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Identification of kafirin film casting solvents

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Abstract

The sorghum prolamin protein, kafirin can be used for making edible films. Several food compatible solvents were examined to identify novel kafirin film casting solvents to replace aqueous ethanol, commonly used for prolamin film casting. Glacial acetic acid and lactic acid were identified as the best primary solvents and 55% (w/w) aqueous isopropanol as a good binary solvent. However, the low volatility of the latter two prevents their use as casting solvents. Films could be cast from glacial acetic acid at 25 °C, a much lower temperature than the 70 °C required with aqueous ethanol. The sensory, tensile, and water barrier properties of the films cast from glacial acetic acid at 25 °C and aqueous ethanol at 70 °C were almost the same. However, the use of glacial acetic acid at 25 °C for casting kafirin films is advantageous as it gave films of more consistent quality.

Keywords: Kafirin; Solubility; Film casting; Film properties

1. Introduction

Research into the development of edible and biodegradable films from natural renewable sources has accelerated recently due to growing environmental concerns and stress on limited resources (Cuq, Gontard, & Guilbert, 1998; Debeaufort, Quezada-Gallo, & Voilley, 1998; Krochta, 2002). Zein, the prolamin protein of maize has been extensively studied for film making and is used commercially (reviewed by Lawton, 2002; reviewed by Shukla & Cheryan, 2001). Kafirin, the prolamin of sorghum has the potential to be an alternative to zein in film production (Buffo, Weller, & Gennadios, 1997). It is more hydrophobic (Wall & Paulis, 1978) and can be less digestible than zein (reviewed by Duodu, Taylor, Belton, & Hamaker, 2003), which may enable more stable films with better barrier properties to be produced.

The solvent most often used to form free-standing prolamin films is aqueous ethanol with aqueous acetone being used less frequently (Cuq et al., 1998). It has long been known that kafirin does not dissolve as easily as zein in aqueous ethanol (Johns & Brewster, 1916) and that elevated temperatures are required for its solvation (Buffo et al., 1997; Haikerwal & Mathieson, 1971; Johns & Brewster, 1916). Kafirin like zein is also prone to gelation, when dissolved in aqueous ethanol (Johns & Brewster, 1916; Jones & Beckwith, 1970). Additionally, ethanol needs a government license for its use and in some communities there are religious objections to its food use. Thus an alternative to ethanol for kafirin solvation would be beneficial if kafirin were to be utilised as an edible film for food use.

The solubility of zein in various solvents has been extensively studied, as reviewed by (Shukla & Cheryan, 2001) and Lawton (2002). The most comprehensive work on zein solubility was carried out by Evans and Manley in the early 1940s. They studied primary (Evans & Manley, 1941), binary (Manley & Evans, 1943) and ternary solvents (Evans & Manley, 1944) for zein and produced a list of some 70 different solvents and solvent combinations. No equivalent study on kafirin solubility

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has been made. The objective of the work reported here was to identify novel food compatible solvents for kafirin and to determine whether such solvents are effective as kafirin film casting solvents in comparison to aqueous ethanol.

2. Materials and methods

2.1. Materials

A mixture of two white condensed tannin free sorghum varieties (PANNAR 202 and 606, ex. Mr. B. Koekemoer, Lichtenburg, South Africa, 2001) was used for kafirin extraction. Kafirin was extracted from decorticated grain using a modification (Emmambux & Taylor, 2003) of the method of Carter and Reck (1970). Flour was extracted for 1 h with 70% aqueous ethanol (w/w) containing 0.5% sodium metabisulphite (w/w) and 0.35% sodium hydroxide (w/w) at 70 °C with constant stirring. The extract was separated by centrifugation at 3000 rpm (1000*g*) for 5 min and the solvent allowed to evaporate from the supernatant overnight at ambient temperature from shallow open trays placed in a fume cupboard. The protein was then washed with a minimal amount of cold (<10 °C) distilled water and the pH adjusted to approximately pH 5. The protein was recovered by filtration and freeze dried. The kafirin and commercial zein (Z3625, Sigma–Aldrich) were defatted with hexane at ambient temperature at a protein to solvent ratio of 1:10 (w/w). The defatted sorghum protein was characterised by amino acid analysis and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) and found to have an amino acid composition (Taylor & Schüssler, 1986) and SDS–PAGE band pattern consistent with that of total kafirin (α-, β- and γ-kafirin) (El Nour, Peruffo, & Curioni, 1998).

2.2. Kafirin solubility

A 13% protein to solvent (w/w) ''solution'' of the kafirin was made in ethanol (70% w/w), isopropanol (88%, 70%, 55% w/w), lactic acid, glacial acetic acid, glycerol at 70 °C, in ethanol (70% w/w), isopropanol (55% w/w), acetone (80%, 70% w/w) lactic acid and glacial acetic acid at 40 °C and finally ethanol (70% w/w), isopropanol (55% w/w), lactic acid and glacial acetic acid at 25 °C. Samples were held at the desired temperature in a waterbath for 15 min and mixed by vortexing at 5 min intervals. The insoluble residue was separated by centrifugation at 2300 rpm (500*g*) for 5 min. The nitrogen content of the supernatant was determined using the Dumas method and converted to protein using a factor of 6.25. Experiments were repeated twice with duplicate samples for each repetition.

2.3. Film casting

Defatted protein preparations (1.44 g protein) were weighed into 100 ml Erlenmeyer flasks. Aqueous ethanol (9 g 70% w/w), then plasticiser (0.6 g of a 1:1:1 w/w mixture of glycerol:polyethylene glycol 400:lactic acid) was added. The contents of the flask were heated to 70 °C on a hot plate and held for 10 min with the contents being stirred rapidly. Aliquots (4 g) were then weighed into 9 cm diameter plastic petri dishes and gently swirled to coat the bottom of the dish. The petri dishes without lids were placed on a level surface (checked with a spirit level) in an oven (not forced draught) at 50 °C and dried overnight.

Films were also cast from lactic acid, and glacial acetic acid at 25 °C, 55% (w/w) aqueous isopropanol and 70% (w/w) aqueous ethanol at 40 °C, using the film casting method described above but at the lower solvent temperatures. These films were also dried at 50 °C as described above.

2.4. Film sensory evaluation

The sensory quality of the kafirin and zein films were assessed by three individuals experienced in kafirin and zein films. After removal from the drying oven and cooling to ambient temperature, films were removed from the petri dishes. A scale from 1 to 5 was used in the evaluation, where a clear, flexible odourless polyethylene bag with a smooth surface texture was used to represent one on the scale for all attributes. The scales for the various film properties were as follows: Clarity (1-clear, transparent, 3-translucent, 5-opaque), colour (1-colourless, 3-yellowish, 5-reddish), flexibility (1-flexible, 5-brittle), surface texture (1-smooth, 5-rough) and odour (1-odourless, 5-strong odour (unpleasant-not food compatible)). The evaluations obtained were the consensus of the individuals and therefore should only be regarded as indication of the sensory quality of the films.

2.5. Film tensile properties

These were determined by a modified method based on ASTM D882-97 (American Society for Testing & Materials, 1997) using a TA-XT2 Texture Analyser (Stable Micro Systems, Goldalming, UK) with tensile grips coated with abrasive paper. Films were not conditioned prior to analysis. Strips (60×6 mm, SD $\pm$ 0.05 mm with respect to the width) of film were cut with a sharp scalpel using a pre-marked cardboard template. The thickness of the strips was measured in five places using a micrometer, before mounting between the tensile grips (40 mm apart). Tension was applied with a cross-head speed of 0.4 mm/s. The maximum force and distance at break was recorded and the stress and strain

calculated. Tensile testing was completed within 6 h of removal of films from the drying oven. At least 6 strips were tested from each film and at least 3 films were tested for each treatment.

2.6. Film water vapour transmission (WVT) and water vapour permeability (WVT)

A modified method based on the ASTM method E96-97, (American Society for Testing & Materials, 1997) was used. Circles (40 mm diameter) were cut from cast films and the thickness measured in five places using a micrometer. Schott bottles (100 ml) were modified by accurately drilling a hole (33 mm) in the centre of the plastic screw top. Films were mounted on top of the modified Schott bottles containing distilled water (90 ml). A fibre tap washer (external diameter 39 mm) between the lid of the bottle and the film ensured a watertight seal was maintained. The bottles were placed in a fume cupboard at ambient temperature (20–23 °C) and relative humidity (38–40%), with the fan switched on. Weight loss was recorded daily for up to 14 days. At least three replicates were performed for each treatment. Films were not conditioned before testing.

2.7. Statistical analysis

Analysis of variance using the least squares procedure was applied to the data on solubility of kafirin in different solvents, tensile and water vapour transmission and water vapour permeability tests.

3. Results and discussion

3.1. General

Extensive homology has been demonstrated between kafirin and zein (De Rose et al., 1989). Kafirin polypeptides can be classified into three overlapping groups based on SDS–PAGE and solubility differences. The kafirin groups are designated as α-(M_r 23 and 25×10^3) β- (M_r 16, 18, and 20×10^3) and γ-kafirin (M_r 28×10^3) (Shull, Watterson, & Kirleis, 1991). These are equivalent to α-(M_r 18 and 21–25×10^3), β-(M_r 17 and 18×10^3) and γ-zein (M_r 27×10^3) as described by Esen (1987). Both kafirin and zein are rich in the amino acids glutamine, leucine, alanine and proline and very low in lysine (Chung & Pomeranz, 1985). Kafirin is considered slightly more hydrophobic than zein (Duodu et al., 2003) and is more difficult to solubilise (Wall & Paulis, 1978). Solvents which solubilise zein easily may be expected to solubilise kafirin. Thus the solvents chosen for this work were based on those identified for zein by Evans and Manley, (Evans & Manley, 1941, 1944; Manley & Evans, 1943) and selected for food compatibility using the Swiss Poisons Class (Merck, 2001) as a guide.

In their study, Evans and Manley (1941) considered that solvents had to make at least a 10% solution to be classed as a primary solvent for zein. Other workers have used lower concentrations. In their review article, Shukla and Cheryan (2001) define zein as soluble in a solvent if >0.5% (w/v) zein dissolves in the solvent and results in a transparent solution at room temperature (20–25 °C). In this investigation, a 13% protein to solvent (w/w) solution in the various solvents was chosen as this was the concentration used for kafirin film casting in the European Union project of which this work was a part.

3.2. Kafirin solubility in food compatible solvents

Lactic acid and glacial acetic acid were the best primary solvents for kafirin at 70 °C and were effective solvents for kafirin at 40 and 25 °C (Table 1). This is in agreement with the work of Evans and Manley (1941) on zein. According to these workers organic acids such as lactic acid and acetic acid are effective solvents due to their amphoteric nature (Manley & Evans, 1943). Thus these solvents have the capacity to donate and accept electrons and so form hydrogen bonds between themselves and the protein. Evans and Manley (1941) found that a 10% (w/w) solution of zein in lactic acid went cloudy at less than 40 °C and in acetic acid at 14 °C.

The worst primary solvent for kafirin at 70 °C was glycerol (Table 1). Lawton (2002) describing the work of Osborne in 1897 reported that zein was soluble in glycerol but only at 150 °C. Possibly kafirin would also be soluble in glycerol if sufficiently high temperature had been used. Glycerol is probably a too hydrophilic alcohol to be a solvent for kafirin, as solutions of higher alcohols such as 55% (w/w) isopropanol (Jambunathan & Mertz, 1973; Taylor, Schüssler, & Van der Walt, 1984) and 60% (w/w) aqueous *tert*-butanol (Taylor et al., 1984) are effective solvents for kafirin.

Comparison of effective binary solvents for kafirin and zein revealed some differences. Those most effective for kafirin were 55% (w/w) aqueous isopropanol and 70% (w/w) aqueous ethanol both at 70 and 40 °C (Table 1). At 25 °C only 55% (w/w) aqueous isopropanol was an effective solvent for kafirin. Binary solvents effective for zein include 70% (w/w) aqueous ethanol, 70% (w/w) aqueous acetone and 70% (w/w) aqueous isopropanol, all solutions going cloudy at temperatures of less than 10 °C (Manley & Evans, 1943). The finding that 55% (w/w) aqueous isopropanol is an effective solvent for kafirin is not surprising, as described above.

Aqueous acetone (70% or 80% w/w) was not a good solvent for kafirin (Table 1), possibly due to the hydrophilic nature of acetone. Probably, the more hydrophobic nature of kafirin makes it more difficult to

Table 1
Solubility of kafirin[a] in various solvents at different temperatures

Solvent	Temperature (°C)	Description	Solubility (as % of protein added)[b]
70% Ethanol	70	Small gelled pellet, clear supernatant	90.9fg (2.8)[c]
	40	Very viscous clear supernatant	94.9hij (2.8)
	25	Large white pellet, clear yellow supernatant	38.2c (1.3)
88% Isopropanol	70	Large white pellet, clear supernatant	41.5d (2.6)
70% Isopropanol	70	Small gelled pellet, clear supernatant	85.7e (1.7)
55% Isopropanol	70	Small gelled pellet, clear supernatant	90.9fg (1.0)
	40	Least viscous solution at 40 °C but slightly cloudy, similar to glacial acetic acid	93.1ghi (1.8)
	25	Slightly darker and less viscous solution than 70% ethanol at 25 °C, but few bits of undissolved kafirin	88.7ef (1.7)
80% Acetone	40	Large white pellet, clear supernatant	4.4a (3.0)
70% Acetone	40	Large white pellet, clear supernatant	27.1b (4.6)
Lactic acid	70	Clear viscous solution, more viscous on cooling	94.4hij (2.7)
	40	Clear viscous solution, more viscous but clearer than glacial acetic acid, 70% ethanol or 55% isopropanol at 40 °C	93.2ghi (3.5)
	25	Clear dark yellow very viscous solution with few bits of kafirin on surface	92.4gh (7.0)
Glacial acetic acid	70	Clear solution, less viscous than lactic acid at 70 °C, slightly cloudy on cooling	95.7ij (2.4)
	40	Least viscous solution at 40 °C but slightly cloudy, similar to 55% isopropanol	96.9j (4.7)
	25	Least viscous solution at 25 °C, slightly cloudy but all protein dissolved	94.4hij (1.4)
Glycerol	70	Gelled completely, not possible to sample	Not determined

[a] Kafirin extracted from sorghum endosperm with 70% ethanol containing 0.35% sodium hydroxide and 0.5% sodium metabisulphite at 70 °C.
[b] Values with different letters are significantly different at the 95% level.
[c] Figures in parentheses indicate standard deviations.

dissolve than zein (Wall & Paulis, 1978) and elevated temperatures are often required (Johns & Brewster, 1916). Because of its volatility it is not possible to raise the temperature of aqueous acetone sufficiently to determine whether it would be an effective solvent for kafirin at a higher temperature.

The dielectric constant of a solvent can be used to give an indication of that solvent's hydrophobicity (Morrison & Boyd, 1992). The higher the dielectric constant the more hydrophilic is the solvent and vice versa. Considering the dielectric constants of the solvents used to dissolve kafirin, that of acetic acid is very low, 6.1 at 20 °C (Merck, 2001), whereas lactic acid (19.4), isopropanol, (18.3), ethanol (24.3) and acetone (20.7) (Merck, 2001) are somewhat higher. Generally 'like dissolves like', (Morrison & Boyd, 1992), so a hydrophobic compound such as kafirin would be expected to dissolve in a more hydrophobic solvent. When a solvent is added to water the dielectric constant of the water is reduced, and the resulting binary solvent is more hydrophobic than water alone (Cheftel, Cuq, & Lorient, 1985). The dielectric constants of the binary solvents, which dissolve kafirin were estimated by calculating the mean dielectric constants of the primary solvent and that of water in the proportions they occur in the binary solvent. This gave 41.1 for 70% (w/w) ethanol, 38.6, for 70% (w/w) acetone, 46.6 for 55% (w/w) isopropanol and 36.6 for 60% (w/w) *tert*-butanol. The best binary solvent for kafirin is 60% (w/w) *tert*-butanol (Taylor et al., 1984), which has the lowest calculated dielectric constant. *Tert*-butanol is not food compatible, rating 4, (not completely harmless) on the Swiss Poison Class (Merck, 2001) and so was not investigated but is included here for comparison. Aqueous ethanol has a slightly higher calculated dielectric constant than aqueous *tert*-butanol and is a poorer solvent for kafirin, since elevated temperatures are needed before it is effective. Values obtained for aqueous acetone and aqueous isopropanol are anomalous and do not help explain the differential solubilities of kafirin and zein in these solvents. It has been suggested by Iversen, Kharkats, and Ulstrup (1995) that the dielectric properties of a total biological system differ dramatically from those of the individual components of that system due to the existence of dielectric boundaries. So just considering the individual dielectric properties of the solvent in isolation may not be sufficient to explain the differential solubilities of kafirin and zein in specific binary solvents and rather other factors might also be involved.

The composition of the kafirin used may have also affected its solubility in the different solvents when compared to commercial zein which was used by Evans and Manley (1941) and Manley and Evans (1943). Commercial zein is predominately α-zein (Lawton,

2002). In contrast, the kafirin used in this study contained β- and γ-kafirin in addition to α-kafirin. The relative hydrophobicities of kafirin and zein have been determined by calculating their free energies of hydration (Duodu et al., 2003), the higher and more negative the free energy of hydration, the less hydrophobic the protein. α-kafirin of sorghum (−140.4 kcal/mol) and the α-zein of maize (−139.8 kcal/mol) have virtually the same free energy of hydration and so the same level of hydrophobicity. In contrast, the free energy of hydration for γ-kafirin (−124.5 kcal/mol) is more negative than that calculated for γ-zein (−113.6 kcal/mol), indicating that γ-kafirin is more hydrophobic than γ-zein. There is no published amino acid sequence for β-kafirin and so no comment on the relative hydrophobicity of β-kafirin to β-zein can be made. The presence of γ-kafirin in the kafirin used in this study would result in it being more hydrophobic than commercial zein and so less soluble in the same solvents.

Conditions used to extract zein affect the ability of that zein to be re-solubilised (reviewed by Lawton, 2002). The same would be expected of kafirin. Reduction resistant oligomers have been identified in both uncooked and cooked sorghum and maize protein body enriched samples (Duodu et al., 2002), which probably contain γ-kafirin (El Nour et al., 1998). Cooked sorghum samples showed the largest proportion of these oligomers. It is possible that as the kafirin was extracted at 70 °C, with 70% aqueous ethanol containing a reducing agent and alkali, reduction resistant oligomers of kafirin may have been formed. It would be expected that these oligomers would be more difficult to dissolve in the same solvents than commercial zein, which is predominantely α-zein.

3.3. Film casting properties of the kafirin solvents

The most effective solvents were investigated for their kafirin film casting properties. Free-standing films of good quality (Fig. 1) could be made from kafirin dissolved in glacial acetic acid at 25 and 40 °C. The sensory properties of these kafirin films appeared to be almost the same as kafirin films cast from aqueous ethanol at 70 °C, having good clarity (2), a yellowish colour (3) and smooth surface texture (1), compared to the aqueous ethanol films cast at 70 °C (2, 3 and 1, respectively). The films cast from glacial acetic acid at 25 °C were slightly more flexible (1) but had a slightly stronger odour (3) than did kafirin films cast from aqueous ethanol at 70 °C (2 and 2, respectively), and were only slightly inferior in clarity to zein film cast from aqueous ethanol (1). An advantage of using glacial acetic acid as a casting solvent is that it can be used at 25 °C instead of 70 °C. Although acetic and lactic acid have been used to reduce the pH of film forming solutions (reviewed by Cuq et al., 1998) and in the case of lactic acid to act as a plasticiser (reviewed by Lawton, 2002), nothing has been published on the casting of protein films from glacial acetic acid. However, Lawton (2002) referring to work of Osborne on zein, published in 1897, mentioned that glacial acetic acid and phenol both leave a clear film of unchanged zein after evaporation.

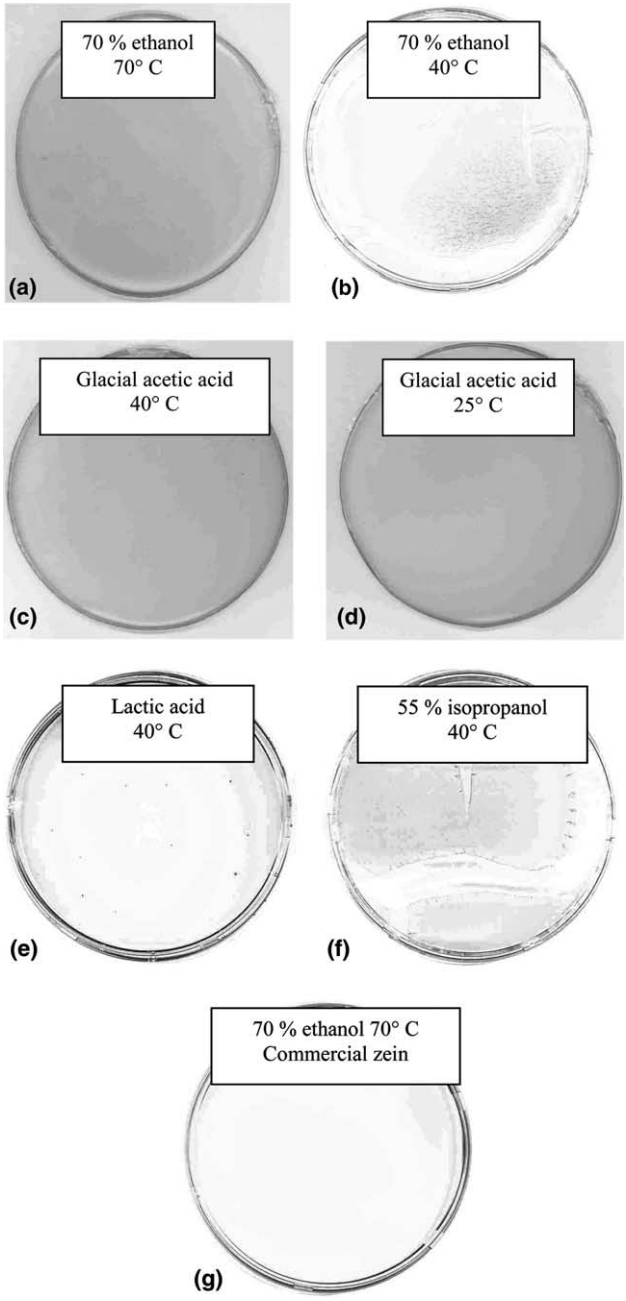

Fig. 1. Kafirin films cast from different solvents at different temperatures: (a) 70% ethanol at 70 °C; (b) 70% ethanol at 40 °C; (c) glacial acetic acid at 40 °C; (d) glacial acetic acid at 25 °C; (e), lactic acid at 40 °C; (f) 55% isopropanol at 40 °C; (g) 70% ethanol at 70 °C – commercial zein.

Concerning the effectiveness of other alternative solvents for kafirin film casting, although lactic acid has been shown to be an excellent solvent for kafirin,

its lack of volatility prevents it being used as a casting solution. The lactic acid solution remained clear (Fig. 1) but sticky even after a week. It was not possible to cast good films from 55% (w/w) aqueous isopropanol at 40 °C, (Fig. 1). These films were opaque and split into a number of pieces. Isopropanol is a higher alcohol than ethanol and so is less volatile than ethanol (boiling point: isopropanol 82.4 °C, ethanol 78.3 °C) (Merck, 2001). In addition, the proportion of water in the aqueous isopropanol binary solvent was higher than that of the aqueous ethanol, again reducing its volatility. This lack of volatility was the likely reason that aqueous isopropanol was unable to form free-standing films of good quality. To produce a good free-standing film the cohesive strength of the protein, molecule to molecule, must be relatively high and the continuous surface of the film material must coalesce on contact, ensuring the disappearance of boundary layers between adjacent polymer molecules (Banker, 1966). Thus, when a solvent evaporates slowly as in the case of aqueous isopropanol, there would possibly be less cohesion and the kafirin molecules would aggregate into clumps and precipitate before a continuous film matrix could be formed.

3.4. Film functional properties

A number of factors are known to influence the functional properties of free-standing films. These include: Protein purity and concentration in solution, casting solvent, pH of the casting solution, plasticisers or additives used, film drying temperature, and environmental factors, temperature and relative humidity (reviewed by Cuq et al., 1998). Due to this large number of variable factors it is not possible to compare functional film properties directly and so in this investigation the objective was to see whether kafirin films had different functional properties from zein films made in the same way and tested under the same conditions.

There was no significant difference in the tensile strength (maximum force) or extensibility (% strain) of any of the kafirin films, regardless of the solvent that the films were cast from (Table 2). There were however, differences in film thickness. Kafirin films cast from aqueous ethanol were the thickest. This was probably due to this solution having a higher viscosity than kafirin dissolved in glacial acetic acid. This may result in films that are less uniform, possibly containing pinholes and cracks and that could be slightly thicker, as observed. Glacial acetic acid cast films were more consistent in their tensile properties, as shown by the lower standard deviation in comparison to the aqueous ethanol cast films. This is presumably because it is much easier, from a practical standpoint, to cast films at a lower temperature. Kafirin films had the same tensile strength and extensibility as zein films cast from aqueous ethanol at 70 °C. This is in contrast to the findings of Buffo et al. (1997) who reported that kafirin films had lower tensile strength and higher elongation than films cast from zein. These workers attributed this to the lower protein content of the kafirin compared to that of the zein, which would have the effect of disrupting the homogeneity and continuity of the kafirin protein film network. Direct comparison with the work of Buffo et al. (1997) is difficult as the kafirin extraction method was different to that used in this present study and the kafirin composition was not given. However, the protein content of the kafirin (93.7% db) used in this investigation was similar to that of zein (92.5% db) and so, as found, similar functional properties would be expected between kafirin and zein films.

Water vapour transmission (WVT) and water vapour permeability (WVP) properties were the same for all kafirin films regardless of the casting solvent and temperature of casting (Table 3). These properties were slightly inferior to those of zein films, whereas Buffo et al. (1997), found them to be the same. This difference is probably related to the fact that Buffo et al. (1997) did not defat their protein preparations.

Possibly the poorer solubility of the kafirin may have been responsible for its slightly lower water barrier properties than zein films. The kafirin films were generally thicker than films cast from commercial zein (Tables 2, 3). Park and Chinnan (1995) reported that the water permeability of gluten and zein films increased as the thickness of the film increased. The reasons given were possible changes in structure of the film due to its

Table 2
Effect of different casting solvents on the thickness and tensile properties of kafirin films

Protein	Casting solvent	Casting temperature (°C)	Thickness mean (μm)	Force maximum (N)	Elongation (%)
Kafirin	70% Ethanol	70	113c[a] (12)[b]	4.1a (2.0)	63.5a (47.9)
Kafirin	Glacial acetic acid	40	94b (4)	2.7a (1.0)	55.5a (14.6)
Kafirin	Glacial acetic acid	25	90b (7)	2.8a (0.1)	41.6a (3.7)
Commercial zein	70% Ethanol	70	59a (2)	2.0a (1.0)	90.9a (72.7)

[a] Values in the same column but with different letters are significantly different at the 95% level.
[b] Figures in parentheses indicate standard deviations.

Table 3
Effect of different casting solvents on the thickness and water vapour barrier properties of kafirin films

Protein	Casting solvent	Casting temperature (°C)	Thickness mean (μm)	WVT[c] (g/h/m^2)	WVP[d] (gmm/m^{2}hkPa)
Kafirin	70% Ethanol	70	94b[a] (13)[b]	10.9ab (0.6)	0.43b (0.06)
Kafirin	Glacial acetic acid	40	90b (14)	10.9ab (0.5)	0.41b (0.05)
Kafirin	Glacial acetic acid	25	90b (6)	11.8b (0.5)	0.44b (0.04)
Commercial zein	70% Ethanol	70	57a (10)	10.6a (1.5)	0.24a (0.03)

[a] Values in the same column but with different letters are significantly different at the 95% level.
[b] Figures in parentheses indicate standard deviations.
[c] WVT, water vapour transmission.
[d] WVP, water vapour permeability.

increased thickness, and the swelling of hydrophobic films altering film structure.

4. Conclusions

Glacial acetic acid and lactic acid have been identified as the best food compatible primary solvents for kafirin at temperatures ranging from 25 to 70 °C. Aqueous isopropanol (55% w/w) is the best binary solvent for kafirin over the same temperature range. Lack of volatility prevents lactic acid and aqueous isopropanol (55% w/w) from being used as casting solvents for kafirin films. Glacial acetic acid can be substituted for aqueous ethanol as a casting solvent for kafirin films. Kafirin films cast from glacial acetic acid are of good quality with the same tensile and water barrier properties as those cast from aqueous ethanol. Glacial acetic acid has the advantage that films can be cast at a much lower temperature (25 °C) than aqueous ethanol (70 °C), which allows films of more consistent quality to be produced.

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