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Physical, mechanical, barrier, and thermal properties of polyol-plasticized biodegradable edible film made from kefiran

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ABSTRACT

The feasibility of using kefiran as a new film-forming material was studied. Edible kefiran films were produced using glycerol and sorbitol as plasticizers at the ratio of 10, 15, and 25% (w/w) by the casting method. Physical, mechanical and water vapor permeability (WVP) properties were determined as a function of plasticizer concentration. Dynamic mechanical thermal analysis was used to determine the glass transition temperature. Increased glycerol concentration caused an increase in flexibility and WVP, as expected. However, the plasticizing effect of glycerol became less significant, particularly for puncture deformation and tensile strength. Glycerol was found to be a more suitable plasticizer for kefiran films than sorbitol. The properties of the films were related to their microstructure, which was observed by scanning electron microscopy. It was determined that plasticizers can play an important role in adapting the properties of these films to make them more suitable for food technology applications.

1. Introduction

In recent decades, food and packaging industries have been joining their efforts to find new ways to protect food from environmental conditions like oxygen, light, moisture, microbes, and mechanical stresses. The use of synthetic polymers and plastics for packaging has grown tremendously in the last century; however, this increase has created serious environmental problems due to the materials' inability to biodegrade. Moreover, the insecurity of oil and petroleum resources – the raw materials from which such packaging is derived – is encouraging the food industry to explore the use of natural bio-based materials and polymers in packaging (Debeaufort, Quezada-Gallo, & Voilley, 1998). There is growing interest in edible biopolymer packaging: it is environmentally friendly and naturally biodegradable. Moreover, it may provide superior support and improve the mechanical integrity of food products, and flavor components.

Several studies have reported the use of polysaccharides from different sources to prepare films and coatings with different properties, and have indicated that these carbohydrates are promising materials (Mali, Sakanaka, Yamashita, & Grossmann, 2005). Less attention has been paid to microbial exopolysaccharides, mainly due to their low production levels compared with other polysaccharides, even though these materials can form gels and viscous solutions at low concentrations (Paul, Morin, & Monsan, 1986).

Kefiran, a microbial polysaccharide obtained from the flora of kefir grains, is finding increasing use in the food industry as a texturing and gelling agent. It is a water-soluble polysaccharide containing approximately equal amount of glucose and galactose (Micheli, Uccelletti, Palleschi, & Crescenzi, 1999). Recent studies have shown that high yields of these exopolysaccharides can be easily isolated from the grains in deproteinized whey (Rimada & Abraham, 2001). Thus exopolysaccharides from kefir grains might be an affordable alternative to synthetic packaging in food applications.

In addition, compared with other polysaccharides, kefiran has several important advantages, such as antibacterial, antifungal, and antitumor properties (Maeda, Zhu, Omura, Suzuki, & Kitamura, 2004; Murofushi, Shiomi, & Aibara, 1983; Shiomi, Sasaki, Murofushi, & Aibara, 1982). The literature data and preliminary studies in our laboratory have shown that kefiran can produce films with good appearance and satisfactory mechanical properties: it appears to have excellent potential as a film-forming agent. However, to date there has been little information available on its film characteristics. In general, natural packaging films exhibit several disadvantages, such as a strong hydrophilic character and poor mechanical properties, compared with synthetic packaging films. These drawbacks make it unsatisfactory for some applications such as packaging (John & Thomas, 2008).

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Many researchers have studied the effects of various plasticizers on film-based biopolymers as a way to overcome the films' brittleness. Plasticizers reduce intermolecular forces and increase the mobility of polymer chains, decreasing the glass transition temperature (T_g); unfortunately, they also decrease the film's water vapor permeability (Gontard, Guilbert, & Cuq, 1993).

A variety of commonly used polyol-plasticizers, such as glycerol, polyethylene glycol (PEG) and sorbitol, propylene glycol (PG), and ethylene glycol (EG), have been used to make edible films. Preliminary experiments have found that only glycerol and sorbitol significantly plasticized kefiran films at reasonably low concentrations. There is only one paper about the use of kefiran as edible film (Piermaria, Pinotti, Garcia, & Abraham, 2009), and, to the best of our knowledge, there is no specific study on the effect of various concentrations of plasticizer on film properties. In the recent years, a major emphasis has been placed on the search for new microbial biopolymers with different compositions and properties, and several of them have been under investigation. This study aims to develop a new edible film based on kefiran, with potential applications as an edible film and a biodegradable food packaging material, and to examine in detail the physical, mechanical, thermal, barrier, and microstructural properties of the resulting films as a function of plasticizer type and concentration.

2. Materials and methods

2.1. Starter culture

Kefir grains, used as a starter culture in this study, were obtained from a household in Tehran, Iran. The grains were kept in skimmed milk at room temperature for short periods and the medium was exchanged daily for fresh to maintain the grains' viability. After the culture was continued for seven subsequent days, the grains were considered active.

2.2. Isolation and purification of kefiran

Exopolysaccharides in the kefir grains were extracted by the method of Piermaria et al. (2009). In brief, a weighed amount of kefir grains was treated in boiling water (1:100) for 1 h and stirred vigorously. The mixture was centrifuged (Sigma 3-16k Frankfurt, Germany) at 10,000 × g for 15 min at 20 °C and an equal volume of chilled ethanol was added to precipitate the polysaccharide and kept at −20 °C overnight. The pellets were collected by centrifuging at 10,000 × g for 20 min at 4 °C. The precipitates were re-dissolved in hot distilled water and the precipitation method was repeated twice. The resulting solution was concentrated, yielding crude polysaccharide. The samples were tested for the absence of other sugars and proteins by high-performance liquid chromatography and the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), respectively.

2.3. Preparation of films

Aqueous solutions of 1, 2, and 3% kefiran were prepared by weighing the amount of film-forming solution under constant stirring via the use of a magnetic stirrer for 15 min. Preliminary experiments had showed that filmogenic solutions containing 2% kefiran were easily removed from the plate. One the other hand, films formulated with 1% had low thickness values and were difficult to handle. Also, the films prepared without plasticizer were brittle, and cracked on the casting plates during drying. Thus, plasticizer was incorporated into the film-forming solutions to achieve more-flexible films.

Polyols such as glycerol and sorbitol (Sigma Chemical Co., St. Louis, MO, USA) were added as plasticizers at various concentrations (15–35% (w/w), with respect to the dry weight of the biopolymer). Following the addition of plasticizers, stirring was continued for a further 15 min. The film solution was left for several minutes to naturally remove most of the air bubbles incorporated during stirring. Films were cast by pouring the mixture onto Teflon-coated plates resting on a leveled granite surface for approximately 18 h at room temperature and room relative humidity. Dried films were peeled off the casting surface and stored inside desiccators at 25 ± 1 °C until evaluation. Saturated magnesium nitrate (50% *RH*, Merck, Germany) solution was used to meet required relative humidity.

2.4. Determination of physical properties of films

2.4.1. Film thickness

Thickness of the films was measured using a manual digital micrometer (Mitutoyo No. 293-766, Tokyo, Japan) to the nearest 0.001 mm. Measurements were made in at least ten random locations for each film, and an average value was calculated. The average value was used in calculations for tensile properties and WVP tests.

2.4.2. Moisture content

The films' moisture content (approximately 1 cm × 3 cm) was determined by measuring the weight loss of films before and after drying in a laboratory oven (Blue M Electric Co., Blue Island, IL) at 103 ± 2 °C until constant weight was reached (dry sample weight). Three replications of each film treatment were used for calculating the moisture content.

2.4.3. Film solubility in water

For this study, solubility in water was defined as the ratio of the water-soluble dry matter of film that is dissolved after immersion in distilled water (Gontard, Duchez, Cuq, & Guilbert, 1994). A circular film sample was cut from each film, dried at 103 ± 2 °C for 24 h in a laboratory oven, and weighed to determine the initial dry weight. The solubility in water (SW) of the different kefiran films was measured from immersion assays in 50 ml of distilled water with periodic stirring for 6 h at 25 °C. After that period, the remaining pieces of films were taken out and dried at 103 ± 2 °C until constant weight (final dry weight).

The solubility in water of the films was calculated using Eq. (1):

$$\%\text{SW} = \frac{\text{initial dry weight} - \text{final dry weight}}{\text{initial dry weight}} \times 100 \qquad (1)$$

SW tests for each type of film were carried out in three replicates.

2.5. Water vapor permeability

The films' water vapor permeability (WVP) was measured gravimetrically according to the standard method E96 (ASTM, 1995) and corrected for the stagnant air gap inside test cups according to the equations of Gennadios, Weller, and Gooding (1994). Special glass cups with wide rims were used to determine WVP. The cups, which contained approximately 50 g anhydrous calcium chloride desiccant (0% *RH*, assay cup) or nothing (control cup), were covered with films of varying plasticizer concentration. Films without pinholes or defects were cut circularly (0.00287 m^2 film area) and sealed to the cup mouths using melted paraffin. Each cup was placed in a desiccator maintained at 75% *RH* with a sodium-chloride-saturated solution (Merck, Darmstadt, Germany). This difference in *RH* corresponds to a driving force of 1753.55 Pa, expressed as water vapor partial pressure. After the films were mounted, the weight gain of the whole assembly was recorded every 1 h during the first 9 h and finally after 24 h (with an accuracy of 0.0001 g). The cups were shaken horizontally after every weighing. The slope of the weight-versus-time plot (the lines' regression coefficients were >0.998) was divided by the effective film area to obtain the water vapor transmission rate. This was multiplied by the thickness of the film and divided by the pressure difference between the inner outer surfaces to obtain the WVP (Eq. (2)).

$$\mathrm{WVP} = \frac{\Delta m}{A\,\Delta t}\frac{X}{\Delta p} \tag{2}$$

where $\Delta m/\Delta t$ is the weight of moisture gain per unit of time (g/s), X is the average film thickness (mm), A is the area of the exposed film surface (m^2), and Δp is the water vapor pressure difference between the two sides of the film (Pa). WVP was measured for three replicated samples for each type of film.

2.6. Determining the mechanical properties of films: tensile and puncture tests

The mechanical properties of the film samples were determined through tensile and puncture tests using a Testometric Machine M350-10CT (Testometric Co. Ltd., Rochdale, Lancs., England) following the guidelines of ASTM standard method D882 (2001).

For the tensile test, films were cut in rectangular strips of 38 mm long and 5.79 mm wide. All the film strips were equilibrated at 51% *RH* for 48 h in a desiccator using saturated magnesium nitrate solution. The films were fixed with an initial grip separation of 50 mm and stretched at a cross-head speed of 50 mm/min. A microcomputer was used to record the stress–strain curves. Tensile strength was calculated by dividing the maximum load on the film before failure by the cross-sectional area of the initial specimen. Percentage elongation was defined as the percentage change in the length of the specimen to the original length between the grips. At least five replicates of each film were tested.

For the puncture test, three specimens (3 cm diameter discs) were cut from each pre-conditioned film. Each disc was mounted on the top of a test cup and a smooth-edged cylindrical probe (2 mm diameter) was moved perpendicularly onto the film surface at a cross-head speed of 50 mm/min until the film broke. Force-deformation data collected by a microcomputer was used to determine the puncture strength (PS) and deformation (PD) of the film at rupture.

2.7. Dynamic mechanical thermal analysis (DMTA)

The determination of the glass transition temperature of kefiran films was performed in a dynamic mechanical thermal analyzer (DMTA, Triton Technology, UK) working with liquid nitrogen and film grip clamps that allowed possible uniaxial traction tests. All the necessary thermal and mechanical calibrations of the instrument were performed before the experiments according to its operation manual. The tested film strips were 4.95 mm long, 8.87 mm wide, and 0.07 thick; they were clamped in the instrument with the initial grip separation of 5.5 mm. The films were subjected to a sinusoidal strain on top of a static deformation. The test was conducted at a constant frequency of 1 Hz and a strain of 0.02% over a temperature range of −69 to 159.6 °C, at a heating ramp rate of 5 °C/min. To prevent water loss during the experiment, the exposed surface of each sample was partially wrapped with aluminum foil, leaving the bottom and top in direct contact with the clamp holders (Pereira & Oliveira, 2000). The storage modulus (E') and loss tangent ($\tan\delta$) of each film sample were obtained as a function of temperature. The glass transition was defined as the midpoint between the onset of the drop in $\Delta E'$ (obtained from the intercept of the “glassy” baseline and the tangent to the point of the steepest drop in modulus) and the peak in $\tan\delta$. All the measurements were conducted at least in duplicate.

2.8. Scanning electron microscopy (SEM)

Microstructural analysis of the surface and cross sections of the dry films were observed by scanning electron microscopy (Oxford Instruments INCA Penta FET×X3). The films containing 25% plasticizer were fractured in liquid nitrogen; mounted on aluminum stubs using a double-sided adhesive tape and then sputtered with a thin layer of gold using a BAL-TEC SCD 005 sputter coater (BAL-TEC AG, Balzers, Liechtenstein). All the samples were examined using an accelerating voltage of 20.0 kV. Samples were photographed with an angle of 90° to the surface to allow observation of the films cross section.

2.9. Statistical analysis

The experiments were factorial with a completely randomized design. The type and concentration of plasticizers were the independent variables. Data was analysed by the analysis of variance (ANOVA) procedure using SAS software (version 9.1; Statistical Analysis System Institute Inc., Cary, NC, USA). Duncan's multiple range tests were used to compare the difference among the mean values for the films' properties at the level of 0.05.

3. Results and discussion

3.1. Film formulation

Preliminary studies were carried out to determine the plasticizer content range for film formulation. For this purpose, films having different glycerol and sorbitol concentrations were prepared. The lowest effective glycerol concentration was 10% (w/w) (film dry weight basis); below this concentration, the films tended to be brittle and difficult to handle, whereas films with more than 35% glycerol were flexible but sticky. Stickiness of glycerol-plasticized films may have resulted from phase separation and diffusion of glycerol to the surface of film. On the other hand, films containing sorbitol at less than 10% were prone to cracking and required care in peeling from the casting surface. Sorbitol-plasticized films with more than 35% sorbitol concentration developed white spots on the film surface after 72 h conditioning in 50% *RH*. The white residue may have been due to an excess amount of sorbitol. A similar situation was observed by Jangchud and Chinnan (1999), who explained that this occurred when the plasticizer content was more than its compatibility limit. All sorbitol-containing films were more opaque than the glycerol-containing films. Unplasticized films displayed a rough surface appearance, whereas sorbitol- and glycerol-plasticized films were relatively transparent, homogeneous, and flexible (see Fig. 1).

3.2. Physical properties of films

When constant ratio of film-forming solution (kefiran) was used, glycerol-containing films showed similar thickness values, ranging from 0.058 to 0.067 mm. However, the sorbitol-plasticized films with a thickness of 0.069–0.074 mm were significantly thicker than other films ($p < 0.05$) (Table 1).

Sorbitol can penetrate the polymer network more quickly and easily than glycerol, forming a stronger and thicker film. The kefiran film-thickness values were much higher than those reported by Piermaria et al. (2009), who reported a thickness value of 0.018–0.021 mm. This difference may be due to the differences in film-forming solution formulations and in the film-making procedures used.

The glycerol-containing films had higher moisture-content values than the sorbitol-containing films; this difference was significant ($p < 0.05$). Increasing the level of glycerol led to increases in the

Fig. 1. Illustrative picture of kefiran edible film (containing 2% kefiran and 25% (w/w) glycerol).

films' moisture content. It is often reported in the literature that the moisture content of hydrocolloid films increases with increasing plasticizer concentration (Hernández-Munoz, Kanavouras, Perry, & Gavara, 2003; Kristo & Biliaderis, 2006; Zhang & Han, 2006). However, the moisture content of sorbitol-plasticized kefiran films was exceptionally constant with increases in sorbitol concentration (Table 1). These results were similar to those achieved by Arvanitoyannis and Biliaderis (1999), whose starch films tended to become more hydrophilic with an increase in plasticizer content. These authors explained this behavior by the fact that glycerol acts as a water-holding agent, with the higher number of water molecules in glycerol-plasticized films increasing plasticizing activity, while sorbitol acts as a plasticizer with a minimum contribution from water molecules (Zhang & Han, 2006).

Solubility in water is an important property of edible films because potential food applications may require good water insolubility to enhance product integrity and water resistance (Perez-Gago & Krochta, 1999). However, in some cases it may be beneficial for a film to be designed to be water-soluble before consumption of the product. Generally, the solubility of the films should increase with increases in the concentration of plasticizer. However, in this study, solubility decreased with increases in sorbitol concentration. Plasticizer can diminish interactions between biopolymer molecules and increase solubility due to its hydrophilic nature, giving the polymer molecules higher affinity to attract water (Chick & Ustunol, 1998). Because the same amount of plasticizer was used in formulating all films, the effectiveness of glycerol over sorbitol in increasing the water-solubility of kefiran films was most likely due to its lower molecular weight, which allows it to be more readily inserted between the polymer chains (Cuq, Gontard, Aymard, & Guilbert, 1997).

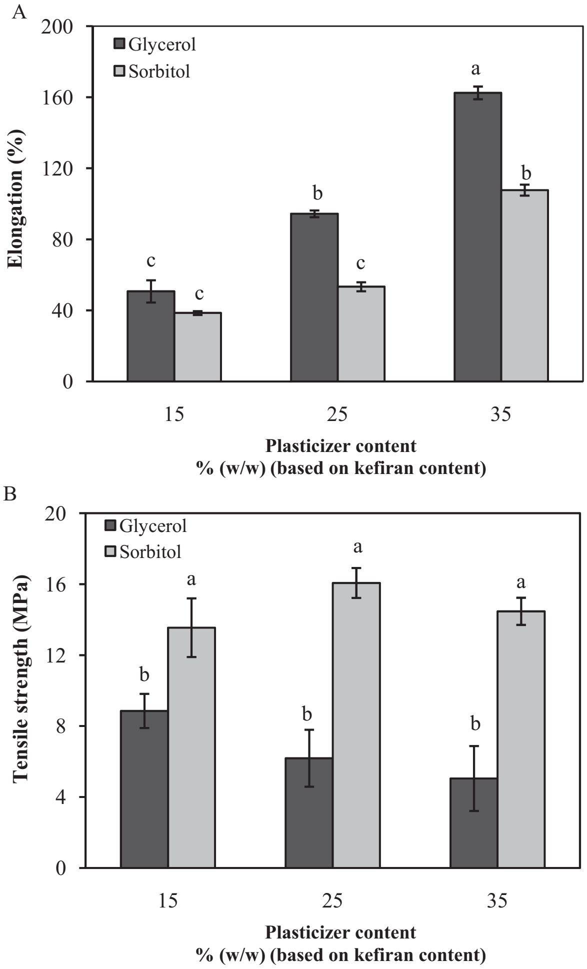


Fig. 2. Effect of plasticizer level and type on tension properties of kefiran films: tensile strength (A) and elongation (B). Columns with the same letter are not significantly different ($p < 0.05$).

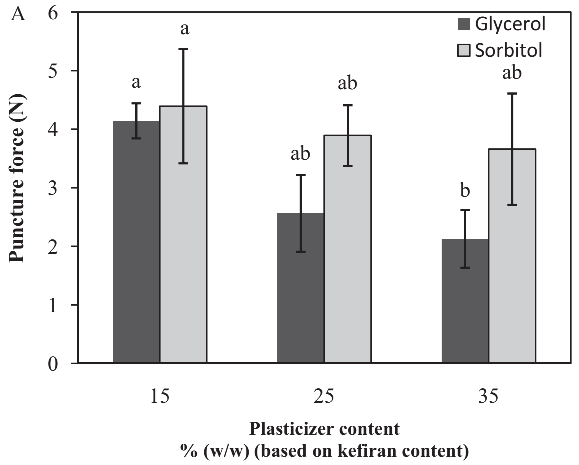
3.3. Water vapor permeability (WVP)

Table 1 shows the water vapor permeability values (WVP) for different kefiran-plasticized films. There was a significant difference between the WVP values of films made with different plasticizer concentrations ($p < 0.05$). WVP values were in the range of those reported by other authors for films based on kefiran (Piermaria et al., 2009). For each concentration studied, the films made with glycerol were more permeable ($p < 0.05$) than the films made with sorbitol. This was assumed to be because the structure of films plasticized by sorbitol was more compact or less favorable to water molecules than the structure of glycerol-plasticized films. In addition, increased proportion of glycerol in the solution sig-

Table 1
Effect of plasticizer level and type on films' WVP and physical properties.[a,b]

Plasticizer type	Content % (w/w) (based on kefiran content)	Thickness (mm)	Moisture content (%)	Solubility in water (%)	WVP ($\times 10^{-11}$ g m^{-1} s^{-1} Pa^{-1})
Kefiran		0.058 ± 0.0041 c	17.95 ± 1.69 d	21.28 ± 1.33 c	4.95 ± 0.13 b
Kefiran + glycerol	15	0.064 ± 0.0098 bc	23.59 ± 2.11 c	25.30 ± 0.64 b	5.04 ± 0.18 b
Kefiran + glycerol	25	0.062 ± 0.0070 bc	32.47 ± 2.89 b	27.91 ± 1.11 a	5.55 ± 0.21 a
Kefiran + glycerol	35	0.067 ± 0.0095 ab	37.04 ± 1.56 a	28.88 ± 0.85 a	5.88 ± 0.10 a
Kefiran + sorbitol	15	0.069 ± 0.0025 ab	17.02 ± 1.28 d	21.37 ± 0.94 c	4.11 ± 0.37 c
Kefiran + sorbitol	25	0.072 ± 0.0020 a	17.62 ± 0.93 d	19.33 ± 0.50 c	3.61 ± 035 d
Kefiran + sorbitol	35	0.074 ± 0.0045 a	18.21 ± 0.47 d	19.45 ± 2.19 c	3.67 ± 0.11 d

[a] Means within each column with same letters are not significantly different ($p < 0.05$).
[b] Data are means ± SD.



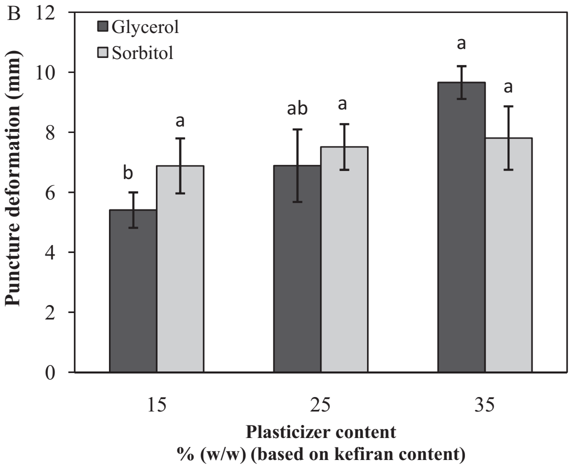


Fig. 3. Effect of plasticizer level and type on puncture properties of kefiran films: puncture force (A) and puncture deformation (B). Columns with the same letter are not significantly different ($p < 0.05$).

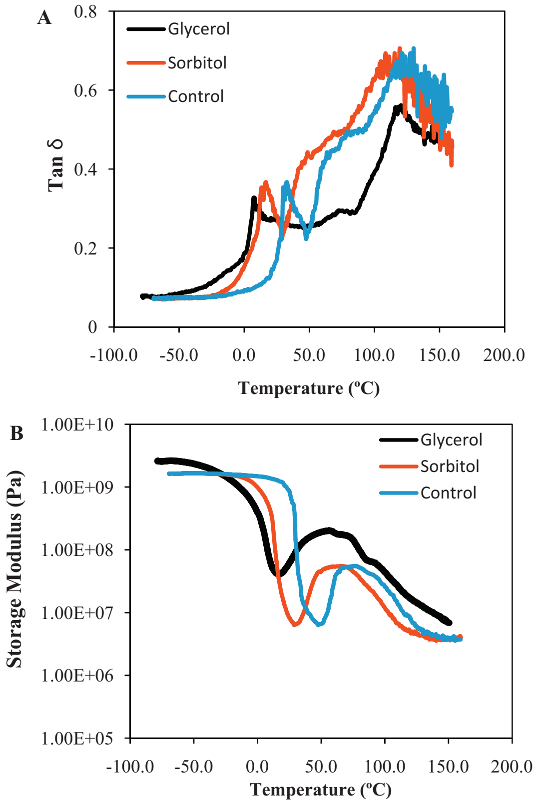


Fig. 4. Temperature dependence of tan δ (A) storage modulus (E') (B) for plasticizer (25% (w/w), based on kefiran content) and unplasticized (control curve) kefiran-based films.

nificantly increased the permeability of the films ($p < 0.05$): film plasticized with sorbitol exhibited lower WVP values compared with films with glycerol. This may arise from differences in their structures and hydrophilic properties. The cyclic conformation of sorbitol molecules may be the main factor that lowered the WVP of sorbitol-plasticized films (Zhang & Han, 2006).

Increasing plasticizer concentration decreased the intermolecular forces between polymer chains and increased free volume and segmental motions (Sothornvit & Krochta, 2001), allowing water molecules to diffuse more easily and giving a higher WVP. The same behaviors were shown in the work of Ghanbarzadeh et al. (2006), who found that increasing the glycerol concentration increased the WVP of zein films. The reported WVP values for films made from corn starch, sodium casein, myofibrillar fish, high-density polyethylene, cellophane, wheat gluten, and chitosan are 8.68, 1.51, 6.4, 0.23, 8.4, 4.52, and 2.25×10^{-11} g s^{-1} m^{-1} Pa s^{-1} (Cuq et al., 1997; McHugh & Krochta, 1994; Ojagh, Rezaei, Razavi, & Hosseini, 2010; Smith, 1986). The WVP values of the kefiran films were lower than those of corn starch, cellophane, myofibrillar fish, and wheat gluten but higher than those of the other films.

3.4. Mechanical properties

Mechanical properties reflect the durability of films and their ability to enhance the mechanical integrity of foods. Figs. 2 and 3 present the effects of incorporating different plasticizer types and concentrations on the mechanical properties of kefiran films. The glycerol-plasticized films were significantly less resistant ($p < 0.05$) and more extendible ($p < 0.05$) than the sorbitol-plasticized films, suggesting that glycerol could be a more effective plasticizer in kefiran films. However, increasing glycerol content did not significantly affect TS of the formed films ($p > 0.05$) (Fig. 2).

As shown in Fig. 3, there was little change in either puncture force (PS) or puncture deformation (PD) with different glycerol concentrations.

The effect of plasticizer concentration increment on the film specimens' mechanical properties was expected and has been broadly discussed in the literature (Butler, Vergano, Testin, Bunn, & Wiles, 1996; Cuq et al., 1997; McHugh & Krochta, 1994). For example, Lourdin, Coignard, Bizot, and Colonna (1997) have discussed it in detail. They stated that a small quantity of plasticizer could be easily inserted between polymer chains, producing a "cross-linker" effect that would decrease the free volume and the segmental mobility of the polymer, decreasing the mechanical strength of the films and enhancing their extensibility. Sorbitol has been extensively used in other biopolymer films, but it appeared to have no significant effect ($p > 0.05$) on plasticizing kefiran film, at least at the concentrations tested. As described earlier, this was due to the differences in molecular size and structure, the number of active hydroxyl groups, polarity, and some other properties that distinguish sorbitol from glycerol. The TS values of kefiran-plasticized films were 5.04–16.18 MPa, which were lower than those of synthetic polymers such as LDPE (9–17 MPa), polystyrene (35–55 MPa), and cellophane (114 MPa) (Smith, 1986). However, kefiran film plasticized with glycerol showed extremely high elongation values – for example, they were higher than cellophane (20%) and polystyrene (1%) – but could not be stretched nearly LDPE (500%).

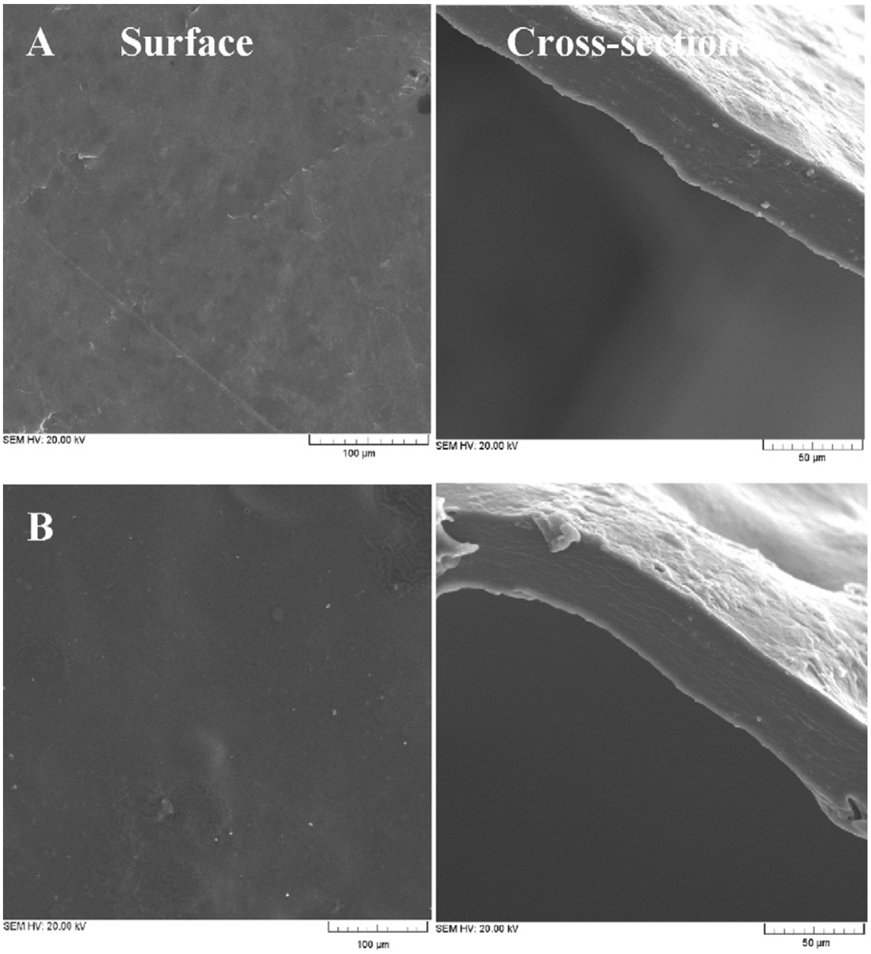


Fig. 5. Scanning electron micrographs of the surface (left) of kefiran-based films viewed at a magnification of 500× and cross-section (right) viewed at a magnification of 1000×: (A) kefiran-sorbitol and (B) kefiran-glycerol.

3.5. Dynamic mechanical thermal analysis (DMTA)

The thermo-mechanical behavior of kefiran films, either alone or with sorbitol and glycerol, was studied by DMTA. Fig. 4 shows typical DMTA curves (E' and $\tan\delta$) of kefiran film with and without sorbitol and glycerol. Because the values for glass transition temperature (T_g) were very close for all plasticized films, the figure shows only the T_g of the 25% concentration level. The glass transition temperature (also denoted as α-relaxation) was defined by the $\tan\delta$ peak or large drop in storage modulus (E'); the $\tan\delta$ peak is generally found at a temperature higher than onset or the midpoint temperature of the modulus (E') drop (Lazaridou & Biliaderis, 2002).

The value of the T_g of a polymer mixture is often claimed as a criterion to establish its miscibility (Ghanbarzadeh & Oromiehi, 2009). Many researchers also suggested that there is no phase separation between the polymer and the plasticizer in the films if there is no evidence for two individual α-relaxations occurring in $\tan\delta$ trace. In our case, a one-step drop in elastic modulus (E') as well as a single peak in $\tan\delta$ was observed in the DMTA traces; separate transition was not detectable. The kefiran-glycerol, kefiran-sorbitol, and kefiran-only films showed $\tan\delta$ peaks at 7.1, 13.1, and 29.8 °C, respectively. It was found that glycerol significantly affected the T_g of the films. Also, a high plasticizing effect of glycerol on the mechanical properties and WVP was observed in various works (Vanin, Sobral, Menegalli, Carvalho, & Habitante, 2005). For all films, those without plasticizer had the highest T_g; moreover, the T_g decreased with increased glycerol or sorbitol concentration due to the plasticization process. Other works available in the literature corroborate these findings (e.g., Quijada-Garrido, Iglesias-Gonzalez, Mazon-Arechederra, & Barrales-Rienda, 2007). Sorbitol did not change the T_g of the film as much as glycerol. The depression of T_g with the addition of plasticizer may be explained by a number of theoretical approaches such as free volume or classical thermo-dynamic theories proposed by Couchman and Karaz (1978). The fact that glycerol molecules are much smaller and have a higher ratio of hydroxyl number to molecular weight may also be factors. Although sorbitol has a hydroxyl number ratio similar to glycerol, its plasticization efficiency was not as good. This could be due to the fact that sorbitol has a bigger molecule size that could reduce its efficiency.

3.6. Film microstructure

In an attempt to study microstructural changes in the films, scanning electron microscopy (SEM) was conducted to visualize the surface and cross-section topography of all prepared kefiran films. Scanning electron microscopy of the plasticized films showed smooth and uniform surfaces without pores or cracks; however, there were no distinguishable differences in any of the films, regardless of which plasticizer or concentration was used. Fig. 5 shows SEM micrographs of the outer surface (left) and cross-section (right) for the kefiran films with 25% glycerol or sorbitol as examples.

A more regular and compact structure was expected from the sorbitol-containing film than those containing glycerol. However, comparison of the films did not present any obvious structural differences using various plasticizers. Sorbitol at tested concentrations might not be as effective as glycerol, but provided a lubricant effect on the films, as demonstrated in the measurements of WVP and tensile properties.

4. Conclusions

This study demonstrated that kefiran could be used as the basis for new film-forming materials to create edible films. The physical, mechanical, and thermal properties of these films, which are important in food packaging applications, were examined as a function of plasticizer concentration. Overall, the best properties for kefiran-based films produced in the present study were observed in glycerol-plasticized films. At high plasticizer concentrations, phase separation occurred, resulting in a sticky surface on the films plasticized with glycerol and crystallization in the films plasticized with sorbitol. Generally, the TS of the films decreased and *%E* increased with increasing concentrations of plasticizer. The resulting films exhibited a potential application as edible food films and coatings. The properties of kefiran-based film can be further improved to be applied to food products. The methods investigated and developed in this study are expected to be very useful for future research.

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