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## Starch films from a novel (*Pachyrhizus ahipa*) and conventional sources: Development and characterization

Olivia V. López\*, María A. García

CIDCA (Centro de Investigación y Desarrollo en Criotecnología de Alimentos), Facultad de Ciencias Exactas Universidad Nacional de La Plata, Centro Científico Tecnológico La Plata (CCT-La Plata) CONICET, 47 y 116 S/No, La Plata B1900AJJ, Buenos Aires, Argentina

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## ABSTRACT

Biodegradable films from ahipa, cassava and corn native starches were developed by casting method and their physicochemical, mechanical and barrier properties were analyzed taking into account the different starch botanical sources. Filmogenic suspensions were prepared; their rheological behaviors were studied and all of them exhibited film-forming ability. However, mechanical assays demonstrated that unplasticized films were too rigid, limiting their technological applications. Thus, 1.5% w/w of glycerol as plasticizer was added to filmogenic suspensions and film flexibility and extensibility were improved, this effect was more significant for ahipa and cassava starch films. Furthermore, thickness, moisture content and water solubility of the developed films were increased when plasticizer was incorporated. Glycerol addition reduced film water vapor permeability and the lowest reduction corresponded to cassava starch films due to the high viscosity of its filmogenic suspensions. Plasticized starch films resulted to be UV radiation barriers; ahipa starch films had the lowest light absorption capacity and higher transparency than cassava and corn starch films. Dynamic-mechanical analysis indicated that plasticized films were partially miscible systems exhibiting two relaxations, one attributed to the starch-rich phase and the other to the glycerol-rich one. Likewise, it could be demonstrated that glycerol exerted a major plasticizing effect on ahipa starch matrixes.

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### 1. Introduction

Interest in biodegradable and edible films development from renewable resources is growing due to increased environmental awareness. Thus, a great interest has been shown in the production of plastics from renewable resources, which are commonly known as bioplastics [1–7]. Bio-based materials are promising as their feedstocks are renewable, theoretically they can be composted or recycled, and their production process may be more energy efficient than petroleum-based plastics processing [7].

Among natural polymers, native starch has been considered as one of the most promising materials because of an attractive combination of availability, price, performance and good film-forming ability [8]. Thus, the functional and nutritional properties of starch edible films, in conjunction with its biodegradability compared with other polymers, could also ensure their consideration in the near future. The most significant sources of starch are cereal grains such as corn, wheat and rice; and tubers, such as potato and cassava [9]. Current starch research is focused on searching for non-conventional starch sources since the actual starch portfolio does not cover the industry entire spectrum requirements [10]. In this regard, food applications related to gluten free

products require novel starch sources to overcome both technological and sensorial problems, associated to traditional starch sources like corn [11]. Likewise, other bio-industrial uses are technically feasible like bio-ethanol production, their use of co-products as bioplastics and nutraceuticals.

Cassava (*Manihot esculenta*) is an important starch source in some countries like Brazil, which is the largest cassava-producing country [12], and also in Thailand, Malaysia, Indonesia and some regions of Africa [13]. Even though raw cassava contains cyanogenic glucosides, particularly linamarin and lotaustralin (methyl linamarin), an efficient starch isolation process reduces the cyanide levels, and acute cyanide toxicity with cassava starch rarely occurs [14,15].

On the other hand, some leguminous plants also produce starch rich tuberous roots, such as *Pachyrhizus ahipa*. This specie grows in a very small scale by native communities in the Andes of Bolivia and northern Argentina. Results referred to physicochemical characteristics of this starch are diverse; in a previous work, an integral study on *P. ahipa* (Wedd. Parodi), including field studies, root characteristics analysis, storage conditions assessment and starch extraction and characterization was performed [16]. Likewise, ahipa is easily adapted to different ecological conditions, and this is a promising alternative that secures the expansion and diversification of this crop in different countries. In the literature, few analyses evaluating the insecticidal and fungicidal properties of *P. ahipa* have been conducted, several authors studied other conspecific species and informed that generic characteristic of

\* Corresponding author. Fax: +54 221 4254853, +54 221 4249287.  
 E-mail address: [ovlopez75@yahoo.com.ar](mailto:ovlopez75@yahoo.com.ar) (O.V. López).

*Pachyrhizus* is the presence of an insecticidal compound called rotenone ( $C_{23}H_{22}O_6$ ), this ingredient can be found in mature seeds, but not in toxic amounts in the tuber itself or in any other part of the plant [17]. Moreover, in the Andean region, ahipa roots are consumed raw by the local people and is being considered rich in energy and easily digestible [18]. They are valued for their high water content, considerable amounts of carbohydrate, crude fiber and protein, and negligible lipid levels.

According to their origin, starches present diverse physicochemical, structural and functional characteristics; they can be processed under different conditions and therefore they have many diverse end-uses. Furthermore, starches from different botanical sources present varied amylose/amylopectin ratio and several studies have demonstrated that these variations lead to obtain biodegradable films with different mechanical properties [19–25].

Films development and characterization from native starches of diverse botanical sources have been widely studied by several authors (refs. [16,19,24] and [26–43]). Numerous studies can be found in the literature about corn starch films and their properties (refs. [21,22,26] and [31–33]). On the other hand, among starches obtained from tuberous roots, the most widely used for developing biodegradable films is cassava starch [29,30]. Alternatively, Torres et al. [44] had studied the development of biodegradable films from different Andean crops for biomedical applications. It was demonstrated that starches present good film forming capacity; the obtained materials exhibit distinctive properties due to the different amylose/amylopectin relations and the structural characteristics of these macromolecules. The current trend is to look for alternative sources of starch with better physicochemical and functional characteristics [40]. To the best of our knowledge there are no reports about films obtained from ahipa starch, thus the characteristics of this starch should be studied in relation to its filmogenic capacity as well as the properties of the developed materials. This one is an interesting application for this non-conventional starch since it could impulse its crop and grant added value to this raw commodity.

The objectives of the present work were to develop and characterize biodegradable films from ahipa, cassava and corn native starches. Furthermore, several relationships between physicochemical, mechanical and barrier film properties and the relevant starch characteristics, due to its different botanical sources, were established.

## 2. Materials and methods

### 2.1. Starch samples

*M. esculenta* (cassava) and *P. ahipa* (ahipa) roots were provided by the INTA Montecarlo Experimental Station farm, located at Colonia Laharrage, Montecarlo, Misiones, Argentina. For both botanical sources, starch was isolated following the procedure described in a previous work [16]. Native corn starch was provided by Misky, Arcor (Tucumán, Argentina).

### 2.2. Starch characterization

Amylose concentration was spectrophotometrically quantified at 635 nm using a Beckman DU650 (Palo Alto, CA, USA) spectrophotometer, according to the method proposed by Morrison and Laignelet [45]. Determinations were performed in triplicate.

Starch granules' morphology and size distribution were studied by SEM with a JEOL 35 CF electron microscope (Japan) [36]. At least 700 starch granules were measured and the following morphological parameters were evaluated: mean diameter; Martin's mean radius, equivalent circle diameter (ECD) and shape factor (or sphericity). Martin's mean radius was calculated considering the gravity center of the particle; it corresponds to the radius at the point that divides randomly oriented particle into two equal projected areas. ECD was calculated by measuring the area of the granule and back-calculating the diameter

of a circle with the same area. Shape factor highlights the difference between the shape granules compared to a spherical particle; a perfect circle has a shape factor of 1 while a very irregular granule has a value closer to 0.

Starch powders were analyzed in an X-ray diffractometer X'Pert Pro-Analytical Model PW 3040/60 (Almelo, The Netherlands) provided with a tube with a copper anode and a detector operated at 40 kV and 40 mA. Diffractograms were obtained from  $2\theta = 3^\circ$  to  $2\theta = 60^\circ$  to acquire information about the crystalline structure of the granules. The crystallinity degree (CD) was evaluated on the basis of the area of the main peaks; it was calculated as the ratio between the absorption peaks area and the diffractogram total area and expressed as percentage (%). The reported values corresponded to the average of two determinations.

Starch gelatinization was determined on 20% w/w starch aqueous suspensions with a Differential Scanning Calorimeter (DSC) model Q100 controlled by a TA 5000 module (TA Instruments, New Castle, Delaware, USA), with a quench-cooling accessory, under a  $N_2$  atmosphere (20 ml/min), using the technique described by López et al. [36]. Approximately 8 mg of starch suspensions were weighed in aluminum pans and closed hermetically; an empty pan was used as reference. Samples were scanned at temperatures between 10 and 120 °C, at a rate of 10 °C  $min^{-1}$ . The DSC assays were carried out in duplicate for each formulation.

### 2.3. Filmogenic suspensions: preparation and characterization

Aqueous suspensions of 5% w/w starch were gelatinized at 90 °C for 20 min. Furthermore, glycerol (J.T. Baker, México) was added as plasticizer to gelatinized suspensions at a concentration of 1.5 g of glycerol per 100 g of starch suspension (1.5 G).

Starch gel clarity was measured spectrophotometrically according to Piyachomkwan et al. [10]. Starch suspensions (1% w/v) were placed in a boiling bath for 30 min, left to cool to room temperature and then transmittance (%T) at 650 nm was determined in a Hitachi U 1900 spectrophotometer (Japan).

The rheological characterization of filmogenic suspensions was performed in a Rheo Stress 600 ThermoHaake (Haake, Germany) rheometer using a plate–plate system PP35 (gap size 1 mm) at controlled temperature (25 °C). Rotational mode was used to investigate time-dependent behavior of starch suspensions as described in a previous work [36]. The resulting curves were mathematically modeled as Ostwald de Waele fluids by the following equation:

$$\tau = k\dot{\gamma} n \quad (1)$$

where  $\tau$  is the shear stress,  $\dot{\gamma}$  is the shear rate,  $k$  is the consistency coefficient, and  $n$  is the flow behavior index.

Viscoelastic behavior of starch pastes was studied by performing dynamic assays. The linear viscoelasticity range was determined in a stress sweep (0–20 Pa) assay at constant frequency (1 Hz). Then, frequency sweeps (0.01 to 10 Hz) were performed at constant stress. The dynamic rheological parameters recorded were the storage modulus ( $G'$ ), the loss modulus ( $G''$ ), the tangent of the phase angle ( $\tan \delta = G''/G'$ ) and the complex shear stress ( $G^*$ ). Mechanical spectra were obtained by plotting  $G'$  and  $G''$  versus frequency ( $\omega$ ).

Rheological (rotational and dynamical) tests were performed in triplicate for each formulation.

### 2.4. Film forming capacity

To evaluate the film forming capacity of ahipa starch, in comparison with both cassava and corn starches, approximately 20 g of filmogenic suspensions were cast onto Petri dishes (diameter 8.7 cm). They were dried in a ventilated oven at 60 °C for 2 h and then they were maintained at 20 °C during a week until constant weight was achieved.

Films were removed from the plates and stored at 20 °C and 65% relative humidity (RH) in a controlled temperature and humidity room. Homogeneity and appearance of the films were examined as well as the film remotion easiness.

### 2.5. Film properties

Film moisture content was determined by measuring weight loss of films, upon drying in an oven at 105 °C until constant weight (dry sample weight) was achieved. Samples were analyzed at least in duplicate and results were expressed as percentage (%).

Thickness of the films was evaluated using a digital coating thickness gauge Check Line DCN-900 (New York, USA) for non-conductive materials on non-ferrous substrates. Ten measurements were randomly taken at different locations for each specimen and the mean value was reported.

Film solubility in water at 25 and 100 °C was determined according to the method described by López et al. [36]. The reported results corresponded to the mean of two replicate assays.

For film optical and UV barrier properties' evaluation, films were cut into rectangles and placed on the internal side of a quartz spectrophotometer cell. The absorbance spectrum (200–700 nm) was recorded for each sample using a Hitachi U1900 spectrophotometer (Japan). Film opacity (AU nm) was defined as the area under the recorded curve determined by an integration procedure according to Piermaría et al. [46] and the standard test method for haze and luminous transmittance of transparent plastics recommendations (ASTM D1003-00). Film transparency was calculated as the ratio between absorbance at 600 nm ( $A_{600}$ ) and film thickness and was expressed as  $A_{600}/\text{mm}$ .

Homogeneity and appearance of the films were examined by visual observation and scanning electron microscopy (SEM) with a JEOL JSM 6360 electron microscope (Japan). For cross-section observations films were cryofractured by immersion in liquid nitrogen. Film pieces were mounted on bronze stubs using a double-sided tape and coated with a layer of gold (40–50 nm), allowing surface and cross-section visualization. All samples were analyzed using an accelerating voltage of 25 kV.

Water vapor permeability (WVP) tests were conducted using ASTM method E96 with several modifications according to López et al. [36]. After steady-state condition was reached, the acrylic permeation cells were weighed (0.0001 g) at initial time and at 1 h interval for 8 h and, the WVP (g/m s Pa) was calculated using the thickness of each tested film. The informed values are the mean of two determinations.

Mechanical profiles (stress–strain curves) were obtained using a texturometer TA.XT2i-Stable Micro Systems (England) with a tension grip system A/TG. Probes of  $7 \times 0.7$  cm were cut; at least ten probes were used for each film formulation. Tensile strength (TS, MPa), elongation at break (E, %) and elasticity modulus (Young's modulus, MPa) were calculated as described in a previous work [37].

Dynamic mechanical analyses (DMA) were conducted in a Q800 (TA Instruments, New Castle, USA) equipment with a liquid N<sub>2</sub> cooling system, using a clamp tension and rectangular film probes (length: 30 mm, width: 6 mm and thickness: determined as described previously). To select the viscoelasticity range for further frequency sweeps, amplitude sweeps were performed from 1 to 20  $\mu\text{m}$  at fixed frequency (5 Hz). Multi-frequency sweeps (1 to 15 Hz) at fixed amplitude from  $-100$  to 100 °C at 2 °C/min were carried out. Temperatures of the relaxation processes associated to dynamic glass transition temperatures were determined through the inflection point of the storage modulus ( $E'$ ) curve as well as the maximum peak in both the loss modulus ( $E''$ ) and  $\tan \delta$  curves [47], using the software Universal Analysis 2000. All measurements were performed at least in triplicates and reported values are the mean of measurements.

On the other hand, starch films were stored for 180 days under controlled conditions (20 °C and 65% RH) and the effect of the storage on their dynamic-mechanical behavior was evaluated. Furthermore, starch

films' crystallinity degree (at initial time and after storage) was also determined using the same equipment and conditions previously described for starch powders analysis.

### 2.6. Statistical analysis

All experiments were performed at least in duplicates, with individually prepared and casted films as replicated experimental units, as described previously in each determination. Systat-software (SYSTAT, Inc., Evanston, IL, USA) version 10.0 was used for multifactor analysis of variance. Differences in the properties of the films were determined by Fisher's least significant difference (LSD) mean discrimination test, using a significance level of  $\alpha = 0.05$ .

## 3. Results and discussion

### 3.1. Extraction and physicochemical characterization of starches

The extraction procedure used allowed to obtain starch yields of 56.5 and 80.9% (dry basis) for ahipa and cassava, respectively. Although starch yield of cassava roots was 30% higher than those of ahipa ones, it should be noted that ahipa cultures are propagated through seeds while cassava requires the use of propagules obtained from the roots. Thus, the whole ahipa production could be used for starch isolation. On the other hand, with regard to cereal starches like corn, the starch extraction procedure requires more technology and generally is carried out at industrial scale. The advantages of the extraction method used in the present work are its low cost and simplicity, thus it could be implemented by agricultural cooperatives, contributing to the development of economical depleted regions.

Table 1 shows the amylose content of starches, with the lowest one corresponding to ahipa. Similar results were reported by Forsthy et al. [48] who stressed that ahipa starch amylose content varied between 11.6 and 16.8% while Leonel et al. [12] found a value of  $12.9 \pm 0.6\%$ . With regard to cassava starch amylose content, the obtained value (15.5%) was lower than those reported by Swinkels [49] and Hung and Morita [50]. Differences in the results reported by different authors are related to root varieties employed, growing conditions as well as amylose quantification method [24]. For example, Aryee et al. [51] working with 31 varieties of cassava found amylose contents ranging between 10.9 and 44.3%. Furthermore, corn, the main commercial botanical source of starch, exhibited an amylose content of 23.9%, which was significantly ( $p < 0.05$ ) higher than those of tuber and root starches.

From the low amylose content values of ahipa and cassava starches, high digestibility and stability of starch pastes would be expected [52]. Additionally, this characteristic makes them attractive as food additives and alternative to corn starch in the formulations of products where starch paste retrogradation limits their shelf life.

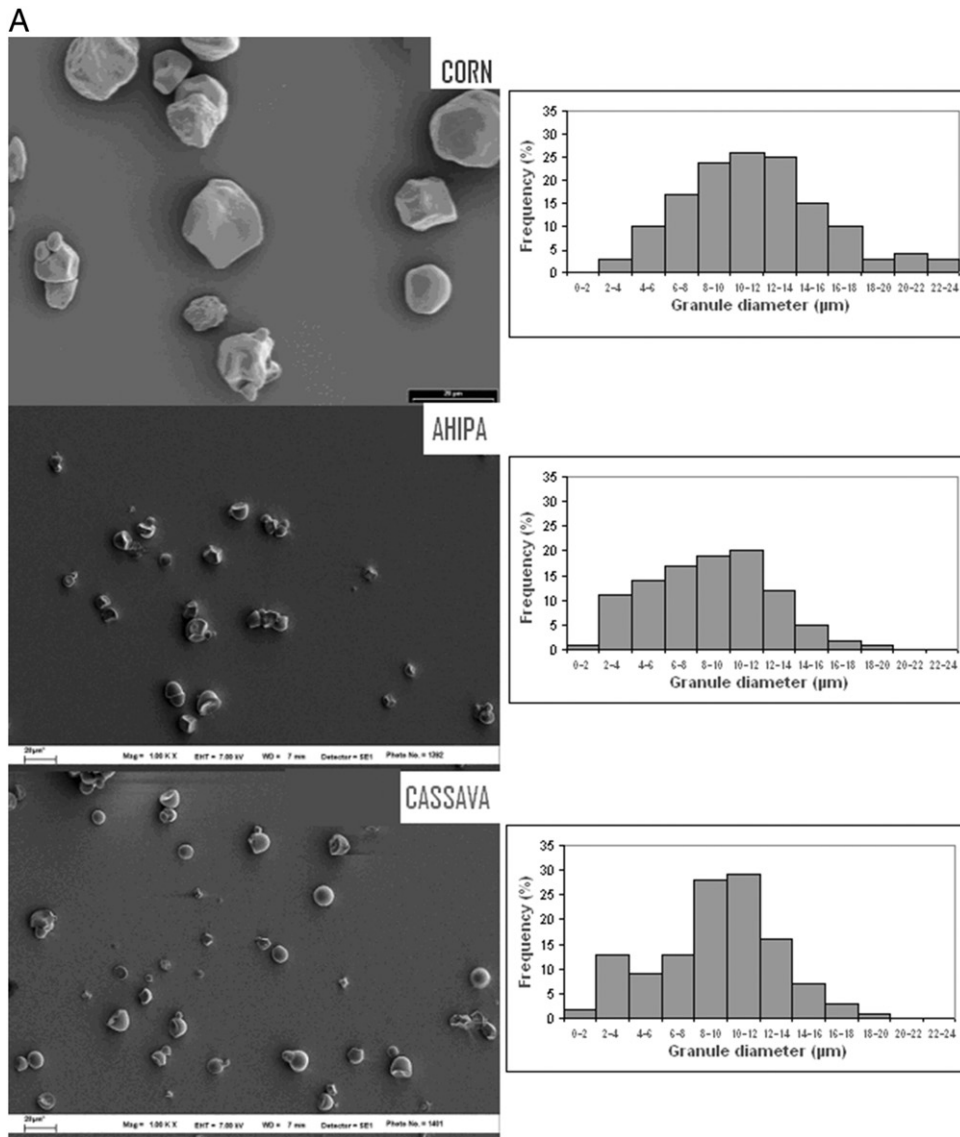
SEM micrographs, the histograms corresponding to the granule size distribution as well as the morphological parameters are shown in Fig. 1A. With regard to morphology, cassava starch granules exhibited oval to round shapes, with a small population having a truncated end. These observations are in agreement with those reported by Gomand et al. [53].

**Table 1**

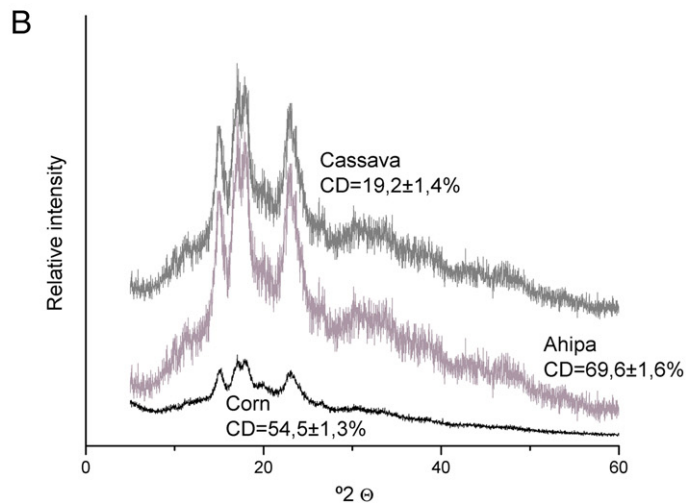
Amylose content and thermal properties of ahipa, cassava and corn starches.

Starch source	Amylose content [%]	Thermal parameters			
		Onset temperature, $T_o$ [°C]	Peak temperature, $T_p$ [°C]	Final temperature, $T_f$ [°C]	Enthalpy, $\Delta H$ [mJ/mg, dry basis]
Ahipa	$11.6 \pm 0.6^a$	$64.2 \pm 0.2$	$67.2 \pm 0.1$	$70.5 \pm 0.2$	$9.5 \pm 0.6$
Cassava	$15.5 \pm 0.8$	$51.7 \pm 0.8$	$69.9 \pm 0.1$	$84.9 \pm 0.5$	$9.7 \pm 0.4$
Corn	$23.9 \pm 0.7$	$67.9 \pm 0.6$	$72.2 \pm 0.1$	$76.9 \pm 0.3$	$11.4 \pm 0.1$

<sup>a</sup> Value  $\pm$  standard deviation.



Native starch	Mean diameter (µm)	Martin's mean radius (µm)	Equivalent circle diameter (µm)	Shape factor
Corn	11.5±4.3	4.6±1.6	9.2±3.3	0.82±0.03
Ahipa	8.8±3.7	4.0±1.7	8.2±3.5	0.90±0.05
Cassava	9.4±3.7	4.3±1.8	8.8±3.6	0.89±0.09





Ahipa starch granules were not associated as clusters; they exhibited round and polygonal shapes with irregular borders. Additionally, in the case of ahipa and cassava, SEM micrographs indicated that granules were not damaged during starch extraction procedure, since smooth surfaces without cracks were observed. Likewise, root and tuber starch granules exhibited a more rounded shape and consequently the corresponding shape factors were higher than that of corn and closer to 1, which correspond to spherical shaped granules (Fig. 1A).

Corn and ahipa starch granules exhibited straight monomodal size distributions which varied between 4 and 22  $\mu\text{m}$ , with a mean value of  $11.5 \pm 4.3 \mu\text{m}$  for corn and varying between 2 and 20  $\mu\text{m}$ , with a mean value of  $8.8 \pm 3.7 \mu\text{m}$ , for ahipa. Granule shape, size and surface are simple physical characteristics which have an impact on starch functionality and they are critical factors for applications that require starch as surface carrier of materials such as pigments, flavors and seasonings.

Fig. 1B shows the diffractograms and crystallinity degrees of the studied native starches. Corn starch granules presented a diffraction pattern Type A, having peaks at  $2\theta = 15.2$  and  $23^\circ$  for those with the major intensities [24]. For ahipa and cassava starches, the pattern assignment was not easy; their diffractograms presented peaks at  $2\theta = 5.5, 15.4, 17.0, 18.0, 20.0$  and  $23.5^\circ$ , thus a Type C pattern was assigned [6]. In the literature there is no homogeneity with respect to the diffraction pattern assignment for starches from non-traditional roots and tubers [54]. Moreover, it was observed that the starch botanical source affected the granules' crystallinity degree; this variation could be inversely related with the amylose content.

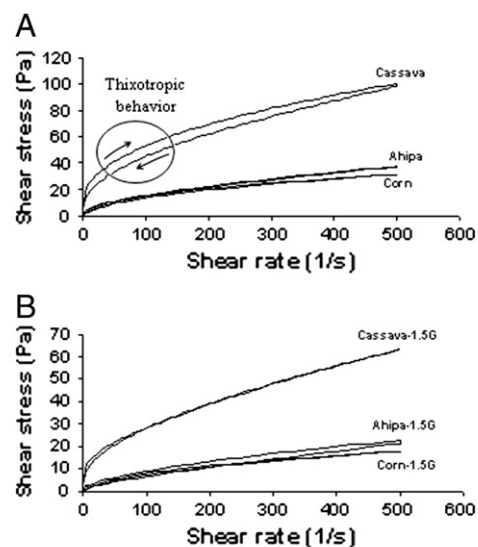
### 3.2. Starch gelatinization

It is well known that the gelatinization process of starch depends on several factors such as the molecular architecture of crystalline region, the amylose to amylopectin ratio, the chain length distribution, and the amount of double-helical orders, as well as the botanical source [6]. Starch suspensions' gelatinization temperature and enthalpy are conveniently evaluated by DSC since the measurement process can be accurately controlled and the sample moisture content can be adjusted [55].

Aqueous suspensions of the studied starches exhibited thermograms with a single endothermic transition, typical of the gelatinization process of high water content suspensions. This process is of primary importance in the food industry because of its high impact on the texture of starch-based products.

Thermal properties of starches depended on botanical source, with gelatinization temperature significantly ( $p < 0.05$ ) different between them. Ahipa and cassava extracted starches presented lower gelatinization temperatures than corn starch, indicating a better aptitude for cooking [56] and a minor energy cost associated with it. These results indicate that the intra-molecular associative forces which maintain starch granule integrity in roots and tubers starches were lower than those of cereals ones.

Ahipa starch exhibited a gelatinization range significantly ( $p < 0.05$ ) narrower than that of the other starches, which could be related with a highly cooperative process [54] and a narrower amylose and amylopectin size distributions. The thermal parameters obtained were similar to those reported by other authors for starches from different botanical sources (refs. [56] and [57]). In general, cereal starches required higher temperatures and involved higher enthalpies than tuber or root starches (Table 1). Gelatinization enthalpy of cassava starch was similar to those reported by Betancur-Ancona et al. [9]. Thermal parameters of native corn starch were similar to those reported by other authors [58–61].



Native starch	Glycerol (% w/w)	$r^2$	Flux behavior index (n)	Consistency index (k) (Pa/s)	Apparent viscosity at 500 s (mPa s)
Cassava	0	0.9985	$0.60 \pm 0.01$	$11.78 \pm 1.77$	$220.4 \pm 36.4$
	1.5	0.9990	$0.51 \pm 0.02$	$1.26 \pm 0.08$	$126.3 \pm 6.2$
Ahipa	0	0.9722	$0.45 \pm 0.03$	$2.59 \pm 0.70$	$81.4 \pm 6.8$
	1.5	0.9963	$0.66 \pm 0.05$	$0.45 \pm 0.03$	$44.0 \pm 2.6$
Corn	0	0.9982	$0.51 \pm 0.01$	$1.24 \pm 0.09$	$60.0 \pm 2.4$
	1.5	0.9950	$0.64 \pm 0.04$	$0.42 \pm 0.01$	$34.9 \pm 1.2$

Fig. 2. Flow curves and rheological parameters of ahipa, cassava and corn starch filmogenic suspensions: A) without glycerol and B) with 1.5% w/w glycerol (1.5 G). Arrows indicate the up and down flow curves.

### 3.3. Characterization of filmogenic suspension

Rheological behavior of filmogenic starch suspensions determines the processing conditions to obtain films at an industrial scale. Furthermore, rheological properties are related with the presence of some defects (bubbles and pores) in polymeric matrixes [62].

Rotational assays led to analyze starch pastes' rheological behavior when they are submitted under conditions close to industrial processing parameters such as high shear stress. Fig. 2A shows flow curves corresponding to gelatinized starch suspensions without glycerol. All of them presented a pseudoplastic behavior ( $n < 1$ ), which was satisfactorily adjusted by the Ostwald de Waele model. Moreover, gelatinized starch suspensions were characterized as thixotropic ones, indicating that rheological behavior of these systems was time dependent, (Fig. 2A). Thixotropic materials exhibit decreasing shear stress and apparent viscosity over time at a fixed shear rate [48]. The corresponding thixotropic indexes were calculated as the area between the flux curves, with 4123, 673.5 and 335.9 Pa/s for cassava, ahipa and corn starch suspensions, respectively.

The rheological properties of starch pastes determine their best use in food processing and depend on botanical species. Cassava starch filmogenic suspensions presented significantly ( $p < 0.05$ ) higher apparent viscosity values indicating that the remainder amylopectin inside starch granules reinforces the amylose network. Furthermore, macromolecules' structural characteristics (molecular weight, polymerization degree, chains size distribution, etc.) determine network reinforcement degree.

Fig. 2B shows that glycerol addition to filmogenic suspensions did not modify their flow behavior although the apparent viscosity at  $500 \text{ s}^{-1}$  values decreased significantly ( $p < 0.05$ ).

Oscillatory assays evaluate starch paste viscoelastic behavior within linear viscoelasticity range where the sample did not suffer structural damage. From stress sweeps, it was determined that linear

Fig. 1. A) Scanning electronic microscopy (SEM), morphological parameters and granule size distribution histograms of ahipa, cassava and corn starch; B) X-ray diffraction patterns and crystallinity degree (CD) of ahipa, cassava and corn starch granules.

viscoelasticity range was extended up to 1.4 Pa for all starch pastes, so for frequency sweeps, 1 Pa was the shear stress value selected.

Fig. 3 shows mechanical spectra obtained from frequency sweeps. Corn starch filmogenic suspensions presented a typically viscous fluid behavior since higher viscous module values compared to elastic ones ( $G'' > G'$ ) were registered over almost all the frequency range, approaching each other at high frequency values (Fig. 3C). While, for ahipa and cassava starch pastes,  $G'$  values were significantly higher ( $p < 0.05$ ) than  $G''$  ones, and remained practically constant throughout all the analyzed frequency ranges, behaving like a weak gel.  $G'$  and  $G''$  values for cassava starch gelatinized suspensions were higher than those of ahipa and corn starch pastes. For example, at 1.1 Hz,  $G'$  and  $G''$  of cassava starch pastes were 20 and 60% higher, respectively, than the corresponding values to ahipa starch pastes. These results are in agreement with the apparent viscosities reported previously (Fig. 2A). When glycerol was incorporated to starch matrices, both  $G'$  and  $G''$  decreased; however the tendency of the curves were similar to those corresponding to unplasticized films (Fig. 3). Similar trend was reported by Rodríguez-González et al. [63] for wheat thermoplastic starch films plasticized with different glycerol concentrations.

Starch gel clarities, determined spectrophotometrically, were significantly different ( $p < 0.05$ ) between each other with the obtained values of  $1.3 \pm 0.0$ ,  $7.9 \pm 0.4$  and  $15.0 \pm 2.6\%$  for corn, ahipa and cassava, respectively. Corn starch gel clarity value is in agreement with those reported by Sandhu and Singh [60] for different corn variety starch pastes, although Mélo et al. [56] reported a significantly higher value (5.6%). These authors also reported clarity gel values of 21.8 and 11.4% for cassava and yam (*Dioscorea* spp.) starches, respectively, analyzed by an equivalent technique.

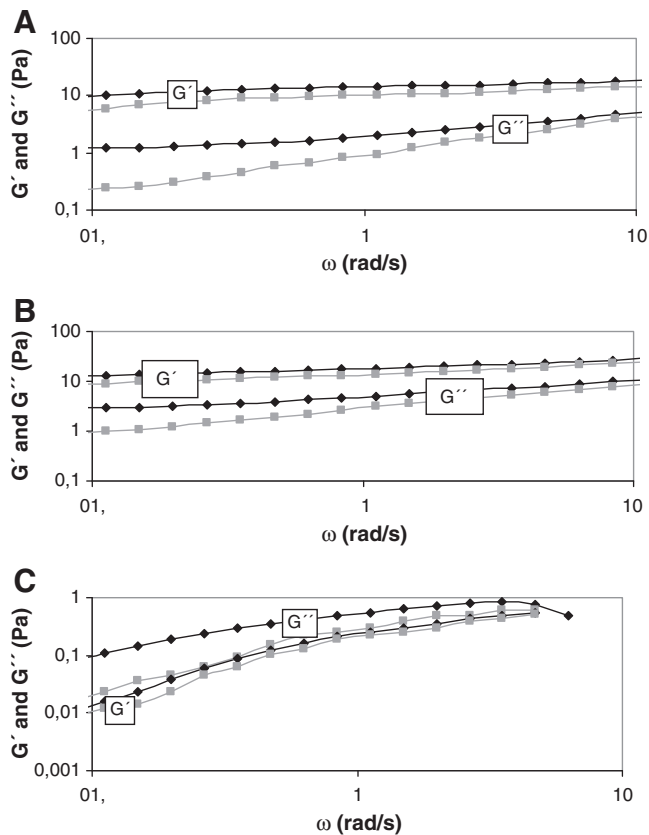


Fig. 3. Mechanical spectra, at constant amplitude = 1 Pa, and viscoelastic behavior of starch filmogenic suspensions without ( $\blacklozenge$ ) and with 1.5% w/w of glycerol ( $\blacksquare$ ): A) ahipa; B) cassava and C) corn.

### 3.4. Film forming capacity

Film forming capacity is related to granule starch amylose concentration as well as the architecture of amylose and amylopectin molecules, mainly chains size distribution [38].

Filmogenic suspensions of the three native starches were able to form films which were easily removed from the casting plates. Cassava starch films were slightly colored; besides, an additional step for bubbles' removal was necessary, due to its high filmogenic suspension apparent viscosity. Perdomo et al. [64] developed cassava starch films and also incorporated one step to avoid bubble forming inside the matrix polymeric.

### 3.5. Film properties

#### 3.5.1. Thickness, moisture content and water solubility

Regardless of native starch type, unplasticized filmogenic suspensions led to films with approximately 85  $\mu\text{m}$  thickness, employing a casting relation of 2 g of filmogenic suspension per  $\text{cm}^2$  of casting area (Table 2). Films with glycerol presented higher thickness due to structural modifications caused by plasticizer incorporation. Zhang and Han [42] reported that thickness variation due to plasticizers incorporation depends on the molecular weight of the plasticizer used. The comparison of film thickness with those reported in the literature for hydrocolloid-based films is difficult since this property depends on the casting relation, as data rarely mentioned.

The moisture content in starch films can affect significantly their physical and barrier properties; Table 2 summarized the obtained values for starch films based on the three native starches studied. As it can be seen, the moisture content was influenced by starch type; presenting cassava and corn starch films as the significantly ( $p < 0.05$ ) higher and lower values, respectively. Glycerol addition increased the films moisture affinity and these results could be attributed to the hydrophilic character of the plasticizer. The addition of glycerol provides more active sites into polymeric matrix by exposing its hydrophilic hydroxyl groups in which the water molecules could be absorbed and kept linked by hydrogen bonds. Additionally, the molecules of this plasticizer are small and present high capacity to interact with starch chains, enhancing the molecular mobility and increasing free volume in the film matrix [39].

Starch films' water solubility determines the application of these materials; in some cases, water insoluble films are required to enhance product integrity and package humidity resistance; in others, total or partial film dissolution before product consumption could be useful, such in food or additive encapsulation [26]. The obtained films' water solubility results are shown in Table 2. The starch type did not affect film water solubility, regardless of the assayed temperature. The values increased significantly ( $p < 0.05$ ) with temperature and glycerol addition due to plasticizer hydrophilic characteristic. Similar results were reported by Shen et al. [65] and by Piermaría et al. [66] for sweet potato starch films with glycerol and for plasticized kefir films, respectively.

Table 2

Thickness, moisture content and water solubility of ahipa, cassava and corn starch films without and with 1.5% w/w of glycerol (1.5 G).

Film composition	Thickness ( $\mu\text{m}$ )	Moisture content (g water/100 g film, dry basis)	Water solubility (%)	
			At 25 °C	At 100 °C
Ahipa	$86.7 \pm 3.2^a$	$12.9 \pm 0.3$	$3.8 \pm 0.4$	$17.2 \pm 0.7$
Ahipa–1.5 G	$110.4 \pm 2.9$	$19.8 \pm 0.3$	$13.2 \pm 0.1$	$26.6 \pm 1.1$
Cassava	$84.0 \pm 3.8$	$15.8 \pm 0.1$	$3.9 \pm 0.1$	$17.0 \pm 0.6$
Cassava–1.5 G	$106.2 \pm 4.7$	$26.3 \pm 0.5$	$13.6 \pm 0.3$	$27.7 \pm 1.3$
Corn	$84.7 \pm 4.9$	$9.0 \pm 0.1$	$4.0 \pm 0.3$	$16.4 \pm 2.9$
Corn–1.5 G	$107.4 \pm 0.8$	$15.3 \pm 0.9$	$13.6 \pm 0.1$	$26.3 \pm 0.5$

<sup>a</sup> Value  $\pm$  standard deviation.

**Table 3**

Water vapor permeability (WVP) and mechanical properties of ahipa, cassava and corn starch films without and with 1.5% w/w of glycerol (1.5 G).

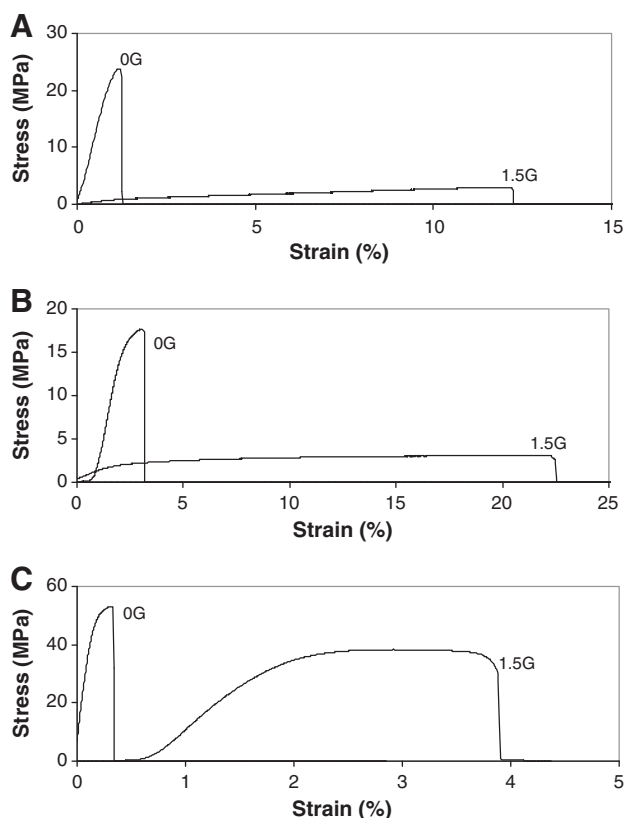
Starch base film composition	WVP $\times 10^{10}$ (g/s m Pa)	Tensile strength (MPa)	Elongation at break (%)	Young's modulus (MPa)
Ahipa	2.6 $\pm$ 0.1 <sup>a</sup>	36.7 $\pm$ 2.2	3.5 $\pm$ 0.3	16.2 $\pm$ 0.5
Ahipa–1.5 G	1.6 $\pm$ 0.1	4.7 $\pm$ 0.3	21.8 $\pm$ 0.6	2.6 $\pm$ 0.1
Cassava	1.6 $\pm$ 0.1	29.7 $\pm$ 0.5	4.3 $\pm$ 0.4	10.5 $\pm$ 1.6
Cassava–1.5 G	1.4 $\pm$ 0.1	3.5 $\pm$ 0.5	28.9 $\pm$ 2.7	1.2 $\pm$ 0.1
Corn	3.5 $\pm$ 0.1	53.5 $\pm$ 0.4	2.5 $\pm$ 0.3	51.5 $\pm$ 2.7
Corn–1.5 G	1.2 $\pm$ 0.1	31.8 $\pm$ 2.4	4.1 $\pm$ 0.3	25.2 $\pm$ 1.0

<sup>a</sup> Value  $\pm$  standard deviation.

### 3.5.2. Water vapor permeability and mechanical properties

WVP values for starch films are presented in Table 3. Our results are of the same order of magnitude with those reported by other authors for different starch types (refs. [21,22], [24,30] and [39]). WVP of plasticized films were lower ( $p < 0.05$ ) than unplasticized ones. Glycerol interferes with polymeric chain association decreasing the rigidity of the network; thus a less ordered and compact film structure is developed, decreasing WVP [36]. Similar tendency were reported by Galdeano et al. [67] for oat starch films plasticized with glycerol (20 g/100 g starch). The WVP reduction caused by glycerol addition resulted in 38, 9 and 65% for ahipa, cassava and corn starch films, respectively. The lower WVP reduction for cassava starch films could be attributed to the high apparent viscosity of its filmogenic suspensions that led to develop more cohesiveness in matrices, with good barrier properties, even without plasticizer addition.

Fig. 4 shows the stress–strain curves and Table 3 shows the films' mechanical properties obtained from tensile assays. Films without glycerol were more brittle compared to those with plasticizer; with



**Fig. 4.** Stress–strain curves of starch films without (0 G) and with 1.5% w/w of glycerol (1.5 G): A) ahipa starch; B) cassava starch and C) corn starch.

unplasticized corn starch films being the most resistant and less flexible (Table 3). It could be related to the higher amylose content of corn starch (Table 1); there is broad agreement in the literature regarding the positive effect of the increase of the amylose content on the elastic modulus of starch films (refs. [19,22] and [67]). According to Rindlav-Westling et al. [68], the structure of the amylose network is very stable, with strong molecular orientation, forming denser films compared to the amylopectin one. Furthermore, Zobel [68] found that linear amylose molecules formed strong films whereas branched amylopectin-based films were weak and brittle.

When glycerol was incorporated into starch matrices the mechanical behavior of these materials changed considerably (Fig. 4) and tensile strength decreased, as well as the elastic modulus; while elongation at break increased significantly ( $p < 0.05$ ; Table 3). When the plasticizer was added, the proximity between starch chains was reduced, thus under tensile forces, movements of starch chains were facilitated [67]. For ahipa and cassava plasticized films, tensile strength and elongation at break values were within the range of those recorded by Mali et al. [39] for yam starch films plasticized with a similar concentration of glycerol with those used in the present work. As it can be observed for unplasticized films, corn starch films with glycerol remained more resistant and less flexible than those of tuberous starches (Table 3). When glycerol was incorporated to starch network, the elastic modulus values were reduced at 51.1, 84.0 and 88.9% for corn, ahipa and cassava starch films, respectively (Table 3). These outcomes indicate that glycerol improved cassava and ahipa starch films' flexibility and extensibility better than those of corn. It could be related with the low amylose content of these starches since, according to Myllarinen et al. [69], amylopectin was more susceptible to plasticizing effect than amylose. Additionally, the characteristics of both amylose and amylopectin determine their ability to interact with plasticizer molecules and the necessary polymer chain rearrangements that lead to enhance film mechanical behavior [22]. The starch type influence on films' mechanical behavior (Fig. 4, Table 3) is in agreement with the films' humidity content shown in Table 2; it is well known, that water is an excellent plasticizer for biopolymers [70]. Thus, films' moisture content correlates directly with films' flexibility and inversely with their tensile strength and Young's modulus.

It should be taken into account that mechanical behavior of films is the compromise between the degree of compaction of the network influence by amylose:amylopectin ratio, chains characteristics and glycerol interactions.

### 3.5.3. SEM observations and UV barrier properties

Since unplasticized films were rigid and exhibited poor mechanical properties they have limited technological applications; so for SEM observation, UV barrier properties evaluation and DMA analysis, only plasticized films were studied.

Fig. 5 shows the SEM micrographs of the cryo-fractured surfaces of plasticized films based on ahipa, cassava and corn starch, respectively. In this figure, it can be seen that corn starch films show a more homogeneous and compact structure than the films obtained from tuberous starches. These observations correlate with the higher tensile strength of corn starch films associated to the rigidity of the developed network.

The study of the UV light absorption capacity of the biodegradable films is relevant to determine their possible applications for food packaging. If these materials are able to absorb UV light they could be useful to package and extend shelf life of fatty foods which are susceptible to the oxidative degradation catalyzed by UV rays. However, if these films cannot absorb in this spectral region, they could be used to develop packages for foods which will be later sanitized by UV light exposure to reduce their microbial load. Fig. 6 shows the obtained spectrum and the opacity calculated from absorption spectrum in the visible zone for the studied plasticized films. All of them resulted



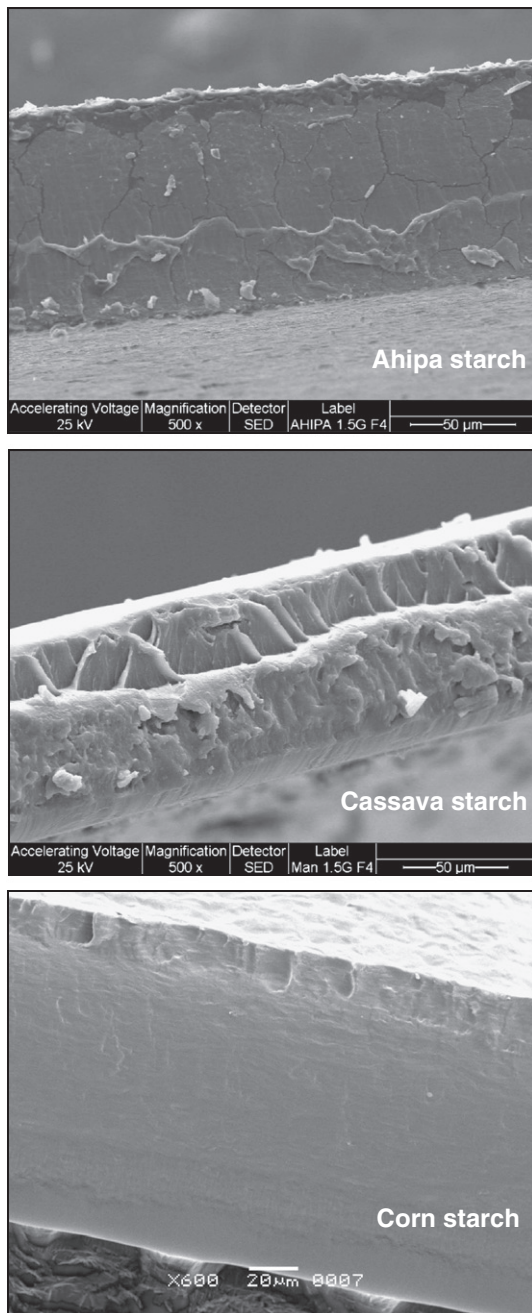


Fig. 5. SEM micrographs of ahipa, cassava and corn starch films plasticized with 1.5% w/w of glycerol.

as being barriers of UV light since a characteristic peak at 270–300 nm was observed. Ahipa led to develop films with lower capacity to absorb UV light since the peak intensity were lower compared with cassava and corn starches (Fig. 6). Furthermore, ahipa starch films were less opaque and more transparent than cassava and corn starch ones; these characteristics can be visualized in the film photographs presented in Fig. 6. The transparency values of the cassava and corn films were closer to those corresponding to some synthetic films, such as polyvinyl dichloride (PVDC, 4.58  $A_{600}/\text{mm}$ ).

#### 3.5.4. DMA assays

Temperatures and intensities of the relaxation phenomena in the films were determined by DMA. The linear viscoelastic range was around 12  $\mu\text{m}$ ; thus, frequency sweep assays as a function of temperature were performed at constant amplitude of 5  $\mu\text{m}$ .

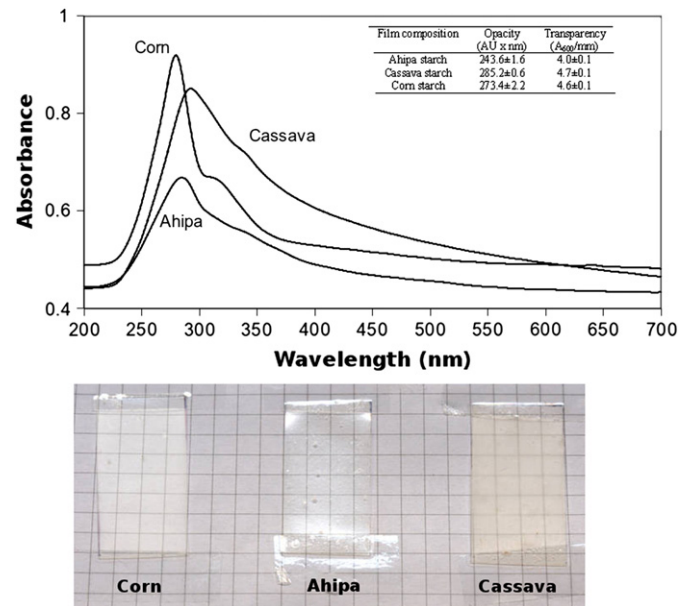


Fig. 6. UV–vis light absorption spectrum and optical properties of starch films (ahipa, cassava and corn) plasticized with 1.5% w/w of glycerol.

Several authors stressed that starch–glycerol mixtures are partially miscible systems, giving rise to starch-rich phase and others rich in glycerol (refs. [22,37] and [70–72]). Fig. 7 shows the  $\tan \delta$  curves of the three plasticized starch films studied and it can be observed that their dynamic-mechanical response showed two relaxation peaks. The low temperature peak is related to the main relaxation of the plasticizer-rich phase, while the high temperature relaxation peak is attributed to the glass transition temperature of the starch-rich phase. Table 4 shows the relaxations temperatures of the glycerol-rich phase, determined through the inflection point of the storage modulus ( $E'$ ) curve as well as the maximum peak in both the loss modulus ( $E''$ ) and  $\tan \delta$  curves. For ahipa starch film this relaxation occurs at higher temperatures compared to the cassava and corn ones. Considering this and the fact that the glass transition temperature,  $T_g$ , of pure glycerol is located at  $-78^\circ\text{C}$  [73], suggest that glycerol interacts more effectively with the ahipa starch matrix, remaining less free glycerol and leading to higher  $T_g$  values for the system. The higher interaction of glycerol with the ahipa starch compared to cassava and corn can be explained by the higher formation of

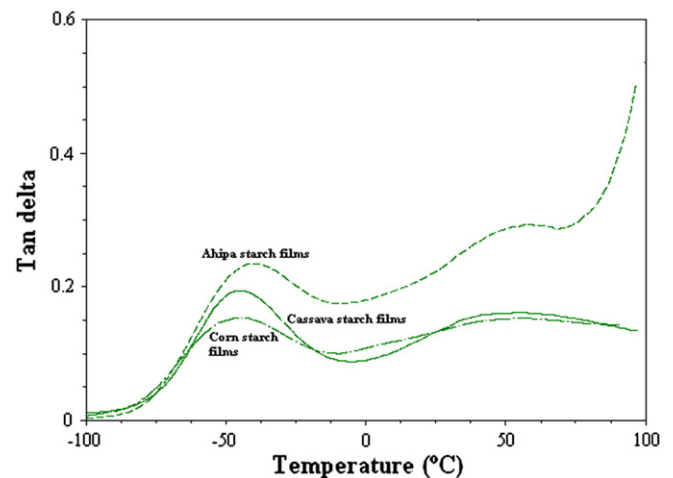


Fig. 7. Dynamic-mechanical analysis (DMA): isochronal evolution of loss factor ( $\tan \delta$ ) as a function of temperature of starch-based films (ahipa, cassava and corn) plasticized with 1.5% w/w of glycerol.



**Table 4**

Relaxation temperatures associated to the glass transition of the glycerol and starch-rich phases of starch films plasticized with 1.5% (w/w) glycerol determined by DMA (frequency: 10 Hz; amplitude: 5  $\mu$ m).

Plasticized film composition	Relaxation temperatures ( $^{\circ}$ C)			
	Glycerol-rich phase			Starch-rich phase
	Inflection of storage modulus ( $E'$ )	Maximum of loss modulus ( $E''$ )	Maximum of $\tan \delta$	
Ahipa	$-59.9 \pm 1.7^a$	$-58.81 \pm 0.08$	$-44.96 \pm 0.01$	$42.9 \pm 6.9$
Cassava	$-61.0 \pm 3.4$	$-58.0 \pm 1.5$	$-48.5 \pm 3.3$	$38.1 \pm 7.0$
Corn	$-60.92 \pm 0.01$	$-61.1 \pm 4.1$	$-52.2 \pm 0.3$	$48.3 \pm 1.4$

<sup>a</sup> Value  $\pm$  standard deviation.

hydrogen bonding between glycerol and its amylose chains. Similar results were reported by García et al. [22] who were working on cassava and waxy maize starch films.

The corresponding peak of glycerol-rich phase relaxation were more defined with a higher associated intensity change than the corresponding to starch rich phase, thus the relaxations temperatures associated to starch-rich phase only could be evaluated through  $\tan \delta$  curves (Table 4). Our results are in agreement with those reported by Jiang et al. [74] who were working on thermoplastic acetylated tapioca starch films. A similar trend was also reported by other authors for films formulated with native starches from different sources such as barley [75], waxy maize [22], pea [76], mungbean [77], corn [37], cassava [28] and cara [8] roots. This tendency was also reported for plasticized protein films [78].

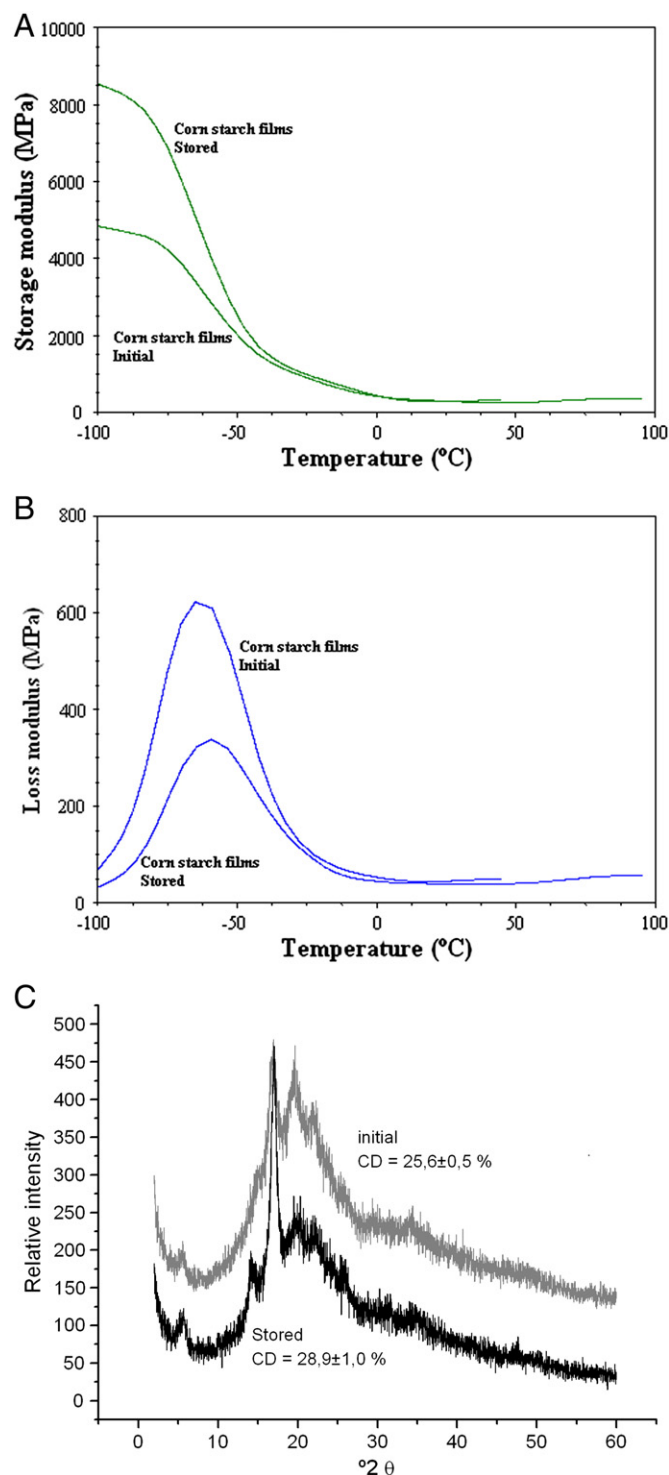
Likewise, plasticized films were stored for 180 days under controlled conditions (20  $^{\circ}$ C and 65% RH) to evaluate the effect of the storage on their dynamic-mechanical behavior. Fig. 8A and B shows the influence of storage time on loss and storage moduli curves of corn starch plasticized films. It could be observed that the inflection point of the  $E'$  curve was shifted to higher values while the maximum of the  $E''$  curve decreased; a similar trend was obtained for ahipa and cassava plasticizer starch films. On the other hand, it was also determined that during storage, the crystallinity fraction increased (approximately 15%, Fig. 8C), non significant differences were observed for films formulated from tuberous roots. This could be attributed to the retrogradation process of starch-based films during storage which causes a molecular restructuring and affecting the ability of the chains to form crystals as well as their mobility [26,43]. Furthermore, this crystallinity increase during storage was also correlated with the material dehydration and their stiffness increase.

#### 4. Conclusions

It was demonstrated that by employing a simple and economic technique, it was possible to extract representative starch quantities from ahipa and cassava tuberous roots. These starches showed some advantages with respect to corn starch: they exhibited low amylose contents, making them attractive additives for food formulations in which the retrogradation process is undesirable and their aqueous suspensions gelatinization required less time and energy. Corn starch gelatinized suspensions behaved like a viscous fluid; while ahipa and cassava ones behaved like a weak gel. Moreover, it was evidenced that cassava starch gelatinized suspensions were more susceptible to severe processing conditions like high shear stress.

All the formulations studied were able to form films which could be easily removed from cast plate. The addition of 1.5% w/w of glycerol increased film thickness, moisture content and water solubility. Plasticizer also reduced WVP values of starch films and the lowest reduction corresponded to cassava ones due to the high viscosity of its filmogenic suspensions. On the other hand, glycerol enhanced the mechanical properties, thus improving films' ductility and flexibility; this effect is

more marked for ahipa and cassava starch films. Plasticized starch films presented UV barrier properties; ahipa starch ones exhibited the lowest capacity to absorb UV radiation. Ahipa starch films were more transparent than cassava and corn ones. Starch films with glycerol resulted in being partially miscible systems since two relaxations were observed in the dynamic-mechanical patterns. Moreover, the major plasticizer effect of glycerol on ahipa starch matrixes was established since the relaxation temperatures associated to the plasticizer rich phase was higher than the cassava and corn ones. During



**Fig. 8.** A) Storage moduli; B) loss moduli and C) X-ray diffraction patterns and crystallinity degree (CD) of corn starch films plasticized with 1.5% w/w of glycerol at initial time and after 180 days of storage at 20  $^{\circ}$ C and 65% RH.

storage, starch-based matrixes increase their stiffness and crystallinity due to the retrogradation process.

Thus, it was verified that biodegradable films based on starches from different botanical sources exhibited diverse physicochemical and functional properties which are related with the starch characteristics. In conclusion, it is possible to use starches from non-conventional sources like *ahipa*, promoting its crops and satisfying the wide demand of different functionally materials, to develop food packages.

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**Olivia V. López** She received her degree in Chemistry in 1999 and holds a Masters degree in Food Science and Technology in 2006, both from Southern National University (UNS) of Bahía Blanca. In 2011, she got a degree of Doctor (PhD) of the Exact Science Faculty of National University of La Plata (UNLP). Her research focuses on the development, characterization and application of starch biodegradable films and it is carried out at the Center for Research and Development in Food Cryotechnology (CIDCA) and at the Chemical Engineering Pilot Plant (PLAPIQUI), both are institutes of the National Council of Technical and Scientific Research (CONICET).

**María A. García** She received her degree in Chemistry in 1990 from National University of Mar del Plata (UNMP). In 1999, she got a degree of Doctor (PhD) at the Exact Science Faculty of National University of La Plata (UNLP). She is a member of the scientific staff of the National Council of Technical and Scientific Research CONICET since 1994. She is a Professor of the subjects in Analytical Chemistry area at the UNLP since 2007. Her research focuses on the development, characterization and application of hydrocolloid-based films. Her workplace is the Center for Research and Development in Food Cryotechnology (CIDCA).