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Physico-chemical properties and *in vitro* digestibility of edible films made from plantain flour with added *Aloe vera* gel

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ABSTRACT

The aim of this study was to relate the physicochemical properties of edible films derived from plantain flour-glycerol containing different concentrations of *Aloe vera* (Av) gel to both their *in vitro* digestibility and sensory attributes, in order to contribute to the very few studies found in the literature. Films were prepared by casting. Degree of substitution, thickness, water solubility, water activity, swelling behavior, thermogravimetric analysis, X-ray diffraction, resistant starch, *in vitro* digestibility, color parameters and sensory evaluation were the tests carried out in this study. A clear relationship between the results demonstrated the effect of the Av gel on the cross-linking of the starchy matrix (plantain flour). This resulted in films with a higher resistant starch content and lower *in vitro* digestibility. The presence of Av gel slowed down starch digestion in the films, a feature which makes it potentially useful as a dietetic.

1. Introduction

The development of new and improved packaging concepts is currently focused on extending the shelf-life of foods, and creating environmentally friendly materials. Nonetheless, traditional packaging is made from polymers derived from petroleum, which are expensive, not renewable and may cause pollution (Wilpiszewska, Antosik, & Spychaj, 2015). An alternative to these are thermoplastic starch materials which are compostable, cheap, biodegradable, non-toxic, and available worldwide (Dang & Yoksan, 2015). Nevertheless, some properties of these materials, such as their hydrophilic nature (water sensitivity), mechanical strength and barrier properties, are not as good as those of petroleum-derived polymer films (Dhakal & Zhang, 2012; Gutiérrez, Tapia, Pérez, & Famá, 2015a, 2015b; Hansen & Plackett, 2008). Different alternatives have been proposed to improve these properties, among which are chemical and/or physical modifications to the starch materials which seem to have been very effective (García-Tejeda et al., 2013).

Organic acids, such as citric acid found in Av gel, represent potential cross-linking agents and could improve both the physicochemical properties and nutritional value of edible films (Reddy & Yang, 2010). According to Majzoobi and Beparva (2014) one of the most important applications of organic acids is in the food industry. A greater understanding of the cross-linking activity of these acids on other food constituents such as starches is thus important since it may alter their physicochemical properties.

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In addition, Av gel is of interest to the scientific community and food industry due to its anti-carcinogenic, anti-inflammatory, anti-oxidant, anti-diabetic and antibiotic characteristics (Boudreau & Beland, 2006; Grover, Yadav, & Vats, 2002; Krishnan, 2006; Pereira et al., 2013; Sharrif & Verma, 2011; Xiao, Guo, Liu, & Zhang, 2007; Xu et al., 2008; Yu, Jin, Xin, & JianMin, 2009). Av gel shows potential activities against allergies, ulcers and AIDS, among others (Eshun & He, 2004; Reynolds & Dweck, 1999; Valverde et al., 2005), and can also aid in wound healing (Boudreau & Beland, 2006; Sharrif & Verma, 2011). It is also worth noting that Av gel may act as a reducing agent of cholesterol and triglyceride levels in the blood and could thus help to prevent gastrointestinal, renal and cardiovascular problems (Pereira et al., 2013; Yu et al., 2009). The potential curative properties of Av gel are mainly attributed to polysaccharides present in the plant, while its anti-septic and antimicrobial activities have been related to the presence of antiseptic agents such as lupeol, salicyclic acid, urea nitrogen, cinnamonic acid and sulfur, which inhibits bacteria, viruses and fungi (Boudreau & Beland, 2006; Pereira et al., 2013; Sharrif & Verma, 2011).

Although Av gel is principally used in the cosmetology and pharmaceutical industries, it has also recently found its place in the food industry as a functional food in beverages and ice-cream (Khoshgozaran-Abras, Azizi, Hamidy, & Bagheripoor-Fallah, 2012; Moore & MacAnalley, 1995). In addition, due to its antifungal activity, it has been applied as an edible coating (solely or in combination with other components) to extend the post-harvest storage of "Arctic Snow" nectarines (Ahmed, Singh, & Khan, 2009), table grapes (Serrano et al., 2006; Valverde et al., 2005), sweet cherries (Martínez-Romero et al., 2006), apple slices (Chauhan, Raju, Singh, & Bawa, 2010) and papayas (Marpudi, Abirami, Pushkala, & Srividya, 2011).

Av-based coatings have also reduced weight loss and ethylene production in raw peaches and plums (Guillén et al., 2013). In a recent study with fresh-cut kiwifruit, Av gel was effective in reducing pectin depolymerization and microbial proliferation, while enhancing sensorial quality (Benítez, Achaerandio, Sepulcre, & Pujolà, 2013). Finally, a practical advantage of Av gel use at industrial level is its easy preparation.

With regard to plantain flour, it is of note that their yield is far higher than starch obtained from plantain. This translates into lower costs and makes plantain flour very competitive compared to synthetic materials (Pelissari, Andrade-Mahecha, do Amaral Sobral, & Menegalli, 2013). In addition, recent studies have demonstrated the potential of plantain flour as a renewable food packaging resource (Gutiérrez, Guzmán, Medina, & Famá, 2016; Gutiérrez, Suniaga, Monsalve, & García, 2016; Pelissari et al., 2013). Nonetheless, Gutiérrez et al. (2016) observed a low compatibility between the flour and glycerol, which led to phase separation. This is obviously undesirable as it may compromise the stability of these materials during storage. The cross-linking of films made from plantain flour with added Av gel could improve glycerol–starch compatibility, which would impact favorably on the physicochemical behavior of the developed films. Despite their potential benefits, however, these physicochemical properties–digestibility–sensory evaluation relationships have been little studied.

The susceptibility of starch to enzyme digestion may be modified with the addition of chemical agents (Tovar, Melito, Herrera, Laurentín, & Pérez, 1999). For example, Han and BeMiller (2007) were able to produce modified starches that modulated both the rate and extent of *in vitro* amylolysis. Likewise, the addition of plasticizers in edible and biodegradable films can modify not only film flexibility but also their nutritional, functional and organoleptic properties (Donhowe & Fennema, 1993). Starch supramolecular arrangement, degree of crystallinity and retrogradation have been identified as the main determinants of the extent of starch digestion and absorption in the small intestine (Hernández, Emaldi, & Tovar, 2008). In addition, changes to the nutritional properties of starch-containing foodstuffs have been related to the bioavailability of the polymer (Björck & Asp, 1994), a characteristic that has been poorly evaluated for edible and biodegradable films based on starch. With this in mind it is worth considering the study carried out by Hernández et al. (2008) who reported that the addition of glycerol in edible films slowed down starch digestion.

From a nutritional point of view, starch has been classified into three groups: rapidly digestible, slowly digestible and indigestible or resistant starch (Englyst, Kingman, & Cummings, 1992). According to Asp (1992) resistant starch is defined as the sum of starch plus the starch degradation products not absorbed by the small intestine of healthy individuals.

In this study we set out to evaluate the effect of Av gel on starch–glycerol compatibility, and relate the physico-chemical behavior of the films developed to both *in vitro* digestibility and some sensory quality attributes of these formulated materials.

2. Experimental

2.1. Materials

Plantain flour (*Musa* ssp., group AAB, sub-group clone Harton) was obtained by the method described by Pacheco (2001). The plantains were acquired at a local market in Caracas, Venezuela, and had a degree of maturation of 1 according to the scale put forward by Loesecke (1950) i.e. unripe fruit. Av gel was obtained from *Aloe vera* leaf pulp. Av leaves were cut from the plant, and then left to allow a yellowish liquid to drain from the leaf, which according to the U.S. Food and Drug Administration (FDA) is a natural laxative. The leaf epidermis was removed with a knife to obtain the Av gel, which was then liquefied for 10 min at room temperature (~25 °C) without the addition of water. The gel was maintained refrigerated at 5 °C in a dark container while the films were developed which was done on the same day the Av gel was prepared in order to avoid oxidative damages to the gel. According to the FDA Av gel is a "dietary supplement" in the United States and Europe (European Commission Annex I of Regulation No. 1831/2003). It is also included among the "flavoring compounds" in the food industry (WHO, 1999; Franz et al., 2005). Glycerol from Prolabo, Sweden, was used as a plasticizer for the formation of the films.

2.2. Chemical composition and average molecular weight of the plantain flour and the Av gel

The chemical composition of both the plantain flour and the Av gel was determined. Moisture content, ash, fat and crude

protein (N × 6.25) (obtained by the micro-Kjeldahl method) were calculated using the gravimetric method (AACC, 2003). Determination of crude fiber was performed by the method proposed by Van Soest and Wine (1967). Total amylose content was measured by the differential scanning calorimetry (DSC) method described by Amani, Buléon, Kamenan, and Colonna (2004) and Mestres, Matencio, Pons, Yajid, and Fliedel (1996). Total carbohydrate content of the flour and the Av gel was calculated by subtracting the percentages of water content, ash, crude protein and fatty materials from one hundred percent of samples. Total polyphenols content in the plantain flour and the Av gel was determined using the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999) with modifications by Dewanto, Wu, Adom, and Liu (2002). Absorbance was measured at 760 nm. Total polyphenols content was calculated from a standard gallic acid equivalent (GAE) curve within a concentration range of 5–100 ppm. Results were expressed as mg of GAE/100 g of total polyphenols. Citric acid content was estimated using the acetic anhydride and pyridine method described by Marier and Boulet (1958). The average molecular weight of the plantain flour and the Av gel was determined by capillary viscosity using an Ostwald viscometer and employing the Mark–Houwink–Sakurada–Staudinger equations (Gutiérrez & González, 2016). All measurements were done using three replicates and results are given as the mean ± standard deviation (SD).

2.3. Film formation

Films were prepared following the methodology described by Gutiérrez et al. (2016) and Gutiérrez et al. (2016). Edible films were prepared from a film forming solution (*FFS*) made by mixing 2% w/v of native plantain flour and 1.5% w/v of glycerol in 500 mL distilled water. Different concentrations of Av gel (0, 2, 4 and 6% v/v) were then added to the developed films. The solutions were then heated in a water bath with constant stirring at 90 °C for 30 min to ensure starch gelatinization and inactivate the enzymes contained in Av gel (Hernández et al., 2008). The gels obtained were poured into stainless steel trays 40 × 30 cm, and dried in a Mitchell dehydrator (Model 645 159) for 24 h at 45 °C. The resulting thermoplastic flour films: plantain flour with 0% of Av gel (*TPF-PF*), plantain flour with 2% of Av gel (*TPF-PF + 2% Av*), plantain flour with 4% of Av gel (*TPF-PF + 4% Av*) and plantain flour with 6% of Av gel (*TPF-PF + 6% Av*) were then carefully removed from the casting molds. They were then conditioned with a saturated solution of NaBr (a_w ~0.575 at 25 °C) for seven days prior to each test. Films used for determining water activity (a_w) were not conditioned.

2.4. Characterization

2.4.1. Determination of degree of substitution (DS) in the plantain flour films esterified with Av gel

The amount of citric acid esterified in films with added Av gel was determined by the method described by Klaushofer, Berghofer, and Steyrer (1978). This is based on the reaction of citric acid and Cu^{2+}, which forms a stable complex during titration with a solution of copper sulfate. DS was calculated based on 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)] × 100, and M = molecular weight (175.1 g/mol) of the citric acid substituent. Each sample was analyzed in triplicate.

2.4.2. Determination of film thickness

Film thickness was measured using an electronic digital micrometer with an accuracy of 0.001 mm (Micromaster®). Film strips were placed between the jaws of the micrometer and the gap was reduced until the minimum friction was measured. Mean thickness (mm) was determined from fifty measurements at different locations on the films.

2.4.3. Water solubility

Water solubility (WS) is defined as the percentage of film dry matter dissolved after 24 h of immersion in distilled water (Gontard, Guilbert, & Cuq, 1992). To determine the initial dry matter of the films, ~0.5 g strips were dried in an oven at 105 ± 1 °C for 24 h. The films were then weighed and immersed in 50 mL distilled water for 24 h at 25 °C. The final dry matter of the films was measured after drying at 105 ± 1 °C for 24 h. All tests were conducted in triplicate and the means reported. Water solubility was calculated by the following equation (Romero-Bastida et al., 2005):

$$\%\ solubility = \frac{\text{initial dry weigh}_{(g)} - \text{final dry weigh}_{(g)}}{\text{initial dry weigh}_{(g)}} \times 100 \quad (2)$$

2.4.4. Water activity (a_w)

A psychrometric a_w meter AquaLab Cx-2 (Decagon Devices, Pullman, USA) previously calibrated with water at 25 °C was used to determine the water activity of the films. The average value from three measurements was reported for each film.

2.4.5. Swelling behavior

The swelling behavior of the films was determined by their immersion in acidic and alkaline solutions. To evaluate the influence of pH on swelling behavior, pieces of film were immersed in plastic petri dishes with 20 mL of standard solutions of HCl (pH 1 / 0.1 M) or NaOH (pH 13 / 0.1 M). The containers were sealed and maintained for 24 h at 25 °C in a dark room. Swelling was defined as the percentage of deformation of diameter before (ø = 25 mm) and after immersing films in the standard solutions. The swelling percentage was calculated using Eq. (3).

$$\%\ swelling = \frac{\phi - \phi_o}{\phi_0} \times 100\% \quad (3)$$

where $ø_o$ and ø (mm) are the initial and final diameters of the films before and after immersion, respectively. To estimate swelling behavior, three samples were tested for each treatment. Experiments were performed in triplicate and the data obtained reported as mean values ± SD.

2.4.6. Thermogravimetric analysis (TGA)

TGA measurements were carried out in N_2 atmosphere (flow 30 mL/min) using a Shimadzu DTG-60. Samples were weighed

at approximately 5–10 mg of dry matter. Measurements started at 25 °C and continued up to 500 °C with a linear increase of 10 °C/min. The weight loss of the edible films was recalculated on dry basis and the different degradation phases noted. Measurements were performed in triplicate to ensure repeatability.

2.4.7. *X-ray diffraction (XRD)*

The X-ray diffraction patterns of the film samples were analyzed using an X-ray diffractometer (Siemens D 5000). Samples were prepared by placing square cutouts of each film (2.5 × 2.5 cm) on a glass slide and the spectra were recorded using radiation Cu $\alpha K = 1.5406$ Å and a copper monochromator filtering wave at 40 kV and 30 mA with scanning at $2\theta = 3$–33°, step size 0.02° and scan speed 2 sec. Percent crystallinity was determined by measuring the relative intensities of the main peaks from the scattering spectrum according to Hermans and Weidinger (1961).

2.4.8. *Determination of resistant starch (RS)*

Resistant starch content was calculated from the Insoluble Dietary Fiber (IDF) values, which were obtained according to the method described by Saura-Calixto, Goñi, Bravo, and Mañas (1993). The IDF residues were mixed with 6 mL of a solution of KOH (2 M), and the resulting residues were then continuously agitated for 30 min at room temperature (~25 °C) before adding 3 mL of an acetate buffer (0.4 M, pH = 4.75) and 5 mL of HCl (2 M). The pH was then adjusted to 4.75 using HCl (2 M) if required and 60 mL of amyloglucosidase suspension (E.C. 3.2.1.3) added. The solution obtained was mixed and incubated for 30 min at 60 °C with continuous agitation.

Samples were centrifuged for 15 min and the supernatants collected. The pellet was then re-suspended in 10 mL distilled water and centrifugation repeated. The supenatants were then combined with the water washes and adjusted to a final volume of 100 mL.

Total glucose was analyzed using a GOD-POD reagent (Glucose Oxidase/Peroxidase). A glucose standard solution (10–50 mg/mL) was used. For this, 0.5 mL of supernatant was pipetted into a glass tube and 1 mL of the reagent solution was added from the combined kit for Glucose determination. The solution was mixed thoroughly and the tubes were placed in a water-bath for 30 min at 37 °C. Absorbance was measured at 500 nm. Resistant starch was calculated as glucose (mg) × 0.9. All tests were conducted in triplicate and the means ± SD were reported.

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

The *in vitro* rate of starch hydrolysis was evaluated using the methodology described by Granfeldt, Bjorck, Drews, and Tovar (1992), Hernández et al. (2008) and Zamora-Gasga, Bello-Pérez, Ortíz-Basurto, Tovar, and Sáyago-Ayerdi (2014). Square pieces, 16 cm^2 (~2 g), of edible films were given to five healthy individuals who then chewed the edible films at a frequency of 1 chew per second for 15 sec. The chewed material was carefully expectorated into a 20 mL beaker containing 0.05 mol/L phosphate buffer adjusted to pH 1.5 with HCl. The mixture was incubated with bovine pepsin provided by Sigma-Aldrich (P-7000, St. Louis MO, USA) for 30 min at 37 °C then neutralized at pH = 7.0 and incubated with porcine pancreatic α-amylase (A3176, Sigma-Aldrich) in a dialysis bag (D9652 – 30.480 m avg., Sigma Aldrich). The reducing amylolysis products that appeared in the dialysate were measured by colorimetry and expressed as maltose equivalents. Data were plotted as the degree of hydrolysis *versus* time curves, and the hydrolysis index (HI) was calculated as the area under the curve (0–60 min) for the test product. This was then expressed as a percentage of the corresponding area for commercial white bread chewed by the same person. The average value ± SD of three measurements was reported.

2.4.10. *Color*

Color parameters of the films were measured according to standard test methods (ASTM D-1992, 1995) using a Macbeth® colorimeter in reflectance mode (Model Color-Eye 2445, illuminant D65 and 10° observer) and the Hunter scale used to express the values obtained: $L^* = 0$ (black) to $L^* = 100$ (white), $-a^*$ (greenness) to $+a^*$ (redness), $-b^*$ (blueness) to $+b^*$ (yellowness) (Francis & Clydesdale, 1975). Color differences (ΔE^*) were calculated according to Eq. (4) as described by Gennadios, Weller, Hanna, and Froning (1996):

$$\Delta E = \sqrt{\Delta a^2 + \Delta b^2 + \Delta L^2} \tag{4}$$

where $\Delta L = L^*_{standard} - L^*_{sample}$, $\Delta a = a^*_{standard} - a^*_{sample}$ and $b^*_{standard} - b^*_{sample}$. The white standard plate ($L^* = 93.52$, $a^* = -0.81$ and $b^* = 1.58$) was used as a standard.

The whiteness index (*WI*) was calculated according to Atarés, Bonilla, and Chiralt (2010) and the yellowness index (*YI*), which as its name suggests determines the degree of yellowness of a substance, according to ASTM D-1925 (ASTM D-1992, 1995; MacFarlane, MacFarlane, & Billmeyer, 1936) using the CIE-$L^*a^*b^*$ coordinates:

$$YI = \frac{100(1277X - 1067Z)}{Y} \tag{5}$$

Nine measurements were taken for each film.

2.4.11. *Sensory evaluation*

The sensory qualities of the film pieces (2.5 × 2.5 cm) were evaluated after 7 days of storage. For the hedonic tests coded (3 digit) samples were presented at random to a panel comprised of thirty judges between 20 and 35 years of age (50% females, 50% males). The panelists were recruited among the students and personnel at the Department of Analytical Chemistry (Specialization Food Science and Technology) of the Faculty of Pharmacy at the Central University of Venezuela. The panelists evaluated several quality attributes of the films using a scale with anchors from 0 to 10, where 0 indicated "extremely dislike" and 10 "extremely like". The attributes evaluated were: color by visual observation under white lightning, texture when bitten with front teeth, taste when chewing, oral dissolution when chewing and overall acceptability. Analyses were performed in a sensory analysis laboratory with individual booths for each panelist in accordance with ISO Standard 8589:2007, 2007. The panelists' average response was calculated for each attribute.

2.5. *Statistical analysis*

Experimental data were analyzed using the Statgraphics Plus 5.1 software (Manugistics Corp., Rockville, MD). A one-way

Table 1 – Chemical composition on dry basis and average molecular weight of the plantain flour and Av gel.

Parameter	Plantain flour	Av gel
Moisture (%)	9.4 ± 0.2[a]	98.5 ± 0.01[b]
Total protein (%)	2.64 ± 0.03[b]	0.013 ± 0.003[a]
Crude fat (%)	0.36 ± 0.08[b]	0.06 ± 0.03[a]
Ash (%)	2.41 ± 0.03[b]	0.47 ± 0.01[a]
Crude fiber (%)	0.297 ± 0.002[b]	0.08 ± 0.01[a]
Total carbohydrates (%)	84.9 ± 0.2[b]	0.88 ± 0.01[a]
Starch content (%)	85 ± 1[b]	0 ± 0[a]
Apparent amylose (%)	24 ± 1[b]	0 ± 0[a]
Total polyphenols (mg of GAE/100 g)	21 ± 1[a]	79 ± 1[b]
Citric acid (mg/100 g)	0 ± 0[a]	151 ± 5[b]
Average molecular weight ($\times 10^6$ g/mol)	70 ± 1[b]	0.3 ± 0.1[a]

Similar superscript letters in the same row indicate no statistically significant difference ($p \leq 0.05$).

ANOVA followed by Tukey's multiple range tests were used to determine any significant differences ($p < 0.05$) between treatments, and the results were expressed as means ± SD.

3. Results and discussion

3.1. *Chemical composition and average molecular weight of the plantain flour and Av gel*

Table 1 shows the chemical composition of the carbohydrate polymers used for developing the edible films. The plantain flour obtained showed a similar composition to that recently published by our research group (Gutiérrez et al., 2016), and the molecular weight was comparable to that reported by other authors such as Rolland-Sabaté, Amani, Dufour, Guilois, and Colonna (2003) and Yoo and Jane (2002). As regards the Av gel, the chemical composition showed high moisture content and the average molecular weight was at least 230 times lower than that of the starchy matrix. In addition, the Av gel contained 3 times more polyphenol than the plantain flour. It is also worth noting the citric acid content determined for the Av gel.

3.2. *Characterization of the films*

3.2.1. *Degree of substitution (DS) by the titration method*

Table 2 shows the degree of substitution of films with added Av gel. The highest degree of substitution was found for films with the highest Av gel content, suggesting that films derived from plantain flour are susceptible to crosslinking with the citric acid contained in the gel. The cross-linking of starches by citric acid has also been reported by Kapelko-Żeberska, Buksa, Szumny, Zięba, and Gryszkin (2016).

3.2.2. *Thickness*

Table 2 shows the thicknesses of the different systems studied. It can be observed that film thickness increased with a higher Av gel content. Similar results have been reported by Pereira, Tojeira, Vaz, Mendes, and Bártolo (2011) for films based on alginate and Av. According to Gutiérrez, Morales, Tapia, Pérez, and Famá (2015) a greater interaction between the starch and plasticizer could result in thicker films probably due to the formation of hydrogen bonds between the glycerol and starch. Pérez, Segovia, Tapia, and Schroeder (2012) reported a significant increase in the thickness of cross-linked starch-based films derived from *Dioscorea trifida*. Cross-linking apparently strengthens internal bonds in starch which increases molar volume (Gutiérrez, Pérez, Guzmán, Tapia, & Famá, 2014; Sívoli, Pérez, Rodríguez, De Abrisqueta, & Raymúndez, 2005). This factor, together with a greater interaction between the starch and plasticizer during gelatinization, could result in thicker films (Gutiérrez et al., 2015, 2015b). These interactions appear to become stronger when Av gel content was increased.

3.2.3. *Water solubility*

According to Romero-Bastida et al. (2005) the solubility of the edible films provides an indication of their integrity in an aqueous medium, whereby higher solubility values indicate a lower resistance to water.

Table 2 shows the water solubility values at 25 °C for the different systems studied. It can be seen that films with lower

Table 2 – Degree of substitution (*DS*), thickness (*e*), solubility, water activity (a_w), crystallinity, resistant starch and color parameters of the different films.

Parameter	TPF-PF	TPF-PF + 2% Av	TPF-PF + 4% Av	TPF-PF + 6% Av
DS	–	0.017 ± 0.001[a]	0.028 ± 0.001[b]	0.042 ± 0.001[c]
e (mm)	0.17 ± 0.01[a]	0.20 ± 0.01[b]	0.23 ± 0.01[c]	0.26 ± 0.01[d]
Solubility (%)	63 ± 1[d]	60 ± 1[c]	57 ± 1[b]	55 ± 1[a]
a_w	0.630 ± 0.004[d]	0.606 ± 0.002[c]	0.573 ± 0.003[b]	0.562 ± 0.001[a]
Crystallinity (%)	9 ± 1[a]	12 ± 1[b]	13 ± 1[b]	16 ± 1[c]
Resistant starch (%)	1.99 ± 0.02[a]	2.12 ± 0.04[b]	2.55 ± 0.07[c]	3.05 ± 0.04[d]
L^*	31.6 ± 0.1[d]	29.0 ± 0.2[c]	28.71 ± 0.02[b]	27.3 ± 0.2[a]
a^*	0.52 ± 0.02[c]	0.51 ± 0.02[c]	0.35 ± 0.04[b]	0.13 ± 0.05[a]
b^*	2.13 ± 0.06[d]	1.68 ± 0.08[c]	0.73 ± 0.05[b]	0.60 ± 0.09[a]
Color difference (ΔE)	62.0 ± 0.1[a]	64.5 ± 0.2[b]	64.85 ± 0.01[c]	66.2 ± 0.2[d]
Whiteness index (*WI*)	–15.5 ± 0.7[c]	–12.8 ± 0.7[b]	–2.5 ± 0.5[a]	–1.8 ± 0.9[a]
Yellow index (*YI*)	10.3 ± 0.3[d]	8.8 ± 0.3[c]	4.2 ± 0.2[b]	3.2 ± 0.3[a]

Equal letters in the same row indicate no statistically significant differences ($p \leq 0.05$).

Table 3 – Swelling behavior of the films evaluated in alkaline medium.

Material	Swelling (%)
TPF-PF	95 ± 5^{d}
TPF-PF + 2% Av	75 ± 6^{c}
TPF-PF + 4% Av	45 ± 5^{b}
TPF-PF + 6% Av	24 ± 2^{a}

Equal letters in the same column indicate no statistically significant difference ($p \leq 0.05$).

Av gel content were significantly more water-soluble ($p \leq 0.05$) than those with higher Av gel content. These results agree with the trend reported by Pérez et al. (2012) for native and phosphated *Dioscorea trifida* white starch-based edible films. According to Almasi, Ghanbarzadeh, and Entezami (2010) a strong hydrogen bond between the hydroxyl groups of the starch chains and the glycerol molecules decreases sensitivity to water. Films with a higher Av gel content interact more strongly with the glycerol which would be water-insoluble. This increases the water resistance of the films thus decreasing their solubility.

The results of the water-solubility of the films with lower Av gel content are promising, as stated by Sothornvit and Krochta (2000) after obtaining similar results, for their possible application as edible candy wrappers, since they easily dissolve and melt in the mouth. In contrast, films with a higher Av gel content could be used to wrap foods with high water content.

3.2.4. *Water activity* (a_w)

The water activity of the films is reported in Table 2. As can be seen, the highest a_w value was obtained for the sample made from plantain flour with the lowest Av gel content. According to Gutiérrez, Morales, Pérez, Tapia, and Famá (2015) a_w is directly proportional to the moisture content of the edible films. Thus, higher a_w values for the plantain flour-derived films were caused by a lower interaction between the glycerol and starch, which allowed water absorption from the environment. This could be related to the cross-linking of the starch since a stronger starch–glycerol interaction could compromise the Lewis sites (polar sites) thus preventing water adsorption and absorption from the environment. Similar results have been found in the literature for native and cross-linked white *Dioscorea trifida* edible coatings (Pérez et al., 2012). Microbiological growth in these systems is unlikely since even the highest the a_w values obtained are relatively low.

3.2.5. *Swelling behavior*

The swelling behavior of the films is reported in Table 3. To investigate the influence of pH on swelling behavior, films were immersed in standard HCl (0.1 M – pH 1.0) and NaOH (0.1 M – pH 13.0) solutions. The films immersed in HCl showed no swelling thus suggesting that they are stable in an acid medium. This means that all the films developed here could be used for packaging slightly acidic food such as meats and citrus fruits (Gutiérrez et al., 2016). Similar results were obtained by Pereira et al. (2013) for alginate-based hydrogel films with added Av. In contrast, films immersed in NaOH did swell, increasing from 24 to 95% in size as the Av gel content decreased. Thus, the swelling behavior of the films was negatively dependent on Av gel content in the alkaline medium employed. What occurs is that the hydroxyl groups in the starch remain in an ionizable form (CO–) thus increasing electrostatic repulsion between them and resulting in swelling. This reduces or destroys intra and intermolecular interactions (hydrogen-bonding interactions) between the starch macromolecules, thus facilitating further swelling of the starch (Hu, Chen, & Gao, 2009). Based on this and knowing that cross-linking of starch takes place due to the nucleophilic character of the hydroxyl groups within it, we suggest that an increase in the degree of starch cross-linking due to higher Av gel content possibly reduces the number of likely sites (hydroxyl groups) that can cause swelling in the developed films.

The biomatrices described here mean that these edible films could act as a vehicle for the transport of active substances to the small and large intestines and their subsequent release through the swelling process. As it is well known, the pH of the intestines is approximately 5–8 whereas that of the stomach is between 1 and 3 (Evans et al., 1988). The films could thus tolerate the pH of the stomach without swelling, and deliver active substances straight to the intestines. This has already been accomplished for active compounds and probiotic bacteria which have been transported in these systems (Arancibia, Alemán, López-Caballero, Gómez-Guillén, & Montero, 2015; Colak et al., 2015; González & Igarzabal, 2015; Jafari et al., 2015; Piermaria, Diosma, Aquino, Garrote, & Abraham, 2015; Soukoulis, Singh, Macnaughtan, Parmenter, & Fisk, 2016).

In fact, during the swelling of the films derived from plantain flour with the lowest Av gel content, some of the active compounds found in Av gel could also be diffused from the films to the intestines. This could then be very important for the transportation and controlled release of active substances.

3.2.6. *Thermogravimetric analysis (TGA)*

Thermogravimetric analysis (*TGA*) was performed in order to analyze the thermal stability of the four film systems (Fig. 1A). According to Ayala, Agudelo, and Vargas (2012), Gutiérrez et al. (2015), Gutiérrez et al. (2016), Marques et al. (2006), and Pelissari et al. (2013), the thermal decomposition of glycerol–starch-containing films occurs in three main stages. During the first stage weight loss starts at around 100 °C. In Fig. 1 the first stage of thermal degradation is not observed, since the weight loss of the materials was recalculated on dry basis in order to avoid distortions as a result of the variable moisture content of the films. The second stage of degradation takes place between 160 and 290 °C and is associated with the evaporation of the glycerol-rich phase, which also contains starch. Finally, the third stage occurs from 330 °C due to the degradation of the partially decomposed starch (Liu, Xie, Yu, Chen, & Li, 2009; Sanyang, Sapuan, Jawaid, Ishak, & Sahari, 2015; Wilhelm, Sierakowski, Souza, & Wypych, 2003).

We can observe that all the films made from plantain flour showed a double weight loss (Fig. 1A) related to phase separation caused by low flour–glycerol compatibility (Liu et al., 2009; Liu, Yu, Liu, Chen, & Li, 2008; Sanyang et al., 2015; Shi et al., 2007). In addition, the DTGA curves (Fig. 1B) of the evaluated systems clearly show the decomposition of two different compounds. We can thus conclude that the films developed are

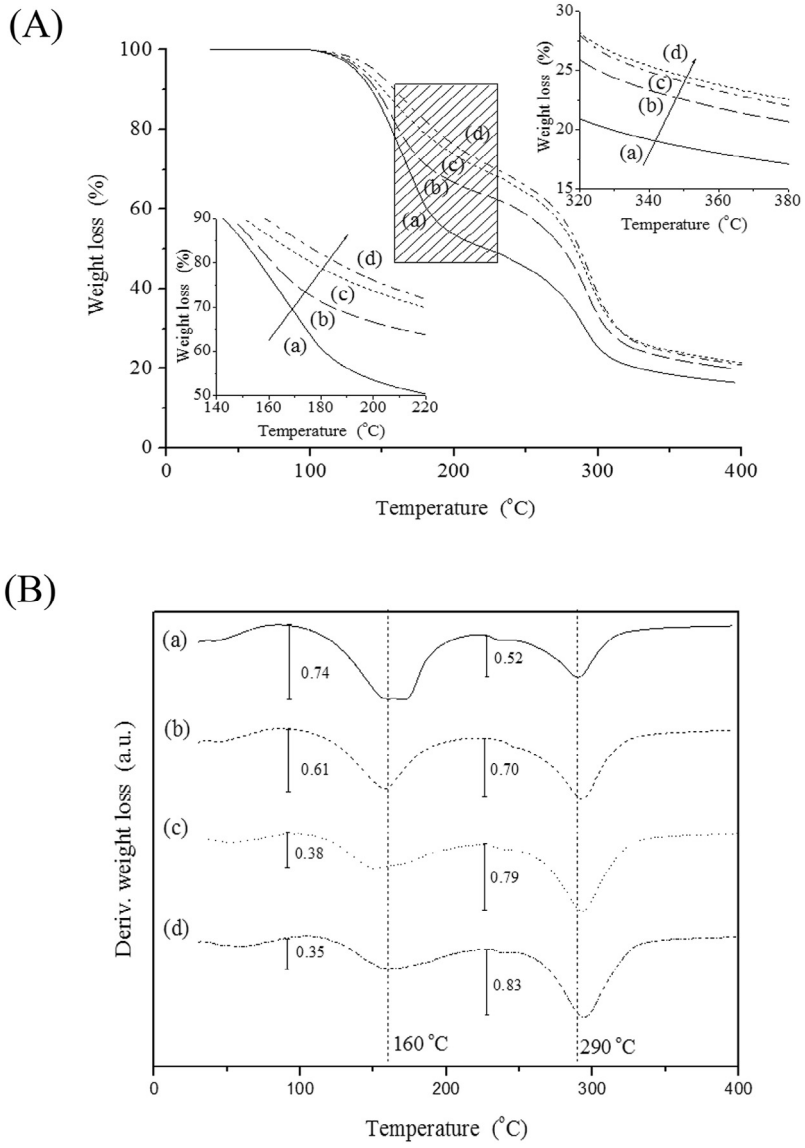

Fig. 1 – (A) TGA and (B) DTGA curves of the different films studied: (a) plantain flour (TPF-PF), (b) plantain flour with incorporation of 2% Aloe vera (TPF-PF + 2% Av), (c) plantain flour with incorporation of 4% Aloe vera (TPF-PF + 4% Av) and (d) plantain flour with incorporation of 6% Aloe vera (TPF-PF + 6% Av).

heterogeneous composite materials. Similar results were reported by Gutiérrez et al. (2016) for native plantain flour-based films.

An increase in resistance to thermal degradation in films with a higher Av gel content can also be observed, manifested by a lower weight loss at the same temperature (Fig. 1A). It is worth remembering that cross-linking reactions increase the average molecular weight of the starch and introduce chemical bonds between different molecules (Rutenberg & Solarek, 1984). These results agree with those reported by Gutiérrez and González (2016) in their study on films derived from taro starch cross-linked by pulsed light. Similar behavior has also been recently reported by Gutiérrez et al. (2015), Gutiérrez et al. (2015a) and Gutiérrez et al. (2016) for edible films based on corn, cassava and cush-cush yam starch, as well as plantain flour chemically modified by cross-linking with sodium trimetaphosphate.

The evaporation temperature of glycerol is well known (290 °C). According to the DTGA curves (Fig. 1B) a slight increase in this temperature can be observed. This is due to a greater glycerol–flour interaction in systems with higher Av gel content.

Although we demonstrated cross-linking of the starchy source, this did not effectively improve the compatibility of these materials compared to films made from plantain flour chemically modified by phosphation (Gutiérrez et al., 2016).

Finally, in the third stage we observed stable curves of thermal degradation up to 500 °C. Here, as for previous stages, a lower weight loss was observed for films with a higher Av gel content. This is possibly due to a lower contribution of minerals and oxidation products of aromatic rings (coal residues) of active compounds such as polyphenols, contained in the Av gel (Patel et al., 2010; Ruiz, 2006).

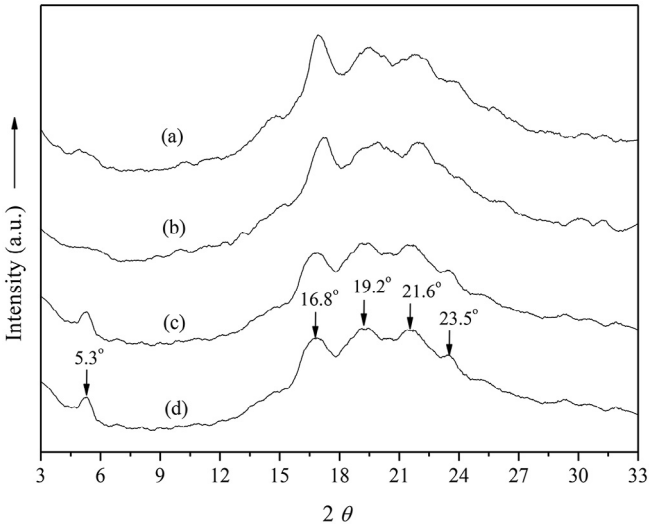

Fig. 2 – X-ray diffraction pattern of the different films studied: (a) plantain flour (TPF-PF), (b) plantain flour with incorporation of 2% Aloe vera (TPF-PF + 2% Av), (c) plantain flour with incorporation of 4% Aloe vera (TPF-PF + 4% Av) and (d) plantain flour with incorporation of 6% Aloe vera (TPF-PF + 6% Av).

3.2.7. *X-ray diffraction (XRD)*

Fig. 2 shows the X-ray diffraction patterns of the developed films. The diffraction curves of all the materials developed exhibited mainly amorphous patterns with a small crystalline fraction. This is consistent with that reported in the literature for starch-based films (Angellier, Molina-Boisseau, Dole, & Dufresne, 2006; Kristo & Biliaderis, 2007).

The crystallinity percentage values were calculated from the area under the curves (Table 2). Higher values were observed for samples with greater Av gel content probably due to the crosslinking of starch. It is well known that starch crosslinking produces the alignment of the carbohydrate polymeric chains, thus converting the amorphous state into a more crystalline one (Gutiérrez et al., 2015a). A similar phenomenon was observed by Gutiérrez et al. (2015) for films derived from cassava and cush-cush yam starches cross-linked with sodium trimetaphosphate.

Furthermore, peaks at $2\theta \cong 16.8$ and 23.5° were observed (Fig. 2). These are characteristic of an A-type crystalline structure associated with the glycerol–amylose complex (Farhat, Oguntona, & Neale, 1999; Manzocco, Nicoli, & Labuza, 2003; Zobel, 1994).

Peaks at $2\theta \cong 5.3$ and 21.6° can also be seen in Fig. 2, corresponding to a B-type structure (Pelissari et al., 2013) which, according to García-Tejeda et al. (2013), is associated with interactions between the short external amylopectin chains and the glycerol. This B-type structure was more developed in films with a higher Av gel content (TPF-PF + 4% Av and TPF-PF + 6% Av) and can be seen particularly by the development of the peak located at $2\theta \cong 5.3°$. This leads us to infer that the degree of substitution of the amylose increased with a higher Av gel content resulting in a greater interaction between the glycerol and the amylopectin chains.

In addition, the observation of a peak at $2\theta \cong 19.2°$ corresponds to a type V crystalline structure (Zobel, French, & Hinkle, 1967). Zobel et al. (1967) suggest that a more developed type V structure is related to an increase in the number of amylose–glycerol interactions. This agrees with that previously established, namely that glycerol–amylose interactions are limited by the cross-linking of amylose chains, resulting in amylopectin–glycerol interactions.

3.2.8. *Resistant starch (RS)*

The results for resistant starch content are shown in Table 2. The first aspect we wish to highlight is the positive relationship between resistant starch content and the crystallinity of the developed films. From this we can infer that film crystallinity may also be a product of resistant starch content, at least type 3 RS, which is a product of the retrogradation of starch polymers. Furthermore, the increase in the crosslinking of the starchy matrix when Av gel is added would result in the alignment of the starch macromolecules thereby increasing crystallinity and resistant starch content. This would fit well with that indicated by Hernández et al. (2008).

One of the attributes of resistant starch is that it can be fermented by a wide variety of bacteria that are found in the colon, producing short chain fatty acids which play an important role in human nutrition and well-being (Björck & Asp, 1994; García-Alonso & Goñi, 2000). Thus, films based on plantain flour with higher Av gel content could also be beneficial from a health perspective.

Jenkins et al. (1981) suggested that the glycemic index can be used to rank foods according to their blood glucose raising potential. In this regard, resistant starch content constitutes a nutritional variable that may be linked to low-glycemic-index foods. Films with a high Av gel content could thus be considered as a potential tool for improving the dietary control of diabetes (Björck & Asp, 1994; Björck, Granfeldt, Liljeberg, Tovar, & Asp, 1994). For this to have a significant impact, however, we would need to substantially increase the resistant starch content in these systems, since the maximum value obtained in this study (3.05%) was too low to produce this beneficial effect.

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

Fig. 3 shows the results of the *in vitro* digestibility of the developed films. A significant decrease ($p \leq 0.05$) in the degree of enzymatic hydrolysis can be observed in films with a higher Av gel content, suggesting that starch crosslinking decreases the rate of hydrolysis of the carbohydrate polymers. These results fit well with the discussion on the swelling behavior of the developed films. In addition, the *in vitro* digestibility of the films was negatively correlated with their stability resulting from the incorporation of Av gel (also see the TGA results, section 3.2.6). In other words, the greater the stability of these food matrices is, the lower the rate of digestion. Thus, using Av gel as an additive decreases the digestion rate of the starch films, a feature that is worthwhile exploring for the production of foods with a low glycemic index. It is also worth noting that a lower starch digestion rate can promote a moderated *in vivo* glycemic response: an important parameter to be considered in the dietary management of diabetes (Björck et al., 1994). Studies on the digestibility of edible films are scarce,

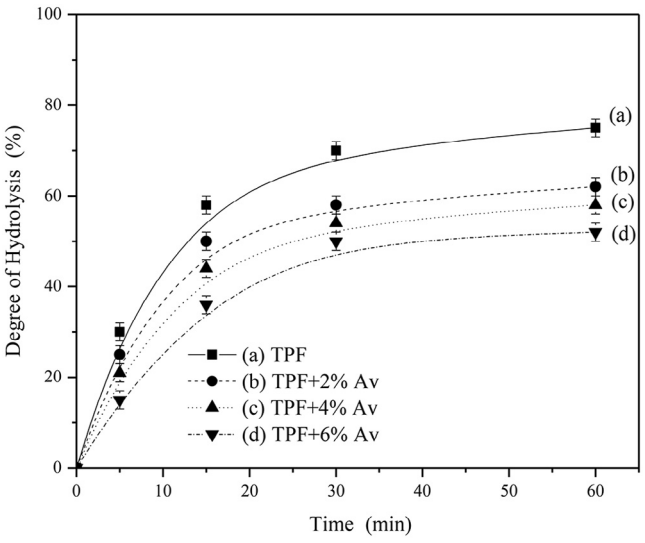

Fig. 3 – *In vitro* α-amylolysis curves of the different films studied: (a) plantain flour (TPF-PF), (b) plantain flour with incorporation of 2% Aloe vera (TPF-PF + 2% Av), (c) plantain flour with incorporation of 4% Aloe vera (TPF-PF + 4% Av) and (d) plantain flour with incorporation of 6% Aloe vera (TPF-PF + 6% Av).

however, similar results have been reported by Hernández et al. (2008) and Ou, Kwok, and Kang (2004) for edible films based on protein and starch, respectively. These results are consistent with a high resistant starch content resulting from the chemical stability produced by the Av gel. Thus, Av gel has additional positive effects related to the *in vitro* digestibility in starch-containing foodstuffs such as edible films based on starch and flour.

3.2.10. *Color*

Table 2 shows the results of the color parameters of the films studied. The L^* values for the films tended to decrease with a higher Av gel content, indicating a lower opacity in these systems. Fakhouri et al. (2007) indicated that opacity can vary as a function of the amylose content of starch as well as the structure of the molecules in solution, which tend to align themselves in parallel close enough to form hydrogen bonds between the hydroxyl groups of adjacent chains. This usually occurs during starch retrogradation, thus films with a lower Av gel content should show a tendency to retrograde.

On the other hand, higher *WI* indexes were observed for films with a higher Av gel content. All samples evaluated showed a^* values of around zero, however, films with a higher Av gel content showed lower values, indicating a tendency towards green in these systems. This may be due to the chlorophyll (green pigment) found in vegetable products such as Av gel. In contrast, films with a lower Av gel content showed a trend away from green. A positive b^* value indicates a trend towards yellow. The results of the *YI* were consistent with the b^* values, which were positive in all cases, indicating a degree of yellowing for all the materials evaluated. This is possibly due to carotenoids (yellow pigment) that are found in plantains. The combined differences observed for the L^*, a^* and b^* chromatographic parameters (total color difference (ΔE)) were lower in the films with lower Av gel. This is consistent with the *WI* indexes obtained, since ΔE was calculated with the standard white plate as a reference.

3.2.11. *Sensory evaluation*

Fig. 4 shows the results of the sensory evaluation of the developed films. In general, none of attributes evaluated were acceptable to the panelists. This leads us to suggest that these films could be developed as secondary food packaging. This would limit the possible health benefits of the developed materials, although formulations used as food coatings could improve the sensory attributes. Longares, Monahan, O'riordan, and O'sullivan (2004) indicated that results of sensory evaluations cannot be extrapolated from one food system to another. This means that the films could be employed as coatings for certain foods, such as kiwifruit slices, which do have favorable attributes (Benítez, Achaerandio, Pujolà, & Sepulcre, 2015). Despite this, the sensory evaluation results could be related to physicochemical properties of the films in interesting ways,

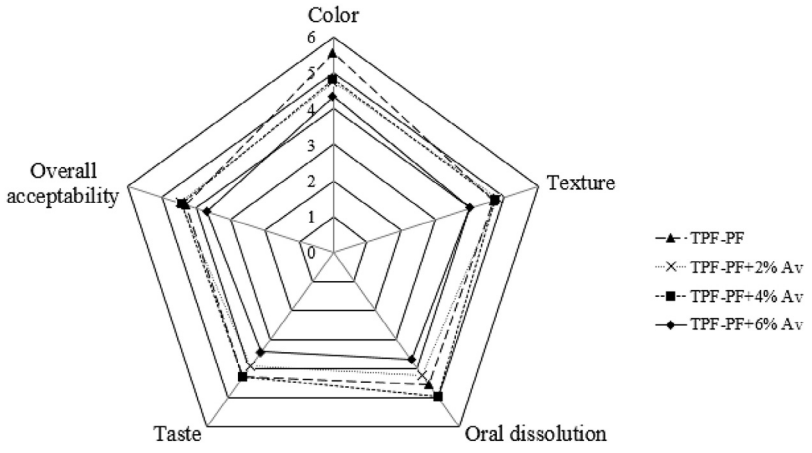

Fig. 4 – Sensory evaluation of the different films studied: plantain flour (TPF-PF), plantain flour with incorporation of 2% Aloe vera (TPF-PF + 2% Av), plantain flour with incorporation of 4% Aloe vera (TPF-PF + 4% Av) and plantain flour with incorporation of 6% Aloe vera (TPF-PF + 6% Av).

such as (1) A lower solubility in films with a higher Av gel content was related to a lower acceptability as regards the oral dissolution of the films. So, desirable solubility percentages in edible films and coatings could be established, (2) Thicker films were described as difficult to chew, which negatively influenced the texture attribute. One possible explanation for this result is that a greater force is required over a larger area to achieve the maximum stress at break of the films. Similar results have been reported by Longares et al. (2004) for films based on whey protein isolate and plasticized with glycerol, (3) The color of the films with a lower Av gel content was less acceptable, possibly because they were more opaque. Similar results were obtained by Pereira et al. (2011) for films derived from alginate and *A. vera*, and (4). There was no noticeable change in the taste of the films with added Av gel, possibly due to the preference *per se* of each panelist.

4. Conclusions

All the tests we undertook in this study (degree of substitution, thickness, water solubility, a_w, swelling behavior, TGA and DRX) showed that Av gel acted as a cross-linking agent for the starchy matrix (plantain flour) resulting in thicker, more stable and drier films. Cross-linking of the starchy matrix also improved compatibility between the flour and the glycerol, enabling us to obtain films with a lower *in vitro* digestion rate. Hence, the presence of Av gel in the films decreased starch digestion, a feature which makes it potentially useful as a dietetic.

An important point that has emerged from this study is that the experimental conditions we used to carry out *in vitro* digestibility tests in these systems were not ideal. This is because using the equivalent areas or fresh weight of the films does not guarantee the equivalent starch substrate concentrations required for the enzymatic assay, as the phase separation that occurs in these systems does not guarantee the homogeneous distribution of the substrate. The situation was exacerbated by the use of white bread as a reference material which could have affected the data and hence the final conclusions. Nevertheless, this study remains a valuable contribution to the limited literature on the nutritional aspects of edible films. For future work on the *in vitro* digestibility of edible films we recommend the use of materials that ensure homogenous substrate content, and the matrix, i.e. starch or flour that has not undergone the gelatinization process, as a reference.

Finally, as regards the sensory evaluation: none of the attributes of the developed films were acceptable to the panelists. Nevertheless, correlations between the physicochemical parameters and the attributes analyzed were obtained.

Conflict of interest

The authors declare no conflict of interest.

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