



Structural properties and *in vitro* digestibility of edible and pH-sensitive films made from guinea arrowroot starch and wastes from wine manufacture

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ABSTRACT

A non-conventional starch obtained from guinea arrowroot tubers (*Calathea allouia*) grown in the Amazon was used as a polymeric matrix for the development of edible films. The films were manufactured by blending/thermo molding and plasticized with glycerol. Agro-industrial wastes from wine manufacture (grape waste flour and grape waste extract) were used as natural fillers of the thermoplastic starch (TPS) matrices. The results showed that the natural fillers caused cross-linking in the TPS matrix. This led to the production of films with higher resistant starch (RS) content, especially RS type 4 (RS4), although the DSC results showed that the films developed also contained RS type 3 (RS3). As expected, the presence of RS reduced the *in vitro* digestibility rate. Films made with the natural fillers were also less hydrophilic, had a greater thermal resistance, and tended towards ductile mechanical behavior. Finally, the edible film containing grape waste flour as a natural filler proved to be pH-sensitive, although this material disintegrated under alkaline conditions.

1. Introduction

Starch is one of the most abundant polysaccharides in nature and plays an important role in the food industry as an ingredient or additive. Non-conventional starches have attracted great interest worldwide since their properties are influenced by their botanical source (Pérez et al., 2011). Guinea arrowroot (*Calathea allouia*) tubers represent a non-conventional starch source. However, according to the FAO (1994) this crop species is increasingly rare and in danger of becoming extinct. The abandonment of guinea arrowroot seems to have been caused by two main factors (FAO, 1994): 1) a very long vegetative cycle (ten to twelve months) and 2) its replacement in the diet of small rural producers by other types of food (sweet potato, care, yam or other industrialized products such as wheat biscuits and bread).

Venezuela, in particular, has a large number of starchy plant species many of which originate in the Amazon. Guinea arrowroot tubers are cultivated by peasants in small plantations or “conucos” in this region, and are used both for direct consumption or the extraction of the starch on a small scale. Recently, Gutiérrez, 2018 characterized and analyzed the *in vitro* digestibility of this non-conventional starch. The results showed that it could be applied as a potential food source for

individuals on special diet regimens, e.g. those suffering from obesity or diabetes, due to its relatively low *in vitro* digestibility and high resistant starch (RS) content. Teixeira et al. (2016) reported that guinea arrowroot flour obtained from these tubers grown in the Brazilian Amazon has a positive effect on the growth of *Bifidobacterium* and *Lactobacillus* populations in the intestinal microbiota of male rats, thus showing itself to be a potential source of prebiotics. According to the authors (Teixeira et al., 2016), the consolidation of products derived from guinea arrowroot tubers could contribute to the economy of the Amazon as well as providing a processed food with promising positive health benefits. However, further research is required to determine human therapeutic doses.

In contrast, research into, and the development of, edible and biodegradable films from biopolymers, especially starch, have been extensively studied in recent years due to their film-forming capacity. In some cases, starch films have been partially or totally used to replace synthetic polymers. Starch-based films are one of the most effective forms of bio-based packaging materials in terms of their performance, adaptability to different products, production operations and cost (Liu, 2005). Nonetheless, they do have some drawbacks including low mechanical strength and high water sensitivity (Gutiérrez, Tapia, Pérez, &

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Famá, 2015). For these reasons, additives and natural fillers are often incorporated into starch-based edible films in order to improve their physicochemical and mechanical properties.

A potential source of natural fillers for edible films is the agro-industrial waste from wine manufacture. Wine is generally produced from grapes, and several studies have highlighted the health benefits of consuming either the fresh fruits or their derivatives (Brenes, Viveros, Chamorro, & Arija, 2016). These include vasorelaxation, anticancer effects, cardiovascular protection, antioxidant and antimicrobial activity, anti-inflammatory activity, lipid metabolism regulation, and protection against the oxidation of membranes (Bagchi, Swaroop, Preuss, & Bagchi, 2014; Friedman, 2014).

It is worth noting that the wine market is dominated by just three countries, France, Italy and Spain which between them account for roughly half the total wine production worldwide (FAO, 2014), with France being the single largest wine producing country (6.5 million tonnes in 2011 and 5.3 million tonnes in 2012). Argentina is the ninth largest producer in the world wine market (FAO, 2014). A huge amount of waste, mostly the skin and seeds, are generated during the transformation of grapes into wine. These agro-industrial waste products contain important nutrients such as citric acid, polyphenols, sugars and anthocyanins, which could be used to improve the physicochemical properties of starch-based films (Vodnar et al., 2017).

Polyphenols and sugars can act as plasticizers (Gutiérrez, Guzmán, Medina Jaramillo, & Famá, 2016), and the citric acid (Kafkas, Koşar, Türemiş, & Başer, 2006) could generate cross-linking reactions between starch chains (Olsson, Hedenqvist, Johansson, & Järnström, 2013; Menzel et al., 2013; Majzoobi & Beparva, 2014; Wang, Ren, Li, Sun, & Liu, 2014; Altamirano-Fortoul, Hernández-Muñoz, Hernando, & Rosell, 2015). Cross-linking reactions build chemical bridges between different molecules, thus reducing the high moisture sensitivity of these starchy materials whilst also helping to enhance both their mechanical and barrier properties (Olsson et al., 2013). In addition, anthocyanins contained in grape wastes could function as an indirect indicator of food quality due to the formation of either the flavylium cation (red color) or the quinoidal structure (blue color) under different pH conditions (Mateus & de Freitas, 2009). Therefore, wine industry waste materials could be used as intelligent natural fillers for the production of pH-sensitive packaging.

The formulation of intelligent films has undergone significant developments since the early of 21st century. These materials have been mainly developed to inform consumers about the state of perishable foodstuffs as well as to avoid their adulteration. According to The Commission of the European Communities (2004) the legal concept of “intelligent food contact materials and articles” is as follows: “materials and articles that monitor the condition of packaged food or the environment surrounding the food”. Nevertheless, a far more detailed academic-scientific definition for intelligent packaging materials was proposed by Rhim & Kim (2014) who described them as substances that “may respond to environmental conditions, alert a consumer to contamination by pathogens, detect harmful chemicals or degradation products caused by food deterioration, indicate food quality, and initiate self-healing”. Restuccia et al. (2010) have also mentioned that intelligent first-generation indicator packaging can be used as an “indirect indicator” of external or internal changes in the environment surrounding a food product, such as the polymerization rate, diffusion, and chemical or enzymatic reactions. The most commonly used of these visual indicators are critical pH indicators, temperature indicators, time/temperature indicators and leak indicators. Although the production of these types of food packaging is booming, the nutritional properties of edible and intelligent films have been sparsely studied. Recently, Gutiérrez & Álvarez (2016) and Gutiérrez (2017) have shown that the addition of natural fillers resulted in the development of edible film systems with lower *in vitro* digestibility rates due to the cross-linking reactions between these fillers and the starch. These authors suggested that the above mentioned films could have applications for

individuals suffering from obesity or diabetes. Readers may obtain more details about the nutritional and nutraceutical properties of edible films in the papers published by Hernández, Emaldi, & Tovar (2008), Gutiérrez & Álvarez (2016), and Gutiérrez (2017).

In previous papers published by our research group we discussed some of the nutritional and structural properties of edible and pH-sensitive starch-based films processed by casting (Gutiérrez & Álvarez, 2016; Gutiérrez, 2017). In this study, however, we investigate two main hypotheses in order to generate new scientific and technological knowledge for the development of carbohydrate polymers, as follows: 1) the anthocyanins contained in grape wastes could be used to develop pH-sensitive films processed by blending followed by thermo molding. It is worth pointing out that the development of pH-sensitive films by extrusion has not so far been possible, as the high pressures inside the extruder result in the degradation of the natural pigment (anthocyanins) (Gutiérrez & Alvarez, 2018), and 2) the citric acid contained in the grape wastes could generate the cross-linking of the starch matrix, thus leading to an increase in the RS content. This would decrease the *in vitro* digestibility rate, thus improving the nutritional quality of the films developed. In this study, we analyze some nutritional properties of edible starch-based films in terms of their RS and *in vitro* digestibility.

The aim of this study was thus to characterize the physicochemical, structural and mechanical properties, and *in vitro* digestibility of food packaging materials prepared from a non-conventional starch source with the addition of natural fillers obtained from wine production waste, in order to assess their potential use in intelligent packaging systems.

2. Experimental

2.1. Materials

Guinea arrowroot (*Calathea allouia*) was harvested in Amazonas State, Venezuela, near the banks of the Orinoco River. Starch was extracted from the guinea arrowroot tubers using the methodology described by Pérez, Bahnassey, & Breene (1993), with a yield of approximately 20% and starch purity 99.48%. Total amylose content was determined by the differential scanning calorimetry (DSC) method described by Pérez et al. (2013), which is based on the energy required to generate the amylose/lyso-phospholipid complex formation, and was estimated to be 17.97%. Ripe grapes (*Vitis vinifera*) were purchased from a local market in Mar del Plata, Buenos Aires, Argentina. The grapes were selected, discarding any diseased or stained fruits. Glycerol from Aurum, Argentina, was employed as a plasticizer in the formation of the films.

2.2. Preparation of natural fillers from wine industry waste

The selected grapes (1 kg) were manually crushed in a beaker maintaining good manufacturing practices. The resulting grape paste was then passed through a sieve, and the skin and seeds retained. Normally, these are byproducts of wine manufacture and are called grape pomace (Mattos, Tonon, Furtado, & Cabral, 2017). From this fresh waste (220 g) the following natural fillers were prepared:

2.2.1. Preparation of grape waste flour

From the grape waste retained in the sieve, 100 g were weighed out and then dried in an oven at 100 °C for 24 h in order to remove moisture. The seeds contained in the dried material were manually removed. After this the dry material was milled and passed through a 60-mesh sieve. A final weight of 22.4 g (22.4% yield) of grape waste flour was obtained from this process.

2.2.2. Preparation of grape waste extract

Grape waste extract was obtained according to the methodology proposed by Dai, Gupte, Gates, & Mumper (2009) using ethanol as a

solvent, since it maintains the properties of the fruit. Briefly, 100 g of the fresh waste were washed with 100 mL of ethanol (Aldrich – product code: 34923). The grape waste extract was prepared the same day the films were developed, and then maintained under refrigeration at 5 °C in a dark container until its use in order to avoid oxidative damages.

2.3. Characterization of the wine waste-derived fillers

Total sugars in both natural fillers (grape waste flour and grape waste extract) were determined by the methodology proposed by Lane & Eynon (1923). The citric acid contents of the grape waste flour and grape waste extract were estimated using the acetic anhydride and pyridine method described by Marier and Boulet (1958). Thermogravimetric analysis (TGA) was performed using the procedure reported by Gutiérrez (2018).

2.4. Film formation

The edible films were developed using the same plasticizer/starch ratio as Gutiérrez, Morales, Tapia, Pérez, & Famá (2015), i.e. a 1:1.4 (w/w) (glycerol/matrix) ratio. To evaluate the effect of each natural filler used, 4 g of grape waste flour or 4 mL of grape waste extract were added *per* 100 g to selected samples of starch film systems, maintaining the same glycerol:starch ratio. The films were processed by blending followed by thermo molding. This procedure was employed as it is as efficient as extrusion processing and consumes far less energy than the casting methodology as there is no solvent (water) to evaporate. This makes it an appropriate methodology for processing the large amounts of materials required by the food and polymer industry (Gutiérrez & Alvarez, 2017a). Briefly, the films were developed as follows: for each sample the compounds were pre-mixed in a beaker. Each mixture was then introduced into a Brabender type blender and blended for 30 min at 60 rpm and 130 °C. The resulting paste was poured out onto steel sheets and preheated for 5 min at the same temperature. Films were obtained by compressing at 100 bar for 15 min at 130 °C, thereafter a cooling cycle was applied until reaching a temperature of 30 °C. The resulting thermoplastic starch (TPS) films were: native guinea arrowroot starch (TPS-G), native guinea arrowroot starch plus grape waste flour (TPS-G/F), and native guinea arrowroot starch plus grape waste extract (TPS-G/E). Before characterization, the films were conditioned for a week at 20 °C and ~57% relative humidity (RH).

2.5. Film characterization

2.5.1. Determination of degree of substitution (DS) in the starch films esterified with citric acid from the added natural fillers

The amount of citric acid esterified in the films was determined by the method described by Klaushofer, Berghofer, & Steyrer (1978). This is based on the reaction of citric acid with Cu^{2+} which forms a stable complex during titration with a solution of copper sulfate. The DS was then calculated from the average number of substituent groups per anhydroglucose unit, as follows (Mei, Zhou, Jin, Xu, & Chen, 2015):

$$DS = (162 \times W)/(100 \times M) - (M - 1) \times W \quad (1)$$

where W (% by weight of substituent) = [bound citrate (g)/sample (g) bound citrate (g)] \times 100, and M = molecular weight of the citric acid substituent (175.1 g/mol). Each sample was analyzed in triplicate.

2.5.2. Moisture content (MC)

The moisture content of the films after conditioning (one week at 20 °C and ~57% RH) was determined by measuring weight loss upon drying in an oven at 105 °C until a constant weight was achieved (dry sample weight). Samples were analyzed in triplicate. Equilibrium moisture content (%) was calculated, considering the initial weight of each conditioned sample and the dry weight (Gutiérrez, Morales, Pérez, Tapia, & Famá, 2015).

2.5.3. Thermogravimetric analysis (TGA)

Thermogravimetric analyses were carried out with a TA Instrument Model TGA Q500. Film samples were weighed and approximately 15–40 mg of dry matter processed per sample. The samples were then heated at a constant rate of 10 °C/min from room temperature (approx. 30 °C) to 600 °C under nitrogen atmosphere (flow rate 30 mL/min). Derivative thermogravimetric analysis (DTGA) was then performed in order to identify the temperatures at which the maximum thermal degradation rates of each component occurred. Each sample was analyzed in triplicate.

2.5.4. X-ray diffraction (XRD)

XRD diffractograms of the samples were obtained using a PAN analytical X'Pert PRO diffractometer (Netherlands) equipped with a monochromatic $\text{Cu K}\alpha$ radiation source ($\lambda = 1.5406 \text{ \AA}$). The generator voltage was 40 kV and the current was 40 mA. Film crystallinity was evaluated from $2\theta = 3^\circ$ to 33° (2° per min) at room temperature. The thicknesses of the samples on the slides were ~1 mm. The percent crystallinity of films was then determined from the scattering spectrum as the ratio of the integrated crystalline intensity to the total intensity following Hermans & Weidinger (1961). The crystalline fraction was estimated by measuring the area above the smooth curve drawn from the main peaks (main *d*-spacing).

2.5.5. Differential scanning calorimetry (DSC)

Differential scanning calorimetry was carried out using a DSC Pyris 1, Perkin Elmer (Massachusetts, USA) to determine the melting temperatures (T_m) and melting enthalpies (ΔH_m) of the films under analysis. About 5 mg of each sample was weighed and placed into an aluminum pan and sealed hermetically. An empty aluminum pan was used as a reference. Initially, samples were heated at a rate of 20 °C/min from 30 °C to 150 °C under nitrogen atmosphere to remove previous thermal history and moisture (Pedroso & Rosa, 2005; Othman, Azahari, & Ismail, 2011). To study the non-thermal crystallization of starch film systems, the samples were cooled down to -60°C using an intracooler (Perkin Elmer, Massachusetts, USA) and then heated to 240 °C at a scan rate of 10 °C/min under nitrogen atmosphere. Three samples of each material were tested to ensure repeatability. All the thermograms shown refer to the second heating.

2.5.6. Uniaxial tensile tests

The mechanical properties of the films were determined using an INSTRON 4467 machine (High Wycombe, UK) with a 100 N load cell and operating at a constant crosshead speed of 1 mm/min. To determine the force-distance curves films were cut into strips (20 \times 80 mm), mounted between the tensile grips (A/TG model) and stretched until they broke. The force-distance curves obtained in the tests were then transformed into stress-strain curves to determine the following parameters: Young's modulus (*E*), maximum stress (σ_m), strain at break (ϵ_b) and toughness, all of which were calculated following the ISO 527-2 norm. At least 10 trials for each film system were performed at 25 °C.

2.5.7. Response to pH changes

In order to observe the responses of the films to changes in pH, samples of each film system were cut into 12 mm diameter discs and immersed in solutions of pH equal to 1, 7 and 13, prepared from NaOH and HCl. The response of the films was then evaluated from photos of each one, taken with an 8.1 mega pixel Cyber-shot Sony camera, model DSC-H3 (Tokyo, Japan).

2.5.8. Determination of resistant starch (RS)

Resistant starch (RS) content was determined following the official AOAC method (AOAC, 2003). RS yield was determined as:

$$RS(\%) = \frac{\text{Residue mass(g)}}{\text{Sample mass(g)}} \times 100(\text{dry weight basis}) \quad (2)$$

Analyses were performed in triplicate to ensure repeatability. Results were reported as mean \pm SD.

2.5.9. *In vitro* digestibility tests – starch hydrolysis index

The *in vitro* rate of starch hydrolysis was evaluated using the methodology described by Hernández et al., 2008 and Zamora-Gasga, Bello-Pérez, Ortiz-Basurto, Tovar, & Sáyago-Ayerdi (2014), with modifications proposed by Gutiérrez, & Álvarez (2016), e.g. guinea arrowroot starch was used as reference. Data were plotted as degree of hydrolysis versus time curves.

2.6. Statistical analysis

The data were analyzed using the software OriginPro 8 (Version 8.5, Northampton, USA). Tukey's test at a confidence level of 95% was used to examine significant differences among the bioplastics tested.

3. Results and discussion

3.1. Characterization of the wine waste-derived fillers

The total sugar content was 15.7% and 2.3% for the grape waste flour and grape waste extract, respectively. Citric acid content was 10.8 mg/100 g and 6.7 mg/100 g for the grape waste flour and grape waste extract, respectively. The TGA and DTGA (derivative TGA) curves (Fig. 1) for the grape waste flour showed two mass losses, the first at 198 °C and the other at 319 °C, associated with the melting point of the citric acid and the decomposition of the fiber content (cellulose and hemicellulose), respectively (Brenes et al., 2016). The grape waste extract showed a first mass loss at 76 °C, associated with the evaporation of the solvent (boiling point of the ethanol, 78 °C), and a second loss at 192 °C, again related to the melting point of the citric acid (175 °C). It should be noted that the peak associated with the citric acid melting point was more intense for the grape waste flour than the grape waste extract. This is possibly because of the higher citric acid content of the grape waste former (10.8 mg/100 g) compared to the latter (6.7 mg/100 g). The starch itself showed a unique loss of mass at 314 °C, associated with its decomposition temperature. A single well-defined mass loss is a consequence of the high purity of the starch.

3.2. Film characterization

3.2.1. Determination of the degree of substitution (DS) in the starch films esterified with the citric acid contained in the added natural fillers

The degrees of substitution (DS) of the films containing the natural fillers were as follows: 0.062 ± 0.001 (TPS-G/F) and 0.089 ± 0.005 (TPS-G/E). Obviously the DS of the TPS-G film was indeterminate, since this film system did not contain any source of citric acid that would lead to the cross-linking of the starch matrix. These results suggest that the citric acid contained in the natural fillers tested (grape waste flour and grape waste extract) causes cross-linking reactions in the thermoplastic starch (TPS) matrix. Similar results were reported by Gutiérrez (2017) for films prepared from plantain starch and pre-gelatinized plantain flour that were cross-linked with the citric acid contained in the incorporated blackberry pulp.

It should be noted that the grape waste extract generated a statistically significant ($p \leq 0.05$) higher degree of cross-linking than the grape waste flour. Thus, although the grape waste flour had a higher citric acid content (10.8 mg/100 g) than the grape waste extract (6.7 mg/100 g), apparently the citric acid in the former is more compromised and cannot easily migrate into the TPS matrix to generate the cross-linking reaction. In contrast, the grape waste extract with a lower citric acid content produced a greater DS in the TPS matrix, suggesting

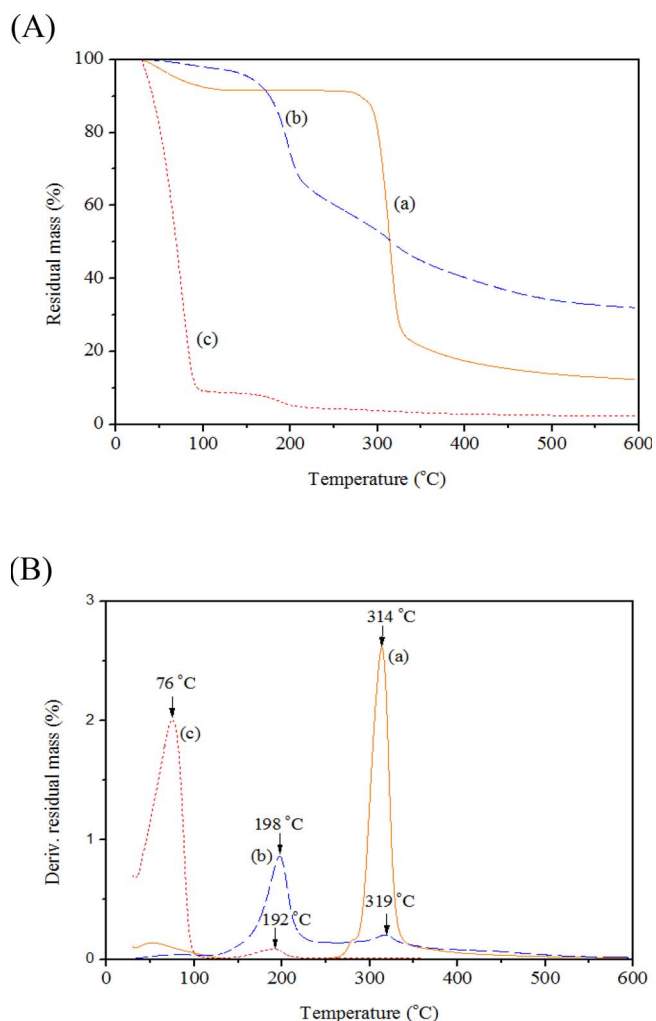


Fig. 1. (A) TGA and (B) DTGA (derivative TGA) curves of the matrix and the different fillers: (a) guinea arrowroot starch (matrix), (b) grape waste flour and (c) grape waste extract.

that the citric acid contained in this filler was more available resulting in stronger cross-linking reactions.

3.2.2. Moisture content (MC)

The moisture content (MC) of the films was recorded as follows: TPS-G ($26.7 \pm 0.8\%$) > TPS-G/F ($22.36 \pm 0.05\%$) \approx TPS-G/E ($23 \pm 2\%$). Thus, the natural fillers fulfilled their purpose, i.e. they reduced the water sensitivity of the starch films tested. No statistically significant differences ($p \geq 0.05$) in MC were observed between the films containing the natural fillers (grape waste flour and grape waste extract).

It is well known that interactions in these types of systems occur mainly through the hydroxyl (OH) groups contained in starch (hydrogen bond interactions). The polarity compensation of these OH groups leads to a reduction in the moisture sensitivity of the films. This would explain the decrease in the MC in the films containing the natural fillers. Another possible explanation could be the cross-linking of the TPS matrix with the grape waste citric acid. This produces strong hydrogen bond interactions between the carbohydrate polymer and the plasticizer, thus limiting water absorption by the polar groups of the TPS matrix (Olsson et al., 2013). It is also worth remembering that the cross-linking of the starch chains occurs through the hydroxyl groups which become nucleophilic after the addition reaction with the carboxyl groups of the citric acid. This results in the loss of the hydroxyl groups, thereby decreasing the susceptibility of the polymer to moisture

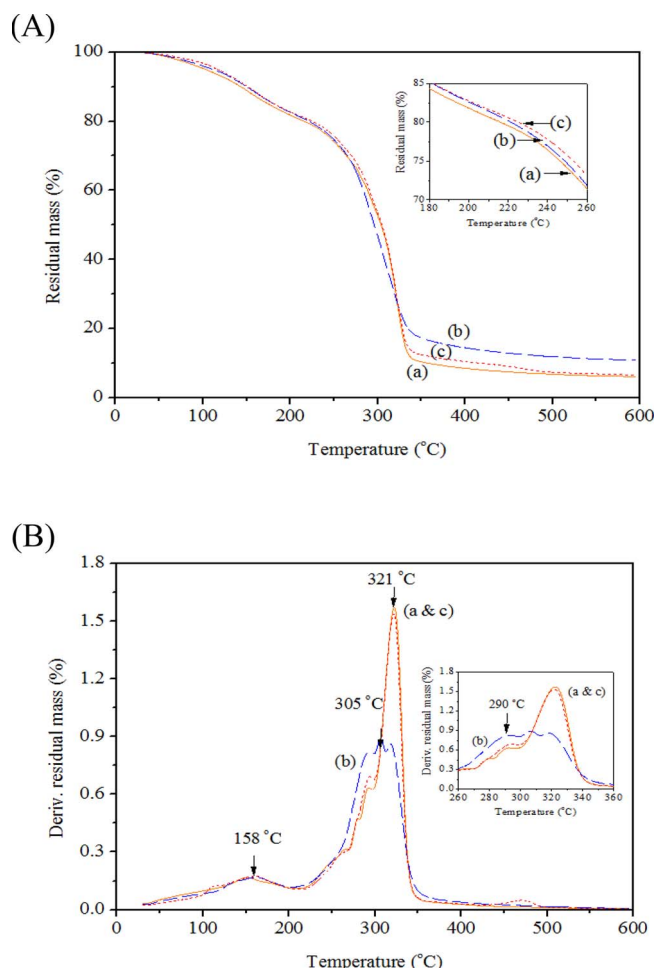


Fig. 2. (A) Thermogravimetric analysis (TGA) and (B) DTGA (derivative TGA) curves of the different films studied: (a) native guinea arrowroot starch (TPS-G), (b) native guinea arrowroot starch plus grape waste flour (TPS-G/F) and (c) native guinea arrowroot starch plus grape waste extract (TPS-G/E).

absorption (Gutiérrez & González, 2017).

3.2.3. Thermogravimetric analysis (TGA)

The TGA and DTGA (derivative TGA) curves of the three film systems evaluated are shown in Fig. 2. Starch-based films have three main stages of thermal degradation: evaporation of the adsorbed water or available (free) water (from 100 °C); evaporation of the glycerol-rich phase (about 290 °C); and the thermal degradation of the starch-rich phase (around 330 °C) (Gutiérrez, Morales, Pérez et al., 2015). All these stages were observed in the films tested.

It can be seen from Fig. 2A that the films with the natural fillers incorporated into the TPS matrix had a slightly higher thermal degradation temperature than the pure TPS. This behavior is typical in systems where cross-linking or grafting reactions have occurred (Gutiérrez & Álvarez, 2016), and agrees with the DS results for the films tested. In addition, there was a positive relationship between the DS and the thermal degradation temperature of the starch-rich phase: the TPS-G/E film showed the highest thermal resistance among the films analyzed.

It can also be observed from the TGA curves (Fig. 2A) that after 330 °C no further thermal degradation occurred, as demonstrated by the unchanged state of the film residual mass. The highest residual mass content at this stage was recorded for the TPS-G/F film followed by the TPS-G/E and TPS-G films, in that order. It is worth remembering that in these systems the final stage of the thermal degradation of the starch-rich phase occurs at temperatures above 330 °C, and involves the

elimination of the polyhydroxyl groups accompanied by the depolymerization and decomposition of the starch matrix and finally the production of carbon. Thus, the highest residual mass content recorded for the TPS-G/F film can be associated with the additional carbon material content resulting from the decomposition of the cellulose and hemicellulose contained in the grape waste flour. As expected, the lowest residual mass content at this stage was registered for the TPS-G film, i.e. the film with no added filler.

The DTGA curves (Fig. 2B) showed that the films suffered a maximum moisture loss at 158 °C in all cases. In addition, the peaks observed at 290 and 321 °C (all the films) (Fig. 2B) demonstrate that these systems underwent a weak phase separation. The TPS-G/F film also showed an additional peak at 305 °C, probably due to the thermal degradation of the cellulose and hemicellulose contained in the grape waste flour (Dorez, Taguet, Ferry, & Lopez-Cuesta, 2013; Brenes et al., 2016), and is consistent with the DTGA curves obtained for the grape waste flour alone (319 °C, Fig. 1). The slightly lower thermal degradation peak for the cellulose and hemicellulose contained within the TPS-G/F film may be due to the depolymerization of these compounds during processing (Dorez et al., 2013).

On the other hand, the peaks at 192 and 198 °C associated with the citric acid found in the natural fillers (see DTGA curves, Fig. 1) were not observed from the DTGA curves of the film systems (Fig. 2B). This confirms that after the cross-linking reaction occurs, the citric acid becomes part of the chemical structure of the TPS matrix. In this sense, the unprocessed arrowroot guinea starch had a degradation temperature of 314 °C, which after plasticization and gelatinization rose to 321 °C. This increase in thermal resistance is closely related to the hydrogen bonding interactions established between the starch and the plasticizer (glycerol) as well as the cross-linking reactions. It is worth remembering that cross-linking results in an increase in the molecular weight of starch, which in turn leads to an increase in its thermal degradation temperature. This would be in line with the results reported here.

3.2.4. X-ray diffraction (XRD)

From the X-ray diffractograms of the studied films (Fig. 3), diffraction peaks corresponding to $2\theta = 15.7^\circ$, 18.1° , 20.8° , and 23.1° , associated with the d -spacings $\cong 5.6 \text{ \AA}$, 4.9 \AA , 4.3 \AA , and 3.9 \AA , respectively, were observed in all the films. Specifically, the peak located at $2\theta = 20.8^\circ$ (4.3 \AA) is associated with the type V structure, reported in the literature as being related to hydrogen bond interactions (García-Tejeda et al., 2013). The remaining peaks correspond to the type A structure. In addition, the TPS-G/E film showed an additional diffraction peak at $2\theta = 21.5^\circ$ (4.1 \AA), demonstrating another type of

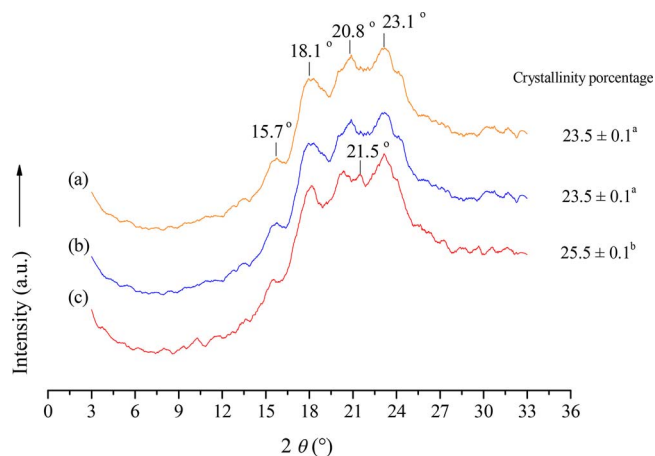


Fig. 3. X-ray diffraction pattern of the different films studied: (a) native guinea arrowroot starch (TPS-G), (b) native guinea arrowroot starch plus grape waste flour (TPS-G/F) and (c) native guinea arrowroot starch plus grape waste extract (TPS-G/E).

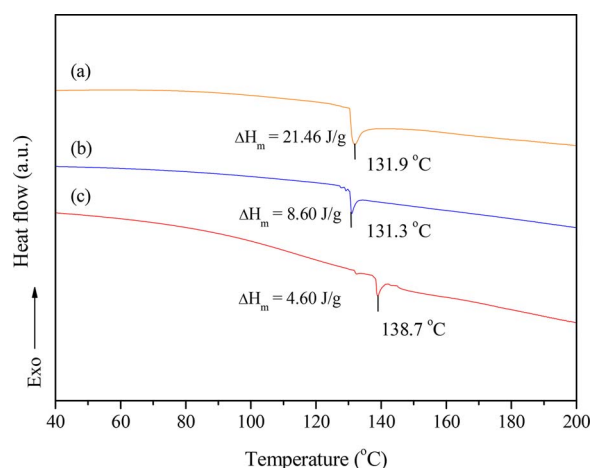


Fig. 4. Heating thermograms of the films based on: (a) native guinea arrowroot starch (TPS-G), (b) native guinea arrowroot starch plus grape waste flour (TPS-G/F) and (c) native guinea arrowroot starch plus grape waste extract (TPS-G/E).

interaction in this film typical of a B-type structure. Gutiérrez & Álvarez (2016) observed a stronger expression of this type B structure in films derived from plantain flour cross-linked with the citric acid contained in the *aloe vera* gel. This would be in agreement with the other results obtained for the TPS-G/E film.

In general, the films characterized showed a behavior typical of semicrystalline materials, which is in line with that observed by other researchers for starch-based films (García-Tejeda et al., 2013). The TPS-G and TPS-G/F films both had crystallinity percentages of about 23.5%, with no significant difference ($p \geq 0.05$) registered between them. The TPS-G/E film did, however, show a significantly higher ($p \leq 0.05$) crystallinity percentage. An increase in the crystallinity of this type of material has been associated with the starch retrogradation phenomenon that occurs during storage (aged films) as well as the cross-linking reactions in the TPS (García-Tejeda et al., 2013; Gutiérrez, Morales, Pérez et al., 2015). Thus, so far the results confirm the cross-linking of the citric acid contained in the grape waste extract with the guinea arrowroot starch.

3.2.5. Differential scanning calorimetry (DSC)

The heating thermograms (Fig. 4) show endothermic melting peaks for all the film samples tested. These are related to the melting of the crystalline phase formed during the starch retrogradation process. Medina Jaramillo, Gutiérrez, Goyanes, Bernal, & Famá (2016) also reported endothermic melting peaks at around 90 °C for cassava starch films containing different concentrations of *yerba mate* extract. According to Medina Jaramillo et al. (2016) the melting endotherms in starch films could originate from the co-crystallized amylose and amylopectin generated during the film-forming process, which may lead to the continuous crystallization of amylopectin.

The TPS-G and TPS-G/F films showed almost equal melting temperatures (T_m), 131.9 °C and 131.3 °C, respectively. However, the T_m of the TPS-G/E film was higher than the other systems evaluated. It is well known that an increase in T_m is associated with a reduction in the mobility of the polymer chains as well as a decrease in their free volume (Gutiérrez & Alvarez, 2017b). Cross-linking reactions can cause a decrease in the mobility of starch chains, thus the increase in the T_m of the TPS-G/E film could be explained by the high DS observed for this system (see Section 3.1).

The melting enthalpy (ΔH_m) of the film systems increased in the following order: 4.60 J/g (TPS-G/E) < 8.60 J/g (TPS-G/F) < 21.46 J/g (TPS-G). Thus, the TPS-G film showed the most endothermic behavior.

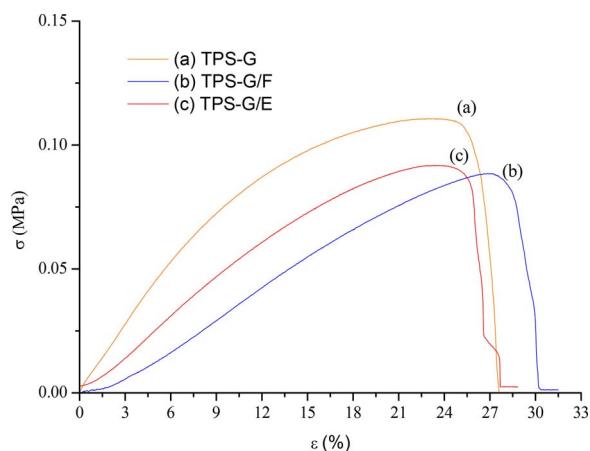


Fig. 5. Stress (σ)–strain (ϵ) curves of the films based on: (a) native guinea arrowroot starch (TPS-G), (b) native guinea arrowroot starch plus grape waste flour (TPS-G/F) and (c) native guinea arrowroot starch plus grape waste extract (TPS-G/E).

3.2.6. Uniaxial tensile tests

Fig. 5 shows the stress–strain curves of each film system studied. A small linear zone followed by a nonlinear zone until breaking point was observed. Similar behavior for corn starch-based films has been reported elsewhere in the literature (Gutiérrez & González, 2017).

Table 1 shows the values of the uniaxial tensile strength parameters tested for each film system: Young's modulus (E), maximum stress (σ_m), strain at break (ϵ_b) and toughness (T). The TPS-G film showed the highest E , σ_m , and T values, i.e. this film system showed a more fragile behavior than the other films. Possibly the retrograded starch-related crystalline phase (see DSC results) in this film system acted as a stress concentrator, resulting in the embrittlement of the material. In contrast, ductile behavior (plastic deformation) was observed for the TPS-G/F film: lowest E , σ_m , and T values, and highest ϵ_b values. The TPS-G/E film displayed mechanical behavior intermediate between the TPS-G and TPS-G/F films, although statistically speaking there were no differences ($p \geq 0.05$) between the ϵ_b and T values of the TPS-G/E and TPS-G/F films. Finally, films prepared from cross-linked starches tend to have higher ϵ_b values than their non-crosslinked analogues (Gutiérrez, Tapia et al., 2015; Gutiérrez & Alvarez, 2017b). This trend was observed in the TPS-G/F and TPS-G/E films compared to the TPS-G film, although statistically there was no difference ($p \geq 0.05$) between the TPS-G/E and TPS-G films.

Equal letters in the same column indicate no statistically significant difference ($p \leq 0.05$). Thermoplastic starch (TPS) films: native guinea arrowroot starch (TPS-G), native guinea arrowroot starch plus grape waste flour (TPS-G/F) and native guinea arrowroot starch plus grape waste extract (TPS-G/E).

3.2.7. Response to pH changes

The anthocyanins contained in the grape waste flour imparted the TPS-G/F film with intelligent (pH-sensitive) properties (Fig. 6). At pH = 1 this film showed a slight pink tinge due to the flavylium cation (red color) of the anthocyanins (Mateus & de Freitas, 2009), whereas at pH = 7 it showed a bluish coloration due to the quinoidal structure of the anthocyanins (Mateus & de Freitas, 2009). It should be noted,

Table 1
Parameters of the uniaxial tensile strength tests: Young's modulus (E), maximum stress (σ_m), strain at break (ϵ_b) and toughness (T).

Material	E (KPa)	σ_m (KPa)	ϵ_b (%)	T ($\times 10^3$) (J/m ³)
TPS-G	9.3 \pm 0.7 ^c	123.3 \pm 0.3 ^c	27.2 \pm 0.5 ^a	2.4 \pm 0.2 ^b
TPS-G/F	4.9 \pm 0.4 ^a	88.7 \pm 0.6 ^a	31 \pm 2 ^{b,a}	1.5 \pm 0.1 ^a
TPS-G/E	5.8 \pm 0.4 ^b	90.9 \pm 0.2 ^b	29 \pm 2 ^a	1.6 \pm 0.2 ^a

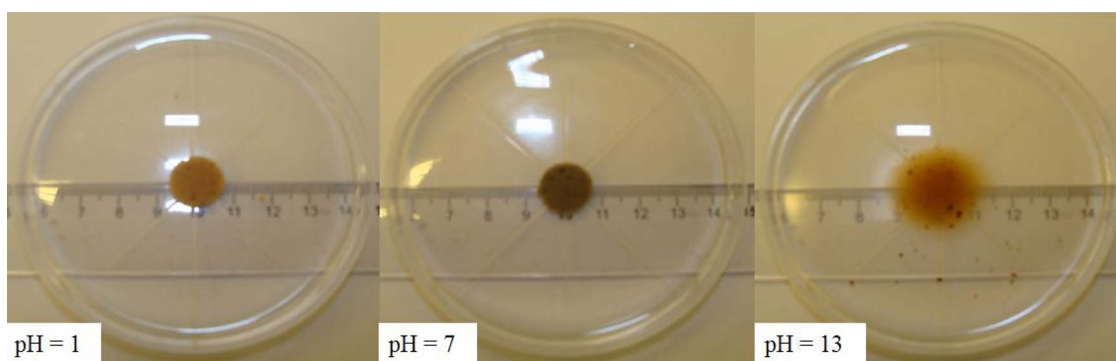


Fig. 6. Response of the guinea arrowroot starch-based films containing grape waste flour obtained as a byproduct from the wine industry (TPS-G/F) at different pH conditions.

however, that the TPS-G/F film system was disintegrated and dissolved in the alkaline medium ($\text{pH} = 13$). This would limit its application to pH intervals between 1 and 7. According to Gutiérrez, Morales, Pérez et al. (2015) the dissolution and disintegration of starch films under alkaline conditions could be due to reactions between the sodium hydroxide and the hydroxyl groups of the starch molecules. This reduces and destroys the intermolecular hydrogen bonding interactions between the starch macromolecules and the glycerol, resulting in swelling and starch gelatinization. The other film systems did not show pH-sensitive behavior, including the TPS-G/E film containing the grape waste extract. This may be because the anthocyanins are more available in the grape waste extract than in the grape waste flour. Thus, apparently the matrix of the grape waste flour gives protection to the pigment during processing (blending/thermo molding – high shear forces and pressures within the mixer). The availability of the anthocyanins in starch-based film systems could represent a limitation of the use of these natural pigments for the development of pH-sensitive food packaging on an industrial scale. In other words, if the pigment is very available it may be degraded by the processing conditions. If it is too protected, however, it may not give the desired pH-sensitive response. Similar results have been reported by Gutiérrez & Alvarez (2018) for films prepared from corn starch containing blueberry extract and processed by extrusion.

3.2.8. Determination of resistant starch (RS)

Currently, resistant starch (RS) is classified into five groups: physically inaccessible starch (RS1), resistant granules (RS2), retrograded starch (RS3), physically or chemically modified starch (RS4 – starch with non-starch bonds), and starches containing amylose-lipid complexes (RS5) (Lovera, Pérez, & Laurentin, 2017). The RS content obtained for the TPS-G film ($12.5 \pm 0.3\%$, Fig. 7A) suggests that it is an RS3. This would explain the melting of the observed crystalline phase in this system (see Section 3.5 – DSC results, Fig. 4).

However, according to the DSC results, all the film systems studied showed the melting of the crystalline phase. This suggests that both the TPS-G/F and the TPS-G/E films also contain RS3, and that the observed increase in the RS content of these films (Fig. 7A) is due to the contribution of RS4, i.e. the fraction of starch chemically cross-linked with the citric acid found in the grape wastes. No statistically significant differences in RS content were observed between the TPS-G/F and TPS-G/E films.

The importance of obtaining starch-based food products with higher RS contents is that these materials are capable of escaping enzymatic digestion. This means that they cannot be adsorbed into the small intestines of healthy individuals, and thus lower rates of glucose are released into the blood. This results in products with low glycemic indexes, which are beneficial for obese and diabetic individuals. In addition, since RS escapes direct digestion it is fermented by several bacterial species in the colon producing short chain fatty acids (e.g. butyric acid) that can then be absorbed into the colon. These short

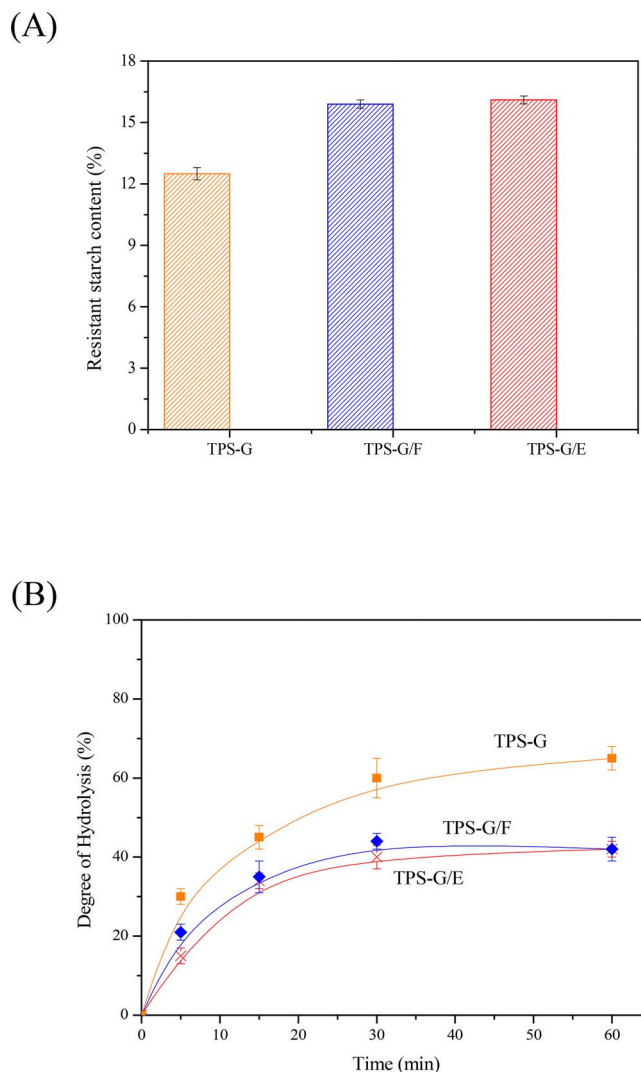


Fig. 7. (A) Resistant starch content and (B) *in vitro* α -amylolysis curves of the different films studied: native guinea arrowroot starch (TPS-G), native guinea arrowroot starch plus grape waste flour (TPS-G/F) and native guinea arrowroot starch plus grape waste extract (TPS-G/E).

chain fatty acids play an important role in reducing cholesterol levels in the blood, thus having a positive effect on people with cardiovascular problems (Hernández et al., 2008; Gutiérrez & Álvarez, 2016; rrez, 2017, 2018;). This means that the films containing grape wastes (TPS-G/F and TPS-G/E) represent a healthy alternative in the development of edible and intelligent packaging materials.

It is worth pointing out that Gutiérrez (2018) reported a RS content

of around 25% for native guinea arrowroot starch, i.e. starch that has not undergone plasticization or gelatinization. This corresponds to RS1, and would be initially lost during the processing of the films. Small fractions of RS1 could remain within the films, but their detection would be impossible. Thus, once starch is gelatinized the RS obtained in the food products developed from is assigned to the RS3 type. Finally, the films studied here had higher RS contents than other films prepared by our research group (Gutiérrez & Álvarez, 2016; Gutiérrez, 2017).

3.2.9. *In vitro* digestibility tests – starch hydrolysis index

The *in vitro* digestibility rates of the films cross-linked with the citric acid contained in the natural fillers used (grape waste flour and grape waste extract) were significantly lower ($p \leq 0.05$) than that of the TPS-G film (Fig. 7B). This means that films with added natural fillers could be used in foods for people suffering from obesity or diabetes. However, more specialized studies such as the determination of the glycemic index and *in vivo* digestibility should be performed in order to verify the possible health effects on these sectors of the population.

The decrease in the *in vitro* digestibility rate of the films containing the natural fillers (TPS-G/F and TPS-G/E) could be positively associated with the RS4 contained in these systems as this type of starch escapes direct digestion. It should be noted that the digestibility rate for guinea arrowroot starch-based films is higher than that of native arrowroot starch (see results reported by Gutiérrez (2018)). This confirms that processed starch products are more digestible than the raw material.

Similar results were reported by Gutiérrez & Álvarez (2016) for films derived from plantain flour chemically cross-linked with different concentrations of *aloe vera* gel (0, 2, 4 and 6%). As a final comment: obviously the RS3 content of the films developed plays an important role in their reduced *in vitro* digestibility rates.

4. Conclusions

Edible films were developed by blending followed by thermo molding from a non-conventional source of carbohydrate polymer (guinea arrowroot starch). Glycerol and by-products from the wine industry (grape waste flour and grape waste extract) were used as the plasticizer and natural fillers, respectively. Edible and pH-sensitive films were obtained from the arrowroot starch containing from grape waste flour (a common waste product of wine manufacture). We have shown that edible and pH-sensitive films could be manufactured on an industrial scale by blending followed by thermo molding. This provides a useful alternative to methodologies such as extrusion which has been shown to degrade pH-sensitive pigments (anthocyanins) mainly due to the high pressures generated inside the extruder (Gutiérrez & Alvarez, 2018). The natural fillers caused cross-linking reactions between the citric acid contained in them and the starch. This resulted in films with a higher resistant starch (RS) content, particularly RS4 (modified starch). In addition, all the films contained RS3 (retrograded starch) demonstrated by the melting temperatures (T_m) observed. Lower *in vitro* digestibility rates and a decrease in the hydrophilic nature of the films were achieved by the addition of the natural fillers.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.carbpol.2017.12.039>.

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