



Original research

Characterisation of biocomposite film made of kefiran and carboxymethyl cellulose (CMC)

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ABSTRACT

This study was carried out to characterize the physical, mechanical and barrier properties of kefiran-carboxy methyl cellulose (CMC) biocomposite films in function with CMC content. Films were prepared by combining these two biopolymers and adding glycerol as plasticizer. Film-forming solutions of different ratios of kefiran to CMC were cast at room temperature. Increasing CMC content from 0 to 50 % (v/v) increased the tensile strength and extensibility of the composite films; however, with further CMC addition these mechanical properties decreased. The water-vapor permeability (WVP) of the films first decreased and then increased with CMC addition. The microstructure of the films was observed by scanning electron microscopy and atomic-force microscopy. The morphology study of the films showed rougher surfaces with increasing CMC amounts. Dynamic mechanical thermal analysis (DMTA) curves showed a single T_g, and addition of CMC at all levels increased the glass-transition temperature of films. These results indicated that these two film-forming components were compatible, and that an interaction existed between them.

Keywords: Kefiran, Carboxy methyl cellulose, composite films, Mechanical Properties, Water-vapor permeability

Received 10 April 2018; Revised 18 July 2018; Accepted 5 August 2018

1. Introduction

Synthetic films and petro-based plastics have long been used in the food packaging industry due to their desirable features such as softness, lightness and transparency. However, their negative environmental impact caused by their total non-biodegradability is a serious disadvantage for these materials. The development of biopolymer-based edible films has become a topic of great interest as a potential alternative to synthetic polymer-based food packaging materials (Abbasi & Askari, 2018). Edible films are generally produced from renewable abundant and natural biodegradable polymeric materials such as polysaccharides, proteins, lipids or the combination of these components. These materials have the possibility to create thin films and coatings for covering fresh or processed foods to extend their shelf life (Campos et al., 2011).

Kefiran is a microbial exopolysaccharide obtained from the flora of kefir grains. It contains glucose and galactose, is water-soluble and improves the viscosity and viscoelastic properties of acid milk gels. It can also form gels with interesting viscoelastic

properties at low temperatures (Ghasemlou et al., 2012). Furthermore, compared to other polysaccharides, kefiran has several important advantages, such as immunomodulation, antibacterial, antifungal, and antitumour properties (Murofushi et al., 1983; Motedayen et al., 2013). Recent studies have reported that high yields of these exopolysaccharides can be easily isolated from the grains in deproteinized whey (Rimada & Abraham, 2001). Kefiran can produce films with satisfactory mechanical properties and good appearance (Ghasemlou et al., 2011; Kazazi et al., 2017). However, generally, edible films produced from only one kind of natural film-forming polymer display good properties in some aspects but poor in others. Blending of biopolymers is a promising strategy to improve the properties of edible films and coatings. Also, by incorporating other compatible polymers that are abundant and lower in cost as filler in the film matrix, the cost of composite film will reduce. Biocomposite films are prepared by various methods, and are usually composed of two or three biopolymers (Campos et al., 2011).

Cellulose is the structural material of plant cell walls and it is composed of linear chains of (1→4)-β-D-glucopyranosyl units.

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Carboxymethyl cellulose (CMC) is the most important water-soluble cellulose derivative with a wide range of applications in foods, pharmaceuticals, cosmetics, detergents, etc. (Olaru et al., 1998). Cellulose derivatives films are flexible, tough, totally transparent and highly sensible to water presence but resistant to oils and fats (Campos et al., 2011). CMC is used primarily because of its high viscosity, non-allergenic and non-toxic characteristics. This semi-synthetic derivative of cellulose is produced by partial substitution of the 2, 3 and 6 hydroxyl groups of cellulose by carboxymethyl groups. Purified CMC has a color between white and yellowish and is a tasteless, odorless, free-flowing powder (Keller, 1986; Hattori et al., 2004). The numerous hydroxyl and carboxylic groups in CMC enable water binding and moisture sorption properties. CMC hydrogel has a good biodegradability, high water content and many different applications due to its low cost. Because of its high molecular weight and polymeric structure, it can be used as filler in biocomposite films (Almasi et al., 2010).

Polysaccharide-based films are relatively stiff. To improve film flexibility, it is necessary to add plasticizer agents. Different types of plasticizers are widely used in hydrocolloid-based films; these include water, oligosaccharides, polyols and lipids (Sothornvit & Krochta, 2001). Plasticizers generally increase film hydrophilicity, of which in turn promotes water-vapor permeability. Because of its stability and compatibility with hydrophilic bio-polymeric packaging chains, glycerol is one of the most popular plasticizers used in film-making. Normally, plasticizers are added to the film-forming solution before casting and drying (Krochta & Mulder-Johnston, 1997).

In recent years, the search for new microbial biopolymers with different compositions and properties has become a topic of great interest, and several of them have been under investigation. There are a few studies on kefiran films, but to the best of our knowledge, there is no specific study on the effect of blending kefiran and CMC to produce biocomposite or blend films. In current research, various levels of CMC were used to produce kefiran/CMC blend films and glycerol was added as plasticizer. The goal of this work was to study the compatibility of these two biopolymers and to characterize the physical, mechanical, and thermal properties of the composite films.

2. Material and Methods

2.1. Materials

CMC with an average molecular weight of 41,000 (practical grade) was purchased from Caragum Parsian Corporation (Tehran, Iran). Kefir grains, used as a starter culture in this study, were obtained from a household in Tehran, Iran. Glycerol, magnesium nitrate ($Mg(NO_3)_2 \cdot 6H_2O$) and calcium chloride (analytical grade) were purchased from Merck (Darmstadt, Germany).

2.2. Starter culture

Kefir grains were kept in skimmed milk at room temperature for short periods and the medium was exchanged daily for new culture to maintain the grains' viability. After the culture was continued for seven subsequent days, the grains were considered active (Ghasemlou et al., 2012a).

2.3. Isolation and purification of kefiran

Kefiran exopolysaccharides were extracted from the kefir grains by the method of Piermaria et al. (2009). In brief, a weighed amount of kefir grains was added to boiling water (1:10) and stirred for 1 h discontinuously. After reaching uniformity, the mixture was centrifuged (Sigma 3-16 k Frankfurt, Germany) at 10,000 g for 15 min at 20°C. To precipitate the polysaccharides secreted in the supernatant of the centrifuged samples, an equal volume of chilled ethanol was added. Then the mixture was kept at -20°C overnight. After that time, 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 a crude polysaccharide. The samples were tested for the absence of other sugars and proteins by high-performance liquid chromatography and the phenol-sulphuric acid method (Dubois et al., 1956), respectively.

2.4. Preparation of films

A CMC solution with a concentration of 2 % (w/v) was prepared by dispersing 2 g of CMC in 100 mL of distilled water with constant stirring. The solution was heated at 75 °C for 10 minute and then cooled to around 20-25 °C. Kefiran solution 2 % (w/v) was prepared by weighing the amount of film-forming solution under constant magnetic stirring for 15 min. Preliminary experiments and studies had shown that filmogenic solutions containing 2 % kefiran were easily removed from the plate. On the other hand, films formulated with 1 % kefiran had low thickness values and were difficult to handle (Ghasemlou et al., 2011). Kefiran/CMC composite films were prepared by mixing various levels of 2 % kefiran solutions with various levels of 2 % CMC solutions (100/0, 70/30, 50/50, 30/70). The films prepared without plasticizer were brittle, and cracked on the casting plates during drying. Thus, plasticizer was incorporated into the film-forming solution to achieve more-flexible films. Glycerol (Merck, Darmstadt, Germany) was added (30 % of the total solid weight). Following the addition of plasticizer, stirring was continued for a further 15 min. The film solutions were transferred into a vacuum oven for about 30 minutes at 30 °C to remove most of the air bubbles incorporated during stirring. The mixtures were cast onto flat, leveled, non-stick Teflon plates and were held at room temperature and room relative humidity for 18 h to set. Once set, they were peeled off the casting surface and stored in plastic bags inside desiccators at $25 \pm 1^\circ C$ for further testing. All treatments were made in triplicate.

2.5. Film Conditioning

According to the standard method D618-61 (ASTM, 1993), all films were conditioned prior to subjecting them to permeability and mechanical tests. Films used for testing water vapor permeability (WVP), tensile strength (TS) and elongation-at-break (E) were conditioned at 51 % RH and $25 \pm 2^\circ C$ by placing them in desiccators over a saturated solution of magnesium nitrate for 48 h or more. For other tests, film samples were transferred to plastic bags after peeling and placed in desiccators.

2.6. Determination of physical properties of films

2.6.1. Film thickness

Film thickness was measured (to an exactness of 0.001 mm) using a manual digital micrometer (Mitutoyo No. 293-766, Tokyo, Japan) at 10 different points of the film, and an average value was calculated. The average value was used in calculations for tensile properties and WVP tests (Martins et al., 2012).

2.6.2. Moisture content

The films' moisture content (approximately $1 \times 3 \text{ cm}^2$) 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 $103 \pm 2^\circ\text{C}$ until constant weight was reached (dry sample weight). Three replications of each film treatment were used for calculating the moisture content.

2.6.3. Film solubility in water

Solubility in water was defined as the percentage of the total soluble matter (%TSM) of film that is solubilized after immersion in distilled water (Gontard et al., 1994). A circular film sample was cut from each film, dried at $103 \pm 2^\circ\text{C}$ for 24 h in a laboratory oven, and weighed to determine the initial dry weight. Then, to measure the solubility in water of the different kefir/CMC films, each film sample was immersed in 50 ml of distilled water with periodic stirring for 6 h at 25°C . After that time, the remaining pieces of films were taken out and dried at $103 \pm 2^\circ\text{C}$ until constant weight (final dry weight).

The %TSM of the films was calculated using the following equation:

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

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

2.6.4. Contact angle measurements

The contact angle is defined as the angle between the baseline of the drop and the tangent line at the point of contact of the water droplet with the surface (Ojagh et al., 2010). Water contact angle (WCA) measurements were estimated with a goniometer (Kruss G10, Germany). To measure the contact angles, a syringe was filled with 5 ml of distilled water and a droplet was injected on to the surface of the film. The WCA was averaged from five measurements.

2.7. Water vapor permeability

The WVP of the films was measured gravimetrically according to the standard method E96 (ASTM, 1995), with some modifications according to the equations of Gennadios et al. (1994). To determine the films' WVP, special glass cups with wide rims were used. Films were cut into discs with a diameter slightly larger than the diameter of the cup. After placing approximately 50 g of anhydrous calcium chloride desiccant ($\sim 0\%$ RH, assay cup) or nothing (control cup) in the cups, they were covered with edible films of varying kefir/CMC contents. Films were 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. At first, cups were weighed every hour for a total of 9 h; after that, measurement was carried out every 24 h for 4 days, and water-vapor transport was determined by the weight gain of the cup. The cups were shaken horizontally after every weighing. Changes in the weight of the cup were recorded as a function of time. Slopes were calculated by linear regression (weight change vs. time). The water-vapor transmission rate (WVTR) was obtained by dividing the slope (g/h) by the transfer area (m^2). 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)).

$$WVP = \frac{\Delta m}{A \Delta t} \frac{X}{\Delta p} \quad (2)$$

where $\frac{\Delta m}{\Delta t}$ 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 ($\text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$) was measured for three replicated samples for each type of film.

2.8. Determination of films mechanical properties

The mechanical properties of films prepared were evaluated by conducting TS and E tests at 25°C with a Testometric M350-10CT (Testometric Co., Ltd., Rochdale, Lancs., England) according to ASTM standard method D882 (ASTM, 2001). Film-specimen strips ($25 \text{ mm} \times 100 \text{ mm}$) were cut and conditioned in a desiccator containing saturated magnesium nitrate solution with 51 % RH for 48 h prior to testing. Samples were clamped between the grips of the machine. The initial grip separation was set at 50 mm and the crosshead speed was set at 50 mm/min. The recording of the stress-strain curves was done using a microcomputer. TS was expressed in MPa and calculated by dividing the maximum load by the initial cross-sectional area (m^2) of the film specimen. E was defined as the ratio of the final length at the point of sample rupture to the initial length of a specimen (50 mm) and expressed as a percentage. At least five replicates of each film were tested.

For the puncture test, 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 (3 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. Puncture tests for each type of film were carried out in three replicates.

2.9. Dynamic mechanical thermal analysis (DMTA)

The glass-transition temperatures (T_g) of kefir/CMC films were determined using 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 mechanical and thermal calibrations specified in the instrument's operating manual were performed before the experiments. Film samples were clamped in the instrument with an initial grip separation of 5.5 mm. Samples were subjected to a sinusoidal strain on top of a static deformation. The testing was conducted at a constant frequency of 1 Hz and a strain of 0.02 % and over a temperature range of -69 to 159.6°C , at a heating rate of $5^\circ\text{C}/\text{min}$. The exposed surface of each film sample was partially

wrapped with aluminum foil, leaving the bottom and top in direct contact with the clamp holders to prevent any water loss during the experiment (Pereira & Oliveira, 2000). The storage modulus (E') and loss tangent ($\tan \delta$) of each sample were obtained as a function of temperature. The T_g was determined 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 done at least in triplicate.

2.10. Scanning electron microscopy (SEM)

Microstructural analysis of the surface and cross-sections of the films was observed using a scanning electron microscope (Model S-4160 SEM, Hitachi Ltd., Tokyo, Japan). Dried film samples were fractured in liquid nitrogen, mounted on aluminum stubs using a double-stick tape, and coated with a layer of gold using a BALTEC SCD 005 sputter coater (BALTEC AG, Balzers, Liechtenstein), allowing surface visualization using an accelerated voltage of 20.0 kV. Samples were photographed at an angle of 90° to the surface to allow observation of the films' cross-section (Swapna et al., 2011).

2.11. Atomic-force microscopy (AFM)

AFM (Dualscope/Rasterscope C26, DME, Denmark) with a 200 $\mu\text{m} \times 200 \mu\text{m}$ scan size and a 6 μm vertical range was used to study the surface morphology of the film samples that had been previously equilibrated at 50 % RH. Film specimens were cut into thin pieces that fit into AFM imaging, and double-sided tape was used to stick them onto the sample stage. All film samples were scanned in noncontact mode. A sharpened cantilever with a spring constant of 25 N/m was positioned over the samples, and 50 $\mu\text{m} \times 50 \mu\text{m}$ images were obtained. For each sample, data results were transformed into 3D images (Fabra et al., 2009). To make the results comparable, all the images were obtained from the center area of each surface. Dualscope/Rasterscope SPM software (Version 2.1.1.2) was used to calculate the roughness value of the films. Different roughness parameters can be measured by AFM. Some of them are reported in this study; the definitions of these parameters follow in Eqs. (3) to (5) (Khulbe et al., 2008).

S_a is the average roughness evaluated over the complete surface, and is defined as:

$$S_a = \int \int_a |Z(x, y)| \cdot dx \cdot dy \quad (3)$$

where Z is the height function of the area.

S_q is the root mean square (rms) roughness expressed as follows:

$$S_q = \sqrt{\int \int_a |Z(x, y)|^2 \cdot dx \cdot dy} \quad (4)$$

S_dq (root mean square gradient) defines the slope for each point of area excluding points on the edge and is calculated as follows:

$$S_dq = \sqrt{\frac{1}{A} \int_0^x \int_0^y \left(\left(\frac{\partial Z(x, y)}{\partial x} \right)^2 + \left(\frac{\partial Z(x, y)}{\partial y} \right)^2 \right) \cdot dx \cdot dy} \quad (5)$$

2.12. Statistical analysis

Statistics on a completely randomized design were performed with the analysis of variance (ANOVA) procedure using SAS (Version 9.1; Statistical Analysis System Institute Inc., Cary, NC, USA) software. Duncan's multiple range tests ($p < 0.05$) were used to detect differences among mean values of films properties.

3. Results and Discussion

3.1. Physical properties of films

The thickness of the composite films showed close thickness values, ranging from 0.055 to 0.060 mm with increase in CMC content (Table 1). However, a significant decrease ($p < 0.05$) could be observed between the thicknesses of the composite films and kefir film (100/0). This could be related to the differences in the molecular sizes and structure of these two biopolymers affecting the thickness of the composite films. All the films appeared clear, smooth, and transparent. As shown in Table 1, the combination of kefir with CMC increased the composite films' moisture content compared to kefir film. With the increase of the CMC content from 30 % to 50 %, the moisture content increased from 38.34 % to 43.05 %. Both kefir and CMC biopolymer are hydrophilic, but these results could be related to the lower water affinity of kefir compared to cellulose fibers.

The water resistance of edible films is an important property in food applications, especially when the film must be in contact with water or the water activity is high. However, in some cases the water solubility of films might be useful before product consumption; such as the encapsulation of food ingredients or additives (Bertuzzi et al., 2007; Mirzakhani et al., 2018). The water solubility of the kefir/CMC films as a function of CMC content is shown in Table 1. As can be seen, addition of CMC, at all levels, significantly increased the water solubility of the composite films ($p < 0.05$). The %TSM was 28.42 % for the samples without CMC, which increased to 33.60 % and 35.54 % for the films containing 30 and 50 % (v/v) CMC, respectively. As for the higher hydrophilicity of CMC, the higher solubility behavior of the composite films could be caused by the more open structure induced by the added CMC, making the composite film matrices more accessible to water compared to the plasticized kefir film. Also, plasticizer can diminish interactions between biopolymers molecules and increase solubility due to its hydrophilic nature, giving the polymers molecules higher affinity to attract water (Chick & Ustunol, 1998).

3.2. Water contact angle

Surface hydrophobicity was evaluated by means of contact angle determination. In this study, a significant decrease could be observed between the water contact angles of the composite films and kefir film (100/0). However, with increase in CMC amounts the decreasing contact angles value of the composite films was not significant ($p < 0.05$) (Table 1). These data indicate that the wettability of the composite films increased with an increase in the CMC content. This phenomenon was attributed to the higher hydrophobicity of kefiran polymer compared to CMC. These results agree with Ghasemlou et al. (2011) and other authors who reported that kefiran films have a higher contact angle with water,

suggesting a higher surface hydrophobicity compared to other polysaccharide-based films.

Table 1. Effect of CMC content on composite films' water contact angle and physical properties.^{a,b}

Films (Kefiran/CMC)	Thickness (mm)	Moisture content (%)	Solubility in water (%)	Water contact angle (degree)
100/0	0.074 ± 0.0045 ^a	33.14 ± 1.54 ^c	28.42 ± 1.12 ^c	96.71 ± 1.52 ^a
70/30	0.060 ± 0.0041 ^b	38.34 ± 2.11 ^b	33.60 ± 1.15 ^b	76.40 ± 1.54 ^b
50/50	0.058 ± 0.0058 ^b	43.05 ± 1.56 ^a	35.54 ± 0.95 ^b	74.92 ± 0.87 ^b
30/70	0.055 ± 0.0036 ^b	45.46 ± 1.22 ^a	39.39 ± 0.86 ^a	72.41 ± 1.12 ^c

^a Values with the same superscript letters within a column are not significantly different ($p < 0.05$).

^b Values were given as mean ± standard deviation

Table 2. Effect of various contents of CMC on the mechanical properties of kefiran-CMC composite films.^{a,b}

Films (Kefiran/CMC)	Tensile strength (MPa)	Elongation at break (%)
100/0	4.78 ± 1.13 ^a	56.83 ± 7.12 ^c
70/30	4.83 ± 1.65 ^a	73.93 ± 6.76 ^b
50/50	5.23 ± 1.72 ^a	95.08 ± 5.47 ^a
30/70	3.57 ± 0.84 ^a	67.59 ± 7.21 ^{bc}

^a Values with the same superscript letters within a column are not significantly different ($p < 0.05$).

^b Values were given as mean ± standard deviation.

Table 3. Mean values (and standard deviation) of roughness parameters obtained from atomic force microscopy images; S_a , S_q , and S_{dq} (four images were analyzed in each case).^a

Films (Kefiran/CMC)	Roughness Parameters		
	S_a (nm)	S_q (nm)	S_{dq}
100/0	194(19.1) ^c	241.3(24) ^c	0.159(0.016) ^c
70/30	323(34.7) ^b	382.8(42) ^b	0.178(0.021) ^b
50/50	374(40.2) ^b	425.5(49) ^b	0.181(0.024) ^b
30/70	501.4(56) ^a	576.5(65) ^a	0.191(0.002) ^a

^a Reported values correspond to the mean (standard deviation). Values within each column followed by different letters indicate significant differences ($P < 0.05$).

3.3. Water vapor permeability

WVP is one of the most important properties in edible films. The WVP of the kefiran/CMC films first decreased with the increase in CMC amounts from 0 to 50 %, and then significantly increased with further CMC addition ($p < 0.05$) (Fig. 1). The WVP was $3.13 \times 10^{-11} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$ for the kefiran film (100/0), which decreased to 2.86×10^{-11} and $2.79 \times 10^{-11} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$ for the films that contained 30 and 50 % CMC, respectively. The WVP maximum value ($3.56 \times 10^{-11} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$) occurred at the kefiran-to-CMC ratio of 30/70. These results suggest that at the kefiran-to-CMC ratio of 30/70, CMC does not disperse well enough in the kefiran-CMC matrix to block the water vapor. Also, due to the higher hydrophilicity of CMC compared to kefiran, the WVP of the composite film containing 70 % CMC increased. However, at lower CMC amounts, the hydroxyl and carboxyl group of CMC

can form strong hydrogen (Ghanbarzadeh et al., 2010) and ester bonds (Li et al., 2008) respectively with the active groups of kefiran. The interactions between kefiran and CMC molecules have the effect of preventing for water molecules from diffusing through the films, thus decreasing the WVP values. The different interaction intensities between CMC and kefiran were responsible for the differences. The decrease in WVP values by incorporation of fibers is in agreement with results usually reported for biocomposites which are studied for packaging applications (Arvanitoyannis & Biliaderis, 1999; Ma et al., 2008; Fama et al., 2009). Low WVP results in an improvement of the functional properties of the composite packaging film, especially in a highly humid environment, considering the hydrophilic characteristics of the matrix.

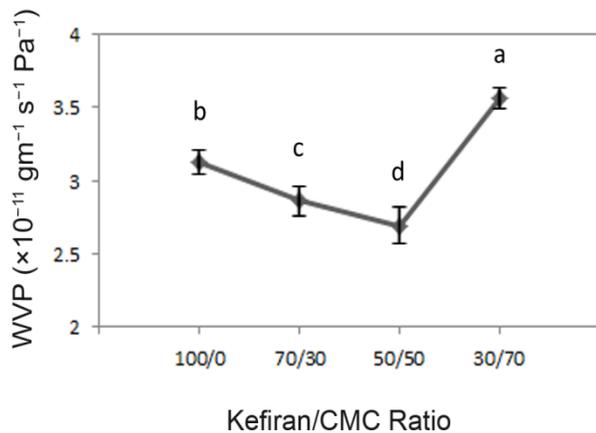


Fig. 1. WVP of the kefiran-CMC blended films as a function of CMC content. Different letters show significantly difference ($p < 0.05$).

3.4. Mechanical properties

TS, E, PD and PS were studied as the mechanical properties of the films. Freddi et al. (1995) observed that because polymer materials, such as films, may be subjected to various kinds of stress during use, the determination of the mechanical properties involves not only scientific but also technological and practical aspects. There is a wide range of reported data for the mechanical properties of hydrocolloid films in other studies; differences may be attributed to composition and structure of these biopolymers and suppliers, as well as film-preparation techniques. TS (MPa) and E (%) values of the kefiran/CMC composite films with different CMC ratios are shown in Table 2. As can be seen, the tensile strength of films increased from 4.78 to 5.23 MPa with the increase in CMC amounts from 0 to 50 %, and then decreased with further CMC addition. However, the TS values variations were not significant ($p < 0.05$). The mechanical properties of composite films depend on the intermolecular forces, chain stiffness, and molecular symmetry of the individual polymer used for blend or the constitutive properties obtained during blending process (Swapna et al., 2011). The increasing TS values of the composite films, with the CMC-to-kefiran ratio increasing from 0/100 to 50/50, are probably due to the formation of inter-molecular hydrogen bonds between the two main components and the plasticizer. With increasing CMC ratios in the film-forming solutions, the number of hydroxyl groups increased. Based on the results, the CMC to kefiran ratio of 50/50 was the critical value for the TS value of the composite films and showed the greatest integrity of the two main film-forming components. At the kefiran-to-CMC ratio of 30/70, the TS of the composite films decreased suddenly. The decrease in TS with increasing CMC ratio may occur because CMC intra-molecular hydrogen bonds rather than kefiran-CMC inter-molecular hydrogen bonds are formed, resulting in a phase separation between kefiran and CMC biopolymer. E and TS evaluations show collinear variations in the results, as shown in Table 2. The maximum value for E (95.08 %) occurred at the CMC-to-kefiran ratio of 50/50. As discussed above, CMC contains free hydroxyl groups that can form hydrogen bonds with kefiran biopolymer, and increase the flexibility of the film matrix. However, at the kefiran-to-CMC ratio

of 30/70, E decreased compared to lower ratios. This reduction showed that high levels of CMC weakened the polymer structure and then E value decreased. It seems that at low levels, CMC could improve the composite films' flexibility without reducing the tensile strength. The TS values of kefiran/CMC composite films (3.57–5.23 MPa) were lower than those of synthetic polymers such as LDPE (9–17 MPa), polystyrene (35–55 MPa) and cellophane (114 MPa). However, these composite films 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 as much as LDPE (500 %) (Smith, 1986). As shown in Fig. 2, there were no significant changes in PD values for different CMC contents. However, the PS values of the composite films first increased significantly and then decreased with increasing CMC content. As described earlier, this was due to the differences in molecular size and structure, the number of active hydroxyl groups and some other properties in the composite film matrix.

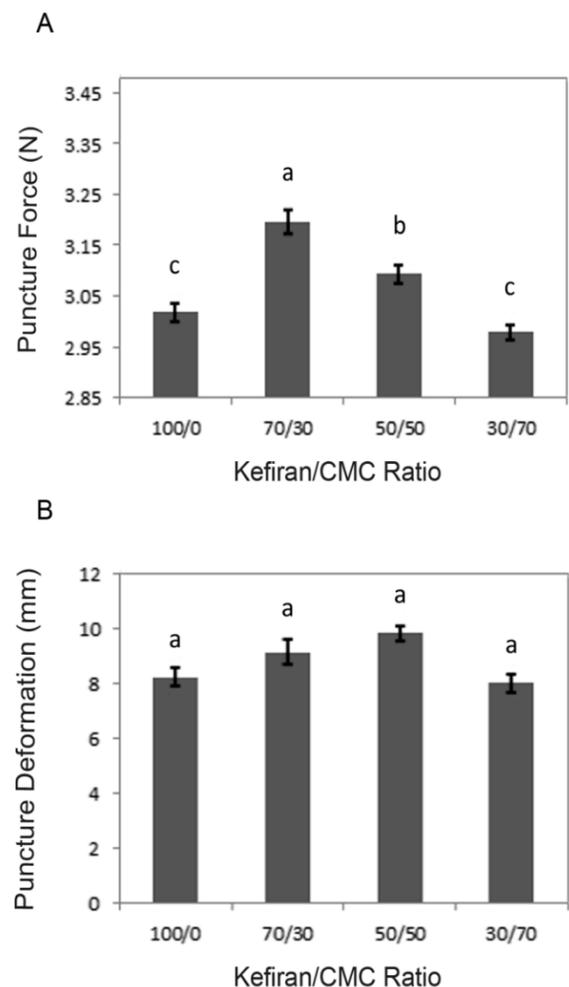


Fig. 2. Effect of CMC content on puncture properties of kefiran-CMC films: puncture force (A) and puncture deformation (B). Columns with the same letter are not significantly different ($p < 0.05$).

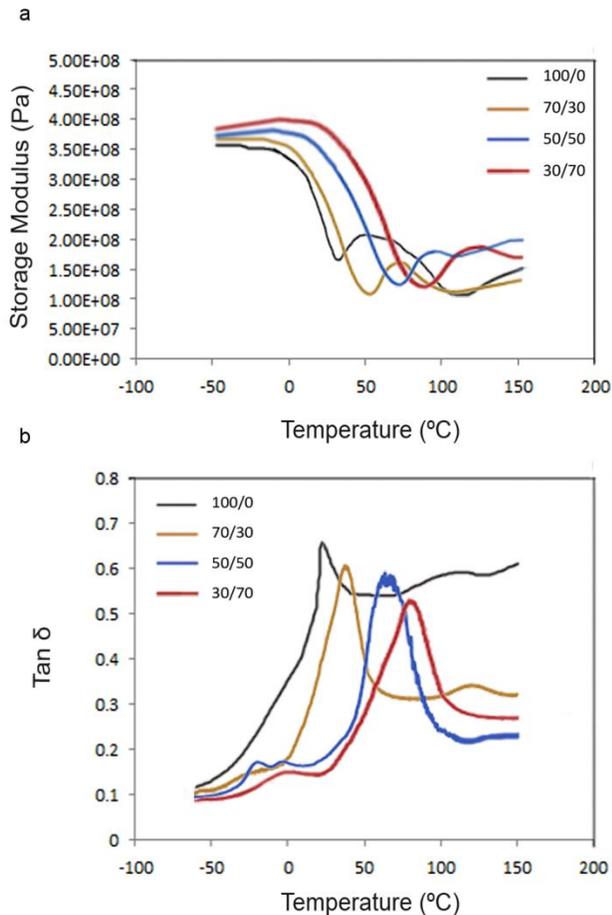


Fig. 3. Temperature dependence of storage modulus (E') (A) $\tan \delta$ (B) for kefiran/CMC composite films with various CMC content.

3.5. Dynamic mechanical thermal analysis

The dynamic storage modulus (E'), loss modulus (E''), and mechanical loss tangent ($\tan \delta = E''/E'$) with changes in temperature were measured by DMTA. Various researchers (Chen et al., 2007; Laaksonen & Roos, 2000; Mano et al., 2000) reported that DMA was very effective in evaluating the viscoelastic properties of biopolymers. The inflections in the E' and E'' temperature were accompanied by peaks in $\tan \delta$, which was the representation of a material molecular transition, such as the glass-transition temperature (T_g). T_g is the temperature indicating the relaxation in a polymer where a material changes from a glassy to a rubber state. The change in T_g is another effective indicator of the compatibility of polymers. The E' and the $\tan \delta$ curves of the kefiran/CMC composite films as a function of temperature are shown in Fig. 3a and Fig. 3b, respectively. T_g were observed in

kefiran/CMC polymer blend films in the range of 34.6 °C (100/0) to 91 °C (30/70) depending on CMC content. Adding CMC to kefiran/CMC films showed an increase in T_g for all ratios (100/0, 70/30, 50/50 and 30/70), respectively. These results could be attributed to increases in the mean molecular weight of polymers in amorphous regions of the composite matrix. By adding CMC to kefiran, the functional group of kefiran forms interpenetrating polymer networks with the CMC matrix that can be believed to cause a change in the polymer structure. The molecular structure of the polymer profoundly affects the T_g (Aklonis & MacKnight, 1983). DMTA curves indicated partial miscibility of kefiran and CMC in the amorphous phase at the molecular level for any given compositional ratio, and both biopolymers remained a homogeneous material, because phase separation (separated glass-transition temperatures) between two biopolymers in composite films was not observed. If two different polymers or blend of polymer and plasticizers are immiscible, the mixture will show two T_g corresponding to the two pure phases. Su et al. (2010) also reported that normally, a single T_g for blended polymers in a DMTA curve indicates good compatibility of the component polymers.

3.6. Film microstructure

The morphology of composite films is a very important characteristic, as it ultimately determines many properties of biodegradable materials. The representative electron scanning micrographs of the outer surfaces and cross-sections of kefiran/CMC composite films plasticized with glycerol are shown in Fig. 4. As can be seen, the surface and cross-section of kefiran film are relatively smooth, homogeneous and free of air bubbles, and represent a continuous matrix without any pores or cracks, and with good structural integrity. However, with increasing CMC content, the micrographs of the films showed different morphologies. The 70/30 and 50/50 kefiran-to-CMC ratio showed a compact structure and no pores can be seen in the film matrix. As more CMC was added to the composite, the micrographs exhibited rougher matrix morphologies. A continuous surface with porous structure can be seen in the 30/70 kefiran-to-CMC ratio where small pores and irregular cavities are observed on the film's surface and cross-section. That could be related to the formation of channels and this may be because at this level, CMC does not disperse well enough in the kefiran-CMC matrix. Also, the pores present in the microstructure of film with 70 % CMC can act as stress concentration factor and therefore lower the mechanical properties of the composite film. This is in regard with the mechanical tests results in this work that showed a significant decrease in the mechanical properties of the composite film in the 30/70 kefiran-to-CMC ratio. Similar results were reported for the surface and cross-section micrographs of starch-chitosan composites (Salleh & Muhamad, 2007). Overall, the kefiran/CMC composite blends were flat and compact with very small pores in high CMC content, and without any phase separation.

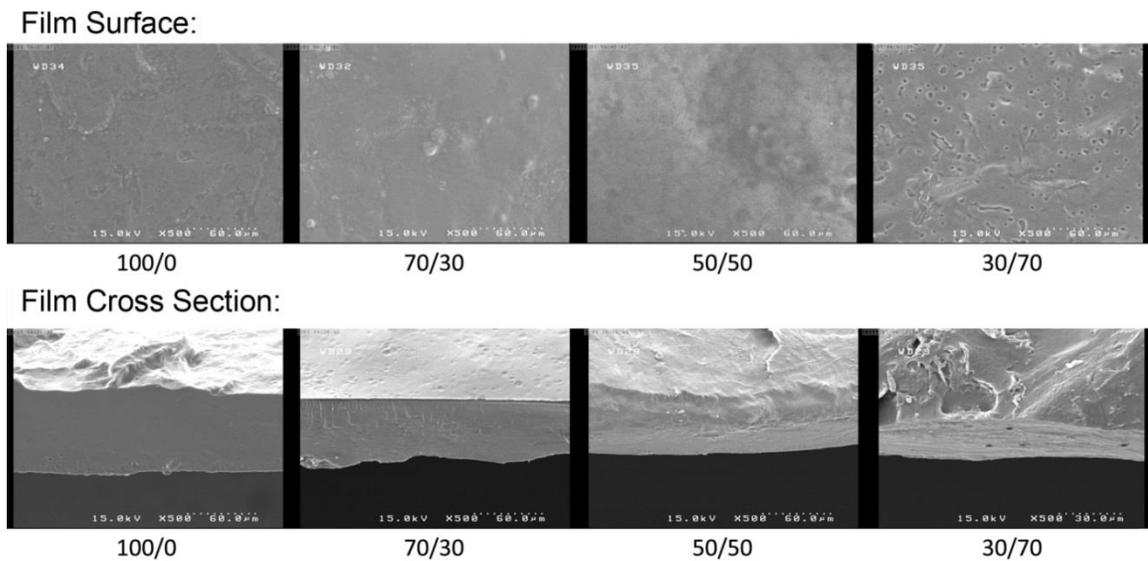


Fig. 4. Surface and cross-section morphologies of kefiran/CMC blended films viewed at a magnification of 500 \times .

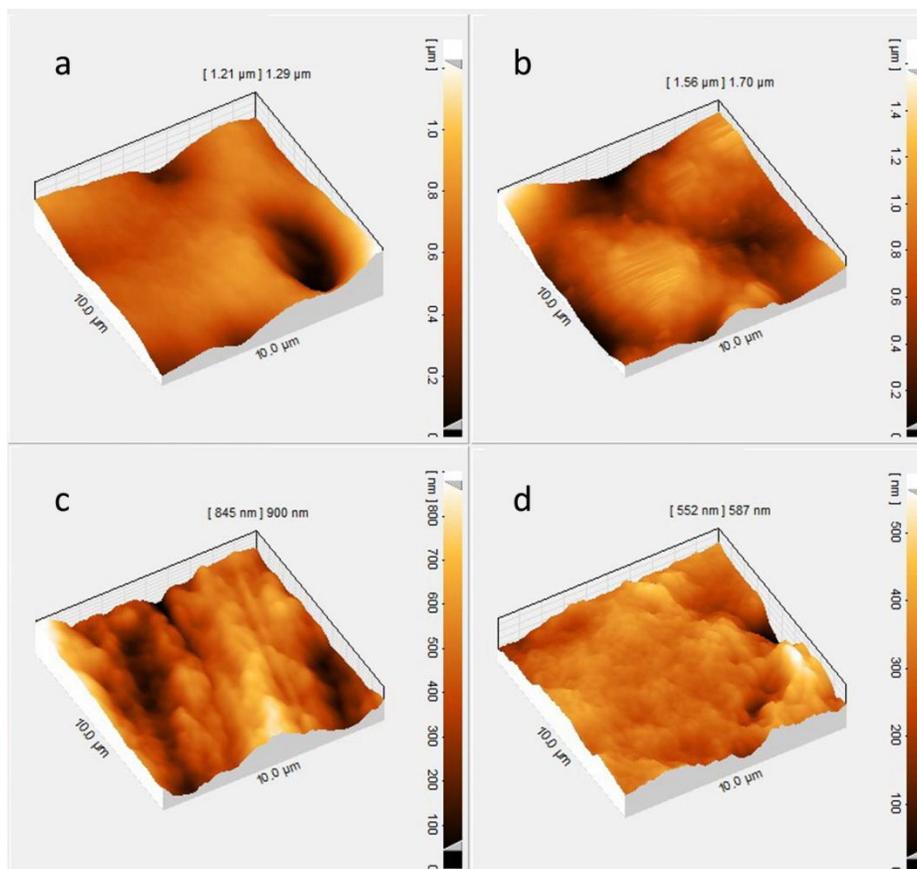


Fig. 5. AFM topographic images of kefiran/CMC composite films (a) kefiran/CMC ratio of 100/0 (v/v) (plasticized kefiran film), (b) kefiran/CMC ratio of 70/30 (v/v), (c) kefiran/CMC ratio of 50/50 (v/v), and (d) kefiran/CMC ratio of 30/70 (v/v).

3.7. Atomic force microscopy

AFM is a powerful tool to study surfaces. It has been used to provide qualitative and quantitative information about biopolymers at the nanometer scale that could not usually be obtained by any other experimental technique. Previously, it has been used to study isolate edible films (Bergo et al., 2010).

3D images of kefiran/CMC films with various ratios of CMC are shown in Fig. 5; Table 3 shows the corresponding results of roughness parameters. The kefiran film (Fig. 5a) presented the smoothest surface, showing an S_a roughness of approximately 194 nm. The roughness of films' surfaces increased significantly as CMC was incorporated ($P < 0.05$). According to the AFM images, kefiran/CMC films with 50 % CMC were rougher than those with 30 % CMC. However, a comparison of roughness parameters of these films did not show a significant difference ($P < 0.05$) between them (Table 3). At a CMC content of 70 %, the composite film showed a rougher film surface than the two previous films examined, and roughness parameters increased significantly ($P < 0.05$). In this study, all the parameters showed a sharp increase ($P < 0.05$) when CMC was added to the film, compared to the control film (plasticized kefiran film). The increase in the surface roughness was principally due to the mixture of CMC in the composite films' structures.

4. Conclusions

The results of this study showed that kefiran and CMC could be easily blended together to form a new edible biocomposite film. The physical, mechanical and barrier properties, which are important in food packaging applications, were examined as a function of CMC content. Increasing amounts of CMC resulted in increases in the water solubility and decreases in the water contact angle of the composite films. Increasing CMC content from 0 to 50 % decreased the films' WVP, but with additional CMC the WVP increased. Changing the ratio of two polymers can modify the strength and extensibility of the composite films. The TS and E of the films increased by increasing CMC content from 0 to 50 % (v/v), but decreased at higher CMC content. It can be concluded that when added to kefiran, CMC could improve the composite films' flexibility without reducing effect on tensile strength. DMTA curves revealed a single T_g which maybe an indication of the compatibility of CMC and kefiran polymers in this study. The glass-transition temperature (T_g) shifted towards higher temperatures with increasing amounts of CMC. AFM images indicated that the surface roughness of the films was affected by the addition of CMC; the maximum surface roughness occurred at the ratio of 30/70 in the composite kefiran/CMC film. Based on the microstructures observed in SEM analysis, the micrograph for the two-component film was homogeneous without signs of phase separation between the components. It can be concluded that these two film-forming components were compatible and an interaction existed between them. By considering these results, it seems that Kefiran-CMC biocomposite films show good physical-mechanical properties and have the potential to replace conventional packaging.

Acknowledgements

The authors wish to express their gratitude to University of Tehran for supporting the facilities for this research work and technical assistance.

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