

# Physicochemical characterization of starch based films

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Received 13 October 2006; received in revised form 13 December 2006; accepted 19 December 2006  
Available online 13 January 2007

## Abstract

In the present study, high amylose corn starch (HACS) based films were obtained with a low temperature method consisted on reduce gelatinization temperature by an alkaline pretreatment of starch. Effects of film composition and preparation method on film properties were investigated to identify HACS film elaboration conditions to improve film properties. Rheological behavior of film-forming solutions is reported. Films were physical and chemically characterized through film solubility in water, water sorption isotherms, opacity and crystallinity of films. Film forming suspensions show pseudoplastic and thixotropic behavior. Apparent viscosity of film forming gel increases exponentially with HACS concentration and Arrhenius law adequately represents variation with temperature. Alkaline treatment time of HACS previous to gelatinization, also affects film properties. It was found that film solubility and opacity decrease while crystallinity increases although it reaches an asymptotic value after treatment time of 60 min. Above 30% of glycerol, an increase in plasticizer content conduces to a looser network, as a result film opacity and water sorption increase.

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**Keywords:** Starch films; Rheological behavior; Water solubility; Film solubility; Opacity; Crystallinity

## 1. Introduction

There is an increasing interest in utilizing renewable resources as food packaging. Polysaccharides such as starches, cellulose derivatives and plant gums are being studied as edible films and coatings in food packaging and preservation (Baldwin, Nisperos-Carriedo, & Baker, 1995; Donhowe & Fennema, 1994; Debeaufort, Quezada-Gallo, & Voilley, 1998; Guilbert, 1986; Kester & Fennema, 1986).

Many studies have been reported on starch based films cast from solutions or gels since 1950 (Famá, Rojas, Goyanes, & Gerschenson, 2005; Liu & Han, 2005; Lourdin, Coignard, Bizot, & Colonna, 1997; Parra, Tadini, Ponce, & Lugao, 2004; Zhang & Han, 2006). HACS is known to lead to strong and flexible films probably due to amylose crystallization (Myllärinen, Partanen, Seppälä, & Forsell, 2002; Van Soest, Hulleman, de Wit, & Vliegenthart, 1996). Film based on amylose or HACS were obtained,

in general, by dissolution at very high temperature (120–155 °C) (Bader & Göritz, 1994a, 1994b; Whistler, Bemiller, & Paschall, 1984). It is known that an alkaline treatment of HACS favors starch dissolution and the expansion of the amylose coil due charge repulsion (Chen & Jane, 1994; Whistler et al., 1984), which could facilitate film formation. However, information concerning HACS based films obtained by a low temperature method through an alkaline pretreatment of starch, and how this treatment affects film characteristics are not available in the current bibliography.

The extended configuration of starch molecules obtains through an alkaline treatment favors the formation of crystalline zones inducing more dense and resistant films. Recrystallization or retrogradation of starch after melting or gelatinization is known to occur in foods as well as in thermoplastic materials. The spontaneous recrystallization in water excess leads to 'B' type crystalline structure (Myllärinen et al., 2002; Rindlav-Westling, Hulleman, & Gatenholm, 1997; Rindlav-Westling, Standing, Hermanson, & Gatenholm, 1998). Furthermore amylose and native

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lipids, which mainly consist of palmitic acid, form single helical inclusion complexes that crystallize in a 'V' pattern (Bader & Göritz, 1994b; Chen & Jane, 1994; Godet, Bouchet, Colonna, Gallant, & Buleon, 1996).

To apply coatings in liquid form directly onto food products by dipping, brushing or spraying, accurate data on rheological properties of film forming dispersions are needed (Peressini, Bravin, Lapasin, Rizzotti, & Sensidoni, 2003). Process operation design requires good knowledge of the influence of temperature, starch concentration and shear rate on rheological properties. During heat treatment of starch dispersion, a very significant change in the apparent viscosity is observed. Gelatinization induces an increase in viscosity as a result of structural changes such as, irreversible swelling of starch granules, melting of crystallites and leaching of starch granules components (mainly amylose). When maximum swelling is reached granule rupture starts, which is associated with viscosity decrease, that could be enlarged under severe shear stress (Lagarrigue & Alvarez, 2001; Li & Yeh, 2001). During cooling, retrogradation occurs when solubilized starch polymers and remaining granules reassociate in an ordered structure (Lagarrigue & Alvarez, 2001; Miles, Morris, & Ring, 1985). Flow properties of starch dispersion are interpreted on the basis of the rheological behavior of swollen granules immersed in a continuous phase composed by water and amylose leached from starch granules. Starch dispersion rheology is thus a combination of the effect of swollen granules concentration, of their deformability and also of the continuous phase behavior related to its composition. Gelatinized starch dispersions are usually represented by Ostwald-de-Waele model (power law) or Herschell–Bulkley model in the range  $1\text{--}1500\text{ s}^{-1}$  (Lagarrigue & Alvarez, 2001; Nurul, Azzerrri, & Manan, 1999; Peressini et al., 2003).

Amylose is responsible for the film-forming capacity of starch based films. In the absence of plasticizer, films made from starch or amylose are brittle. The addition of plasticizing agent to edible films is required to overcome film brittleness caused by extensive intermolecular forces, thereby improving flexibility and extensibility of films (Koskinen, Suortti, Autio, Myllärinen, & Poutanen, 1996). Plasticizers extend, dilute and soften the structure, increasing chain mobility. Usually the main functional properties of hydrophilic materials such as starch, strongly depend on their water content and therefore on the surrounding humidity. Since water acts as a plasticizer for these materials their mechanical and barrier properties strongly depend on water content (Gontard, Guilbert, & Cuq, 1993; Gennadios & Weller, 1994; Mehryar & Han, 2004). On these materials, multilayer adsorption is accompanied by swelling and conformational changes in the macromolecular structure (Bader & Göritz, 1994a; Cuq, Gontard, Aymard, & Guilbert, 1997; Debeaufort, Voilley, & Meares, 1994; Lievonen & Roos, 2002). Water solubility in films, that can be obtained from sorption isotherms and film solubility in water give valuable information for film characterization and application procedures.

HACS films were produced by alkaline hydrolysis of starch and low temperature gelatinization instead of the traditional high temperature process. The purpose of the present contribution was to gain a better understanding of relationships between film forming composition and conditions, and physicochemical properties of resulting films (water and film solubilities, opacity and crystallinity).

## 2. Materials and methods

### 2.1. Film preparation

High amylose corn starch (Sigma Chemical Co., St. Louis, MO, USA) with amylose content of about 70% and native lipid content of 1.2%, was used. To prepare its dispersion in water, HACS and 0.125 M sodium hydroxide were used. All films studied were prepared at 5% HACS dispersion, but film forming dispersions at 5%, 7.5% and 10% HACS were prepared to analyze rheological behavior. The resulting dispersion was kept 60 min under magnetic stirring. To study the effect of alkaline treatment time on film properties, stirring time varying between 15 and 75 min were used. After, the dispersion was gelatinized in a shaker water bath at  $78\text{--}80\text{ }^{\circ}\text{C}$  during 10 min. This procedure ensures disintegration of starch granules and formation of a homogeneous dispersion. When needed, glycerol (Merck, Whitehouse Station, NJ, USA) was added as plasticizer. A glycerol concentration range from 0% to 60% of starch was used. The resulting dispersion, while still hot, was poured on plastic Petri dishes (9 cm diameter). Then, they were placed in an air-circulating oven at  $35\text{ }^{\circ}\text{C}$  and 50% RH for 15 h. After that, dishes were removed from the oven and films peeled off.

### 2.2. Rheological characterization of starch suspensions

Rheological characterization of the film-forming suspension was performed in a Haake RV2 (Haake, Germany) rotational viscosimeter, at controlled constant temperature. A MV1P type sensor system of coaxial cylinders was used in all measurements of suspensions. Gels obtained with 5%, 7.5% and 10% HACS concentration were analyzed at  $30\text{ }^{\circ}\text{C}$ . Effect of temperature on rheological properties of 5% HACS gel was investigated at 30, 40 and  $50\text{ }^{\circ}\text{C}$ . Different Rheological curves were obtained after a stabilization time of 10 min. Shear stress was determined as a function of shear rate. An acceleration of  $100\text{ s}^{-2}$  was used to increase shear rate from 0 to  $1200\text{ s}^{-1}$  and the same but negative acceleration value to decrease shear rate until 0. Rheological behavior was correlated by Ostwald-de-Waele model (power law):

$$\tau = -\kappa D^n$$

where  $\tau$  is the shear stress (Pa),  $\kappa$  is the consistency index ( $\text{Pa s}^n$ ),  $D$  is the shear rate ( $\text{s}^{-1}$ ) and  $n$  is flow index (dimensionless). When samples are non-Newtonian, apparent viscosity is highly dependent on the shear rate at which shear

stress is measured (Steffe, 1992). Then, apparent viscosity ( $\eta$ ) for power law model could be calculated as follow:

$$\eta = \kappa D^{n-1}$$

Apparent viscosities [mPa s] were calculated at 37, 74, 150, 300, 424, 600, 847 and 1200 s<sup>-1</sup> for each combination of starch concentration and temperature.

The variation of apparent viscosity with temperature was evaluated by using Arrhenius law:

$$\eta = A \exp\left(\frac{E_a}{RT}\right)$$

where  $A$  is frequency factor,  $E_a$  is the activation energy,  $R$  is the gas constant and  $T$  the absolute temperature.

### 2.3. Film solubility in water

Film solubility in water was measured as percentage of dry matter of the film solubilized in water during a period of 24 h. Films prepared with different alkaline treatment time (15–75 min) and a glycerol concentration of 30% HACS were analyzed. The initial dry matter of each film was obtained after drying film specimens in desiccators containing P<sub>2</sub>O<sub>5</sub> during a week. Samples were weighed and immersed in 50 mL distilled water at 30 °C, sealed and periodically agitated. Films not solubilized in water were separated by centrifugation (Sigma 4K10, Germany) and dried to determine the weight of dry matter. Test were performed in triplicate and the solubility was calculated as follows:

$$\text{Solubility (\%)} = \left( \frac{\text{Initial dry weight} - \text{Final dry weight}}{\text{Initial dry weight}} \right) \times 100$$

### 2.4. Water sorption isotherms

Water solubility in films is obtained from moisture sorption isotherms. Constant relative humidity environments were established inside sorbostats, glass jars, using salt solutions. The chemicals used (LiCl, CH<sub>3</sub>COOK, MgCl<sub>2</sub>, K<sub>2</sub>CO<sub>3</sub>, Mg(NO<sub>3</sub>)<sub>2</sub>, NaBr, SrCl<sub>2</sub>, NaCl, KCl, BaCl<sub>2</sub>) were the 10 different salts recommended by COST-90 project, to cover a water activity range from 0.10 to 0.90. All salts used were analytical grade. Film samples were cut into pieces approximately 2 cm<sup>2</sup>. They were brought to zero moisture by freeze-drying during 24 h (Thermovac Industries Corp, USA). Samples were weighed and placed on a stainless steel lattice by holding it on a tripod inside the sorbostats that contain the saturated salt solutions and then the sorbostats were sealed. The sorbostats were kept inside an environmental chamber maintained at constant temperature. Film samples were equilibrated in the sorbostats for 4 days before their weights were recorded. The weight of the samples was checked during 3 days more. Equilibrium was judged to have been attained when the dif-

ference between two consecutive samples weighings was less than 1 mg/g dry solid. Results at equilibrium were reported for each relative humidity as gram water sorbed/100 g dry film. Absorption tests were done in triplicate at each  $a_w$ . The moisture sorption determination was done at 30 °C. Glycerol content was varied from 0% to 60% of starch weight.

### 2.5. Film opacity

Opacity of HACS films was determined according Gontard, Guilbert, and Cuq (1992) procedure and ASTM D 1003-00 “Standard test method for haze and luminous transmittance of transparent plastics” recommendations.

Films prepared with different alkaline treatment time (15–75 min) and different glycerol content (0–60%) were studied. Film samples were cut into a rectangle and placed on the internal side of a spectrophotometer cell. The absorbance spectrum (400–800 nm) was recorded for each sample using a Spectronic Unicam Genesys 10UV (USA) spectrophotometer. Film opacity was defined as the area under the recorded curve determined by an integration procedure. The opacity was expressed as Absorbance Units × nanometers (AU nm).

### 2.6. X-ray diffraction

X-ray diffraction spectra were carried out on a Rigaku Denki D-Max IIC diffractometer, using a Cu K<sub>α</sub> radiation ( $\lambda = 0.1542$  nm), 40 kV and 20 mA over an angular range 3–40° with step size 0.02. Films prepared with different alkaline treatment time ranged between 15 and 75 min were studied. Samples were previously conditioned at 50% RH and 25 °C. The area of the crystalline peak diffraction relative to the total area of the diffractogram was taken as a measure of the relative crystallinity.

## 3. Results and discussion

### 3.1. Rheological characterization of starch suspensions

Fig. 1 shows the effect of starch concentration (at constant temperature) and Fig. 3 the effect of temperature (at constant starch concentration) on rheological behavior of gelatinized HACS suspensions used as film forming gel to obtain films. As can be seen, gelatinized starch suspension is non-Newtonian. They present a rheological behavior that can be adequately described by Ostwald-de-Waele model. All experimental data fitted the model with correlation coefficients greater than 0.99 (Figs. 1 and 3). All samples presented pseudoplastic or shear-thinning behavior ( $n < 1$ ), as previously reported in starch gelatinized dispersions (Lagarrigue & Alvarez, 2001; Nurul et al., 1999; Simmons, Weigand, Albalak, Armstrong, & Thomas, 1993; Tako & Hizukuri, 2000). Flow index for all analyzed samples were calculated using non-linear regression and ranged between 0.46 and 0.79. These values

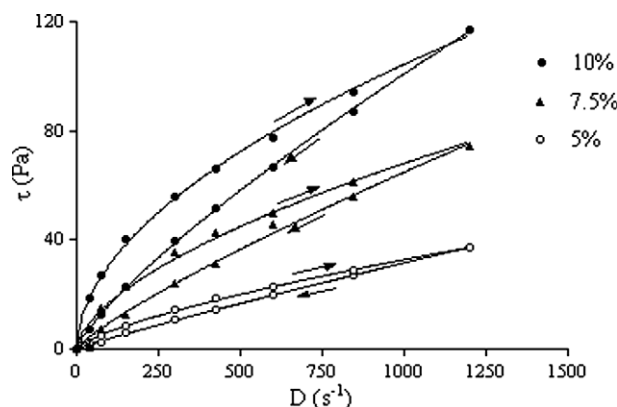


Fig. 1. Rheograms of HACS based film forming gels evaluated at 30 °C. Experimental data for: (▲) 5%, (○) 7.5% and (●) 10% of HACS concentration.

are within values mentioned in the literature. Marcotte, Taherian, Trigui, and Ramaswamy (2001) reported in their study on rheological properties of hydrocolloids,  $n$  values ranging between 0.51 and 0.65 for 4% starch gelatinized solutions in a temperature range 20–80 °C.

Samples showed a time-dependent flow. The hysteresis loops, typical of the suspending systems of macromolecules, were found to increase their area as starch concentration increased. As a result thixotropy increases with starch concentration. It can be caused by applied shear that break or deform hydrate granules or molecule aggregates. When suspension is subjected to an increasing deforming effect, there is a high resistance to deformation in the first stage of the process. At low shear rate values, time needed for structure relaxation and reorganization is greater than the rate of shear rate change. So, shear stress shows a significant increase. As shear rate increases, swollen starch granules break, molecular arrangements disintegrate and molecules take an extended configuration. Internal structure rearranges to support that stress decreasing their apparent viscosity. As a consequence, starch molecules mobility is greater and the relaxation structure time reduces. Finally, the reached structure remains in equilibrium with the imposed shear rate and apparent viscosity becomes almost constant. In returning curves, molecules maintain that structure and curves descend with practically constant slope (Figs. 1 and 3). Hysteresis loop area decreases as starch concentration decreases and temperature increases.

A decrease in flow behavior index with starch concentration was observed. As starch concentration is lowered very dilute solution will exhibit almost Newtonian behavior ( $n = 1$ ) while departure is noticed as soon as free water molecules become to interact with starch granules. The same behavior was detected in starch–methylcellulose film forming dispersions by Peressini et al. (2003).

The increase of apparent viscosity with starch concentration reflects the effects of individual starch molecules on dilute dispersion. As concentration increases, starch–

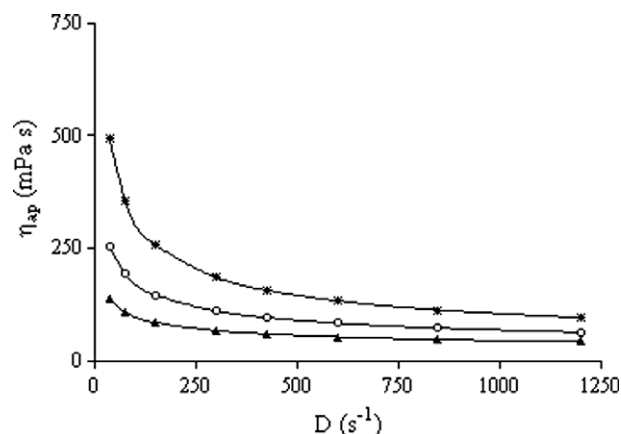


Fig. 2. Effect of HACS concentration on apparent viscosity of film forming gels. Experimental data for: (▲) 5%, (○) 7.5% and (●) 10% of HACS concentration.

starch interactions become dominant and a greater number of water molecules are immobilized, resulting in additional increase of apparent viscosity (Fig. 2). As previously stated, at high shear rate values, apparent viscosity reaches a constant value related to completion of structure reorganization. Marcotte et al. (2001) found apparent viscosity values, for 4% starch solutions, ranging between 58 and 200 mPa s, calculated at shear rate values between 100 and 300 s<sup>−1</sup> at 20 °C. Nurul et al. (1999), for 4% sago starch, found apparent viscosity values of 30–200 mPa s over a wide range of shear rate (13.6–704 s<sup>−1</sup>) at 40 °C. These results compares fairly well with our findings shown in Fig. 2. At 5% starch concentration the minimum appeared viscosity is about 40 mPa s and its maximum value is 75 mPa s at 30 °C.

The effect of temperature on rheological behavior of starch suspension is shown in Fig. 3. As temperature increases, starch molecules vibrate more vigorously, thus breaking the intermolecular hydrogen bond and allowing penetration of water. As a result, there is more free water

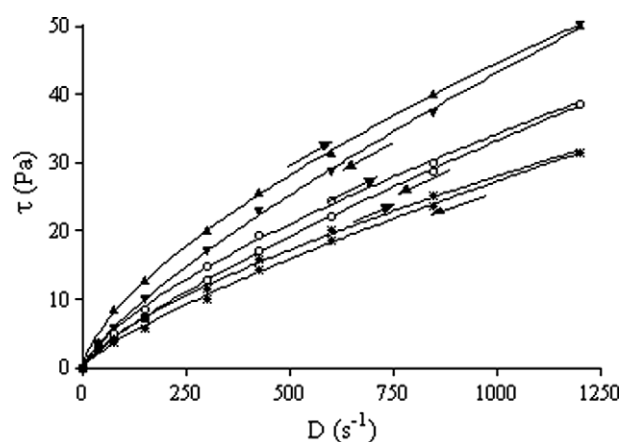


Fig. 3. Rheograms of 5% HACS based film forming gels evaluated at different temperatures. Experimental data for: (▲) 30, (○) 40 and (●) 50 °C.



available to facilitate the movement of particles in suspension yielding low shear stress values. At all temperature studied, no significant differences exists in flow index values. Apparent viscosity values were examined as a function of temperature and it was observed that apparent viscosity decrease with increasing temperature (Fig. 4). Apparent viscosity variation with temperature was correlated with an Arrhenius type law. Upon fitting the data into the model a significant relationship was found. Activation energies, frequency factors and correlation coefficients are shown in Table 1. Activation energies and frequency factors varied at different shear rates. It should be noted that a decrease in activation energy is associated with an increase in the shear rate. This is a consequence of the progressive alignment of the molecules with the shearing force. In general, higher activation energies are indicative of greater effect of temperature on viscosity. Activation energy values founded by Marcotte et al. (2001) for 4% starch solutions were 11, 10 and 9.9 kJ mol<sup>-1</sup> at 100, 200 and 300 s<sup>-1</sup>, respectively. Nurul et al. (1999) found activation energies for sago starch ranging from 3.07 to 7.35 kJ mol<sup>-1</sup>. They used starch concentrations ranging from 3.0% to 5.5% and shear rates between 13.6 and 704 s<sup>-1</sup>. Greater values of activation energy found in our study could be related to higher dissolution, amylose unfolding and charge repulsion produced by alkaline treatment.

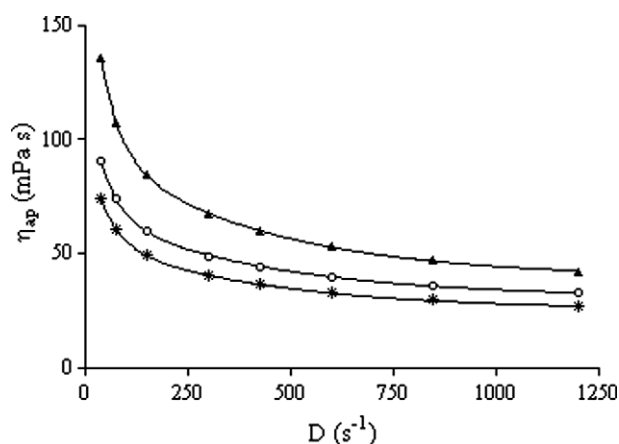


Fig. 4. Effect of temperature on apparent viscosity of film forming gels. Experimental data for: (▲) 30, (○) 40 and (\*) 50 °C.

Table 1  
Arrhenius parameters as a function of shear rate for 5% HACS suspensions

$D$ (s <sup>-1</sup> )	$A$ (mPa s)	$E_a$ (kJ mol <sup>-1</sup> )	$r^2$
150	12.107	22.248 ± 2.1	0.988
300	15.192	21.134 ± 1.8	0.992
450	18.079	20.344 ± 1.4	0.995
600	20.651	19.737 ± 1.1	0.997
750	22.641	19.305 ± 0.9	0.998
900	23.969	19.014 ± 0.7	0.998
1050	24.773	18.823 ± 0.6	0.999

### 3.2. Film solubility in water

Solubility in water is an important property of starch based films. Potential applications may require water insolubility to enhance product integrity and water resistance. However, in other cases, water solubility of the film before product consumption might be useful as in encapsulation of food or additives.

Solubility of HACS films in water depends on pretreatment time with NaOH solution before gelatinization. It was found that film solubility decreases with the increase in alkaline treatment time before gelatinization (Fig. 5). After approximately 60 min, solubility reaches the lowest value and it is not practically affected by further increase in treatment time. The amylose polymer is more flexible in alkaline solution than in neutral water. As pH increases, a progressive increase in negative charge on the polymer molecule (due to enhanced ionization degree) is expected which results in an expansion of the amylose coil from charge repulsion (Whistler et al., 1984). In a large measure, the crystallinity of the film is dependent upon the completeness of dissolution of starch and the extent of hydrogen bonding in the film. An extended configuration favors the formation of more dense and water resistant films.

Mehyar and Han (2004) found film solubility in water of 44.4% and 32.0% for rice starch and pea starch films, respectively. The glycerol concentration used in those experiments was 50% of starch weight. Besides, they found 1.5% solubility for both non-plasticized pure starch films. They concluded that glycerol contributes towards an increase in solubility of starch films. In our contribution, the glycerol concentration was 30%. Our results are in the same range of those obtained by Mehyar and Han (2004) and Peressini et al. (2004) in starch based films. The decline in film solubility observed in the same solvent (water) used during film elaboration, is a strong indication that some crystallization occurs as was confirmed in X-ray diffractograms.

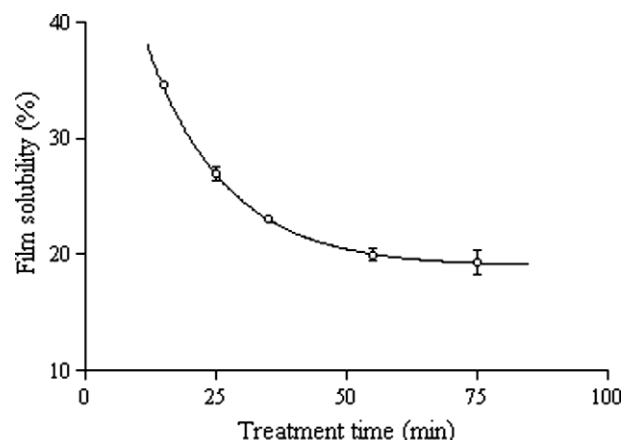


Fig. 5. Effect of alkaline treatment time on film solubility at 30 °C.

### 3.3. Water sorption isotherms (water solubility in film)

Experimental data of moisture sorption at 30 °C for high amylose corn starch films plasticized with different glycerol content, as a function of water activity are shown in Fig. 6. When the surrounding relative humidity raises film takes up water, which then plasticizes the amorphous areas of the starch network. Sorption data at all glycerol concentration studied was adequately fitted by GAB model ( $r^2 > 0.99$ ). GAB parameters for starch films prepared with different levels of glycerol content are shown in Table 2. Sorption curves are typical of water vapor sensitive polymers, and sorption levels are within the range reported for starch based films (Bader & Göritz, 1994a; Stading, Rindlav-Westling, & Gatenholm, 2001), cellulose films and protein based films (Chinnan & Park, 1995; Cuq et al., 1997; Gennadios & Weller, 1994; Velásquez, Torres, & Martín-Polo, 2001).

The effect of glycerol concentration on moisture content, at different relative humidity values, can be seen in Fig. 7. As glycerol concentration is increased a small drop on water sorption is first observed and a further increase produces

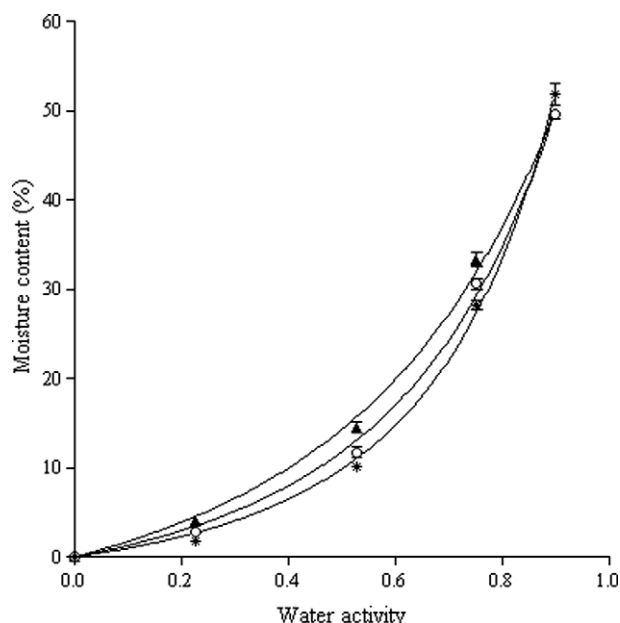


Fig. 6. Sorption isotherms of HACS films as a function of water activity at 30 °C. Experimental data for: (▲) 60%, (○) 40% and (\*) 20% of glycerol content.

Table 2  
GAB parameters for isotherms of HACS films for different percentage of glycerol

	0%	10%	20%	40%	60%
$w$	13.75	8.878	39.43	47.78	62.20
$c$	1.097	0.8822	0.3147	0.3912	0.4754
$k$	0.8707	0.9398	0.6851	0.6090	0.5245
$r^2$	0.9824	0.9954	0.9961	0.9955	0.9970

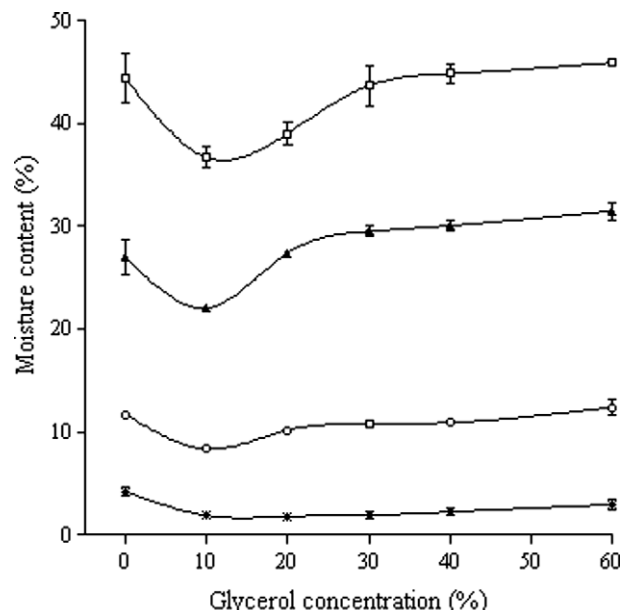


Fig. 7. Water uptake of plasticized HACS films as a function of plasticizer content, conditioned at: (□) 90%, (▲) 75%, (○) 53% and (\*) 22% relative humidity.

water sorption increase. At lower glycerol content, there is a competition between glycerol and water for hydrophilic sites on film surface. Glycerol present in film formulation reduces the amount of active sites available for water fixing and film moisture content declines. When glycerol content reaches 10–12% of starch, film moisture content increases. These results could be related to structural modifications of starch network produced by the plasticizer. The soft matrix favors water sorbed mobility within the structure. When the surface available for water sorption are saturated, an increase in glycerol content produces very slight water sorption increase for all surrounding relative humidity analyzed (glycerol content greater than 30%), probably due to the hydrophilic character of glycerol.

Other authors also reported this behavior of starch based films although no explanation was offered. Lourdin et al. (1997) reported the minimum film hydration between 10% and 15% of plasticizer content for a variety of substances studied for potato starch based films. Gaudin, Lourdin, Le Botlan, Ilari, and Colonna (1999) found the minimum water content value at 25% of sorbitol in wheat starch based films.

### 3.4. Film opacity

As shown in Fig. 8, opacity of HACS films depends on pretreatment time, with NaOH solution, before gelatinization. It was found that film opacity decreases with alkaline treatment time. It could be due that in alkaline medium starch molecules exhibit an extended configuration that favors the formation of crystalline zones within the film. The increase in crystalline zone decrease the absorbance and increase the film transparency. Similar observations

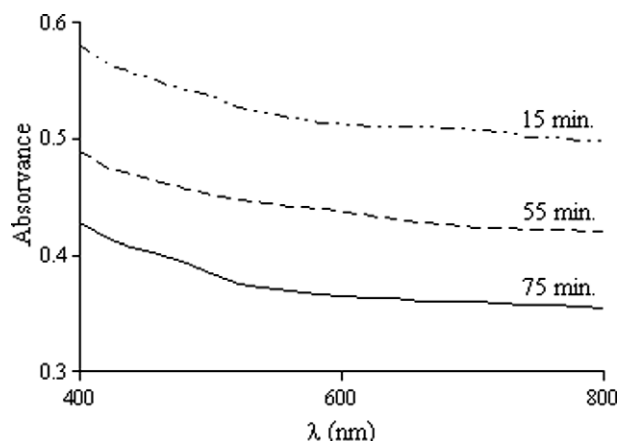


Fig. 8. Effect of alkaline treatment time on absorbance curves of HACs films.

(Gontard et al., 1992) were made with gluten proteins based films. Such conditions allowed sufficient unfolding and dispersion of the main gluten proteins in the film solution, resulting in a homogeneous and transparent gluten film.

Miles et al. (1985) explained that opacity development appears to be related to the irreversible phase separation into polymer-rich and polymer-deficient regions. The development of a phase-separated system and formation of a network structure of the gel are strongly correlated (Liu & Han, 2005). Crystallization, presumably within the polymer-rich phase, slowly develops with time.

Glycerol plasticization leads to a more flexible network and allows possibilities for rearrangements in the network structure. As was seen in Fig. 7, when glycerol content overcomes 15%, some structural changes occur and chain mobility increases. After 30% glycerol content and with further increase in the surrounding relative humidity, the network swell, intermolecular forces are reduced and starch matrix take up more water, therefore, films loose transparency and develop opacity.

As can be seen in Fig. 9, at lower glycerol content (<15%) films present high transparency. The glycerol con-

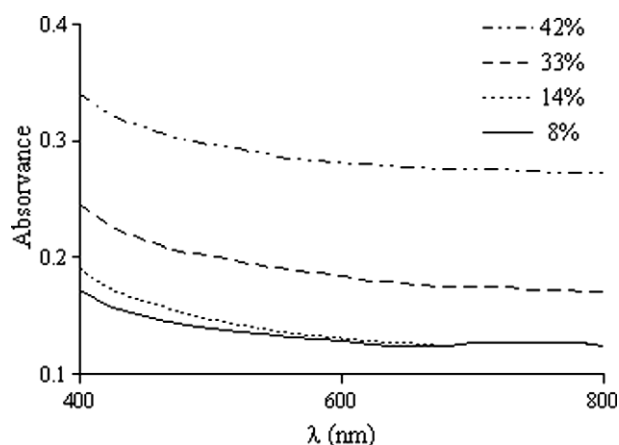


Fig. 9. Effect of glycerol content on absorbance curves of HACs films.

tent increase coincided with a marked large increase in the area under the curves showing an increase in film opacity. In agreement with the present findings, Shaw, Monahan, O'Riordan, and O'Sullivan (2002) also found an important increase in opacity when glycerol concentration ranged between 50% and 70% in weight of whey protein isolate.

### 3.5. X-ray diffraction

Starch based film crystallinity depends on grade of amylose dissolution and amount of hydrogen bond formed within the film (Bader & Göritz, 1994b; Chen & Jane, 1994; Liu & Han, 2005).

X-ray diffraction patterns of native HACs and HACs based film are shown in Fig. 10. They are characterized by sharp peaks associated with the crystalline diffraction and an amorphous zone mainly due to amylopectin. Lower peak intensity of films in comparison with HACs is due to in the film starch concentration is lower than in HACs powder.

Diffraction patterns recorded can be explained by the superposition of different kind of crystals: crystallites 'A' and 'B' formed by an aggregation of amylose chains with different double helical conformations and 'V' crystals formed by amylose-lipid complexes units. 'B' crystallographic patterns are characterized by greater interplanar space and high energy and 'A' type crystals are more compact, energetically more favorable and thermodynamically more stable. 'B' type structure is described as a more loosely packed hexagonal assembly of the helices capable of incorporate a great amount of water (Rindlav-Westling et al., 1997; Van Soest et al., 1996). At high temperature and moisture content the transition from 'B' to 'A' forms is favored (Bader & Göritz, 1994b). Crystallographic patterns of amylose present the higher intensity peaks at  $d$ -spacing 15.6, 5.2 and 3.7 Å.

In Fig. 10, diffraction patterns reveal that the original crystalline structure was slightly modified in the film elaboration process. In the crystallographic pattern of HACs

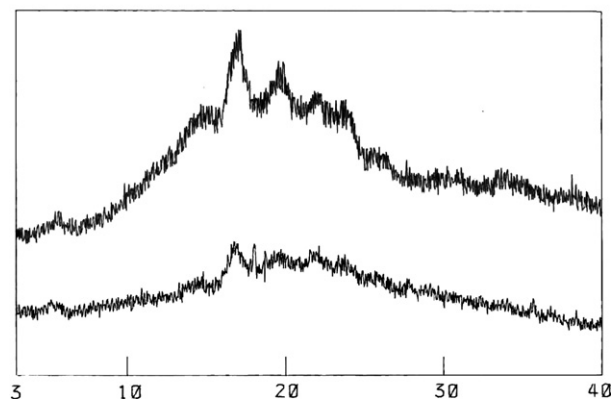


Fig. 10. X-ray diffractograms of HACs (up) and HACs based films (down).

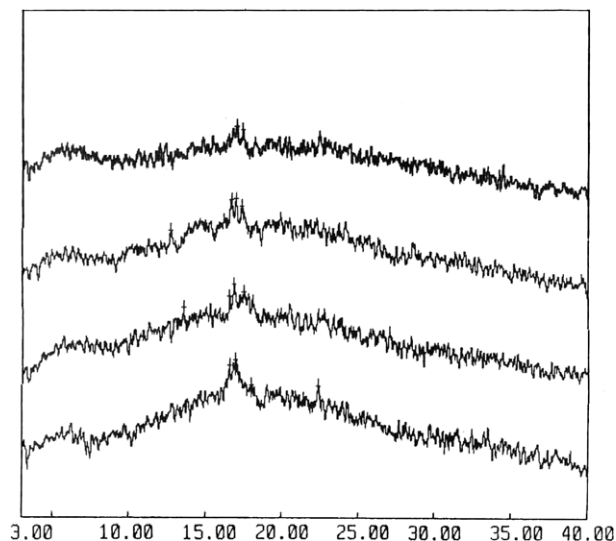


Fig. 11. Effect of alkaline treatment time on X-ray diffraction spectra of HACS based films (15 min, 30 min, 45 min and 60 min from up to down).

based film the amylose crystalline structure, characterized by the previously mentioned peaks, can be observed. Besides, new peaks, related to 'V' structure, appear ( $d$ -spacing 6.2, 4.9, 4.6 and 3.2 Å). Bader and Göritz (1994b) found similar patterns for films obtained by autoclaving HACS suspensions between 130 and 155 °C. Famá et al. (2005); Rindlav-Westling et al. (1998) and Van Soest et al. (1996) reported similar X-ray diffraction patterns for different starch based films.

Effect of alkaline treatment on film crystallinity was also studied. In Fig. 11 an increase in peak intensity and a slight decrease in peak width with treatment time previous to gelatinization can be observed. A tendency to increase crystallinity as time of alkaline treatment increases is shown. This effect is more noticeable by observing peak  $2\theta = 17^\circ$  ( $d = 5.2$  Å). These findings reinforce results discussed in film solubility and opacity tests.

Alkaline treatment produces swelling of the starch granules (Chen & Jane, 1994). When starch molecules are placed in an alkaline solution, protons of the –OH group are dissociated and leave negative charges on starch molecules. The repulsion between negative charges favors swelling of starch granules, which facilitates amylose lixiviation. Amylose chains lixiviated from the starch granule form a semicrystalline network, in which swelling granules are immersed. The final degree of crystallinity in the film depends on the ability of the chains to form crystals as well as on the mobility of the chains during the crystallization process. A long alkaline treatment time leads to higher mobility of the starch polymer chains.

Bader and Göritz (1994b) and Rindlav-Westling et al. (1997) showed that degree of crystallinity of starch films depends on temperature, air humidity and time required for film drying. A lower drying rate gives the chains a longer time to arrange into more favorable conformations,

for example in the form of crystals, and higher crystallinity is thereby achieved. Then, it could be concluded that crystallinity is related to chain mobility.

#### 4. Conclusions

In this work physical properties of HACS based films obtained by a low temperature gelatinization procedure were studied. This contribution provides detailed knowledge of the effect of formulation and methodology on the non-Newtonian behavior of film-forming dispersions and solubility, opacity and crystallinity of HACS films.

Rheological behavior of film forming gels was conveniently described by Ostwald-de-Waele model. All samples investigated exhibited pseudoplastic and thixotropic behavior. Apparent viscosity, flow index and thixotropy increase with HACS concentration while the effect of temperature was successfully modeled by Arrhenius law.

It was clearly shown that film crystallinity is highly related to chain mobility. Preparation procedures that enhance starch solubilization and chain mobility, will improve resulting film properties. Appropriate alkaline treatment applied before HACS gelatinization produces films with low solubility and opacity and high crystallinity. However, no further improvements are noticed after 60 min of alkaline treatment time.

Film glycerol content affects opacity and water sorption. Opacity decreases with decreasing glycerol content. We found a minimum film water sorption at about 10% of glycerol content, which suggests that a competing mechanism, between glycerol and water by hydrophilic sites on film surface, occurs. It was found glycerol concentrations higher than 30% lead to looser network as revealed by observed increasing levels of opacity and water sorption.

Alkaline treatment of HACS increases amylose dissolution, unfolding and charge repulsion in comparison with usual autoclaved gelatinization. Activation energies of apparent viscosity of film forming suspension clearly support these findings.

These results may lead to the optimization of technological conditions for any given application of starch based films.

#### Acknowledgements

Authors are grateful to Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) for financing PICT No. 14–11700 and Facultad de Ingeniería and Consejo de Investigación de la Universidad Nacional de Salta for additional support.

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