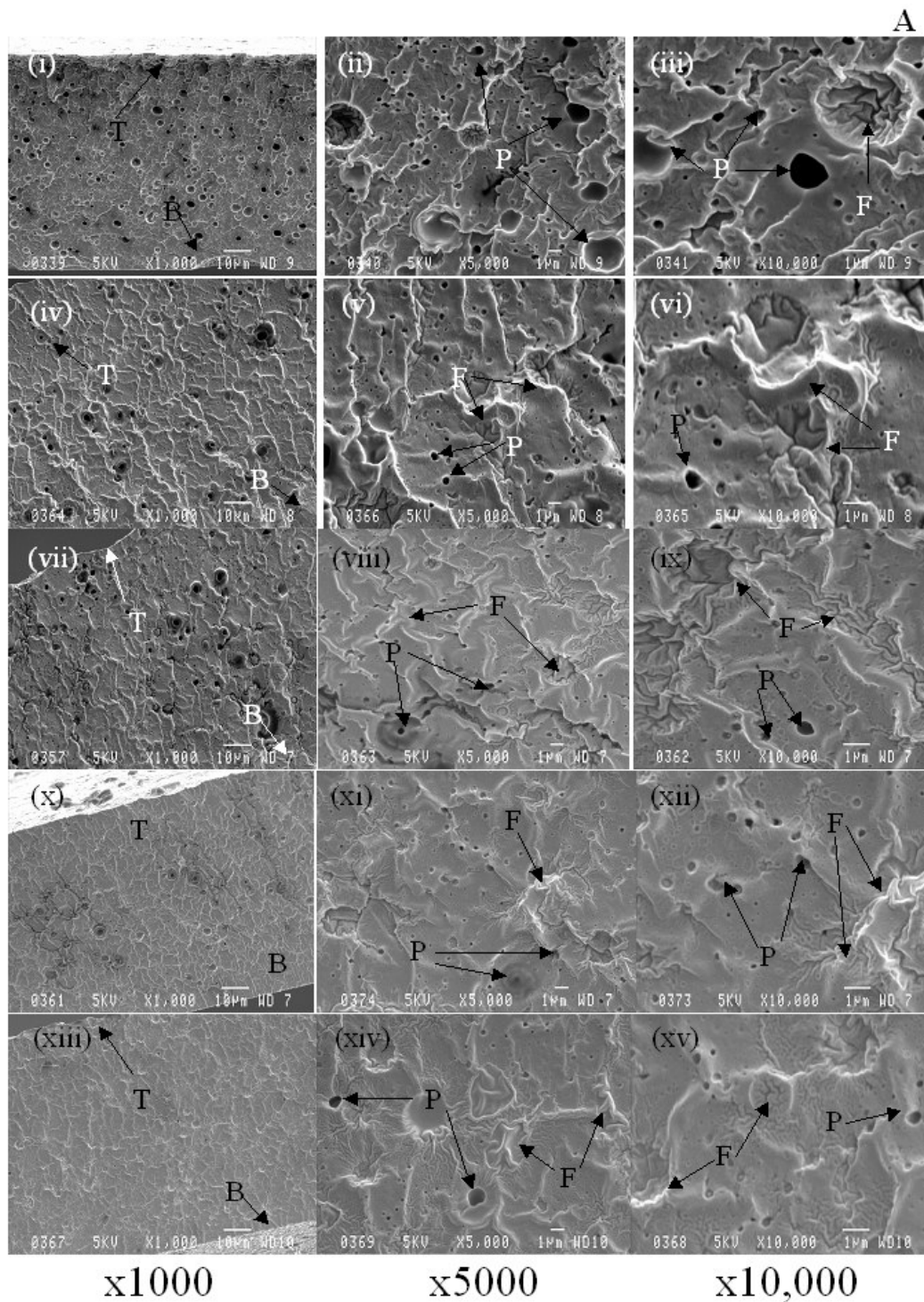
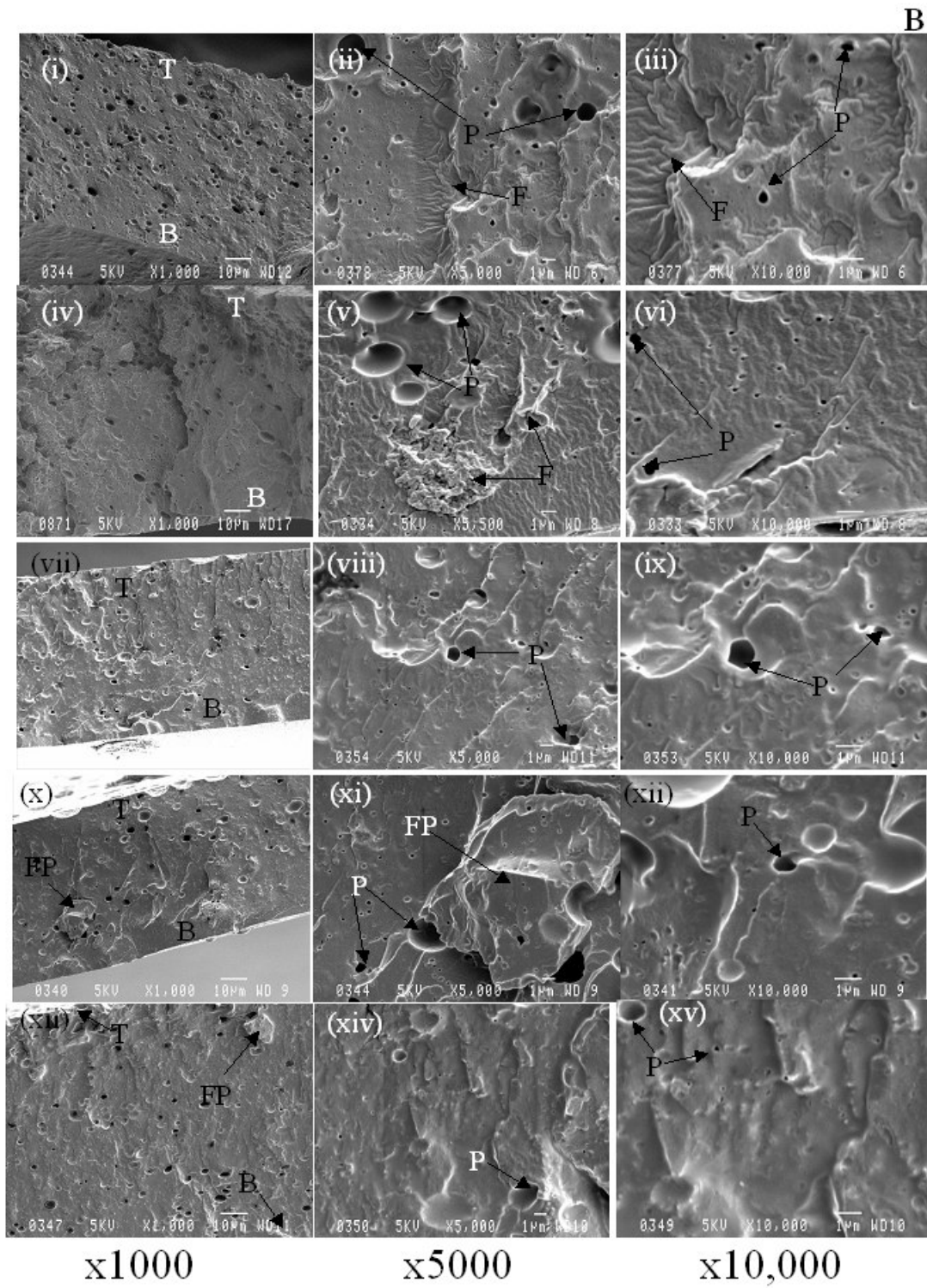


Fig. 3.2.4. Scanning electron micrographs of freeze fracture surface of kafirin films microwave heated at 80 W for different times. (A) plasticised and (B) non-plasticised films. (i-iii) = control film, (iv-vi) = 1 min, (vii-ix) = 2 min, (x-xii) = 3 min, (xiii-xv) = 4 min. T = top side of the film in contact with atmosphere, B = bottom side of film in contact with Petri dish, FP = fractured piece, P = pore, F = folded or wrinkled structure.



3.2.4. Conclusions

Microwave heating of cast films can be used to modify the functional properties of kafirin films, such as tensile strength, strain, digestibility and biodegradability. The plasticisers in films increase the effectiveness of microwaving on the film functional properties, probably because of their high dielectric constant. Microwave induced intermolecular disulfide cross-links seem to be responsible for the modification of kafirin film properties. Microwaving after film casting appears to be more effective than microwaving before film casting since it caused a higher increase in the tensile strength of kafirin films.

3.2.5. Literature cited

- Ali, Y., Ghorpade, V. M., and Hanna, M. A. 1997. Properties of thermally-treated wheat gluten films. *Ind. Crops. Prod.* 6:177-184.
- Argos, P., Pedersen, K., Marks, M. D., and Larkins, B. A. 1982. Structural model for maize zein proteins. *J. Biol. Chem.* 257: 9984-9990.
- Chapiro, A. 1988. Polymers in radiation-related technologies: radiation modified polymers. Pages 3-17 in: *Polymers for Advanced Technologies*. IUPAC International Symposium. M. Lewin, ed. VCH: Weinheim, Germany
- Cheftel, J. C., Cuq, J. L., and Lorient, D. 1985. Amino acids, peptides and proteins. Pages 245-369 in: *Food Chemistry 2nd ed.* O. R. Fennema, ed. Marcel Dekker: New York.
- Chiue, H., Kusano, T., and Iwami, K. 1997. Deamidation-induced fragmentation of maize zein, and its linked reduction in fatty acid binding capacity as well as antioxidative effect. *Food Chem.* 58:111-117.
- Damodaran, S. 1996. Amino acids, peptides and proteins. Pages 321-429 in: *Food Chemistry 3rd ed.* O. R. Fennema, ed. Marcel Dekker: New York.
- Di Gioia, L., Cuq, B., and Guilbert, S. 1998. Effect of hydrophilic plasticizers on thermomechanical properties of corn gluten meal. *Cereal Chem.* 75:514-519.
- Duodu, K. G., Nunes, A., Delgadillo, I., Parker, M. L., Mills, E. N. C., Belton, P. S., and Taylor, J. R. N. 2002. Effect of grain structure and cooking on sorghum and maize *in-vitro* protein digestibility. *J. Cereal Sci.* 35:161–174.
- El Nour, I. N. A., Peruffo, A. D. B., and Curioni, A. 1998. Characterisation of sorghum kafirins in relation to their cross-linking behaviour. *J. Cereal Sci.* 28:197-207.

- Emmambux, N. M., Stading, M., and Taylor, J. R. N. 2004. Sorghum kafirin film property modification with hydrolysable and condensed tannins. *J. Cereal Sci.* 40:127-135.
- Gennadios, A., Ghorpade, V. M., Weller, C. L., and Hanna, M. A. 1996. Heat curing of soy protein films. *Trans. Am. Soc. Agric. Eng.* 39:575-579.
- Gennadios, A., Rhim, J. W., Handa, A., Weller, C. L., and Hanna, M. A. 1998. Ultraviolet radiation affects physical and molecular properties of soy protein films. *J. Food Sci.* 63:225–228.
- Gontard, N., Guilbert, S., and Cuq, J. L. 1993. Water and glycerol as plasticizers affect mechanical and water barrier properties of an edible wheat gluten film. *J. Food Sci.* 58:206-211.
- Hamaker, B. R., Kirleis, A. W., Butler, L. G., Axtell, J. D., and Mertz, E. T. 1987. Improving the in vitro protein digestibility of sorghum with reducing agents. *Proc. Natl. Acad. Sci. USA* 84:626-628.
- Irissin-Mangata, J., Bauduin, G., Boutevin, B., and Gontard, N. 2001. New plasticizers for wheat gluten films. *Eur. Polymer. J.* 37:1533-1541.
- Kaya, S., and Kaya, A. 2000. Microwave drying effects on properties of whey protein isolate edible films. *J. Food Eng.* 43:91-96.
- Kester, J. J., and Fennema, O. R. 1986. Edible films and coatings: A review. *Food Technol.* 40(12):47-59.
- Kim, K. M., Weller, M. A., and Gennadios, A. 2002. Heat curing of soy protein films at atmospheric and sub-atmospheric conditions. *J. Food Sci.* 67:708-713.
- Lacroix, M., Le, T. C., Ouattara, B., Yu, H., Letendre, M., Sabato, S. F., Mateescu, M. A., and Patterson, G. 2002. Use of γ -irradiation to produce films from whey, casein and soy proteins: structure and functional characteristics. *Radiation Physics Chem.* 63:827-832.
- Lakkis, J., and Villota, R. 1992. Comparative performance of chemically and enzymatically modified whey proteins. *Food Chem.* 43:93-105.
- Lawton, J. W. 2004. Plasticizers for zein: Their effects on tensile properties and water absorption of zein films. *Cereal Chem.* 81:1-5.
- Le Tien, C., Letendre, M., Ispas-Szabo, P., Mateescu, M. A., Delmas-Patterson, G., Yu, H. L., and Lacroix, M. 2000. Development of biodegradable films from whey proteins by cross-linking and entrapment in cellulose. *J. Agric. Food Chem.* 48:5566-5575.

- Lens, J.-P., De Graaf, L. A., Stevels, W. M., Dietz, C. H. J. T., Verhelst, K. C. S., Vereijken, J. M., and Kolster, P. 2003. Influence of processing and storage conditions on the mechanical and barrier properties of films cast from aqueous wheat gluten dispersions. *Ind. Crops Prod.* 17:119-130.
- Madeka, H., and Kokini, J. L. 1996. Effect of glass transition and cross-linking on rheological properties of zein: development of a preliminary state diagram. *Cereal Chem.* 73:433-438.
- Matveev, Y. I., Grinberg, V. Y., and Tolstoguzov, V. B. 2000. The plasticizing effect of water on proteins, polysaccharides and their mixtures. Glassy state of biopolymers, food and seeds. *Food Hydrocolloids* 14:425-437.
- Miller, K. S., Chiang, M. T., and Krochta, J. M. 1997. Heat curing of whey protein films. *J. Food Sci.* 62:1189-1193.
- Mimouni, B., Raymond, J., Merle-Desnoyers, A. M., Azanza, J. L., and Ducastaing, A. 1994. Combined acid deamidation and enzymic hydrolysis for improvement of the functional properties of wheat gluten. *J. Cereal Sci.* 21:153-165.
- Nunes, A., Correia, I., Barros, A., and Delgadillo, I. 2004. Sequential *in-vitro* pepsin digestion of uncooked and cooked sorghum and maize samples. *J. Agric. Food Chem.* 52:2052-2058.
- Oria, M. P., Hamaker, B. R., and Shull, J. M. 1995b. *In vitro* protein digestibility of developing mature sorghum grain in relation to α -, β - and γ -kafirin disulphide cross-linking. *J. Cereal Sci.* 22:85-93.
- Park, H. J., and Chinnan, M. S. 1995. Gas and water vapor properties of edible films from protein and cellulosic materials. *J. Food Eng.* 25:497-507.
- Park, H. J., Bunn, J. M., Weller C. L., Vergano, P. J., and Testin, R. F. 1994c. Water vapor permeability and mechanical properties of grain protein-based films as affected by mixtures of polyethylene glycol and glycerin plasticisers. *Trans. Amer. Soc. Agric. Eng.* 37:1281-1285.
- Parris, N., and Coffin, D. R. 1997. Composition factors affecting the water vapor permeability and tensile properties of hydrophilic zein films. *J. Agric. Food Chem.* 45:1596-1599.
- Quinn, G., Monahan, F. J., O'Riordan, E. D., O'Sullivan, M., and Longares, A. 2003. Role of covalent and non-covalent interactions in the formation of films from unheated whey protein solutions following pH adjustment. *J. Food Sci.* 68:2284-2288.

- Rhim, J. W., Gennadios, A., Fu, D., Weller, C. L., and Hanna, M. A. 1999. Properties of ultraviolet irradiated protein films. *Lebens. Wissen. Technol.* 32:129-133.
- Ringe, D., and Petsko, G. A. 2003. The 'glass transition' in protein dynamics: What is it, why it occurs and how to exploit it. *Biophys. Chem.* 105:667-680.
- Sastry, L. V. S., and Virupaksha, T. K. 1969. Alcohol soluble proteins of grain sorghum. *Cereal Chem.* 46:284-293.
- Sharma, G. P., and Prasad, S. 2002. Dielectric properties of garlic (*Allium sativum* L.) at 2450 MHz as a function of temperature and moisture content. *J. Food Eng.* 52:343-348.
- Shrewsbury, R. P. 2004. Factors affecting the solubility of drugs. <http://pharmlabs.unc.edu/solubility/text.htm> [30 November 2004].
- Shull, J. M., Watterson, S. J., and Kirleis, A. W. 1991. Proposed nomenclature for the alcohol-soluble proteins (kafirins) of *Sorghum bicolor* (L. Moench) based on molecular weight, solubility and structure. *J. Agric. Food Chem.* 39:83-87.
- Sothornvit, R., Reid, D. S., and Krochta, J. M. 2002. Plasticizer effect on the glass transition temperature of beta-lactoglobulin films. *Trans. Amer. Soc. Agric. Eng.* 45:1479-1484.
- Stading, M., Rindlav-Westling, Å., and Gatenholm, P. 2001. Humidity-induced structural transitions in amylose and amylopectin films. *Carbohydrate Polymers* 45:209-217.
- Taylor, J., Taylor, J. R. N., Dutton, M. F., and De Kock, S. 2005a. Identification of kafirin film casting solvents. *Food Chem.* 90:401-408.
- Vachon, C., Yu, H. L., Yefsah, R., Alain, R., St-Gelais, D., and Lacroix, M. 2000. Mechanical and structural properties of milk protein edible films cross-linked by heating and γ -irradiation. *J. Agric. Food Chem.* 48:3202-3209.
- Van Eck, H. 2004. Plasticization of kafirin films. MSc. (Agric) Dissertation. University of Pretoria, Pretoria, South Africa.
- Wohlfarth, C. 1974. Permittivity (dielectric constants) of pure liquids. Section 6, pages 155-188, in: *CRC Handbook of Chemistry and Physics* 75th ed. D. R. Lide and H. P. R. Frederikse, eds. CRC Press: Boca Raton, USA.
- Yamauchi, K., Takeuchi, N., Kurimoto, A., and Tanabe, T. 2001. Films of collagen cross-linked by disulphide bonds: Preparation and characterisation. *Biomaterials* 22:855-863.
- Yoshino, T., Isobe, S., and Maekawa, T. 2002. Influence of preparation conditions on the physical properties of zein films. *J. Amer. Oil Chem. Soc.* 79:345-349.

3.3. EFFECT OF MICROWAVE HEATING ON THE STRUCTURE OF KAFIRIN AND KAFIRIN FILMS

Abstract

The molecular basis for the changes in properties of cast kafirin film due to microwave heating was investigated. SDS-PAGE data suggested that microwaving of both kafirin and cast kafirin films induced intermolecular SS and possibly non-SS covalent cross-linking of the protein. Compared to the controls, SS groups increased in microwave heated kafirin, plasticised films made from microwave heated kafirin, non-plasticised and plasticised films that were microwave heated cast, confirming the involvement of SS cross-links in the kafirin cross-linking. FTIR indicated an increase in the β -sheet conformation accompanied by a decrease in the α -helical structure in microwaved kafirin and films made from it. Although there was a slight increase in the β -sheet conformation of the kafirin in the films microwave heated after casting, this protein was essentially α -helical in conformation. It appears the stage at which microwave heating is applied could be an important determinant of the kafirin structure in the film and of film functional properties. Cross-linking and changes in protein secondary structure induced by microwave heating were probably responsible for the differences in the functional properties of kafirin films as described earlier in this thesis.

3.3.1. Introduction

Kafirin, the prolamin protein of sorghum, has been used to make films of tensile strength similar or superior to those made from zein (Buffo et al 1997; Da Silva and Taylor 2005; Taylor et al 2005a; Emmambux et al 2004). However, a wide range of packaging requirements exists in the food industry, and in some cases it is necessary to chemically or physically modify the functional properties of protein films to achieve definite packaging goals. For example, Emmambux et al (2004) increased the tensile strength and decreased oxygen permeability of kafirin films through complexation with tannins.

Regarding physical methods of modification, thermal energy has been used to increase the tensile strength of protein films, such as those made from soy protein (Gennadios et al 1996), gluten (Ali et al 1997; Roy et al 1999) and whey (Perez-Gago et al 1999; Perez-Gago and Krochta 2001). Treatment with microwaves was used to modify the tensile and barrier properties of whey protein films (Kaya and Kaya 2000). It has also been shown in Chapters 3.1 and 3.2 that microwaving of kafirin or cast kafirin films modified film properties, i.e. increased tensile strength and decreased strain, decreased water vapour permeability in the case of films made from microwaved kafirin, decreased digestibility and slowed biodegradability. Changes in functional properties of protein films appear to be a result of intermolecular cross-links and changes in protein secondary structure induced by the physical or chemical modifying agents used (Perez-Gago et al 1999; Emmambux et al 2004). However, it is not known how, specifically, kafirin is modified when subjected to microwave heating. In this work, the effects of exposure to microwave heating on SS cross-linking and the secondary structure of kafirin and kafirin films were examined.

3.3.2. Materials and methods

3.3.2.1. Extraction of kafirin

Kafirin was extracted as described in Chapter 3.1.

3.3.2.2. Microwave heating of kafirin

This was done as described in Chapter 3.1. Wetted kafirin powder that was not microwaved was used as the control.

3.3.2.3. Film casting and microwave heating of cast films

Films were cast from microwaved kafirin as described in Chapter 3.1. Films were also cast from non-microwaved kafirin with and without plasticiser and microwaved after casting, as described in Chapter 3.2. Non-microwaved film was used as control.

3.3.2.4. SDS-PAGE

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) under both reducing and non-reducing conditions was conducted in 12% acrylamide gels, as described in Chapter 3.1.

3.3.2.5. Determination of SH and SS groups

3.3.2.5.1. Determination of free SH groups

The solid-state direct colorimetric method described by Thannhauser et al (1987) was used. Kafirin and kafirin film samples were first washed three times in 12% (w/w) trichloroacetic acid to remove the dithiothreitol. The washed protein was recovered by centrifugation at 7200 *g* for 20 min. Washed samples (30 mg) were then suspended in 1 mL reaction buffer, consisting of 8*M* urea, 10 mM 5, 5-dithiobis 2-nitrobenzoic acid (DTNB), 3 mM ethylene-diaminetetraacetic acid (EDTA) 0.2 *M* Tris-HCl and 1% SDS at pH 8.0. Samples were flushed with nitrogen gas the incubated for 30 min in the dark. To remove particulate matter, samples were centrifuged at 10,000 *g* for 15 min. A 0.1 mL aliquot of supernatant was removed and diluted with the reaction buffer minus DTNB. This suspension was centrifuged at 10,000 *g* for 15 min and its absorbance read at 412 nm.

3.3.2.5.2. Determination of total SH groups

Washed samples (30 mg) were suspended in 1 mL reaction buffer consisting of 8*M* urea, 0.1 *M* sodium sulphite, 3 mM EDTA, 0.2 *M* Tris-HCl, pH 9.5 and 10 mM 2-nitro-5-thiosulphobenzoate (NTSB²⁻). The NTSB²⁻ was synthesized from DTNB in the presence of sodium sulphite and oxygen, as described by Thannhauser et al (1987). The samples were subsequently incubated and treated as described for determination of free SH groups, except in this case the dilution was done using reaction buffer containing sodium sulphite minus the NTSB²⁻. The SS group content was calculated as the difference between SH group content before and after reduction of SS bonds with

sodium sulphite and expressed as $\mu\text{mole SH/g protein}$. Total cysteine content was calculated as $(-\text{SH}) + 2(-\text{S-S-})$.

3.3.2.6. Fourier transform infra-red (FTIR) spectroscopy

FTIR spectra of microwaved kafirin were obtained from 100 mg KBr discs containing approximately 2 mg sample. Spectra (43 scans at 2 cm^{-1} resolution) were obtained using a Bruker Infra-red Spectrophotometer (Bruker Instruments, Billerica, USA) from 4000 to 500 cm^{-1} . Background spectra were subtracted using the Opus software (Bruker Instruments, Billerica, USA). Triplicate samples were analysed and the spectra averaged. Fourier self deconvolution was conducted on the average spectra using resolution enhancement factor of 1.8 and full height band width of 13 cm^{-1} .

FTIR spectra of kafirin films were obtained using a FTS 175 spectrometer (Bio-Rad, Hemel Hempstead, UK) with a Golden Gate diamond horizontal attenuated total reflectance (ATR) crystal (Specac, Hemel Hempstead, UK). A kafirin film sample (10 mm x 10 mm) was placed on the ATR crystal to cover the crystal surface area. The sample was gently squeezed using screws to promote contact with the crystal. The spectra (128 scans at 2 cm^{-1} resolution) were collected within the frequency range 4000 to 800 cm^{-1} . The empty crystal was used as background. The FTIR spectra were Fourier deconvoluted using a resolution enhancement factor of 1.5 and full height band width of 15 cm^{-1} .

3.3.2.7. Fourier Transform-Raman (FT-Raman) spectroscopy

The Raman spectra of kafirin samples were collected using a FT-Raman spectrometer (Bio-Rad, Hemel Hempstead, UK). About 2 mg was placed in the cavity in a circular aluminium stub. The sample was aligned to the incoming laser. Near infrared laser (1065 nm, 300-500 mW power) was used for excitation. The number of recorded spectra was 256 at a resolution of 8 cm^{-1} .

3.3.2.8. Statistical analysis

Mean values for SS and SH groups were obtained from three independent microwaving experiments. One-way analysis of variance (ANOVA) was performed on the data to determine the effect of different microwave treatments on the individual kafirin film properties. The least significant difference test was performed at $p < 0.05$ on treatment effects for which significant differences were found.

3.3.3. Results and discussion

Figures 3.3.1 to 3.3.4 show the SDS-PAGE pattern of kafirin and kafirin films subjected to microwave heating. All samples showed bands that were consistent with the α -, β -, and γ -kafirin species (Shull et al 1991). In addition, bands of M_r 40,000 to 50,000 were observed. Such bands have been identified as dimers (El Nour et al 1998). However, the M_r of the α 1-kafirin (23,000), α 2-kafirin (21,000) and γ -kafirin (25,000) appeared to be somewhat underestimated. The M_r for α 1-, α 2- and γ -kafirins are 25,000, 23,000 and 28,000, respectively (Shull et al 1991). Possible reasons for this apparent underestimation are discussed in Chapter 4.1.

Under non-reducing conditions, microwave heated samples showed a higher intensity of oligomer bands as compared to the controls of kafirin (Fig 3.3.1A, track 1), plasticised films made from microwaved kafirin (Fig 3.3.2A, track 1) and non-plasticised films that were microwaved cast (Fig 3.3.3A, track 1). However, this was less clear in the plasticised films that were microwaved cast (Fig 3.3.4A, track 1). The lower intensity of oligomer bands in the controls suggests less cross-linking of the kafirin. This is not surprising since the kafirin was extracted at ambient temperature in a solvent system containing a reducing agent. The increase in oligomer band intensity is consistent with earlier research where microwave induced protein polymerisation has been reported in rapeseed protein (Valentinova et al 2000) and beef liver protein (Farag et al 2001). Protein polymerisation due to wet cooking has also been reported in kafirin (Duodu et al 2002; Nunes et al 2004).

With SDS-PAGE under reducing conditions, the intensity of the oligomer bands in the samples subjected to microwave heating was lower than under non-reducing conditions (Figs. 3.3.1-3.3.4). In the controls, the intensity of oligomer bands was generally lower as compared to the microwave treated samples and in some cases the oligomers were almost non-existent. Once again this effect was not clear in the plasticised films that were microwaved cast (Fig 3.3.4B, track 1). The decrease in oligomer band intensity after reduction indicates the involvement of SS cross-links in the formation of oligomers. However, the fact that oligomers remained in the microwave treated kafirin and kafirin films, indicates that not all the cross-links could be cleaved by mercaptoethanol. This was possibly because of the presence of hidden SS cross-links or non-SS covalent cross-links. Duodu et al (2003) suggested that wet

cooking of sorghum can cause both intermolecular SS and non-SS covalent cross-links between kafirin molecules and that some of the SS bonds may be inaccessible to reducing agents such as mercaptoethanol.

In addition, compared to non-reducing conditions, there was an increase in the intensity of β -kafirin bands following reduction in all the samples (Figs 3.3.1-3.3.4). The increase in intensity of β -kafirin bands, after reduction, paralleled the decrease in intensity of oligomer bands, suggesting that β -kafirin was a component of the oligomers. This agrees with the findings of El Nour et al (1998) who reported that β -kafirin is involved in the polymerisation of kafirins as a chain extender. Although it was hypothesised that the effects of microwaving would be different from those of conventional heating, these results suggest that the effects of microwave heating on wet kafirin are the same as those of wet cooking reported by Duodu et al (2002) and Nunes et al (2004).

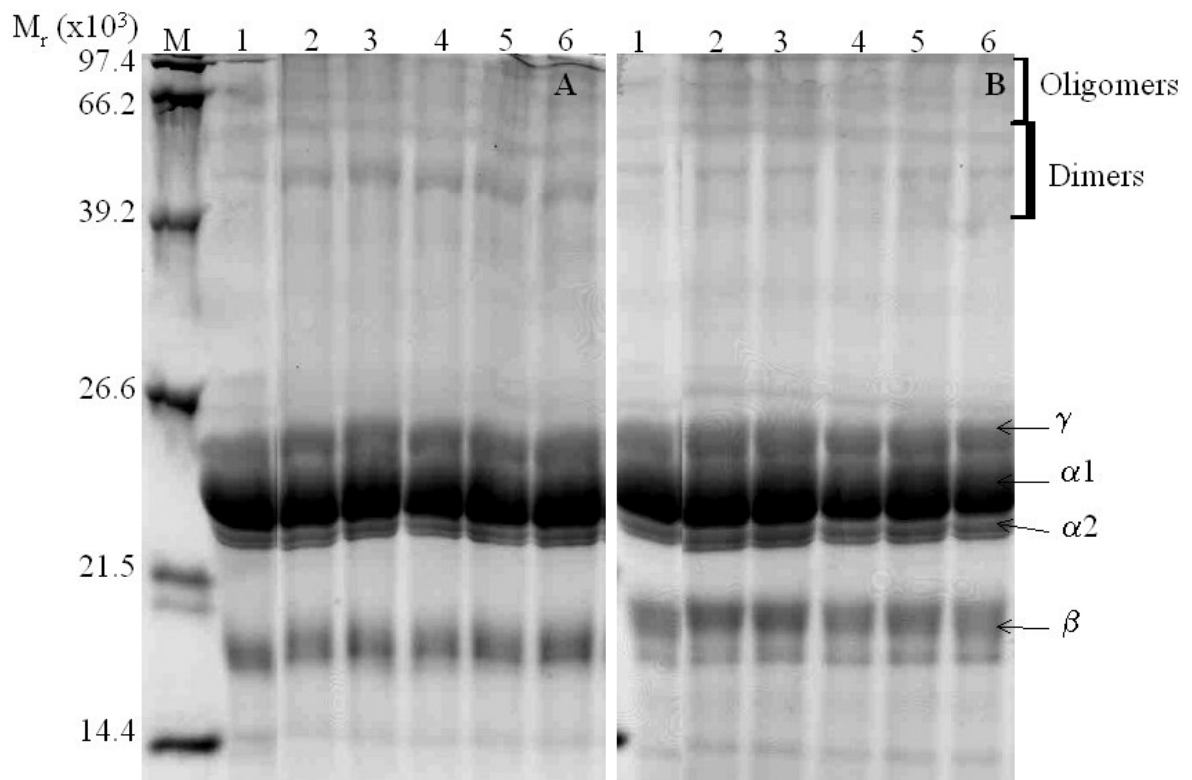


Fig. 3.3.1. SDS-PAGE of kafirin that was microwaved at 96°C for different time periods; A) non-reducing, B) reducing conditions. Tracks M = molecular markers, 1 = control (untreated kafirin), 2 = 1 min, 3 = 2 min, 4 = 3 min, 5 = 4 min, 6 = 10 min.

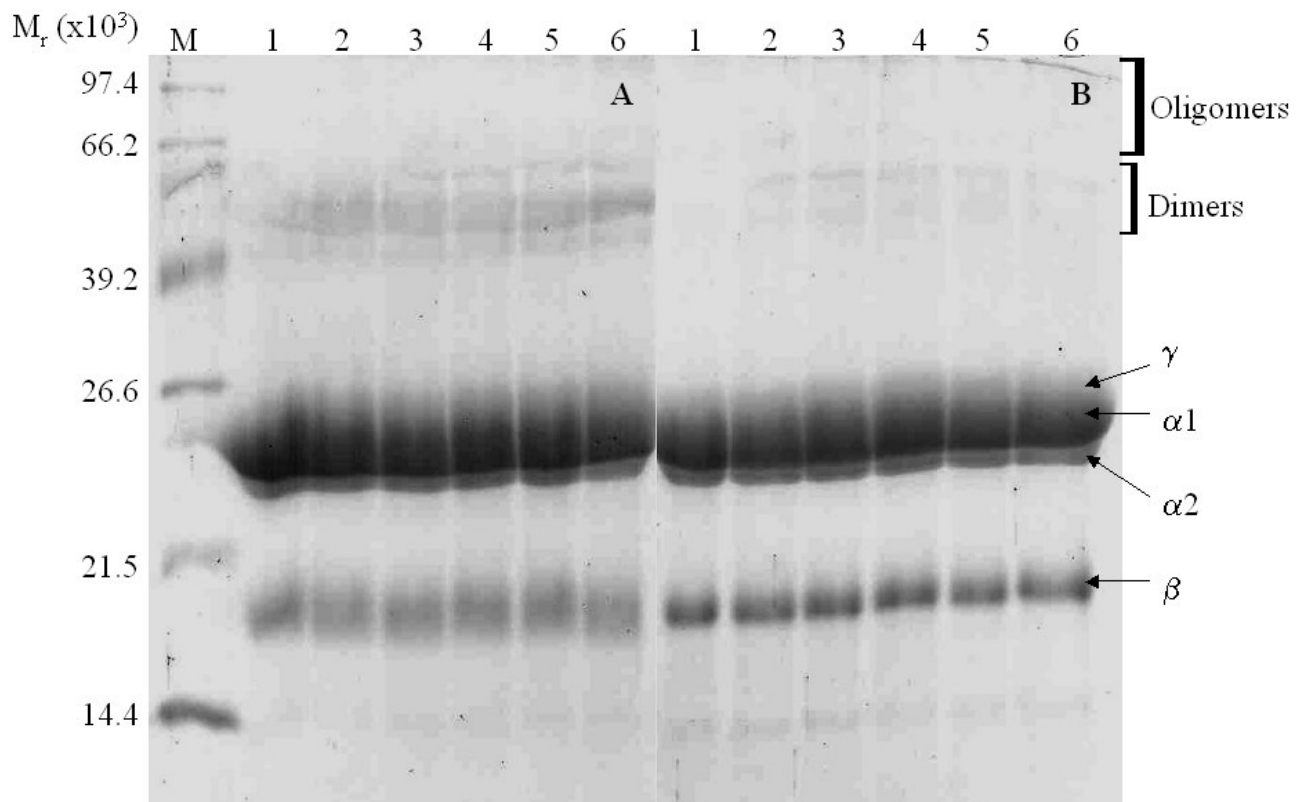


Fig. 3.3.2. SDS-PAGE of plasticised films made from kaffir that was microwaved at 96°C for different time periods; A) non-reducing, B) reducing conditions. Tracks M = molecular markers, 1 = control (untreated kaffir), 2 = 1 min, 3 = 2 min, 4 = 3 min, 5 = 4 min, 6 = 10 min.

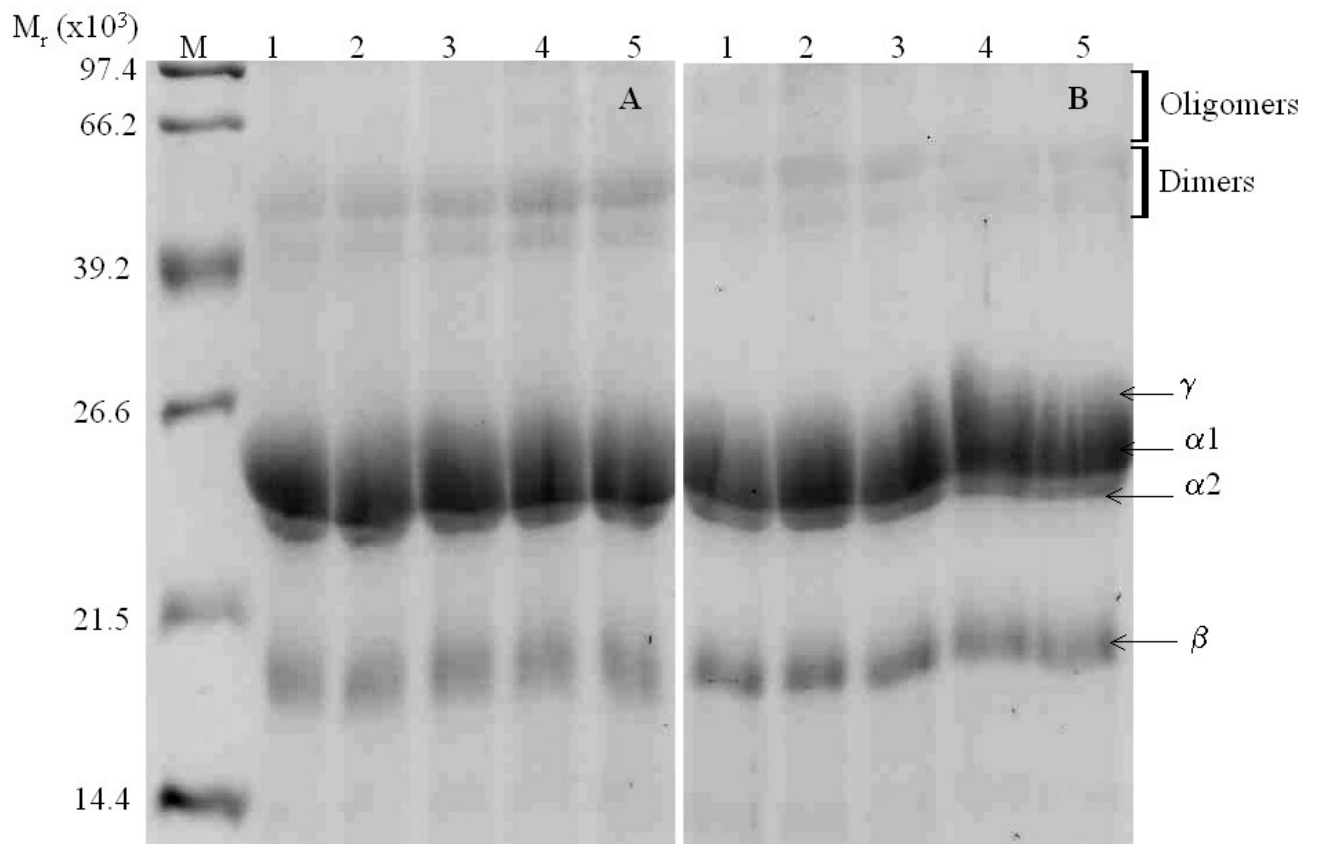


Fig. 3.3.3. SDS-PAGE of non-plasticised kafirin films that were microwaved at 80 W for different time periods; A) non-reducing, B) reducing conditions. Tracks M = molecular markers, 1 = control, 2 = 1 min, 3 = 2 min, 4 = 3 min, 5 = 4 min.

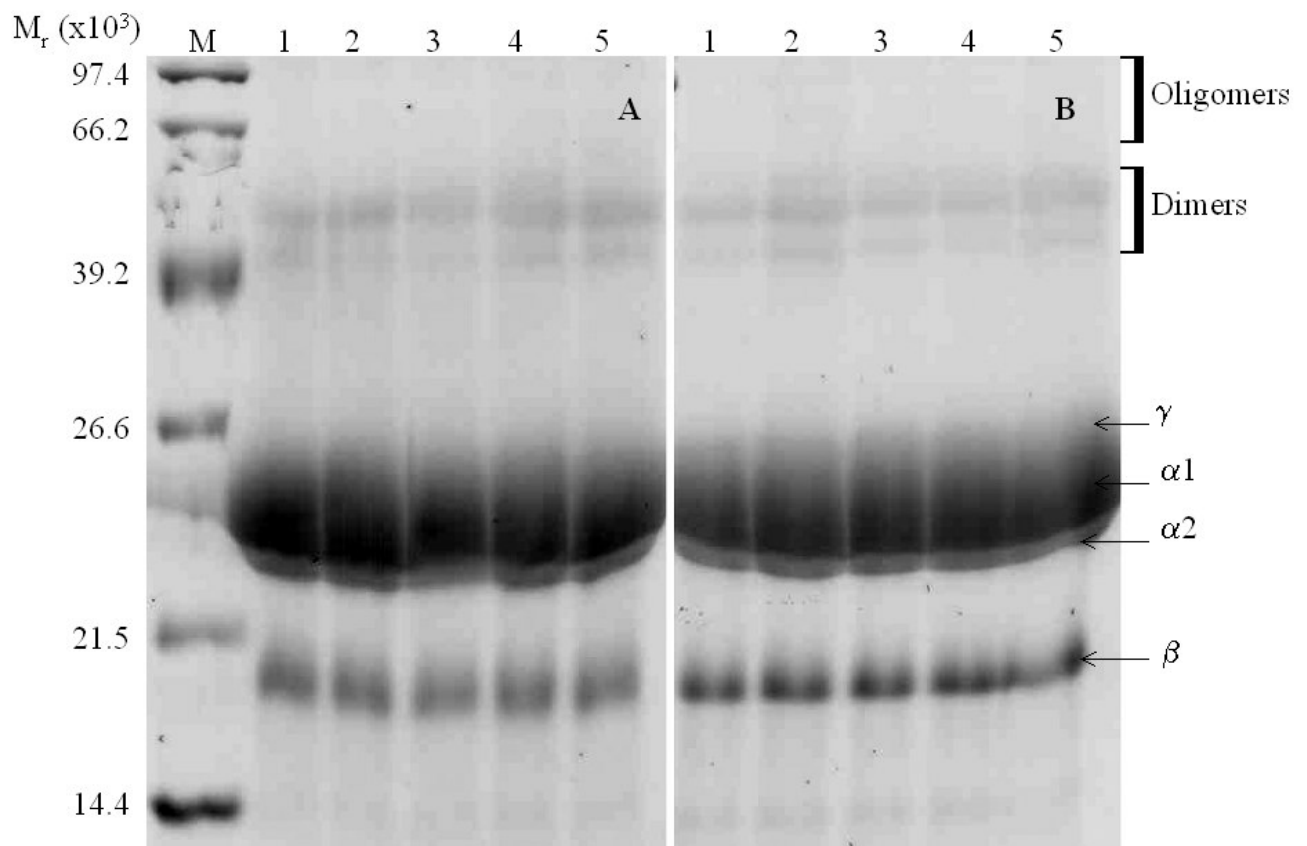


Fig. 3.3.4. SDS-PAGE of plasticised kafirin films that were microwaved at 80 W for different time periods; A) non-reducing, B) reducing conditions. Tracks M = molecular markers, 1 = control, 2 = 1 min, 3 = 2 min, 4 = 3 min, 5 = 4 min.

Table 3.3.1 shows a significant decrease in free SH groups of microwaved kafirin and plasticised films made from microwaved kafirin compared to their respective controls. A similar decrease in free SH groups, with microwaving, was also observed in non-plasticised and plasticised films that were microwaved cast (Table 3.3.2). In all cases, the decrease in free SH groups was accompanied by increases in SS groups. This indicates that microwaving induced the formation of SS bonds, as suggested by SDS-PAGE data. This is consistent with research which showed significant decreases in the free SH group content with concomitant increases in the SS group content after heat treatment of film forming solutions of whey protein (Quinn et al 2003). Similar results were reported in heated soy proteins (Shimada and Cheftel 1988a) and whey proteins (Shimada and Cheftel 1989).

It is apparent that there was no dose response between increasing microwaving time and the free SH groups and SS bonds of kafirin and kafirin films (Tables 3.3.1 and 3.3.2). Similarly, it was found that there was no significant difference found in the free SH groups and SS bonds content of whey proteins subjected to increasing temperature (Li-Chan 1983). The lack of a dose response was possibly because most or all the free SH groups available for reaction had reacted. Any additional reactions could have involved the exchange between SH and SS groups as reported by Owusu-Apenten et al (2003) in whey protein and/or the conversion of intramolecular SS bonds to intermolecular SS bonds, as suggested by Morel et al (2000) in gluten films. Such reactions would cause a zero net effect on the free SH groups and SS bond content with increasing exposure to microwave energy.

Compared to the controls, there was a large decrease in the total SH groups after reduction for the plasticised films made from microwaved kafirin (Table 3.3.1) and a smaller, but still significant, decrease in the microwaved kafirin (Table 3.3.1) and plasticised films that were microwaved after casting (Table 3.3.2). It is possible that the decrease in SH groups after reduction was due to the inaccessibility of some SH and SS groups. Such inaccessible SH and SS groups were possibly a result of conformational changes due to protein polymerisation. This supposition is corroborated by the SDS-PAGE data (Figs. 3.3.1 to 3.3.4) in which reduction resistant oligomers were found. As stated earlier, heat induced reduction resistant oligomers presumably resulting from hidden SS and/or non-SS bonds have been reported in kafirin (Duodu et al 2002; Nunes et al 2004). It is apparent that the measured levels of SS groups and total cysteine in the plasticised films made from microwaved kafirin was very low as compared to that of microwaved kafirin (Table 3.3.1) and the non-plasticised and plasticised films that were microwaved cast (Table 3.3.2). This could be attributed to heat induced changes in protein structure, which can limit access of the reagents to the SH and SS groups of all cysteine residues (Chan and Wasserman 1993). According to Wedemeyer et al (2000) access to such hidden SH and SS groups requires global unfolding of the protein to expose them.

The low SS groups content of the plasticised films made from microwaved kafirin (Table 3.3.1) as compared to the SS group content of both plasticised and non-plasticised films that were microwaved cast (Table 3.3.2) suggests that the stage at

which microwaving is applied could affect the extent of SS cross-linking of kafirin. This could be related to the different effects of microwaving before and after film casting on film tensile strength. Microwaving of kafirin caused nearly two times increase in film tensile strength (Chapter 3.1) as compared to about four times increase in tensile strength observed in plasticised films that were microwaved after casting (Chapter 3.2). The high tensile strength in the plasticised films that were microwaved cast paralleled a high SS bond content in the films. A similar relation between SS bond content and film tensile strength is also seen when non-plasticised and plasticised films that were microwaved cast are compared. This suggests that microwave induced SS cross-linking probably directly contributed to the modification of kafirin film properties as described in Chapters 3.1 and 3.2.

TABLE 3.3.1 Sulphydryl (SH), Disulphide (SS) Group and Cysteine Content ($\mu\text{mole SH/g protein}$) of Kafirin that was Microwaved to 96°C and Held at 96°C for Different Time Periods and Films Made from the Protein

Microwaving Time (min)	0 (Control)	1	2	3	4	10
KAFIRIN						
Free SH Groups	21.7a ^a (0.5) ^b	16.3b (0.4)	16.9b (0.6)	16.5b (0.6)	15.8b (0.5)	14.7b (0.7)
SH Groups After Reduction	27.9a (0.2)	26.1b (0.7)	24.8b (0.3)	24.9b (0.3)	23.9b (0.6)	23.4b (0.5)
SS Groups	6.2a (0.1)	9.8b (0.7)	7.9b (0.5)	8.3b (0.6)	8.1b (0.4)	8.7b (0.5)
Total Cysteine	34.1a (0.5)	35.8a (0.8)	32.6b (0.5)	33.2b (0.5)	32.1b (0.5)	32.1b (0.6)
PLASTICISED FILMS						
Free SH Groups	22.0a (0.5)	8.9b (0.4)	8.6b (0.6)	8.2b (0.7)	7.9b (0.8)	8.4b (0.7)
SH Groups After Reduction	23.9a (0.5)	12.7b (0.7)	11.7b (0.5)	12.1b (0.3)	11.3b (0.6)	12.1b (0.5)
SS Groups	1.9a (0.4)	3.8b (0.5)	3.2b (0.2)	3.9b (0.5)	3.4b (0.4)	3.6b (0.4)
Total Cysteine	26.7a (0.5)	16.6b (0.7)	14.9b (0.6)	15.0b (0.5)	14.7b (0.6)	15.7b (0.5)

a. Values in the same row with different letters are significantly different ($p < 0.05$).

b. Values in parentheses are standard deviation of means.

TABLE 3.3.2 Sulphydryl (SH), Disulphide (SS) Group and Cysteine Content ($\mu\text{mole SH/g protein}$) of Plasticised and Non-plasticised Kafirin Films that were Microwaved at 80 W for Different Time Periods

Microwaving Time (min)	0 (Control)	1	2	3	4
NON-PLASTICISED FILM					
Free SH Groups	19.4a ^a (0.2) ^b	16.2b (0.3)	16.3b (0.3)	16.9b (0.5)	17.1b (0.4)
SH Groups After Reduction	22.9a (0.6)	22.0a (0.5)	21.7a (0.6)	22.2a (0.3)	21.9a (0.6)
SS Groups	3.5a (0.4)	5.8b (0.5)	5.4b (0.6)	5.3b (0.3)	4.8b (0.5)
Total Cysteine	26.4a (0.7)	27.8a (0.6)	27.0a (0.5)	27.5a (0.6)	26.6a (0.6)
PLASTICISED FILMS					
Free SH Groups	22.0a (0.5)	12.5b (0.6)	12.5b (0.4)	13.1b (0.7)	13.1b (0.6)
SH Groups After Reduction	23.9a (0.5)	21.0b (0.5)	20.7b (0.6)	21.7b (0.5)	21.6b (0.7)
SS Groups	1.9a (0.2)	8.5b (0.5)	8.2b (0.5)	8.6b (0.4)	8.5b (0.5)
Total Cysteine	26.7a (0.5)	29.5b (0.6)	28.9b (0.6)	30.2b (0.6)	30.1b (0.7)

a. Values in the same row with different letters are significantly different ($p < 0.05$).

b. Values in parentheses are standard deviation of means

The Raman spectrum obtained for the control kafirin showed peaks which could be associated with C-H stretching, amide I, C-H bending, amide III, phenylalanine, C-S stretching and S-S stretching regions (Fig 3.3.5, spectrum a) as identified by Ma et al (2000) and Chung et al (2005). This is consistent with what has been reported for other proteins such as red bean globulin protein (Meng et al 2003). However, the spectrum for the microwave heated kafirin showed a lot of fluorescence (Fig 3.3.5, spectrum b). This was possibly because microwave heating caused changes in the tryptophan residues that led them to fluoresce, as suggested by Shore and Parde (1956). Therefore, because of the high fluorescence of the microwaved samples, FT-Raman spectroscopy was not continued further.

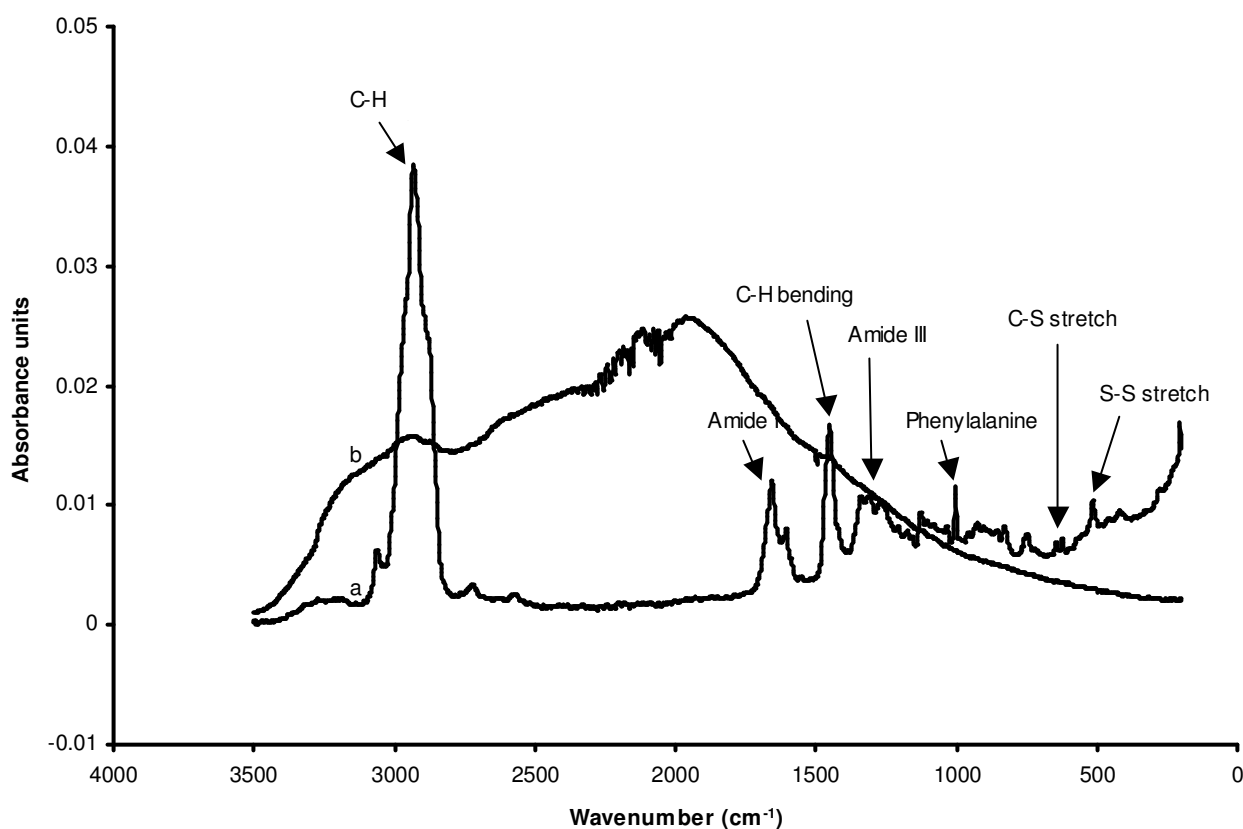


Fig. 3.3.5. FT-Raman spectroscopy of a, the control and b, microwaved kafirin

The FTIR spectra of microwaved kafirin (Fig. 3.3.6), plasticised films made from microwaved kafirin (Fig. 3.3.7), non-plasticised kafirin films that were microwaved cast (Fig. 3.3.8) and plasticised kafirin films that were microwaved cast (Fig. 3.3.9) had two broad bands, one between 1680–1600 cm^{-1} and another between 1550–1500 cm^{-1} . The bands appearing between 1680–1600 cm^{-1} and 1550–1500 cm^{-1} are termed Amide I and Amide II, respectively (Surewicz and Mantsch 1988). The amide I vibrations correspond mostly to C=O stretching, coupled with some contribution from CN stretch, CCN deformation and in plane NH bending modes of groups in the polypeptide chain. The amide I vibrations are directly related to the polypeptide backbone conformation and are therefore, highly sensitive to protein secondary structure (Bandekar 1992). Besides this, the protein signal is very strong in the amide I region (Singh 2000). Therefore, the protein conformational assignments, in this work, are based mainly on the spectral peaks in the amide I region. The amide II vibration is believed to be an out of phase combination of CN stretch and in plane NH deformation mode of groups in the polypeptide chain (Bandekar 1992). Although the vibrations in the amide II region are not as sensitive to secondary structure as those in amide I, they offer information that can be complementary to amide I (Bandekar 1992). In the amide I, the peak in the frequency range of 1650 to 1658 cm^{-1} can be assigned to protein α -helices, while that in frequency range 1620 to 1640 cm^{-1} has been assigned to the β -sheet structures (Duodu et al 2001). Assignment of the shoulder appearing at 1670–1690 cm^{-1} is unresolved. Peaks in this frequency range have been assigned to β -strands or β -turns (Surewicz and Mantsch 1988), β -turns (Subirade et al 1998; Rahmelow and Hubner 1996) or random coils (Haris 2000). Although the assignment of peaks in the 1670–1690 cm^{-1} range is unresolved, their appearance indicates the presence of other protein conformations apart from the α -helix and β -sheet structures. In the amide II region, α -helices can be assigned to 1545 to 1547 cm^{-1} and β -sheets to about 1515 to 1524 cm^{-1} (Duodu et al 2001)

The spectra of the kafirin samples (Fig 3.3.6) showed absorbance peaks for α -helical conformation (1650 cm^{-1}) and β -sheet conformation (1625 cm^{-1}) in the amide I region. In the amide II region, absorbance peaks for α -helical conformation (1545 cm^{-1}) and β -sheets (1524 cm^{-1}) appeared. The ratios of α -helical: β -sheet conformation of the

samples was estimated from the peak intensities in the spectra (Table 3.3.3). The ratio of these intensities is an indication of the relative amounts of α -helical and β -sheet structures. When wet kafirin was microwave heated, the ratio decreased from 1.49 in the untreated kafirin (control) to 1.04-1.10 in the kafirin that was microwave heated. Similarly, in the amide II region, the ratio of peak intensities for α -helices (1545 cm^{-1}) to β -sheets (1520 cm^{-1}) decreased from 1.92 in the control to 1.10-1.22 in the kafirin that was microwave heated. This indicates that microwave heating induced an increase in β -sheet formation accompanied by a decrease in α -helical conformation. This is consistent with earlier research in which wet cooking of sorghum was reported to increase the β -sheet with concomitant decrease in α -helical conformation of kafirin (Duodu et al 2001). In another study, Gao et al (2005) found that high extraction and drying temperatures used to isolate kafirin also increased the β -sheet structures with an accompanying decrease in α -helical conformation. Increase in β -sheet structure is in agreement with microwave induced kafirin polymerisation showed by SDS-PAGE (Figs. 3.3.1-3.3.4) as β -sheet formation is indicative of protein polymerisation (Haris 2000; Fink et al 2000; Ma et al 2001). Although these researchers (Haris 2000; Ma et al 2001; Gao et al 2005) used the formation of β -sheet structures as an indicator of protein cross-linking, they did not say whether it is the structural change that led to protein cross-linking or vice versa.

The protein conformation of the plasticised films made from microwave heated kafirin (Fig. 3.3.7) was slightly different from that of the microwaved kafirin (Fig. 3.3.6). The amide I region of the spectrum for the control film showed a broad peak with maximum absorbance for α -helical conformation (1656 cm^{-1}) (spectrum a). The broadening of this peak is an indication of presence of overlapping peaks possibly arising from other conformations apart from α -helices (Haris 2000). The peak showed a shoulder at about 1627 cm^{-1} , which can be assigned to β -sheets (Duodu et al 2001) and another shoulder at about 1675 cm^{-1} . As stated earlier, assignment of the peak at $1670\text{-}1690\text{ cm}^{-1}$ is unresolved i.e. it may be assigned to random coils, β -turns or β -strands. Spectra for plasticised films made from microwaved kafirin showed a high absorbance peak for β -sheets (1627 cm^{-1}), while the α -helical conformation (1650 cm^{-1}) appears as a shoulder (spectra b-f). The amide II region showed a high absorbance peak for α -helical conformation (1544 cm^{-1}) (Duodu et al 2001) and a lower absorbance peak for

β -sheets (1518 cm^{-1}) (Duodu et al 2001). In the amide I region, the estimated ratio of α -helices: β -sheet conformation decreased from 1.33 in the control to 0.69-0.79 in the films made from microwaved kafirin (Table 3.3.3). Similarly, in the amide II region, the ratio decreased from 6.14 in the control to 1.17-1.38 in the films made from microwaved kafirin. These data indicate an increase in β -sheets accompanied with a decrease in α -helices in the films, as was observed in the kafirin itself (Fig. 3.3.6, Table 3.3.3). It appears that the structure of the films made from microwaved kafirin was predominantly of β -sheet conformation as opposed to the control, which was predominantly α -helical in conformation. The predominantly β -sheet structure of the plasticised films made from microwaved kafirin could be related to the apparently low SS group content of these films as compared to the kafirin from which they were made (Table 3.3.1). As stated earlier, β -sheet formation is indicative of protein polymerisation. Thus, the predominance of this structure could mean folding of the protein in a manner that limits reagent accessibility to all SH and SS groups.

Comparison of the spectra of the plasticised films made from microwaved kafirin (Fig. 3.3.7) to those of the microwaved kafirin (Fig. 3.3.6) suggests that the film making procedure altered the secondary structure of kafirin, by increasing the proportion of β -sheets (Table 3.3.3). This is consistent with research, which showed that the secondary structure of soy protein changed from a mixture of β -sheet and unordered structures in the native state to predominantly β -sheet conformation in the film state (Subirade et al 1998). Although the film making procedure affected the kafirin secondary structure, the structure of the films showed a trend similar to that observed in the kafirin before film casting, i.e. a reduction in the α -helical conformation accompanied by an increase in the β -sheet conformation with microwaving.

The spectra for non-plasticised films that were microwaved cast showed a high absorbance peak for α -helical conformation (1650 cm^{-1}) and a shoulder for the β -sheet conformation (1620 cm^{-1}) (Fig. 3.3.8). The amide II region showed a high absorbance peak for α -helices (1542 cm^{-1}) and a low absorbance peak for β -sheet structure (1516 cm^{-1}). The ratio of α -helix to β -sheet in the amide I decreased from 1.78 in the control to 1.27-1.56 in the non-plasticised microwaved films (Table 3.3.3). In this case, there appeared to be a clear dose response effect, similar to that indicated by the SDS-

PAGE data (Fig. 3.3.3). Similarly, in the amide II region, the ratio decreased from 1.78 in the control to 1.22-1.56 in the non-plasticised microwaved films. These high ratios of α -helix to β -sheet indicate a slight increase in the β -sheet conformation with microwaving. However, the kafirin in the films was predominantly of α -helical conformation.

Spectra for the plasticised films that were microwaved cast in amide I region showed a high absorbance peak for α -helix conformation (1656 cm^{-1}) and a shoulder for β -sheet conformation (1620 cm^{-1}) spectra b-e (Fig. 3.3.9). The spectra for the control film showed a broad peak with maximum absorbance for α -helices (1656 cm^{-1}), a shoulder for β -sheets (1620 cm^{-1}) and another at about 1675 cm^{-1} , which can be assigned to β -turns, β -strands or random coils (spectrum a). As stated earlier, peak broadening is indicative of overlapping peaks attributable to α -helical, β -sheet and other conformations possibly β -turns, β -strands and/or random coils. In the amide II region, all samples showed a high absorbance peak for α -helical conformation (1545 cm^{-1}) and a shoulder for β -sheet (1519 cm^{-1}). In the amide region, the estimated ratio of α -helical: β -sheet conformation decreased from 1.33 in the control to 1.17-1.27 in the microwaved plasticised films. Again, in this case there was a dose-response effect, although this was not apparent in the SDS-PAGE data (Fig. 3.3.4). Similarly, in the amide II region, the ratio decreased from 6.14 in the control to 1.50-2.23 in the microwaved film (Table 3.3.3). The ratios indicate a slight increase in β -sheet conformation with microwaving although the films were also predominantly of α -helical conformation. The disappearance of the shoulder at about 1675 cm^{-1} in the control spectrum after microwaving (Fig. 3.3.9), suggests a decrease in the conformations such as β -strands, β -turns and random coils, with microwaving.

A comparison of the protein structure of the plasticised (Fig. 3.3.9) and non-plasticised films (Fig. 3.3.8) that were microwaved cast, shows that although they were both predominantly α -helical, there were differences. The amide I peaks for the plasticised films were broader than those of the non-plasticised ones, suggesting that the plasticised films were composed of more conformations such as β -strands, β -turns and random coils, in addition to the α -helical and β -sheet structures, while the non-plasticised films were mainly composed of the α -helical and β -sheet structures. This

was possibly due to the presence of plasticiser, which increases intermolecular free volume leading to increased molecular mobility (Sothornvit et al 2002), thus allowing formation of more secondary structures.

Microwaving of kafirin before film casting (Fig. 3.3.7) and microwaving after casting of plasticised films (Fig. 3.3.9) resulted in different protein structures in the films. The predominance of α -helical conformation following microwave heating of cast films contrasts with the predominance of β -sheet conformation in the plasticised films that were made from microwaved kafirin. A similar predominance of α -helical structure has been reported in zein that was heated in presence of linolenic acid ethyl ester (Mizutani et al 2004). It has been reported that factors such as water activity (Mizutani et al 2003) and pH (Lefevre and Subirade 1999; Meng and Ma 2001) influence heat effects on the secondary structure of proteins. It is therefore, possible that the environment in which the kafirin was at the time of microwaving affected the final protein structure in the film. Microwaving before film casting was done on kafirin wetted with water and the structure of this protein was consistent with what is reported in literature (Duodu et al 2001; Gao et al 2005). In fact, the kafirin structure reported by Duodu et al (2001) and Gao et al (2005) was after heating sorghum or kafirin in essentially a water medium. On the other hand, microwaving of cast films was, in effect, done on kafirin that had been exposed to glacial acetic acid during making of the film. Acids are known to deamidate proteins (Mimouni et al 1994). It is also possible that the difference in pH and water activity of the two microwaving environments contributed to the different protein conformation of the kafirin films following microwaving before and after film formation.

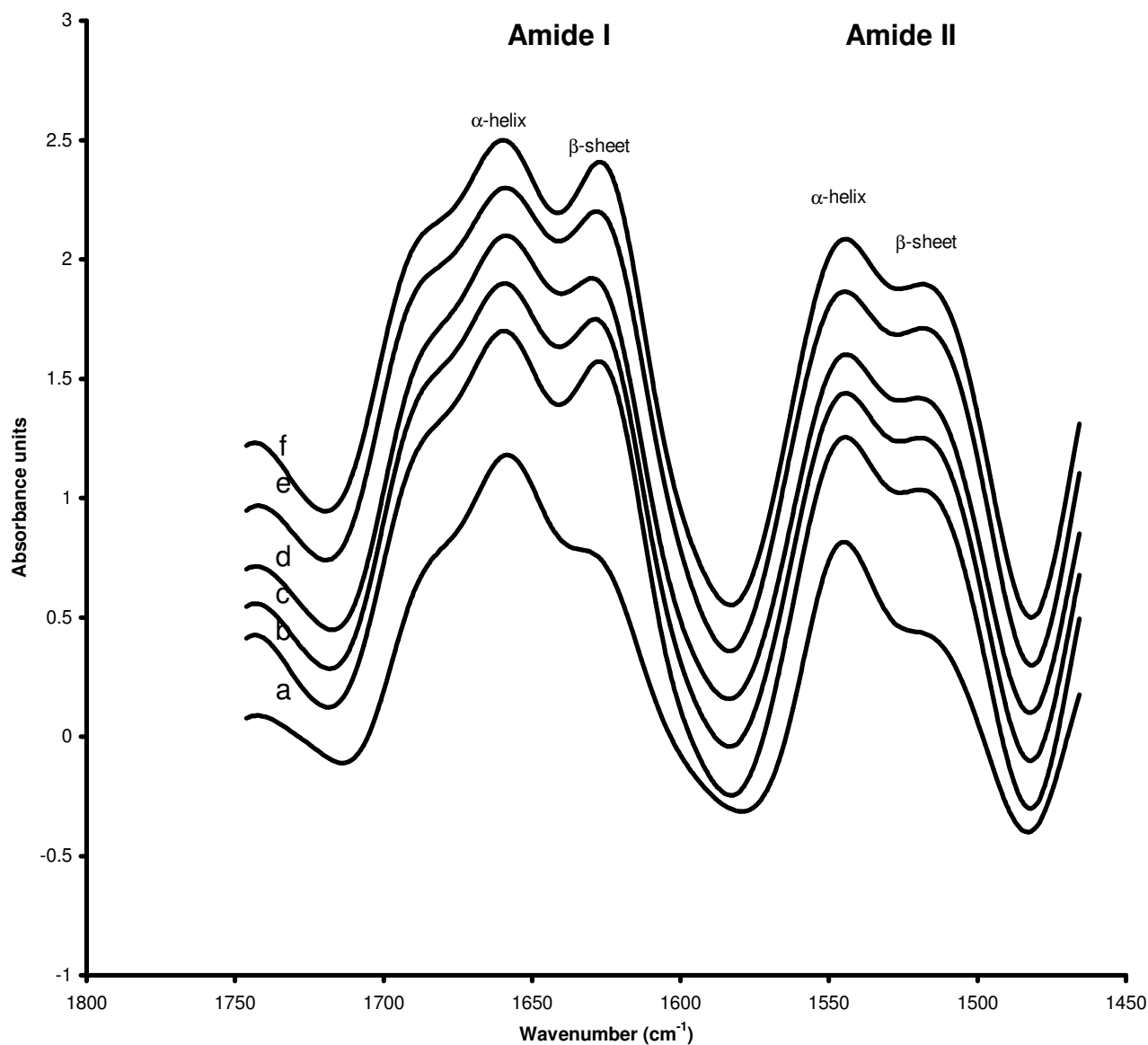


Fig. 3.3.6. FTIR spectra of kafirin that was microwaved at 96°C for different time periods. Spectrum a = control, b = 1 min, c = 2 min, d = 3 min, e = 4 min, f = 10 min.

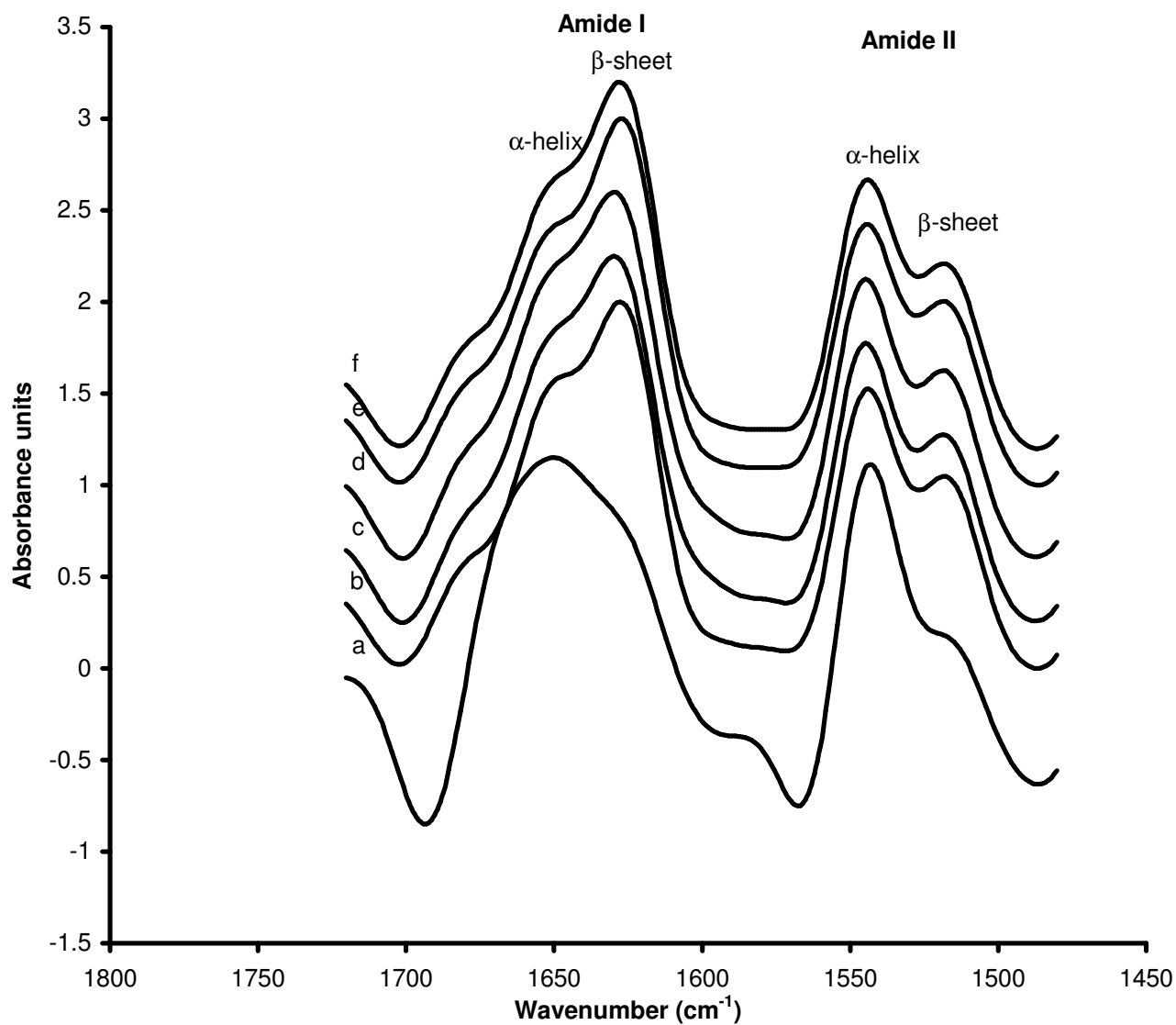


Fig. 3.3.7. FTIR spectra of plasticised films made from kafirin that was microwaved at 96°C for different time periods. Spectrum a = control, b = 1 min, c = 2 min, d = 3 min, e = 4 min, f = 10 min.

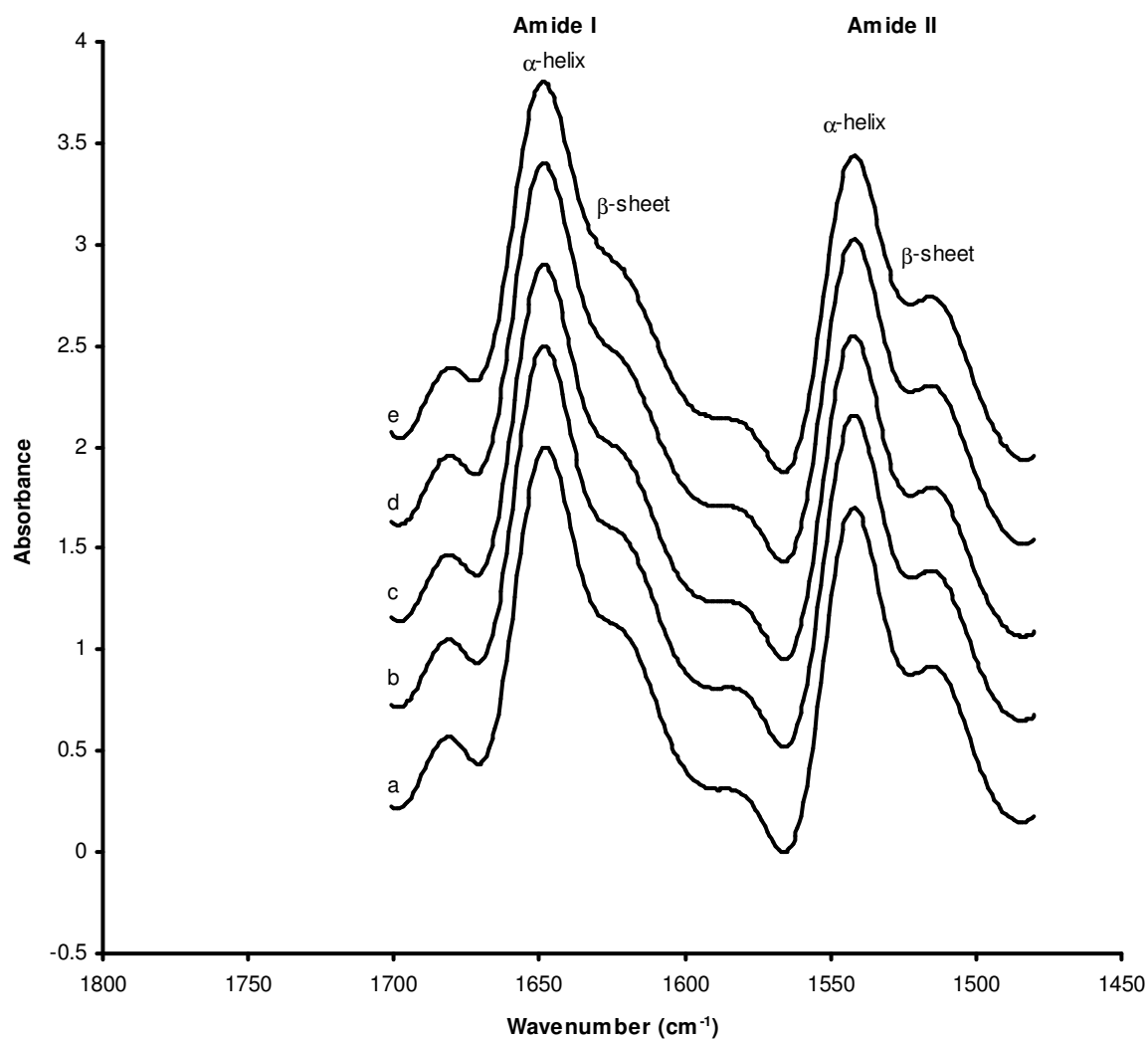


Fig. 3.3.8. FTIR spectra of non-plasticised kafirin films that were microwaved at 80 W for different time periods. Spectrum a = control, b = 1 min, c = 2 min, d = 3 min, e = 4 min.

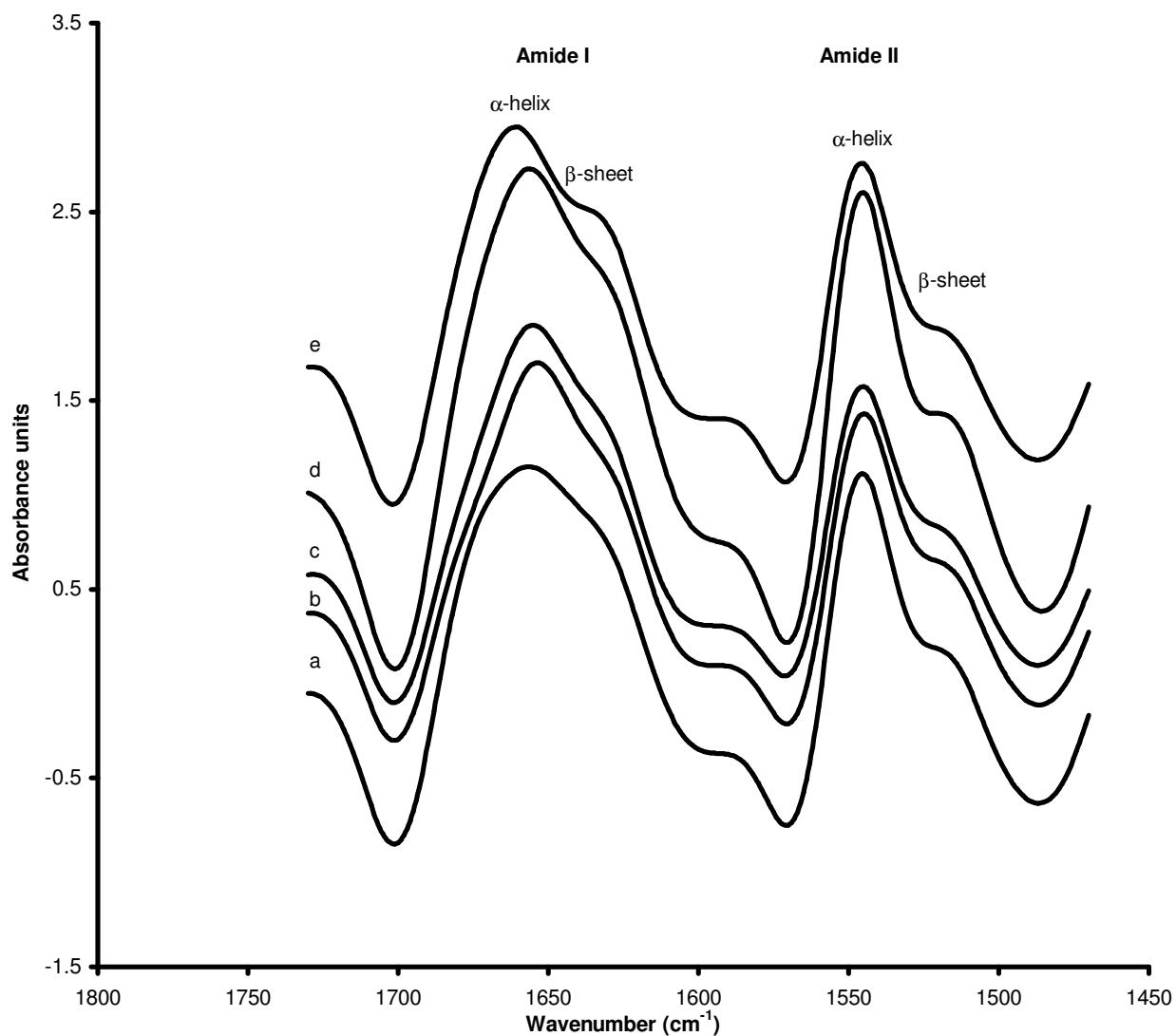


Fig. 3.3.9. FTIR spectra of plasticised kafirin films that were microwaved at 80 W for different times. Spectrum a =control, b = 1 min, c = 2 min, d = 3 min and e = 4 min.

TABLE 3.3.3 Estimated Ratio of α -Helical to β -Sheet Conformation of Kafirin that was Microwaved at 96°C, Plasticised Films Made from the Microwaved Kafirin and Non-plasticised and Plasticised Films that were Microwaved cast at 80 W

Microwaving time (min)	Amide I	Amide II
	α -Helices (1658 cm ⁻¹) to β - Sheets (1620 cm ⁻¹)	α -Helices (1545 cm ⁻¹) to β - Sheets (1516 cm ⁻¹)
KAFIRIN		
0 (Control)	1.49	1.92
1	1.10	1.22
2	1.10	1.12
3	1.10	1.12
4	1.04	1.10
10	1.04	1.10
FILMS MADE FROM MICROWAVED KAFIRIN		
0 (Control)	1.33	6.14
1	0.75	1.38
2	0.79	1.27
3	0.75	1.17
4	0.79	1.17
10	0.69	1.17
NON-PLASTICISED FILMS		
0 (Control)	1.78	1.78
1	1.56	1.56
2	1.44	1.38
3	1.38	1.27
4	1.27	1.22
PLASTICISED FILMS		
0 (Control)	1.33	6.14
1	1.33	2.23
2	1.27	1.86
3	1.17	1.86
4	1.17	1.50

3.3.4. Conclusions

Microwaving induces increases in the SS cross-linking of kafirin and changes the secondary structure of the protein in the film state from α -helical to β -sheet conformation. However, its effects depend on the stage when microwaving is applied during the film making process. Microwaving of kafirin before and after film casting caused a decrease in α -helical conformation. However, the films made from microwaved kafirin were predominantly of β -sheet conformation while those microwaved after casting were predominantly α -helical in conformation. The kafirin conformation in films depends not only on microwaving and when it is applied, but also on the plasticiser and solvent effects on intermolecular free volume and on protein unfolding. The effects of wet microwave heating on kafirin cross-linking and secondary structure are not fundamentally different from those of conventional wet heating methods.

3.3.5. Literature cited

- Ali, Y., Ghorpade, V. M., and Hanna, M. A. 1997. Properties of thermally-treated wheat gluten films. *Ind. Crops. Prod.* 6:177-184.
- Bandekar, J. 1992. Amide modes and protein conformation. *Biochimica et Biophysica Acta* 1120:123–143.
- Buffo, R. A., Weller, C. L., and Gennadios, A. 1997. Films from laboratory-extracted sorghum kafirin. *Cereal Chem.* 74:473-475.
- Chan, K-Y., and Wasserman, B. P. 1993. Direct colorimetric assay of free thiol groups and disulfide bonds in suspensions of solubilized and particulate cereal proteins. *Cereal Chem.* 70:22-26.
- Chung, M. W. Y., Lei, B., and Li-Chan, E. C. Y. 2005. Isolation and structural characterisation of the major protein fraction from NorMan flaxseed (*Linum usitatissimum* L.). *Food Chem.* 90:271-279.
- Da Silva, L. S., and Taylor, J. R. N. 2005. Physical mechanical and barrier properties of kafirin films from red and white sorghum milling fractions. *Cereal Chem.* 82:9-14.

- Duodu, K. G., Taylor, J. R. N., Belton, P. S., and Hamaker, B. R. 2003. Factors affecting sorghum protein digestibility. *J. Cereal Sci.* 38:117–131.
- Duodu, K. G., Tang, H., Grant, A., Wellner, N., Belton, P. S., and Taylor, J. R. N., 2001. FTIR and solid-state ^{13}C NMR spectroscopy of proteins of wet cooked and popped sorghum and maize. *J. Cereal Sci.* 33:261–269.
- Duodu, K. G., Nunes, A., Delgadillo, I., Parker, M. L., Mills, E. N. C., Belton, P. S., and Taylor, J. R. N. 2002. Effect of grain structure and cooking on sorghum and maize in vitro protein digestibility. *J. Cereal Sci.* 35:161–174.
- El Nour, I. N. A, Peruffo, A. D. B., and Curioni, A. 1998. Characterisation of sorghum kafirins in relation to their cross-linking behaviour. *J. Cereal Sci.* 28:197–207.
- Emmambux, N. M., Stading, M., and Taylor, J. R. N. 2004. Sorghum kafirin film property modification with hydrolysable and condensed tannins. *J. Cereal Sci.* 40:127-135.
- Farag, R. S., Hegazy, R. A., El-Khawas, K. H., and Farag, M. M. 2001. Electrophoretic patterns of microwaved and γ -irradiated beef liver proteins. *J. Sci. Food Agric.* 81:975-982.
- Fink, A. L., Seshadri, S., Khurana, R., and Oberg, K. A. 2000. Determination of secondary structure in protein aggregates using attenuated total reflectance FTIR. Pages 132-144 in: *Infrared Analysis of Peptides and Proteins: Principles and Applications*. B. R. Singh, ed. American Chemical Society: Washington DC, USA.
- Gao, C., Taylor, J., Wellner, N., Byaruhanga, Y. B., Parker, M. L., Mills, E. N. C., and Belton, P. S. 2005. Effect of preparation conditions on protein secondary structure and biofilm formation of kafirin. *J. Agric. Food Chem.* 53:306-312.
- Gennadios, A., Ghorpade, V. M., Weller, C. L., and Hanna, M. A. 1996. Heat curing of soy protein films. *Trans. Amer. Soc. Agric. Eng.* 39:575-579.
- Haris, P. I. 2000. Fourier transform infrared spectroscopic studies of peptides: potentials and pitfalls. Pages 54-95 in: *Infrared Analysis of Peptides and Proteins Principles and Applications*. B. L. Singh, ed. American Chemical Society: Washington DC, USA.
- Kaya, S., and Kaya, A. 2000. Microwave drying effects on properties of whey protein isolate edible films. *J. Food Eng.* 43:91-96.
- Lefevre, T., and Subirade, M. 1999. Structural and interaction properties of β -lactoglobulin as studied by FTIR spectroscopy. *Int. J. Food Sci. Technol.* 34:419-428.

- Li-Chan, E. 1983. Heat induced changes in the proteins of whey protein concentrate. J. Food Sci. 48:47-56.
- Ma, C. Y., Rout, M. K., and Mock, W. Y. 2001. Study of oat globulin conformation by Fourier transform infrared spectroscopy. J. Agric. Food Chem. 49:3328-3334.
- Ma, C. Y., Rout, M. K., Chan, W. M., and Phillips, D. L. 2000. Raman spectroscopic study of oat globulin conformation. J. Agric. Food Chem. 48:1542-1547.
- Meng, G. T., and Ma, C. Y. 2001. Fourier-transform infrared spectroscopic study of globulin from *Phaseolus angularis* (red been). Int. J. Biol. Macromol. 29:287-294.
- Meng, G. T., Ma, C. Y., and Phillips, D. L. 2003. Raman spectroscopic study of globulin from *Phaseolus angularis* (red bean). Food Chem. 81:411-420.
- Mimouni, B., Raymond, J., Merle-Desnoyers, A. M., Azanza, J. L., and Ducastaing, A. 1994. Combined acid and enzymic hydrolysis for improvement of functional properties of wheat gluten. J. Cereal Sci. 21:153-165.
- Mizutani, Y., Matsumura, Y., Murakami, H., and Mori, T. 2004. Effects of heating on the interaction of lipid and zein in a dry powder system. J. Agric. Food Chem. 52: 3570-3576.
- Mizutani, Y., Matsumura, Y., Imamura, K., Nakanish, K., and Mori, T. 2003. Effects of water activity and lipid addition on secondary structure of zein in powder systems. J. Agric. Food Chem. 51:229-235.
- Morel, M-H., Bonicel, J., Micard, V., and Guilbert, S. 2000. Protein insolubilization and thiol oxidation in sulfite treated wheat gluten films during aging at various temperatures and relative humidities. J. Agric. Food Chem. 48:186-192.
- Nunes, A., Correia, I., Barros, A., and Delgadillo, I. 2004. Sequential in vitro pepsin digestion of uncooked and cooked sorghum and maize samples. J. Agric. Food Chem. 52:2052-2058.
- Owusu-Apenten, R. K., Chee, C., and Hwee, O. P. 2003. Evaluation of a sulphhydryl-disulphide exchange index (SEI) for whey proteins: β -lactoglobulin and bovine serum albumin. Food Chem. 83:541-545.
- Perez-Gago, M. B., and Krochta, J. M. 2001. Denaturation time and temperature effects on solubility, tensile properties and oxygen permeability of whey protein edible films. J. Food Sci. 66:705-710.
- Perez-Gago, M. B., Nadaud, P., and Krochta, J. M. 1999. Water vapor permeability, solubility and tensile properties of heat denatured versus native whey protein films. J. Food Sci. 64:1034-1037.

- Quinn, G., Monahan, F. J., O’Riordan, E. D., O’Sullivan, M., and Longares, A. 2003. Role of covalent and non-covalent interactions in the formation of films from unheated whey protein solutions following pH adjustment. *J. Food Sci.* 68:2284-2288.
- Rahmelow, K., and Hubner, W. 1996. Fourier self-deconvolution: parameter determination and analytical band shapes. *Appl. Spectrosc.* 50:795-804.
- Roy, S., Weller, C. L., Gennadios, A., Zeece, M. G., and Testin, R. F. 1999. Physical and molecular properties of wheat gluten films cast from heated film-forming solutions. *J. Food Sci.* 64:57–60.
- Shimada, K., and Cheftel, J. C. 1988a. Determination of sulfhydryl groups and disulfide bonds in heat-induced gels of soy protein isolate. *J. Agric. Food Chem.* 36:147-153.
- Shimada, K., and Cheftel, J. C. 1989. Sulfhydryl group/disulfide bond interchange reactions during heat-induced gelation of whey isolate. *J. Agric. Food Chem.* 37:161-168.
- Shore, V. G., and Parde, A. B. 1956. Fluorescence of some proteins, nucleic acids and related compounds. *Arch. Biochem. Biophys.* 60:100-107.
- Shull, J. M., Watterson, S. J., and Kirleis, A. W. 1991. Proposed nomenclature for the alcohol-soluble proteins (kafirins) of *Sorghum bicolor* (L. Moench) based on molecular weight, solubility and structure. *J. Agric and Food Chem.* 39:83-87.
- Singh, B. R. 2000. Basic aspects of the technique and applications of infrared spectroscopy of peptides and proteins. Pages 2-37 in: *Infrared Analysis of Peptides and Proteins: Principles and Applications*. B. R. Singh, ed. American Chemical Society: Washington DC, USA.
- Sothornvit, R., Reid, D. S., and Krochta, J. M. 2002. Plasticizer effect on the glass transition temperature of beta-lactoglobulin films. *Trans. Amer. Soc. Agric. Eng.* 45:1479-1484.
- Subirade, M., Kelly, I., Gueguen, J., and Pezolet, M. 1998. Molecular basis of film formation from soybean protein: comparison between the conformation of glycinin in aqueous solution and in films. *Int. J. Biol. Macromol.* 23:241-249.
- Surewicz, W. K., and Mantsch, H. H. 1988. New insight into protein secondary structure from resolution-enhanced infrared spectra. *Biochimica et Biophysica Acta* 952:115–130.

- Taylor, J., Taylor, J. R. N., Dutton, M. F., and De Kock, S. 2005a. Identification of kafirin film casting solvents. *Food Chemistry* 90:401-408.
- Thannhauser, T. W., Konishi, Y., and Scheraga, H. A., 1987. Analysis for sulphide bonds in peptides and proteins. *Methods Enzymol.* 143:115-119.
- Valentinova, O., Novotina, Z., Svoboda, Z., Schwarz, W., and Kas, J. 2000. Microwave heating and γ -irradiation treatment of rapeseed (*Brassica napus*). *J. Food Lipids* 7:237–245.
- Wedemeyer, W. J., Welker, E., Narayan, M., and Scheraga, H. A. 2000. Disulfide bonds and protein folding. *Biochem.* 39:4209-4216.

4. DISCUSSION

This chapter first discusses the methods used in this work, followed by a discussion of the microwave induced conformational changes and cross-linking in the kafirin and kafirin films. The effectiveness of microwaving in the modification of kafirin film properties is then compared to that of other methods of film modification. Lastly, some potential uses of microwave modified kafirin films are explored.

4.1. Methodological considerations

Much of the work done on the effect of microwaving on proteins has involved investigation of microwave-induced changes on the electrophoretic pattern of the proteins (Farag et al 2001; Valentinova et al 2000). Little work has been reported on the use of microwaves to modify protein films. Kaya and Kaya (2000) applied microwaves to whey protein films and found an increase in the film tensile strength. This being the first work on the application of microwaves to modify kafirin film properties, two microwaving procedures, namely microwaving of dry and wetted kafirin powder before film casting and microwaving of kafirin films after casting were investigated. The implications of the procedures are discussed in terms of process control and effectiveness in modification of film properties. The major analytical methods used are discussed in terms of their principles, advantages and limitations. Any modifications to the methods that were used are also discussed.

The use of microwaves to modify kafirin film properties posed a number of challenges, for example control of the amount of energy to which the samples were exposed. Microwave heating of dry kafirin powder resulted in undesirable heating effects, as shown by the burnt areas in the powder and had no effect on the tensile properties of kafirin films (Table 3.1.2). A major problem with microwave heating of food products is its inherently non-uniform heating effect, probably because of the low thermal conductivity of some food materials and changes in the microwave penetration into the food (Giese 1992).

Microwave heating of wetted kafirin appeared to allow better control of the microwave energy to which a sample was exposed, as compared to microwaving of kafirin powder. This was probably because of the increased thermal conductivity and dielectric constant in the wetted kafirin. Water has a high thermal conductivity (Szczesniak 1983) and dielectric constant (Wohlfarth 1974), as compared to other food components. Control of the amount of microwave energy that the samples received was achieved by applying fixed microwave power levels or by microwave heating of the samples at fixed temperatures for fixed time periods. However, the exact amount of microwave energy that was absorbed and utilised to effectively heat and modify the sample was not known.

Ideally the power or energy absorbed by a sample in a microwave heating process is estimated using the equation $P = 55.61 \times 10^{-14} E^2 f \epsilon' \epsilon''$, where P is the power absorbed by sample (W/cm^{-3}), E is the electrical field strength (V/cm), f is the microwave frequency (Hz), ϵ' is the dielectric constant of sample and ϵ'' is the loss factor of sample (Singh and Heldman, 1993). However, the E for the samples could not be determined due the lack of an appropriate probe. Being an important factor in the equation, the absorbed energy could not be determined using this proper approach. Therefore, the absorbed energy was estimated using the equation for conventional heating processes, as described in section 3.1.2.3. The value of the conventional method to estimate energy absorbed by a sample in a microwaving process is limited since it depends on the mass, specific heat capacity and temperature change of the sample. Yet, as stated above, in a microwaving process, energy absorbed by the sample depends on the electric field strength, the dielectric constant and loss factor and the frequency of the microwaves. The limitation of the conventional method is illustrated in the following example: 247.1 J/g absorbed by wet kafirin increased the film tensile strength from 1.14 to 1.69 MPa (Table 3.1.4). Conversely, lower energy of 236.3 J/g absorbed by wet kafirin increased the film tensile strength from 1.37 to 2.43 MPa (Table 3.1.5). Such limitations notwithstanding, the estimated absorbed energy (EAE) was related to the film tensile strength and can help explain the effects of microwave heating.

To achieve control of microwave energy applied to wetted kafirin, the following three microwaving procedures were used: (1) microwave heating at increasing power levels

for 1 min, (2) microwave heating at a fixed power to selected temperature targets and holding at these temperatures for 1 min, (3) microwave heating to selected temperatures and holding at these temperatures, in the microwave unit, for different time periods (Chapter 3.1). With the first procedure, the linear increase in microwave power did not necessarily result in a linear increase in the temperature of the sample (Table 3.1.4). This was possibly because increase in temperature changes the dielectric constant and loss factor of different food materials, which variably affects heat generation as the microwaving progresses (Miller et al 1991; Sipahioglu and Barringer 2003; Wang et al 2003b). With the second procedure, the target temperatures in a sample could not easily be achieved, since there was a 2-7°C variation from the target temperatures (Table 3.1.3). This can be attributed to the variation of the actual amount of power delivered by the microwave unit. The limitations in process control notwithstanding, microwave power applied to wet kafirin did relate to increase in kafirin temperature and to the film functional properties (Chapter 3.1). The third procedure, where microwave power was electronically controlled to achieve fixed temperatures in the samples for given time periods, gave the most repeatable settings, which can be attributed to the fast and automated nature of the electronic control system.

Other challenges with using microwave energy to modify kafirin film properties were related to the equipment design and suitability. The wave-guide for microwave heating kafirin before film casting was specifically designed for that purpose. Hence, the microwaves were easily concentrated on to the sample. Further, the sample temperature was monitored and controlled during microwaving using a fibre optic temperature sensor and recording system. The temperature sensor was immersed in the sample during microwave heating, hence temperature monitoring was on line and in real time. The recording and regulating system automatically regulated the microwave power to maintain the desired temperature. However, the equipment used for microwaving cast films (Fig. 3.2.1) was designed for other purposes. Therefore, the microwaving of cast films had to be adapted to fit the equipment. The films had to be cut to a size (18 x 66 mm) that the quartz tube of the microwaving unit could take. This adaptation had implications in the determination of WVP of the kafirin films that were microwaved cast.

Regarding film temperature determination, the nature of the film samples and the design constraints of the microwaving apparatus did not allow the film temperature to be measured during microwaving. This was because adequate contact between the film and the temperature sensor could not be ensured. Therefore, the temperature was determined using a non-contact infrared thermometer immediately after microwaving. Thus, even the short delay (10-20 sec) between the end of microwaving and temperature measurement could have led to underestimation of the film temperatures. It is therefore probable that the films were microwaved to higher temperatures than those reported (Table 3.2.1). However, even with this limitation, the temperature readings obtained showed a meaningful trend with the microwave power used.

WVP was determined according to the procedure described by Taylor et al (2005a) who used Schott bottles (100 mL). However, the small size of films that were microwaved cast (18 x 66 mm) necessitated the use of a smaller vessel. Therefore, for plasticised films that were microwaved cast, small glass vials (4 mL screw neck, 45 x 14.7 mm) were used. The diameter of the neck of both types of vessels was smaller than the rest of the vessel body. This could have affected the rate of water evaporation from the vessels. To minimise this source of error, the water was filled up to the neck of the vessels that had the same diameter as the opening. An additional problem with WVP measurement was that as the moisture was lost during the test, the stagnant air gap between the film and water surface in the vessels increased. According to Gennadios et al (1994a), the stagnant air gap can resist water vapour transmission through the film, resulting in underestimation of the film WVP. Besides this, the WVP of films is affected by film thickness (Park and Chinnan 1995). Therefore, the procedure described by Gennadios et al (1994a) to compensate for the effects of the stagnant air gap and film thickness was followed in calculating the film WVP.

The thickness of the films was determined before mounting them onto the vials and Schott bottles. However, prolamins can absorb moisture and swell (Gontard et al 1993). It is therefore, possible that during the test period, the kafirin in the films absorbed moisture and swelled, thus altering film thickness. Such unforeseen changes in film thickness were not accounted for and could have affected the WVP values.

The WVP in the vials was higher than that in the Schott bottles (Table 3.1.6). In the method used to determine WVP, the relative humidity inside the bottle is taken to be 100% (Gennadios et al 1994a). Therefore, as a result of the smaller volume between the film and the water surface in the vials as compared to the Schott bottle, the internal vapour pressure in the vials could have been higher than that in the Schott bottles. In addition, the difference in height to volume ratio of the air gaps for the two vessels was large. The ratio for the vials and Schott bottles was 7 mm/ 352mm³ and 22 mm/ 4009 mm³, respectively. This difference could have affected the dynamics of moisture evaporation in the respective vessels. It is probably because of these differences in internal vapour pressure and height to volume ratio that the vials gave a higher WVP than the Schott bottles. Nonetheless, the WVP readings obtained from both the vials and Schott bottles showed the same trend. This indicates that the use of both types of vessels was valid.

The tensile properties of the films were determined essentially according to the procedure described by Taylor et al (2005a). The method is based on the ASTM method D882-97 (ASTM 1998). The ASTM method requires that the films be conditioned and their properties determined in a room where the temperature and RH are controlled. Although the room used had its temperature maintained at 22°C, its RH was not controlled. Therefore, to minimize the variations arising from RH fluctuations, the filmstrips were first conditioned at 50% RH and 22°C for 48 hr. The filmstrips were kept at this RH and only exposed to ambient RH, which was generally lower than 50%, during the actual measurement of tensile properties. Measurement of the tensile properties was done within 1-2 min of removal from the 50% RH dessicator. However, it is possible that the small changes in RH during the actual measurements could have led to variations in the determinations of tensile properties. Notwithstanding this, the tensile properties obtained using this procedure could be meaningfully related to the microwave treatments applied.

Protein digestibility would ideally be determined by conducting *in vivo* studies using humans. However, *in vivo* assays are expensive, time consuming and they raise a lot of ethical issues. Therefore, an *in vitro* method that is rapid and correlates with *in vivo* studies would be the method of choice. *In vitro* methods for determining protein digestibility may involve the use of single or multiple proteolytic enzymes. The multiple

enzyme systems include pepsin-pancreatin (Youssef 1998), pepsin-trypsin (El Maki et al 1999) and pepsin and α -chymotrypsin (Yamada et al 1995). However, multiple enzyme systems are cumbersome due to the involvement of multiple digestions and washings. Also, this may not correlate well with the pepsin method (Fombang 2005). Some single enzyme assays make use of trypsin (Le Tien et al 2000) or pronase (Hahn et al 1982). However, the most widely used single enzyme application involves the use of pepsin (Hamaker et al 1987; Duodu et al 2002; Ou et al 2004; Nunes et al 2004). The nitrogen solubilisation values of the *in vitro* assay using pepsin correlate with the *in vivo* findings of Maclean et al (1981) who investigated sorghum protein digestibility in humans. The digestibility values here were obtained using the pepsin digestion method described by Duodu et al (2002). The procedure was slightly modified by using a smaller sample size (10 mg protein) instead of 20 mg protein used by Duodu et al (2002). The modification was done so as to obtain digestion residues that were small enough to fit in the sample port for the LECO Nitrogen/Protein Analyser instrument, which was used for determination of protein content. The results obtained for microwaved kafirin show a reduction in digestibility, which is the same as that reported by Duodu et al (2002) and Nunes et al (2004) after wet heating of sorghum. This indicated that modification of the procedure did not affect the validity of the results.

An appropriate standard method to evaluate the biodegradability of protein films has not been established. Researchers have used various methods including subjecting the films to single- or multiple-enzyme digestions as an indicator of film biodegradability. Enzymes such as pancreatin (Le Tien et al 2000) and pepsin and α -chymotrypsin (Yamada et al 1995) have been used. Other methods involve subjecting the films to microbial cultures. For example, Mezgheni et al (1998) and Lacroix et al (2002) used a pure culture of *Pseudomonas aeruginosa* to determine protein film biodegradability. The main limitation with the use of proteolytic enzymes and microbial cultures in determining film biodegradability is that none of these methods represent the conditions in landfills, the most common waste disposal method.

In this work, biodegradability of kafirin films was determined using a method based on the ASTM method D5512-96 for exposing plastics to a simulated compost environment (ASTM 1996). The method involved burial of the kafirin film samples in a

well-rotted horse manure and sawdust-based compost, at 37 and 60% moisture content. This was, perhaps, a closer representation of landfill conditions as compared to the enzyme or pure culture based methods. Ideally, it would have been useful to obtain data on changes in weight, size, moisture and nitrogen content of the buried films. However, such data could not be obtained because a thorough cleaning of the buried films, to rid them of all adhering compost material, was not possible and the amount of compost adhering to the films could not be estimated. Therefore, the films could only be scanned and qualitatively described. Nonetheless, the qualitative data obtained showed differences between the treated and the control film samples.

Electrophoresis refers to the migration of charged particles in an electric field. SDS-PAGE is a widely used analytical technique for the separation and characterisation of complex mixtures of proteins and for the estimation of their relative molecular weight (M_r) (Makowski and Ramsby 1997). SDS, an anionic detergent is used to denature the proteins giving them all the same conformational properties and to prevent protein interaction during electrophoresis. It also masks the intrinsic protein charge and gives all proteins a similar negative charge so that they migrate toward the anode. Proteins are then separated solely based on size (Makowski and Ramsby 1997). Unlike single concentration gels, gradient gels comprise a gradient of polyacrylamide concentration. Thus, these gels contain a range of pore sizes and therefore, are capable of resolving a wider range of protein M_r than single concentration gels. In addition, gradient gels give sharper protein bands for lower molecular weight proteins, since the proteins are about the same size as the gel pores (Bollag et al 1996). In this work single concentration gels of 12% acrylamide and gradient gels of 3.9-12% and 3.9-15% acrylamide were used. The 3.9-15% gradient gels (Fig. 3.1.3) showed somewhat better resolution of the protein species as compared to the first two (Figs. 3.1.2 and 3.3.1).

In the SDS-PAGE work it appears that the M_r of some kafirin species was underestimated. The γ -, $\alpha 1$ - and $\alpha 2$ -kafirin appeared at M_r 25,000, 23,000 and 21,000, respectively. According to Shull et al (1991), the M_r of γ -kafirin is 28,000 and that of α -kafirins is 23,000-25,000. However, the β -kafirin appeared at M_r 18,000, which is in agreement with that reported by Shull et al (1991). This apparent underestimation of the γ - and α -kafirin was possibly because the molecular weight marker at 26,600 was

not correct. The apparent underestimation could also have been a result of the difference in heating times of the samples and molecular markers. The molecular markers were heated for 3 min, while the samples were heated for up to 20 min to ensure complete dissolution. As stated, in SDS-PAGE, the detergent, SDS is used to give the protein molecules an overall net negative charge so that they all migrate towards the anode (Hames 1990). Therefore, uniform SDS binding is critical for appropriate electrophoretic migration. However, some SDS bound proteins have unequal charge density or atypical shapes and therefore, demonstrate anomalous electrophoretic behaviour, possibly due to differences in SDS binding (Makowsky and Ramsby 1997). Heating of the samples in SDS improves the capacity of the protein to bind with the detergent (Hames 1990). It is therefore, possible that due to the long heating time during sample dissolution, the kafirins bound more SDS compared to the markers thus attaining a higher net negative charge density resulting in a higher mobility as compared to the molecular markers. In spite of the apparent underestimation of the α - and γ -kafirins, the SDS-PAGE patterns of the kafirin species were consistent with those reported by other workers, for example Duodu et al (2002).

The most commonly used techniques to determine the glass transition temperature (T_g) of a polymeric material are differential scanning calorimetry (DSC) and dynamic mechanical analysis (DMA). DSC is a technique whereby the difference in energy input into a substance and a reference material is measured as a function of temperature while both materials are subjected to a programmed heating or cooling cycle (Karim et al 2000). With DSC, the glass transition is detected by a step change of the heat capacity on heating or cooling. The temperature at the midpoint of the slope of the DSC heat capacity change is taken as the T_g (Hohne et al 1996). In DMA, a periodic or oscillating stress or strain is applied to a sample and the resulting strain or stress response is measured at given temperatures. The storage modulus of the sample is determined from the measured stress and strain and plotted against temperature. The T_g of the sample is determined from the point of intersection of the lines interpolated from the maximum storage modulus and the section of logarithmic decrease in the storage modulus (Rieger 2001).

DMA of microwaved and non-microwaved kafirin films was done with temperature scans of -10 to 60°C at $5^\circ\text{C}/\text{min}$ to determine the T_g of the films. DMA is more

sensitive at measuring T_g of heteropolymers and amorphous homopolymers such as kafirin films as compared to DSC. This is possibly because DMA is able to measure small changes in the modulus and damping effect in such polymers over a wide temperatures range (Gearing 1999). Only samples of non-plasticised kafirin films were subjected to DMA. This is because the DMA was done at SIK in Sweden and only a few samples could be analysed for this work. Since plasticisation lowers the T_g of protein films (Lawton 2004), non-plasticised films were chosen for analysis to determine the effect of microwaving on film T_g without plasticiser influence.

Protein conformation would have been best determined by X-ray diffraction, which can give the precise static three-dimensional structure of proteins (Glusker et al 1994). However, it requires that the protein sample be in crystal form and proteins are not easily crystallised (Drenth 1994). Besides, functionality of proteins is not realised in crystal form but in colloids, emulsions, gels or solutions. Another method widely used for studying protein structure is circular dichroism (CD) spectroscopy. However, CD is limited by the requirement that the protein be in an optically clear solution (Singh 2000), yet kafirin samples for this study were in film form. Thus, FTIR and FT-Raman spectroscopy were used to determine microwave induced structural changes in kafirin films.

Infrared spectroscopy can be used to study protein conformation because the vibrational modes of atoms in the polypeptide chain are sensitive to changes in chemical structure, conformation and environment (Lin-Vien et al 1991). Thus, the frequency associated with these vibrations can yield information about conformation and orientation of the main polypeptide chain (Fraser and Suzuki 1970). The three major spectral regions for proteins are around 1550 cm^{-1} (Amide II) 1650 cm^{-1} (Amide I) and 3300 cm^{-1} (Amide A). Since the amide bands of proteins are conformation sensitive, they can be associated with structures such as the α -helices, β -sheets, β -turns and unordered structures of the polypeptide (Surewicz and Mantsch 1988). Regarding kafirin, FTIR spectroscopy has been used to study the effects of wet heating (Duodu et al 2001), tannin complexation (Emmambux 2004) and different extraction and drying methods (Gao et al 2005) on protein secondary structure.

FTIR spectroscopy can be applied to solid or liquid samples. However, sample preparation is important in the application of FTIR. Commonly used sample preparation for solid samples are the KBr (potassium bromide) discs and the attenuated total reflectance (ATR) techniques. ATR is particularly suitable for samples in form of pastes, films and coatings (Westra and Van Woerkom 1987). Proper use of KBr discs requires that the sample is dry, finely ground and evenly distributed in the disc. In addition, there should be no reaction between the sample and KBr to form new molecular species (McClure 1987). In the ATR technique the sample is held as a thin layer pressed onto the surface of a material with high refractive index like a diamond crystal. Infrared light is launched through the crystal where it is reflected many times to form an evanescent wave before it enters the sample. This makes the ATR technique sensitive to small changes in protein structure (Fink et al 2000).

Microwaved then freeze-dried kafirin powder samples meant for FTIR were prepared in KBr discs. These samples produced spectra that agreed with those reported by Duodu et al (2001). Therefore, the spectra were used in determining the conformation of microwaved kafirin. However, film samples prepared in KBr discs produced weak and inconsistent spectra, possibly because of the poor distribution of the ground film in the KBr discs. When the kafirin film samples were prepared and spectra collected using the ATR technique, the spectra obtained showed peaks that could be used to determine the conformation of the kafirin in the films. Consequently, the spectra of film samples reported here were obtained using the ATR technique. It is possible that the plasticiser (composed of lactic acid, polyethylene glycol-400 and glycerol) and moisture in the films interfered with the spectra. However, the spectra of lactic acid (Salvador et al 2001) and polyethylene glycol-400 (Wang et al 2003a) do not show absorbance peaks in the Amide I and II regions. Only, water (Singh 2000) and glycerol (Nieuwoudt et al 2004) have absorbance peaks between 1680 and 1430 cm^{-1} , which could therefore, interfere with protein peaks in the Amide I region. In addition, according to Singh (2000), the peaks assigned to α -helix and random coils overlap at about 1650 cm^{-1} thus creating difficulty in assigning these two conformations. Nonetheless, the Amide I region is still considered useful in studying conformational changes in proteins (Singh 2000) and it gave meaningful spectra in this work.

Raman spectroscopy is another form of vibrational spectroscopy used in studying protein secondary structure. It is based on scattering of light by vibrating protein molecules. The scattered light is transformed into Raman spectra, i.e. spectra showing peaks that can be associated with the vibration of particular molecular groups (Schrader 1995a). Among other molecular groups, Raman spectroscopy provides information about the conformational changes involving the C-S, S-H and S-S groups. The absorption for the S-containing groups is weak in infrared spectroscopy but strong in Raman spectroscopy. Therefore, infrared and Raman spectroscopy are used complimentary to one another so as to get a better understanding of protein secondary structure (Grasselli et al 1981). Since SDS-PAGE had indicated an involvement of microwave induced SS bonds in kafirin (Figs. 3.1.2 and 3.1.3), their nature and extent of involvement was investigated further. Therefore, FT-Raman spectroscopy was used to study the changes in the C-S, S-S and S-H groups of kafirin resulting from microwave treatments. However, while the control kafirin yielded useful spectra, the microwave-heated kafirin exhibited a lot of fluorescence (Fig. 3.3.5). This was possibly because microwave heating affected some amino acid residues such that they produced a lot of fluorescence. Heat treatments cause certain molecular systems to exhibit high fluorescence that is strong enough to totally obscure the Raman signal (Kiefer and Spiekermann 1995; Schrader 1995b). Such molecular systems include amino acid residues that contain aromatic groups especially tryptophan (Shore and Parde 1956).

Since FT-Raman spectroscopy did not yield useful information about the effect of microwaving on SS bonds in kafirin, an alternative method was sought. A method to quantify the SS bonds was used since these cross-links play an important role in the cross-linking and structural changes of kafirin during heating (Duodu et al 2002). Li-Chan (1983) described a method to measure SS groups by difference, between the free SH groups and the total SH groups after reduction of SS groups. However, the limitation with this method is that it requires complete dissolution of the protein before assaying for SH or SS groups. Complete protein dissolution takes a long time at ambient temperature. Since kafirin is a hydrophobic protein (Duodu et al 2003), it could be difficult to solubilise at ambient temperature. In addition, mercaptoethanol, which is used as reducing agent, reacts with DTNB, the reagent used to quantify SH groups in a sample. Thus, mercaptoethanol has to be washed out thoroughly before

measuring the concentration of SH groups, since its residues resulting from improper washing could lead to an overestimation of the SH groups. Other errors with the Li-Chan procedure may arise from protein loss during washing and oxidation of the reduced SH groups (Chan and Wesserman 1993).

To avoid these problems, the solid-state direct colorimetric method described by Thannhauser et al (1987) was used since it can determine SH and SS groups in samples containing soluble and insoluble protein. In addition, the Thannhauser method does not require washing out of the reducing agent, sodium sulphite. Besides, there is no oxidation of the reduced SH groups like it is the case in the Li-Chan procedure (Chan and Wasserman 1993). The method involves the use of 2-nitro-5-thiosulphobenzoate (NTSB) which reacts with the SH and reduced SS groups. This reaction produces a chromophore whose concentration can be spectrophotometrically determined at 412 nm. Here, the Thannhauser et al procedure was first tested with bovine serum albumin (BSA) as a standard. The results showed that BSA had a cysteine group content of 35.6 mole/mole, which is in agreement with that calculated from amino acid sequence of BSA (35 mole/mole) (NiceProt 2004). Since no work has been reported on the SS bond assay for kafirin using this procedure, sodium sulphite at three concentrations (0.1, 0.2 and 0.4 M) was tested to determine a concentration that would be most effective at reducing SS bonds. The 0.2 and 0.4 M sodium sulphite concentrations resulted in less reduction and 0.1 M was found to be the most effective for reduction of SS groups.

It does, however, appear that not all SS groups were exposed for assay since there was a decrease in SH groups after reduction in the microwaved kafirin, the plasticised films made from microwaved kafirin and the plasticised films that were microwaved cast (Tables 3.3.1 and 3.3.2). This is possibly because of conformational changes in kafirin that limited reagent access to all SH and SS groups in the protein. Although the number of cysteine residues in each of the different kafirin species is known, the amount of those involved in SS bonds in a kafirin sample composed of all the species is not known. However, the results obtained were in agreement with SDS-PAGE data (Figs. 3.3.1-3.3.4) in that not all the kafirin oligomers were reduced to monomers by mercaptoethanol, which suggested that some SS cross-links were not accessible to

the reducing agent. In addition, the SS bond assay results could be related to changes in the functional properties of kafirin films.

Scanning electron microscopy (SEM) was used to determine the effect of microwaving on the microstructure of kafirin films. SEM uses electrons instead of light to create an image. As a result of the much shorter wavelength of electrons than light, image resolution of electron microscopy is much higher than that of light microscopy (Gabriel 1982). Although SEM generated high-resolution images, interpretation of structures seen in the micrographs was difficult. It is reasonable to think that the kafirin film samples should contain the protein, plasticiser and some residual solvent. However, it was difficult to distinguish between these components in the film micrographs. The micrographs showed varied structures such as wrinkles or folds, pores and fractured pieces depending on the microwave treatment. Emmambux (2004) suggested that the pores seen in kafirin films were possibly due to the vacuum used in SEM which could have evaporated the phase separated plasticiser and solvent. However, as stated earlier, the pores were possibly due to presence of air bubbles. Thus, although the various structures such as pores, wrinkles and fractured pieces indicated in the micrographs may have been influenced by the SEM procedure, they were probably related to the microwave treatments.

4.2. Effect of microwaving kafirin and cast kafirin films

TABLE 4.1. Summary of Results on the Effects of Different Microwave Treatments on the Functional and Structural Properties of Cast Kafirin Films

Treatment	Functional or structural property of kafirin film										
	Tensile Strength	Strain	Young's Modulus	Water Vapour Permeability	Digestibility	Biodegradability	Protein Polymerisation	SH Groups	SS Groups	α -Helix	β -Sheet
Microwaving Before Film Casting											
Plasticised Films from Microwaved Kafirin ^a	↑ ^c	↓	↑	↓	↓	↓	↑	↓	↑	↓↓	↑↑
Microwaving After Film Casting											
Non-Plasticised Films ^b	↑	↓	↑	- ^d	-	↓	↑	↓	↑	↓	↑
Plasticised Films ^a	↑↑	↓↓	↑↑	-	-	↓	↑	↓↓	↑↑	↓	↑

a. Control was plasticised kafirin films that were not subjected to microwaving

b. Control was non-plasticised kafirin films that were not subjected to microwaving.

c. Arrows indicate degree of increase (↑) or decrease (↓) relative to the control. Double arrows indicated a relatively higher decrease or increase in a given parameter.

d. (-), Same as control

Table 4.1 summarises the results of the effect of microwave heating before and after film casting on the functional and structural properties of kafirin films. Two major microwave induced changes, namely conformational and cross-linking changes were observed in the structure of the kafirin in the films. While the two changes could indeed be related, it is not clear what the relationship is or which of the two comes first. Nonetheless, it has been shown that the conformation (Subirade et al 1998) and cross-linking (Quinn et al 2003) of the proteins in films contribute to the functional properties of protein films. It is therefore, probable that the changes in kafirin conformation and cross-linking due to applying microwaves before and after casting of the films were responsible for the modification of kafirin film functional properties.

The reasons why a protein adopts a particular conformation are not well understood, though it has been postulated that conformations that minimise space and energy requirements are favoured (Ptitsyn 1992). However, conformational changes in proteins following heat treatment seem to depend on a number of factors. These include diverse protein-protein and protein-solvent interactions, conformational entropy of polypeptide (Thornton 1992), pH (Lefevre and Subirade 1999; Meng and Ma 2001), water activity (Mizutani et al 2003), plasticiser (Subirade et al 1998), as well as the free energy of formation for the different conformational structures (Damodaran 1996). It is not clear to what extent each of these factors contributes in determining protein conformation and how their interaction affects the conformation (Darby and Creighton 1993). However, it can be speculated that the conformational entropy of polypeptides (i.e. the conformational disorder of a polypeptide or its ability to adopt so many conformations) and the free energy for the formation of different conformations play an important role in directing the conformational changes in proteins as affected by process conditions. It seems that all the other factors impact in different ways, on the conformational entropy and the free energy for the formation of various protein conformations such that a particular set of conditions favours the formation of a given protein conformation.

The foregoing speculation is based on the fact that the major difference between applying microwave heating before and after film casting lies in the medium in which the kafirin was held. As stated earlier, microwave heating before film casting was done

on hydrated kafirin, while in the film state, microwaves were applied on kafirin that had been exposed to glacial acetic acid. Water and glacial acetic acid differ in their properties e.g. polarity, ionic strength, dielectric constant and hydrophobicity. Such differences in the properties would therefore impact differently on the effect of microwaving on kafirin conformation. Regarding water, hydrophobic proteins such as kafirin tend to expel water when heated (Belton et al 1994), suggesting that as the protein is heated it folds to keep the hydrophobic residues in the interior of the protein molecule. Such folding of the molecules could increase the proximity of the different kafirin molecules so that they cross-link easily to form aggregates. According to El Nour et al (1998), close proximity of kafirin molecules is required for cross-linking to occur. Concerning glacial acetic acid, as explained in Chapter 3.2 it is probable that the acid deamidated the kafirin, which could have altered its structural conformation.

As shown in Table 4.1, microwaving of kafirin increased SS cross-links in the protein. SS cross-links stabilise protein conformations through decreasing the conformational entropy of the polypeptides by imposing distance and angular constraints on the polypeptide backbone. This limits the motion of the polypeptide chain, making it stiffer, and thus reduces the number of possible conformations (Creighton 1992; Abkevich and Shakhnovich 2000). In addition, protein folding simulation studies by Abkevich and Shakhnovich (2000) indicate that inclusion of SS cross-links affects the free energy barrier for the folding and unfolding of proteins. It is therefore, possible that the SS bond formation in microwaved wet kafirin decreased the free energy barrier for the formation of β -sheets and/or increased the free energy barrier for the formation of α -helices in the kafirin films made from microwaved kafirin. Such effects of SS cross-links on the conformational entropy and the free energy for the formation of the different protein conformations possibly explain the apparent relationship between conformational changes and protein cross-linking in kafirin. This, however, does not tell whether it is the SS cross-linking that influences the conformation or vice versa.

It has been suggested that conformational changes in protein molecules can promote or discourage the formation of SS cross-links in certain positions since SS bond formation requires that the S atoms of the participating cysteine residues be held in a closely-defined conformation with respect to each other (Darby and Creighton 1993). In relation to this, Livney et al (2003) investigated the steric effects governing SS bond

interchange in heated solutions of β -lactoglobulin and α -lactalbumin. They found that the participation of different SH groups in SS bonds depended on their location in the native structure, with surface-located cysteine residues more involved than internally located ones. It seems that the participation of SH groups in SS bond formation is predetermined by the position of the cysteine residues on the polypeptide in relation to the conformation of the protein molecule. It can therefore be inferred that the conformation of the hydrated kafirin and the presumably deamidated kafirin in the film state influenced the extent and position of SS cross-linking following the application of microwaving. As stated, protein conformation contributes to the functional properties of protein films (Subirade et al 1998). It is proposed that the contribution of the conformational changes in kafirin following microwaving before or after film casting is to facilitate the cross-linking of the kafirin in the films.

The predominance of β -sheet conformation in the plasticised films made from microwaved kafirin (Fig. 3.3.7) was probably related to the microwave induced kafirin aggregates. According to Pezolet et al (1992), protein aggregation can influence the extent of unfolding of protein polypeptides on dissolution. Regarding kafirin, Gao et al (2005) reported that the kafirin fraction that was insoluble in aqueous ethanol was composed mainly of aggregated protein, suggesting that the cross-linked kafirin could not be easily solubilised. Therefore, it is possible that when the microwaved kafirin was dissolved in glacial acetic acid, some of the protein molecules unfolded forming random coils and the conformation of the aggregated protein remained conserved. It is conceivable that the conserved conformation of the kafirin aggregates led to the predominance of the β -sheet conformation in films made from microwaved kafirin.

Concerning microwaving of cast kafirin films, it seems that dissolving the untreated kafirin in glacial acetic acid unfolded the protein, probably yielding an almost entirely random coil structure. Gao et al (2005) found that kafirin that, as in this work, had been extracted in aqueous tert-butanol dissolved completely in aqueous ethanol. According to Damodaran (1996), low pH creates strong intramolecular electrostatic repulsions, which alter the linkages like hydrogen bonds and hydrophobic interactions that stabilise the protein structure. This leads to protein unfolding. In addition, as stated earlier, glacial acetic acid probably deamidated the kafirin polypeptides thus, breaking more intermolecular hydrogen bonds. Deamidation is known to induce conformational

changes in proteins such as decreasing β -sheet and β -turns (Popineau et al 1994). It is conceivable that such effects of glacial acetic acid led to the exposure of more SH and other functional groups on the polypeptides such that they could easily take part in intermolecular cross-linking.

Of all protein conformations, random coils seem to exhibit the greatest conformational entropy. This is because of the absence of net interactions between atoms on the polypeptide chain and with the solvent (Darby and Creighton 1993). It appears that if the high conformational entropy is not substantially curtailed, random coils more readily form α -helices as compared to β -sheets, possibly because of differences in free energy of formation between the two conformations. Since free energies of formation and propagation of α -helices and β -sheets in kafirin are not available, the calculated free energy values for a model protein, polyalanine are used here. The calculated free energy changes for initiation and propagation of α -helix in polyalanine are 4 kcal/mol/turn and 0.1-0.9 kcal/mol/residue, respectively (Yang and Honig 1995b). In contrast, the calculated free energy changes for the initiation and propagation of a β -sheet in polyalanine are 5.9-7.6 kcal/mol/four residues and 0.7-1.5 kcal/mol/residue, respectively (Yang and Honig 1995a). It is possible that a similar scenario explains the predominantly α -helical conformation of the kafirin films before they were microwaved.

Microwaving of the cast films caused an increase in β -sheets but the films remained predominantly α -helical in conformation (Figs. 3.3.8 and 3.3.9). This suggests that while microwaving increased the β -sheet structure, the condition of the polypeptides in the cast film, seems to have favoured formation of α -helical conformation. It is proposed that once the protein adopts a specific conformation inside the film matrix, it will not readily change because the film matrix has low solvent content thus making the protein molecules less mobile. This, therefore, could allow SS bond formation but not enough molecular mobility and interaction to form substantial proportions of β -sheets. According to Yang and Honig (1995b), the formation of β -sheets requires a paired interaction between side chains on two polypeptides. It is possible that microwaving of the films cross-linked the polypeptides in such a way that substantial paired interaction between polypeptides required for β -sheet formation could not be achieved. Since there can be SS cross-linked α -helices, as has been found in insulin

(Williams et al 1978), the predominant α -helices in the microwaved kafirin films were at least in part stabilised by SS cross-links.

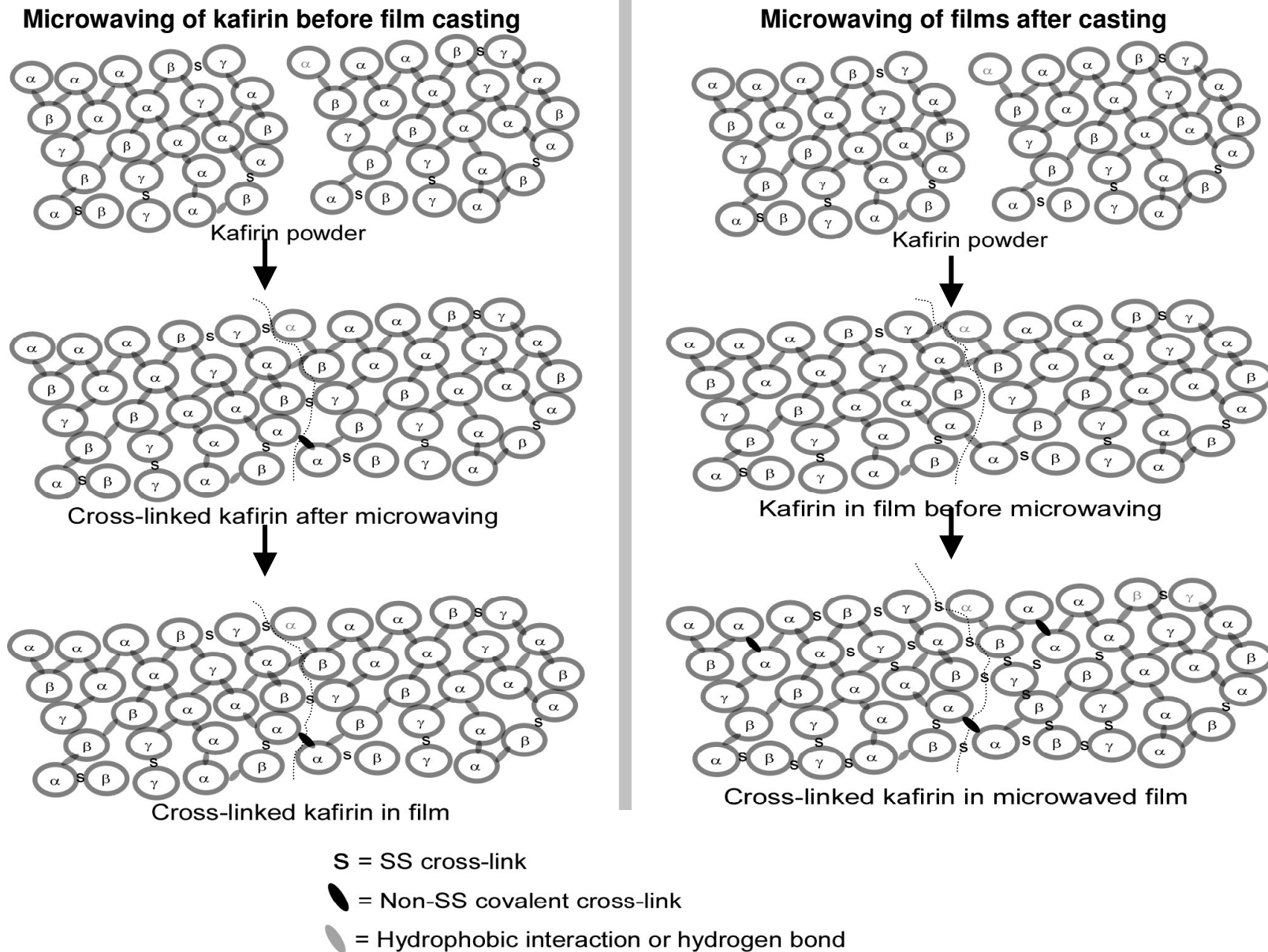


Fig. 4.1. Model for microwave induced cross-linking in kafirin and kafirin films.
 Note: the model does not include conformational changes in the polypeptides.

The probable main reason for the increase in tensile strength and decrease in strain of kafirin films following microwaving before (Table 3.1.5) or after film casting (Table 3.2.1) was the increase in microwave induced intermolecular SS cross-links, as is indicated by the SS bond assay (Tables 3.3.1 and 3.3.2), SDS-PAGE (Figs. 3.3.1-3.3.4) and glass transition temperature (T_g) (Table 3.2.2) results. The results suggest an apparent direct relation between the increase in SS bond content of films and the increase in film tensile strength. In kafirin, wet heat is known to cause intermolecular SS cross-linking (Duodu et al 2002; Nunes et al 2004). Although heat induced SS cross-links have been implicated in the modification of the functional properties of soy protein films (Rangavajhyala et al 1997) and whey protein films (Perez-Gago and Krochta 2001; Quinn et al 2003), this is the first clear evidence of their involvement.

Figure 4.1 is a proposed model to explain the effects of microwaving on the cross-linking of kafirin in the films. As mentioned, the conformational changes in kafirin following microwaving probably facilitated the cross-linking of kafirin in the films. However, the model does not show the microwave induced conformational changes in kafirin. In the model, α , β and γ represent the three species of kafirin that could be cross-linked through SS bonds and possibly hydrogen bonds, hydrophobic interactions and/or non-SS covalent cross-links. The model illustrates how two kafirin complexes, composed of the different kafirin species, are cross-linked when subjected to microwaving before or after film casting. The dotted line indicates the boundary along which the two kafirin complexes are cross-linked. It is proposed that in the film system, the different kafirin species cross-link in the same manner as suggested by El Nour et al (1998), where the β -kafirins act as chain extenders between the α - and γ -kafirins through SS bond formation. Plasticiser may form a lubricating layer between the different kafirin species in the film network. The microwaves provide the energy to cause conformational changes that expose functional groups at which cross-links occur, thus facilitating the cross-linking between the different kafirin species.

As stated, the SS bond assay and SDS-PAGE results suggest that microwave induced SS cross-linking directly contributed to the modification of kafirin film functional properties like increased tensile strength and reduced strain, WVP, digestibility and biodegradability. Perhaps in the case of tensile properties, such intermolecular cross-

links create a more dense protein network in the film, which creates higher resistance against applied stress, thus increasing film tensile strength. Regarding digestibility and biodegradability, it has been suggested that SS cross-links can reduce accessibility of proteolytic enzymes to some peptide bonds (Yamauchi et al 2001, Duodu et al 2003). In a study of the biodegradation of poly(hydroxyalkanoate) films, Doi et al (1992) found that the enzymatic degradation of the films depended on the molecular structure and orientation of the polymer in the film form. It is proposed that as the kafirin conformation changed and facilitated the cross-linking of protein molecules, the kafirin aggregates became bigger (Fig 4.1) making polypeptide bonds in the interior of the aggregates less accessible to the enzyme. The decreased enzyme accessibility probably led to the reduced digestibility of microwaved kafirin and films made from microwaved kafirin and the slowed biodegradability of microwaved films.

The relationship between SS cross-links and WVP, however, seems not to be as direct as that with tensile strength, strain and digestibility of the films. While microwaving of kafirin before film casting decreased WVP of the films (Table 3.1.6), microwaving of cast films had no effect on their WVP (Table 3.2.4). Yet films made from microwaved kafirin showed less SS cross-links as compared to films that were microwaved cast. It is hypothesised that microwave induced SS cross-links in the films made from microwaved kafirin decreased the amount of free volume across which water vapour diffused. However, this hypothesis is not supported by the film thickness data. According to Park and Chinnan (1995), WVP of protein films increases with film thickness. The mean film thickness for the plasticised films made from microwaved kafirin and those microwaved after casting were 100 and 94 μm , respectively.

SDS-PAGE of films and their RPDs indicated a relationship between kafirin cross-linking and film digestibility (Figs. 3.1.3 and 3.2.2). The high intensity of oligomers in the RPDs of films compared to the films before pepsin digestion suggests that the oligomeric kafirin was less digestible than the monomeric kafirins. This sheds more light on the probable involvement of oligomers in the reduced kafirin digestibility associated with cooking. Other researchers (Hamaker et al 1987; Duodu et al 2002; Nunes et al 2004) postulated that presence of kafirin oligomers in cooked sorghum could be related to the reduction in digestibility of kafirin after heating. According to Carbonaro et al (1998), protein digestibility depends on enzyme access to peptide

bonds. Hurrell and Finot (1982) suggested that cross-linking of proteins reduces their digestibility by preventing enzyme access to peptide bonds or by masking sites of enzyme attack.

The increase in T_g of cast non-plasticised films after microwaving indicated reduced molecular mobility, which suggests microwave induced cross-linking of the kafirin in the films. This agrees with the SDS-PAGE, SS bond and FTIR data, which further points to kafirin cross-linking as the main reason for the modification of kafirin film properties. Microwaving is a heating process (Ohlsson and Bengtsson 2001) and heating of polymer materials above their T_g increases the efficiency of cross-linking of the polymer molecules (Chapiro 1988). Perhaps microwaving of cast kafirin films had a similar effect on the cross-linking of kafirin molecules. However, it is not known whether microwaving heated kafirin films to temperatures above their T_g .

Although experiments were not conducted to determine the role of hydrogen bonding and hydrophobic interactions, it is probable that they contributed to the kafirin film properties. Kafirin has a high content of hydrophobic amino acid residues such as proline, alanine and leucine and is therefore, classified as a hydrophobic protein (Taylor and Belton 2002). In this case, intra- and/or inter-molecular interactions between the hydrophobic side chains of the residues were possibly involved. It is therefore, conceivable that such hydrophobic interactions contributed to the stability and functional properties of kafirin films. Hydrogen bonds are known to contribute to the formation and stabilisation of α -helices and β -sheets in proteins (Darby and Creighton 1993). Since kafirin films had varying proportions of α -helix and β -sheet conformations (Table 3.3.3), it is probable that hydrogen bonding was involved in kafirin film formation. Hence, it is reasonable to think that hydrogen bonding and hydrophobic interactions, possibly through intra-molecular associations, also contributed to the formation and stabilisation of kafirin films subjected to different microwaving treatments as illustrated in Fig 4.1. However, the effect of microwaving on hydrogen bonding and hydrophobic interactions in kafirin is not known.

The fact that kafirin oligomers remained after reduction with mercaptoethanol when the microwaved films were subjected to SDS-PAGE (Figs. 3.3.1-3.3.4) suggests the involvement of microwave induced non-SS covalent cross-links. Such cross-links

could include the dityrosyl (Fig 4.2) and to a lesser extent, the glutamyl-lysyl bonds. Figure 4.2 illustrates how dityrosyl bonds could have cross-linked the kafirin in films after microwaving. Duodu (2000) suggested that wet heat could induce dityrosyl cross-links in kafirin. According to Damodaran (1996), heating of proteinaceous foods that are low in carbohydrate content can result in the formation of the glutamyl-lysyl cross-links. However, the formation of the glutamyl-lysyl cross-link could have been to a limited extent, since kafirin contains little amounts of lysine (Shull et al 1992). Non-SS covalent cross-links are known to affect protein functional properties such as digestibility by preventing proteases from reaching the sites of hydrolysis (Cheftel et al 1985). Thus microwave induced dityrosyl and the glutamyl-lysyl cross-links could have contributed to the modification of functional properties of kafirin films.

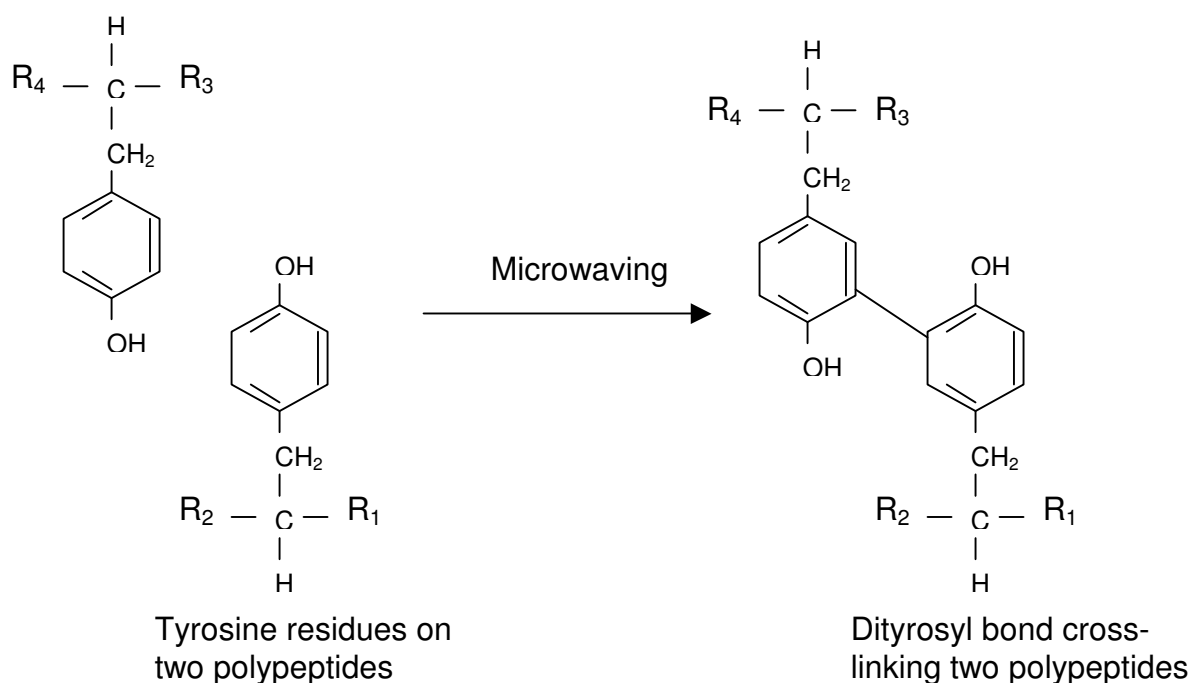


Fig. 4.2. The dityrosyl cross-link likely to have been induced by microwave heating in kafirin films. R_1 to R_4 represent the rest of the polypeptide chains. Adapted from Otterburn (1989).

It was hypothesized that the effects of microwave heating on kafirin would be different from those of conventional heating (Chapter 1). However, from Chapters 3.1 and 3.3, it is clear that applying microwave heating before or after film casting promoted the formation of β -sheet and SS cross-linking of kafirin. As mentioned, wet heating causes similar effects in kafirin (Duodu et al 2001, 2002; Nunes et al 2004). It can be inferred that in spite of the differences in the mode of heat generation and transfer for microwaving and conventional heating, the effects of microwave heating on kafirin are essentially the same as those of conventional wet heating.

Table 4.2 summarises the proposed relationship between chemical/structural properties and the functional properties of kafirin films microwaved before and after casting.

TABLE 4.2 Summary of Proposed Relationship Between Chemical/Structural Properties and Functional Properties of Kafirin Films Microwaved Before and After Casting.

Film Property	Effect of Microwaving on film property	Proposed Explanation
Tensile strength	Increased with microwaving of kafirin and cast kafirin films	A stronger film network resulting from increased microwave-induced intermolecular SS and non-SS cross-linking causing increased resistance to stress.
Strain	Decreased with microwaving of kafirin and cast films	A stronger film network resulting from increased microwave-induced intermolecular SS and non-SS cross-linking which limit molecular mobility thus causing more resistance to extension
Water vapour permeability (WVP)	- WVP decreased with microwaving of kafirin - WVP not affected by microwaving of cast films	- Microwaving of kafirin before film casting decreased free volume across which moisture diffuses thus decreased WVP. - Presumably no effect at molecular level.
Digestibility	- Digestibility decreased with microwaving of kafirin and non-plasticised films cast from aqueous ethanol - No effect on plasticised films cast from aqueous ethanol - No effect on films cast from glacial acetic acid	- Microwave induced intermolecular SS cross-linking caused some peptide bonds and sites of enzyme attack to be inaccessible to pepsin - Presumably no effect at molecular level - Presumably no effect at molecular level
Biodegradability	Biodegradability slowed with microwaving of kafirin and cast kafirin films.	Microwave induced cross-linking caused some of the polypeptide bonds to be inaccessible to microbial proteolytic enzymes

TABLE 4.3 Tensile Properties and Water Vapour Permeability of Kafirin Films Modified with Microwaves and Tannins and Other Protein Films Modified by Various Methods.

Protein Film	Method of Modification	Tensile Strength (MPa)	Strain (%)	Water Vapour Permeability (g mm m ⁻² d ⁻¹ kPa ⁻¹)
Kafirin ^a	Unmodified	1.8	136	2.50 ⁱ
Kafirin ^a	Microwaving of kafirin at 96°C for 2.min	3.13	83	1.33 ^j
Kafirin ^a	Microwaving of cast film at 80 W for 2 min.	6.9	22.8	2.50 ^j
Kafirin (non-plasticised)	Unmodified	12.8	1.5	0.93 ^j
Kafirin (non-plasticised)	Microwaved at 80 W for 2 min	20.8	1.7	0.93 ^j
Kafirin ^b	Unmodified	2.4	109	0.98
Kafirin ^b	10% tannic acid (w/w)	3.0	65	0.90
Kafirin ^b	20% tannic acid (w/w)	5.0	24	0.94
Kafirin ^b	10% sorghum condensed tannin	3.6	49	1.03
Kafirin ^b	20% sorghum condensed tannin	4.9	24	0.98
Zein (15% glycerol) ^c	Unmodified	5.2	4.4	Nd
Zein (15% glycerol) ^c	50% glutaraldehyde	19.8	3.0	Nd
Whey protein isolate (50% glycerol) ^d	Unmodified	1.94	26.1	5.66
Whey protein isolate (50% glycerol) ^d	Microwaved 700 W, 5 min	2.43	35.9	5.40
Soy protein isolate (60% glycerol) ^e	Unmodified	3.25	111.9	3.61
Soy protein isolate (60% glycerol) ^e	Heat curing of film at 95°C for 24 hr	14.3	21.0	1.64
Gluten (34% glycerol) ^f	Unmodified	6.0	100.0	1.93
Gluten (34% glycerol) ^f	Heat curing of film at 95°C for 24 hr.	15.5	5.0	1.62
Soy protein isolate (50% glycerol) ^g	Unmodified	3.7	124	7.2
Soy protein isolate (50% glycerol) ^g	Ultraviolet radiated at 103.7 J/m ²	6.1	86	7.2
Low density polyethylene	Non-modified	8.6-17 ^h	500 ^h	0.08 ⁱ
High density polyethylene	Non-modified	17-35 ^h	300 ^h	0.02 ^j
Polyvinylidene chloride	Non-modified	48-138 ^h	20-40 ^h	Nd
Polyethylene terephthalate	Non-modified	175 ^h	70-100 ^h	Nd

a. Kafirin films 42% plasticiser (glycerol: lactic acid: poly ethylene glycol in a 1:1:1 ratio), b. Emmambux et al (2004), c. Parris and Coffin (1997), d. Kaya and Kaya (2000), e. Gennadios et al (1996), f. Ali et al (1997), g. Gennadios et al (1998), h. Briston (1988), i. Smith (1986); j = WVP values were determined in vials therefore higher than those of Emmambux et al (2004); Nd = not determined.

Table 4.3 compares the effect of modification of kafirin and other protein films achieved by different means on their properties. Comparison of modifications should be done with caution due to the different samples of the same protein, the different conditions under which the modifications were achieved and differences in assay conditions. This notwithstanding, some valid comparisons can be made. Increase in tensile strength and a reduction in strain and WVP of kafirin films can be achieved using microwaving just like it has been achieved in kafirin and other protein films using chemical and other physical modifying agents like heat and ultraviolet light. Microwaving of kafirin before film casting increased film tensile strength by about two fold and decreased strain and WVP by about one and half times each. Cross-linking of kafirin with tannins before film casting increased their tensile strength by about two fold but had no effect on the WVP (Emmambux et al 2004). Microwaving of cast kafirin films increased their tensile strength by about four fold but had no effect on WVP. Similarly, microwaving of cast whey protein films increased their tensile strength and had no effect on WVP (Kaya and Kaya 2000). The tensile strength of the microwaved non-plasticised kafirin films was in the same range as those of heat cured soy protein films (Gennadios et al 1996), heat cured gluten films (Ali et al 1997) glutaraldehyde treated zein films (Parris and Coffin 1997) and the high density polyethylene films (Briston 1988). However, the WVP of the modified kafirin films was still higher than that of high-density polyethylene films. This suggests that if modified, kafirin films can be as strong as other protein and synthetic polymer films but to achieve additional reduction in the WVP will require further modification. Although microwaving of cast plasticised kafirin films showed the highest percent increase in tensile strength, the absolute tensile strength value (6.9 MPa) was still lower than that of heat cured soy protein films (Gennadios et al 1996) heat cured gluten films (Ali et al 1997) and the synthetic polymer films (Briston 1988). However, the tensile strength of the plasticised films that were microwaved cast was higher than that reported by Emmambux et al (2004), suggesting that microwaving cast films could be more effective at increasing tensile strength as compared to tannin cross-linking.

It is also apparent that the effect of modifying agents can vary among similar or different protein films and can depend on the stage at which the modifying agent is applied. Although both microwaving of kafirin before film casting and microwaving of

cast films increased film tensile strength and decreased strain, the latter seemed to be more effective. However, microwaving of kafirin before film casting reduced film WVP but microwaving of cast films had no effect on their WVP (Table 4.3). This suggests that, the choice of a modifying agent and the extent of modification could depend on the type of protein and intended use of the film. In addition, a single modification treatment may not necessarily modify all the functional properties of kafirin films.

Although microwaving of kafirin or cast plasticised kafirin films modified the tensile properties and WVP of the films, these properties were still inferior to those of synthetic polymer films (Table 4.3). This means that if tensile strength and WVP similar to those of synthetic polymer films are to be achieved in plasticised kafirin films, further modification is required. It is probable that further film property modification could be achieved if microwaving is used in combination with some of the food compatible chemical cross-linkers like tannins. Nonetheless, the modification of kafirin films so far achieved by microwaving can offer improved barrier and mechanical properties and hence better protection of certain products.

Use of microwaving to improve the functional properties of kafirin films is possible at industrial scale, just like γ -irradiation (Chapiro 1988) and ultraviolet light (Seidel 1988) are used in cross-linking of synthetic films and plastics to achieve properties for various industrial and biomedical applications. As stated, the efficiency of the cross-linking process is greatly increased if the process temperature is above the T_g of the polymer so as to increase molecular mobility. This requires an external source of heating which increases costs (Chapiro 1988). However, with microwaving, increasing the process temperature is part of the process and hence does not require an external heating source since microwaves generate heat internally within the kafirin or kafirin films to bring the temperature above their T_g .

Both microwaving of wet kafirin and cast kafirin films can be commercially viable. Microwaving of cast kafirin films involves fewer operations than microwaving of wet kafirin and may, therefore, appear more viable. However, microwaving of the cast films would require proper distribution of the microwaves onto the films to ensure uniform heating. Conversely, microwaving of wet kafirin requires that the water be removed before film casting or somehow be used in the film formulation. These challenges

would have to be thoroughly addressed in the design and development of the process and microwaving equipment meant for kafirin film modification before or after film casting.

In addition, the problem with industrial application of microwave technology is that, in most cases, the microwave equipment has to be custom designed and tailored to a particular process (Giese 1992). Although this problem still exists, the newer industrial microwave systems used for heat initiated polymerisation reactions (Microdry 2005) can be adapted to the production of modified kafirin films with slight design alterations. Such industrial microwave systems include those used for glue-line curing of laminates (Microdry 2005) and curing of thermoplastic and thermoset polymers (Fathi et al 2005). Such commercial microwave ovens could be adapted to the modification of either kafirin or cast kafirin films at industrial scale. This, however, would require a parallel development of an industrial scale process for the casting of kafirin films.

This work was part of the EU INCO-DEV ENVIROPAK project under which the functional properties of kafirin films were to be improved for coating of nuts and fresh fruits and vegetables to extend their shelf life (ENVIROPAK, 2002). Microwave modification of kafirin film properties like tensile strength, WVP, digestibility and biodegradability increases the film's potential for food applications such as coating of fruits and vegetables, nuts and non-food applications such as pharmaceuticals. For example, because of their increased tensile strength and decreased strain, microwaved kafirin films can be used to provide better mechanical protection to food and/or pharmaceutical products.

In the postharvest distribution and marketing of fresh fruits and vegetables, some of the major factors that lead to quality loss and shorter shelf life are moisture loss, respiration and ripening, physical damage or injury and microbial spoilage (Kader 1992). Coating of fruits and nuts with a kafirin-based film has been shown to retard some of the factors that lead to spoilage of these products. Goburdhun (2004) found that coating litchis with kafirin and storing them at 4°C decreased the fruits respiration although it did not reduce moisture loss from the fruit. In another study, kafirin coating of Packham's Triumph pears and storing them at 20°C reduced fruit respiration and moisture loss and increased shelf life (Buchner 2004). Kafirin coating of pears was

also shown to retard the growth of *Botrytis cinerea* (which causes the grey mould rot) inoculated onto sterile fruits (Nilsson, 2004). Since they have increased tensile strength, microwaved kafirin films should provide increased protection against e.g. bruising of the fruits or vegetables. In addition, the low oxygen permeability that is characteristic of protein films (Krochta 2002) and the reduced WVP of films made from microwaved kafirin could decrease the rate of respiration and moisture loss from the product, thus increase its shelf life. However, such applications of microwaved kafirin films require more research since fruits and vegetables vary in their post-harvest physiology.

Concerning nuts, the major factors leading to spoilage and quality loss are moisture absorption, lipid oxidation and mould growth (Trezza and Krochta 2002). Khan (2004) found that kafirin coated cashew nuts absorbed less moisture than the uncoated ones during 45 days of storage. This shows that kafirin coating increases product shelf life and slows quality loss in nuts. Application of microwave modified kafirin films on nuts could provide increased protection against mechanical damage and oxidation. Although the WVP of films made from microwaved kafirin was reduced, there could still be moisture uptake by the nuts that are coated with such kafirin films. Moisture uptake by the nuts, in time, could promote mould growth on the nuts, which is undesirable. Hence, as a moisture barrier for nuts, microwaved kafirin films require more modification to further reduce their WVP.

The application of microwaves before or after film casting to modify kafirin films presents new challenges and opportunities in the application of the technology. For example, can microwaving be used to improve the properties of kafirin coatings already formed on nuts, fresh fruits and vegetables? If microwaving were used on already coated products, it may result in the heating of the product leading to reactions within the product itself and between the product and the coating. These effects may result in desirable or undesirable quality changes in the product. The undesirable effects would have to be eliminated or minimized while the desirable ones are harnessed. Microwaving could then be used to meet two objectives with a single operation e.g. to improve the properties of the coating and at the same time heating the product to decrease its microbial load or reduce the activity of undesirable endogenous enzymes. It is possible that in situations where a product needs short

heat treatment e.g. in blanching or inactivation of the fruit fly eggs (Sommer and Arpaia 1992), a fruit that is already coated with kafirin can be microwaved to improve film properties and provide the short heat treatment at the same time. The modification of functional properties of kafirin coating using microwave energy needs to be tailor-made to meet requirements of particular products. The possibility and practicality of such potential application need researching.

Regarding pharmaceutical applications, coating of tablets is often used to mask the taste or odour of unpalatable drugs, to physically and chemically protect the drug from water, oxygen and light (Brink 1995) and in controlled drug release applications (Bott et al 2004). Liu et al (2005) used zein to produce microspheres for the drug ivermectin and found it suitable for use in a sustained drug release system. Although proteins are not commonly used in the coating of pharmaceutical drugs, the properties of kafirin and the developments in protein film production and modification such as those discussed here indicate a potential use of the microwave modified kafirin films in such applications. For example, the variation in digestibility of kafirin films due to the type of solvent and plasticiser used, may have implications in situations where the film is applied in environments with proteolytic activity. In such situations, the release of substance or drug in a kafirin film coated tablet could be controlled in a proteolytic environment. For example, timed drug release may be required in the stomach or small intestine where the environment is proteolytic. Hence, microwave modified kafirin films could potentially be applied in coating of pharmaceutical tablets. This, however, requires more research to further modify the kafirin film properties.

5. CONCLUSIONS AND RECOMMENDATIONS

The extraction method used to isolate kafirin can affect its behaviour during modification and subsequent film functional properties. An effect of microwaving on kafirin film properties could only be established with kafirin that was extracted using a procedure that minimised protein aggregation and maximised native α -helical structure in the kafirin. Microwave modification of kafirin film properties depended on the content of polar molecules from water and plasticiser in the kafirin.

Contrary to the hypothesis that microwave heating will have a different effect to conventional heating, the results here suggest that the effects of the two heating methods on kafirin are essentially the same.

Microwave induced SS cross-linking was probably the primary cause of increased tensile strength and Young's modulus, the decreased strain and digestibility and the slowed biodegradability of the kafirin films. The conformational changes seem to have facilitated the cross-linking of kafirin.

Although both microwaving before and after film casting modified kafirin film properties, microwaving after film casting was better since it was more effective at increasing film tensile strength and involved less processing steps than microwaving kafirin before film casting. The stage at which microwaving is applied probably affected the kafirin conformation, which influenced the nature and extent of polypeptide interaction and cross-linking.

A careful selection and manipulation of process conditions is required to achieve the desired properties of kafirin films, since factors such as microwave power and time, the solvent and plasticiser can singly or together affect the functional properties of kafirin films. Moderate microwave power levels resulted in cross-linking and modification of properties of kafirin films, while high microwave power levels seemed to have adverse effects on the tensile properties possibly because of the negative effects of thermal denaturation of the protein. Use of plasticisers can increase the digestibility of microwaved kafirin films.

Compared to most synthetic polymer films, the tensile strength and WVP of microwaved kafirin films were still inferior. However, the modification of kafirin film properties achieved with microwaving can be of use in packaging or coating situations that do not necessarily require the properties offered by synthetic polymer films. Further modification of kafirin films could be achieved by using other microwaving approaches e.g. microwaving of the film forming solution and/or combining microwaving with food grade chemical cross-linking agents.

Microwave modified kafirin films can potentially provide increased protection to coated fruits, vegetables and nuts against mechanical damage and moisture loss due to their increased tensile strength and decreased WVP. The decreased digestibility of microwaved films suggests that they have potential in the coating of pharmaceutical tablets for controlled drug release applications.

6. REFERENCES

- Abkevich, V. I., and Shakhnovich, E. I. 2000. What can disulfide bonds tell us about protein energetics, function and folding: simulation and bioinformatics analysis. *J. Mol. Biol.* 300:975-985.
- Ali, Y., Ghorpade, V. M., and Hanna, M. A. 1997. Properties of thermally-treated wheat gluten films. *Ind. Crops. Prod.* 6:177-184.
- Andrews, D. C., Caldwell, R. A., and Quail, K. J. 1995. Sulfhydryl analysis. I. Determination of free sulfhydryls in wheat flour doughs. *Cereal Chem.* 72:326-329.
- Argos, P., Pedersen, K., Marks, M. D., and Larkins, B. A. 1982. Structural model for maize zein proteins. *J. Biol. Chem.* 257:9984-9990.
- Arvanitoyannis, I., Kalichevsky, M., Blanshard, J. M. V., and Psomiadou, E. 1994. Study of diffusion and permeation of gases in undrawn and uniaxially drawn films made from potato and rice starch conditioned at different relative humidities. *Carb. Polymers* 24:1-15.
- ASTM. 1996. American Society for Testing and Materials. Standard practice for exposing plastics to a simulated compost environment using an externally heated reactor; Designation D5512-96. Annual Book of the American Society for Testing and Materials Standards: West Conshohocken, USA.
- ASTM. 1998. American Society for Testing and Materials. Standard method for tensile properties of thin plastic sheeting, Designation D882-97. Annual Book of the American Society for Testing and Materials Standards: West Conshohocken, USA.
- Aydt, T. P., Weller, C. L., and Testin, R. F. 1991. Mechanical and barrier properties of edible corn and wheat protein films. *Trans. Amer. Soci. Agric. Eng.* 34:207-211.
- Bandekar, J. 1992. Amide modes and protein conformation. *Biochimica et Biophysica Acta* 1120:123-143.
- Banerjee, R., Chen, H., and Wu, J. 1996. Milk protein-based edible film mechanical strength changes due to ultrasound process. *J. Food Sci.* 61:824-828.
- Banker, G. S. 1966. Film coating theory and practice. *J. Pharm. Sci.* 55:81-89.

- Biser, A. 1987. Concepts in Modern Physics, 4th Edition. Pages 87-117. McGraw-Hill Book Company: New York, USA.
- Belton, P. S., Colquhoun, I. J., Field, J. M., Grant, A., Shewry, P. R., and Tatham, A. S. 1994. ¹H and ²H NMR relaxation studies of a high M_r wheat glutenin and comparison with elastin. J. Cereal Sci. 19:115-121.
- Bircan, C., and Barringer, S. A. 1998. Salt starch interactions as evidenced by viscosity and dielectric property measurements. J. Food Sci. 63:983-986.
- Bollag, D. M., Rozycki, M.D., and Edelstein, S. J. 1996. Protein Methods. Page 110. John Wiley and Sons: New York.
- Bott, C., Rudolph, M. W., Schneider A. R. J., Schirrmacher, S., Skalsky, B., Petereit, H. U., Langguth, P., Dressman, J. B., and Stein J. 2004. *In vivo* evaluation of a novel pH- and time based multiunit colonic drug delivery system. Aliment. Pharmacol. Ther. 20:347-353.
- Brault, D., D'aprano, G., and Lacroix, M. 1997. Formation of free standing sterilized edible films from irradiated caseinates. J. Agric. Food Chem. 45:2964-2969.
- Brink, D. J. D. 1995. The development of a model to predict the release rate of Ibuprofen from aqueous coating systems for sustained release dosage forms. MPharm. Dissertation, University of Pretoria, Pretoria, South Africa.
- Briston, J. H., 1988. Plastic Films, 3rd Ed. John Wiley and Sons: New York.
- Buchner, S. 2004. Extending the shelf life of export quality Packham triumph pears. www.sik.se/enviropak [29 December 2004]
- Buffo, R. A., Weller, C. L., and Gennadios, A. 1997. Films from laboratory-extracted sorghum kafirin. Cereal Chem. 74:473-475.
- Carbonaro, M., Bonomi, F., Iametti, S., Cappelloni, M., and Carnovale, E. 1998. Aggregation of proteins whey from raw and heat-processed milk: formation of soluble macroaggregates and nutritional consequences. Leben. Wiss. Technol. 30:522-529.
- Chan, K. Y., and Wasserman, B. P. 1993. Direct colorimetric assay of free thiol groups and disulfide bonds in suspensions of solubilized and particulate cereal proteins. Cereal Chem. 70:22-26
- Chapiro, A. 1988. Polymers in radiation-related technologies: radiation modified polymers. Pages 3-17 in: Polymers for Advanced Technologies. IUPAC International Symposium. M. Lewin, ed. VCH: Weinheim, Germany.

- Cheftel, J. C., Cuq, J. L., and Lorient, D. 1985. Amino acids, peptides and proteins. Pages 245–369 in: Food Chemistry 2nd Ed. O. R. Fennema, ed. Marcel Dekker: New York, USA.
- Cherian, G., Gennadios, A., Weller, C., and Chinachoti, P. 1995. Thermo-mechanical behavior of wheat gluten films: effect of sucrose, glycerin and sorbitol. Cereal Chem. 72:1-6.
- Chiue, H., Kusano, T., and Iwami, K. 1997. Deamidation-induced fragmentation of maize zein, and its linked reduction in fatty acid-binding capacity as well as antioxidative effect. Food Chem. 58:111-117.
- Cho, S-Y., Park, J-W., and Rhee, C. 1998. Edible films from protein concentrates of rice wine meal. Korean J. Food Sci. Technol. 30:1097-1106.
- Choi, W.-S., and Han, J. H. 2001. Physical and mechanical properties of pea-protein-based edible films. J. Food Sci. 66:319-322.
- Chung, M. W. Y., Lei, B., and Li-Chan, E. C. Y. 2005. Isolation and structural characterisation of the major protein fraction from NorMan flaxseed (*Linum usitatissimum* L.). Food Chem. 90:271-279.
- Coleman, C.E., Dannehofer, J.M., and Larkins, B.A. 1990. The prolamin proteins of maize, sorghum and coix. Pages 257-288, in: Cellular and Molecular Biology of Plant Seed Development, B. A. Larkins and I. K. Vasil, eds. Kluwer Academic Publishers: Dordrecht, The Netherlands.
- Copson, D. A. 1975. Microwave Heating 2nd Ed. Pages 1-615 AVI Publishing Company: Westport, USA.
- Creighton, T. E. 1992. Folding pathways determined using disulfide bonds. Pages 301-349 in: Protein Folding. T. E. Creighton, ed. WH Freeman and Company: New York, USA.
- Cuq, B., Gontard, N., and Guilbert, S. 1998. Proteins as agricultural polymers for packaging production. Cereal Chem. 75:1-9.
- Curnutte, B. 1980. Principles of microwave radiation. J. Food Protection 43:618–624.
- Da Silva, L. S., and Taylor, J. R. N. 2004. Sorghum bran as a potential source of kafirin. Cereal Chem. 81:322-327.
- Da Silva, L. S., and Taylor, J. R. N. 2005. Physical mechanical and barrier properties of kafirin films from red and white sorghum milling fractions. Cereal Chem. 82:9-14.

- Damodaran, S. 1996. Amino acids, peptides and proteins. Page 321-429 in: Food Chemistry 3rd Ed. O. R. Fennema, ed. Marcel Dekker: New York, USA.
- Darby, N. J., and Creighton, T. E. 1993. Protein Structure. Pages 1-99. IRL Press: Oxford, UK.
- De Graaf, L. A., 2000. Denaturation of proteins from a non-food perspective. J. Biotechnol. 79:299-306.
- Debeaufort, F., Quezada-Gallo, J. A., and Voilley, A. 1998. Edible films and coatings: tomorrow's packaging: A review. Critical Rev. Food Sci. 38:299-313.
- Decareau, R. V. 1985. Microwaves in the Food Processing Industry. Pages 1-234. Academic Press: Orlando, USA.
- Demicheli, M. 1996. Biodegradable plastics from renewable sources. The Institute for Prospective Technological Studies Report 10:28-34.
- DeRose, R. T., Ma, D. P., Kwon, I. S., Hasnain, S. E., Klassy, R. C., and Hall, T. C. 1989. Characterization of the kafirin gene family from sorghum reveals extensive homology with zein from maize. Plant Mol. Biol. 12:245-256.
- Di Gioia, L., Cuq, B., and Guilbert, S. 1998. Effect of hydrophilic plasticizers on thermomechanical properties of corn gluten meal. Cereal Chem. 75:514-519.
- Dickinson, E. 1997. Enzymic cross-linking as a tool for food colloid rheology control and interfacial stabilization. Trends Food Sci. Technol. 8:334-339.
- Doi, Y., Kumagai, Y., Tanahashi, N., and Mukai, K. 1992. Structural effects on biodegradation of microbial and synthetic poly(hydroxyalkanoates). Pages 139-148 in: Biodegradable Polymers and Plastics. M. Vert, J. Feijen, A. Albertsson, G. Scott and E. Chiellini, eds. Royal Society of Chemistry: Cambridge, UK.
- Drenth, J. 1994. Principles of Protein X-ray Crystallography. Pages 1-128. Springer-Verlag: Berlin, Germany.
- Duodu K. G. 2000. Role of grain organisational structure in sorghum protein digestibility. PhD. Thesis, University of Pretoria, Pretoria, South Africa.
- Duodu, K. G., Taylor, J. R. N., Belton, P. S., and Hamaker, B. R. 2003. Factors affecting sorghum protein digestibility. J. Cereal Sci. 38:117-131.
- Duodu, K. G., Tang, H., Grant, A., Wellner, N., Belton, P. S., and Taylor, J. R. N. 2001. FTIR and solid-state ¹³C NMR spectroscopy of proteins of wet cooked and popped sorghum and maize. J. Cereal Sci. 33:261-269.

- Duodu, K. G., Nunes, A., Delgadillo, I., Parker, M. L., Mills, E. N. C., Belton, P. S., and Taylor, J. R. N. 2002. Effect of grain structure and cooking on sorghum and maize *in-vitro* protein digestibility. *J. Cereal Sci.* 35:161–174.
- El Maki, H. B., Babiker, E. E., and El Tinay, A. H. 1999. Changes in chemical composition, grain malting, starch and tannin contents and protein digestibility during germination of sorghum cultivars. *Food Chem.* 64:331-336
- El Nour, I. N. A., Peruffo, A. D. B., and Curioni, A. 1998. Characterisation of sorghum kafirins in relation to their cross-linking behaviour. *J. Cereal Sci.* 28:197-207.
- Elkhalifa, A. E. O., Chandrasheker, A., Mohamed, B. E., and El Tinay, A. H. 1999. Effect of reducing agents on the *in vitro* protein and starch digestibilities of cooked sorghum. *Food Chem.* 66:323-326.
- Emmambux, N. M. 2004. Tannin binding of kafirin and its effects on kafirin films. PhD. Thesis. University of Pretoria, Pretoria, South Africa.
- Emmambux, N. M., and Taylor J. R. N. 2003. Sorghum kafirin interaction with various phenolic compounds. *J. Sci. Food Agric.* 83:402-407.
- Emmambux, N. M., Stading, M., and Taylor, J. R. N. 2004. Sorghum kafirin film property modification with hydrolysable and condensed tannins. *J. Cereal Sci.* 40:127-135.
- ENVIROPAK. 2002. www.sik.se/enviropak [29 November 2004]
- Evans, D. J., Schüssler, L., and Taylor, J. R. N. 1987. Isolation of reduced-soluble protein from sorghum starchy endosperm. *J. Cereal Sci.* 5:61–65.
- FAO. 2004. Food and Agriculture Organization Statistical databases (<http://apps.fao.org>) [30 November 2004]
- Farag, R. S., Hegazy, R. A., El-Khawas, K. H., and Farag, M. M. 2001. Electrophoretic patterns of microwaved and γ -irradiated beef liver proteins. *J. Sci. Food Agric.* 81:975 - 982.
- Fathi, Z., Wander, J., and Ahmad, I. 2005. Microwave curing. <http://www.ammagazine.com/CDA/ArticleInformation/features/BNP> [12 May 2005].
- Fayle, S. E., Gerrard, J. A., Simmons, L., Meade, S. J., Reid, E. A., and Johnston, A. C. 2000. Crosslinkage of proteins by dehydroascorbic acid and its degradation products. *Food Chem.* 70:193-198.

- Feeney, R. E., and Whitaker, J. R. 1988. Importance of cross-linking reactions in proteins. Pages 21-46 in: *Advances in Cereal Science and Technology* Vol. IX. Y. Pomeranz, ed. American Association of Cereal Chemists: St Paul, USA.
- Fink, A. L., Seshadri, S., Khurana, R., and Oberg, K. A. 2000. Determination of secondary structure in protein aggregates using attenuated total reflectance FTIR. Pages 132-144 in: *Infrared Analysis of Peptides and Proteins: Principles and Applications*. B. R. Singh, ed. American Chemical Society: Washington DC, USA.
- Finley, J. W. 1985. Environmental effects on protein quality. Pages 443-482 in: *Chemical Changes in Food During Processing*. T. Richardson and J. W. Finley, eds. AVI Publishing Company: Westport, USA.
- Fombang, E. N. 2005. Protein digestibility of sorghum and maize flours and porridges as affected by gamma-irradiation. PhD. Thesis, University of Pretoria, Pretoria, South Africa.
- Foster, R. 1986. Ethylene-vinyl alcohol copolymers (EVOH). Pages 270-275 in: *The Wiley Encyclopaedia of Packaging Technology*, M. Bakker ed. John Wiley and Sons: New York, USA.
- Fraser, R. D. B., and Suzuki, E. 1970. Infrared methods. Pages 251-273 in: *Physical Principles and Techniques of Protein Chemistry*. S. J. Leach ed. Academic Press: London, UK.
- Gabriel, B. L. 1982. *Biological Scanning Electron Microscopy*. Pages 1-31. Van Nostrand Reinhold: New York, USA.
- Gallawa, J. C. 2005. The Magnetron Tube Used in Microwave Ovens. <http://www.gallawa.com/microtech/magnetron.html> [21 October 2005].
- Gao, C., Taylor, J., Wellner, N., Byaruhanga, Y. B., Parker, M. L., Mills, E. N. C., and Belton, P. S. 2005. Effect of preparation conditions on protein secondary structure and biofilm formation of kafirin. *J. Agric. Food Chem.* 53:306-312.
- Gearing J., 1999. Dynamic mechanical (thermal) analysis. Pages 501-531 in: *Handbook of Polymer Testing*. R. Brown, ed. Marcel Dekker: New York.
- Gennadios, A., Park, H. J., and Weller, C. L. 1993a. Relative humidity and temperature effects on tensile strength of edible protein and cellulose ether films. *Trans. Amer. Soc. Agric. Eng.* 36:1867-1872.
- Gennadios, A., Weller, C. L., and Testin, R. F. 1993b. Property modification of edible wheat gluten based films. *Trans. Amer. Soc. Agric. Eng.* 36:465-470.

- Gennadios, A., Weller, C.L., and Gooding, C.H. 1994a. Measurement errors in water vapor permeability of highly permeable, hydrophilic films. *J. Food Eng.* 21:395-409.
- Gennadios, A., Ghorpade, V. M., Weller, C. L., and Hanna, M. A. 1996. Heat curing of soy protein films. *Trans. Am. Soc. Agric. Eng.* 39:575-579.
- Gennadios, A., McHugh, T. H., Weller, C. L., and Krochta, J. M. 1994b. Edible coatings and films based on proteins. Pages 201–277 in: *Edible Coatings and Films to Improve Food Quality*. M. J. Krochta, E. A. Baldwin and M. N. Carriedo, eds. Technomic Publishing Company: Lancaster, USA.
- Gennadios, A., Rhim, J. W., Handa, A., Weller, C. L., and Hanna, M. A. 1998. Ultraviolet radiation affects physical and molecular properties of soy protein films. *J. Food Sci.* 63:225-228.
- Giese, J. 1992. Advances in microwave food processing. *Food Technol.* 46 (9):118-123.
- Glusker, J. P., Lewis, M., and Rossi, M. 1994. *Crystal Structure Analysis for Chemists and Biologists*. Pages 1-104. VCH Publishers: Weinheim, Germany.
- Goburdhun, D., 2004. Extending the shelf life of export quality litchis. www.sik.se/enviropak [29 December 2004]
- Gontard, N., and Ring, S. 1996. Edible wheat gluten film: Influence of water content on glass transition temperature. *J. Agric. Food Chem.* 44:3474-3478.
- Gontard, N., Guilbert, S., and Cuq, J. L. 1993. Water and glycerol as plasticisers affect mechanical and water barrier properties of an edible wheat gluten film. *J. Food Sci.* 58:206-211.
- Grasselli, J. G., Snavely, M. K., and Bulkin, B. J. 1981. *Chemical Applications of Raman Spectroscopy*. Pages 1-79. John Wiley and Sons: New York, USA.
- Hahn, D. H., Faubion, J. M., Ring, S. H., Doherty, C. A., and Rooney, L. W. 1982. Semiautomated *in vitro* analysis of sorghum protein availability via pronase hydrolysis. *Cereal Chem.* 59:132-136.
- Hamaker, B. R., Kirleis, A. W., Mertz, E. T., and Axtell, J. D. 1986. Effect of cooking on the protein profiles and *in vitro* protein digestibility of sorghum and maize. *J. Agric. Food Chem.* 34:647–649.
- Hamaker, B. R., Kirleis, A. W., Butler, L. G., Axtell, J. D., and Mertz, E. T. 1987. Improving the *in vitro* protein digestibility of sorghum with reducing agents. *Proc. Natl. Acad. Sci. USA* 84:626-628.

- Hamaker, B. R., Mohamed, A. A., Habben, J. E., Huang C. P., and Larkins, B. A. 1995. Efficient procedure for extraction of maize and sorghum kernel proteins reveals higher prolamin contents than the conventional method. *Cereal Chem.* 72:583-588.
- Hames, B. D. 1990. One-dimensional polyacrylamide gel electrophoresis. Pages 1-147 in: *Gel Electrophoresis of Proteins: A Practical Approach* 2nd Ed. B. D. Hames and D. Rickwood, eds. IRL Press: Oxford, UK.
- Haris, P. I. 2000. Fourier transform infrared spectroscopic studies of peptides: potentials and pitfalls. Pages 54-95 in: *Infrared Analysis of Peptides and Proteins Principles and Applications*. B. L. Singh, ed. American Chemical Society: Washington DC, USA.
- Harrison, D. L. 1980. Microwave versus conventional cooking methods: effects on food quality attributes. *J. Food Protection* 43:633-637.
- Hattori, M., Numamoto, K., Kobayashi, K., and Takahashi, K. 2000. Functional changes in β -lactoglobulin by conjugation with cationic saccharides. *J. Agric. Food Chem.* 48:2050-2056.
- Hohne, G. W. H., Hemminger, W., and Flammersheim, H. J. 1996. *Differential Scanning Calorimetry: An Introduction for Practitioners*. Pages 1-171, Springer-Verlag: Berlin.
- Hurrell, R. F., and Finot, P. A. 1982. Effects of food processing on protein digestibility and amino acid availability. Page 233-246, in : *Digestibility and amino acid availability in cereals and oilseeds*. J. W. Finley and D. T. Hopkins, eds. American Association of Cereal Chemists: St Paul, USA.
- ICRISAT/FAO. 1996. *The World Sorghum and Millet Economies: Facts Trends and Outlook*. Pages 1-2. International Crops Research Institute for the Semi-Arid Tropics. ICRISAT/FAO: Patancheru, India/ Rome, Italy.
- Irissin-Mangata, J., Bauduin, G., Boutevin, B., and Gontard, N. 2001. New plasticizers for wheat gluten films. *Eur. Polymer. J.* 37:1533-1541.
- Joly, M. 1965. *A Physico-Chemical Approach to the Denaturation of Proteins*. Pages 1-350. London: Academic Press: London, UK.
- Kader, A. A. 1992. Postharvest biology and technology: An overview. Pages 15-20 in: *Postharvest Technology of Horticultural Crops*, 2nd Ed. A. A. Kader, ed. University of California, Davis, California, USA.

- Karim, A. A, Norziah, M. H., and Seouw, C. C. 2000. Methods for the study of starch retrogradation. *Food Chem.* 54:9-36
- Kaya, S., and Kaya, A. 2000. Microwave drying effects on properties of whey protein isolate edible films. *J. Food Eng.* 43:91-96.
- Kester, J. J., and Fennema, O. R. 1986. Edible films and coatings: A review. *Food Technol.* 40:(12) 47-59.
- Khan, M. 2004. Extending the shelf life of export quality cashew nuts. www.sik.se/enviropak [29 December 2004].
- Kiefer, W., and Spiekermann, M. 1995. Application of non-classical Raman Spectroscopy: Resonance Raman, surface enhanced Raman and non-linear coherent Raman spectroscopy. Pages 465-516 in: *Infrared and Raman Spectroscopy*. B. Schrader, ed. VCH: Weinheim, Germany.
- Kim, K. M., Weller, M. A., and Gennadios, A. 2002. Heat curing of soy protein films at atmospheric and sub-atmospheric conditions. *J. Food Sci.* 67:708-713.
- Krochta, J. M. 2002. Proteins as raw materials for films and coatings: definitions, current status and opportunities. Pages 1–41 in: *Protein-based Films and Coatings*. A. Gennadios, ed. CRC Press: Boca Raton, USA.
- Krochta, J. M., and De Mulder-Johnston, C. 1997. Edible and biodegradable polymer films: challenges and opportunities. *Food Technol.* 51:(2) 61-74.
- Krochta, J. M., and McHugh, T. H. 1996. Water-insoluble protein-based edible barrier coatings and films. US Patent 5 543 164.
- Lacroix, M., Le, T. C., Ouattara, B., Yu, H., Letendre, M., Sabato, S. F., Mateescu, M. A., and Patterson, G. 2002. Use of γ -irradiation to produce films from whey, casein and soy proteins: structure and functional characteristics. *Rad. Phys. Chem.* 63:827-832.
- Lai, H. M., and Chiang, I. C. 2003. Effects of MTGase on the properties of gluten films. www.ac.ntu.edu/ [20 July 2004].
- Lai, H. M., and Padua, G. W. 1998. Water vapor barrier properties of zein films plasticized with oleic acid. *Cereal Chem.* 75:194-199.
- Lakkis, J., and Villota, R. 1992. Comparative performance of chemically and enzymatically modified whey proteins. *Food Chem.* 43:93-105.
- Lambert, J. P. 1980. Biological hazards of microwave radiation. *J. Food Protection* 43:625-628.

- Larre, C., Desserre, C., Barbot, J., and Gueguen, J. 2000. Properties of deamidated gluten films enzymatically cross-linked. *J. Agric. Food Chem.* 48:5444-5449.
- Lawton, J. W. 2002. Zein: A history of processing and use. *Cereal Chem.* 79:1-18.
- Lawton, J. W. 2004. Plasticizers for zein: their effect on tensile properties and water absorption of zein films. *Cereal Chem.* 81:1-5.
- Le Tien, C., Letendre, M., Ispas-Szabo, P., Mateescu, M. A., Delmas-Patterson, G., Yu, H. L., and Lacroix, M. 2000. Development of biodegradable films from whey proteins by cross-linking and entrapment in cellulose. *J. Agric. Food Chem.* 48:5566-5575.
- Lefevre, T., and Subirade, M. 1999. Structural and interaction properties of β -lactoglobulin as studied by FTIR spectroscopy. *Int. J. Food Sci. Technol.* 34:419-428.
- Lending, C. R., and Larkins, B. A. 1989. Changes in the zein composition of protein bodies during maize endosperm development. *Plant Cell* 1:1011-1023.
- Lens, J.-P., De Graaf, L. A., Stevels, W. M., Dietz, C. H. J. T., Verhelst, K. C. S., Vereijken, J. M., and Kolster, P. 2003. Influence of processing and storage conditions on the mechanical and barrier properties of films cast from aqueous wheat gluten dispersions. *Ind. Crops Prod.* 17:119-130.
- Li-Chan, E. 1983. Heat-induced changes in the proteins of whey protein concentrate. *J. Food Sci.* 48: 47-56.
- Lim, L. T., Mine, Y., and Tung, M. A. 1999. Barrier and tensile properties of transglutaminase cross-linked gelatin films as affected by relative humidity, temperature and glycerol content. *J. Food Sci.* 64:616-622.
- Lindsay, M. P., and Skerritt, J. H. 1999. The glutenin macropolymer of wheat flour doughs: Structure-function perspectives. *Trends Food Sci. Technol.* 10:247-253.
- Lin-Vien, D., Colthup, N. B., Fateley, W. G., and Grasselli, J. G. 1991. *The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules*. Pages 1-7. Academic Press: San Diego, USA.
- Liu, X., Sun, Q., Wang, H., Zhang, L., and Wang, J. Y. 2005. Microspheres of corn protein, zein, for an ivermectin drug delivery system. *Biomaterials* 26:109-115.
- Livney, Y. D., Verespej, E., and Dalgleish, D. G. 2003. Steric effects governing disulfide bond interchange during thermal aggregation in solutions of β -lactoglobulin B and α -lactalbumin. *J. Agric. Food Chem.* 51:8098-8106.

- Ma, C. Y., Rout, M. K., and Mock, W. Y. 2001. Study of oat globulin conformation by Fourier transform infrared spectroscopy. *J. Agric. Food Chem.* 49:3328-3334.
- Ma, C. Y., Rout, M. K., Chan, W. M., and Phillips, D. L. 2000. Raman spectroscopic study of oat globulin conformation. *J. Agric. Food Chem.* 48:1542-1547.
- Macleane, W. C., De Romana, G. L., Placko, R. P., and Graham, G. G. 1981. Protein quality and digestibility of sorghum in pre-school children: balance studies and plasma free amino acids. *J. Nutr.* 111:1928-1936.
- Madeka, H., and Kokini, J. L. 1996. Effect of glass transition and cross-linking on rheological properties of zein: Development of a preliminary state diagram. *Cereal Chem.* 73:433-438.
- Makowsky, G. S., and Ramsby, M. L. 1997. Protein molecular weight determination by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Pages 1-57 in: *Protein Structure: A Practical Approach*, 2nd Ed. T. E. Creighton, ed. IRL Press: Oxford, UK.
- Mate, J. I., and Krochta, J. M. 1996. Comparison of oxygen and water vapor permeabilities of whey protein isolate and β -lactoglobulin edible films. *J. Agric. Food Chem.* 44:3001-3004.
- Matveev, Y. I., Grinberg, V. Y., and Tolstoguzov, V. B. 2000. The plasticizing effect of water on proteins, polysaccharides and their mixtures. Glassy state of biopolymers, food and seeds. *Food Hydrocolloids* 14:425-437.
- McClure, G. L. 1987. Quantitative analysis from infrared spectrum. Pages 145-202 in: *Laboratory Methods in Vibrational Spectroscopy*, 3rd Ed. H. A. Willis, J. H. van der Maas and R. G. J. Miller eds. John Wiley and Sons: Chichester, UK.
- Meng, G. T., and Ma, C. Y. 2001. Fourier-transform infrared spectroscopic study of globulin from *Phaseolus angularis* (red bean). *Int. J. Biol. Macromol.* 29:287-294.
- Meng, G. T., Ma, C. Y., and Phillips, D. L. 2003. Raman spectroscopic study of globulin from *Phaseolus angularis* (red bean). *Food Chem.* 81:411-420.
- Mezgheni, E., D'aprano, G., and Lacroix, M. 1998. Formation of sterilized films based on caseinate: effects of calcium plasticizers. *J. Agric. Food Sci.* 46:318-324.
- Microdry, 2005. Microdry Industrial Microwave Technology. <http://www.microdry.com> [12 May 2005]
- Miller, K. S., and Krochta, J. M. 1997. Oxygen and aroma barrier properties of edible films: A review. *Trends Food Sci. Technol.* 8:228-237.

- Miller, K. S., Chiang, M. T., and Krochta, J. M. 1997. Heat curing of whey protein films. *J. Food Sci.* 62:1189-1193.
- Miller, L. A. Gordon, J., and Davis, E. A. 1991. Dielectric and thermal transition properties of chemically modified starches during heating. *Cereal Chem.* 68:441-448.
- Mimouni, B., Raymond, J., Merle-Desnoyers, A. M., Azanza, J. L., and Ducastaing, A. 1994. Combined acid deamidation and enzymic hydrolysis for improvement of functional properties of wheat gluten. *J. Cereal Sci.* 21:153-165.
- Mizutani, Y., Matsumura, Y., Murakami, H., and Mori, T. 2004. Effects of heating on the interaction of lipid and zein in a dry powder system. *J. Agric. Food Chem.* 52: 3570-3576.
- Mizutani, Y., Matsumura, Y., Imamura, K., Nakanishi, K., and Mori, T. 2003. Effects of water activity and lipid addition on secondary structure of zein in powder systems. *J. Agric. Food Chem.* 51:229-235.
- Mohsenin, N. N. 1984. *Electromagnetic Radiation Properties of Foods and Agricultural Products*. Pages 401-435. Gordon and Breach Science Publishers: New York, USA.
- Morel, M-H., Bonicel, J., Micard, V., and Guilbert, S. 2000. Protein insolubilization and thiol oxidation in sulfite treated wheat gluten films during aging at various temperatures and relative humidities. *J. Agric. Food Chem.* 48:186-192.
- Motoki, M., and Seguro, K. 1998. Transglutaminase and its use for food processing. *Trends Food Sci. Technol.* 9:204-210.
- Mudgett, R. E. 1982. Electrical properties of foods in microwave processing. *Food Technol.* 36(2):109-115.
- Nelson, S. O., and Stetson, L. E. 1975. 250 Hz – 12 GHz dielectric properties of grain and seed. *Trans. Amer. Soc. Agric. Eng.* 18:714-715, 718.
- NiceProt 2004. NiceProt view of TrEMBL: Q6KB71, O8205 and Q41506. <http://ca.expasy.org/cgi-bin/niceprot.pl> [30 November 2004]
- Nieuwoudt, H. H., Prior, B. A., Pretorius, I. S., Manley, M., and Bauer, F. F. 2004. Principal component analysis applied to Fourier transform infrared spectroscopy for the design of calibration sets for glycerol prediction models in wine and for the detection and classification of outlier samples. *J. Agric. Food Chem.* 52:3726-3735.

- Nilsson, K. 2004. Migration and release of added substances from kafirin coatings to fruits. www.sik.se/enviropak [29 December 2004]
- Nunes, A., Correia, I., Barros, A., and Delgadillo, I. 2004. Sequential *in-vitro* pepsin digestion of uncooked and cooked sorghum and maize samples. J. Agric. Food Chem. 52:2052-2058.
- Ohlsson, T., and Bengtsson, N. 2001. Microwave technology and foods. Pages 65-140. in: Advances in Food and Nutrition Research Vol. 34. S. L. Taylor, ed. Academic Press: San Diego, USA.
- Oria, M. P., Hamaker, B. R., and Shull, J. M. 1995a. Resistance of sorghum α -, β -, and γ -kafirins to pepsin digestion. J. Agric. Food Chem. 43:2148-2153.
- Oria, M. P., Hamaker, B. R., and Shull, J. M. 1995b. *In vitro* protein digestibility of developing mature sorghum grain in relation to α -, β - and γ -kafirin disulphide cross-linking. J. Cereal Sci. 22:85-93.
- Otterburn, N. S. 1989. Protein crosslinking. Pages 247-289, in: Protein Quality and the Effects of Processing, R. D. Phillips and J. W. Finley, eds. Marcel Dekker: New York, USA.
- Ou, S. Kwok, K. C., and Kang Y. 2004. Changes in *in-vitro* digestibility and available lysine of soy protein isolate after formation of films. J. Food Eng. 64:301-305.
- Owusu-Apenten, R. K., Chee, C., and Hwee, O. P. 2003. Evaluation of a sulphhydryl-disulphide exchange index (SEI) for whey proteins: β -lactoglobulin and bovine serum albumin. Food Chem. 83:541-545.
- Padua, G. W. 1993. Proton NMR and dielectric measurements on sucrose filled agar gels and starch pastes. J. Food Sci. 58:603-604, 626.
- Padua, G. W., and Wang, Q. 2002. Formation and properties of corn zein films and coatings. Pages 43-67 in: Protein Based Films and Coatings. A. Gennadios, ed. CRC Press: Boca Raton, USA.
- Park, H. J., and Chinnan, M. S. 1995. Gas and water vapor barrier properties of edible films from protein and cellulosic material. J. Food Eng. 25:497-507.
- Park, H. J., Chinnan, M. S., and Shewfelt, R. L. 1994a. Edible corn-zein film coatings to extend storage life of tomatoes. J. Food Processing Preservation 18:317-331.
- Park, H. J., Chinnan, M. S., and Shewfelt, R. L. 1994b. Edible coating effects on the storage life and quality of tomatoes. J. Food Sci. 59:568-570.

- Park, H. J., Bunn, J. M., Weller C. L., Vergano, P. J., and Testin, R. F. 1994c. Water vapor permeability and mechanical properties of grain protein-based films as affected by mixtures of polyethylene glycol and glycerin plasticizers. *Trans. Amer. Soc. Agric. Eng.* 37:1281-1285.
- Parris, N., and Coffin, D. R. 1997. Composition factors affecting the water vapor permeability and tensile properties of hydrophilic zein films. *J. Agric. Food Chem.* 45:1596-1599.
- Pascat, B. 1986. Study of some factors affecting permeability. Pages 2-24 in: *Food Packaging and Preservation: Theory and Practice*. M. Mathlouthi, ed. Elsevier Applied Science Publishers: New York, USA.
- Perez-Gago, M. B., and Krochta, J. M. 2001. Denaturation time and temperature effects on solubility, tensile properties and oxygen permeability of whey protein edible films. *J. Food Sci.* 66:705-710.
- Perez-Gago, M. B., Nadaud, P., and Krochta, J. M. 1999. Water vapor permeability, solubility and tensile properties of heat denatured versus native whey protein films. *J. Food Sci.* 64:1034-1037.
- Pezolet, M., Benonfant, S., Dousseau, F., and Popineau, Y. 1992. Conformation of wheat gluten proteins: comparison between functional and solution states as determined by infrared spectroscopy. *Fed. Eur. Biochem. Soc.* 299:247-250.
- Popineau, Y., Bonenfant, S., Cornec, M., and Pezolet, M. 1994. A study by infrared spectroscopy of the conformations of gluten proteins differing in their gliadin and glutenin compositions. *J. Cereal Sci.* 20:15-22.
- Ptitsyn, O. B. 1992. The molten globule state. Pages 243-300 in: *Protein Folding*. T. E. Creighton, ed. WH Freeman and Company: New York, USA.
- Quinn, G., Monahan, F. J., O'Riordan, E. D., O'Sullivan, M., and Longares, A. 2003. Role of covalent and non-covalent interactions in the formation of films from unheated whey protein solutions following pH adjustments. *J. Food Sci.* 68:2284-2288
- Rahmelow, K., and Hubner, W. 1996. Fourier self-deconvolution: parameter determination and analytical band shapes. *Appl. Spectrosc.* 50:795-804.
- Rakotonirainy, A. M., Wang, Q., and Padua, G. W. 2001. Evaluation of zein films as modified atmosphere packaging for fresh broccoli. *J. Food Sci.* 66:1108–1111.
- Rangavajhyala, N., Ghorpade, V., and Hanna, M. 1997. Solubility and molecular properties of heat-cured soy protein films. *J. Agric. Food Chem.* 45: 4204-4208

- Reiger, J., 2001. The glass transition temperature of polymers, comparison of values from differential thermal analysis (DTA, DSC) and dynamic mechanical measurements (torsion pendulum). *Polymer Testing* 20:199-204.
- Rhim, J. W., Gennadios, A., Fu, D., Weller, C. L., and Hanna, M. A. 1999. Properties of ultraviolet irradiated protein films. *Lebensmittel-Wiss. Technol.* 32:129-133.
- Ringe, D., and Petsko, G. A. 2003. The 'glass transition' in protein dynamics: What it is, why it occurs and how to exploit it. *Biophysical Chem.* 105:667-680.
- Rom, D. L., Shull, J. M., Chandrashekar, A., and Kirleis, A. W. 1992. Effects of cooking and treatment with sodium bisulfite on in vitro protein digestibility and microstructure of sorghum flour. *Cereal Chem.* 69:178-181.
- Roy, S., Weller, C. L., Gennadios, A., Zeece, M. G., and Testin, R. F. 1999. Physical and molecular properties of wheat gluten films cast from heated film-forming solutions. *J. Food Sci.* 64:57-60.
- Salame, M. 1986. Barrier polymers. Pages 46-54 in: *The Wiley Encyclopaedia of Packaging Technology*. M. Bakker, ed. John Wiley and Sons: New York, USA.
- Salmoral, E. M., Gonzalez, M. E., and Mariscal, M. P. 2000a. Biodegradable plastic made from bean products. *Ind. Crops Products* 11:217-225.
- Salmoral, E. M., Gonzalez, M. E., Mariscal, M. P., and Medina, L. F. 2000b. Comparison of chickpea and soy protein isolate and whole flour as biodegradable plastics. *Ind. Crops Products* 11:227-236.
- Salvador, A., Pena, M. C., and De la Guardia, M. 2001. Stopped-flow Fourier-Transform Infrared spectrometric speciation of glycolic and lactic acids in cosmetic formulations. *Analyst* 126:1428-1431.
- Sastry, L. V. S., and Virupaksha, T. K. 1969. Alcohol soluble proteins of grain sorghum. *Cereal Chem.* 46:284-293.
- Schrader, B. 1995a. General survey of vibrational spectroscopy. Pages 7-61 in: *Infrared and Raman spectroscopy*. B. Schrader, ed. VCH: Weinheim, Germany.
- Schrader, B. 1995b. Tools for infrared and Raman spectroscopy. Pages 63-188 in: *Infrared and Raman spectroscopy*. B. Schrader, ed. VCH: Weinheim, Germany.
- Seidel, J. R. 1988. Industrial radiation curing. Pages 123-143 in: *Polymers for Advanced Technologies*. IUPAC International Symposium. M. Lewin, ed. VCH: Weinheim, Germany
- Selke, S. 1996. *Biodegradation and Packaging* 2nd Ed. Pages 1-20. Pira International, Surrey, UK.

- Sharma, G. P., and Prasad, S. 2002. Dielectric properties of garlic (*Allium sativum* L) at 2450 MHz as a function of temperature and moisture content. J. Food Eng. 52:343-348.
- Shewry, P. R. 1995. Plant storage proteins. Biol. Rev. 70:375-426.
- Shewry, P. R. 2002. The major seed storage proteins of spelt wheat, sorghum, millets and pseudocereals. Pages 1-24 in: Pseudocereals and Less Common Cereals: Grain Properties and Utilization Potential. P. S. Belton and J. R. N. Taylor, eds. Springer: Berlin, Germany.
- Shewry, P. R., and Halford, N. G. 2002. Cereal seed storage proteins: structures, properties and role in grain utilisation. J. Exp. Bot. 53:(370) 947-958.
- Shewry, P. R., and Tatham, A. S. 1997. Disulphide bonds in wheat gluten proteins. J. Cereal Sci. 25:207-227.
- Shimada, K., and Cheftel, J. C. 1988a. Determination of sulfhydryl groups and disulfide bonds in heat-induced gels of soy protein isolate. J. Agric. Food Chem. 36:147-153.
- Shimada, K., and Cheftel, J. C. 1988b. Texture characteristics, protein solubility and sulfhydryl group/disulfide bond contents of heat-induced gels of whey protein isolate. J. Agric. Food Chem. 36:1018-1025.
- Shimada, K., and Cheftel, J. C. 1989. Sulfhydryl group/disulfide bond interchange reactions during heat-induced gelation of whey isolate. J. Agric. Food Chem. 37:161-168.
- Shore, V. G., and Parde, A. B. 1956. Fluorescence of some proteins, nucleic acids and related compounds. Arch. Biochem. Biophys. 60:100-107
- Shrewsbury, R. P. 2004. Factors affecting the solubility of drugs. <http://pharmlabs.unc.edu/solubility/text.htm> [30 November 2004].
- Shukla, R., and Cheryan, M. 2001. Zein: The industrial protein from corn. Ind. Crops Products 13:171-192.
- Shull, J. M. Watterson, S. J., and Kirleis, A. W. 1991. Proposed nomenclature for the alcohol-soluble proteins (kafirins) of *Sorghum bicolor* (L. Moench) based on molecular weight, solubility and structure. J. Agric. Food Chem. 39:83-87.
- Shull, J. M., Watterson, J. J., and Kirleis, A. W. 1992. Purification and immunocytochemical localization of kafirins in *Sorghum bicolor* (L. Moench) endosperm. Protoplasma 171:64-74.

- Sikorski, Z. E. 1997. Proteins. Pages 119-160 in: Chemical and Functional Properties of Food Components. Z. E. Sikorski, ed. Technomic Publishing Company: Lancaster, USA.
- Singh, B. R. 2000. Basic aspects of the technique and applications of infrared spectroscopy of peptides and proteins. Pages 2-37 in: Infrared Analysis of Peptides and Proteins: Principles and Applications. B. R. Singh, ed. American Chemical Society: Washington DC, USA.
- Singh, P., and Heldman, D. R. 1993. Introduction to Food Engineering, 2nd Edition. Pages 1-499. Academic Press, London.
- Sipahioglu O., and Barringer S. A. 2003. Dielectric properties of vegetables and fruits as a function of temperature, ash, and moisture content. J. Food Sci. 68:234-239.
- Skerritt, J. H. 1988. Immunochemistry of cereal grain storage proteins. Pages 263-338 in: Advances in Cereal Science and Technology, Vol. IX. Y. Pomeranz, ed. American Association of Cereal Chemists: St Paul, USA.
- Smith, S. A. 1986. Polyethylene: Low density. Pages 48-54 in: The Wiley Encyclopaedia of Packaging Technology. M. Bakker, ed. John Wiley and Sons: New York, USA.
- Sommer, N. F., and Arpaia, M. L. 1992. Postharvest handling systems: Tropical fruits. Pages 241-256 in: Postharvest Technology of Horticultural crops. 2nd Ed. A. A. Kader, ed. University of California, Davis, California, USA.
- Sothornvit, R., Reid, D. S., and Krochta, J. M. 2002. Plasticizer effect on the glass transition temperature of beta-lactoglobulin films. Trans. Amer. Soc. Agric. Eng. 45:1479-1484.
- Spahioglu, O., and Barringer, S. A. 2003. Dielectric properties of vegetables and fruits as a function of temperature, ash and moisture content. J. Food Sci. 68:234-239.
- Stading, M., Rindlav-Westling, A., and Gatenholm, P. 2001. Humidity-induced structural transitions in amylose and amylopectin films. Carb. Polymers 45:209-217.
- Stuchell, Y. M., and Krochta, J. M. 1994. Enzymatic treatments and thermal effects on edible soy protein films. J. Food Sci. 59:1332-1337.

- Subirade, M. Kelly, I. Gueguen, J., and Pezolet, M. 1998. Molecular basis of film formation from a soybean protein: comparison between the conformation of glycinin in aqueous solution and in films. *Int. J. Biol. Macromol.* 23:241-249.
- Surewicz, W. K., and Mantsch, H. H. 1988. New insight into protein secondary structure from resolution-enhanced infrared spectra. *Biochimica et Biophysica Acta* 952:115–130.
- Szczesniak, A. S. 1983. Physical properties of foods: what they are and their relation to other food properties. Pages 1-41, in: *Physical Properties of Foods*, M. Peleg and E. B. Bagley, eds. AVI Publishing Company: Westport, USA.
- Taylor, C. C. 1986. Cellophane. Pages 159-163 in: *The Wiley Encyclopaedia of Packaging Technology*. M. Bakker, ed. John Wiley and Sons: New York.
- Taylor, J., Taylor, J. R. N., Dutton, M. F., and De Kock, S. 2005a. Identification of kafirin film casting solvents. *Food Chem.* 90:401-408.
- Taylor, J., Taylor, J. R. N., Dutton, M. F., and De Kock, S. 2005b. Glacial acetic acid-a novel food-compatible solvent for kafirin extraction. *Cereal Chem.* In press.
- Taylor, J. R. N., and Belton, P. S. 2002. Sorghum. Pages 25-91 in: *Pseudocereals and Less Common Cereals: Grain Properties and Utilization Potential*. P. S. Belton and J. R. N. Taylor, eds. Springer: Berlin, Germany.
- Taylor, J. R. N., and Evans, D. J. 1989. Action of sorghum proteinase on the protein bodies of sorghum starchy endosperm. *J. Exp. Bot.* 40:763-768.
- Taylor, J. R. N., Novellie, L., and Liebenberg, N. v. d. W. 1984a. Sorghum protein body composition and ultrastructure. *Cereal Chem.* 61:69-73.
- Taylor, J. R. N., Schüssler, L., and Van der Walt, W. H. 1984b. Fractionation of proteins from low tannin sorghum grain. *J. Agric. Food Chem.* 32:149-154.
- Thannhauser, T. W., Konishi, Y., and Scheraga, H. A., 1987. Analysis of disulphide bonds in peptides and proteins. *Methods Enzymol.* 143:115-119.
- Tharanathan, R. N. 2003. Biodegradable films and composite coatings: Past present and future. *Trends Food Sci. Technol.* 14:71-78.
- Thornton, J. M. 1992. Protein structures: The end point of the folding pathway. Pages 59-81 in: *Protein Folding*. T. E. Creighton, ed. WH Freeman and Company: New York, USA.
- Toufeili, I, Lambert, I. A., and Kokini, J. L. 2002. Effect of glass transition and cross-linking on rheological properties of gluten: development of a preliminary state diagram. *Cereal Chem.* 79:138-142.

- Trezza, T. A., and Krochta, J. M. 2002. Application of edible protein coatings to nuts and nut-containing food products. Pages 527-549 in: Protein Based Films and Coatings. A. Gennadios, ed. CRC Press: Boca Raton, USA.
- Uvarov, E. B., Chapman, D. R., and Isaacs, A. 1971. A Dictionary of Science 4th ed. Pages 109-110. Penguin: Harmondsworth, UK.
- Vachon, C., Yu, H. L., Yefsah, R., Alain, R., St-Gelais, D., and Lacroix, M. 2000. Mechanical and structural properties of milk protein edible films cross-linked by heating and γ -irradiation. J. Agric. Food Chem. 48:3202-3209.
- Valentinova, O., Novotina, Z., Svoboda, Z., Schwarz, W., and Kas, J. 2000. Microwave heating and γ -irradiation treatment of rapeseed (*Brassica napus*). J. Food Lipids 7:237-245.
- Van de Velde, K., and Kiekens, P. 2002. Biopolymers: overview of several properties and consequences to their applications. Polymer Testing 21:433-442.
- Van Eck, H. 2004. Plasticization of kafirin films. MSc. (Agric) Dissertation. University of Pretoria, Pretoria, South Africa.
- Villamiel, M., Corzo, N., Martinez-Castro, I., and Olano, A. 1996. Chemical changes during microwave treatment of milk. Food Chem. 56:385-388.
- Wall, J. S., and Paulis, J. W. 1978. Corn and sorghum proteins. Pages 135-219 in: Advances in Cereal Science and Technology Volume II. Y. Pomeranz, ed. American Association of Cereal Chemists: St. Paul, USA.
- Wang, Q., and Padua, G. W. 2001. Effect of ionizing radiation on tensile properties of zein films. http://ift.confex.com/ift/2001/techprogram/paper_8169.htm. [20 August 2003]
- Wang, Y., and Padua, G. W. 2004. Water sorption properties of extruded zein films. J. Agric. Food Chem. 52:3100-3105.
- Wang, Y., Wig, T. D., Tang, J., and Hallberg, L. M. 2003b. Dielectric properties of foods relevant to RF and microwave pasteurization and sterilization. J. Food Eng. 57:257-268.
- Wang, Y., Sommers, E. B., Manolache, S., Denes, F. S., and Wong, A. C. L. 2003a. Cold plasma synthesis of poly(ethylene glycol)-like layers on stainless-steel surfaces to reduce attachment and biofilm formation by *Listeria monocytogenes*. J. Food Sci. 68:2772-2779.

- Watterson, J. J., Shull, J. M., and Kirleis, A. W. 1993. Quantitation of α -, β -, γ -kafirins in vitreous and opaque endosperm of *Sorghum bicolor*. *Cereal Chem.* 70:452-457.
- Watterson, J., Shull, J. M., Mohamed, A. A., Reddy, V., and Kirleis, A. W. 1990. Isolation of high-cysteine kafirin protein and its cross-reactivity with gamma-zein antiserum. *J. Cereal Sci.* 12:137-144.
- Wedemeyer, W. J., Welker, E., Narayan, M., and Scheraga, H. A. 2000. Disulfide bonds and protein folding. *Biochem.* 39:4209-4216.
- Wells-Knecht, K. J., Zyzak, D. V., Litchfield, J. E., Thorpe, S. R., and Baynes, J. W. 1995. Mechanism of autoxidative glycosylation: identification of glyoxal and arabinose as intermediates in the autoxidative modification of proteins by glucose. *Biochem.* 34:3702-3709.
- Westra, J. G., and Van Woerkom, P. C. M. 1987. Reflection spectroscopy. Pages 229-250 in: *Laboratory Methods in Vibrational Spectroscopy*, 3rd Ed. H. A. Willis, J. H. van der Maas and R. G. J. Miller, eds. John Wiley and Sons: Chichester, UK.
- Williams, V. R., Mattice, W. L., and Williams, H. B. 1978. *Basic Physical Chemistry for the Life Sciences* 3rd Ed. Pages 130-131. WH Freeman and Company: San Francisco, USA.
- Wohlfarth, C. 1974. Permittivity (Dielectric constants) of pure liquids. Section 6, pages 155-188 in *CRC Handbook of Chemistry and Physics* 75th Ed. D. R. Lide and H. P. R. Frederikse, eds. CRC Press: Boca Raton, USA.
- Wong, S. S., and Wong, L. J. C. 1992. Chemical cross-linking and the stabilisation of proteins and enzymes. *Enzyme Microbial Technol.* 14:866-874.
- Yamada, K., Takahashi, H., and Noguchi, A. 1995. Improved water resistance in edible zein films and composite for biodegradable food packaging. *Int. J. Food Sci. Technol.* 30:599-608.
- Yamauchi, K., Takeuchi, N., Kurimoto, A., and Tanabe, T. 2001. Films of collagen cross-linked by S-S bonds: preparation and characterization. *Biomaterials* 22:855-863.
- Yang, A. S., and Honig, B. 1995a. Free energy determinants of secondary structure formation: I. α -helices. *J. Mol. Biol.* 252:351-365.
- Yang, A. S., and Honig, B. 1995b. Free energy determinants of secondary structure formation: II. Antiparallel β -sheets. *J. Mol. Biol.* 252:366-376.

- Yen, G. C., Lee, T. C., and Chichester, C. O. 1989. Effect of Maillard browning reaction on the chemical properties of various proteins. Pages 273-289 in: Protein Quality and the Effects of Processing. R. D. Philip and J. W. Finley, eds. Marcel Dekker: New York, USA.
- Yildirim, M., and Hettiarachchy, N. S. 1998. Properties of films produced by cross-linking whey proteins and 11S globulin using transglutaminase. J. Food Sci. 63:248-252.
- Yoshino, T., Isobe, S., and Maekawa, T. 2002. Influence of preparation conditions on the physical properties of zein films. J. Amer. Oil Chem. Soc. 79:345-349.
- Youssef, A. M. 1998. Extractability, fractionation and nutritional value of low and high tannin sorghum proteins. Food Chem. 63:325-329.

7. LIST OF PUBLICATIONS FROM THIS RESEARCH

Gao, C., Taylor, J., Wellner, N., Byaruhanga, Y. B., Parker, M. L., Mills, E. N. C., and Belton, P. S. 2005. Effect of preparation conditions on protein secondary structure and biofilm formation of kafirin. *J. Agric. Food Chem.* 53:306-312.

Byaruhanga, Y. B., Erasmus, C., and Taylor, J. R. N. 2005. Effect of microwave heating of kafirin on the functional properties of kafirin films. *Cereal Chem.* In press.

Byaruhanga, Y. B., Erasmus, C., and Taylor, J. R. N. 2005. Effect of microwave heating of cast kafirin films on their functional properties. Submitted to *J. Sci. Food Agric.*