Vol. 21, No. 3(2022) Alim2917
Ingeniería de alimentos
Revista Mexicana de Ingeniería Química

Effect of the storage relative humidity on the physicochemical properties of corn starch edible films obtained by a combination of extrusion process and casting technique

Efecto de la humedad relativa de almacenamiento sobre las propiedades fisicoquímicas de películas comestibles de almidón de maíz obtenidas por la combinación de la tecnología de extrusión y técnica de casting

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Received: August 12, 2022; Accepted: October 1, 2022

Abstract

Starch is one of the most used polysaccharides in the formulation of Edible Films (EFs). This work aimed to develop starch-based EFs employing extrusion technology as a pre-treatment to casting technique and evaluate the storage relative humidity (RH) effect to determine their possible application as food coating. Corn starch and a mixture of plasticizers (sorbitol and glycerol) were processed in a twin-screw extruder. The casting technique was used for EFs formation. The EFs were conditioned at different storage RH (53, 75, and 100%) for ten weeks. The studied response variables were: puncture resistance (PR), puncture deformation (PD), water vapor permeability (WVP), water solubility (WS), X-ray diffraction patterns, and relative crystallinity. EFs at 53 and 75% RH showed the most stable behavior in PR and PD. Concerning the barrier properties, it was found that EFs stored at 53% RH had the lowest value of WVP and XRD patterns showing that storage time produces crystalline zones by the aging effect. Starch-based EFs obtained by the combination of extrusion-casting and stored at an RH of 53 and 75% presented the best physicochemical properties. Hence, these EFs could be used as coatings of intermediate moisture foods.

Keywords: corn starch, edible films, extrusion technology-casting technique, storage relative humidity, physicochemical properties.

Resumen

El almidón es uno de los polisacáridos más utilizados en la formulación de Películas Comestibles (PC). Este trabajo tuvo como objetivo desarrollar PC a base de almidón empleando tecnología de extrusión como pretratamiento a la técnica de casting y evaluar el efecto de la humedad relativa (HR) de almacenamiento para determinar su posible aplicación como recubrimiento de alimentos. Utilizando un extrusor de doble tornillo, se procesó almidón de maíz y una mezcla de plastificantes (sorbitol y glicerol). Se utilizó la técnica de casting para la formación de PC. Las PC se acondicionaron a diferentes HR de almacenamiento (53, 75 y 100%) durante diez semanas. Las variables de respuesta estudiadas fueron: resistencia a la punción (RP), deformación por punción (DP), permeabilidad al vapor de agua (PVA), solubilidad en agua (SA), patrones de difracción de rayos X y cristalinidad relativa. Las PC al 53 y 75% de HR mostraron el comportamiento más estable en RP y DP. Con respecto a las propiedades de barrera, se encontró que las PC almacenadas a una HR de 53% registraron el valor más bajo de PVA. Los patrones XRD mostraron que el tiempo de almacenamiento produce zonas cristalinas por efecto del envejecimiento. Las PC a base de almidón obtenidas por la combinación de extrusión-casting y almacenadas a una HR de 53 y 75% presentaron las mejores propiedades fisicoquímicas. Por lo tanto, estas PC podrían emplearse como recubrimientos en alimentos de humedad intermedia.

Palabras clave: almidón de maíz, películas comestibles, tecnología de extrusión-técnica de casting, humedad relativa de almacenamiento, propiedades fisicoquímicas.

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https://doi.org/10.24275/rmiq/Alim2917
ISSN:1665-2738, *issn-e*: 2395-8472

1 Introduction

The increasing environmental pollution and plastic disposal have generated significant interest in replacing synthetic plastics with environmentally friendly alternatives from natural resources (Piñeros-Guerrero *et al*., 2021). Edible films (EFs) are thin layers of biodegradable materials applied to food products that play an important role in their preservation, distribution, and marketing (Fakhouri *et al*., 2015). These materials act as barriers producing modified atmospheres, reducing moisture exchange, controlling microbial growth, and carrying functional ingredients. The most common polymers used in the formulation of EFs are proteins, lipids, and polysaccharides which are used alone or combined (Dai *et al*., 2015; Fitch-Vargas *et al*., 2016; Valdez-Fragoso *et al*., 2015).

Starch has been widely used in the formulation of EFs due to its availability, biodegradability, and relatively low price (Fakhouri *et al*., 2015; Garcia *et al*., 2000b; Medrano-de Jara *et al*., 2020). However, further modification is usually necessary to overcome several disadvantages of starch-based EFs, such as their hydrophilicity and poor mechanical properties compared to synthetic polymers (Dai *et al*., 2015). Chemical, enzymatic and physical processes are frequently used to modify starches' molecular structure (Shah *et al*., 2016). Physical treatments generally change starch polymer molecules packing arrangements (BeMiller & Huber, 2015). Physical modification has been gaining wider acceptance because of the absence of chemical reagents in the modified starch. In this sense, an adequate technology to produce physically modified starch is the extrusion process.

Twin-screw extruder could be used to produce modified starch in a continuous process with a consistent product of better quality. The processing of starch implicates its transformation from a native granular state to a molten state (Xie *et al*., 2012). Under specific extrusion conditions and with the help of a plasticizer, such as glycerol or sorbitol, thermoplastic starch (TPS) can be obtained after the disruption and plasticization of native starch. TPS is considered one of the most attractive materials due to its low cost and biodegradability (Piñeros-Guerrero *et al*., 2021). Several authors have investigated the use of TPS in producing EFs and reported that these presented better properties than those obtained from the native polymers (Bohórquez-Ayala *et al*., 2021; Mendes *et al*., 2016; Piñeros-Guerrero *et al*., 2021). On the other hand, studies on obtaining films suggest that the casting technique is the traditional procedure applied in research areas (Cheng *et al*., 2021). The casting technique begins with a solution containing the polymer former, and heating with excess water is applied. The solution is spilled out into plates. After that, it is dried under specific temperature conditions and Relative Humidity (RH) (Cazón & Vázquez, 2020). Therefore, according to Fitch-Vargas *et al*. (2016) and Calderón-Castro *et al*. (2018), combining the casting technique with the extrusion process as a pre-treatment for the native starch physical modification is an alternative to obtain EFs with good functional properties.

In addition, it has been reported that EFs, when exposed to specific relative humidity or storage time, may undergo physical and chemical changes, resulting in alterations in their barrier and mechanical properties (Akbari *et al*., 2007; Chinma *et al*., 2015). Acosta *et al*. (2015) explained that starch-based films are greatly affected by aging (defined by the storage time), promoting significant physicochemical changes in their functionality. Aging involves chain rearrangement, reduces mobility, and is an inherent characteristic of the amorphous phase of polymers. Physical aging can be understood as a slow reorganization of polymer chains. The free volume of the thermoplastic matrix changes with time because of the alteration in packing density, segmental mobility, and interstitial space among the chains (Ranganathaiah *et al*., 2002). The rate and length of aging depend on factors such as chain length, the concentration of starch, system pH and composition, time, temperature, and storage RH (Famá *et al*., 2007). In previous works, it was found that crystallization or reorientation of starch molecules produces changes in the physical and mechanical properties, glass transition temperature, water content, and plasticizer migration of EFs (Apriyana *et al*., 2016; Famá *et al*., 2007; Garcia *et al*., 2000b). Nevertheless, more information is needed concerning the alterations in the physicochemical properties of starch-based EFs during storage time and different humidity conditions.

Previous research found that cornstarch-based EFs with improved mechanical and barrier properties can be obtained by employing the extrusion process and the casting technique (Fitch-Vargas *et al*., 2016). Hence, to give continuity to this research, this work aimed to evaluate the effect of RH and storage time on starch-based EFs obtained by employing the combination of extrusion technology and casting technique to determine their possible application as food coatings.

2 Materials and methods

2.1 Materials

Corn starch with an amylose/amylopectin ratio of 25.58/74.72 was employed as thermoplastic material and provided by Ingredion (IL, USA). Glycerol and sorbitol were employed as plasticizers and supplied by JT Baker® (PA, USA) and Cedrosa S.A. de CV (Edo. Mexico, Mexico), respectively.

2.2 Starch-based EFs preparation

The starch-based EFs were obtained by employing the extrusion process and the casting technique according to the previous research by Fitch-Vargas *et al*. (2016). The blend was obtained by mixing 80% corn starch and 20% plasticizers (sorbitol and glycerol, 79.7 and 20.3%, respectively) and adjusted to the moisture of 20.0±1.0%. A twin-screw extruder (Shandong Light M&E, Model LT32L, China) with a screw compression ratio of 2:1, L/D=18.5, and a circular die with 42 mm diameter, 19 mm length, and 5 mm of output was employed. The feed rate was maintained constant at 22.8 g/min. The temperature profile was 70-90-89 °C (the three heating zones were air-cooled and independently electrically heated), and the screw speed was 66 rpm. The TPS was collected in water (ratio of 1:4) to avoid its retrogradation. The final product was named extruded mixture (EM).

A casting technique was employed to obtain the EFs. 300 mL of EM were heated on a plate (Fisher Scientific, Waltham, MA, USA) at 80°C and stirred for 10 min. Subsequently, 25 mL of EM were spilled out for drying into acrylic molds and placed in a convection oven at 60°C for two hours. Finally, the film's thickness was measured by a digital micrometer (Digital Insize, Model 3109-25A, Spain), recording values of 50±5 μm.

2.3 Stability analysis

In order to know the stability and the possible application of the starch-based EFs, the effect of the relative humidity (RH) and storage time were evaluated. The EFs were conditioned in different desiccators containing $Mg(NO_3)_2$ saturated solution (RH 53%), NaCl saturated solution (RH 75%), and deionized water (RH 100%) for ten weeks. The storage time was defined based on preliminary experiments. There were four treatments, EFs53, EFs75, EFs100, and EFsT0 (EFs at time zero). The studied variables were: puncture resistance, puncture deformation, water vapor permeability, water-solubility, X-ray diffraction patterns, and relative crystallinity.

2.4 Physicochemical and microstructural characterization

2.4.1 Puncture test

Puncture resistance (PR) and puncture deformation (PD) were evaluated according to the methodology reported by Socaciu *et al*. (2020). An universal texture analyzer (INSTRON, Model 3342, Mass., U.S.A.) was employed to evaluate the maximum force changes before the break (Newtons, N) and the distance from the sample contact until its break (millimeters, mm). The puncture head with a cylindrical rod of 10 mm diameter and a constant speed of 1 $mm \cdot s^{-1}$ was employed. Twenty replicates were made per treatment.

2.4.2 Water vapor permeability (WVP)

The WVP was determined using the gravimetric method described by Fakhouri *et al*. (2015). EFs were fixed on the top of glass containers with 15 g of anhydrous calcium chloride (JT Baker®, PA, USA) to produce an RH of 0%. The containers were placed into a desiccator (Dry Keeper, Sanplatec Corp., Osaka, Japan) with a saturated solution of sodium chloride (RH=75%). The weight gained by the calcium chloride was recorded by quintuplicate every 12 hours for four days. WVP was calculated according to Eq. (1):

$$WVP = \frac{Mp \times E}{A \times t \times \Delta p} \tag{1}$$

Where: Mp = absorbed moisture mass (g), E = EFs thickness (m), t = time (s), A = exposed film area (m^2) and Δp = partial pressure difference through the film (Pa).

2.4.3 Water solubility (WS)

The water solubility of EFs provides information about their integrity in an aqueous medium. The WS was determined following the methodology reported by Chiumarelli and Hubinger (2014). Per treatment, five repetitions were done. The WS was calculated by employing Eq. 2:

$$WS = \frac{w_i - w_f}{w_i} \times 100 \tag{2}$$

Where: w_i = initial sample weight, and w_f = final sample weight.

2.4.4 X-ray diffraction (XRD)

Small pieces of edible films were packed into a glass holder with 0.5 mm depth and placed on an X-ray diffractometer (Rigaku Model Last D/Max-2100, Rigaku Denki Co. Ltd., Japan). Employing a sweep angle of Bragg of 4-60° over a scale of 2θ with intervals of 0.02, operating at 16 mA and 30 kV, with a wavelength $\lambda = 1.5406$ Å and CuKα radiation, diffractograms were obtained. The relative crystallinity (RC) was calculated as crystalline area/total area × 100 according to Herman's method, described by Gomez *et al*. (1989)

2.5 Statistical analysis

For PR and PD data analysis, a completely randomized factorial experimental design with two factors was used, RH (Factor A) and Storage Time (Factor B). Factor A levels were 53, 75, and 100%, and Factor B levels were 0, 2, 4, 6, 8, and 10 weeks. For WVP, S, and RC, a unifactorial design was employed. The factor for WVP and S was RH (53, 75, and 100%). The factor for RC was Storage Time (0 and 10 weeks). Data statistical analysis was performed through analysis of variance (ANOVA) with Statgraphics plus 6.0 (Manugistics, Rockville, MD, USA). Means were compared using Fisher's minimum significant difference test with a 95% confidence level.

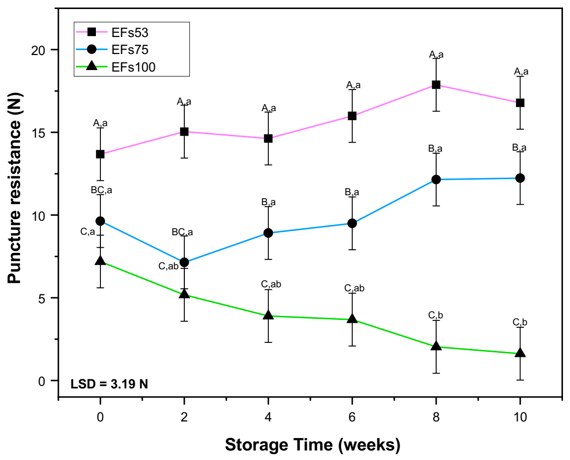

Figure 1. Effect of storage time and relative humidity (RH) on puncture resistance (PR) of edible films (EFs) stored at 53% RH (EFs53), 75% RH (EFs75), and 100% RH (EFs100). According to Fisher's test, different uppercase letters (A, B, C) and lowercase letters (a, b, c) show statistical difference (P≤0.05) among treatments at different RH and storage time, respectively.

3 Results and discussion

3.1 *Puncture resistance (PR)*

The mechanical properties of EFs are widely studied due to their influence on food product performance during transport and storage. PR is the maximum force required to penetrate through a material. Figure 1 shows the effect of RH (53, 75, and 100%) and storage time on the PR. For ten weeks of storage, EFs53 and EFs75 recorded PR ranges of 13.8-16.9 N and 9.6-12.2 N, respectively. EFs53 showed the highest PR values performing better resistance to penetration and a significant difference (P≤0.05) concerning the other two treatments throughout the storage time. EFs53 and EFs75 showed similar behavior with a slight increase in PR over time. This behavior can be explained by the microstructural reorganization phenomenon that affects polymeric chains and needs time to occur. Thermoplastic materials are well known to be susceptible to starch retrogradation and crystallization. In the retrogradation stage, a spontaneous increase in the order state occurs. The hydrogen bonds are rearranged, and the amylose and amylopectin molecules are reoriented, forming ordered crystalline structures (Van Soest *et al.*, 1996a). Kerch and Korkhov (2011) reported that the mechanical properties of chitosan films improve with the increase in storage time due to a decrease in the free volume of polymer chains during storage.

On the other hand, EFs100 showed the lowest values (7.2-1.6 N), recording a trend of decreasing PR throughout the storage. The moisture sensitivity of starch-based films modifies their physical properties, which are influenced by different temperatures or relative humidity (Galus & Lenart, 2019). Therefore, this downward behavior probably is due to the decomposition caused by the high RH storage. It is known that water is an effective plasticizer for starch-based EFs. The water molecule is positioned between the polymeric matrix components, diminishing the hydrogen-bonding interactions and decreasing the film's strength and rigidity (Bonilla-Lagos *et al.*, 2015). For this reason, at low storage RH, intermolecular interactions between starch and plasticizers could remain stable without significantly affecting the EFs' mechanical properties.

Regarding the effect of storage time on PR, no significant differences (P≥0.05) were observed in EFs53 and EFs75 through ten weeks. However, the PR of EFs100 was statistically lower (P≤0.05) at weeks 8 and 10 concerning time zero. The resistance loss can be attributed to the high relative humidity that, in conjunction with the plasticizer, could have facilitated the molecular mobility of the components of the polymeric matrix, decreasing the intermolecular interactions (Zhong & Li, 2014).

Similar results were obtained by Zhong and Li (2014) in kudzu starch-based EFs with 20% of glycerol and stored at different RH (53, 75, and 100%). The authors registered the highest values of PR at RH 53% (7.82 N) compared to RH 75% (5.12 N) and RH 100% (4.97 N), attributing these results to the significant increase of the storage RH and possible hydration of the films. Similarly, Mali *et al.* (2005) observed in cassava-based-starch EFs an increment in their moisture content and a decrease in tensile stress when the storage RH increased. Likewise, Chinma *et al.* (2015) and Osés *et al.* (2009) reported a decrease in tensile strength when the RH increased for edible films based on cassava starch with 20% glycerol and soy and whey protein EFs, respectively.

3.2 *Puncture deformation (PD)*

The EFs must be materials that provide structural integrity and reinforce the food product. PD evaluates the EFs elasticity under stress and is expressed as the relationship between the sample length to the breaking point and its original length. This property is strongly influenced by the concentration of plasticizer used (Guilbert & Gontard, 2005; Socaciu *et al.*, 2020).

Figure 2 shows the behavior of PD at different RH (53, 75, and 100%) regarding storage time (10 weeks). Stable deformation was recorded for EFs53 and EFs75 during the entire storage time, whereas EFs100 showed a downward trend. After ten weeks of storage, EFs53 and EFs75 did not present significant differences (P≥0.05), recording final values of 9.84 and 9.69 mm, respectively. While, EFs100 registered a final PD of 6.16 mm, presenting a statistical difference (P≤0.05) concerning the other two treatments. For this reason, to maintain good deformation in the corn starch-based EFs, it is advisable to use them in foodstuffs stored at

RH in the range of 53 to 75%.

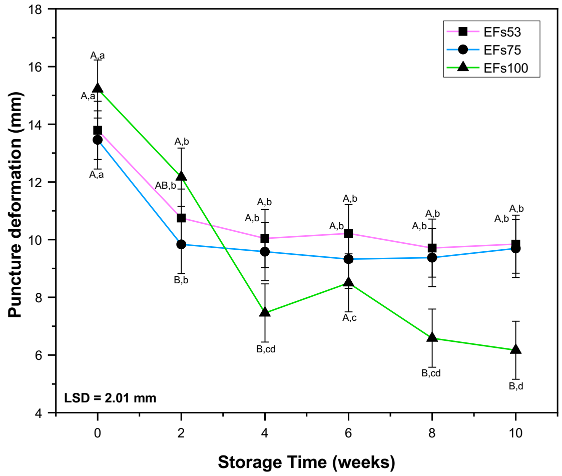

Figure 2. Effect of storage time and relative humidity (RH) on puncture deformation (PD) of edible films (EFs) stored at 53% RH (EFs53), 75% RH (EFs75) and 100% RH (EFs100). According to Fisher's test ($P \leq 0.05$), different uppercase letters (A, B, C) and lowercase letters (a, b, c) show statistical difference ($P \leq 0.05$) among treatments at different RH and storage time, respectively.

Plasticizers can reduce hydrogen bonds and increase intermolecular spaces (López-Chavez *et al.*, 2017). Considering the water as a plasticizer, it could interact with the starch molecules, resulting in wide spaces, lower cohesion between starch-plasticizer, and minor deformation. For this reason, the excessive hydration of films caused by the storage RH at 100% could have produced the polymeric matrix's swelling, increased the mobility of molecular chains, weakened the binding forces, and reduced the PD. In the same way, Zhong and Li (2014) reported that 100% RH and 40% glycerol in kudzu starch-based films produced an elongation decrease. The authors explained that this is due to an overplasticization effect which caused a weak and heterogeneous structure.

Regarding the storage time effect, EFs53 and EF75 registered a statistical difference ($P \leq 0.05$) between week zero and the other assessment weeks, showing a downward trend. This difference may be due to the retrogradation phenomenon that causes a reorganization of the starch molecules, decreasing the mobility of the polymeric matrix and the PD. It is well known that when a material is resistant, it tends to have less deformation or elongation. Hence, this behavior agrees with the obtained in PR since, from week two, an increase in the EFs resistance was registered (Van Soest *et al.*, 1996a).

For its part, EFs100 registered a significant statistical difference ($P \leq 0.05$) between weeks zero and 2. While weeks 4, 6, 8, and 10 were statistically lower ($P \leq 0.05$) than weeks zero and 2, showing a clear decrease in PD from week 2. This effect may be due to the high RH and accumulation of plasticizer, which could have interfered with the hydrogen interactions, increasing the spaces between the molecular starch chains and generating a polymeric matrix with poor mechanical properties. This supports the obtained in PR since a decrease in resistance was also observed due to a possible weakening between intermolecular interactions and overplasticization (Basiak *et al.*, 2017; Zhong & Li, 2014).

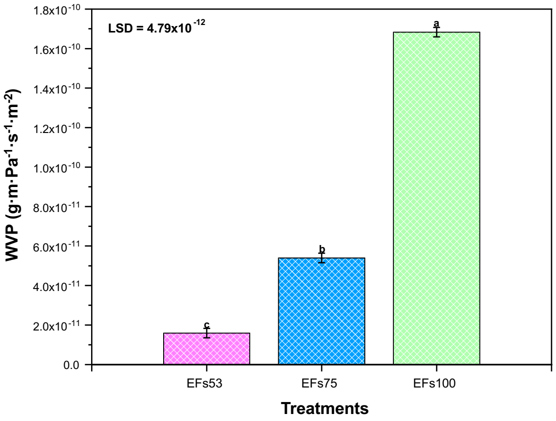

Figure 3. Water vapor permeability (WVP) of edible films (EFs) stored at 53% RH (EFs53), 75% RH (EFs75), and 100% RH (EFs100). According to the Fisher's test ($P \leq 0.05$), different letters indicate significant differences among treatments.

On the other hand, Figure 2 shows the behavior of PD of corn starch-based EFs regarding storage time. A PD decrease of 29.63, 27.96, and 59.49% was recorded for EFs53, EFs75, and EFs100, respectively. Nonetheless, the PD loss of EFs53 and EFs75 was significantly less ($P \leq 0.05$) than EFs100, probably indicating higher stability and minor plasticizers migration (glycerol and sorbitol) under these storage conditions. Similar behavior was reported by Apriyana *et al.* (2016), where the elongation at break in starch-based EFs with 40% glycerol decreased from 65.85±0.80% to 24.72±7.25% after eight weeks of storage. The elongation loss was attributed to the plasticizer migration and the rearrangement of amylose and linear portions of amylopectin to form crystalline structures.

3.3 Water vapor permeability (WVP)

WVP is one of the most critical properties of EFs since they must prevent or reduce moisture transfer between the surrounding environment and food products. This barrier property can be affected by several factors, such as the thickness of films, water activity, humidity, temperature, plasticizer content, and components' proportion (Bertuzzi *et al.*, 2007). The biopolymers used in the EFs' formulation are commonly hydrophilic, so their polar groups can interact with water molecules inducing plasticization. The water absorption during the permeation process allows the polymeric chains to increase mobility due to swelling, which leads to higher WVP (Gontard *et al.*, 1994). Therefore, it is crucial to control the environmental RH during the manufacturing and storage of starch-based EFs since water could change their structure and physicochemical properties.

Figure 3 shows that as the RH storage increased from 53 to 100%, the WVP increased, where the three treatments were statistically different ($P \leq 0.05$). Final values of 1.59×10^{-11}, 5.39×10^{-11} and 1.68×10^{-10} $g \cdot m \cdot Pa^{-1} \cdot s^{-1} \cdot m^{-2}$ for EFs53, EFs75, and EFs100 respectively were obtained. WVP is a phenomenon that involves water solubility and water diffusion through the film (Osés *et al.*, 2009). For this reason, the increment of storage RH could have generated greater water absorption in the polymeric matrix resulting in swelling and expansion of the biopolymer matrix, higher water vapor diffusion, and an increment of WVP. Zhong and Li (2014) obtained similar results studying the effect of storage RH (53, 75, and 100 %) on the WVP in kudzu-based EFs. They found that EFs stored at 75 and 100% RH had the highest values, attributing this to the presence of water in conjunction with the plasticizer, which facilitated the starch matrix's molecular mobility and water diffusion through the film. Chinma *et al.* (2015) reported a WVP increment in EFs based on cassava starch and soy protein concentrate stored at 50 and 80% RH due to the moisture adsorption at high RH. For its part, Basiak *et al.* (2017) found that EFs of wheat starch and whey protein stored at RH of 30-75% showed higher WVP when humidity increased, explaining that when the water content is high enough, the water plasticization is induced.

In addition, EFs53 and EFs75 recorded similar WVP values than those reported for synthetic films, such as cellulose acetate ($0.5\text{-}1.6 \times 10^{-11}$ $g \cdot m \cdot Pa^{-1} \cdot s^{-1} \cdot m^{-2}$), cellophane ($7.7\text{-}8.4 \times 10^{-11}$ $g \cdot m \cdot Pa^{-1} \cdot s^{-1} \cdot m^{-2}$), polyester ($1.2\text{-}1.5 \times 10^{-12}$ $g \cdot m \cdot Pa^{-1} \cdot s^{-1} \cdot m^{-2}$) and ethylene-vinyl acetate ($2.4\text{-}4.9 \times 10^{-12}$ $g \cdot m \cdot Pa^{-1} \cdot s^{-1} \cdot m^{-2}$) (Garcia *et al.*, 2000a; Gennadios *et al.*, 1994). As a pre-treatment to the casting technique, the extrusion process could have improved the interactions among starch and plasticizers, decreasing the free water adsorption sites and the WVP values. Nonetheless, to maintain the barrier properties, it is recommended that corn starch-based EFs plasticized with sorbitol and glycerol be employed as food packing at RH from 53 to 75%.

3.4 Water solubility (WS)

WS is an essential property in starch-based packaging. The WS indicates the film's integrity in an aqueous medium, where higher solubility represents lower water resistance. Chiumarelli and Hubinger (2014) reported that the plasticizer type and concentration influence the solubility of EFs. For most food applications, EFs with good water insolubility are desired to provide water resistance and enhance the shelf-life (Bertuzzi *et al.*, 2007; Gutiérrez *et al.*, 2015b; Sanyang *et al.*, 2016).

Figure 4 shows the effect of storage RH on EFs solubility. There were no significant differences ($P \geq 0.05$) among the treatments. After 48 h of storage, there were recorded WS values of 30.85, 30.70, and 30.63% for EFs53, EFs75, and EFs100, respectively. Müller *et al.* (2008) found that RH and the plasticizer type affected the WS of cassava starch-based films; as the RH increased, the WS was higher. Similar trends were reported in previous studies regarding the effect of plasticizer content on WS (Calderón-Castro *et al.*, 2018; Chiumarelli & Hubinger, 2014). Sanyang *et al.* (2016) reported that the high content of plasticizers in sugar palm starch films significantly increased film solubility. Contrariwise, a definite trend was not observed in this work due to the effect of storage RH. The films were probably in the process of being adapted to the storage environment, which kept them in good integrity. Hence, it would have been desirable to wait a longer storage time to conduct the evaluation and appreciate the effect of RH on the WS of EFs.

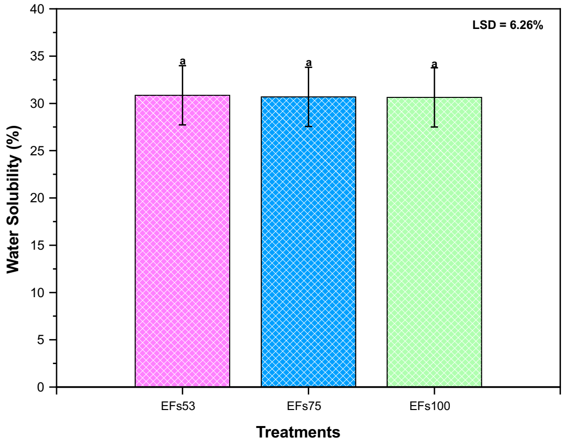

Figure 4. Water solubility of edible films (EFs) stored at 53% RH (EFs53), 75% RH (EFs75), and 100% RH (EFs100) after 48 h of storage. According to the Fisher's test ($P \leq 0.05$), different letters indicate significant differences among treatments.

On the other hand, it is essential to highlight that WS values of corn starch-based EFs stored at different RH were lower (<32%) than those reported in the literature for native starch-based films (Calderón-Castro *et al.*, 2018; Chiumarelli & Hubinger, 2014; Fakhouri *et al.*, 2015; Medrano-de Jara *et al.*, 2020; Quequezana-Bedregal *et al.*, 2021; Sanyang *et al.*, 2016). It is known that physical treatments, like the extrusion technique, commonly produce changes in the semi-crystalline structure of starch. These molecular changes could have produced strong hydrogen interactions between starch and plasticizers, resulting in more water-resistant films (Fitch-Vargas *et al.*, 2019).

3.5 X-ray diffraction (XRD)

Figure 5 shows the edible films' XRD patterns at time zero (EFsT0) and different RH (53, 75, and 100%) after ten weeks of storage. All the EFs showed a significant amorphous phase and a crystalline fraction. Two types of crystallinity can be identified in starch-based films: a) residual crystallinity (A, B, or C crystallinity by incomplete melting of starch) and b) processing-induced crystallinity, VA (not hydrated), V_H (hydrated), and E_H (Van Soest *et al.*, 1996b). Moreover, the films' crystallinity depends on the following processing conditions: (i) biopolymer source and plasticizers, (ii) biopolymer's water dissolution, (iii) film drying conditions, and (iv) samples' final moisture content (Bader & Göritz, 1994).

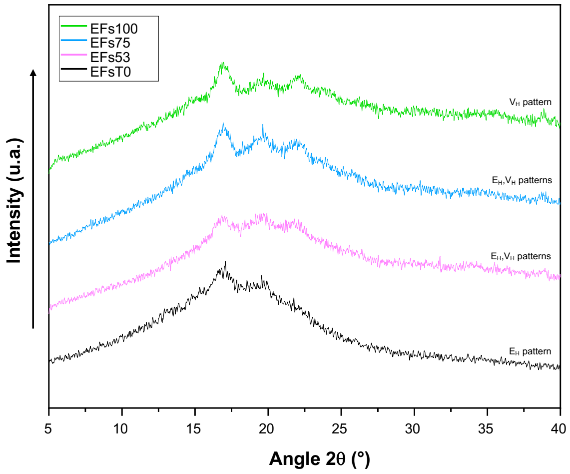

Figure 5. X-ray diffractograms of edible films (EFs) stored at 53% RH (EFs53), 75% RH (EFs75), and 100% RH (EFs100) after ten weeks of storage and EFs at time zero (EFsT0).

XRD diffraction patterns showed that EFsT0 has an E_H polymorph with two peaks 2θ at ≈17 and 20°; according to Singh *et al.* (1998), the E_H complex is formed under lower humidity conditions. EFs53, EFs75, and EFs100 presented three principal peaks at ≈17, 20, and 22°. The peaks at 20 and 22° correspond to a V_H-type crystalline structure, indicating the existence of amylose-glycerol interactions and increased moisture content in films. EFs53 and EFs75 presented a combination of V_H and E_H polymorphisms, and EFs100 showed a V_H form.

Comparing the XRD diffraction patterns of EFsT0 and EFs after ten weeks of storage, it was found that the intensity and number of peaks for EFs53, EFs75, and EFs100 were higher than EFsT0, indicating a crystallinity increment and amylose-glycerol complex formation during storage. This behavior could result from the rearrangement of starch molecules due to the effect of storage time and retrogradation. Gutiérrez *et al.* (2015a) and Perez *et al.* (2012) reported that in starch-based EFs, the amylose is mainly responsible for the crystallinity. Zhong and Li (2014) obtained XRD patterns with peaks 2θ at 17 and 20° in kudzu starch-based EFs, implying the formation of double-helical B and helical V-type crystalline. Meanwhile, Mali *et al.* (2002) found in the XRD patterns of yam starch-based EFs with 90 days of storage an increment in the peaks' intensities which could correspond to a slow recrystallization process.

On the other hand, EFs53 and EFs75 presented greater intensity at peak 2θ = 20° than EFs100, which is related to a higher proportion of amylose-glycerol complex. This difference indicates that the water adsorbed by EFs100 diminished the formation of the complex and decreased the RC. Iftikhar and Dutta (2019) found in starch from three rice varieties that a dual retrogradation-annealing modification contributed to the formation of a combination of polymorphisms A, B, and V_H, which was correlated with a decrease in starch digestibility and a greater presence of resistant starch. At the same time, Castro *et al.* (2020) establish that resistant starch can be produced due to the intact structure of starch granules or the formation of lipid-amylose complexes. Therefore, it could be hypothesized that starch with lower digestibility could be generated under storage conditions of 53 and 75% RH due to a greater formation of starch-glycerol complexes. This behavior could support the mechanical and barrier properties of EFs53 and EFs75, which were more stable than EFs100.

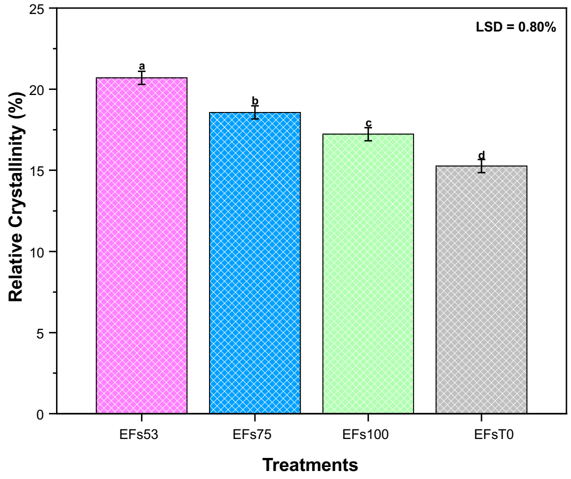

Figure 6- Relative crystallinity of edible films (EFs) stored at 53% RH (EFs53), 75% RH (EFs75), and 100% RH (EFs100) after ten weeks of storage and EFs at time zero (EFsT0). According to the Fisher's test (P≤0.05), different letters indicate significant differences among treatments.

Figure 6 shows the relative crystallinity (RC) of EFsT0 and EFs after ten weeks of storage, where treatments were statistically different (P≤0.05) among them. EFsT0 recorded a RC of 15.26%, while EFs53, EFs75, and EFs100 presented values of 20.69, 18.57 and 17.22%, respectively. Acosta *et al.* (2015) explained that starch films are greatly affected by the storage time, inducing physical and chemical changes in film properties, affecting their functionality as a consequence of reassociation into crystalline segments. For its part, Mali *et al.* (2006) evaluated the storage effect on RC in starch-based EFs and obtained an increase in RC of EFs stored for 90 days regarding time zero. Therefore, probably due to storage time and recrystallization, EFs53, EFs75, and EFs100 presented a higher RC than EFsT0.

Regarding the effect of the storage RH on EFs, an increase in RC as the humidity decreased was recorded. EFs53, after ten weeks of storage, presented the highest RC and a significant difference (P≤0.05) concerning EFs75 and EFs100. Zhong and Li (2014), Gutiérrez *et al*. (2015b), and Sanyang *et al*. (2016) reported that the increase in crystallinity of starch-based films is strongly related to a decrease in film moisture content. It is known that plasticizers can reduce starch recrystallization, leading to lower enthalpy and peak melting temperatures (Versino *et al*., 2016). Hence, increasing the storage RH, water may act as a plasticizer, interacting with hydroxyl groups of starch and positioning it between sorbitol and glycerol, increasing the spatial distance among polymeric chains and avoiding the molecular rearrangement and crystallization.

Conclusions

The results found that starch-based EFs stored at RH of 53 and 75% showed more stable mechanical properties than those stored at 100% RH since the environment water could have acted as an extra plasticizer, reducing the interactions between starch, sorbitol, and glycerol. Concerning the barrier properties, it was found that EFs stored at 53% RH had the lowest values of WVP because the low moisture content could decrease molecular mobility and water diffusion through the film.

Regarding the results of XRD patterns, EFsT0 at time zero and stored at 53% RH showed an E_H polymorphism due to their low water content. On the other hand, the EFs stored for ten weeks presented different diffraction patterns, recording $V_H E_H$, $V_H E_H$, and E_H polymorphisms for EFs53, EFs75, and EFs100, respectively. EFs53 recorded the highest RC concerning storage time, indicating recrystallization of the polymeric matrix due to aging. It can be concluded that the starch-based EFs obtained by a combination of extrusion-casting and stored at RH of 53 and 75% during ten weeks presented the best mechanical and barrier properties. Hence, these EFs could be used as coatings of intermediate moisture foods, such as dried fruits and vegetables, baked foods, candies, or cheeses.

Acknowledgments

The authors thank Universidad Autónoma de Sinaloa and CINVESTAV Querétaro for microstructural analysis techniques.

References

Acosta, S., Jiménez, A., Cháfer, M., González-Martínez, C. and Chiralt, A. (2015). Physical properties and stability of starch-gelatin based films as affected by the addition of esters of fatty acids. *Food Hydrocolloids 49*, 135-143. https://doi.org/https://doi.org/10.1016/j.foodhyd.2015.03.015

Akbari, Z., Ghomashchi, T. and Moghadam, S. (2007). Improvement in food packaging industry with biobased nanocomposites. *International Journal of Food Engineering 3*(4), 1-24. https://doi.org/10.2202/1556-3758.1120

Apriyana, W., Poeloengasih, C. D., Hernawan, Hayati, S. N., Pranoto, Y., Nuringtyas, T. R., *et al*.Kusumaadmaja, A. (2016). Mechanical and microstructural properties of sugar palm (*Arenga pinnata* Merr.) starch film: Effect of aging. *AIP Conference Proceedings*.

Bader, H. G. and Göritz, D. (1994). Investigations on high amylose corn starch films. Part 3: Stress strain behaviour. *Starch-Stärke 46*(11), 435-439. https://doi.org/10.1002/star.19940461106

Basiak, E., Lenart, A. and Debeaufort, F. (2017). Effects of carbohydrate/protein ratio on the microstructure and the barrier and sorption properties of wheat starch-whey protein blend edible films. *Journal of the Science of Food and Agriculture 97*(3), 858-867. https://doi.org/10.1002/jsfa.7807

BeMiller, J. N. and Huber, K. C. (2015). Physical modification of food starch functionalities. *Annual Review of Food Science and Technology 6*, 19-69. https://doi.org/10.1146/annurev-food-022814-015552

Bertuzzi, M. A., Armada, M. and Gottifredi, J. C. (2007). Physicochemical characterization of starch based films. *Journal of Food Engineering 82*(1), 17-25. https://doi.org/10.1016/j.jfoodeng.2006.12.016

Bohórquez-Ayala, M., Rojano-Quiroz, D., González-Cuello, R., García-Zapateiro, L. and Ortega-Toro, R. (2021). Application of modified vegetable oil for improvement of biodegradable materials based on thermoplastic starch and polylactic acid. *Revista Mexicana de Ingeniería Química 20*(1), 423-433. https://doi.org/10.24275/rmiq/Poly2164

Bonilla-Lagos, J., Vicentini, N. M., Dos Santos, R. M. C., Bittante, M. Q. B. and Sobral, P. J. A. (2015). Mechanical properties of cassava starch films as affected by different plasticizers and different relative humidity conditions. *International Journal of*

Food Studies 4(1), 116-125. https://doi.org/10.7455/ijfs/4.1.2015.a10

Calderón-Castro, A., Vega-García, M. O., Zazueta-Morales, J. J., Fitch-Vargas, P. R., Carrillo-López, A., Gutiérrez-Dorado, R., *et al.*Aguilar-Palazuelos, E. (2018). Effect of extrusion process on the functional properties of high amylose corn starch edible films and its application in mango (*Mangifera indica* L.) cv. Tommy Atkins. *Journal of Food Science and Technology 55*(3), 905-914. https://doi.org/10.1007/s13197-017-2997-6

Castro, L. M., Alexandre, E. M., Saraiva, J. A. and Pintado, M. (2020). Impact of high pressure on starch properties: A review. *Food Hydrocolloids 106*, 105877. https://doi.org/https://doi.org/10.1016/j.foodhyd.2020.105877

Cazón, P. and Vázquez, M. (2020). Mechanical and barrier properties of chitosan combined with other components as food packaging film. *Environmental Chemistry Letters 18*(2), 257-267. https://doi.org/10.1007/s10311-019-00936-3

Cheng, H., Chen, L., McClements, D. J., Yang, T., Zhang, Z., Ren, F., *et al.*Jin, Z. (2021). Starch-based biodegradable packaging materials: A review of their preparation, characterization and diverse applications in the food industry. *Trends in Food Science & Technology 114*, 70-82. https://doi.org/10.1016/j.tifs.2021.05.017

Chinma, C. E., Ariahu, C. C. and Alakali, J. S. (2015). Effect of temperature and relative humidity on the water vapour permeability and mechanical properties of cassava starch and soy protein concentrate based edible films. *Journal of Food Science and Technology 52*(4), 2380-2386. https://doi.org/10.1007/s13197-013-1227-0

Chiumarelli, M. and Hubinger, M. D. (2014). Evaluation of edible films and coatings formulated with cassava starch, glycerol, carnauba wax and stearic acid. *Food Hydrocolloids 38*, 20-27. https://doi.org/10.1016/j.foodhyd.2013.11.013

Dai, L., Qiu, C., Xiong, L. and Sun, Q. (2015). Characterization of corn starch-based films reinforced with taro starch nanoparticles. *Food Chemistry 174*, 82-88. https://doi.org/10.1016/j.foodchem.2014.11.005

Fakhouri, F. M., Martelli, S. M., Caon, T., Velasco, J. I. and Mei, L. H. I. (2015). Edible films and coatings based on starch/gelatin: Film properties and effect of coatings on quality of refrigerated Red Crimson grapes. *Postharvest Biology and Technology 109*, 57-64. https://doi.org/10.1016/j.postharvbio.2015.05.015

Famá, L., Goyanes, S. and Gerschenson, L. (2007). Influence of storage time at room temperature on the physicochemical properties of cassava starch films. *Carbohydrate Polymers 70*(3), 265-273. https://doi.org/10.1016/j.carbpol.2007.04.003

Fitch-Vargas, P. R., Aguilar-Palazuelos, E., Vega-García, M. O., Zazueta-Morales, J. J., Calderón-Castro, A., Montoya-Rodríguez, A., *et al.*Camacho-Hernández, I. L. (2019). Effect of a corn starch coating obtained by the combination of extrusion process and casting technique on the postharvest quality of tomato. *Revista Mexicana de Ingeniería Química 18*(3), 789-801. https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n3/Fitch

Fitch-Vargas, P. R., Aguilar-Palazuelos, E., Zazueta-Morales, J. J., Vega-García, M. O., Valdez-Morales, J. E., Martínez-Bustos, F. and Jacobo-Valenzuela, N. (2016). Physicochemical and microstructural characterization of corn starch edible films obtained by a combination of extrusion technology and casting technique. *Journal of Food Science 81*(9), 2224-2232. https://doi.org/10.1111/1750-3841.13416

Galus, S. and Lenart, A. (2019). Optical, mechanical, and moisture sorption properties of whey protein edible films. *Journal of Food Process Engineering 42*(6), 1-10. https://doi.org/10.1111/jfpe.13245

Garcia, M. A., Martino, M. N. and Zaritzky, N. E. (2000a). Lipid addition to improve barrier properties of edible starch-based films and coatings. *Journal of Food Science 65*(6), 941-944. https://doi.org/10.1111/j.1365-2621.2000.tb09397.x

Garcia, M. A., Martino, M. N. and Zaritzky, N. E. (2000b). Microstructural characterization of plasticized starch-based films. *Starch-Stärke 52*(4), 118-124. https://doi.org/10.1002/1521-379X(200006)52:4<118::AID-STAR118>3.0.CO;2-0

Gennadios, A., Brandenburg, A. H., Park, W., Weller, C. L. and Testin, R. F. (1994). Water vapor permeability of wheat gluten and soy protein isolate films. *Industrial Crops and Products 2*(3), 189-195. https://doi.org/10.1016/0926-6690(94)90035-3

Gomez, M. H., McDonough, C. M., Rooney, L. W. and Waniska, R. D. (1989). Changes in corn and sorghum during nixtamalization and tortilla baking. *Journal of Food Science 54*(2), 330-336. https://doi.org/10.1111/j.1365-2621.1989.tb03074.x

Gontard, N., Guilbert, S., Marchesseau, S. and Cuq, J. L.(1994).Edible wheat gluten films: optimization of the main process variables and improvement of water vapor barrier properties by combining gluten proteins with lipids. In: *Developments in Food Engineering*, (T. Yano, R. Matsuno and K. Nakamura, eds), Pp. 790-792. Springer, Boston, MA.

Guilbert, S. and Gontard, N.(2005).Agro-polymers for edible and biodegradable films: review of agricultural polymeric materials. In: *Innovations in Food*

Packaging, (J. H. Han, eds), Pp. 263. Elsevier Academic Press, Winnipeg, Manitoba.

Gutiérrez, T. J., Morales, N. J., Pérez, E., Tapia, M. S. and Famá, L. (2015a). Physico-chemical properties of edible films derived from native and phosphated cush-cush yam and cassava starches. *Food Packaging and Shelf Life 3*, 1-8. https://doi.org/10.1016/j.fpsl.2014.09.002

Gutiérrez, T. J., Tapia, M. S., Pérez, E. and Famá, L. (2015b). Structural and mechanical properties of edible films made from native and modified cush-cush yam and cassava starch. *Food Hydrocolloids 45*, 211-217. https://doi.org/10.1016/j.foodhyd.2014.11.017

Iftikhar, S. A. and Dutta, H. (2019). Status of polymorphism, physicochemical properties and *in vitro* digestibility of dual retrogradation-annealing modified rice starches. *International Journal of Biological Macromolecules 132*, 330-339. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2019.03.206

Kerch, G. and Korkhov, V. (2011). Effect of storage time and temperature on structure, mechanical and barrier properties of chitosan-based films. *European Food Research and Technology 232*(1), 17-22. https://doi.org/10.1007/s00217-010-1356-x

López-Chavez, M. C., Osorio-Revilla, G., Arellano-Cárdenas, S., Gallardo-Velázquez, T., Flores-Valle, S. O. and López-Cortez, M. S. (2017). Preparation of starch/clay/glycerol nanocomposite films and their FTIR, XRD, SEM and mechanical characterizations. *Revista Mexicana de Ingeniería Química 16*(3), 793-804.

Mali, S., Grossmann, M. V. E., Garcia, M. A., Martino, M. N. and Zaritzky, N. E. (2002). Microstructural characterization of yam starch films. *Carbohydrate Polymers 50*(4), 379-386. https://doi.org/10.1016/S0144-8617(02)00058-9

Mali, S., Grossmann, M. V. E., García, M. A., Martino, M. N. and Zaritzky, N. E. (2006). Effects of controlled storage on thermal, mechanical and barrier properties of plasticized films from different starch sources. *Journal of Food Engineering, 75*(4), 453-460. https://doi.org/10.1016/j.jfoodeng.2005.04.031

Mali, S., Sakanaka, L. S., Yamashita, F. and Grossmann, M. V. E. (2005). Water sorption and mechanical properties of cassava starch films and their relation to plasticizing effect. *Carbohydrate Polymers 60*(3), 283-289. https://doi.org/10.1016/j.carbpol.2005.01.003

Medrano-de Jara, E., García-Hernández, E., Quequezana-Bedregal, M., Arrieta-González, C., Salgado-Delgado, R., Lastarria-Tapia, H. and Castañón-Vilca, J. (2020). Potato starch-based films: Effects of glycerol and montmorillonite nanoclay concentration. *Revista Mexicana de Ingeniería Química 19*(2), 627-637. https://doi.org/10.24275/rmiq/Poli779

Mendes, J. F., Paschoalin, R. T., Carmona, V. B., Neto, A. R. S., Marques, A. C. P., Marconcini, J. M., *et al.*Oliveira, J. E. (2016). Biodegradable polymer blends based on corn starch and thermoplastic chitosan processed by extrusion. *Carbohydrate Polymers 137*, 452-458. https://doi.org/10.1016/j.carbpol.2015.10.093

Müller, C. M. O., Yamashita, F. and Laurindo, J. B. (2008). Evaluation of the effects of glycerol and sorbitol concentration and water activity on the water barrier properties of cassava starch films through a solubility approach. *Carbohydrate Polymers 72*(1), 82-87. https://doi.org/10.1016/j.carbpol.2007.07.026

Osés, J., Fabregat-Vázquez, M., Pedroza-Islas, R., Tomás, S. A., Cruz-Orea, A. and Maté, J. I. (2009). Development and characterization of composite edible films based on whey protein isolate and mesquite gum. *Journal of Food Engineering 92*(1), 56-62. https://doi.org/10.1016/j.jfoodeng.2008.10.029

Perez, E., Segovia, X., Tapia, M. S. and Schroeder, M. (2012). Native and cross-linked modified *Dioscorea trifida* (cush-cush yam) starches as bio-matrices for edible films. *Journal of Cellular Plastics 48*(6), 545-556. https://doi.org/10.1177/0021955X12445603

Piñeros-Guerrero, N., Marsiglia-Fuentes, R. and Ortega-Toro, R. (2021). Improvement of the physicochemical properties of composite materials based on cassava starch and polycaprolactone reinforced with sodium montmorillonite. *Revista Mexicana de Ingeniería Química 20*(3), 1-12. https://doi.org/10.24275/rmiq/Alim2416

Quequezana-Bedregal, M. J., Castillo-Rosales, K. A., Mederano-de Jara, E., Vargas-Galarza, Z., Lastarria-Tapia, H., Castañón-Vilca, J. and García-Hernández, E. (2021). Rice flour-based films: effects of type and solvent and plasticizer concentration. *Revista Mexicana de Ingeniería Química 20*(1), 323-332. https://doi.org/10.24275/rmiq/Poly1829

Ranganathaiah, C., Ramani, R. and Ramachandra, P. (2002). Influence of vinylidenefluoride on the physical ageing of poly (chlorotrifluoroethylene). *European Polymer Journal 38*(11), 2285-2294. https://doi.org/10.1016/S0014-3057(02)00110-6

Sanyang, M. L., Sapuan, S. M., Jawaid, M., Ishak, M. R. and Sahari, J. (2016). Effect of plasticizer type and concentration on physical properties of biodegradable films based on sugar palm (*Arenga pinnata*) starch for food packaging. *Journal of Food Science and Technology 53*(1), 326-336. https://doi.org/10.1007/s13197-015-2009-7

Shah, U., Naqash, F., Gani, A. and Masoodi, F. A. (2016). Art and science behind modified starch edible films and coatings: a review. *Comprehensive Reviews in Food Science and Food Safety 15*(3), 568-580. https://doi.org/10.1111/1541-4337.12197

Singh, N., Cairns, P., Morris, V. J. and Smith, A. C. (1998). Physical properties of extruded wheat starch-additive mixtures. *Cereal Chemistry 75*(3), 325-330. https://doi.org/10.1094/CCHEM.1998.75.3.325

Socaciu, M. I., Fogarasi, M., Semeniuc, C. A., Socaci, S. A., Rotar, M. A., Mureşan, V., *et al.*Vodnar, D. C. (2020). Formulation and characterization of antimicrobial edible films based on whey protein isolate and tarragon essential oil. *Polymers 12*(8), 1748. https://doi.org/10.3390/polym12081748

Valdez-Fragoso, A., Welti-Chanes, J., Mújica-Paz, H. and Hernández-Carrillo, J. (2015). Tracing phenolic compounds through manufacturing of edible films from orange and grapefruit peels. *Revista Mexicana de Ingeniería Química 14*(3), 567-578.

Van Soest, J. J. G., Benes, K., De Wit, D. and Vliegenthart, J. F. G. (1996a). The influence of starch molecular mass on the properties of extruded thermoplastic starch. *Polymer 37*(16), 3543-3552. https://doi.org/10.1016/0032-3861(96)00165-6

Van Soest, J. J. G., Hulleman, S. H. D., De Wit, D. and Vliegenthart, J. F. G. (1996b). Crystallinity in starch bioplastics. *Industrial Crops and Products 5*(1), 11-22. https://doi.org/10.1016/0926-6690(95)00048-8

Versino, F., Lopez, O. V., Garcia, M. A. and Zaritzky, N. E. (2016). Starch-based films and food coatings: An overview. *Starch-Stärke 68*(11-12), 1026-1037. https://doi.org/10.1002/star.201600095

Xie, F., Halley, P. J. and Avérous, L. (2012). Rheology to understand and optimize processibility, structures and properties of starch polymeric materials. *Progress in Polymer Science 37*(4), 595-623. https://doi.org/10.1016/j.progpolymsci.2011.07.002

Zhong, Y. and Li, Y. (2014). Effects of glycerol and storage relative humidity on the properties of kudzu starch-based edible films. *Starch-Stärke 66*(5), 524-532. https://doi.org/10.1002/star.201300202