

Accepted Manuscript

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PII: S0141-8130(19)30776-7

DOI: <https://doi.org/10.1016/j.ijbiomac.2019.06.004>

Reference: BIOMAC 12510

To appear in: *International Journal of Biological Macromolecules*

Received date: 1 February 2019

Revised date: 23 May 2019

Accepted date: 1 June 2019

Please cite this article as: D. Domene-López, J.J. Delgado-Marín, I. Martín-Gullón, et al., Comparative study on properties of starch films obtained from potato, corn and wheat using 1-ethyl-3-methylimidazolium acetate as plasticizer, *International Journal of Biological Macromolecules*, <https://doi.org/10.1016/j.ijbiomac.2019.06.004>

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Comparative study on properties of starch films obtained from potato, corn and wheat using 1-ethyl-3-methylimidazolium acetate as plasticizer

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Abstract

Starch films are gaining attention as substitutes of synthetic polymers due to their biodegradability and low cost. Some ionic liquids have been postulated as alternatives to glycerol, one of the best starch plasticizers, due to their great capacity to form hydrogen bonds with starch and hence great ability of preventing starch retrogradation and increasing film stability. In this work, [emim⁺][Ac⁻]-plasticized starch films were prepared from potato, corn and wheat starch. The effect of starch molecular structure in terms of granular composition (amylose and phosphate monoester contents) and molecular weight (M_w) on film properties was evaluated. Potato starch films were the most amorphous because of the higher M_w and phosphate monoester content of potato starch, both contributing to a lower rearrangement of the starch chains difficulting the crystallization process. In contrast, corn and wheat starches lead to more crystalline films because of their lower M_w , which may imply higher mobility and crystal growth rate, and lower phosphate monoester content. This more crystalline structure could be the responsible of their better mechanical properties. [emim⁺][Ac⁻] can be considered suitable for manufacturing starch films showing corn and wheat starch films similar properties to synthetic low-density polyethylene, but involving a simple and environmentally-friendly process.

Keywords: starch film, 1-ethyl-3-methylimidazolium acetate, mechanical properties.

1. Introduction

Starch is a biopolymer that has been gaining great attention in the recent years as it is considered one of the best candidates to substitute petroleum-based polymers in a wide range of applications. The increasing consumption of non-renewable polymers by a growing world population has led

to a situation where our ability to treat the produced wastes is being outstripped [1,2]. The non-biodegradable aspect of such polymers has provoked the presence of microplastics in the environment that may reach humans in common table salt [3] or in the trophic chain in general [4,5]. This situation and increased environmental concerns have encouraged the scientific community to develop polymers based on natural resources (starch, cellulose, lignin...) [6–9], since they are considered renewable, biodegradable and biocompatible [10]. Among all the biopolymers studied, starch is the most interesting, because it is readily obtained from different types of plants and hence is very abundant and has a competitive price compared with other commodity polymers [1,11].

Starch is a biopolymer formed mainly by amylose and amylopectin. Amylose is a linear macromolecule of (1→4)-linked α -D-glucopyranosyl units slightly branched by (1→6)- α -linkages, while amylopectin is a highly branched macromolecule composed of hundreds of short (1→4)- α -glucan chains interlinked by (1→6)-linkages [11,12]. The relative weight ratio of these molecules depends on the botanical origin of the starch and, according to recent studies, could be important in the final properties of starch-based applications [13,14]. Properties of the final starch films can be also influenced by the starch molecular weight. This feature is related to the crystallinity of the films. Heavier starch chains may hinder the macromolecular chain movement during the film preparation implying lower rearrangement and crystallization ability as has been previously reported for other polymers [15]. On the other hand, phosphate monoesters are charged groups which are present in starch and seem to play an important role by inducing strain in crystalline starch and resulting in helix unfolding. Therefore, the presence of these groups can also alter the properties of the starch films. Normally, tuber starches contain more phosphate monoesters than cereal starches [16].

In plants, starch is stored in packed granules with both amorphous and crystalline phases [12], a structure that must be disrupted in order to obtain a film by means a gelatinization process. As the starch granules are not soluble in common solvents or cold water [2,17], they are gelatinized in an excess of water at high temperature in the presence of a suitable plasticizer. A plasticizer should

fulfil several different requirements, such as high thermal stability, low volatility, non-toxicity and high compatibility with the starch matrix. Traditionally, water has been used as solvent, dispersion medium and even plasticizer in biological and food systems [18] but, in the case of starch, water presents problems, such as its low processability window (below 100 °C), which limits the processability conditions and properties of starch films. For this reason, water is usually used along with other plasticizers, for example glycerol, which is considered one of the best starch plasticizers, although this, too, has several drawbacks, including rapid aging and retrogradation processes [19,20]. In order to avoid such problems, other types of plasticizer are being considered, among which ionic liquids (ILs), which have received growing attention in recent decades [21], have been postulated as a perfect alternative to conventional plasticizers of starch due to their great capacity to form hydrogen bonds, thus contributing to the destructure phase of starch and acting as glass transition depressors in the final film [22]. In addition, the use of ILs as plasticizers can prevent retrogradation during storage, providing films with more stable physical-chemical and mechanical properties [23–27]. However, not all ILs are suitable for use as starch plasticizers: for example, ILs containing halogenated anions like [Cl⁻], which is corrosive, could provoke starch degradation [23,27]. Kosmotropic ILs containing anions like acetate are the best candidates for use as plasticizers, such as 1-ethyl-3-methylimidazolium acetate ([emim⁺][Ac⁻]) [28–30], which has high thermal stability and an ability to interact with the hydrogen in starch and also due to its low density, vapour pressure, toxicity and viscosity at room temperature [11,23,31,32]. In fact, Ismail *et al.* [23] studied the tapioca starch plasticization by 1-ethyl-3-methylimidazolium chloride and [emim⁺][Ac⁻] concluding that the last one is the best option because acetate anion influenced the gelatinization process. However, they did not report microstructure or mechanical properties. Other authors, such as Zhang *et al.* [25] and Xie *et al.* [27] compared the use of glycerol and [emim⁺][Ac⁻] to obtain starch films from a high-amylose maize starch (82.9%) by careful blending of the components and later compression moulding. This type of starch is a genetically-modified starch product. The results revealed that [emim⁺][Ac⁻] was more effective in plasticization of this starch. Xie *et al.* [26] also compared the use of [emim⁺][Ac⁻] as plasticizer in two maize starches with different amylose contents to obtain films

with the same technique. They found that plasticisation by [emim⁺][Ac⁻] made the effect of amylose content insignificant, contrary to most previous studies when other plasticisers were used. Colomines *et al.* [22] synthesized a series of cholinium cation-based ILs to plasticize corn starch by the casting technique but the mechanical behavior of the films obtained was not reported.

Thus, the aim of this work was to study the properties of starch films obtained from three different starch sources (potato, corn and wheat) using [emim⁺][Ac⁻] as plasticizer. To the best of our knowledge, this is the first time that a systematic study on the plasticizing effect of [emim⁺][Ac⁻] on these three types of starch has been carried out.

2. Experimental

2.1. Materials

Corn starch and wheat starch were purchased from Sigma-Aldrich (Madrid, Spain). Potato starch was provided by Across Organics (Geel, Belgium) and the plasticizer, 1-ethyl-3-methylimidazolium acetate ([emim⁺][Ac⁻]), was supplied by IoLitec (Heilbronn, Germany). The assay kit for the determination of amylose content of the starches was acquired from Megazyme (Wicklow, Ireland). All chemicals were used without further purification.

2.2. Amylose content, molecular weight and phosphorus content of the starches

Amylose content of the starches was quantified using the concanavalin A precipitation method with an amylose/amylopectin assay kit. The procedure was performed by quintuplicate according to Megazyme assay kit instructions. A starch reference sample with specified content of amylose have been also tested.

The weight-average molecular weight of the starches was determined in duplicate by GPC-MALS. About 20 mg of the sample were weighed exactly on an analytical balance. A defined volume of mobile phase (DMSO with 0.1M LiCl) was added to each sample and was also weighed. The concentration of the sample solutions was about 1.0 mg/mL, assuming a polymer content of 100%. Afterwards, the sample solutions were dissolved at 80 °C. Before the

measurement, the sample solutions were filtered through a filter unit with a pore size of 1 μm . Samples were injected into a Size-exclusion Chromatographer (SECurity 1260, Polymer Standard Service, Mainz, Germany) with double detection (MALS and refractive index) and separated using PSS-Suprema precolumn (10 μm , Guard, ID 8.0 mm x 50 mm) and PSS-Suprema analytical column (10 μm , 10000 \AA , ID 8.0 mm x 300 mm) at a flow rate of 0.3mL/min at 80 $^{\circ}\text{C}$. GPC-MALS is an absolute method for the determination of the total weight-average molar mass (M_w). This method requires an instrument constant for the light scattering detector (calibration constant) which has to be determined by means of an isotropic scatterer with known differential refractive index dn/dc and weight-average molar mass. Additionally, the normalization coefficient for the different angles and the inter detector delay have to be calculated using the reference substance. For the determination of the light scattering parameters a pullulan standard with a narrow molecular weight distribution (pullulan p100) was analyzed. The obtained results were verified with a broad dextran standard (dxt70) with known molecular weight. The dn/dc values used were 0.031mL/g for potato and corn starches and 0.034mL/g for wheat starch. The light scattering parameters were used for the calculation of the molecular weights using the light scattering-calculation method "factor x concentration". The GPC-MALS data were processed using WinGPC UniChrom software version 8.3 (Polymer Standard Service, Mainz, Germany). The phosphorus content of the studied starches was obtained with an Agilent 7700x inductively coupled plasma mass spectrometer (ICP-MS, Agilent Technologies, Waldbronn, Germany). The starch samples (around 0.2 g exactly weighed) were previously digested with 5mL of a mixture of nitric acid and hydrogen peroxide (4:1 v/v) for 45 min reaching a maximum temperature of 240 $^{\circ}\text{C}$. After digestion, each sample was diluted to 25 mL with Milli-Q water.

2.3. Preparation of starch films

Starch films were prepared by the casting technique following the procedure previously described by Medina-Jaramillo *et al.* [33]. In summary, the film-forming solution was composed of potato, corn or wheat starch (5 wt.%), [emim⁺][Ac⁻] (1.5 wt. %) and distilled water (93.5 wt. %). Each solution was stirred at room temperature for 45 min and, then heated up to 96 $^{\circ}\text{C}$ for 40 min to guarantee starch gelatinization. The mixture was then degassed under vacuum for 7 min and

poured onto Petri dishes (0.34 g/cm²). These plates were dried in an oven with air circulation at 50°C for 48 h. The dried films were peeled off and stored at room conditions (around 25 °C and 50% relative humidity) for a week before characterization. Each film sample was prepared at least by triplicate.

2.4. Film characterization

2.4.1. Water content and solubility in water

The water content and solubility in water of the films were determined using 1 x 1 cm² samples following previously described procedures with slight modifications [7,33]. The water content was measured by determining the weight loss of films, previously dried in an oven at 110 °C for 5 hours. Four measurements were made for each sample. The absorbed water or moisture content was expressed in terms of percentage (grams of water in 100 grams of sample) using Eq. (1).

$$\text{Water content (\%)} = \left(\frac{m_0 - m_1}{m_0} \right) \times 100 \quad (1)$$

where m_0 and m_1 are the mass before and after drying, respectively.

After that, the solubility in water of each sample was measured placing the above dried films individually in 10 mL tubes filled with 9 mL of distilled water. The tubes were capped and stored at 25, 40 and 60 °C for 24 h, after which the films were taken out and dried again at 110 °C for 5 h in order to determine the final mass of dry matter, m_f . The solubility in water was calculated from the loss of total soluble matter as follows (Eq. 2):

$$\text{Solubility (\%)} = \left(\frac{m_0 - m_f}{m_0} \right) \times 100 \quad (2)$$

Water solubility values were obtained from the average of at least four measurement repetitions.

2.4.2. Opacity

The opacity of the films was determined using a VIS V-1200 spectrophotometer (VWR, Barcelona, Spain) at a wavelength of 600 nm, following the procedure described in [34]. The opacity was determined as shown in Eq. (3):

$$Opacity = Abs_{600}/x \quad (3)$$

where x is the thickness (expressed in mm) of the film and Abs_{600} is the absorbance measured at 600 nm. The film thickness was measured at different locations using a Palmer Electronic digital micrometer (Madrid, Spain). The mean value was calculated and used for the determination of opacity.

2.4.3. Scanning Electron Microscopy (SEM)

Microstructure of the films was observed through SEM images obtained with a Hitachi Scanning Electron Microscope (Hitachi S3000N, USA) using an accelerating voltage of 5 kV, which is sufficiently high to obtain good quality images without provoking damage to the starch matrix. SEM images were taken of the surface and of cross sections of all the studied films. Dry sheet samples were cryofractured by immersion in liquid nitrogen and subsequently coated with gold for a better observation.

2.4.4. Atomic Force Microscopy (AFM)

The surface of the films was analysed using an NT-DMT Atomic Force Microscope (NTEGRA Prima model, Moscow, Russia) operating in tapping mode. The images were post-processed using the software Nova Px (NT-MDT Co., 2013) and 3D images of a film surface of 10 μm x 10 μm were obtained. The surface roughness was evaluated by means of the average roughness (Ra) or average of the height deviations from a mean surface and root-mean-square roughness (Rq) or root-mean-square average of height deviations taken from the mean data plane, which were calculated as follows:

$$Ra = \frac{\sum_{i=1}^N Z_i - Z}{N} \quad (2)$$

$$Rq = \sqrt{\sum_{i=1}^N \frac{(Z_i - Z)^2}{N}} \quad (3)$$

where Z is the arithmetic mean of the height in nm, Z_i is the height of the data point in nm and N is the number of the data point.

2.4.5. Attenuated total reflection-Fourier transform infrared (ATR-FTIR) Spectroscopy

ATR-FTIR spectra of the synthesized films were recorded on a Bruker spectrometer (IFS 66/S model, Ettlingen, Germany) with ATR accessory between 500 and 4000 cm^{-1} . The ATR-FTIR spectra were deconvoluted using FITYK software (0.9.8 version) and the ratio of band intensities at 995 and 1022 cm^{-1} was determined in order to study the molecular rearrangement of the films.

2.4.6. Mechanical properties

The mechanical properties of the films were determined with an Instron 3344 Universal Test instrument (MA, USA) equipped with 2000N load cell and operated at 25 mm/min following ASTM D882-12 (2012) [35] standard recommendations. The samples were cut into dumbbell-shaped specimens. The mechanical properties of each film were calculated using the average thickness of the specimen and at least eight specimens per sample were tested. The tensile properties studied were tensile strength at break, percentage of elongation at break and Young's modulus. Tensile strength (in MPa) was calculated by dividing the maximum tensile force (in N) by the initial cross-sectional area (in mm^2) of the specimen. Elongation at break (in percentage) was calculated by dividing the maximum extension by the initial specimen length and multiplying by 100. Young's modulus was calculated as the slope of the tensile-extension curve at the beginning of the experiment.

2.4.7. Thermo-gravimetric analysis (TGA)

The weight loss (TG) and the weight loss derivative (DTG) curves of films were determined using a Metler Toledo thermogravimetric analyzer (TGA/SDTA851e/SF/1100 model, Barcelona,

Spain). Samples were heated from 30 °C to 700 °C at 5 °C/min using a nitrogen flow of 100 mL/min. The thermal degradation temperature was taken as the maximum of the DTG curves.

2.4.8. X-ray diffraction (XRD) studies

Diffraction patterns of the films were recorded on a Bruker diffractometer (D8-Advance model, Ettlingen, Germany) equipped with a KRISTALLOFLEX K 760-80F X-ray generator (Power=3000 W, Voltage=20-60 kV and Intensity=5-80 mA) which has an X-ray tube with copper anode ($\lambda=1.54056 \text{ \AA}$). The equipment was operated at 40 kV and 40 mA with 2θ varying from 4 to 50° with a step size of 0.05°. The degree of crystallinity was estimated for the corn and wheat starch films by the height ratio between the diffraction peak at $2\theta \approx 19.9^\circ$ and the baseline of the diffractogram at $2\theta \approx 8^\circ$ [36].

2.4.9. Statistical analysis

All samples were analyzed at least in triplicate and mean values and standard deviations were reported. Analysis of variance (ANOVA) was used to determine the significance of differences among the samples. The statistical analysis was carried out with Excel 2016 and Duncan test was used for the multiple comparisons. Differences were statistically significant at the $p < 0.05$ level.

3. Results and discussion

3.1. Starch characterization

Table 1 shows the amylose content, weight-average molecular weight (M_w), water content and phosphorus content of the different native starches. Amylose content and molecular weight are two important features of starch and have influence on starch gelatinization process [16]. Therefore, they are closely related to the physicochemical properties of the synthesized films. In this work, no very significant differences in the amylose content were found. Potato starch showed the lowest amylose content (20.5%) and corn and wheat starches similar values (24-25%). These values are in consonance with those previously published on the literature [19,37,38]. In contrast, regarding to the molecular weight of the starches, we found that potato starch possesses a considerably higher value ($6.95 \cdot 10^7 \text{ g mol}^{-1}$) than corn and wheat starch which have a very similar

molecular weight ($\approx 5.1 \cdot 10^7 \text{ g mol}^{-1}$). These results can be explained having in mind the different molecular weight of the two macromolecules of starch, amylose and amylopectin. Amylose is a linear polysaccharide with an average molecular mass of 10^5 g mol^{-1} and amylopectin is a branched polysaccharide with an average molecular mass of $10^6\text{-}10^7 \text{ g mol}^{-1}$. Therefore, it could be inferred that starches with high amylose content could have low molecular weight and a relatively more linear structure than those with a high content in amylopectin. The concentration of phosphate esters in the starch is typically very low and depends on plant origin. Commonly, potato starch has higher phosphate monoester content than cereal starches [39,40]; in good agreement, in this study the phosphorus content of potato starch was the highest as can be seen in Table 1. The phosphorus monoester content follows the same trend as the phosphorus content since phosphorus exists in the form of phosphorus monoester in starch [16]. The presence of phosphate ester is mainly located in the amylopectin fraction of starch [39,40,41]. Therefore, it could be inferred that potato starch, which has lower amylose content and hence higher amylopectin content, has higher phosphate monoester content. Water content of the native starches is similar for corn and wheat starches (10-11%) and slightly higher (15%) for potato starch. This difference could be related to the fact that potato starch is more phosphorylated and hence has a significant higher hydration capacity [41].

(Insert Table 1 about here)

3.2. Starch film characterization

As can be seen from Figure 1, the resulting films made from potato, corn and wheat starch are homogeneous and transparent. They are also easy to handle.

(Insert Figure 1 about here)

3.2.1. *Water content and solubility in water*

It is well known that water is a good starch plasticizer, and that the water content of films can readily influence the properties of the final films [24,42]. It is also important to bear in mind that the water content may depend on several factors, e.g., the amount of water included in the

formulations and the processing procedure (casting method under an excess of water in our case). We can conclude that the three film samples had a similar water content of about 10-13% (see Table 2). The water content of these films was lower than that found in literature for starch films that include ILs [27,43] or glycerol [38,44] as plasticizers. The solubility in water of starch films is a key parameter closely related with the potential biodegradability of the material [45]. The quantification of the solubility in water of the studied films at 40 and 60 °C was not possible because the samples lost their integrity after their immersion in water at these temperatures. This indicates, on the one hand, that the applications of these films in humid ambient at high temperature are limited but, on the other hand, their great capacity of dissolution in water which could be interesting from the point of view of their management as wastes after their use. As expected from the above results concerning the water content, corn and wheat starch films had similar solubility in water values at 25 °C (about 33-34%), which were lower than the solubility of potato starch film (41.25%) (see Table 2). As far as we know, there are no data in the literature on the water solubility of ionic liquid-plasticized starch films. However, the solubility in water of the studied [emim⁺][Ac⁻]-plasticized starch films was similar or higher than that mentioned in the literature [44,46] for other plasticizers, which points to an interesting advantage in terms of the increased biodegradability of the material.

(Insert Table 2 about here)

3.2.2. *Transparency*

The transparency of a film is an interesting parameter to bare in mind, because it can determine the field of application. For example, high transparency may be required for food packaging because it enables the product to be appreciated visually [47,48] and may reduce the antimicrobial charge of packaged food [49]. By contrast, films of low transparency can increase the shelf life of packed products [50]. In general terms, as can be seen in Figure 1, the transparency of our films was good, although there were slight differences in this respect, as shown in Table 2, which expresses the opacity. Of note was the high transparency obtained by potato starch films compared with corn and wheat starch films. This result is in good agreement with the literature, which

describes the use of ionic liquid or glycerol as starch plasticizers, and attributes the transparency of films to the botanical origin and the level of gelatinization [7,29,38,43]. The greater transparency of potato starch films could be indicative of a bigger amorphous phase in the films and hence a greater plasticization [43] as it will become clearer below with the XRD results. The lower transparency of the films obtained from the other two starches (corn and wheat) could be due to the lower degree of plasticization and also to the presence of some impurities inherent to these starches from cereals: lipids, proteins and colorants [2,38], whose presence provoked certain yellowness and opacity to the films.

3.2.3. Scanning Electron Microscopy (SEM)

SEM micrographs of the cross section and surface can provide information about the microstructure of the obtained films: homogeneity, layer structure, pores, cracks and surface smoothness [51]. The SEM micrographs of the starch films presented in Figure 2 reveal a high degree of internal structural homogeneity, since no pores, no starch granule structure and no proliferation of cracks can be observed. When the microstructure of the obtained films was compared to other starch films described in the literature, our films were more similar to glycerol plasticized-starch films [33,52] (usually quite homogeneous) than ILs plasticized-starch films [27,38,43] (with irregular and/or cracked surfaces). This is indicative that the process conditions and resulting composition of the films produced in this assay led to the complete disruption of the starch granule shape, and good gelatinization.

(Insert Figure 2 about here)

3.2.4. Atomic Force Microscopy (AFM)

In order to further understand the morphology of the starch films synthesized, a topographic study made by AFM to obtain qualitative (morphology) and quantitative (roughness) information of the different samples, based on the atomic interaction of their constituents [53]. Tri- and bi-dimensional topographic AFM images and bi-dimensional contrast phase images are presented in Figure 3. The first are useful for characterizing the surface of films while the second ones

characterize likely differences in mechanical properties due to the segregation and different phase domains. Contrast phase images contribute to understanding the degree of plasticization of the different samples through the observation of lighter and darker regions on the z -axis. As suggested by the SEM analysis, all synthesized films appeared homogeneous without any significant granular structure. Despite that, in all the specimens produced, small amount of unplasticized starch (also known as “ghost” granules) can be identified in topographic images (marked by blue arrows) and contrast phase images (marked by red arrows). However, in all cases the size of the nanoscopic “ghost” granules was insignificant compared to that of the native starch grains (around 15-40 μm [14]), again pointing to the good starch gelatinization process.

Table 3 shows the results obtained for the roughness determination. Films made from potato starch had the lowest roughness values ($Ra=78.9 \pm 3.7$ nm and $Rq=99.9 \pm 6.2$ nm) while films made from corn and wheat starches had similar and higher values ($Ra=151.7 \pm 20.5$ nm and $Rq=183.9 \pm 25.3$ nm, and $Ra=167.6 \pm 1.7$ nm and $Rq=197.2 \pm 4.4$ nm).

(Insert Figure 3 about here)

(Insert Table 3 about here)

3.2.5. Attenuated total reflection-Fourier transform infrared (ATR-FTIR) Spectroscopy

ATR-FTIR spectra for potato, corn and wheat films synthesized and the reference spectra of $[\text{emim}^+][\text{Ac}^-]$ are presented in Figure 4. All spectra showed a similar pattern to the bands, corresponding to the characteristic functional groups of starch and the $[\text{emim}^+][\text{Ac}^-]$. In these spectra, the broad band observed between 3200-3400 cm^{-1} corresponds to the stretching vibration of -OH groups, mainly found in the glucose units and water, and the C-H vibrational mode of the imidazolium ring in $[\text{emim}^+][\text{Ac}^-]$. The peaks found between 2800-3000 cm^{-1} are due to the aliphatic asymmetric C-H stretching vibrations and symmetric and asymmetric stretching modes of -CH₃ and -CH₂ of both the starch and $[\text{emim}^+][\text{Ac}^-]$. The vibration associated to the -CH₂ groups can be observed in the band between 1327 and 1463 cm^{-1} . The small band around 1630

cm^{-1} corresponds to the typical absorption of bound water. The bands between 1400 and 1500 cm^{-1} are assigned to C-C stretching vibrations; the band at around 1300 cm^{-1} to the stretching due to -C=C- in the $[\text{emim}^+][\text{Ac}^-]$ and 925 - 1148 cm^{-1} to the stretching of -C-O located in -C-O-C- and C-O-H bonds of the glucose ring in starch. There were no significant changes in these bands for the different starch samples, but the peaks located at around 995 cm^{-1} and 1022 cm^{-1} , associated to the degree of the rearrangements of the starch after the plasticization process [43], showed slight changes. The absorption band at around 1022 cm^{-1} is related to vibrational modes in the amorphous phase of the film and the band at 995 cm^{-1} is indicative of the presence of hydrogen bonds and the regularity given by molecular rearrangements. To further analyze these peaks, a deconvolution of the spectra was carried out and the ratio of the band intensities at 995 and 1022 cm^{-1} ($R_{1/2}$, being 1 and 2 the peaks located around 995 and 1022 cm^{-1} , respectively) was calculated. Results are shown in Table 3. According to the $R_{1/2}$ values obtained, corn ($R_{1/2} = 1.17$) and wheat ($R_{1/2} = 1.26$) starch films seem to undergo a higher degree of molecular rearrangements than potato films ($R_{1/2} = 1.04$) which had a more amorphous structure. These results agree with the XRD analysis, as will be confirmed later.

(Insert Figure 4 about here)

3.2.6. X-ray diffraction (XRD) studies

The crystalline structure of the synthesized films was studied by XRD. Figure 5 presents the comparison between the XRD diffractogram of the pristine starch granules and the films produced with each one. As can be observed, the crystalline organization of the native starch grains is relatively different, depending on their origin. After the plasticization process, a loss in crystallinity was to be expected due to the disruption of H-bonding between the starch molecules [54]. This fact is clearly observed in the XRD of the films (Figure 5) which confirms that $[\text{emim}^+][\text{Ac}^-]$ is a suitable plasticizer of starch. From an analysis of the results obtained for potato starch films, it can be seen that no peak is clearly identified in the potato starch film diffractogram, indicating the loss of B-type crystals, which are characteristic of tuber starch grains. In the case of cereal starches, the plasticization process was characterized by the disruption of the doublet

formed by the peaks located at 17° and 18.1° [26,43], as observed in Figure 5 for the case of corn and wheat starch films. Therefore, the peak located at around $2\theta=17^\circ$ would be due to the existence of small regions of residual A-type crystallinity in the films [55,56]. The plasticization process induces the formation of a new type of crystallinity, the V-type crystals, centered at 2θ values about $19.6-19.9^\circ$ and $2\theta=22.2-22.3^\circ$, as found by other authors [55]. As happens when glycerol is used as starch plasticizer [19], the V-type structure would consist of six-fold single helices of the amylose-[emim⁺][Ac⁻] complex. In these V-type crystals amylose adopts a single helical conformation with a relatively large cavity. In contrast, A- and B-crystals are formed by double helical structures [57]. The peak at $2\theta \sim 21.5^\circ$ has been described in the literature as being indicative of the dehydration of the V-type crystallinity (named as V_a) [19]. We have estimated the degree of crystallinity of the films based on XRD curves. In the case of potato starch film, the estimation has not been possible because any defined diffraction peak can be observed. It has been assumed that this sample is highly amorphous. As expected, the results for corn and wheat starch films revealed a similar degree of crystallinity of 0.35 and 0.38, respectively, since the respective starches exhibit a relatively similar structure expressed in terms of amylose content and average molecular weight.

According to results obtained in the present work, potato starch films are clearly the most amorphous because no crystalline peak can be identified after the plasticization process. Therefore, potato starch films are also the most plasticized. This could be responsible for the high transparency of potato starch films. According to Zhang *et al.* [43], an abundant plasticization with [emim⁺][Ac⁻] contributes to the disruption of the sophisticated structure of the starch grains, which could promote higher transparency of the final film. In addition, according to Bhagabati *et al.* [58], more crystalline polymeric films, such as corn and wheat starch films, are optically less transparent due to the heterogeneous morphology. They claimed that the density difference in compactly packed crystalline region and loosely held amorphous region cause scattering of incident light in the visible range and lead to development of opacity. In the case of corn and wheat starch films, the resulting XRD diffractograms showed a more crystalline structure than potato starch films. Both samples (corn and wheat starch films) followed the same pattern, with

slight differences in the intensity of the peaks located at around 21.5 and 22.3°. Corn starch films had a more intense peak at 21.5° and a less intense peak at 22.3° than wheat starch films which, as previously mentioned, is indicative of a major presence of a V_a-type structure. Although both structures (V and V_a) are similar [19], these small differences between corn and wheat starch films could be due to the slightly lower water content of corn starch films. The differences observed between the crystallinity of potato starch films *versus* corn and wheat starch films are probably due to the higher molecular weight of potato starch which leads to a lower crystallization ability of the polymer, because the rearrangement starch chains could be hindered by its size. A similar conclusion was reached by Chen *et al.* [15] for poly(trimethylene terephthalate) and by Bhagabati *et al.* [58] for poly(ϵ -caprolactone). In addition, there are more phosphate monoesters on potato starch than in the studied cereal starches (corn and wheat) as can be seen in Table 1. Those charged groups could significantly contribute to a lower starch rearrangement during starch film preparation, and thus reduced the ordered or crystal structures of potato starch film. In conclusion, potato starch showed a lower ordered structure not only attributable to a higher interaction with the plasticizer, but also, could be the result of contribution of two different factors, i.e. a high molecular weight and a high proportion of phosphate monoesters. As will be shown later, the XRD analysis agrees with the analysis of their mechanical properties. More amorphous starch films, such as potato starch films, have lower tensile strength and Young's modulus [7,27,59].

(Insert Figure 5 about here)

3.2.7. Mechanical properties

A knowledge of the mechanical properties of films is necessary for their potential use in future applications, for example, in the food packaging industry. The results obtained for the mechanical properties (Young's modulus, maximum tensile strength and elongation at break) of the films are presented in Figure 6. Young's modulus ranged from 14.65 to 87.64 MPa, the maximum tensile strength from 2.43 to 4.99 MPa, and elongation and break from 37.72% to 75.59%. As stated by other researchers, the amylose content seems to affect the mechanical behavior of the films [60,61]. The order of amylose content of the starches used was: potato (20.5%) < corn (24.8%) \approx

wheat (24.5%). Results obtained reveal that the starches with higher amylose content (corn and wheat starches) lead to more resistant films (higher tensile strength and Young's modulus and at the same time more extensible); similar conclusions can be found in the literature [62,63]. In contrast to glycerol-plasticized starch films [7,59], the use of [emim⁺][Ac⁻] as plasticizer leads to an increase in the elongation at break of the films which could be useful for their future applications. Similar results have been found with other ILs such as 1-butyl-3-methylimidazolium chloride [64]. Crystallinity of the film is also related to its mechanical behavior. In this work, as it was mentioned before, corn and wheat starch films lead to more resistant films which show, according to XRD analysis, a higher quantity of crystalline domains in these films [43,54]. These crystalline domains embedded in the amorphous matrix could be the responsible of the improvement of the mechanical properties. The mechanical properties obtained are similar or even better than those reported in previous works using similar concentrations of [emim⁺][Ac⁻] respect to the starch matrix and other ILs [25–27,43,64]. Films obtained from corn and wheat starches showed mechanical properties comparable to other common polymers currently used for food packaging such as low-density polyethylene [44], making these films potential substitutes for these commonly used polymers and potentially reducing the associated environmental hazards.

(Insert Figure 6 about here)

3.2.8. *Thermo-gravimetric analysis (TGA)*

A thermogravimetric analysis permits the influence of starch type on the thermal stability of the biopolymer films to be studied. Figure 7 shows the TGA and differential thermogravimetric analysis (DTGA) curves obtained in the nitrogen atmosphere for the studied film samples from potato, corn and wheat starch. All samples showed a slight initial weight loss up to about 100 °C, which is associated with a loss of free and bound water in the films [6,14,52]. The degradation of the starch films produced was evident from the pronounced mass loss between 200 and 350 °C. The maximum value of the peak in the DTGA curve was attained at around 265 and 267°C for the potato and corn starch films, respectively, and around 275°C for the wheat starch films. Similar

results were found for [emim⁺][Ac⁻] plasticized-starch films developed by other researchers [27,43], who claimed that [emim⁺][Ac⁻] leads to thermal degradation taking place at lower temperatures than when other starch plasticizers are used. This could be explained if we bear in mind that Zhang *et al.* [43] studied the thermal degradation of pure [emim⁺][Ac⁻] and found a large derivative weight loss peak with a maximum at about 220 °C. Other plasticizers, such as glycerol, show this peak at higher temperature (around 260°C) [25]. Our results confirm that any polymer degradation occurred during the processing of samples using the casting method, in which the maximum temperature reached is around 96°C. At the end of the experiment (700 °C) the total mass loss is around 86.5% and 87.4% for potato and corn starch films, respectively; and 84.28% for the wheat starch film. Nevertheless, despite these small differences, all thermogravimetric patterns for the films were similar and, hence, the thermal resistance is independent of the starch source used for their synthesis, but, as mentioned above, dependent on the plasticizer used.

(Insert Figure 7 about here)

4. Conclusion

Using [emim⁺][Ac⁻] as a plasticizer, starch films from three different botanical sources (potato, corn and wheat) were successfully processed using the casting technique. The starch granular components (amylose and phosphate monoesters, among others) and the molecular weight of starch had a significant influence on some physical and chemical properties in the final [emim⁺][Ac⁻]-plasticized starch films. The crystallinity of the films was greatly affected by the amylose content, molecular weight and phosphate monoester content of starch. On the one hand, films from potato starch were the most amorphous and hence most transparent, because its considerably higher molecular weight and the presence of more phosphate monoesters. The overall growth rate of crystallization and the resulting crystallinity decrease with increasing molecular weight. Phosphate monoester are charged groups which can influence the crystalline arrangement during starch film preparation leading to a lower ordered structure. On the other hand, cereal starches (wheat and corn) lead to films with a more crystalline structure because of

their lower molecular weight and phosphate monoester content which enable a higher rearrangement of the starch chains. This more pronounced crystalline structure would be responsible for their better mechanical properties. The cereal starch films obtained had mechanical properties comparable to other common polymers with high levels of solubility, which is directly related with the biodegradability of films, underlining their potential to replace synthetic and non-biodegradable polymers.

Acknowledgements

This work was partially supported from the European Commission (FEDER/ERDF) and the Spanish MINECO (Ref. CTQ2016-78246-R) and Generalitat Valenciana (Project PROMETEOII/2014/007). M.G. Montalbán acknowledges support from MINECO (Juan de la Cierva-Formación contract, Ref. FJCI-2016-28081).

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Tables:**Table 1.** Properties of starches.

Samples	Amylose content (%)	M_w (g mol⁻¹)	Water content (%)	Phosphorus content (%)
Potato	20.5 ^a	6.95·10 ^{7b}	15.1	0.043
Corn	24.8 ^b	5.12·10 ^{7a}	11	0.020
Wheat	24.5 ^b	5.13·10 ^{7a}	9.6	0.038

^{a,b}Means within each column with different letters are significantly different ($p < 0.05$), Duncan's test.

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Table 2. Properties of starch films obtained.

Sample Film	Water content (%)	Solubility in water (%)	Opacity (A_{600}/mm)
Potato	9.88 ± 0.38^a	33.88 ± 1.95^a	0.20 ± 0.05^a
Corn	10.72 ± 1.11^{ab}	34.37 ± 1.28^a	0.65 ± 0.12^b
Wheat	12.78 ± 1.43^b	33.21 ± 1.33^a	1.37 ± 0.04^c

^{a-c}Mean \pm standard deviation within each column with different letters are significantly different ($p < 0.05$), Duncan's test.

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Table 3. *Ra* and *Rq* values obtained from AFM analysis and ATR-FTIR band intensity ratio ($R_{1/2}$) of the starch films obtained.

Film Sample	<i>Ra</i> (nm)	<i>Rq</i> (nm)	$R_{1/2}$
Potato Starch	78.9 ± 3.7^a	99.9 ± 6.2^a	1.04
Corn Starch	151.7 ± 20.5^b	183.9 ± 25.3^b	1.17
Wheat Starch	167.6 ± 1.7^{bc}	197.2 ± 4.4^{bc}	1.26

Ra is the average roughness and *Rq* is the root-mean-square roughness.

^{a-c}Means within each column with different letters are significantly different ($p < 0.05$), Duncan's test.

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Captions to illustrations:

Figure 1. Visual aspect of starch films made from a) potato, b) corn and c) wheat starch.

Figure 2. SEM micrographs of the cross sections and surfaces of potato, corn and wheat starch films (Magnification 1000x).

Figure 3. AFM three- and bi-dimensional topographic images and bi-dimensional contrast phase images of the surface of potato, corn and wheat films.

Figure 4. ATR-FTIR spectra of the films obtained from potato, corn, and wheat starch, and the [emim⁺][Ac⁻].

Figure 5. XRD of the obtained films.

Figure 6. Mechanical properties of the starch films studied. a) Young's modulus; b) Maximum tensile strength; c) Elongation at break.

Figure 7. TGA and DTGA curves of potato, corn and wheat starch films obtained in nitrogen atmosphere.

Illustrations:**Comparative study on properties of starch films obtained from potato, corn and wheat using 1-ethyl-3-methylimidazolium acetate as plasticizer**

D. Domene-López, J.J. Delgado-Marín, I. Martín-Gullón, J.C. García-Quesada and

*M. G. Montalbán**

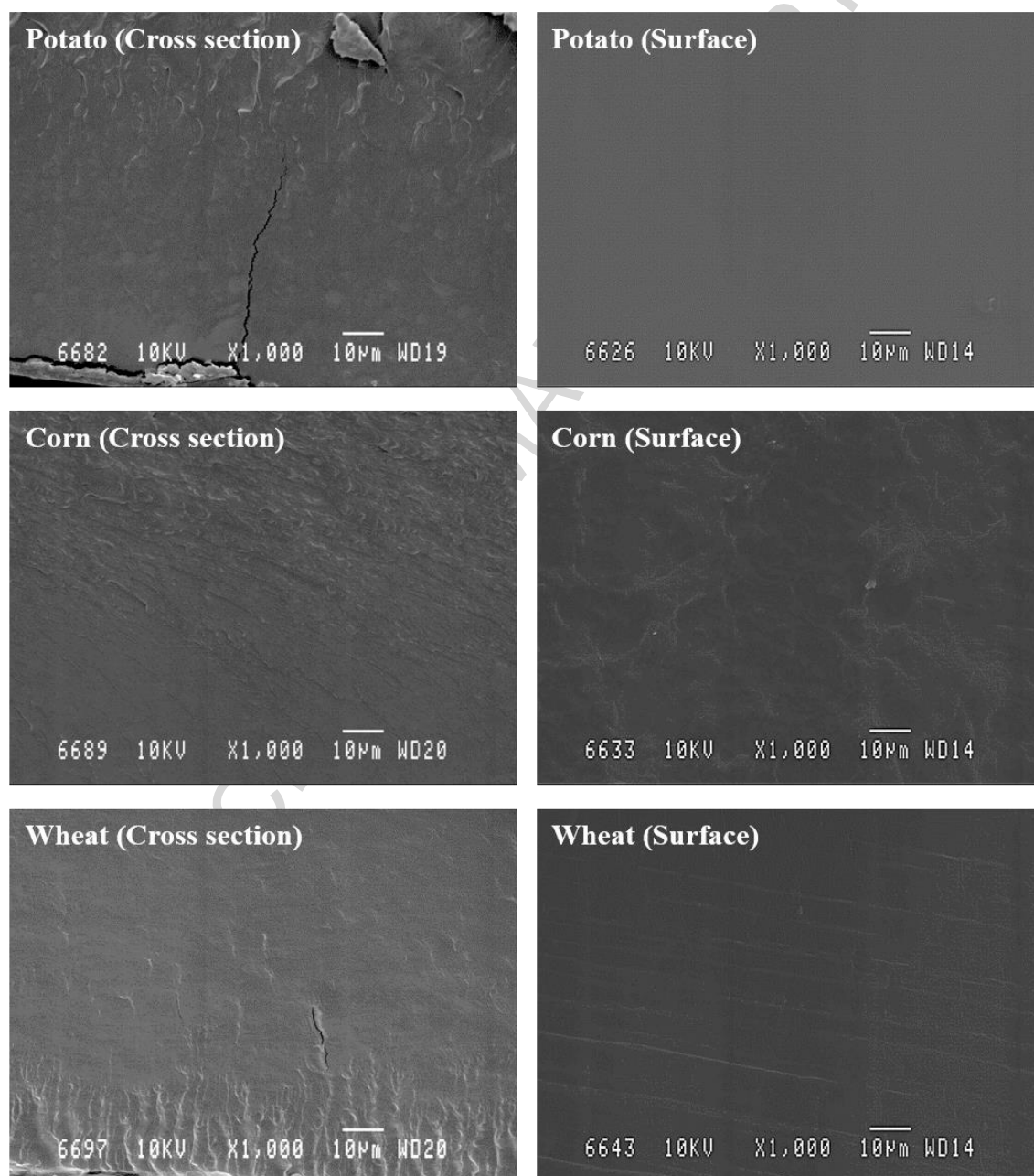
Figure 1

Comparative study on properties of starch films obtained from potato, corn and wheat using 1-ethyl-3-methylimidazolium acetate as plasticizer

D. Domene-López, J.J. Delgado-Marín, I. Martín-Gullón, J.C. García-Quesada and

*M. G. Montalbán**

Figure 2

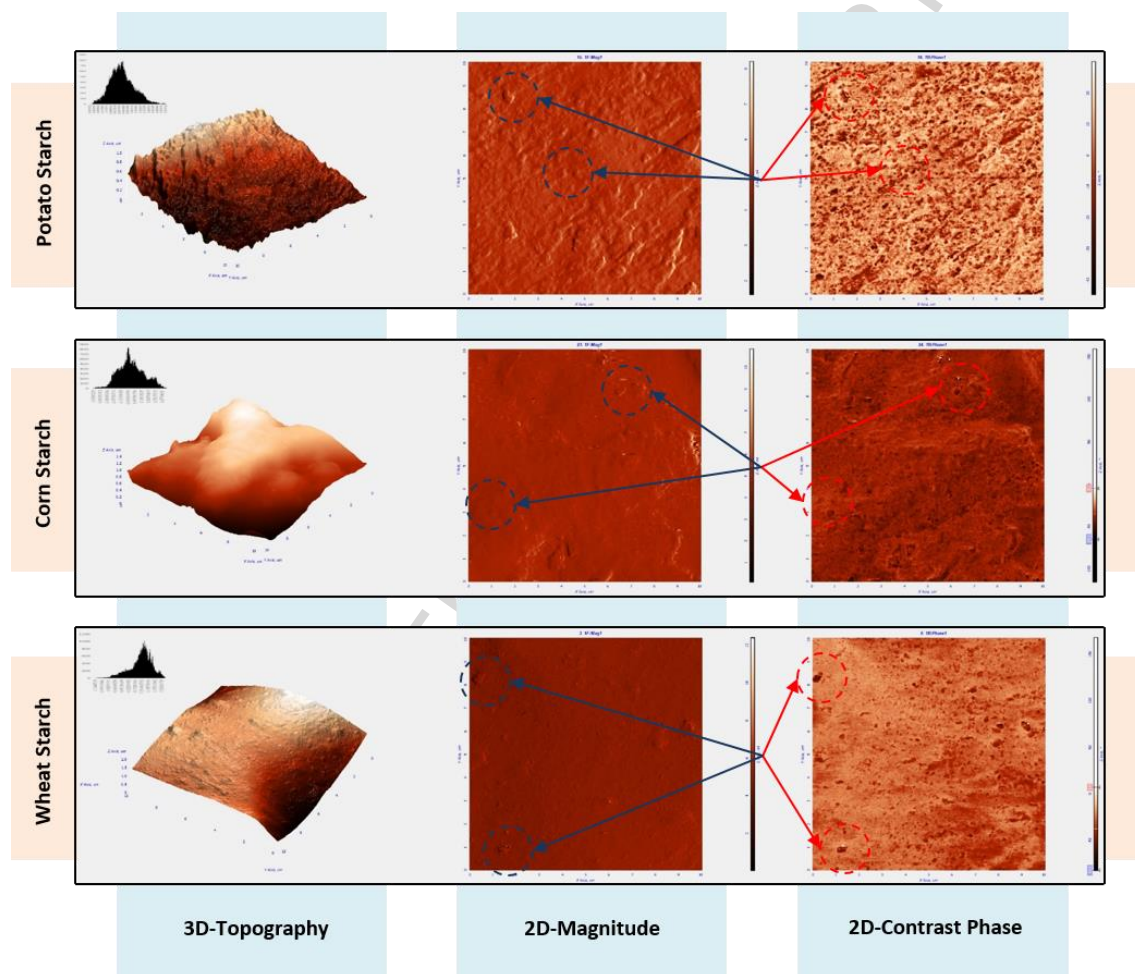


Comparative study on properties of starch films obtained from potato, corn and wheat using 1-ethyl-3-methylimidazolium acetate as plasticizer

D. Domene-López, J.J. Delgado-Marín, I. Martín-Gullón, J.C. García-Quesada and

*M. G. Montalbán**

Figure 3

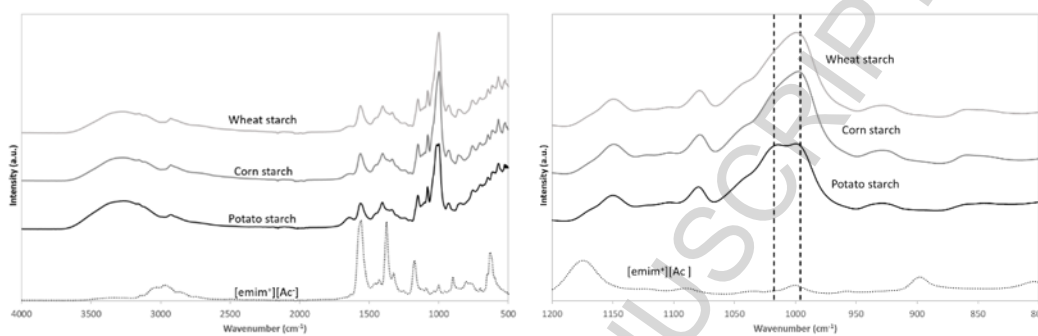


Comparative study on properties of starch films obtained from potato, corn and wheat using 1-ethyl-3-methylimidazolium acetate as plasticizer

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Figure 4

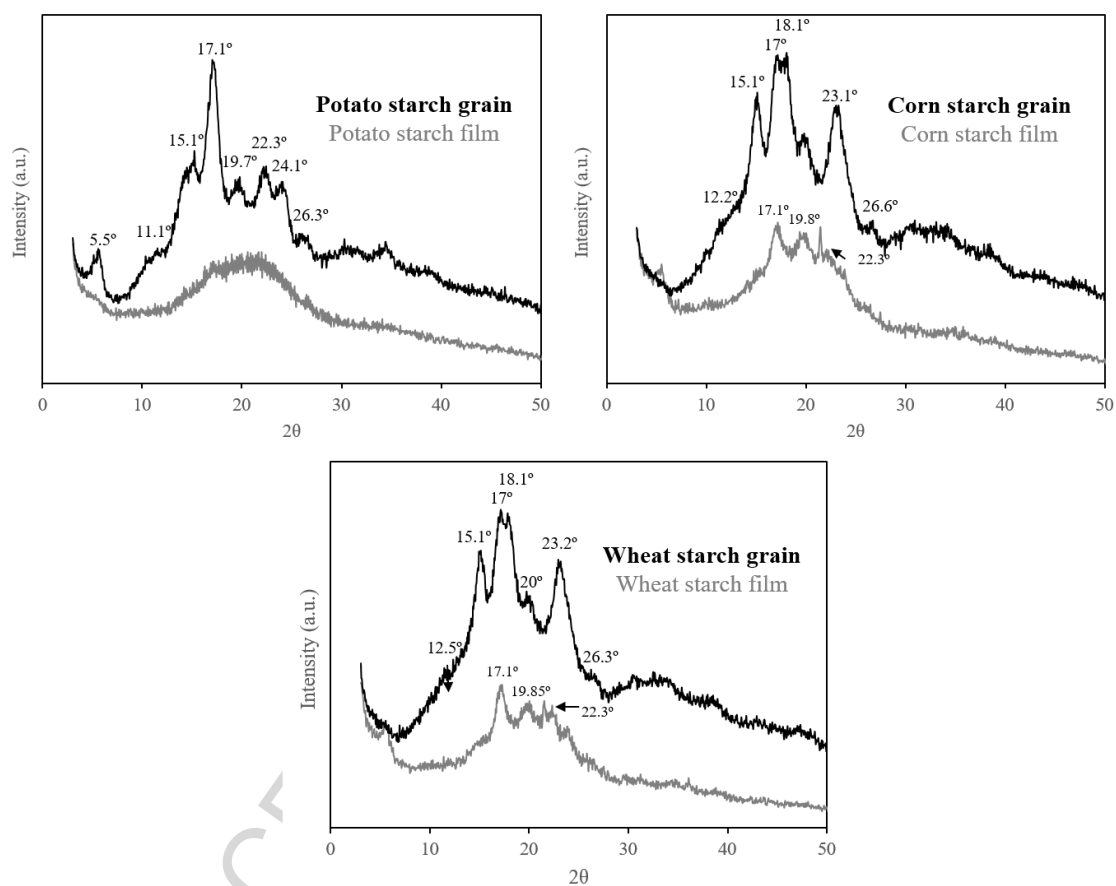


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Figure 5

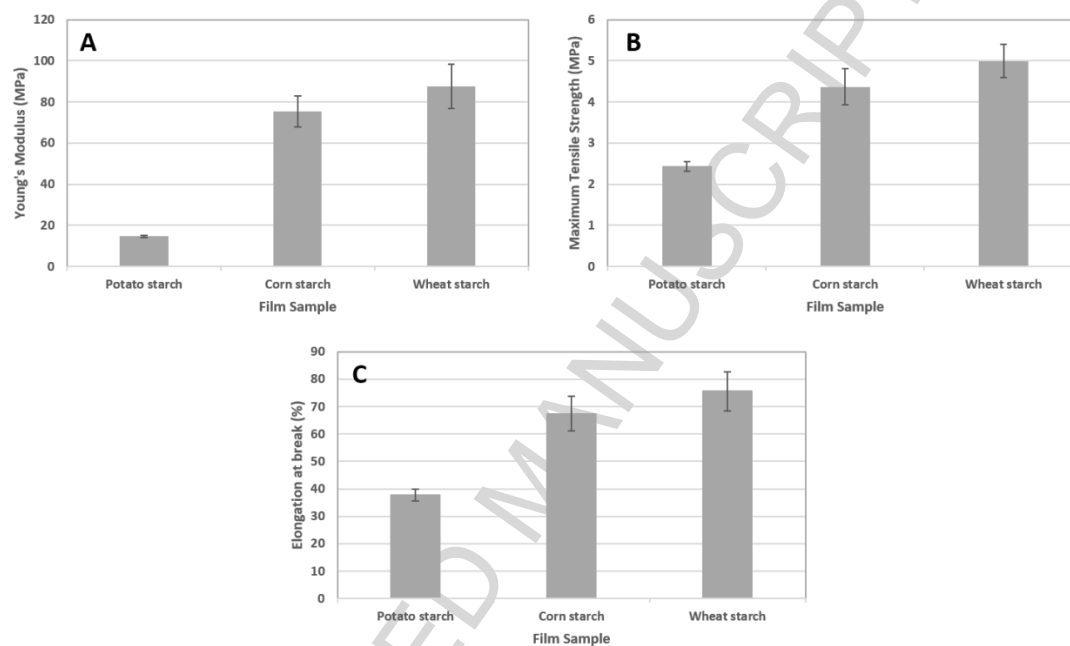


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Figure 6



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Figure 7

