

Effect of heating cast kafirin films on their functional properties

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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 plasticizer, from glacial acetic acid (GAA) and aqueous ethanol (AE), then heated using microwave energy. A power of 80 W for 2 min caused an approximately two- and fourfold increase in the tensile strength of non-plasticized and plasticized films, respectively. Film glass transition temperature increased by about 15%. Film biodegradability was slowed. However, only the digestibility of the non-plasticized films cast from AE heated for the longest time (4 min) was decreased, by about 25%; while that of the other films was not affected. GAA cast films had higher digestibility than AE cast ones, possibly as a result of acid-mediated deamidation of kafirin. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis indicated heat-induced kafirin oligomers. Scanning electron microscopy of heated plasticized films showed more wrinkled structures compared to non-heated films, whereas the non-plasticized films appeared more brittle with heating. The results indicate that heat-induced intermolecular disulfide cross-linking was involved in modifying the properties of kafirin films.

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Keywords: kafirin films; microwave energy; heating; film digestibility; film biodegradability; film tensile properties

INTRODUCTION

Kafirins are the prolamin proteins of sorghum.¹ As they are hydrophobic proteins,² kafirins have the potential of producing biodegradable and/or edible films of good tensile and barrier properties for food products. Although kafirins form stable films,^{3,4} the diverse food packaging requirements necessitate modification of the functional properties of kafirin films.

Physical treatments have been used to modify functional properties of cast protein films. For example, thermal treatment has been used to increase the tensile strength of cast whey protein films,⁵ wheat gluten films⁶ and soy protein films.⁷ Electromagnetic waves such as ultraviolet light have been used to modify the functional properties of soy protein films.⁸ Heating with microwave energy has been used to increase the tensile strength of whey protein films.⁹ Such modifications in film properties have been attributed to intermolecular cross-linking of the protein induced by the physical agent used.⁹

Research by Byaruhanga *et al.*¹⁰ has shown that heating of kafirin using microwave energy before casting of films increases the tensile strength but decreases the strain, water vapor permeability and digestibility of the films. However, to achieve this, the kafirin powder had to be wetted first. To avoid

the step of first wetting the kafirin powder, casting the films then heating them with microwave energy was investigated as a process for modifying kafirin film properties. Here, we report the effects of heating of cast kafirin films using microwave energy on their tensile and barrier properties, digestibility and biodegradability.

MATERIALS AND METHODS

Extraction of kafirin

Kafirin was extracted from milled decorticated sorghum grain (a mixture of two white, condensed tannin-free hybrids, PANNAR 202 and 206) with aqueous *tert*-butanol plus the reducing agent dithiothreitol at room temperature, following the procedure described by Taylor *et al.*¹¹ This extraction procedure was used as it has been shown that extraction with an aqueous ethanol solvent at elevated temperature, as may be done in commercial practice, would have caused thermal damage to the kafirin,¹¹ probably masking any effects of heating with microwave energy. The extracted and defatted kafirin had a protein content of 812 g kg⁻¹ (dry basis, N × 6.25).

Film casting

Films were cast by dissolving kafirin powder (containing 1.44 g protein) in 9 g of glacial acetic acid of

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concentration 980 g kg^{-1} with and without 0.6 g plasticizer (lactic acid:polyethylene glycol 400:glycerol in a 1:1:1 weight ratio). For the digestibility study, films were also cast in aqueous ethanol with and without the plasticizer. The film casting and drying procedure of Taylor *et al.*⁴ was followed.

Dielectric properties of films

Before determining their dielectric properties, non-plasticized and plasticized films were conditioned at 50% relative humidity (RH) for 48 h. The conditioned films were then rolled to fit into a cylindrical quartz tube of the dielectric property measurement cavity. The dielectric constant and dielectric loss factor of the films were then measured at 2450 MHz with a perturbation resonant cavity fixture connected to a HP8753 network analyzer (Delphius Technologies, Pretoria, South Africa).

Heating of films

Before heating, plasticized films were cut into sample pieces of $18 \times 66 \text{ mm}$, while non-plasticized films were not cut. All films were then conditioned for 48 h at 50% RH in a desiccator containing saturated calcium nitrate. Heating was achieved applying microwave energy on the films 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 microwave energy onto the film sample in the microwaving apparatus (Fig. 1). Plasticized film samples were held between two Teflon frames ($19 \times 70 \text{ mm}$) to prevent them from buckling and adhering to the cylindrical quartz tube of the microwaving apparatus (Fig. 1). The frames were fastened together with Teflon screws. Non-plasticized films were rolled up to fit into the quartz tube, since they did not adhere to the tube after microwaving. The film samples were then heated in the quartz tube. The quartz tube could be slid in and out of the wave-guide with the microwaves switched off. Films were exposed to various heating rates, namely 60, 80, 100 and 120 W for periods of 1, 2, 3

and 4 min. Timing of exposure to heating started with switching on and ended with switching off the microwaves. The quartz tube was manually turned through 360° every 30 s to ensure even heating of the sample. The temperature of the films after heating was measured using a Thermalert MID infrared thermometer (Raytek, Santa Cruz, CA, USA). The controls were films cast with and without plasticizer but not heated.

Measurement of film moisture content and tensile and barrier properties

Before measurement, film samples were cut into strips of $60 \times 6 \text{ mm}$ and conditioned at 50% RH for 48 h. For moisture content, film samples of 300–400 mg were weighed into aluminum dishes, then dried in a circulating air oven at 105°C for 24 h, and their moisture content was expressed on a wet basis. The maximum tensile strength, tensile strength at break, strain and Young's modulus of the film strips were determined using a TA-XT2 texture analyzer (Stable Microsystems, Godalming, UK) fitted with tensile grips (code A/TG) following the procedure described by Emmambux *et al.*³ Film treatments that gave the greatest increase in tensile strength were used for determination of other functional properties. The water vapor permeability (WVP) of the films was determined in vials (4 cm^3 screw neck, $45 \times 14.7 \text{ mm}$) following the procedure of Taylor *et al.*⁴

In vitro protein digestibility and biodegradability

The digestibility of the non-plasticized and plasticized films cast from glacial acetic acid and aqueous ethanol was determined by the pepsin method of Hamaker *et al.*¹² Sample portions (10 mg protein basis) were digested with 998 units of pepsin P7000 (Sigma, St Louis, MO, USA) at 37°C for 2 h. Protein ($\text{N} \times 6.25$) was determined by the Dumas combustion method. Digestibility was determined on the basis of the protein content of the kafirin films after treatment. Film biodegradability was determined in compost under aerobic conditions as described by Byaruhanga *et al.*¹⁰

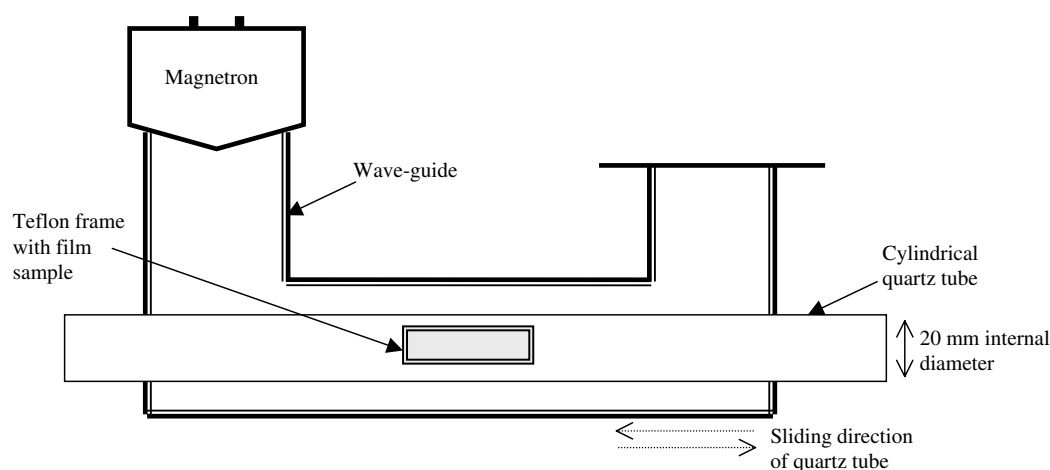


Figure 1. The microwave-heating apparatus.

SDS-PAGE

Gradient sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) under non-reducing conditions was conducted using a discontinuous Tris-HCl/glycine buffer system. An acrylamide:bisacrylamide ratio of 19:1 was used to make gels (125 mm × 160 mm and 1.5 mm thickness) of concentration gradient 39–150 g kg⁻¹. Samples (10 mg protein) were mixed in 1 cm³ of sample buffer in Eppendorf tubes containing glass beads. Sample buffer comprised 0.125 mol L⁻¹ Tris-HCl pH 6.8, 200 cm³ L⁻¹ glycerol, 40 g L⁻¹ SDS and 0.05 g L⁻¹ bromophenol blue. For the digestibility residues, 10 mg of sample was obtained from multiple digestions. The sample preparations were then heated in a boiling water bath for 15 min with periodic vortex mixing. Approximately 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, CA, USA) for 12 h at a constant current of 50 mA. The gels were stained using 1 g L⁻¹ Coomassie Brilliant Blue R250, destained and scanned.

Glass transition temperature (T_g) and scanning electron microscopy (SEM)

The T_g of non-plasticized films was determined by dynamic mechanical analysis (DMA). DMA was performed under tension using a Rheometrics RSA-II (Rheometric Scientific, Piscataway, NJ, USA), as described by Emmambux *et al.*³ SEM was done following the procedure described by Byaruhanga *et al.*¹⁰

Statistical analysis

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.

RESULTS AND DISCUSSION

The mean moisture contents of heated plasticized and non-plasticized kafirin films were 230 and 80 g kg⁻¹ (wet basis), respectively. The moisture contents of the heated plasticized and non-plasticized films were not different from that of the respective control films. Gennadios *et al.*¹³ reported a decrease in moisture content of soy protein films heated at 80 and 95 °C for up to 24 h. The results suggest that the films reabsorbed moisture to equilibrium after heating. However, the moisture content of the plasticized kafirin films was almost three times higher than that of the non-plasticized kafirin films. This was

probably because of the hydrophilic nature of glycerol and polyethylene glycol used as plasticizers. Such hydrophilic plasticizers cause more absorption of moisture into films.¹⁴

There was an increase in the maximum tensile strength, tensile strength at break and Young's modulus ($p < 0.05$) with heating of both plasticized and non-plasticized kafirin films (Table 1). In the non-plasticized 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 plasticized 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-plasticized films, strain decreased from 132% to 23% (nearly six times lower). This indicates that the films became stronger and less extensible with heating. In both the non-plasticized and plasticized films, the tensile strength at break showed the same trend as that of maximum tensile strength. As noted, the non-plasticized films were generally not extensible, with strain of only about 1.5%, far lower than that of the plasticized films. Plasticizers make protein films more extensible by forming a lubricating layer between protein molecules,^{15,16} which tends to decrease the tensile strength, while increasing strain of the films.¹⁴

As stated, the mean increase in the tensile strength in the plasticized films was several times higher than that in the non-plasticized films, although the non-plasticized films were of higher tensile strength (Table 1). Also, the increase in film temperature after heating was nearly twice as great in the plasticized films (from 22 to 45 °C) as in the non-plasticized films (from 22 to 35 °C). This suggests that heating of plasticized films was more effective than heating of non-plasticized ones. Heating of a food material using microwave energy depends on its dielectric properties, i.e. dielectric constant and loss factor, which are largely determined by the amount of polar molecules.¹⁷ Plasticization of the films significantly increased their dielectric loss factor but not their dielectric constant (Table 2). A material with a high dielectric constant coupled with a high dielectric loss factor has high heat generation when subjected to microwave energy.¹⁸ The dielectric constants of the plasticizers (glycerol 46.5,¹⁹ lactic acid 22.0¹⁹ and polyethylene glycol 400 12.4²⁰) are higher than that of the kafirin 2.7–3.0 (Table 2). However, the fact that only the loss factor of the plasticized films was significantly increased implies that the plasticizers have high dissipation factors and that this was a cause of the greater temperature increase in the plasticized films. Additionally, as mentioned, hydrophilic plasticizers increase the amount of moisture absorbed in a protein film.¹⁴ Since water has a high dielectric constant of 80 and a high loss factor of 12,^{8,19} foods

Table 1. Tensile properties of plasticized (P) and non-plasticized (NP) kafirin films heated with microwave energy at different powers for different time periods

Microwave power (W)	Heating time (min)	Film temperature after heating (°C)		Maximum tensile strength (N mm ⁻²)		Tensile strength at break (N mm ⁻²)		Strain (%)		Young's modulus (N mm ⁻²)	
		NP	P	NP	P	NP	P	NP	P	NP	P
0 (Control)	0	22	22	12.8a ^a (2.7) ^b	1.9a (0.5)	12.3a (2.4)	1.6a (0.5)	1.5a (0.6)	132.0e (32.5)	943a (112)	51a (26)
60	1	30	39	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	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	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	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	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	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	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	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	1	36	48	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)
	2	37	48	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	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	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	1	45	56	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 deviations of means.

^c ND, not determined because films were too brittle for testing.

containing substantial amounts of moisture tend to have high dielectric properties and thus are heated more effectively by microwaves than those that are dry.¹⁷ In our previous work, we found that kafirin powder had to be wetted so as to effectively heat it using microwave energy. Heating of wetted kafirin using microwave energy produced plasticized films with tensile strength of about two times higher than that of films produced from non-heated kafirin.¹⁰ Thus, compared to heating of kafirin before film casting,¹⁰ heating of cast kafirin films was more effective at increasing the tensile strength of plasticized kafirin films.

With both the plasticized and non-plasticized films, film temperature was directly related to the

Table 2. Dielectric properties of non-plasticized and plasticized kafirin films determined at 2450 MHz

Sample	Dielectric constant	Dielectric loss factor
Non-plasticized film	2.7 (0.2) ^a	0.08 (0.01)
Plasticized film	3.0 (0.2)	0.24 (0.03)

^a Values in parentheses are standard deviations of means.

microwave power applied (Table 1). However, the highest increase in maximum tensile strength for both the plasticized and non-plasticized films was achieved at 80 W. Heating at 100 W of microwave power 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 heating the films using 80 W of microwave power was probably the most effective. Further, films heated at 60 and 80 W of microwave power did not show a change in color as compared to the non-heated films, whereas films heated at 100 W showed a slight brown color development, while those subjected to 120 W of microwave power were burnt and too brittle for tensile testing. Such color changes are indicative of the beginning of negative effects of thermal treatment on the kafirin films. Excessive heating of proteins causes destruction of protein polymer chains,²¹ which could weaken the protein network in the films.

Table 3 shows that the mean T_g of non-plasticized kafirin films increased significantly ($p < 0.05$) with heating, from 68.6 °C in the control up to 78.9 °C in

the heated films. This is consistent with the results of Emmambux *et al.*³ 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.²² The T_g of biopolymers increases with chain rigidity and the intensity of intermolecular interactions, including hindrance to internal rotation along the macromolecular chain.²³ 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.^{16,24}

The mean WVP of the non-plasticized and plasticized kafirin films was 0.93 and 2.80 g mm m⁻² h⁻¹ kPa⁻¹, respectively (data not shown). Heating had no effect on the WVP of plasticized or non-plasticized films. This is consistent with the results of Emmambux *et al.*,³ 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 RH higher than 60% kafirin changed

Table 3. T_g of non-plasticized kafirin films cast from glacial acetic acid heated with microwave energy at 80 W for different time periods

Heating 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 deviations of means.

Table 4. Protein digestibility of plasticized and non-plasticized kafirin films cast from glacial acetic acid (GAA) and aqueous ethanol (AE) and heated with microwave energy at 80 W for different time periods

Heating time (min)	Protein digestibility (%)			
	Films cast from GAA		Films cast from AE	
	Plasticized	Non-plasticized	Plasticized	Non-plasticized
0 (Control)	87.3a ^a (3.8) ^b	87.9b	24.9ab (4.1)	28.7b (2.9)
1	85.1a (0.3)	89.9b (2.1)	24.8ab (4.3)	24.0ab (3.3)
2	92.9b (4.0)	87.5b (1.7)	25.9ab (1.1)	26.1ab (4.5)
3	88.4ab (3.7)	82.3a (4.3)	20.4a (4.5)	23.2a (4.6)
4	84.7a (3.3)	88.6b (0.4)	29.6b (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 deviations of means.

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. In the work reported here, WVP was determined at an 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 such that the effect of heating could not be detected. Moisture absorption at high RH was also reported to cause extensive swelling of gluten networks, which increased water diffusion through gluten films.²⁵

Table 4 shows that only the digestibility of the non-plasticized films cast from aqueous ethanol heated for the longest time (4 min) was decreased, by about 25%. This is consistent with the finding of Byaruhanga *et al.*¹⁰ that kafirin had to be heated for an extended time before the digestibility of films made from the kafirin was reduced. The digestibility of the plasticized films cast from aqueous ethanol and the non-plasticized and plasticized films cast from glacial acetic acid was not affected. The lack of effect of heating on the digestibility of plasticized films cast from aqueous ethanol was probably due to the presence of plasticizers. As a plasticizer forms a lubricating layer between protein molecules,¹⁶ 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.^{12,26–28} Thus, the decrease in digestibility of the heated kafirin films cast from aqueous ethanol was probably due to heat-induced intermolecular cross-linkssd.

The digestibility of films cast from glacial acetic acid was several times higher than that of films cast from aqueous ethanol (Table 4). 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,²⁹ 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.²¹ Deamidation is known to increase enzyme susceptibility of whey proteins³⁰ and wheat gluten.³¹ Deamidation also reduces protein–protein interactions like hydrogen bonding.^{31,32} Argos *et al.*³³ proposed an α -zein model that shows the involvement of glutamine residues in intra- and intermolecular hydrogen bonding, which plays an important role in the structural stability of zein. Since kafirin and zein are closely similar,³⁴ it is probable that deamidation of kafirin reduced hydrogen bonding and adversely affected its structural stability.

SDS-PAGE of heated kafirin films and their residues after pepsin digestion (RPD) (Fig. 2) shows bands consistent with α -, β - and γ -kafirin,³⁵ except for the RPD of films cast from glacial acetic acid in which the β -kafirin 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.³⁶ The relative concentration of these dimers was much higher in the RPDs (tracks 5–8) than in the films (tracks 1–4). 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 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 compared to those cast in aqueous ethanol (Table 4).

From Fig. 2, it appears that oligomer formation due to heat-induced cross-linking is actually involved in the reduction of protein digestibility following heating of kafirin films (Table 4). 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 heat treatment of the kafirin films (Table 1). Formation of cross-links and subsequent protein polymerization is well-known to occur on heating kafirin.^{27,28} Cross-linking has also been proposed as the cause of increased tensile strength and reduced strain in soy protein films^{7,13} and whey protein films⁵ that were thermally treated.

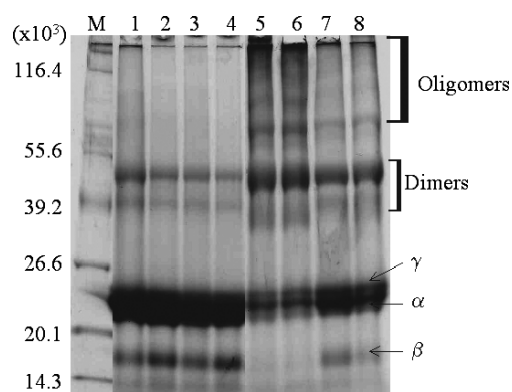


Figure 2. SDS-PAGE under non-reducing conditions of heated (80 W for 3 min) plasticized and non-plasticized kafirin films and their residues of pepsin digestion (RPD). Tracks: M, molecular markers; 1, plasticized film cast from glacial acetic acid; 2, non-plasticized film cast from glacial acetic acid;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

Figure 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 color, 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 heat-treated films were found in the low-moisture content compost. The fragments were black, wrinkled, brittle and had holes in them. 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 throughout the test period with only slight wrinkling occurring.

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 heat-treated films degraded somewhat slowly as compared to the control films, probably because of heat-induced intermolecular cross-links and consequent structural changes, which made some of the protein less accessible to microbial proteolytic enzymes. Similarly, heating of kafirin with microwave energy before film casting also slowed the biodegradability of the films.¹⁰ Slowed biodegradation of films has also been reported in heat-treated whey protein films³⁷ and γ -irradiated whey, casein and soy protein films.³⁸ Heat- and γ -irradiation-induced cross-links were postulated to account for the observed slowed biodegradation of the treated films. Similarly, disulfide cross-linked collagen films were found to be more resistant to degradation by collagenase than the uncross-linked collagen films, suggesting that SS cross-linking made some of the protein less accessible to the enzyme.³⁹

Scanning electron microscopy (SEM) revealed that the heated plasticized films (Fig. 4A iv–vi) had more wrinkled or folded structures (F) and smaller pore sizes (P) as compared to the non-heated control (Fig. 4A i–iii). The top and bottom parts of the films were similar in appearance, with the pores regularly distributed. The pores were possibly due to the presence of air bubbles in the films. A reduction in the number of pores was evident with increasing heating time. This was probably because some of the air bubble collapsed as the films were heated. Compared to the films cast from heated kafirin,¹⁰ the plasticized films that were heated 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 heating was applied during making of the film. However, in both cases, the pores appeared to

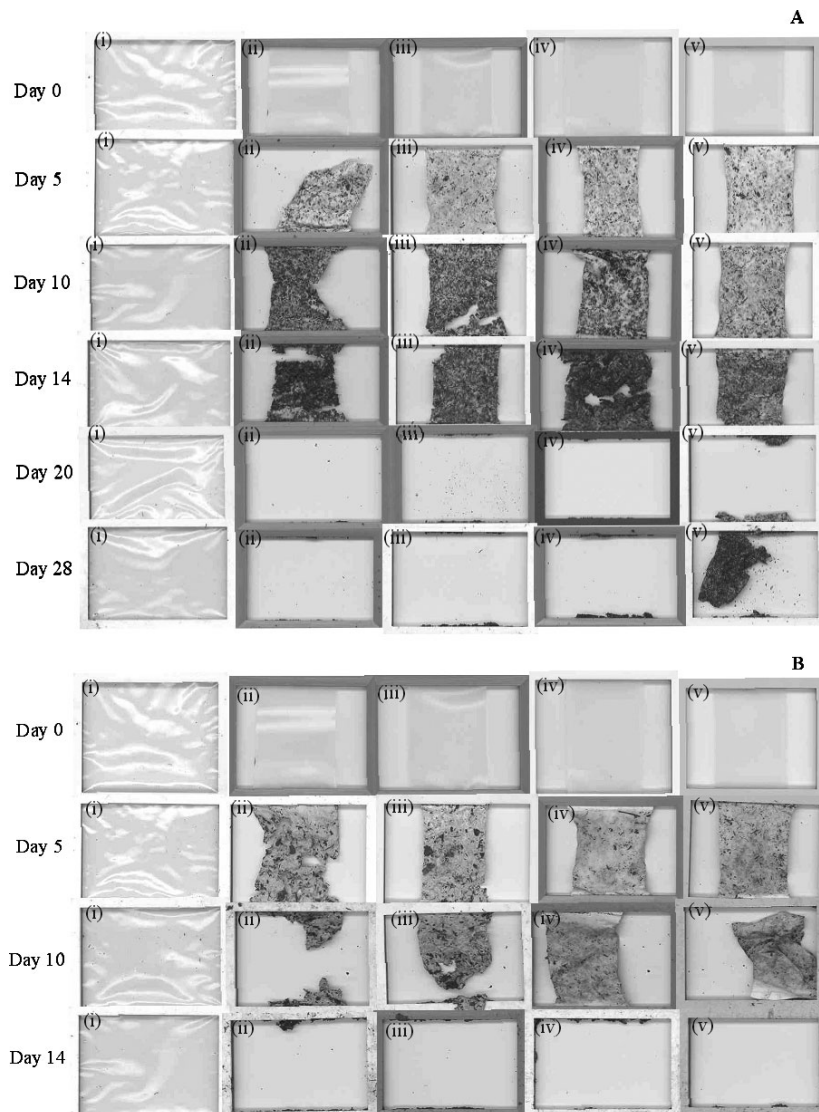


Figure 3. Degradation of kafirin films buried in compost for 28 days at 50 °C: (A) low-moisture compost (37% moisture content); (B) high-moisture compost (60% moisture content); (i) polyethylene film; (ii) non-plasticized untreated film; (iii) plasticized untreated film; (iv) non-plasticized film heated with microwave energy at 80 W for 4 min; (v) plasticized film heated with microwave energy at 80 W for 4 min.

become fewer in number and smaller in size with heating.

The structure of heated non-plasticized films (Fig. 4B iv–vi) appeared to be more brittle, in some cases with fractured pieces (FP), as compared to the control (Fig. 4A i–iii). The brittle appearance could be related to the non-extensible nature of the non-plasticized films (Table 1). As with the plasticized films, the top and bottom parts of the non-plasticized 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.⁷ Also, using SEM, Emmambux *et al.*³ 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 heat-induced cross-linking of kafirin. Therefore, as mentioned, the decrease

in pore size and number was probably caused by heating and its subsequent inducement of cross-links. The decrease in pore size and number was presumably related to the changes in film functional properties, such as biodegradability, tensile strength and strain.

CONCLUSIONS

Heating of cast films with microwave energy can be used to modify the functional properties of kafirin films, such as tensile strength, strain, digestibility and biodegradability. The plasticizers in films increase the effectiveness of microwave heating on the film functional properties, probably because of their high dielectric constant. Heat-induced intermolecular disulfide cross-links seem to be responsible for the modification of kafirin film properties. Heating after film casting appears to be more effective than heating

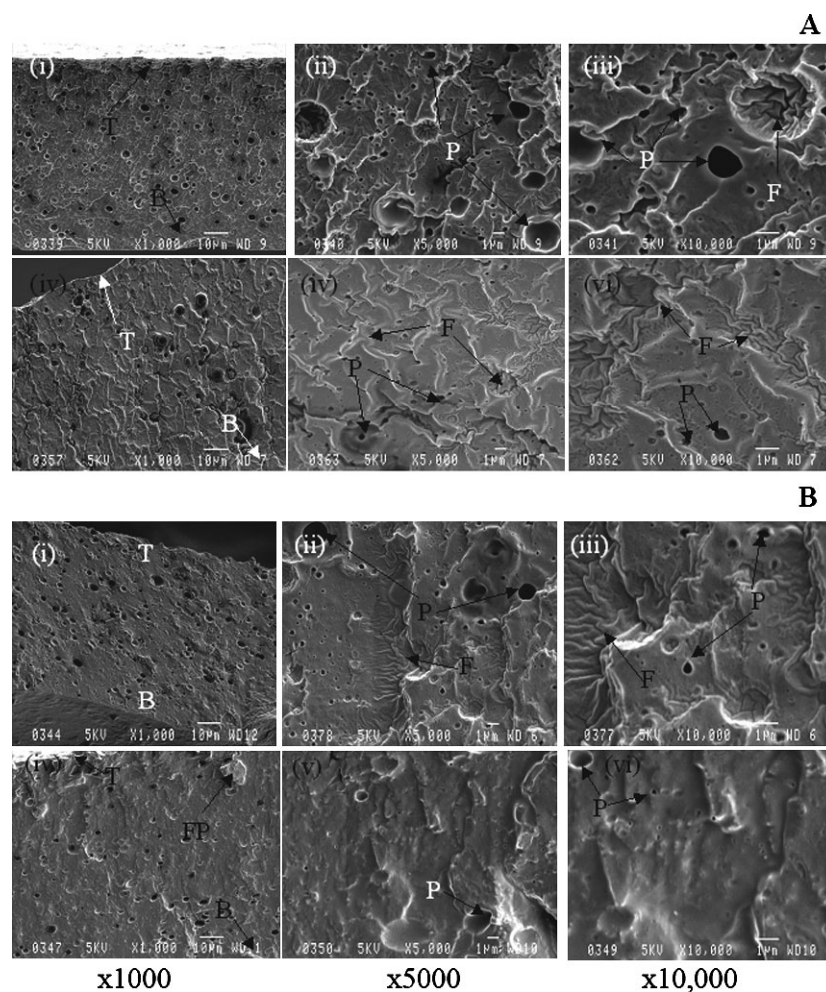


Figure 4. Scanning electron micrographs of freeze–fracture surface of kafirin films heated with microwave energy at 80W for different times: (A) plasticized; (B) non-plasticized films; (i–iii) control film; (iv–vi) heated film. T, top side of the film in contact with atmosphere; B, bottom side of film in contact with Petri dish; FP, fractured piece; P, pore; F, folded or wrinkled structure.

before film casting since it caused a higher increase in the tensile strength of kafirin films.

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