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Water kefir grains as an innovative source of materials: Study of plasticiser content on film properties

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Abstract

Microorganisms' biomass is a sustainable and innovative source of biopolymers, such as proteins and polysaccharides, which are suitable for development of biodegradable films. In this work, an alternative material based on the entire biomass of water kefir grains was developed. The whole biomass was submitted to physical treatments of ultrasonic homogenization separated by a thermal treatment at 90 °C. The effect of glycerol on film properties was evaluated. The films obtained exhibited high transparency and homogeneity without cracks. Infrared spectroscopy revealed that the material is constituted basically by the polysaccharide dextran. The increasing plasticiser content decreased glass transition temperature. Indeed, the presence of plasticiser increased the amount of hydration water and the water vapour permeability. Moreover, the addition of 30 wt% of glycerol improved significantly the elasticity of films to 275 ± 15%. Results revealed that water kefir grains biomass is a viable and innovative source of biodegradable materials.

Keywords: Biobased films; Water kefir grains; Plasticisation; Glass transition; Mechanical properties; Hydration.

1. Introduction

The indiscriminate use of petroleum-based polymers and plastics for short-life food packaging has caused serious environmental problems due to the materials' inability to biodegrade. For this reason, innovation in the packaging industry is focused on the development of new materials more sustainable and economically viable than the traditional ones [1-3]. Particularly, there is a growing interest in renewable and/or biodegradable films obtained from biopolymers such as proteins, polysaccharides and lipids [1,2,4]. These biodegradable films and coatings may be used to cover food surfaces and can act as barriers to control the transfer of moisture, oxygen, carbon dioxide, lipids, and flavour components, and thus maintain the quality and increase the shelf-life of food products [5]. Among other important features, they can be used as carriers of functional agents, as antimicrobials or antioxidants and to improve appearance and handling [6].

According to the type of biopolymers that compose the film, their properties will be different [6]. Some properties of interest include appropriate mechanical and thermal characteristics and serve as a barrier with selective permeability to the transfer of various substances, including gases and water vapour. Polysaccharides and proteins interact strongly with water; therefore, films made from these biopolymers are hydrophilic films [2].

The obtainment of films by casting method involves the use of at least a film-forming agent (biopolymers like polysaccharides or proteins), a solvent and a plasticiser. To form the film matrix, it is necessary to prepare a dispersion containing the biopolymers and remove the solvent by drying in order to decrease the distance between polymers, favouring their interaction. This interaction allows the formation of a polymer network that will be ended with a film conformation [7]. Film characteristics are dependent on the structure and chemical properties of the polymer chains, conditions of casting and the presence of plasticiser agents [4]. Plasticisers are needed to improve the integrity and mechanical properties of films [1,8]. They are generally small molecules that destabilize hydrogen bonds, reducing intermolecular forces and increasing the space and the mobility of polymer chains [9]. Correspondingly, plasticisers increase the amount of water molecules hydrating the film, affecting water barrier properties as well as sorption characteristics [8,10,11]. Therefore, it is important to study the effect of plasticisers in polymer matrices. Glycerol is the most used plasticiser in films based on biopolymers for its good properties of miscibility and low cost [12].

Several studies have reported the use of polysaccharides from different sources to obtain films and coatings [13-17]. However, in recent years, a considerable emphasis has been placed on the research of materials obtained from microbial exopolysaccharides [18].

Water kefir is a sour, alcoholic, and fruity fermented beverage of which the fermentation is started with water kefir grains [19]. These grains consist of exopolysaccharide and contain a consortium of different microorganisms responsible for the water kefir fermentation [20]. For the production of grains, a sucrose solution (up to 80 g L^{-1}) is fermented by the consortium of microorganisms [21]. Water kefir grains contain dextran, which is a glucose polymer, mainly composed of linear α-D-1,6-linked with a low percentage of α-1,3-linked side chains [22]. This is the main difference with the milk kefir, which has more complex heteropolysaccharide called kefiran [23]. Despite these differences, both types of kefir contain the same groups of microorganisms: lactic acid bacteria (LAB), acetic acid bacteria (AAB) and yeasts [19,24]. The symbiotic microorganisms are embedded in the matrix of polysaccharide forming a complex structure. Different sets of yeasts and bacteria have been identified by several authors [25,26]. Beneficial effects of water kefir on human health were declaimed by consumers but have not yet been scientifically substantiated. Nevertheless, probiotic properties are widely spread among LAB and so can be expected in a LAB containing system like water kefir with high probability [21]. The potential health benefits have contributed significantly to the increase in the consumption of kefir and interest in this product in several countries [21].

Several research works have demonstrated the capacity of kefiran from milk kefir grains to form films [18,27-30] but, in contrast, there is no specific literature about the use of water kefir grains as film-forming agents. While films based on kefiran proved to have good water vapour barrier and mechanical properties, the process to produce films implies the isolation and purification of the polysaccharide from milk kefir grains. Traditionally, the most common approach to develop new biodegradable materials was the purification of interesting biopolymer from their original biomass, by using physical or chemical modifications to enhance their capability to form films. In this work, the entire biomass of the water kefir grains will be used, thus this new approach contributes to a more efficient process with less waste.

This study aims to simplify the preparation method and develop a new biodegradable film using the integral biomass of water kefir grains, submitted to successive physical treatments including ultrasonic homogenization and thermal

treatment. Functional characterisation of the material was carried out examining the microstructural, thermal, mechanical, hydration and water vapour barrier properties of the resulting films. Glycerol content was considered as a factor in this study.

2. Materials and methods

2.1. Water kefir grains and culture conditions

Water kefir grains LOMCEM HWK1, was used as starter culture and they were obtained from a household at El Pinar, Uruguay. The grains were stored frozen at −20 °C. Water kefir grains were reactivated by successive subculture at room temperature in 2 L water cultivation medium containing ~100 g of kefir grains, 100 g of muscovado sugar, 50 g of dried white figs, and one lemon cut in four slices. The medium was exchanged every two days with a new culture medium to maintain grain viability. Subcultures were repeated several times in order to increase kefir grain biomass. For film preparation, the grains were separated from the fermented product by filtration through a plastic sieve, washed three times with distilled water, and pressed in order to remove the excess of water. The amount of dry matter of the washed and pressed kefir grains was as 0.14 g per g determined by drying at 105 °C.

2.2. Preparation of films

Washed and pressed kefir grains were used for the preparation of film-forming dispersion of 3 wt% dry matter of water kefir grains. The dispersion was firstly homogenized at 15000 rpm during 5 min by using an Ultraturrax T-25 (IKA, Germany). Then, it was submitted to ultrasonic homogenization at 80 W during 15 min, followed by a thermal treatment in a water bath at 90 °C, for a time lapse of 20 min. Afterwards, a second ultrasonic homogenization was applied at the same conditions of the earlier homogenization process. The first homogenisation process was necessary to disassemble the compact structure of the grains and cell wall of remaining microorganisms, in order to produce a fine dispersion. The heat treatment was made to denature and unfold biopolymers, and the final homogenisation was done to break possible aggregates formed by the heat treatment [15]. With the purpose of study the effect of plasticiser on film properties, pure glycerol was added to dispersion at levels of 0, 10, 20, and 30 wt% respect to dry matter (d.m.). Following the addition of plasticiser, stirring was applied during 15 min. None other thermal treatment was applied to the dispersions after the addition of

glycerol. Finally, the dispersions were subjected to vacuum for about 30 min to remove the air bubbles incorporated during processes. The final pH of the dispersions was 4.5.

In order to obtain films of thicknesses close to 6×10^{-5} m, 17 g of dispersion were placed in 8.6×10^{-2} m diameter plastic Petri dishes. Evaporation of water was done by casting at 40 °C and 40% relative humidity (r.h.) in a ventilated oven (Sanyo MOV 212F, Japan) until the remaining water content of the films was between 10 and 15%. Next, films were stored at 22 °C and 43% r.h. Finally, according to the requirements of the experiment, the films were equilibrated in desiccators at different r.h. using saturated solutions of LiCl, $MgCl_2$, K_2COO_3, NaBr, NaCl, and $BaCl_2$, to generate conditions of 11, 33, 43, 57, 75, and 90% r.h. respectively. Dried atmospheres were obtained using silica gel.

2.3. Thickness measurements, visual appearance and quality evaluation of the films

Films thickness was measured with a digital calliper (± 10^{-6} m; 3109-25-E, Insize Co., China). Measurements were taken at ten different locations of the films, obtaining for each specimen an average value. The visual appearance of films was checked by taken photographs. The quality of the films obtained was assessed considering aspects of handleability, homogeneity and continuity [31]. This evaluation was carried out with three independent replicates.

2.4. Microstructural characterization by scanning electron microscopy (SEM)

Surfaces and cross sections of films were analysed by using a scanning electron microscope SEM–Carl Zeiss NTS–SUPRA 40 at 5 kV. Cross sections were obtained by a cut with a sharp blade at room temperature. For best viewing under a microscope, the samples were coated with a gold layer. Images of the faces (magnification 500×) and the cross sections (magnification 3000×) of unplasticised and plasticised films were obtained.

2.5. Attenuated total reflectance-Fourier transform infrared analyses (ATR-FTIR)

Infrared spectra of films were recorded in the range of 4000-400 cm^{-1} on a Fourier-Transform Infrared Analyzer (FTIR) Shimadzu IR-Affinity (Shimadzu Co., Japan) equipped with an attenuated total reflectance diamond module (GladiATR, Pike Technologies, USA). Spectra were obtained, in duplicate, as an average of 60 scans with 4.0 cm^{-1} resolution and Happ-Genzel apodization. A blank spectrum was obtained before

each test to compensate the humidity effect and the presence of carbon dioxide in the air by spectra subtraction.

2.6. Thermogravimetric analyses (TGA)

Mass loss in samples as a function of temperature was registered in a TA Instruments Q-500 (Delaware, USA) thermo-balance. Samples with different percentages of glycerol were previously conditioned at 90% r.h. Approximately 10 mg of each sample were weighed in a platinum pan and heated from 20 to 110 °C, at 1 °C min^{-1}, in order to study in detail the loss of hydration water. Then, samples were heated at 20 °C min^{-1}, from 110 to 800 °C, to study the thermal degradation of the films. Experiments were carried-out in duplicate under nitrogen atmosphere (flow rate 60 mL min^{-1}). The temperature at the maximum degradation rate (T_{max}) was determined from the peak of derivative curves.

2.7. Differential scanning calorimetry (DSC)

Glass transitions temperature (T_g) of the films were determined using a Differential Scanning Calorimeter (TA Instruments Q200, Delaware, USA), in the range of −85 °C to 210 °C, with a previous equilibration step at −85 °C during 5 min, and then, temperature was increased at 10 °C min^{-1}. Approximately 7 mg of samples were placed into Tzero® aluminum pans and sealed with hermetic lids. Three different DSC experiments were carried out to analyse the influence of different factors. Firstly, the effect of glycerol was studied by previously dehydrating the samples in silica gel for 7 days. Secondly, the effect of hydration water was studied on unplasticised films hydrating the samples until reach equilibrium in environments of 0, 43, 75, and 90% h.r. In the third set of assays, samples with different percentages of plasticiser were previously conditioned at 43% r.h. This last experiment was carried out to correlate with the experimental conditions and results of the mechanical properties tests, which are described in the following section. All the conditioning steps were performed at 22 °C. In all DSC experiments, glass transitions were determined using TA Universal Analysis software (v4.5, TA Instruments, USA), at the mid-point. Experiments were done in triplicates.

2.8. Mechanical properties

Uniaxial tensile tests of films were carried out in a Universal Testing Machine (TC-500 II-Series, Micrometric, Argentina) equipped with a 30 N cell. Samples probes were cut in rectangular shapes of 50 mm of length and 10 mm of width; the effective distance between jaws was 25 mm. Temperature was controlled at 22 °C and specimens of kefir films with different percentages of plasticiser (20 and 30 wt%) were previously conditioned at 43% of r.h. Samples with 0 and 10 wt% glycerol were not assayed due to their brittleness that makes difficult the measurements. The selected speed to perform tests was 5 mm min^{-1} and ten specimens of each composition were tested. Deformation at break (%), maximum tensile strength (MPa), and Young's modulus (MPa) were calculated from the resulting stress-strain curves as average of ten measurements according to ASTM D882, 1997 [32].

2.9. Water sorption isotherms

Water sorption isotherms were determined gravimetrically at 22 °C according to standard procedure described by [15]. Samples of films with different percentages of glycerol and a superficial area of 5.8×10^{-3} m^2 were placed in containers of 1.5 L and equilibrated at different a_w. Samples were periodically weighed using an analytical balance (± 10^{-4} g) and the evolution to equilibrium at each moisture condition was monitored until constant weight. The water content or hydration h, given in units of g of water per g of dry matter (d.m.) was evaluated as a function of water activity a_w (a_w=% r.h./100), taking the difference between the mass of the hydrated film and that of the dried film. Experiments were performed in triplicates.

Isotherms were fitted using Guggenheim-Anderson-De Boer (GAB) model [33] through Eq. (1):

$$h(a_w) = \frac{N.c.k.a_w}{\left[(1+(c-1)k.a_w)(1-k.a_w)\right]} \qquad (1)$$

where N is the monolayer water content (g of water per g of dried mass) related to primary binding sites of water molecules, c is a parameter related to the sorption heat monolayer that represents the force of the water binding to monolayer, and k is a parameter related to sorption heat multilayer that represents the capability of water to bind to the multilayer [34].

2.10. Water vapour permeability

Experimental water vapour permeability (P_w^{exp}) of water kefir films was measured using the cup method described in ASTM-E96, 2016 [35]. Films were sealed on top of the cups containing a saturated solution of $BaCl_2$ that provides 90% r.h. Test cups were placed in desiccators maintained at a constant temperature of 22 °C and 10% r.h., provided by a saturated solution of NaOH. A fan was used to maintain uniform conditions inside the desiccators over the films according to recommendations from previous authors [36,37]. Weight loss measurements were taken by weighing the test cup using an analytical balance (± 10^{-3} g). Weight loss versus time was plotted and when the steady state (straight line) was reached 24 hours further were registered.

The water vapour flux through the film J_w was calculated from the slope ($\Delta m/\Delta t$) of a linear regression of weight loss versus time by Eq. (2):

$$J_w = \frac{1}{A}\left(\frac{\Delta m}{\Delta t}\right) \qquad (2)$$

where A is the effective area of exposed film (2.2×10^{-3} m^2). The experimental water vapour permeability P_w^{exp} was calculated according to Eq. (3):

$$P_w^{exp} = \frac{J_w L}{\Delta p_w} \qquad (3)$$

where P_w^{exp} is given in units of g $s^{-1}m^{-1}Pa^{-1}$, L is the film thickness, and $\Delta p_w=(p_{w2}-p_{w1})$ is the differential water vapour partial pressure across the film, p_{w1} and p_{w2} are the partial pressures (Pa) of water vapour at the film surface outside and inside the cup, respectively, corrected by air gap distance (5×10^{-3} m in the present study) between saturated solution of $BaCl_2$ level and the film position [37]. Experiments were performed in triplicates.

2.11. Statistical analyses

Statistical analyses were performed using OriginPro 8 (OriginLab Corporation). The data were subjected to the analysis of variance, and the means were compared using Student's *t*-test. Differences were considered to be significant at $p<0.05$.

3. Results and discussion

3.1. The visual aspect, quality evaluation, and thickness of films

Water kefir films were homogeneous, with no cracks, and presented high transparency as seen in Fig. 1(a), for films plasticised with 20 wt% glycerol. No differences were found in the visual appearance for the rest of formulations. This is not a minor aspect since transparency and colour of films and coatings can influence the appreciation of the final product. The spectacular transparency of these films was not observed in films made from purified kefiran [29]. On the other hand, the gradual addition of plasticiser increased significantly the flexibility of the films. The small molecules of glycerol intercalate between polymer chains, disrupting inter-polymer' bonds and spreading the chains apart increasing film flexibility [10]. Samples with 0 and 10 wt% glycerol were brittle, difficult to handle and required care in peeling from the casting surface. In contrast, films with 20 and 30 wt% glycerol were flexible and easy to peel and manipulate. Films plasticised with 30 wt% glycerol presented great elasticity (Fig. 1(b)). The average thickness of all films studied was $6 \pm 0.3 \times 10^{-5}$ m.

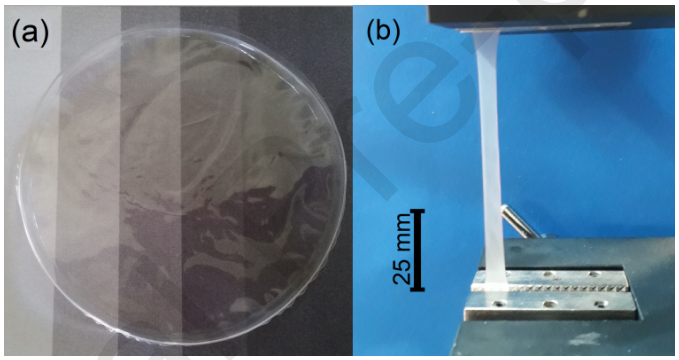

Fig. 1. (a) Photograph of water kefir film with 20 %wt glycerol. (b) The elasticity of a rectangular probe of water kefir film with 30 wt% glycerol (initially, the effective distance between jaws was 25 mm).

3.2. Film microstructure

In an attempt to study film microstructure, micrographs of the faces and cross-sections of water kefir films were obtained by scanning electron microscopy (SEM). Fig. 2 shows the microscopy images of unplasticised and plasticised water kefir films with 30 wt% glycerol.

Both unplasticised and plasticised films presented a continuous and homogeneous cross section, with no agglomerates, pores, faults, or film punctures (Figs. 2(a) and 2(b)). Cross section of the plasticised film (Fig. 2(b)) exhibited rubber-like characteristics due to the effect of glycerol. With regard to the faces of the films, they also showed a continuous and homogenous matrix without pores (Figs. 2(c) and 2(d)).

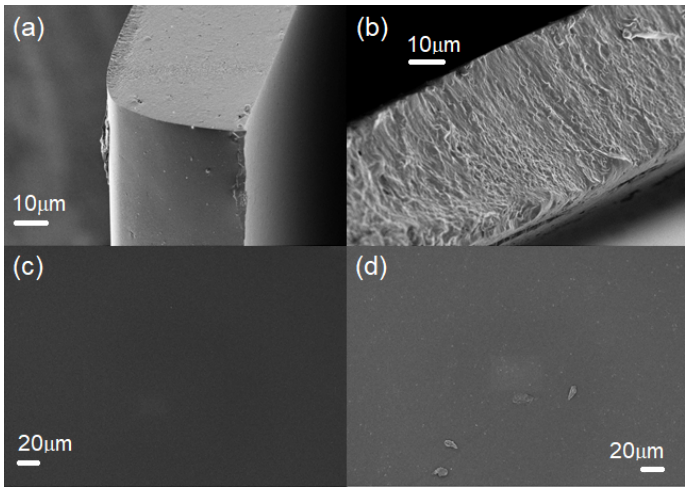

Fig. 2. SEM observations of cross-sections (3000×) of unplasticized (a) and plasticized films with 30 wt% glycerol (b). SEM observations of the surface (500×) of unplasticized (c) and plasticized films with 30 wt% glycerol (d).

3.3. Infrared spectroscopy of the films

Infrared spectra of plasticised and non-plasticised water kefir films can be seen in Fig. 3. All samples showed a quite similar general feature, differing mainly in their band intensities, and were very similar to those obtained for films based on kefiran polysaccharide purified from milk kefir [28].

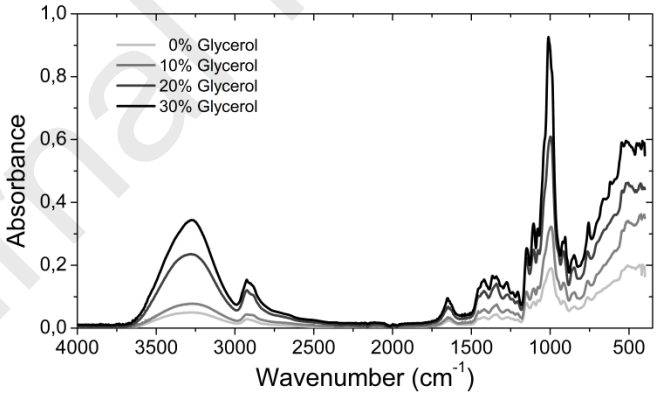

Fig. 3. FTIR spectra of water kefir films with different content of glycerol.

Piermaria et al. (2011) divided into four zones the spectrum of kefiran films [28]. These authors identified a first region attributed to hydroxyl groups (OH) due to water and carbohydrates (3600-3000 cm^{-1}), a next zone related to the symmetric and anti-symmetric stretching modes of C-H in methyl (CH_3) and methylene (CH_2) functional groups (3000-2800 cm^{-1}), a third zone (1700-1580 cm^{-1}) assigned to bending mode of O-H in water molecules and a fourth zone (1200-900 cm^{-1}) which holds the peaks mainly

assigned to stretching modes of carbohydrate rings and side groups (C-O-C, C-OH, C-H). In water kefir films, the peaks in this latter region could be associated with the vibrational modes of glucose, due to dextran structure.

In addition, Fig. 3 allowed studying the effect of glycerol in the spectra profiles of the films. As can be observed, band intensities increased with the gradual addition of glycerol. In particular, the intensity of the bands assigned to water molecules (first and third region) were higher in plasticised films spectra. This could be due to an increase in the water content induced by the plasticiser and also to the presence of glycerol itself.

The spectra of Fig. 3 showed the negligible intensity of bands corresponding to proteins groups of amide I and amide II in the 1650-1500 cm^{-1} zone, which are usually intense even at small protein concentrations. In water kefir grain biomass the protein content, which comes exclusively from the contribution of microorganisms cells, is negligible as compared with the polysaccharide content. Bearing this in mind, evidently it is not convenient to carry out purification processes that can raise costs without a justification from the characteristics of the films.

3.4. Thermogravimetric analysis of films

Fig. 4 shows the thermogravimetric analysis of water kefir films that were previously hydrated in equilibrium in an atmosphere at 90% r.h.

First degradation zone up to 110 °C in Figs. 4(a) and 4(b) was attributed to water evaporation or dehydration. The weight loss due to dehydration raises with the content of glycerol, indicating that the initial water content increases with the amount of plasticiser in the films. As can be seen in Figs. 4(a) and 4(b), at temperatures above 110 °C degradation of plasticised samples occurred at lower temperatures than non-plasticised one. This is due to the presence of glycerol in the film matrix, which increased chain mobility and exposed polymer chains even more to the thermal degradation.

In Fig. 4(a) there is a pronounced degradation event, which is evidenced by an abrupt fall in films weight at 300 °C and the maximum decomposition rate occurred at 317 ± 4 °C (Fig. 4(b)). This pronounced event could be attributed to the thermal degradation of dextran. Other polysaccharides presented degradation steps in these temperatures [38]. Fig. 4(b) showed the presence of another degradation event between 110-270 °C, which could be associated with the degradation of glycerol molecules [12].

The degradation zone between 30-110 °C corresponded to the dehydration of the films (Fig. 4(c)). As seen in Fig. 4(c), water retention values were lower in plasticised

samples, indicating that the addition of glycerol decreased the force of the water binding to the matrix.

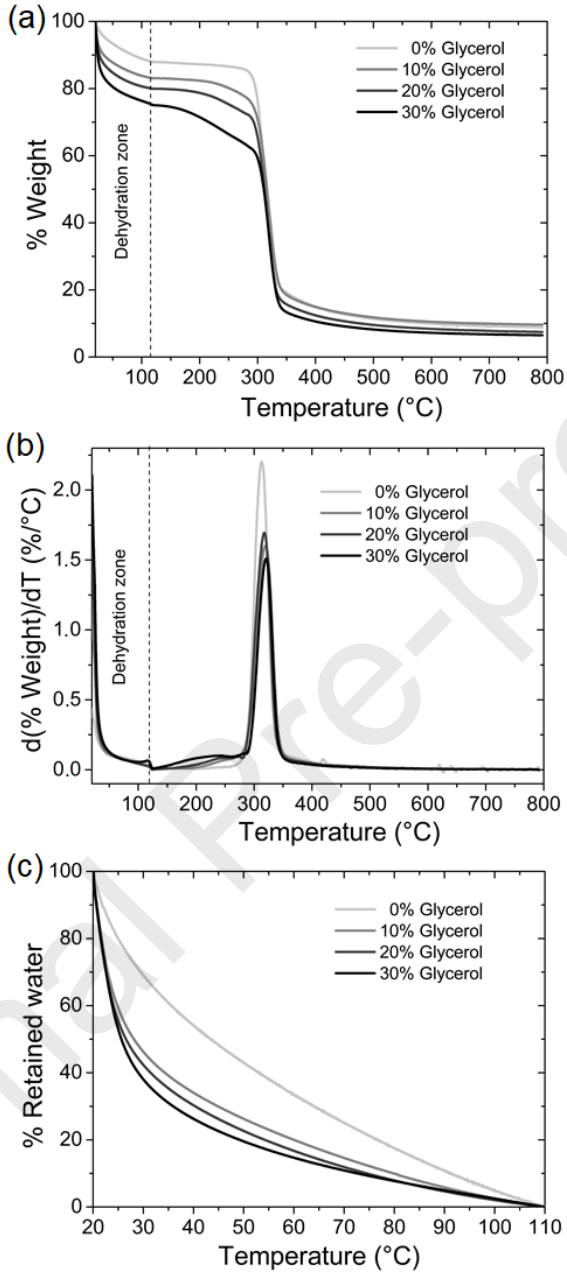

Fig. 4. Thermogravimetric analysis of films with different content of glycerol: (a) Mass loss, (b) derivative of mass loss, and (c) percentage of retained water as function of temperature.

3.5. Differential Scanning Calorimetry

DSC experiments allowed to obtain glass transition temperatures (T_g) of the films and study the effect of glycerol and moisture content. The determination of T_g is important because it greatly affects the thermo-mechanical properties, and physical and chemical stability of the material [39]. In Fig. 5 are shown DSC thermograms of water kefir films.

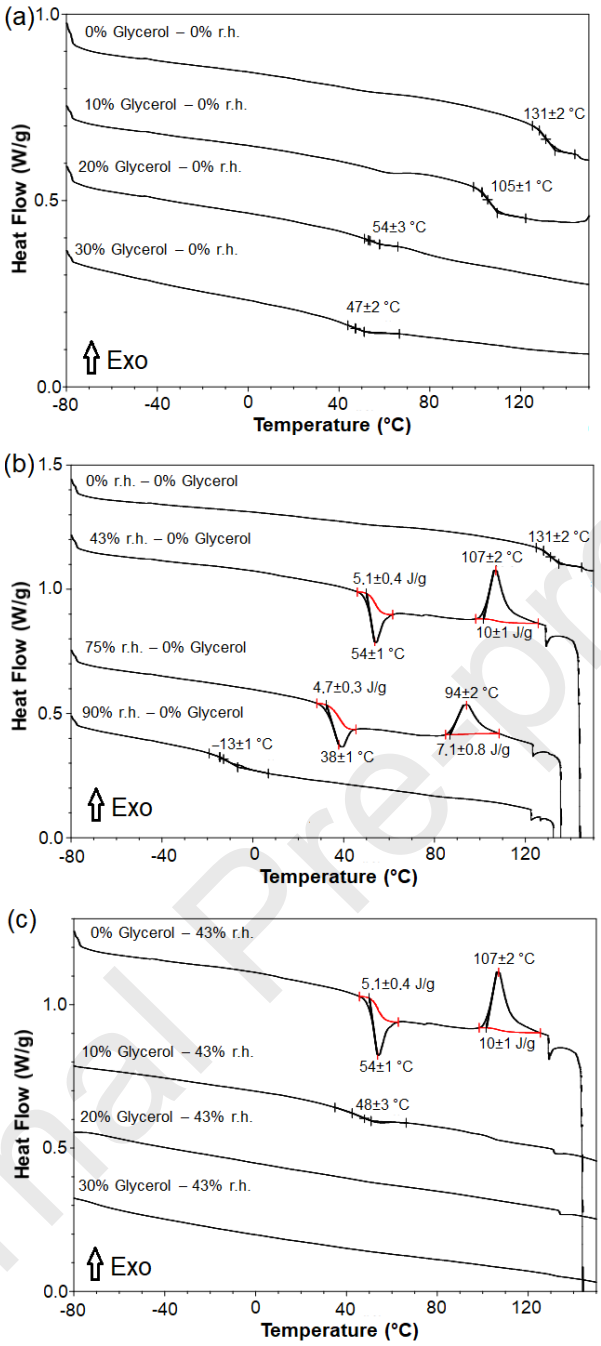

Fig. 5. DSC thermograms of water kefir films. (a) Dried samples with different content of glycerol. (b) Samples without glycerol previously hydrated at 0, 43, 75, and 90% r.h. (c) Samples with different content of glycerol previously hydrated at 43% r.h.

As it could be seen in Fig. 5(a), films without plasticiser had the highest T_g; moreover, the T_g decreased with increased glycerol concentration due to the plasticisation process. The unplasticised sample presented the T_g at 131 ± 2 ºC and was shifted to 105 ± 1 ºC when 10 wt% glycerol was added. Samples with 20 and 30 wt% glycerol presented T_gs at 54 ± 3 and 47 ± 2 °C, respectively. This behaviour agrees with other studies on the

effect of glycerol concentration on T_g values of biopolymeric films [12,29]. The depression of T_g with the addition of plasticiser may be explained by a number of theoretical approaches such as free volume or classical thermodynamic theories proposed by Couchman & Karaz (1978) [40]. In addition, the plasticiser reduces the attractive forces between polymer chains, producing an increase in the molecular space and favouring the transition from glassy to rubbery state.

Fig. 5(b) shows the effect of water in the polymer matrix matrix by the hydration of samples at different r.h. It was found that T_g decreased with increasing hydration water of the unplasticised films, as expected. The obtained T_g values were 131 ± 2, 54 ± 1, 38 ± 1, and −13 ± 1 °C for films previously hydrated at 0, 43, 75, and 90% r.h., respectively. In this way hydration water acts as a plasticiser and films become more fragile in low hydration conditions. The plasticizing effect of water on proteins, polysaccharides and their mixtures was already observed by other authors [39]. Samples that were hydrated at 43% and 75% r.h. exhibited enthalpic relaxation phenomenon, while dry sample 0% and hydrated at 90% r.h. showed no enthalpy relaxation because their T_g were far from storage temperature [41]. Enthalpy relaxation appears as an endothermic process associated to the glass transition in the DSC thermogram [42] and is related to a physical ageing of the sample. Some authors reported enthalpy relaxation in amorphous starch and relate the phenomena with the moisture content of native and gelatinized starch [43]. Other authors that studied the phenomena found that physical ageing was dependent on the storage temperature and cooling rate of DSC assays [44]. Enthalpy relaxation is important in amorphous polymers since is accompanied by a change in macroscopic properties such as mechanical properties and barrier properties. Related to this, Kim et al., (2003) found that the physical ageing below the T_g decreased water vapour permeability due to a decrease in free volume [45]. As mentioned, it was observed that water kefir films suffered from physical ageing and it would be an interesting area to be explored in future studies.

Fig. 5(c) exhibits the DSC profile of samples with different content of glycerol previously hydrated at 43% r.h. The calculated T_gs were at 54 ± 1 and 48 ± 3 ° C for films with 0 and 10 wt% glycerol, respectively. In the films with 20 and 30 wt% glycerol, it was not possible to determine the T_gs. This result suggested that when glycerol and hydration water were both plasticizing the film matrix, it resulted in a substantial reduction on the T_g values. These results indicated that in laboratory conditions (22 °C and 43% h.r.), films with 0 and 10 wt% glycerol were in their glassy state, while films

with 20 and 30 wt% of glycerol had rubber-like characteristics and constituted flexible materials.

3.6. Mechanical properties

The tensile parameters Young's modulus, deformation at break (%) and maximum tensile strength were calculated from the experimental stress-strain curves (Fig. 6).

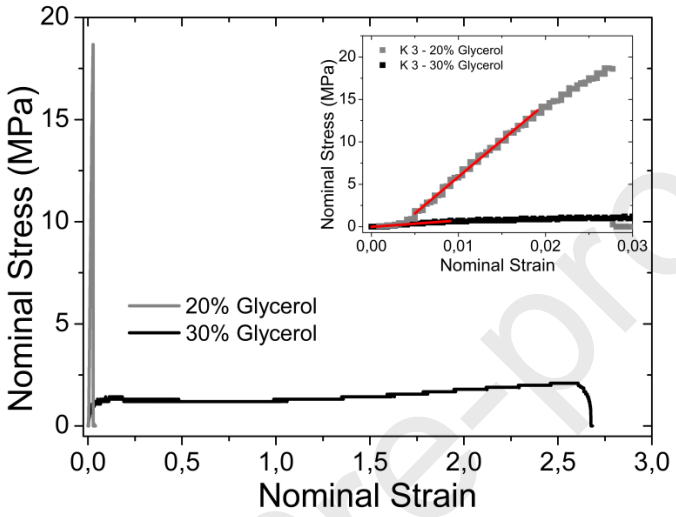

Fig. 6. Stress-strain curves for one of ten replications of the mechanical test performed for films with 20 and 30 wt% of glycerol. Young'a modulus was calculated from the slope in the linear region (red line) and deformation at break (%) from the maximum value of the nominal strain.

Unplasticised water kefir films and those with 10 % of glycerol could not be assayed because they were too brittle to be manipulated at 22°C and 43% r.h. These films were in the glassy state, as confirmed DSC thermograms (Fig. 5(c)), were T_g of the samples were above room temperature (Section 3.5).

When plasticiser concentration in the matrix increased from 20 to 30 wt%, it resulted in a substantial reduction on Young's modulus from 900 ± 15 to 54 ± 6 MPa (p<0.05) and maximum tensile strength values from 13 ± 1 to 1.9 ± 0.3 MPa (p<0.05). Moreover, the rise from 20 to 30 wt% glycerol in the films resulted in a remarkable increase of the deformation at break from 2.5 ± 0.2 to 275 ± 15% (p<0.05). These results showed that the decrease in Young's modulus and maximum strength values with plasticisation was accompanied by an increase in the deformation at break value. A similar trend was reported for other films obtained from different biopolymers [27,46,47]. This behaviour clearly exemplifies plasticiser work at the molecular level which produced a decrease in the cohesive forces between polymer chains, allowing greater mobility.

Plasticisers interfere with polymeric chain association facilitating their slipping and thus enhancing film flexibility.

There is a wide range of reported data for the mechanical properties of edible films in other studies; differences may be attributed to composition and structure of these biopolymers and suppliers, as well as film-preparation techniques [30]. In our work, tensile tests revealed an extremely high deformation at break value of 275 ± 15% in films with 30 wt% glycerol. This value was clearly higher than those values obtained for other biodegradable films with significant properties of elongation at the same plasticiser concentration [27,46,47]. Moreover, this value was higher than the values registered for films based on kefiran plasticised with 35 wt% of glycerol (~170%) [27,29] and high-density polyethylene (150 ± 8%) [48].

3.7. Water sorption isotherms

Sorption isotherms of water kefir films are shown in Fig. 7 and experimental points were fitted with GAB model done by Eq. (1). The addition of glycerol increased the amount of hydration water, preserving the shape of the isotherms. This effect was also observed from whole yeast biomass films [12], yeast cell wall films [17], whey protein films [49] and cassava starch film [50]. Fig. 7 shows that the hydration of water kefir films without glycerol is lower in comparison with other unplasticised biopolymeric films such as whole yeast biomass films [15], yeast cell wall film [17], cellulose-based films [51], starch films [52], myofibrillar protein films [53] and sodium caseinate film [54]. In this way films obtained from water kefir grains were less hydrophilic than other materials based on biopolymers.

All isotherms showed a slight increase in the hydration water content at low values of a_w, and a sharp increase for a_w>0.6. This shape of sorption isotherms suggested the existence of a small amount of water directly bound to the polymeric matrix, forming the monolayer. Then, most of the hydration water was forming multilayers and was indirectly bound to the polymeric matrix [34]. In general, hydrophilic films made from biopolymers show water sorption isotherms with a slight increase in hydration for low values of a_w, and a significant increase for a_w>0.6 [2].

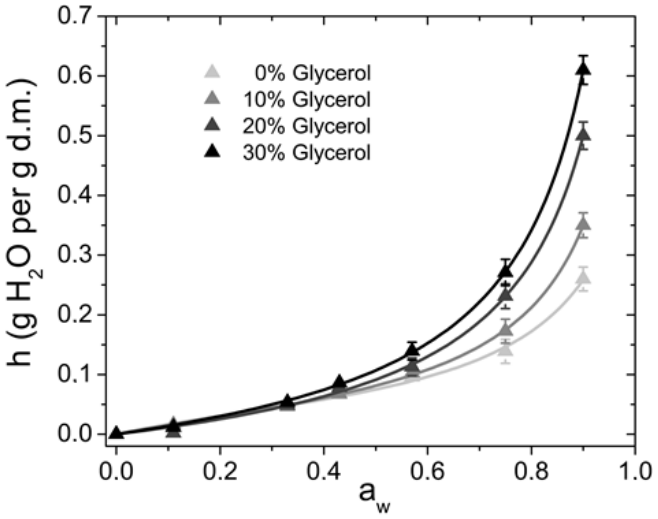

Fig. 7. Water sorption isotherms of water kefir film with different content of glycerol. Experimental data was fitted with Eq. (1) and showed in Table 1.

Table 1. Values of the GAB parameters fitted for the water sorption isotherms displayed in Fig. 7. Units of N are g H_2O per g of d.m. The reported values of the statistical parameter R^2 indicate a very good acceptance of the fit model. Errors are estimated from the fit analysis.

wt% glycerol	N	c	k	R^2
0	0.055 ± 0.007	4 ± 1	0.89 ± 0.03	0.994
10	0.061 ± 0.003	2.8 ± 0.4	0.93 ± 0.01	0.999
20	0.095 ± 0.009	1.3± 0.4	0.93 ± 0.02	0.998
30	0.105 ± 0.007	1.3 ± 0.1	0.94 ± 0.01	0.999

Table 1 displays the parameters obtained from fitting sorption isotherms with the GAB model. Parameter N related to the number of primary binding sites of hydration increased with the glycerol content in the films, while parameter c linked to the force of the water binding to these primary sites decreased. Then, parameter k related to the capability of water to be bounded to the multilayer is independent of glycerol content. Similar behaviour of GAB parameters as a function of glycerol content was observed in yeast cell wall films [17], whey protein films [49] and cassava starch film [50]. We conclude that the addition of glycerol increases the amount of hydration water in the monolayer, but decreases the force of the water binding to the primary sites of hydration. Glycerol interacted with dextran chains by establishing hydrogen bonds with the reactive groups of polymer. Therefore, glycerol incorporated to the film matrix decreased the attractive forces between polymer chains, increased free volume and segmental motions. In this way, the incorporation of glycerol in the film matrix produces a global increase in

hydration water content, allowing greater mobility to water molecules, as also described thermogravimetric studies (Section 3.4).

3.8. Water vapour permeability

Water vapour permeability of biopolymer-based films is an important property, indicating their ability to control water vapour transport between a food system and its surroundings. It was found from Fig. 2 that water kefir films micrographs exhibited a continuous and homogenous matrix, without pores, faults, or film punctures. These studies indicated that the water transport in water kefir films did not occur through pores but by means of the mechanism of sorption-diffusion-desorption. First, the permeant molecules dissolve in the film matrix on the high-concentration side; second, they diffuse through the film, driven by a permeant concentration gradient; and third, they desorb and evaporate from the other side of the film [55,56]. Therefore, water vapour permeability depends on the hydration or water solubility in the film as well as the mobility of water in the matrix [11].

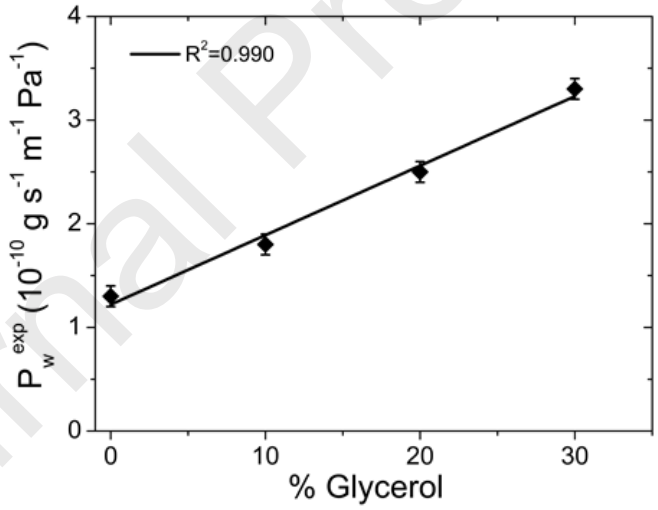

Fig. 8. Dependency of experimental water vapour permeability P_w^{exp} of water kefir films as a function of the content of glycerol.

Experimental water vapour permeability P_w^{exp} was obtained by Eq. (3) measuring water flux through the film. Fig. 8 shows that P_w^{exp} increased linearly with the glycerol content in the film. A linear relation between permeability and glycerol content was also observed in yeast biomass-based films [11], yeast cell wall films [17] and starch films [52]. The effect of glycerol increasing water vapour permeability was observed in the most of protein-based films and polysaccharides-based films [11,52]. However, it was

reported a slight decrease in water vapour permeability with glycerol content for kefiran films [27] and potato starch film [57]. These authors attributed this behaviour to the development of a more compact structure in plasticised films.

Water sorption isotherms (Section 3.7) and thermogravimetric analysis (Section 3.4) showed that the increase in the amount of glycerol in the film produced a global increase in hydration and a greater mobility to water molecules. Consequently, it was expected that water vapour permeability of water kefir films would be increased with the raising percentage of glycerol.

Comparison of water vapour permeability values of hydrophilic films is difficult because of anomalies that occur in matrices strongly interacting with the species that permeates. Permeation of water vapour through hydrophilic biopolymeric films deviates substantially from the ideal behaviour and depend on the experimental conditions such as the differential water vapour partial pressure across the test Δp_w and the thickness L of the film [11,36,55,56]. In the present work experimental water vapour permeability ranged from 1.3 ± 0.1 to $3.3 \pm 0.1 \times 10^{-10}$ g m^{-1} s^{-1} Pa^{-1}. These values were lower than those measured for yeast biomass-based films [11], in similar experimental conditions of Δp_w, L, and glycerol content.

4. Conclusions

Films obtained from whole biomass of the water kefir grains exhibited a great continuity and homogeneity without cracks, and presented high transparency. Infrared spectroscopy and thermal degradation studies revealed that the material is constituted basically by the polysaccharide dextran.

Since the applicability of these films based on water kefir grains depends on their properties, a complete characterization covering relevant aspects as thermal, hydration, water vapour barrier, and mechanical properties was carried out, studying the influence of the addition of glycerol. The presence of plasticizer had a great impact on thermal properties decreasing T_g of the samples, in mechanical properties increasing significantly elongation at break of the films, and barrier properties by increasing the P_w^{exp}. Therefore, the optimal amount of plasticiser to be incorporated comprises a commitment of all these properties depending on the application required.

The traditional approach to develop new biodegradable materials has been the purification of interesting biopolymer from their original biomass, and physical or

chemical modifications to enhance their capability to form films. The results obtained in this work showed the potential of the entire water kefir grains to be used in the development of new biodegradable materials. The material developed here should be considered as an alternative option to traditional sources of biopolymers, without further purification.

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Data availability

The raw/processed data required to reproduce these findings cannot be shared at this time due to technical and time limitations.

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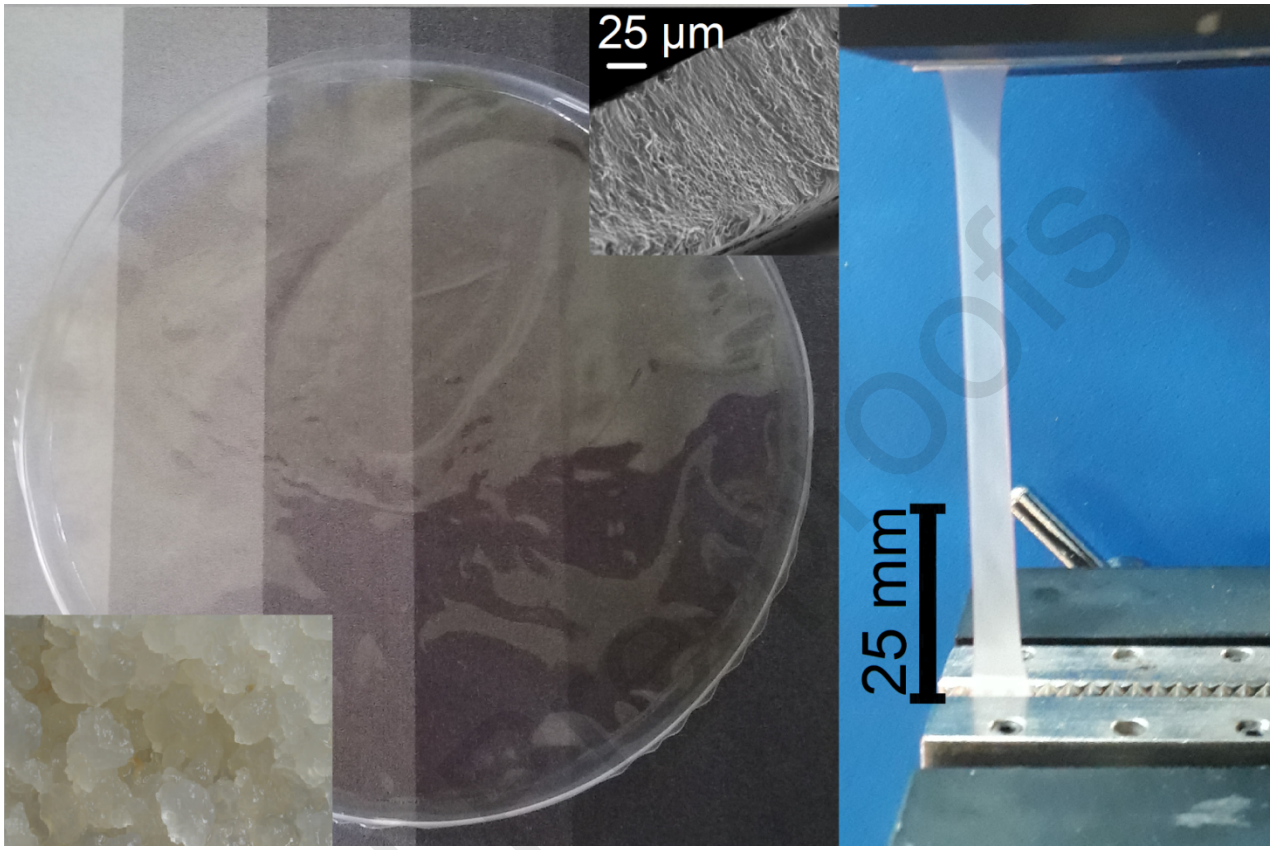
Graphical abstract



Highlights

- Innovative material based on the biomass of water kefir grains was developed
- Water kefir grains are constituted basically by the polysaccharide dextran
- The obtained films exhibited a great continuity, homogeneity and high transparency
- The addition of glycerol affected thermal, hydration, and water barriers properties
- The addition of 30 wt% glycerol improved significantly the elongation of the films