



Enhancing structural properties and antioxidant activity of kefiran films by chitosan addition



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ABSTRACT

Film-forming solutions of different ratios of kefiran to chitosan (100/0, 68/32, 50/50 and 32/68) were cast at oven in 30 °C. The effects of chitosan addition on the resulting films' physical, mechanical, water vapor permeability properties and antioxidant activity were investigated. Increasing chitosan content to composite films significantly ($P < 0.05$) decreased moisture content, solubility in water and water vapor permeability, but significantly ($P < 0.05$) increased tensile strength, elongation at break, puncture strength, puncture deformation and scavenging ability on 1,1-diphenyl-2-picrylhydrazyl radicals. Results related to optical properties of surface composite films showed that with increasing chitosan content, opacity degree, a and b values significantly ($P < 0.05$) increase, but L values significantly ($P < 0.05$) decreases.

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1. Introduction

Films are generally defined as stand-alone thin layers of materials. They are usually consisted of polymers able to provide mechanical strength to the stand-alone thin structure (Hun & Cennadios, 2005). Chemically synthesized polymeric films have long been used in the food packaging industry due to their desirable features such as lightness, softness and transparency. However, their negative environmental impact caused by their total non-biodegradability is a serious disadvantage in using these materials (Motedayen, Khodaiyan, & Salehi, 2013). The major goals of a conventional packaging system include safety assurance, quality maintenance and shelf-life extension (Jin & Zhang, 2008). Since edible films are both a packaging and a food component, they have to satisfy certain requirements, such as good sensory qualities, mechanical efficiencies, enough biochemical, physical-chemical, microbial stability, being free of toxic substances, safe for health and simple technology. Edible films with good mechanical properties can replace synthetic packaging films (Debeaufort, Quezada-Gallo, & Voilley, 1998; Saremnezhad, Azizi, Barzegar, Abbasi, & Ahmadi, 2011).

Kefir is defined as cultured milk containing a variety of an original native starter (kefir grain) that contain both lactic acid bacteria and yeasts. Secondary metabolism of lactic acid bacteria of

kefir produces a polysaccharide matrix which surrounds kefir grains. Lactic acid bacteria are generally recognized as safe and exopolysaccharides isolated from them named kefiran are a water-soluble glucogalactan that have been reported as antibacterial with therapeutical activity (Laws & Marshall, 2001; Rodrigues, Caputo, Carvalho, Evangelista, & Schneedorf, 2005). Kefiran can form transparent films, in spite of its brittleness and rigidity (Piermaria et al., 2011). Chitin is a polysaccharide composed of N-acetyl-D-glucosamine units. It is the second abundant biopolymer on earth and is found mainly in invertebrates, insects, marine diatoms, algae and fungi. Chitin is converted to chitosan by deacetylation in concentrated alkali solution (Maghsoudlou, Maghsoudlou, Khomeiri, & Ghorbani, 2012). Chitosan is a safe, natural, no allergen and biocompatible polymer with health benefits (Bornet & Teissedre, 2011). Because of its antimicrobial activity (Maghsoudlou et al., 2012), antioxidant activity (Kamil, Jeon, & Shahidi, 2002) and good mechanical properties (Arvanitoyannis, Nakayama, & Aiba, 1998), chitosan can be successfully used in food industry to preserve foods (Bal, 2013), compared to medium strength synthetic films (Arvanitoyannis et al., 1998), and its relatively more hydrophobic nature in comparison with other hydrophilic biopolymers that could provide higher moisture barrier and water resistance (Bangyekan, Aht-Ong, & Sriukulkit, 2006; Chillo et al., 2008). In order to improve the performance of biopolymers, blending of two or more biopolymers has gradually been suggested (Chen, Wang, Mao, Liao, & Hsieh, 2008). Several disadvantages include: strong hydrophilic character, poor physical, mechanical and barrier properties. Compared to synthetic

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packaging films, they can be improved by mixing with other biopolymer. Previous researchers studied the effect of blending kefir with starch (Motedayen et al., 2013) and whey protein with nanoparticles (Zolfi, Khodaiyan, Mousavi, & Hashemi, 2014) on improving structural properties of kefir film. Chitosan has been previously added to other polymer such as polylactic acid (Bonilla, Fortunati, Vargas, Chiralt, & Kenny, 2013), starch from different plant sources (Mathew & Abraham, 2008; Vasconez, Flores, Campos, Alvarado, & Gerschenson, 2009; Xu, Kim, Hanna, & Nag, 2005; Zhong, Song, & Li, 2011), methyl cellulose (Pinotti, Garcia, Martino, & Zaritzky, 2007) to improve structure properties of these polymers. The objectives of the present work were to develop edible composite films by blending kefir with chitosan, a by-product obtained from wastes of the fishing industry, to improve physical properties, water vapor permeability (WVP), mechanical properties and antioxidant activity of the kefir films to target purpose use as an applied biopolymer in food packaging.

2. Materials and methods

2.1. Materials

Kefir grains, used as a starter culture in this study, were obtained from a laboratory in University of Agricultural Sciences and Natural Resources Gorgan, Iran. Chitosan (deacetylation degree of 75%) was purchased from Sigma Aldrich Company USA and food grade glycerol, food grade ethanol (96%), magnesium nitrate, sodium chloride, silica gel and acetic acid (100% anhydrous) were purchased from Merck Company Germany.

2.2. Isolation and purification of kefir

The grains were kept in skimmed milk at room temperature 20–22 °C and the medium was exchanged daily for the grains' viability to be maintained fresh. After 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 10,000×g for 20 min at 20 °C (Refrigerated Centrifuge, Combi 514R, Hanil Science Industrial, Korea). The polysaccharide in the supernatant was precipitated by addition of two volume of cold ethanol and left at –20 °C overnight, then the mixture was centrifuged at 10,000×g for 20 min at 4 °C. Pellets were dissolved in hot water and the precipitation procedure according to Rimada and Abraham (2001), 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. Yield of extraction of kefir polysaccharide in supernatant was 1.95 g/L.

2.3. Preparation of films

Kefir (Kef) solution (2%; w/v) was prepared by dispersing 2 g of Kef powder in 100 mL of distilled water (85 ± 3 °C) under stirring condition until Kef powder was completely dissolved (Ghasemlou, Khodaiyan, & Oromiehie, 2011). Chitosan (Chi) solution (2%; w/v) was prepared by dispersing 2 g of Chi powder in 100 mL of acetic acid (1%; v/v) (60 ± 3 °C) under stirring condition until Chi powder was completely dissolved (Siripatrawan & Harte, 2010). Glycerol as plasticizer at ratio of 25% (w/w) dry powder was separately added to Kef and Chi film solutions. Following the addition of plasticizer, stirring was continued for a further 15 min. The solutions were then cooled to room temperature. Film-forming solutions of different ratios of Kef/Chi (100/0, 68/32, 50/50 and 32/68) were prepared. After the blending of two film forming components, they were stirred for 15 min. The film-forming solutions were subsequently degassed to remove air bubbles using a sonicator (Ultrasonic Processor,

Cole-Parmer, Vernon Hills, IL, USA). 15 mL portions from each film forming solution were cast on a 10 cm diameter petri dish and dried in an oven at temperature of 30 °C, after 24 h dried films were peeled and stored in plastic bags in desiccators at 25 ± 2 °C for further testing. The obtained films were conditioned in desiccator at 25 ± 2 °C and 50% relative humidity using saturated magnesium nitrate for 48 h prior to testing (ASTM, 2000). All property measurements were performed immediately after removing film specimens from the desiccator.

2.4. Determination of antioxidant activity of composite films

The ability of film samples to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical was evaluated according to the method of Siripatrawan and Harte (2010). 3 mL of film samples were added to 1 mL of 1 mM methanolic solution of DPPH. The mixture was vortexed (Labinco, L-46, Netherlands) and incubated in dark at ambient temperature for 30 min. The absorbance of each sample was measured at 517 nm and the percentage of scavenging activity calculated from the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{\text{Abs DPPH} - \text{Abs extract}}{\text{Abs DPPH}} \times 100$$

Each sample was assayed at least five times.

2.5. Determination physical properties of films

2.5.1. Film thickness

Film thickness was measured by manual micrometer (Mitutoyo, Japan) having a sensitivity of 1 μm. Ten thickness measurements were taken on each test sample in different randomly chosen points and the mean values were used in permeability and mechanical test calculations.

2.5.2. Moisture content

The films' moisture content (approximately 1 cm²) was determined by measuring the weight loss of films 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.

2.5.3. Film solubility in water

The film solubility in water was determined according to the method reported by Gontard, Duchez, Cuq, and Guilbert (1994). It was defined as the percentage of the total soluble matter (%TSM) of film that is solubilized after 24 h immersion in water. The initial dry matter content (W_1) of each film was determined by drying it to constant weight in an oven at 103 ± 2 °C. Disk samples were immersed in 50 mL of distilled water. After 6 h of immersion at 20 °C with periodic agitation, the pieces of films were taken out and dried to constant weight (W_2) in an oven at 103 ± 2 °C to determine the weight of dry matter which was not solubilized in water.

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

The solubility in water of the disk samples was measured from immersion assays in 50 mL of distilled water with periodic stirring for 6 h at 20 °C.

$$\%TSM = \frac{W_1 - W_2}{W_1} \times 100$$

2.6. Water vapor permeability

Water vapor transmission rate (WVTR) of the films was determined following ASTM standard test method (ASTM, 2003).

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