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# Development and characterisation of a new biodegradable edible film made from kefiran, an exopolysaccharide obtained from kefir grains

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ABSTRACT

This study examined the feasibility of using kefiran, an exopolysaccharide obtained from kefir grains, as a new film-forming material. Kefiran-based films, with and without glycerol as plasticizer, were prepared by a casting and solvent-evaporation method. To study the impact of the incorporation of glycerol into the film matrix, physical, mechanical, and thermal properties of the films were investigated. As expected, the increase of glycerol concentration from 15% to 35% w/w increased extensibility but decreased tensile strength, implying higher mobility of polymer chains by the plasticizing effect of glycerol. Water vapour permeability of films was found to increase as the plasticizer content increased. Glass transition temperatures decreased as a result of plasticization as glycerol content increased. The properties of the films were related to their microstructure, which was observed by scanning electron microscopy. Thus, it was observed that plasticizer is a significant factor in the properties of these films and their food technology applications.



## 1. Introduction

In recent years, the food and packaging industries have been joining their efforts to determine new ways to protect food from environmental conditions 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). Edible, biodegradable films, by acting as barriers to control the transfer of moisture, oxygen, lipids, and flavours, can prevent quality deterioration and increase the shelf life of food products (Gontard, Guilbert, & Cuq, 1993).

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 when compared to 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 amounts 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, when compared with other polysaccharides, kefiran has several important advantages, such as antibacterial, antifungal, and antitumour properties (Maeda, Zhu, Omura, Suzuki, & Kitamura, 2004; Murofushi, Shiomi, & Aibara, 1983). 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, when compared to synthetic packaging films. These drawbacks make it unsatisfactory for some applications such as packaging (Debeaufort et al., 1998).

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

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mobility of polymer chains, decreasing the glass transition temperature ($T_g$); unfortunately, they also decrease the film's water vapour permeability (Gontard et al., 1993). Glycerol is one of the most widely used plasticizers in film-making techniques. It is a high-boiling-point plasticizer that is water-soluble, polar, and non-volatile; these properties make glycerol a suitable plasticizer for use with a compatible water-soluble polymer (Cheng, Karim, & Seow, 2006).

There is only one paper on the use of kefiran as an 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. In the current research, varying levels of glycerol were used in kefiran-based films. The aim of this work was to study some selected characteristics of glycerol plasticized kefiran films intended for use as edible or biodegradable films. These results are not available in the literature but are very important to evaluate possible applications of these films as packaging material.

## 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 new culture 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 3–16 k 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 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, 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. On 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.

Glycerol (Sigma Chemical Co., St. Louis, MO, USA) was added as a plasticizer at various levels (15–35% w/w based on kefiran weight). 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 levelled 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 (Merck, Darmstadt, 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 micrometre (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 $1 \times 3\ cm^2$) 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 ± 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 of the different kefiran films was measured from immersion assays in 50 ml of distilled water with periodic stirring for six hours 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 percentage of the total soluble matter (% TSM) of the films was calculated using Eq.(1):

$$\%\mathrm{TSM} = [(\text{initial dry weight} - \text{final dry weight})/\text{initial dry weight}] \times 100 \quad (1)$$

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

#### 2.4.4. 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, Rezaei, Razavi, & Hosseini, 2010). Contact angle measurements were performed with water using a goniometer (Kruss G10, Germany). To perform the measurements of the contact angles, a syringe was filled with 5 ml of distilled water and a drop was placed on the film surface. For each film type, at least five measurements at different positions on the film surface were taken and the average was calculated.

### 2.5. Surface colour measurements

Film colour was determined using a colourimeter (Minolta CR 300 Series, Minolta Camera Co., Ltd., Osaka, Japan). Film specimens were placed on a white standard plate ($L^* = 93.49$, $a^* = -0.25$ and $b^* = -0.09$) and the lightness ($L$) and chromaticity parameters a (red–green) and $b$ (yellow–blue) were measured. $L$ values range from 0 (black) to 100 (white); a values range from −80 (greenness) to 100 (redness); and $b$ values range from −80 (blueness) to 70