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Original Article

Novel Kefiran-Polyvinyl Alcohol Composite Film: Physical, Mechanical and Rheological Properties

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ABSTRACT

Background and Objectives: Kefiran (Kef) is a water-soluble polysaccharide that can form transparent film; however, it is brittle. Therefore, in order to improve the mechanical properties of kefiran film, a mixture with other polymers can be offered. The expansion of mixed systems can propose kefiran and polyvinyl alcohol (PVOH) as a new composite film.

Materials and Methods: Solutions of 20 gram per liter kef and 40 gram per liter of PVOH were prepared. A mixture of film-forming solutions of different ratios of Kef/PVOH (100/0, 68/32, 50/50, 32/68) was prepared. In this study, different experiments including the physical properties (thickness, moisture content, and film solubility in water), water vapor permeability, and mechanical properties (tensile strength, elongation at break, puncture strength, and puncture deformation) of composite films as well as the rheological properties of film forming solutions were investigated.

Results: The results of physical properties such as thickness, moisture content, solubility in water and mechanical properties such as tensile strength, elongation at break, puncture deformation, puncture strength and water vapor permeability indicated that the mechanical properties, thickness and solubility in water increase with increase in PVOH content; however, moisture content and water vapor permeability decrease. Rheological characterization of different film forming solutions exhibited Newtonian fluid behavior.

Conclusions: These results contribute to the establishment of an approach to optimize films' composition based on the interactions between polymers, aiming at improving the properties of polysaccharide-based films.

Keywords: Kefiran, Polyvinyl alcohol, Physical properties, Mechanical properties, Rheological properties

Introduction

Films are thin layers of materials consisting of polymers that are able to provide mechanical strength with the thin structure (1). Due to the problems associated with the disposal of packaging plastics, there is a growing interest toward the development of biodegradable materials (2). Some microorganisms such as lactic acid bacteria excrete polysaccharides of high molecular weight. These exopolysaccharides are important because they can impart functional properties to food and confer beneficial health effects. Lactic acid bacteria are generally recognized as safe, and exopolysaccharides isolated from them offer an alternative source of microbial polysaccharides for wider use in food formulations. The polysaccharide of kefir, named kefiran, is a water-soluble glucogalactan, which has been reported to have antibacterial and therapeutic activities (3). Kefiran can form transparent films with low opacity, though they have weak mechanical properties (4). Numerous biopolymers have been used to develop biocompatibility of food packaging materials. Usually, films based on biopolymers are highly

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sensitive to the environmental conditions, and generally, present low mechanical resistance. As a result, several studies have been carried out to progress films based on mixtures of biopolymers and synthetic polymers (5). Different biodegradable polymers have been explored for the development of edible films as an effort to reduce packaging waste created by non-degradable petroleum materials (6). PVOH is one of the degradable synthetic polymers with good draw ability as it can be combined with other polymers or fillers to enhance its properties (7). Because of its non-toxicity, biocompatibility, excellent chemical resistance and mechanical strength (8), PVOH has been widely used in biomedical applications such as Starch-PVOH (9), chitosan-PVOH (10) and Bacterial cellulose-PVOH (11). Viscosity is the internal friction that tends to resist the sliding of one element of fluid over another (12). Viscometric method is inexpensive and rapid, and can be used with fairly good accuracy to determine the miscibility of polymer blends (13). The mechanical properties of a polymer describe how it responds to deforming forces. Polymeric item can be considered as a collection of viscous and elastic sub-components. When a deforming force is applied, the elastic elements are reversibly deformed, while the viscous elements flow. The balance between the proportional of different components controls the overall properties (14). To develop biodegradable materials in terms of environmental protection and ecosystem, the enhancement of mechanical properties of kefiran film is very significant for their utility as industrial materials. To pursue the extensive utilities of kefiran, the mixture of kefiran with PVOH is prepared to promote an increase in the Young’s modulus. Block copolymers of kef and PVOH have emerged as one of the highly promising biodegradable materials due to their highly controllable chemical and physical properties. In general, kefiran film suffers from poor mechanical properties and they can be modified by blending with other Biopolymers or synthetic biodegradable polymers. This study is aimed to improve the mechanical structure of kefiran film by blending with PVOH, and generate new biodegradable films.

Materials and Methods

Materials: Polyvinyl alcohol (PVOH) was purchased from Sigma Aldrich (USA), food grade glycerol, magnesium nitrate, sodium chloride and silica gel were purchased from Merck (Germany), and Kefir grains, used as a starter culture in this study, were obtained from a laboratory in Gorgan University of Agricultural Sciences and Natural Resources, Iran.

Isolation and purification of kefiran: The grains were kept in skimmed milk at room temperature (20-22 ◦C) for short periods, and the medium was exchanged daily for the grains’ viability to be maintained fresh. If the culture was continued for seven subsequent days, the grains were considered active. A weighed amount of kefir grains was treated in boiling water (1:10) for 30 min with discontinuous stirring. The mixture was centrifuged at 10,000g for 20 min at 20 ◦C (Combi 514R. centrifuge, Hanil Science Industrial). The polysaccharide in the supernatant was precipitated by adding two volumes of cold ethanol and left at -20 ◦C overnight. The mixture was centrifuged at 10,000g for 20 min at 4 ◦C. The pellets were dissolved in hot water, and the precipitation procedure according to Rimada and Abraham (2006) with slight modification was repeated thrice (instead of twice) to obtain pure product. The precipitate was finally dissolved in hot distilled water and freeze-dried (15).

Preparation of films: PVOH film solution was prepared by dispersing 40 g of PVOH powder in 1000 mL distilled water (95±3 ◦C) under stirring conditions until the powder was completely dissolved in distilled water. The solution was then cooled down to room temperature. Kefiran film solution was prepared by 20 gram per liter of kefiran powder in distilled water (85±3 ◦C) under stirring conditions until the powder was completely dissolved in distilled water. The solution was then cooled down to room temperature (16). Kef/PVOH composite films were prepared by mixing various levels of 20 gram per liter of kefiran solutions with various levels of 40 gram per liter of PVOH solutions (100/0, 68/32, 50/50, 32/68). Glycerol at ratio of 1-4 dry powder was added to the film solution. Plasticizers are nonvolatile high boiling point, non-separating substance. They must be miscible with polymer, indicating similar intermolecular forces between the components (17). The chemical characteristics of plasticizers should be identified to verify their chemical compatibility with biopolymers, and determine the changes in film structure caused by the addition of plasticizers (1).

Following the addition of plasticizer, stirring was continued for a further 15 min. The film-forming solutions were subsequently degassed to remove air bubbles using a sonicator (Ultrasonic Processor, Cole-Parmer, Vernon Hills, Illinois, USA). 15 ml portions of each film forming solution were cast on a petri dish and dried at oven at temperature of 30◦C. The dried films were peeled and stored in plastic bags in desiccators at 25±2 ◦C for further testing.

The obtained films were conditioned in an environmental chamber at magnesium nitrate and 50% relative humidity (RH) using saturated magnesium nitrate for 48 h prior to test. All property measurements were performed immediately after removing the film specimens from the chamber to minimize their moisture variances.

Determination of physical properties of films

Film thickness: Film thickness was measured with a manual micrometer (Mitutoyo, Japan) having a sensitivity of 0.001 mm. Ten thickness measurements were taken on each test sample in different randomly chosen points and the mean values were used in permeability and water vapor permeability test calculations. Three replications of each film treatment were used for calculating the film thickness.

Moisture content: The films' moisture content (approximately 1 cm×3 cm) was determined by measuring their weight loss before and after drying in a laboratory oven at 103±2 ◦C until constant weight was reached (dry sample weight). Three replications of each film treatment were used to calculate the moisture content.

Film solubility in water: The film solubility in water was determined according to the method reported by Gontard et al. (1994), it was defined it as the percentage of the total soluble matter (%TSM) of film that is solubilized after 24 h immersion in water (19). The initial dry matter content (W1) of each film was determined by drying it to constant weight in an oven at 105 ◦C. Two disks of film (2 cm diameter) were cut, weighed, and immersed in 50 mL of water. After 24 h of immersion at 20 ◦C with occasional agitation, the pieces of film were taken out and dried to constant weight (W2) in an oven at 105 ◦C, to determine the weight of dry matter, which was not solubilized in water (Eq. 1):

$$\%TSM = \frac{W1-W2}{W1} \times 100 \qquad \text{Eq. (1)}$$

Solubility-in-water tests were carried out for each type of film treatment in three replicates.

Water vapor permeability: Water vapor transmission rate of the films was determined following the ASTM standard test method (20). The film samples, previously equilibrated at 50% RH for 48h, were sealed to glass cups (with 3 cm internal diameter, 4 cm outer diameter and a depth of 5 cm containing silica gel). Films were sealed to the cups using melted paraffin. Each cup was placed in a desiccator maintained at 25 ◦C and relative humidity of 75% with a saturated solution of sodium chloride. The driving force, expressed as water vapor partial pressure, was 1753.55 Pascal. The cups were weighed periodically using an analytical balance (Satorius GCA8035 CTE3135) until steady state was reached (±0.0001g). The water vapor transmission rate (g m^{-2} d^{-1}) of the films (Eq. 2) was determined from the slope obtained from the regression analysis of moisture weight gain (ΔW) transferred through the film area (A) during a definite time (Δt); once the steady state was reached, the water vapor transmission rate of the films was used to calculate the water vapor permeability coefficient using Eq. (3). At least 3 replications of each film treatment were tested for water vapor permeability:

$$\text{Water vapor transmission rate} = \frac{\Delta W}{A\,\Delta t} \qquad \text{Eq. (2)}$$

$$\text{Water vapor permeability} = \frac{\Delta m}{\Delta t} \times \frac{X}{\Delta P} \qquad \text{Eq. (3)}$$

Where water vapor permeability is (g mm m^{-2} d^{-1} kPa^{-1}), x is the film thickness, and Δp is the partial water vapor pressure gradient between the inner (p1) and outer (p2) surfaces of the film in the chamber.

Mechanical properties: Mechanical properties were measured by using a Texture Analyser TA-XT-plus (Stable Micro Systems, Surrey, UK) with a 50 N load cell equipped with tensile grips (A/TG model). Sample films were cut into 25 mm wide and 100mm long strips according to the ASTM D-882 standard (21). Grip separation was set at 50 mm with a cross-head speed of 50 mm/min. Tensile strength and percentage of elongation at break (%) were evaluated in 9 samples from each type of film. The tensile strength (Eq. (4)) was calculated by dividing the maximum load (F_{max}) by the initial cross-sectional area (∅) of the film sample expressed as MPa. Elongation at break (Eq. (5)) was calculated as the ratio of the film extension (ΔL) at the point of sample rupture to the initial length (I_0) of the sample and expressed as: percentage.

$$\text{Tensile strength} = \frac{\text{Fmax}}{\emptyset} \qquad \text{Eq. (4)}$$

$$\text{Elongation at break (\%)} = \frac{\Delta L}{I_0} \times 100 \qquad \text{Eq. (5)}$$

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 (2 mm diameter) was moved perpendicularly onto the film surface at a cross-head speed of 50 mm/min until the film was broken. Force–deformation data collected by a microcomputer was used to determine the puncture strength and puncture deformation of the film at rupture. Puncture tests for each type of film were carried out in triplicate.

Rheological characterization of film forming solutions: Rheology is a branch of physics concerned with the composition and structure of flowing and deformable materials. In order to determine the effect of blending on the rheological behavior of film solution before casting, viscosities of the solution films were determined using a rotational viscometer (Model RVDV-II, Brookfield, Inc. USA); this viscometer measures multiple factors such as shear rate, shear stress and viscosity automatically. The solution samples were loaded into the coaxial cylindrical chamber (16 ml capacity; ULA-31Y, Brookfield, Inc. USA) for all experiments, and were allowed to equilibrate at the desired temperature using a circulating water jacket (Model ULA-40Y, Brookfield, Inc. USA) at 27◦C. Spindle YULA-15 with six different speeds (30, 40, 50, 60, 70 and 80 rpm) was used to determine the film solutions' viscosity.

Various mathematical models have been proposed for the fluids; one of the most complete models is Herschel Bulkley model:

$$\tau = k\dot{\gamma}^n + \tau_0 \qquad \text{Eq. (6)}$$

Where, τ, is the shear stress (Pa), k, is the consistency index (pa.s^n), γ is the shear rate (s^{-1}), τ_0 is yield stress, and *n* is the flow index (dimensionless).

Rheology measurement for each type of film forming solution was carried out in three replicates.

Scanning electron microscopy (SEM): Scanning electron microscopy (SEM) images of surface were taken using an Ultra-high Resolution Analytical Scanning Electron Microscope (EM-3200) operating at 20-24 kV.

Statistical analysis: Statistics on a completely randomized design (CRD) was performed with the analysis of variance (ANOVA) procedure using SAS software (Version 9.1; Statistical Analysis System Institute Inc., Cary, NC, USA). Duncan tests were used to compare the difference among the mean values the film properties at the level of 0.05.

Results

Physical properties and water vapor permeability of films: With increase in PVOH content, the thickness of composite films was increased due to the increase of dry matter film solution d. Thickness ranged between 0.030-0.034 mm (Table 1). Although the PVOH solution had double concentration of kef film, but there was no significant (P< 0.05) difference between the thicknesses of Kef/PVOH films with increase in its PVOH content.

Table 1. Effect of different concentrations of kefiran and PVOH on the physical and water vapor permeability of the composite films

Kef/ PVOH (%w/w)	Thickness (mm)	Moisture content (%)	Solubility in water (%)	Water vapor permeability ($\times 10^{-11}$gm^{-1}pa^{-1})
100/0	0. 030±0.05^a	0. 034±0.05^a	5.9±0.123^a	2.135±0.018^a
68/32	0.0 31±0.0922^a	0. 032±0.0929^a	7.83±0.029^b	2.056±0.024^b
50/50	0. 032±0.0929^a	0.0 31±0.0922^a	11.66±0. 18^c	1.997±0.044^c
32/68	0. 034±0.05^a	0. 030±0.05^a	21.18±0.16^d	1.937±0.011^d

Values are given as mean ± standard deviation. Different letters in the same column indicate significant differences (*P*< 0.05).
All experiments were performed in triplicate.
Kef/ PVOH: Kefiran/Polyvinyl alcohol

These results indicate that the combination of kefiran with PVOH decreased the film’s moisture content. The control film had the highest moisture content (0.034±0.05). Although the moisture content decreased as the content of PVOH increased; however, it had no significant ($P< 0.05$) effect on moisture content. As shown in Table 1, addition of PVOH at all concentration levels significantly increased the solubility of composite films in water. Table 1 further reveals that the control film has higher water permeability values than the films obtained from different proportions of PVOH and kefiran. Higher PVOH content of Kef/ PVOH (32/68) showed higher barrier water vapor transfer rate.

Mechanical properties: Tensile strength and elongation at break are presented here to characterize the mechanical properties of the Kef/PVOH composite films. Tensile strength is the maximum tension supported by the film until it collapses. Elongation at break is a measure of the flexibility of the film, and can be considered as a characteristic that defines the ability of the film’s deformation until it collapses (22). As shown in Table 2, the tensile strength and elongation at break values of kef/PVOH composite films increase with increase in PVOH ratios, and higher tensile strength and elongation at break are attributed to the higher content of PVOH (Kef 32/PVOH 68). Puncture strength is a measure of the maximum force or energy required to penetrate into a material, and puncture deformation is a measure of deformation required to penetrate into a material. As can be seen in Table 2, the puncture strength and puncture deformation values of the kef/ PVOH composite films increase with increase in PVOH ratios.

Table 2. Effect of different concentrations of kefiran and PVOH on the physical and mechanical properties and water vapor permeability of the composite films

Kef/ PVOH (%w/w)	Tensile strength (Mpa)	Elongation at break (%)	Puncture strength (N)	Puncture deformation (mm)
100/0	0.71 ± 0.014^{a}	59.77 ± 2.76^{a}	2.166 ± 0.0018^{a}	7.214 ± 0.11^{a}
68/32	0.92 ± 0.39^{b}	92.31 ± 1.27^{b}	5.32 ± 0.001^{b}	7.55 ± 0.0138^{b}
50/50	1.49 ± 0.464^{c}	192.35 ± 1.07^{c}	5.899 ± 0.24^{c}	9.15 ± 0.172^{bc}
32/68	2.13 ± 0.475^{d}	272.03 ± 4.54^{d}	13.82 ± 0.054^{d}	13.73 ± 0.05^{c}

Values are given as mean ± standard deviation. Different letters in the same column indicate significant differences ($P< 0.05$).
Nine replications of each film treatment were used for calculating the tensile strength and elongation at break, and three replication of each film treatment were used for calculating the puncture strength and puncture deformation.
Kef/ PVOH: Kefiran/Polyvinyl alcohol

Rheological characterization of film forming solutions**:** Viscosity measurement of different proportions of solution films showed Newtonian fluid behavior; this is in contrast to our result previous research (4) showing that Kefiran film solution exhibited a pseudoplastic behavior. As shown in Figure 1, with increase in PVOH content, viscosity of the film solutions increases. Also based on the results of Hershel-bulkly model (Table 3), since the obtained shear stress and fluid index are near to one, it can be concluded that fluid behavior was Newtonian in the experiment temperature (27◦C). Herschel Bulkley model fitted satisfactorily the experimental data being the correlation coefficient higher than 0.9988 in all cases. As shown in Table 3, increasing the PVOH content to Kef/ PVOH (50/50) had no significant (P< 0.05) effect on the K parameters, but in Kef/ PVOH (32/68), the K parameters experienced significant (P< 0.05) increase. The results showed that PVOH addition had no significant effect on the n parameters (Table 3).

Scanning electron microscopy: The morphology of composite films is a very important characteristic as it ultimately determines many properties of biodegradable materials. Surface section morphology of different Kef/PVOH composite films was evaluated using the SEM micrograph. The control film images revealed a porous structure. As shown in Figure 2, the microstructures obtained by the SEM images for the blended film indicate that the increase of PVOH content does not cause discontinuous or porous structures.

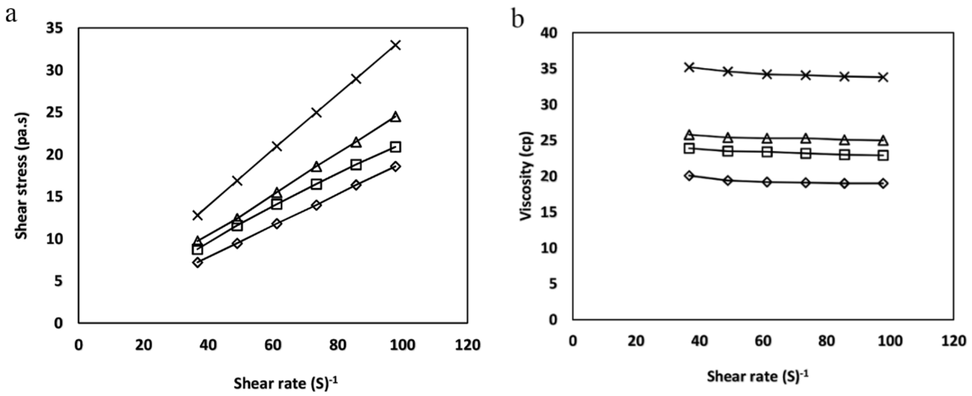

Figure 1. Rheograms of different proportional of Kef/ PVOH solution films: curves' shear stress to shear rate (a), curves' viscosity to shear rate (b).
—◇— (100/0), —□— (68/32), —△— (50/50) and —×— (32/68).

Table 3. The parameters related to Herschel- Bulkley model to different blended films

Kef/ PVOH (%w/w)	K (pa.s^n)	n	τ_0 (Pa)	R^2
100/0	1.5449^a	0.8934^a	1.1897^b	0.9988
68/32	1.0169^a	1.0708^a	1.2491^b	0.9996
50/50	1.8127^a	0.9925^a	5.4212^c	0.9999
32/68	4.8274^b	1.0291^a	0.0313^a	0.9999

Different letters in the same column indicate significant differences ($P < 0.05$).
Where,k, is the consistency index (pa.s^n),n is the flow index, τ_0, is the yield stress (Pa), R^2 is the correlation coefficient.
All experiments were performed in triplicate.
Kef/ PVOH: Kefiran/Polyvinyl alcohol

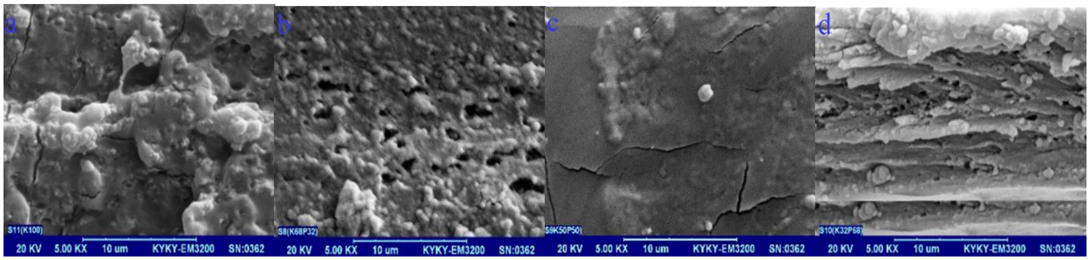

Figure 2. Surface morphology of Kef/PVOH blended films viewed at a magnification of 1000 x. a (100/0), b (68/32), c (50/50) and d (32/68).
Kef/ PVOH: Kefiran/Polyvinyl alcohol

Discussion

In this study, with increase in PVOH content, the thickness of composite films was increased; this could be related to hydrogen bond formation between kef and PVOH that can lead to more closely packed matrices though the PVOH concentration was doubled (24). Both the control and blend films formed homogeneous structure. These results indicate that the combination of kef with PVOH decreased the film's moisture content. Both kef and PVOH polymers are hydrophilic; however, kef hydrophobicity is lower than that of PVOH, which could be a reason for the decrease in moisture content.

Solubility in water of the composite films increased with increase in PVOH content; this is the reason why PVOH is more readily disintegrated by absorbing water. Motedayen et al. (2013) reported that the solubility of kefiran-starch blend film in water increased with increase in its starch content (25). Low solubility in water is important when films are in contact with water during the processing and storage. The opposite is desirable when the intent is to design a package with premeasured dry food amounts to be dissolved in water or in hot food (26).

The results of water vapor permeability showed that with increase of PVOH content in composite films, water vapor transmission rate decreased. Hydrogen bond interactions between Kef and PVOH reduce the availability of the hydrophilic groups, diminishing their interactions with water molecules, and consequently water vapor transmission rate (WVTR) (27). It was not possible to observe the plasticizer's effect on the water vapor permeability (28).

Mechanical properties: Increase in the mechanical properties of composite films with increase in PVOH ratio is probably due to the formation of inter-molecular hydrogen bonds between the two main components. It is evident from the values of the mechanical parameters of kefiran and PVOH that the cohesion forces in the PVOH matrix are more intense than in the kefiran matrix as a result of the higher intermolecular forces. Glycerol as a plasticizer increased with increase in PVOH content, because of its ability to reduce internal hydrogen bonding; while the increasing flexibility of polymer films by decreasing the attractive intermolecular forces along the polymer chains glycerol increases the chains' mobility (29).

Rheological characterization of film forming solutions: As shown in Figure 1, viscosity measurement of different proportions the solution films showed Newtonian fluid behavior. In contrast to our results, Piermaria et al. (2011) reported that kef film solution exhibited a pseudoplastic behavior, probably because they used rheometer for measuring the rheological behavior of the film solution (4). Viscometer is highly suitable for quality control testing and for on-line process control, whereas rheometer is essential for the true simulation of real processes and complete material characterization. At very high shear rates, there are several additional factors such as inertial effect, and viscous heating that impact rheometric measurements (29). At high rotation rates, centrifugal stresses may become large enough to overcome the surface tension stresses that hold the liquid between the plates resulting in liquid being thrown out of the gap; a phenomenon termed euphemistically the 'radial migration effect' (30). Studying the viscosity of solution film is important because when the viscosity is high, homogenization of the film solution (4) and removing their air bubbles (23) are difficult. It is clearly seen from Figure 1 that the viscosity of Kef/PVOH film forming solutions increases with increasing PVOH content. The obtained viscometric results showed that Kef/PVOH blends are miscible in the range of the studied concentrations. The increase in viscosity depends on several factors such as temperature, solvent-polymer interaction, polymer concentration, and the size of the polymer molecules (31). Increase in the viscosity of film blend solutions with increase in the PVOH content is due to the increase in the polymer concentration of PVOH.

Scanning electron microscopy: Study of film microstructure is critical to determine barriers and mechanical properties (32). It is clearly seen from the SEM micrographs that both surfaces of the Kef/PVOH composite films are smooth and compact with very small particles in the films of high PVOH content without any phase separation and thus increase in the mechanical and water vapor barrier properties of the composite films.

The results provided beneficial information on the structural properties of kefiran and PVOH composite films, and structural changes were created in the films' network when different ratios of the two polymers were mixed. The synergistic effect between the two polymers, explained by miscibility between kefiran and PVOH, is one of the main reasons for the differences observed in the films' properties. These results contribute to the establishment of an approach to optimize films' composition based on the interactions between polymers, aiming at improving the properties of polysaccharide-based films. The physical, mechanical, water vapor permeability, rheological film solution and optical properties are significant in food packaging applications. Increase in PVOH content significantly increased the mechanical properties, solubility in water and viscosity of the solution but decreased the water vapor permeability and moisture content of the composite film. Measuring the viscosity of the solution film showed Newtonian fluid behavior. These results suggest that the two film-forming components were compatible and that there was an interaction between them.

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