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Preparation of Free-Standing Films from Kafirin Protein Microparticles: Mechanism of Formation and Functional Properties

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A method of preparing free-standing films using kafirin microparticles made by phase separation from acetic acid is described. Film preparation involved the suspension of the microparticles in acetic acid solution containing plasticizer. On evaporation of the acetic acid, a complete, smooth, flexible, transparent film was formed. A minimum concentration of acid was required to form a cohesive film relative to the concentration of kafirin. This was approximately 10.8:1, percent acetic acid to percent kafirin. Film formation appears to be by controlled aggregation of kafirin microparticles, followed by dissolution of the microparticles in acetic acid and drying into a cohesive film. The functional properties of microparticle films were generally superior to films cast directly from a solution of kafirin, at the same protein content. Kafirin microparticle films were very thin (<15 μm), relatively strong but not very extensible, with better water barrier properties and lower protein digestibility than conventionally cast kafirin films.

KEYWORDS: Kafirin; microparticle; film; functional properties; bioplastic

INTRODUCTION

At present the functional properties of protein bioplastic materials are not as good as those of synthetic polymers. For example, protein bioplastic films generally have much worse water barrier properties and lower tensile strength and elongation (*1*). However, according to Krotcha (*1*) bioplastic films made from zein (maize prolamin) have some of the best functional properties. Cast bioplastic films made from kafirin, the prolamin protein of sorghum, have similar barrier properties to cast zein films, but kafirin films are generally stronger and less extensible than the zein films (*2*−*4*).

Microparticles, also known as microspheres, while not true spheres, can be made from proteins (*5*). Protein microparticles have a variety of applications. Zein microparticles have been reported to have potential for use as food coatings (*6*), encapsulation of essential oils (*7*), drug delivery (*8*), tissue engineering (*9*), drug eluting films (*10*), and delayed release of pesticides (*11*). An ethanol-free method of making kafirin microparticles by phase separation from acetic acid has been devised (*12*). The microparticles formed have a very large surface area.

A novel approach for the preparation of kafirin bioplastics is the use of kafirin microparticles for their preparation. The major objective of the work reported here was to prepare cast free-standing films from kafirin microparticles in acetic acid and compare their functional properties with those of conventionally cast free-standing films from a solution of kafirin in glacial acetic acid. Free-standing films are those which can be released from the casting surface. Free-standing films can be used, for example, as packaging materials.

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MATERIALS AND METHODS

Materials. A mixture of grain from two condensed tannin-free, tan plant, white sorghum cultivars (*Sorghum bicolor* (L.) Moench) PANNAR PEX 202 and 206 was used for kafirin extraction, using the method described by Emmambux and Taylor (*13*). In brief, decorticated, milled grain was extracted with 70% (w/w) aqueous ethanol containing 5% sodium hydroxide (w/w) and 3.5% sodium metabisulfite (w/w) at 70 °C for 1 h with vigorous stirring. The extractant was recovered by centrifugation and the ethanol removed by evaporation. Kafirin was precipitated on pH adjustment of the protein suspension to approximately pH 5 and recovered by filtration under vacuum and freeze-dried. The kafirin was defatted with hexane at ambient temperature and air-dried. The protein content of the kafirin was 88% ($N \times 6.25$) as determined by a Dumas combustion method, AACC Method 46−30 (*14*).

Preparation of Kafirin Microparticles. Kafirin microparticles were made according to the method described by Taylor et al. (*15*). Kafirin was dissolved in glacial acetic acid containing a plasticizer (40% in relation to protein, 1:1:1 lactic acid:polyethylene glycol 400:glycerol (*2*−*4*, *16*)). After a 16 h "rest" (to give time for any changes in protein structure to equilibrate), distilled water at ambient temperature was added slowly, and the microparticles formed. The final concentration of protein was 2% (w/w), with an acetic acid concentration of 5.4% (w/w).

To determine the effect of protein concentration and acid concentration on kafirin microparticle film formation, suspensions of kafirin microparticles were prepared in different acetic acid concentrations as described by Taylor et al. (*15*). Kafirin microparticle concentrations of 2% and 4% (w/w) protein were prepared in 5.4%, 10.8%, and 21.6% (w/w) acetic acid. Kafirin microparticles, 8% (w/w) protein, were prepared in 5.4% and 10.8% (w/w) acetic acid.

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Preparation of Films. *Free-Standing Cast Kafirin Films from a Solution of Kafirin in Glacial Acetic Acid* (*Conventionally Cast Films*). Free-standing kafirin films were cast using the method described by Taylor et al. (*16*) using 2% kafirin, with 40% plasticizer in relation to protein and glacial acetic acid as casting solvent. All films were cast at a weight of 1 g of protein solution/16 cm^2 casting container. Films were dried overnight at 50 °C in an oven (not forced draft).

Free-Standing Films Cast from Kafirin Microparticle in Acetic Acid Solution (*Microparticle Films*). Suspensions of kafirin microparticles in acetic acid (4 g) were mixed at various concentrations with 40% plasticizer in relation to protein and then cast in Perspex Petri dishes (9 cm) (weight of 1 g of protein solution/16 cm^2 casting container) by drying overnight at 50 °C as described. All free-standing films were assessed visually and photographed using a flatbed scanner.

Effects of Protein Concentration and Acid Concentration on Kafirin Microparticle Film Formation. Acetic acid prepared microparticles were cast into free-standing films at 2% and 4% protein in 5.4%, 10.8%, and 21.6% acetic acid. At 8% protein, 5.4% and 10.8% acetic acid was used. For all of these films, plasticizer concentration was constant at 40% (w/w) with respect to protein. To investigate the effects of low protein concentration and low acid concentration on microparticle film formation, kafirin microparticles were prepared as described above but additional water was added to give a concentration of 1% protein in 10.6% acetic acid or 0.5% protein in 5.4% acetic acid. Films were cast and dried as described. Kafirin films containing 0.5% and 1% protein cast in glacial acetic acid were used for comparison.

Effects of Plasticizer Concentration and Acid Concentration on Kafirin Microparticle Film Formation. Acetic acid prepared microparticles were cast into free-standing films at 2% protein in 5.4%, 10.8%, 15%, and 21.6% acetic acid with 0%, 20%, 40%, 60%, 80%, and 100% plasticizer with respect to protein.

Analysis of Films. *Scanning Electron Microscopy* (*SEM*). Film surfaces were examined by mounting the top and underside of the films on a stub with double sided tape. Samples were then sputter coated with gold, and SEM preparations were viewed with a JSM-840 scanning electron microscope (Tokyo, Japan) and photographed.

Atomic Force Microscopy (*AFM*). Kafirin microparticles in either 5.4% or 21.6% acetic acid (5 μL) were dropped onto a piece of polished silica, and the solution was allowed to dry in a desiccator to form a film. Samples were viewed with a Topometrix TMX 2000 Discoverer AFM (Santa Clara, CA) in contact mode and photographed.

Fourier Transform Infrared Spectroscopy (*FTIR*). Film samples were scanned as a double layer using a Perkin-Elmer Spectrum GX FTIR system (Waltham, MA), with a zinc selenide crystal using 32 scans, 8 cm^{-1} bandwidth, and an interval of 1 cm^{-1} in the attenuated total reflectance (ATR) mode. The FTIR spectra were Fourier-deconvoluted with a resolution enhancement factor of 2 and a bandwidth of 12 cm^{-1}.

Water Vapor Transmission (*WVT*) *and Water Vapor Permeability* (*WVP*). A modified method based on the ASTM method E96−97 (*17*) was used as described by Taylor et al. (*16*).

Tensile Properties. These were determined by a modified method based on ASTM D882−97 (*18*) as described by Taylor et al. (*16*) using a TA-XT2 texture analyzer (Stable Micro Systems, Goldalming, U.K.).

Observation of Microparticle Film Formation. Droplets of microparticles (2% kafirin in 5.4% acetic acid and 2% kafirin in 21.6% acetic acid) were placed on microscope slides with no coverslip. The microscope slide was then placed on a Linkam Scientific Instruments CO 102 hot stage (Tadworth, England) and viewed using a Reichert Neovar (Vienna, Austria) light microscope at 100 × magnification and photographed using a still camera. The temperature of the hot stage was set to heat up to a temperature of 50 °C, the temperature used for drying free-standing films. The film- forming process was complete from start to finish in 2 min. Photographs were taken every 5 or 10 s to record the film formation process.

Statistical Analysis. One-way analysis of variance (ANOVA) was applied to the data on FTIR of films, tensile properties, WVT, and WVP. In all cases, tests were carried out at least in duplicate and repeated at least once, giving a total of at least four results for each test.

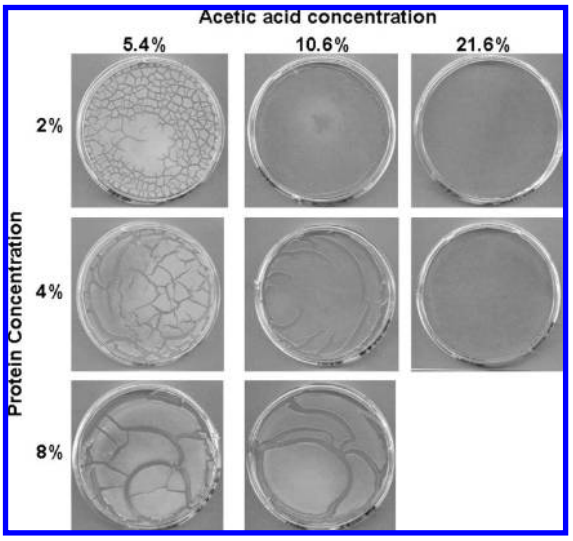

Figure 1. Effects of increasing protein concentration and acetic acid concentration on kafirin microparticle film formation.

RESULTS AND DISCUSSION

Effects of Protein Concentration and Acid Concentration on Kafirin Microparticle Film Formation. Under appropriate conditions preparations of kafirin microparticles suspended in acetic acid were found to dry into clear, transparent films when sufficient acetic acid was present. Below the critical acetic acid concentration complete films were not formed (see **Figure 1**). Protein (2%), with approximately 21.6% acetic acid, formed a complete film which could be released from the Petri dish. At 10.8% acid the film was almost complete and transparent, whereas at 5.4% acid the film was incomplete, formed in small fragments, and was opaque. At higher protein concentrations the same pattern was observed. Increasing protein concentrations from 2% to 4% and 8% also seemed to have an effect on film formation as at a particular acid concentration the fragments of film were larger with increased protein concentration. Protein concentration did not appear to affect the clarity of the film. However, as would be expected, increased protein concentration increased the thickness of the resultant films, and the color changed from colorless (21.6% and 10.8% acid) or white (5.4% acid) at 2% protein to yellow at higher protein concentrations.

It appears that a minimum amount of acid relative to the amount of protein is required in order to form a complete film. For kafirin microparticles made with acetic acid this minimum acid concentration is approximately 21.6% when 2% protein is used. If the relationship between protein concentration and acid concentration holds, it was thought that a complete film or at least a complete coating should form with 1% protein and approximately 10.6% acetic acid and with 0.5% protein and approximately 5.4% acetic acid. This was found to be the case (**Figure 2**). At a protein concentration, 0.5%, with 5.4% acetic acid (**Figure 2a**) a complete, transparent, smooth coating was formed but was too thin to be released from the Petri dish. At the higher protein concentration (1% protein, 10.6% acetic acid) (**Figure 2b**), a complete, transparent, smooth, flexible film was formed which could be released whole from the Petri dish. At 0.5% and 1% kafirin, films could also be cast from glacial acetic acid, but they were not as good sensorially, having a rough surface texture.

There are few references in the literature to the formation of films from protein microparticles and none found using them to make free-standing films. The patent of Cook and Shulman (*6*)

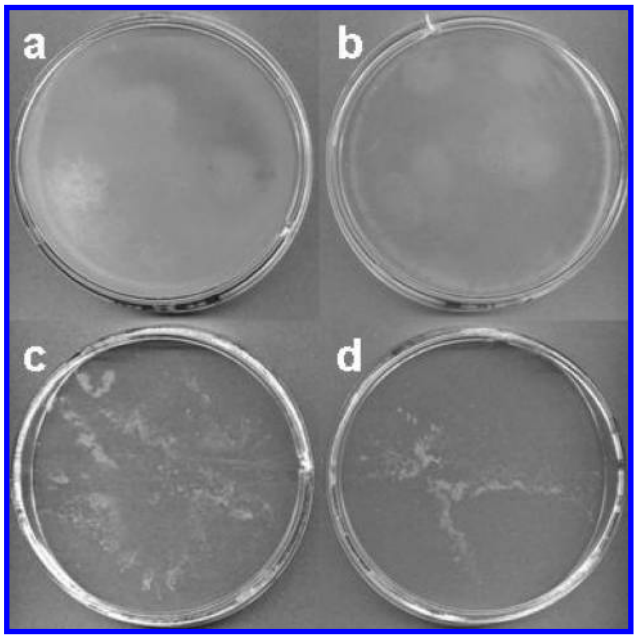

Figure 2. Effect of low protein concentration on kafirin microparticle film formation. Kafirin microparticle films: (**a**) 0.5% kafirin, 5.4% acetic acid; (**b**) 1% kafirin, 10.8% acetic acid. Conventional kafirin films: (**c**) 0.5% kafirin; (**d**) 1.0% kafirin.

described zein colloidal dispersions similar to the microparticle suspensions used in this study and claimed that they could be dried into glossy coatings and "films". According to these workers, a cohesive film could be made from zein microparticles at a much lower acid concentration but higher protein concentrations than was found in this study. Details of the methods of preparation are insufficient to enable valid comparisons to be made, and there was no evidence presented that indicated that the films could be released from the coating surface.

Dong et al. (*9*) and Wang et al. (*10*) described the formation of zein microsphere films cast from 40% aqueous ethanol, a different solvent system to that used in this study. Dong et al. (*9*) showed by SEM that zein microspheres could agglomerate together to form a film at very low zein concentration (< 1% (w/v)). However, the illustrations of microsphere films made by both groups (*9*, *10*) showed very little fusion of the microspheres, and so the material cannot be considered as a continuous film.

Effects of Plasticizer Concentration and Acid Concentration on Kafirin Microparticle Film Formation. Plasticisers are added to cast films to reduce brittleness and increase flexibility and extensibility by decreasing intermolecular forces between polymer chains (*19*). The plasticiser used in this work was a mixture of 1:1:1 (w/w) glycerol:polyethylene glycol:lactic acid. Early work by Buffo et al. (*20*) used glycerol and polyethylene glycol as plasticizers for kafirin films. Lactic acid was added to this combination to help the dissolution of the kafirin during film formation, since lactic acid has been found to be a good solvent for kafirin (*16*).

Figure 3 illustrates the combined effect of varying plasticizer and acetic acid concentration on the formation of kafirin microparticle films cast at constant protein concentration (2%). Only films made with 21.6% acetic acid formed complete films regardless of the concentration of plasticizer. This is not surprising as addition of plasticizer decreases intermolecular forces and increases molecular mobility (*19*), which would have the effect of decreasing the cohesiveness of the microparticle suspension. Cohesiveness is necessary for film formation (*21*). At 21.6% acetic acid, the films with 0% plasticizer were complete but brittle

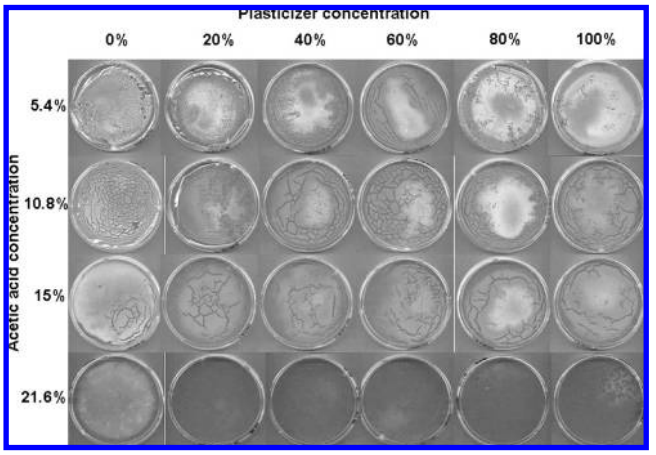

Figure 3. Effects of increasing acetic acid concentration and increasing plasticizer concentration on kafirin microparticle film formation (2% protein).

and opaque (**Figure 3**). All other plasticizer concentrations at 21.6% acetic acid formed clear, transparent, flexible films with smooth surfaces. Films with 40% plasticizer produced films that were odorless, stronger, and less extensible than films made with higher concentrations of plasticizer. Films with 60%, 80%, and 100% plasticizer in relation to the weight of protein were sticky and overplasticized, appearing weaker and more extensible than those with lower concentration of plasticizer. The stickiness was probably due to excess glycerol which is noted to migrate through a film matrix to the surface of the film resulting in loss of flexibility (*19*). Gao et al. (*22*) noted glycerol leaching out of kafirin films at a lower concentration of plasticization (40%) than was found in this study. At 40% (w/w) platicization these workers found two glass transition temperatures, one of which corresponded to that of pure glycerol. They suggested that at this level of plasticization a proportion of the glycerol behaved as a separate phase to the protein. As noted below conventional kafirin films have less β-sheet than the kafirin microparticle films, and this may have allowed more interprotein interactions resulting in an effectively smaller pore size in the microparticle film which would in turn allow better glycerol holding capacity. Certainly, kafirin microparticle films made with 40% plasticizer were still very flexible after several months with no apparent loss of plasticizer.

Microparticle Film Formation. As already stated, it appears that a minimum amount of acetic acid relative to the amount of protein is required in order to form a complete film. This was confirmed by following the film formation process with 2% kafirin microparticles in 5.4% and in 21.6% acetic acid using light microscopy (panels **A** and **B** of **Figure 4**, respectively). **Figure 4A**, 0 s, shows the individual kafirin microparticles in 5.4% acid as small well-defined spheres. As heat was applied to the film-forming suspension via the hot stage on which the slide was mounted, the spheres moved across the slide probably due to convection. The spheres appeared to form into short interlinked chains and separated from the liquid phase (**Figure 4A**, 40−80 s). It is suggested that convection influenced the formation of the strings of spheres. This appears, similar to the effect of molecular combing during conventional zein cast film formation observed by AFM (*23*). These workers described zein film as consisting of globules of nonuniform size. When alignment by molecular combing was applied, these workers suggested that the zein globules formed into joined zein rods. In this present work, as more heat was applied, the interlinked chains of microparticles appeared to aggregate and merge together as some of the solvent

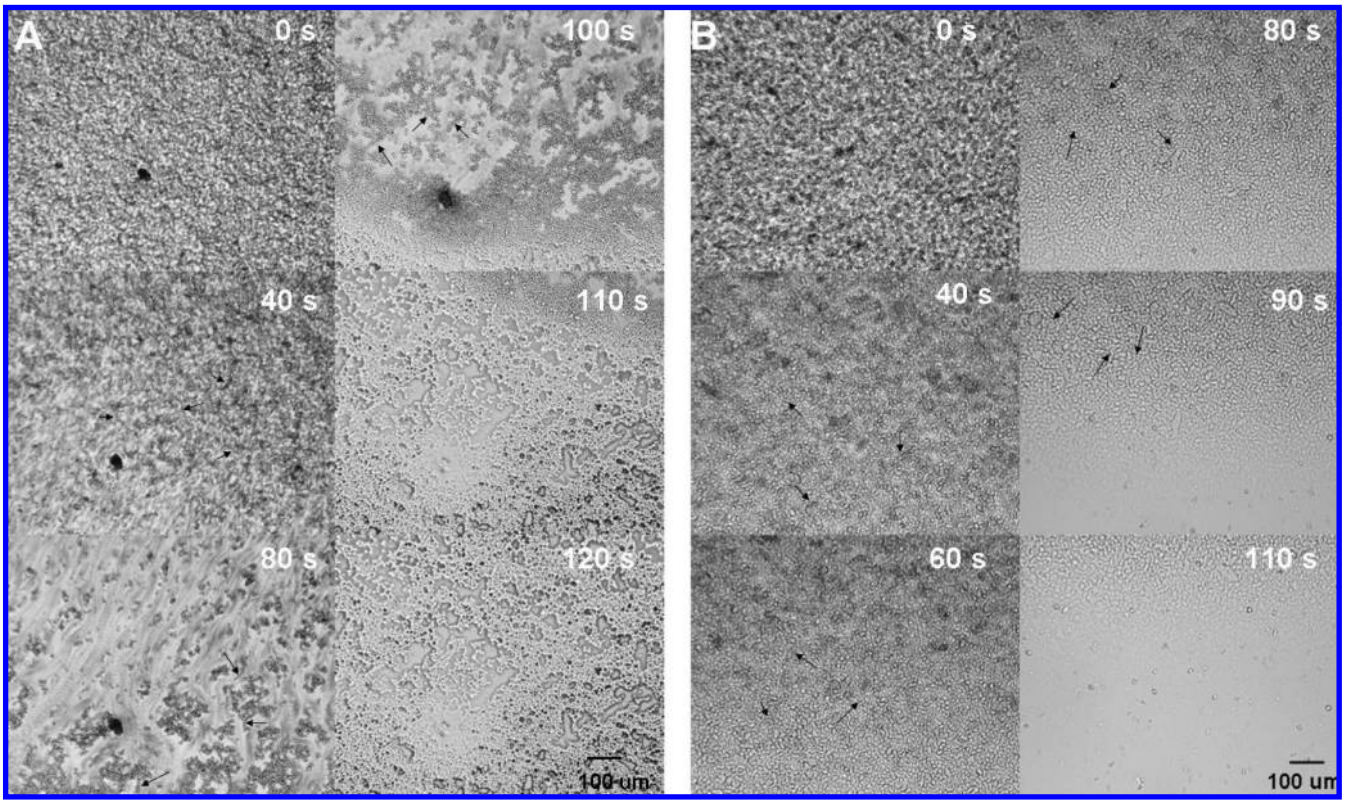

Figure 4. Light microscopy time lapse record of kafirin microparticle film formation: (**A**) 5.4% acetic acid; (**B**) 21.6% acetic acid. Arrows indicate interlinked protein chains.

evaporated (**Figure 4A**, 100 s). This process continued as more liquid evaporated forming an incomplete mesh arrangement (**Figure 4A**, 110 s). When all of the solvent had evaporated (**Figure 4A**, 120 s) the mesh of microparticles was deposited onto the slide and appeared as a rough, opaque, incomplete, and still to some extent particulate film.

Figure 4B illustrates how a complete cohesive film is formed from 2% kafirin microparticles with the higher acetic acid concentration of 21.6%. **Figure 4B**, 0 s, shows the individual kafirin microparticles in 21.6% acid were slightly larger than those in 5.4% acetic acid but still well-defined spheres. The increase in microparticle size with increasing acid concentration may be due to protein aggregation; light scattering studies could be used to confirm this. As heat was applied to the film-forming suspension, the spheres appeared to form into short interlinked chains in the same way as in the presence of 5.4% acid (**Figure 4B**, 40−60 s). However, at the higher acid concentration there did not seem to be a separation from the liquid phase (**Figure 4B**, 40−60 s). As more heat was applied, the interlinked chains of microparticles seemed to aggregate in a controlled manor and merge together (**Figure 4B**, 80 s). It appeared that as the water evaporated from the acetic acid solution, the microparticle aggregates dissolved in the remaining more concentrated acetic acid (**Figure 4B**, 90 s). Finally, as all of the solvent evaporated, a clear, cohesive, transparent film was deposited on the slide (**Figure 4B**, 110 s). Thus, in order for a cohesive film to form, it appears that the kafirin microparticles must dissolve in the concentrated acetic acid.

Fourier Transform Infrared Spectroscopy (FTIR) of Kafirin Films. FTIR spectra of kafirin microparticle films (21.6% acetic acid) and conventional kafirin films were compared and found to show different secondary structure between the two types of films (**Figure 5**). Considering the amide I band of the FTIR spectra of the preparations, two main peaks were observed at wavenumbers

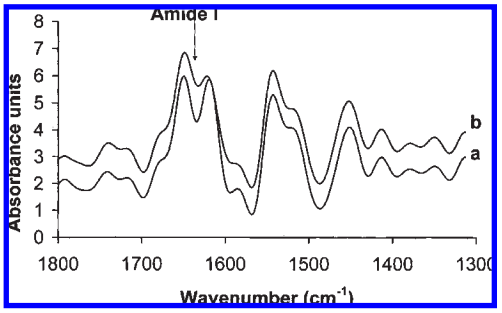

Figure 5. FTIR of kafirin films: (**a**) kafirin microparticle film; (**b**) conventional kafirin film.

1650 and 1620 cm^{-1} (**Figure 5**), the proportions of the peaks differing with the different films. The peak around 1650 cm^{-1} can be assigned to α-helical conformations or random coils and 1620 cm^{-1} to antiparallel β-sheet conformations (*24*, *25*). In the amide I region the conventional kafirin film showed more α-helical than β-sheet conformation, while the kafirin microparticle film had almost equal proportions of α-helical and β-sheet conformation.

To quantify the differences in secondary structure between the two film preparations, the ratio of α-helical to intermolecular β-sheet conformation was calculated for the amide I region. The conventional kafirin film had an approximate ratio of 1.2:1, while the ratio for the kafirin microparticle film was 1.0:1 in the amide 1 region. Byaruhanga et al. (*26*) reported a slightly higher ratio of 1.3:1 in the amide I region for conventional kafirin films, indicating the presence of slightly more α-helical conformation than is found in films prepared in this study. Byaruhanga et al. (*26*) and Gao et al. (*4*) showed that, for kafirin, differences in extraction and drying conditions resulted in slight differences in secondary structure. Thus, the higher ratio of α-helical conformation found by Byaruhanga et al. (*26*) was probably a result of

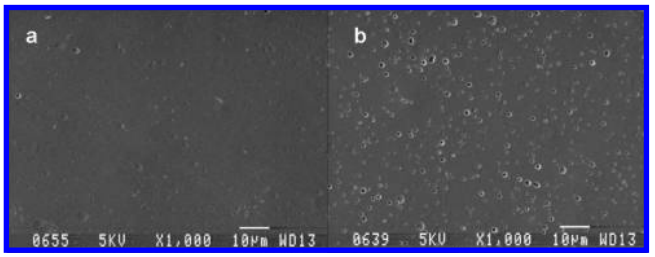

Figure 6. SEM of kafirin film surfaces. Kafirin microparticle film: (**a**) top surface. Conventional kafirin film: (**b**) top surface.

the different extraction procedures being used. No reference could be found in the literature of FTIR of protein microparticle films.

The data indicate that the protein secondary structure of the kafirin microparticle film contained more β-sheet conformation than the glacial acetic acid film. According to Mizutani et al. (*27*) the presence of β-sheet conformation is indicative of protein aggregation. This is in agreement with protein aggregation observed when microparticle film formation was followed microscopically (**Figure 4**). Contrary to these findings, Hsu et al. (*28*) found the secondary structure of zein films to be predominately α-helical. This contradiction may be due to differences in the state of the protein used for film formation. It is known that the state of a protein is a strong function of its history as demonstrated by Gao et al. (*4*).

Subirade et al. (*29*) suggested that β-sheet structures might be essential for protein−protein interactions and network formation in protein films from vegetable origins, intermolecular hydrogen bonding between β-sheets acting as junction zones stabilizing the film network. Thus it would be expected that the higher degree of intermolecular interactions which are present in β-sheet structures would result in the formation of films with better functional properties than films with a predominately α-helical secondary structure (*30*). In the case of kafirin microparticle films, this seems to be the case.

Functional Properties of Kafirin Microparticle Films. *Film Surface Properties.* Kafirin microparticles films cast from 21.6% acetic acid were very smooth to the touch with no apparent imperfections. When observed by SEM, the top surface of the films appeared uniformly smooth with very few small holes (**Figure 6a**). These were of approximately 1 μm in diameter. When the underside of the same film was examined by SEM, it showed patches of roughness, possibly where the film adhered to the plastic of the Petri dish in which it was cast. By comparison, conventional kafirin films at the same protein concentration were slightly rough to the touch. When examined by SEM, these films were extensively pitted on the surface with many holes, which were approximately 1−3 μm in diameter (**Figure 6b**). These holes may have been caused by air bubbles entrapped during the film casting process. The solution of kafirin in glacial acetic acid was very viscous compared to the kafirin microparticle suspension and so would be more likely to retain air bubbles incorporated by stirring during protein dissolution. During the drying process the film is heated to 50 °C. With increasing temperature the viscosity of the kafirin solution would be reduced, allowing escape of air bubbles from the film surface. As the film dried, residual bubbles on the surface would have formed a circular footprint on the film surface, seen as holes by SEM. This problem could possibly have been prevented by sparging the film-forming solution to remove air before the films were cast. The underside of this film observed by SEM showed more patches of roughness than the kafirin microparticle film, but they were smaller in size. This may again have been due to adhesion to the plastic of the Petri dish, and

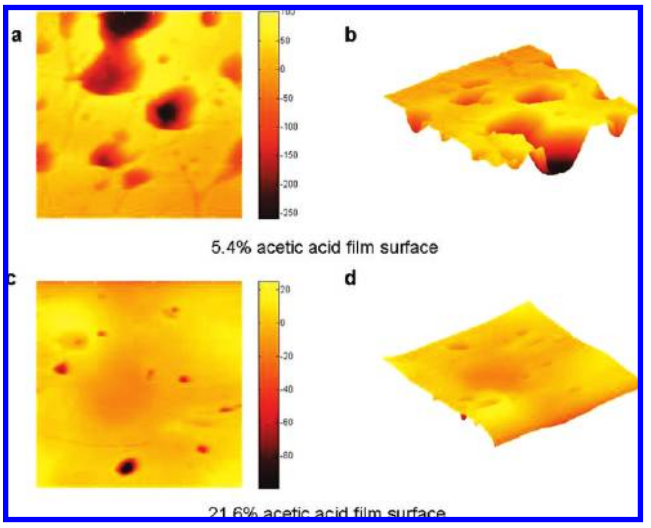

Figure 7. AFM of kafirin microparticle film surfaces. 5.4% acetic acid kafirin microparticle film: (**a**) top surface; (**b**) 3-D image of film surface. 21.6% acetic acid kafirin microparticle film: (**c**) top surface; (**d**) 3-D image of film surface. Scale bar in nm.

possibly the high viscosity of the solution may have increased the amount of adhesion. Byraruhanga et al. (*31*) presented SEM micrographs of the top surface and underside of conventional kafirin films. They were similar in appearance to the conventional kafirin films of this study, being pitted on the top surface and rough on the underside.

Concerning microparticle films, descriptions of acidified colloidal zein "film" surfaces by Cook and Shulman (*6*) appear similar to kafirin microparticle films made in this study. These authors describe the appearance of the colloidal "films" (which by the definitions of this study were actually coatings), when visualized by SEM, as smooth and more dense and homogeneous than aqueous ethanol cast zein films, containing no void spaces or porosity.

Other workers have examined, using SEM, the surfaces of zein films cast from microparticles by various methods based on aqueous ethanol (*9*, *10*, *32*). Some film surfaces, such as those described by O'Donnell et al. (*32*), using zein pseudolatexes (6% protein) as coating material for tablet coatings appeared similar to kafirin microparticle films when plasticizer was used. However, when no plasticizer was used, these films were incomplete with many surface defects and cracks. As stated, both Dong et al. (*9*) and Wang et al. (*10*) observed that their films consisted of spherical particles agglomerated together to a greater or lesser extent.

AFM of kafirin microparticle films cast from 5.4% acetic acid showed in cross section many indentations, some of which were up to 250 nm in depth (**Figure 7a**). The same film had places of elevation of up to 100 nm. This was expected since complete films cannot be cast from the kafirin microparticle in 5.4% acetic acid as illustrated by **Figure 4A**. By comparison the kafirin microparticle films cast from 21.6% acetic acid were more uniform with fewer imperfections covering a maximum depth of 100 nm and elevation of 20 nm (**Figure 7b**). Unfortunately, the AFM used was not sufficiently sensitive to reveal information on the kafirin microparticle film structure at a molecular level.

WVP and WVT. The WVT through all kafirin microparticle films and conventional kafirin films examined was similar except for kafirin microparticle films containing a low level of plasticizer (20%) (**Table 1**). Here the WVT was slightly lower but not substantially so. Irrespective of the level of plasticizer, the WVP through kafirin microparticle films was much lower than through

Table 1. WVT and WVP of Kafirin Microparticle Films Compared with Conventional Kafirin Films at the Same Protein Concentration (2%)[a]

film type	thickness (μm) start of test	thickness (μm) end of test	WVT (g h^{-1} m^{-2})	WVP (g mm m^{-2} h^{-1} kPa^{-1})
kafirin microparticle film, 21.6% acid, 20% plasticizer	15.9 a (2.2)	13.1 a (2.2)	36. 2 a (1.0)	0.19 a (0.03)
kafirin microparticle film, 21.6% acid, 40% plasticizer	19.5 ab (6.7)	14.1 a (3.0)	39.4 ab (3.7)	0.22 a (0.05)
kafirin microparticle film, 21.6% acid, 60% plasticizer	17.7 ab (1.9)	15.4 a (2.3)	41.7 b (2.2)	0.25 a (0.03)
conventional kafirin film, 40% plasticizer	26.4 b (5.5)	31.0 b (5.7)	40.8 b (2.6)	0.56 b (0.11)

[a] Values in the same column but with different letters are significantly different ($p < 0.05$). Numbers in parentheses indicate standard deviations.

Table 2. Tensile Properties of Kafirin Microparticle Films Compared with Conventional Kafirin Films at the Same Protein Concentration (2%)[a]

film type	thickness (μm)	max force (N)	force at break (N)	stress (N/mm^2)	stress at break (N/mm^2)	strain (%)
kafirin microparticle film, 21.6% acid, 20% plasticizer	13.0 a (3.0)	0.66 b (0.34)	0.63 b (0.33)	8.98 b (4.71)	8.53 b (4.48)	1.70 a (1.85)
kafirin microparticle film, 21.6% acid, 40% plasticizer	14.0 a (3.0)	0.38 a (0.44)	0.33 a (0.41)	4.18 a (5.25)	3.72 a (4.87)	2.53 a (3.37)
conventional kafirin film, 40% plasticizer	30.0 b (3.4)	0.79 b (0.26)	0.74 b (0.25)	5.77 a (2.15)	5.39 a (2.07)	2.99 a (1.79)

[a] Values in the same column but with different letters are significantly different ($p < 0.05$). Numbers in parentheses indicate standard deviations.

conventional kafirin films of the same protein concentration (**Table 1**). All of the kafirin microparticle films were thinner (16−19.5 μm) than the conventional kafirin film (26.4 μm) and so had lower WVP. Differences in film thickness at the same protein concentration were reflected in the film surface properties when viewed by SEM as previously described (**Figure 6**). The conventional kafirin films were rougher than kafirin microparticle films with many small holes. Park and Chinnan (*33*) noted that the WVP through a film increases as film thickness increases due to differences in film structure. Since the mechanism of vapor flow through a film is predominantly by diffusion, any holes, cracks, or imperfections as seen in the conventional kafirin films would be expected to result in higher WVP as was observed. The different nature of the film-forming solutions of the kafirin microparticle films and the conventional kafirin films may have affected the rates at which the films dried. This may have resulted in variations in film thickness and the observed defects.

It is difficult to compare WVP for protein microparticle films. Cook and Shulman (*6*) in their patent described the water loss through zein colloidal films as 42 mg/h. Film area was not considered, and no control value was given. Film thickness, at an unspecified solid content, was given as 6 μm for colloidal films compared with 10 μm for ethanol cast zein films. Meaningful comparisons of these values with those obtained in this study are not possible. When comparisons are made with other published data for conventional kafirin films, the kafirin microparticle films almost inevitably have much lower WVP since they are much thinner. Buffo et al. (*20*) reported conventional kafirin film WVP as 5.5 g mm m^{-2} h^{-1} kPa^{-1}, whereas other workers reported lower values ranging from 0.4 to 0.8 g mm m^{-2} h^{-1} kPa^{-1} (*3*, *4*, *16*, *31*). Conventional kafirin films cast by the above workers were approximately 10 times thicker than kafirin microparticle films. However, these values are similar to the conventional kafirin films from this study.

Tensile Properties. Tensile properties of kafirin microparticle films were in the range of 3.7−8.5 N/mm^2 for stress at break (tensile strength) and 1.7−2.5% for strain (extensibility) (**Table 2**). Not surprisingly, the strongest kafirin microparticle films were those containing the least amount of plasticizer (20%). There were no real differences in stress (tensile strength) between any of the films at the same plasticizer concentration, and all of the films had low percentage strain and were not very extensible.

Stress (tensile strength) values for kafirin microparticle films (3.7−8.5 N/mm^2) and conventional kafirin films (5.4 N/mm^2) are similar to published values for kafirin films cast from aqueous ethanol, 6−8 N/mm^2 (*3*) and 1.6−5.9 N/mm^2 (*4*) and 1.4 N/mm^2 for glacial acetic acid cast films (*31*) and 3.6−3.9 N/mm^2 (*16*), but strain (extensibility) values were much lower, ranging from 1.7% to 2.5% for microparticle films to 3.0% for the conventional kafirin films. Published strain (extensibility) values are 5−40% (*3*) and 13.5−142% (*4*) for ethanol cast films both tested at ambient RH and 42−55.5% for glacial acetic acid cast films tested at ambient RH (*16*) and 142% tested at 50% RH (*31*). The very low strain (extensibility) values of the kafirin microparticle films and the conventional kafirin films were probably due to micro defects, the presence of which were shown by SEM (**Figure 6**).

Kafirin microparticles made by phase separation from acetic acid may be used to form very thin free-standing films and coatings. There is a minimum concentration of acetic acid required before a free-standing film can be formed from kafirin microparticles in relation to the concentration of kafirin present. This relative amount was approximately 10.8:1, percent acetic acid to percent kafirin. The mechanism of film formation appears to involve a controlled aggregation of the kafirin microparticles and then dissolution of the microparticles in the acetic acid before drying into a cohesive film matrix. Some of the functional properties, e.g., film surface properties and WVP of these films, are superior to those of conventional kafirin films at the same protein concentration.

DEFINITIONS AND ABBREVIATIONS USED

Conventional kafirin film(s), free-standing film(s) cast from a solution of kafirin in glacial acetic acid; kafirin microparticle film(s), free-standing film(s) cast from a suspension of kafirin microparticles in acetic acid; SEM, scanning electron microscopy; TEM, transmission electron microscopy; FTIR, Fourier transform infrared spectroscopy; WVT, water vapor transmission; WVP, water vapor permeability.

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