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The Effect of Extraction Conditions on the Barrier and Mechanical Properties of Kefiran Films

Carmen Rodica Pop [1], Teodora Emilia Coldea [2], Liana Claudia Salanţă [1,*], Alina Lăcrămioara Nistor [1], Andrei Borşa [2], Anca Corina Fărcaș [1], Vasile Constantin Florian [3] and Ancuţa Mihaela Rotar [1,*]

1 Department of Food Science, Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 400372 Cluj-Napoca, Romania; carmen-rodica.pop@usamvcluj.ro (C.R.P.); alina.nistor@usamvcluj.ro (A.L.N.); anca.farcas@usamvcluj.ro (A.C.F.)
2 Department of Food Engineering, Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 400372 Cluj-Napoca, Romania; teodora.coldea@usamvcluj.ro (T.E.C.); andrei.borsa@usamvcluj.ro (A.B.)
3 Plant Protection Department, Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 400372 Cluj-Napoca, Romania; vasile.florian@usamvcluj.ro
* Correspondence: liana.salanta@usamvcluj.ro (L.C.S.); anca.rotar@usamvcluj.ro (A.M.R.); Tel.: +40-264-596-384 (L.C.S.)

Abstract: Kefiran is an exopolysaccharide classified as a heteropolysaccharide comprising glucose and galactose in equimolar quantities, and it is classified as a water-soluble glucogalactan. This work aimed to investigate the effect of different extraction conditions of kefiran on the structural and physical properties of the edible films obtained. Fourier-transform infrared spectroscopy and scanning electron microscopy were performed, together with a determinations of moisture content, solubility, water vapor permeability and degree of swelling. The kefiran films presented values of the water vapor permeability between 0.93 and 4.38 $\times$ 10^{-11} g/m.s.Pa. These results can be attributed to the development of a more compact structure, where glycerol had no power to increase the free volume and the water vapor diffusion through their structure. The possible conformational changes in the kefiran film structure, due to the interspersing of the plasticizers and water molecules that they absorb, could be the reason for producing flexible kefiran films in the case of using glycerol as a plasticizer at 7.5% *w/w*. Moreover, it was observed that the extraction conditions are a significant factor in the properties of these films and their food technology applications.

Keywords: kefiran; polysaccharide; plasticizer; glycerol; edible film; barrier properties

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1. Introduction

Nowadays, there is a clear trend towards natural and environmentally friendly alternatives for food protection solutions, since aspects such as clean labeling, healthy or organic food concepts are demanded increasingly by the consumers [1,2]. The food packaging industry is now in pursuit of edible or biodegradable packaging that is lightweight for reducing materials used, waste, and transportation costs [3]. Attention has been directed towards biopolymer-based packaging as a potential alternative to synthetic polymer-based food packaging materials. These materials offer the possibility of creating a thin layer of edible films and coatings with many advantages [4] and functions (antioxidant, antibacterial, antifungal, prebiotic) [5]. Furthermore, in the food packaging industry, microbial activities are of great concern. They can form protective layers (wrapping or coating) around fresh or processed foods, regulate their respiration rates, and protect them from loss of water, tissue softening, browning, and microbial contamination [6]. Additionally, they can add commercial value to food products by enhancing their appearance, while acting as carriers of functional ingredients, such as antioxidants, antimicrobial agents and nutraceuticals [7]. Edible films and coatings are defined as flexible films prepared from proteins, polysaccharides and lipids [8]. These serve as a barrier to water and gas transfer

and have multiple applications within the medical, pharmaceutical and food sectors [9]. The main role of these is to protect products from physical, chemical, or microbiological factors, and add functionality to food products, therefore influencing human health [10]. The maintenance of quality and safety, as well as extending shelf life, ensures product integrity throughout the food chain, from purchase to consumption [11,12]. Their use in highly perishable products such as fruits, vegetables, meat and fish is based on some specific properties, such as cost, availability, functional attributes, mechanical properties (flexibility, tension), optical properties (brightness and opacity), and structural resistance to water and microorganisms [13]. Polysaccharides play an important role within the food industry, by enhancing the quality, texture, mouthfeel and flavor of the food as thickeners, stabilizers, and texturizers [14,15]. One of the most interesting polysaccharides is kefiran, an extracellular polysaccharide with many hydroxyl groups, which is obtained from the microorganisms present in kefir grains and produced by several *Lactobacillus* species. These include *L. kefiranofaciens*, *L. kefirgranum, L. parakefiri, L. kefiri* and *L. delbrueckii subsp. Bulgaricus* [16]. Kefiran, mainly produced by *L. kefiranofaciens* [16], contains equal amounts of D-glucose and D-galactose in a chain sequence and improves the viscosity and viscoelastic properties of dairy products [17]. Kefiran is reported to have wound healing, antimicrobial, antifungal and antitumoral properties [18]. An advantage of kefiran compared to other polysaccharides is its antimicrobial activity [19] due to the lactic and acetic acids in its composition [20]. In comparison with other polysaccharides, it has some advantages due to its unique features, such as rheological behavior, biodegradability, biocompatibility, safety, emulsifier effect, stabilizing effect, resistance against hydrolysis, and water vapor permeability (WVP) [16,21,22]. In addition, previous studies have reported that it has excellent potential as a film-forming agent with good mechanical and barrier properties, and it can be an affordable alternative to synthetic packaging in food applications [23–28]. Biodegradable films generally present poor mechanical properties regarding processability and end-use application, because of stiffness and brittleness. To overcome this problem, plasticizers are added to provide the necessary workability to biopolymers [29]. The type and concentration of plasticizer employed have a significant effect on the physical, thermal, mechanical and barrier properties of films [30]. Glycerol is considered non-toxic and is the most common plasticizer in use in the food industry [31]. The concentration of glycerol depends on the targeted applications, and thus an optimal proportion of plasticizer is required in the solution to reduce intermolecular forces and water vapor transmission of the films [32]. Therefore, based on previous experiments conducted by our team [24,33], the objective of this work was to investigate the effect of different extraction conditions of kefiran on the structural and chemical properties of the edible films obtained.

2. Materials and Methods

2.1. Isolation and Purification of Kefiran

Kefiran was extracted from kefir grains (KJ), which were originally from the collection of the Walloon Agricultural Research Centre (CRA-W, Gembloux, Belgium). The kefir grains underwent freezing for at least 24 h at −20 °C prior to utilization. Fresh active kefir grains were dissolved in distilled water (in a weight ratio of 1:10). The extraction of kefiran was performed at four different temperatures: at 70 °C/100 min (F_70.100), 80 °C/30 min (F_80.30), 80 °C/100 min (F_80.100), 90 °C/20 min (F_90.20); 90 °C/100 min (F_90.100); 100 °C/5 min (F_100.5) and 100 °C/100 min (F_100.100).

The extraction parameters were established according to the methodology described by Pop et al. (2016) [33], thus that the temperature levels led to the total solubilization of the kefir grains' biomass. The aim of this study was to select the best combination of time and temperature for the extraction of kefiran used as a new film-forming material without the structure of the film being disrupted.

The mixture was then cooled and centrifuged at 10,000 *g* for 10 min. The polysaccharide dissolved in the supernatant was purified with an equal amount of chilled ethanol [34] by freezing at −20 °C overnight, followed by a slow thawing, avoiding the destruction of

the polysaccharide structure. After centrifugation at 5000 *g* for 10 min at 4 °C, the kefiran-rich pellets were dissolved in distilled water at 60 °C. The precipitation procedure was repeated twice. The kefiran solution obtained showed a high purity [14]. The experimental design for setting the extraction conditions followed that of Pop et al., 2013 [24].

The current experimental design was used to optimize the extraction conditions to obtain new films with properties fit for food purposes: physical, mechanical properties and structural characterization.

2.2. Preparation of Films

The preparation of films protocol was developed based on the protocol described by Piermaria et al., 2011 [35]; Ghasemlou et al., 2011 [21,36] and Motedayen et al., 2013 [37].

Vacuum (5–10 min) was used to degas the purified kefiran solution (10% final concentration), preventing pinholes from forming in the edible film structure. Further, an aqueous solution of kefiran 17–20 g was stirred constantly with the use of a magnetic stirrer for 10 min to a homogenous cast. The glycerol was used as a plasticizer because the edible 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, according to Ghasemlou et al., 2011 [21].

Glycerol by Sigma Chemical Co., St. Louis, MO, USA was incorporated at a concentration of 7.5% *w*/*w* based on the kefiran weight [21,35–38].

The mixture of kefiran and glycerol in a ratio of 7.5% was prepared under continuous agitation and with the casting of 17–20 g into micro-weighing dishes (65 mm diameter × 15 mm height). The Teflon plates were dried in an oven at 45 °C for approximately 18 h using an oven type C4051358 Memmert (Memmert GmbH + Co.KG). The filmogen characteristics of the several kefiran solutions obtained in different extraction conditions were tested for physical properties, barrier properties, and structural characterization.

2.3. Determination of Physical Properties of Films

2.3.1. Moisture Content

The moisture content of the sample was determined by measuring the weight loss of films before and after drying in an oven at 103 ± 2 °C until a constant weight was achieved; a C4051358 Memmert (Memmert GmbH + Co.KG) oven was used. The film samples were cut to approximately 1 cm × 3 cm.

For the samples, an aluminum dish (pan) was used, which had a 50 mm diameter, 40 mm depth and top-loading electronic balance, accurate to 0.1 mg. Two replications of each film treatment were used for calculating the moisture content.

The moisture content was calculated using the following equation:

$$\%\text{Moisture} = \frac{\text{Wi} - \text{Wf}}{\text{Wi}} \times 100 \quad (1)$$

where:

Wi = weight of wet sample in grams;
Wf = weight of dry sample in grams.

2.3.2. Water Solubility

To determine the water solubility, we used the method described by Motedayen et al., 2013 [37] and Ahmadi et al., 2012 [38]. Solubility in water is defined as the percentage of the total soluble matter (%TSM) of film that is solubilized after immersion in distilled water. The film samples (30 mm × 30 mm) were initially tested to determine the dry weight, which was called the initial dry weight. Further, dried film samples were directly immersed in 50 mL of distilled water at 25 °C for 6 h, under stable stirring (300 rpm). The obtained solution was then filtered through (Whatman 1) qualitative filter paper and the remaining pieces of film were removed and dried at 103 ± 2 °C to a constant weight (final dry weight). %TSM tests for each type of film were carried out in two replicates.

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

$$\%TSM = \frac{Wi - Wf}{Wi} \times 100 \quad (2)$$

where:

Wi = initial dry weight in grams;

Wf = final dry weight in grams.

2.3.3. Degree of Swelling

The degree of swelling of the films was determined according to Alves et al., 2011 [39] and Seixas et al., 2013 [8]. The total initial weight (Wi) of dried film samples (30 mm × 30 mm) was determined. The samples were pre-treated at 45 °C for 24 h and then immersed in distilled water for 24 h. After this period, the weight (Wf) of the samples was measured. Excess moisture on the sample surface was removed by placing the film between two sheets of filter paper before weighing. The degree of swelling (DS) was determined in terms of weight and volume, according to Equation (3) below:

$$DS(\%)_W = \frac{Wf - Wi}{Wi} \times 100 \quad (3)$$

2.3.4. Film Thickness

The thickness of the prepared films was measured with a digital caliper (Type POWERFIX Profi, Model No. Z22855F, Version 04/2010, Milomex, Bedfordshire, UK). Five thickness measurements were taken on each film sample at different points and the mean values were calculated as the average.

2.3.5. Water Vapor Permeability

WVP was determined gravimetrically according to Method E95-96 (ASTM, 1995b), as established by Ahmadi et al., 2012 [38], at 25 ± 2 °C. The WVP was calculated (Equation (4)) by using the thickness of each film. Each film sample of 30 mm × 30 mm (0.0009 m^2 film area) was placed in a cup, containing 25 g anhydrous calcium chloride desiccant (0% RH), and the cups mouths were sealed. Inside the desiccator was placed the saturated sodium chloride solution to maintain a gradient of relative humidity of approximately 60 ± 2%. The relative humidity and room temperature inside the desiccator were monitored over time using a thermo-hygrometer (Sper Scientific, Scottsdale, AZ, USA).

$$WVP = \frac{\Delta m}{A\Delta t}\frac{X}{\Delta P} \quad (4)$$

where $\Delta m/\Delta t$ is the weight of moisture gain per unit of time (g/s), A is the area of the exposed film surface (m^2), ΔP is the water vapor pressure difference between the two sides of the film (Pa) and X is the average film thickness (mm).

2.4. Determination of Films' Mechanical Properties

The mechanical properties of the film samples, including tensile strength (MPa) and elongation at break (%), were determined using a TA.TX plusC Texture Analyzer Stable Micro System with support tensile grip A/TG (Vienna Court, Lammas Road, Godalming, Surrey GU7 1YL, United Kingdom) according to the ASTM standard method D882 (ASTM, Annual Book of ASTM Standards, ASTM International, Philadelphia, PA, USA, 2004). The films were cut into strips (20 mm wide and 70 mm height) with an initial grip separation of 50 mm. Five specimens were tested for each sample. The results have been reported as the mean ± SD of 5 measurements.

2.5. Structural Characterization of Edible Films

2.5.1. FTIR Spectroscopy of Films Obtained from Kefiran Solution

The spectral analysis of film samples was recorded on a single reflection attenuated by a total reflection unit at room temperature using the FTIR spectrometer (Shimadzu, Kyoto, Japan). Each spectrum was recorded from 4000 to 800 cm^{-1} and was composed of an average of 64 separate scans with 4 cm^{-1} resolution. The films were analyzed without any preparation, as a layer directly on the ATR crystal. A background spectrum of air was scanned under the same instrumental conditions before each series of measurements. The spectra were processed using an IR solution Software Overview (Shimadzu, Kyoto, Japan) and OriginR 8SR1 Software (OriginLab Corporation, Northampton, MA, USA).

2.5.2. Scanning Electron Microscopy (SEM)

SEM micrographs have a large depth of field, yielding a three-dimensional appearance that is useful for understanding the structure of the sample. Film microstructure analysis was conducted by scanning electron microscopy (SEM), type FEI, Quanta 3D EGF. The samples of the edible film were mounted on carbon stubs using double-sided tape. They were sputter-coated with Pt/Pb to a layer of 10 nm under argon gas flow (Automatic Sputter Coater from Agar Scientific). A cross-section of each film was examined using an accelerating voltage of 30.0 kV. SEM analysis was performed for the kefiran film samples, which presented good physical and mechanical properties, and we derived one sample for each temperature level.

2.6. Statistical Analysis

ANOVA (analysis of variance) was used to compare the means using the SPSS 19.0 statistical analysis system and a Tukey HSD test with a confidence interval of 95 or 99%. Differences were considered significant at $p < 0.05$. The Tukey test was used to determine any significant differences between specific means (the differences among mean values of films' properties).

3. Results and Discussion

3.1. Physical and Mechanical Properties

One of the main challenges in obtaining edible films is the optimization of their physical characteristics. The uniformity of film thickness is evidenced by the low values of standard deviation ($p > 0.05$) presented in Table 1. The film thickness varied between 0.087 and 0.099 mm, and the values were dependent on the structural features of the kefiran solution used for the film preparation. The thickness of the films used as packaging is crucial for the protection of food [30]. The gas permeability and pest resistance of films may increase as the thickness of the films increases [40].

Table 1. Values of moisture content, thickness, swelling index, water solubility and mechanical properties of films.

Kefiran Film Type	Moisture Content (%)	Thickness (mm)	Swelling Index (%)	Solubility in Water (%)	Tensile Strength (MPa)	Elongation at Break (%)
F_70.100	24.03 ± 0.135 ^{d}	0.093 ± 0.008 ^{a}	109.87 ± 0.021 ^{e}	30.96 ± 0.167 ^{c}	12.01 ± 0.365 ^{d}	112.54 ± 0.986 ^{e}
F_80.30	19.72 ± 0.278 ^{a}	0.095 ± 0.010 ^{a}	54.77 ± 0.028 ^{a}	19.63 ± 0.726 ^{a}	18.45 ± 0.045 ^{f}	168.85 ± 0.436 ^{g}
F_80.100	23.29 ± 0.181 ^{c}	0.093 ± 0.015 ^{a}	112.46 ± 0.014 ^{f}	30.32 ± 0.033 ^{c}	8.46 ± 0.021 ^{c}	95.89 ± 1.12 ^{c}
F_90.20	20.65 ± 0.259 ^{b}	0.091 ± 0.007 ^{a}	68.55 ± 0.028 ^{b}	25.60 ± 0.191 ^{b}	14.13 ± 0.036 ^{e}	124.24 ± 0.89 ^{f}
F_90.100	24.74 ± 0.137 ^{d}	0.095 ± 0.013 ^{a}	112.06 ± 0.028 ^{f}	38.51 ± 0.223 ^{e}	6.56 ± 0.223 ^{b}	87.56 ± 1.43 ^{b}
F_100.5	25.83 ± 0.386 ^{e}	0.0.95 ± 0.015 ^{a}	83.04 ± 0.014 ^{c}	32.63 ± 0.214 ^{d}	8.89 ± 0.765 ^{c}	97.65 ± 1.65 ^{d}
F_100.100	26.28 ± 0.315 ^{f}	0.089 ± 0.004 ^{a}	105.78 ± 0.042 ^{d}	31.00 ± 0.306 ^{c}	5.45 ± 0.211 ^{a}	65.85 ± 1.98 ^{a}

Means with different letters within a column indicate significant differences ($p < 0.05$). Data reported are mean values (standard deviation).

The presence of moisture in the film can cause serious problems since it degrades the device reliability and deteriorates the interface adhesion properties between the dielectrics and the cap layer. Moisture resistance is, therefore, a reliability concern for the long-term stability of the edible film's layers [41]. Generally, the moisture of the films was low (Table 1); this helps the films to remain stable, as the capacity to increase the moisture within the films is dependent on the hydrophilicity of the plasticizer used. Our results are comparable with those obtained by Ghasemlou et al., 2011 [21], Sanyang et al., 2016 [30], and Galus and Kadzińska, 2016 [42]. The low value of moisture, a desirable property that could help the formulation to maintain its stability, reduces brittleness during long-term storage, decreases susceptibility to microbial contamination, and reduces bulkiness [43].

The moisture content increased significantly ($p < 0.05$) from 19.72% in sample F_80.30 to 26.28% in samples F_100.100, which indicates the different links between glycerol and kefiran solution, depending on the degree of its degradation due to the high-temperature extraction used. The level of interaction between the absorbed water molecules in the matrix structure is also dependent on the extraction parameters. The percentage of moisture varies due to the formation of hydrogen bonds in kefiran solutions extracted at high temperatures. Under extraction at 70 °C, the kefiran solution presents incomplete solubilization. As mentioned by Ren et al., 2008 [44], the effect of temperature on the properties of the polymer layer is due to the competition between the polymer–water and the water–water hydrogen bonds.

The moisture absorption of kefiran films after dipping them for 24 h in water is shown in Table 1. The results show that the film's capacity to absorb water is dependent on its structural integrity. After this test, all samples of edible films remained intact, which indicates the extraordinary ability of this biopolymer. However, further studies are required to take into consideration the multiple factors involved (e.g., texture of the products, atmosphere, etc.). The movement of water inside the film leads to the creation of osmotic pressure in the film. This concentration of water within the matrix of the film influences the balance between two forces that exist inside the film: extension and retraction. Therefore, the thickness of the films limits the concentration and depth of penetration of the water molecules in the polymer matrix, and also the overall swelling of the films [45]. According to the literature, the kefiran film shows very low swelling capacity. Kanmani and Lim, 2013 [46], reported that the swelling degree of the pure pullulan films was 22.15 ± 0.16% after a few minutes of immersion. For films of alginate and pectin, Seixas et al., 2013 [8], found values ranging from 109% to 218% after 36 min of immersion in water.

The solubility of the plasticized kefiran films obtained from various types of solutions increased significantly ($p < 0.01$) from 19.63% to 38.51%, as shown in Table 1. As presented by Seixas et al., 2013 [8], although in some applications the high solubility of the films may be desirable, a low-solubility edible film is one of the most important requirements for food and pharmaceutical applications. In the case of the kefiran films, a higher solubility was achieved when the kefiran solution used was extracted under aggressive conditions (high temperature and long-term maintenance).

Mechanical properties reflect the durability of films and their ability to enhance the mechanical integrity of foods [47]. Kefiran films showed a tensile strength (TS) ranging between 5.45 ± 0.211 MPa and 14.13 ± 0.036 MPa, and for elongation break (EB), the values were between 65.85 ± 1.98% and 168.85 ± 0.436%. These values are typical for biopolymer films. As can be observed in Table 1, the TS and EB values of the films were negatively affected by the extraction treatments. This factor significantly affected ($p < 0.05$) the films' mechanical properties and can be considered a solid base for the development of smart stimuli-responsive films, with good barriers and mechanical properties.

Ghasemlou et al. (2011) [21] reported that the TS values of the kefiran film extracted at 100 °C are lower than those of synthetic polymers, such as low-density polyethylene (LDPE) (9–17 MPa), polystyrene (35–55 MPa), and cellophane (114 MPa), and in terms of elongation at break, the film showed much higher values than cellophane (20%) and polystyrene (1%), but could not be stretched nearly as far as LDPE (500%). In our experiments, the kefiran films extracted at 80 °C for 30 min showed higher TS values than LDPE, and also presented the same tendency in their EB values as commercial films such as LDPE and HDPE.

3.2. Barrier Property—Water Vapor Permeability

The WVP values were evaluated over 14 days (Table 2), in order to monitor the edible film barrier's properties and establish if it can improve the fresh food stability and offer a shelf-life extension to certain foods. The kefiran films presented values of WVP between 0.93 and 6.39 × 10^{-11} g.m.m/m^2.s.Pa. The quality of the polysaccharide solution might increase the WVP; therefore, the polymer structures significantly affect the water vapor transfer property. The high values of WVP found are explained by the use of kefiran solutions, which show structural changes due to extraction conditions, and the extraction parameters have a significant effect on the barrier properties ($p < 0.05$).

Table 2. Barrier property of kefiran film obtained from the extracted solution under different extraction conditions.

Kefiran Film Type	WVP (×10^{-11} g.mm/m^2.s.Pa)		
	1 Day	7 Days	14 Days
F_70.100	2.15 ± 0.007 ^{cA}	2.80 ± 0.000 ^{cB}	4.028 ± 0.000 ^{dC}
F_80.30	0.93 ± 0.014 ^{aA}	0.99 ± 0.021 ^{aA}	1.05 ± 0.014 ^{aA}
F_80.100	2.27 ± 0.014 ^{cA}	2.56 ± 0.021 ^{cA}	3.46 ± 0.007 ^{cB}
F_90.20	1.77 ± 0.014 ^{bA}	1.87 ± 0.007 ^{bA}	1.90 ± 0.021 ^{bA}
F_90.100	3.09 ± 0.014 ^{dA}	3.35 ± 0.014 ^{dA}	5.89 ± 0.007 ^{eB}
F_100.5	3.43 ± 0.007 ^{eA}	3.63 ± 0.014 ^{eA}	6.17 ± 0.014 ^{fB}
F_100.100	3.54 ± 0.007 ^{eA}	3.81 ± 0.007 ^{eA}	6.39 ± 0.021 ^{fB}

The value of WVP represents the mean of two experiments ± standard deviations. Different capital letters within a row indicate significant differences among the same sample ($p < 0.01$) for 14 days. Different lower-case letters within columns indicate significant differences among the formulations ($p < 0.01$).

The lower value of WVP was obtained for the samples extracted at 80 °C/30 min. This result can be attributed to the development of a more compact structure, where glycerol had no power to increase the free volume and the water vapor diffusion through its structure. A significant increase in WVP values, depending on the time of the test, was observed in film samples obtained from the kefiran solution with the modified chemical structure due to the extraction conditions applied. Films of kefiran present as moderate barriers to WVP, according to the classification given by Ahmadi et al., 2012 [38], and Ghasemlou et al., 2011 [21,36,47]. The values reported by Ghasemlou et al., 2011 [21], for kefiran films tested at 75% RH after 24 h were 4.95 × 10^{-11} g/m.s.Pa for unplasticized film and between 5.55 and 5.88 × 10^{-11} g/m.s.Pa for the film with different glycerol concentrations (15–35%). Piermaria et al., 2009 [23] and Ghasemlou et al., 2011 [21,36] indicated that the kefiran films exhibited good water vapor barrier properties since the obtained values were lower than those reported for cellophane and other hydrocolloid films. The water vapor barrier properties of these films were similar to those of chitosan, a

cationic polysaccharide obtained as the by-product of the seafood industry [48]. Owing to the hydrophilicity of kefiran, the WVP values registered a gradual increase during the testing period. To prevent this drawback, Rad et al. (2017) [34] proposed an improvement in the protection against water vapor by adding a polymeric carbohydrate, such as starch.

3.3. Structural Characterization by Fourier-Transform Infrared Spectroscopy

The FTIR analysis of dried kefiran film samples was performed to evaluate the chemical structure and occurrence of changes in the polysaccharide films' structures in the presence of plasticizer. The IR spectrum of the kefiran film, as seen in Figure 1, contains four major absorption zones: 1—3500–3200 cm^{-1}, 2—3000–2800 cm^{-1}, 3—1700–1540 cm^{-1} and 1230–870 cm^{-1}. These were similar to the results obtained in a previous study [24]. In the first region, the spectrum showed that the relative absorption intensities of the bands assigned to water molecules were higher. The peak at this zone is attributed to the O-H stretching vibration that overlaps the N-H stretching vibration in the same region.

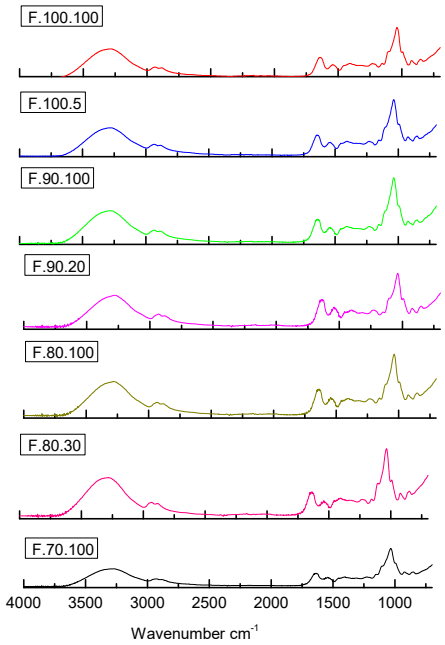

Figure 1. FTIR spectra of the kefiran films.

The area of the bands between 3500 and 3100 cm^{-1} increased with the increase in glycerol concentrations, confirming the higher intensity of O-H bonds. The area of the bands closest to those at ca. 2720 cm^{-1} and ca. 2900 cm^{-1} corresponds to C-H stretching vibration, and in this zone a decrease is also evident [49]. The increase in glycerol concentration leads to higher numbers of O-H bonds and consequently higher band areas, also resulting in a decrease in the areas that correspond to the C-H bonds [34,50]. The next region, 3000–2800 cm^{-1}, is representative of the bands attributed to the symmetric and anti-symmetric stretching modes of C-H in the methyl (CH_3) and methylene (CH_2) functional groups [35]. Additionally, the most relevant peak at 2925 cm^{-1} can be related to the symmetric and asymmetric stretching vibration of the aliphatic group (CH_2) [45]. At about 2850 and 2917 cm^{-1}, the bands are attributed to C-H stretching, and the peak intensity decrease is dependent on the addition of glycerol into kefiran films, which might be due to the disruption on the kefiran film's structure. This is due to the presence of plasticizer molecules or the water molecules that they absorb, which "hide" the C-H bond of the carbohydrate rings and thus reduce the C-H absorbance bands' contribution to the film's spectra. These plasticizer molecules might reduce the interaction between the adjacent chains of the kefiran polymer. The possible conformational changes in kefiran structure, due to the interspersing of the plasticizers, and/or the water molecules that

they absorb, could contribute to producing flexible kefiran films in the case of glycerol or improving the water vapor barrier [35].

The third region (1700–1540 cm^{-1}) is an area specific to water molecules, assigned to the bending mode of O-H.

The relative absorption intensities of the bands assigned to water molecules depend on the concentration in glycerol and also on the function of the quality of the kefiran solution that is shown in Figure 1. The rheological properties of the kefiran solution are given by the extraction conditions, which reflect the quality of the film. The region located between 1230 and 870 cm^{-1} is particular for each polysaccharide. This region is dominated by ring vibrations overlapped with stretching vibrations of C–O-H side groups and C–O–C glycosidic band vibration [35,51]. The presence of glycerol also leads to changes in the band region ranging between 800 and 1200 cm^{-1}, which gives information about the alcoholic (C-O) stretching bands and the asymmetric and symmetric (C-O-C) stretching vibrations bands [19]. Moreover, the broadband at ca. 920 cm^{-1} that also corresponds to the stretching vibration of C-O in the C-O-C bond only appears in the films containing glycerol, confirming the more intense C-O stretching vibration in these films' structures [50,52]. According to Piermaria et al., 2011 [35], the relationship between the physicochemical properties and the FT-IR spectrum of edible films with potential application in the food industry depends on water–matrix and plasticizer interactions.

3.4. Structure Characterization by SEM

A microstructural study of the films offers relevant information about the arrangement of the components, allowing us a better understanding of water vapor transmission mechanisms and improving the structure [53]. The structural composition of films is a very important characteristic, as it also determines many properties of biodegradable materials [54]. Figure 2 shows the scanning electron micrographs of the cross-sections of kefiran films.

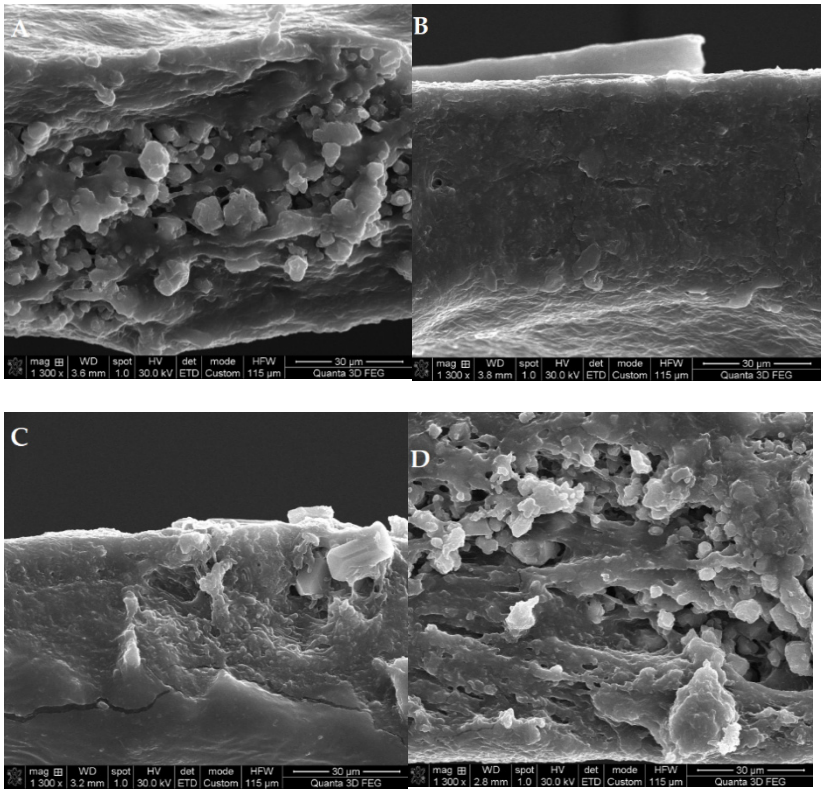

Figure 2. Cross-section of kefiran films plasticized with 7.5% glycerol, as a function of the type of film-forming solution ((**A**)—F_70.100; (**B**)—F_80.30; (**C**)—F_90.20; (**D**)—F_100.5). Magnification is indicated in the micrographs.

As observed by SEM, the kefiran films show structural differences, dependent on the quality of the extract. The kefiran film denoted as F_80.30 (Figure 2B) has a smooth structure, is homogeneous and free of air bubbles, and represents a continuous matrix without any cracks and with good integrity. In the study conducted by Rad et al., 2018, the samples of waterborne polyurethane and pure kefiran films showed uniform morphology without pores or cracks [34]. The rest of the samples have a porous structure. The samples represented in Figure 2A,D displayed more channels able to retain air. This fact can be explained by the temperature profiles that substantiate a structural change in the polysaccharide. Molecular weight plays an important role in obtaining a film with a homogeneous structure. The high-molecular-weight polysaccharides, being hydrophilic, do not have much of a tendency to adsorb at the air–water interface; therefore, they lead to the formation of films with stable and homogeneous structures [55]. Regarding the results obtained in previous research [33], the molecular mass of the kefiran solution varied between 15.20×10^6 Dalton for sample T80.30 and 2.49×10^6 for sample T100.100. As observed previously, kefiran exhibited higher values of molecular mass, which were attributed to kefiran samples that did not undergo degradation. The degradation is mainly due to aggressive extraction conditions, temperatures above 80 °C, and long-term maintenance at such temperatures.

After testing the plasticizing ability of sugars and polyols when obtaining the kefiran films, Piermaria et al., 2011 [35], reported that there were no significant differences between plasticizers. A similar trend was observed by Ghasemlou et al., 2011 [21,36,47], for sorbitol and glycerol, which are plasticizers in kefiran films. Therefore, the kefiran film can be produced with glycerol as a plasticizer, with good effects on the physical and structural properties. Data from this study may provide new formulation options for developing antimicrobial packaging films based on kefiran polysaccharide, using bioactive molecules to improve the microbiological safety and quality of food.

4. Conclusions

Based on the results obtained in this study, the kefiran solution extracted at 80 °C could be used as new film-forming material. Kefiran films plasticized with glycerol (7.5% (*w*/*w*)) were successfully prepared by the casting method, showing good film-forming properties, transparency, and a homogeneous surface. Regarding the water vapor permeability and the physical properties, the results indicate that the extraction conditions have a major influence on the quality of this polysaccharide's structure and, therefore, on the film's structure. Thus, the value of the swelling index and the solubility in water of the edible film based on kefiran increase along with the temperature of the extraction. The FT-IR spectra show that when a high temperature is used for kefiran extraction, the structure of the film disrupts its architecture. Based on the microstructures observed in the SEM analysis, it was concluded that the extraction process is a significant contributing factor in the properties of these films and their applications. The properties of kefiran films used in food packaging applications can be further improved by the incorporation of bioactive compounds, such as phenolic antioxidants.

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References

1. Mihalca, V.; Kerezsi, A.D.; Weber, A.; Gruber-Traub, C.; Schmucker, J.; Vodnar, D.C.; Dulf, F.V.; Socaci, S.A.; Fărcaș, A.; Mureșan, C.I. Protein-Based Films and Coatings for Food Industry Applications. *Polymers* **2021**, *13*, 769. [CrossRef]
2. Socaci, S.A.; Rugină, D.O.; Diaconeasa, Z.M.; Pop, O.L.; Fărcaș, A.C.; Păucean, A.; Tofană, M.; Pintea, A. Antioxidant compounds recovered from food wastes. In *Functional Food-Improve Health through Adequate Food*; Intech Open: Rijeka, Croatia, 2017.
3. Ncube, L.K.; Ude, A.U.; Ogunmuyiwa, E.N.; Zulkifli, R.; Beas, I.N. Environmental impact of food packaging materials: A review of contemporary development from conventional plastics to polylactic acid based materials. *Materials* **2020**, *13*, 4994. [CrossRef] [PubMed]
4. Han, J.W.; Ruiz-Garcia, L.; Qian, J.P.; Yang, X.T. Food Packaging: A Comprehensive Review and Future Trends. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 860–877. [CrossRef]
5. Vodnar, D.C.; Pop, O.L.; Dulf, F.V.; Socaciu, C. Antimicrobial efficiency of edible films in food industry. *Not. Bot. Horti. Agrobot. Cluj-Napoca* **2015**, *43*, 302–312. [CrossRef]
6. Pop, O.L.; Pop, C.R.; Dufrechou, M.; Vodnar, D.C.; Socaci, S.A.; Dulf, F.V.; Minervini, F.; Suharoschi, R. Edible films and coatings functionalization by probiotic incorporation: A review. *Polymers* **2020**, *12*, 12. [CrossRef] [PubMed]
7. Sapper, M.; Chiralt, A. Starch-based coatings for preservation of fruits and vegetables. *Coatings* **2018**, *8*, 152. [CrossRef]
8. Seixas, F.L.; Turbiani, F.R.B.; Salomão, P.G.; Souza, R.P.; Gimenes, M.L. Biofilms composed of alginate and pectin: Effect of concentration of crosslinker and plasticizer agents. *Chem. Eng. Trans.* **2013**, *32*, 1693–1698. [CrossRef]
9. Díaz-Montes, E.; Castro-Muñoz, R. Edible Films and Coatings as Food-Quality Preservers: An Overview. *Foods* **2021**, *10*, 249. [CrossRef]
10. Pop, O.L.; Vodnar, D.C.; Diaconeasa, Z.; Istrati, M.; Bințințan, A.; Bințințan, V.V.; Suharoschi, R.; Gabbianelli, R. An Overview of Gut Microbiota and Colon Diseases with a Focus on Adenomatous Colon Polyps. *Int. J. Mol. Sci.* **2020**, *21*, 7359. [CrossRef]
11. Puscaselu, R.; Gutt, G.; Amariei, S. The use of edible films based on sodium alginate in meat product packaging: An eco-friendly alternative to conventional plastic materials. *Coatings* **2020**, *10*, 166. [CrossRef]
12. Valdés, A.; Ramos, M.; Beltrán, A.; Jiménez, A.; Garrigós, M.C. State of the Art of Antimicrobial Edible Coatings for Food Packaging Applications. *Coatings* **2017**, *7*, 56. [CrossRef]
13. Chawla, R.; Sivakumar, S.; Kaur, H. Antimicrobial edible films in food packaging: Current scenario and recent nanotechnological advancements—A review. *Carbohydr. Polym. Technol. Appl.* **2021**, *2*, 100024. [CrossRef]
14. Ramalingam, C.; Priya, J.; Mundra, S. Applications of microbial polysaccharides in food industry. *Int. J. Pharm. Sci. Rev. Res.* **2014**, *27*, 322–324.
15. Jindal, N.; Singh Khattar, J. *Microbial Polysaccharides in Food Industry*; Elsevier Inc.: Amsterdam, The Netherlands, 2018; ISBN 9780128115015.
16. Moradi, Z.; Kalanpour, N. Kefiran, a branched polysaccharide: Preparation, properties and applications: A review. *Carbohydr. Polym.* **2019**, *223*, 115100. [CrossRef]
17. Bengoa, A.A.; Iraporda, C.; Garrote, G.L.; Abraham, A.G. Kefir micro-organisms: Their role in grain assembly and health properties of fermented milk. *J. Appl. Microbiol.* **2019**, *126*, 686–700. [CrossRef]
18. Bodea, I.M.; Muste, A.; Cătunescu, G.M.; Mureşan, C. Bacterial biofilms as wound healing dressing—A review. *Sci. Work. Ser. C Vet. Med.* **2017**, *63*, 55–64.
19. Nešić, A.; Cabrera-Barjas, G.; Dimitrijević-Branković, S.; Davidović, S.; Radovanović, N.; Delattre, C. Prospect of polysaccharide-based materials as advanced food packaging. *Molecules* **2020**, *25*, 135. [CrossRef] [PubMed]
20. Prado, M.R.; Blandón, L.M.; Vandenberghe, L.P.S.; Rodrigues, C.; Castro, G.R.; Thomaz-Soccol, V.; Soccol, C.R. Milk kefir: Composition, microbial cultures, biological activities, and related products. *Front. Microbiol.* **2015**, *6*, 1–10. [CrossRef]
21. Ghasemlou, M.; Khodaiyan, F.; Oromiehie, A.; Yarmand, M.S. Development and characterisation of a new biodegradable edible film made from kefiran, an exopolysaccharide obtained from kefir grains. *Food Chem.* **2011**, *127*, 1496–1502. [CrossRef]
22. Piermaría, J.; Bengoechea, C.; Abraham, A.G.; Guerrero, A. Shear and extensional properties of kefiran. *Carbohydr. Polym.* **2016**, *152*, 97–104. [CrossRef]
23. Piermaria, J.A.; Pinotti, A.; Garcia, M.A.; Abraham, A.G. Films based on kefiran, an exopolysaccharide obtained from kefir grain: Development and characterization. *Food Hydrocoll.* **2009**, *23*, 684–690. [CrossRef]
24. Pop, C.; Apostu, S.; Rotar, A.M.; Semeniuc, C.; Marianne Sindic, M.; Mabon, N. FTIR spectroscopic characterization of a new biofilm obtained from kefiran. *J. Agroaliment. Process. Technol.* **2013**, *19*, 157–159.
25. Marangoni Júnior, L.; Vieira, R.P.; Anjos, C.A.R. Kefiran-based films: Fundamental concepts, formulation strategies and properties. *Carbohydr. Polym.* **2020**, *246*. [CrossRef]
26. Tan, K.X.; Chamundeswari, V.N.; Loo, S.C.J. Prospects of kefiran as a food-derived biopolymer for agri-food and biomedical applications. *RSC Adv.* **2020**, *10*, 25339–25351. [CrossRef]
27. Matos, R.S.; Ramos, G.Q.; da Fonseca Filho, H.D.; Ţălu, Ş. Advanced micromorphology study of microbial films grown on Kefir loaded with Açaí extract. *Micron* **2020**, *137*, 102912. [CrossRef]
28. Zolfi, M.; Khodaiyan, F.; Mousavi, M.; Hashemi, M. Development and characterization of the kefiran-whey protein isolate-TiO_2 nanocomposite films. *Int. J. Biol. Macromol.* **2014**, *65*, 340–345. [CrossRef] [PubMed]

29. Vieira, M.G.A.; Da Silva, M.A.; Dos Santos, L.O.; Beppu, M.M. Natural-based plasticizers and biopolymer films: A review. *Eur. Polym. J.* **2011**, *47*, 254–263. [CrossRef]
30. Sanyang, M.L.; Sapuan, S.M.; Jawaid, M.; Ishak, M.R.; Sahari, J. Effect of plasticizer type and concentration on tensile, thermal and barrier properties of biodegradable films based on sugar palm (Arenga pinnata) starch. *Polymers* **2015**, *7*, 1106–1124. [CrossRef]
31. Tian, H.; Guo, G.; Xiang, A.; Zhong, W.H. Intermolecular interactions and microstructure of glycerol-plasticized soy protein materials at molecular and nanometer levels. *Polym. Test.* **2018**, *67*, 197–204. [CrossRef]
32. Basiak, E.; Lenart, A.; Debeaufort, F. How glycerol and water contents affect the structural and functional properties of starch-based edible films. *Polymers* **2018**, *10*, 412. [CrossRef]
33. Pop, C.R.; Salanţă, L.; Rotar, A.M.; Semeniuc, C.A.; Socaciu, C.; Sindic, M. Influence of extraction conditions on characteristics of microbial polysaccharide kefiran isolated from kefir grains biomass. *J. Food Nutr. Res.* **2016**, *55*, 121–130.
34. Rad, F.H.; Sharifan, A.; Asadi, G. Miscibility and morphology of kefiran/waterborne polyurethane blend films. *Int. J. Food Prop.* **2018**, *20*, S2764–S2775. [CrossRef]
35. Piermaria, J.; Bosch, A.; Pinotti, A.; Yantorno, O.; Garcia, M.A.; Abraham, A.G. Kefiran films plasticized with sugars and polyols: Water vapor barrier and mechanical properties in relation to their microstructure analyzed by ATR/FT-IR spectroscopy. *Food Hydrocoll.* **2011**, *25*, 1261–1269. [CrossRef]
36. Ghasemlou, M.; Khodaiyan, F.; Oromiehie, A.; Yarmand, M.S. Characterization of edible emulsified films with low affinity to water based on kefiran and oleic acid. *Int. J. Biol. Macromol.* **2011**, *49*, 378–384. [CrossRef]
37. Motedayen, A.A.; Khodaiyan, F.; Salehi, E.A. Development and characterisation of composite films made of kefiran and starch. *Food Chem.* **2013**, *136*, 1231–1238. [CrossRef]
38. Ahmadi, R.; Kalbasi-Ashtari, A.; Oromiehie, A.; Yarmand, M.S.; Jahandideh, F. Development and characterization of a novel biodegradable edible film obtained from psyllium seed (Plantago ovata Forsk). *J. Food Eng.* **2012**, *109*, 745–751. [CrossRef]
39. Alves, V.D.; Ferreira, A.R.; Costa, N.; Freitas, F.; Reis, M.A.M.; Coelhoso, I.M. Characterization of biodegradable films from the extracellular polysaccharide produced by Pseudomonas oleovorans grown on glycerol byproduct. *Carbohydr. Polym.* **2011**, *83*, 1582–1590. [CrossRef]
40. Allahvaisi, S. Polypropylene in the industry of Food Packaging. In *Polypropylene*; Dogan, F., Ed.; IntechOpen: London, UK, 2012; pp. 1–20. ISBN 978-953-51-0636-4.
41. Parreidt, T.S.; Müller, K.; Schmid, M. Alginate-based edible films and coatings for food packaging applications. *Foods* **2018**, *7*, 170. [CrossRef]
42. Galus, S.; Kadzińska, J. Moisture sensitivity, optical, mechanical and structural properties of whey protein-based edible films incorporated with rapeseed oil. *Food Technol. Biotechnol.* **2016**, *54*, 78–89. [CrossRef]
43. Sánchez-Maldonado, A.F.; Lee, A.; Farber, J.M. Methods for the Control of Foodborne Pathogens in Low-Moisture Foods. *Annu. Rev. Food Sci. Technol.* **2018**, *9*, 177–208. [CrossRef]
44. Ren, C.L.; Nap, R.J.; Szleifer, I. The role of hydrogen bonding in tethered polymer layers. *J. Phys. Chem. B* **2008**, *112*, 16238–16248. [CrossRef]
45. Castel, A.; Gutfreund, P.; Cabane, B.; Rharbi, Y. Swelling, dewetting and breakup in thin polymer films for cultural heritage. *Soft Matter* **2020**, *16*, 1485–1497. [CrossRef] [PubMed]
46. Kanmani, P.; Lim, S.T. Development and characterization of novel probiotic-residing pullulan/starch edible films. *Food Chem.* **2013**, *141*, 1041–1049. [CrossRef]
47. Ghasemlou, M.; Khodaiyan, F.; Oromiehie, A. Physical, mechanical, barrier, and thermal properties of polyol-plasticized biodegradable edible film made from kefiran. *Carbohydr. Polym.* **2011**, *84*, 477–483. [CrossRef]
48. Santos, V.P.; Marques, N.S.S.; Maia, P.C.S.V.; de Lima, M.A.B.; Franco, L.D.O.; de Campos-Takaki, G.M. Seafood waste as attractive source of chitin and chitosan production and their applications. *Int. J. Mol. Sci.* **2020**, *21*, 4290. [CrossRef] [PubMed]
49. Cui, F.J.; Qian, L.S.; Sun, W.J.; Zhang, J.S.; Yang, Y.; Li, N.; Zhuang, H.N.; Wu, D. Ultrasound-assisted extraction of polysaccharides from Volvariella volvacea: Process optimization and structural characterization. *Molecules* **2018**, *23*, 1706. [CrossRef]
50. Cerqueira, M.A.; Souza, B.W.S.; Teixeira, J.A.; Vicente, A.A. Effect of glycerol and corn oil on physicochemical properties of polysaccharide films—A comparative study. *Food Hydrocoll.* **2012**, *27*, 175–184. [CrossRef]
51. Figueroa-Lopez, K.J.; Andrade-Mahecha, M.M.; Torres-Vargas, O.L. Development of antimicrobial biocomposite films to preserve the quality of bread. *Molecules* **2018**, *23*, 212. [CrossRef]
52. Pelissari, F.M.; Grossmann, M.V.E.; Yamashita, F.; Pined, E.A.G. Antimicrobial, mechanical, and barrier properties of cassava starch-chitosan films incorporated with oregano essential oil. *J. Agric. Food Chem.* **2009**, *57*, 7499–7504. [CrossRef] [PubMed]
53. Müller, K.; Bugnicourt, E.; Latorre, M.; Jorda, M.; Sanz, Y.E.; Lagaron, J.M.; Miesbauer, O.; Bianchin, A.; Hankin, S.; Bölz, U. Review on the processing and properties of polymer nanocomposites and nanocoatings and their applications in the packaging, automotive and solar energy fields. *Nanomaterials* **2017**, *7*, 74. [CrossRef] [PubMed]
54. Azucena Castro-Yobal, M.; Contreras-Oliva, A.; Saucedo-Rivalcoba, V.; Rivera-Armenta, J.L.; Hernández-Ramírez, G.; Salinas-Ruiz, J.; Herrera-Corredor, A. Evaluation of physicochemical properties of film-based alginate for food packing applications. *E-Polymers* **2021**, *21*, 82–95. [CrossRef]
55. Bealer, E.J.; Onissema-karimu, S.; Rivera-galletti, A.; Francis, M.; Wilkowski, J.; Hu, X. Protein–Polysaccharide Composite Materials. *Polymers* **2020**, *12*, 464. [CrossRef]