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Study of gelatinization process and viscoelastic properties of cassava starch: Effect of sodium hydroxide and ethylene glycol diacrylate as cross-linking agent

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Abstract

The gelatinization of cassava starch was studied by means of differential scanning calorimetry (DSC) and dynamic rheological measurements. The influence of NaOH in the concentration range (2×10^{-3} M < [NaOH] < 0.5 M, corresponding to $0.2\% < [m_{\mathrm{NaOH}}/m_{\mathrm{starch}}] < 7.5\%$) on the gelatinization was investigated. Similar experiments were performed with ethylene glycol diacrylate acting as a cross-linker during gelatinization. The cassava starch was characterized by DSC measurements and static and dynamic light scattering. The influence of starch and NaOH concentrations on the gelatinization temperature, as well as the overshoot of the moduli values accompanying the phenomenon, were studied. An unusual shift in the gelatinization temperature was observed at an [NaOH] concentration of around 0.01 M. As far as the study in presence of cross-linker is concerned, we focused on the effect of the cross-linker/starch ratio on the dynamic viscoelastic properties of the "gel" obtained at a relatively high temperature, 85 °C.

Keywords: Starch; Viscoelastic properties; Gel

1. Introduction

Starch represents an important part of many agricultural products like legumes (bean, pea, and faba), cereals (wheat, corn, and rice) and tubers (potato and tapioca). It is composed of two polysaccharides, amylose and amylopectin. Both polysaccharides are based on 1 → 4 linked α-D-glucose chains. The amylose is essentially linear whereas the structure of amylopectin is highly branched, in the 1 → 4 → 6 positions. The branching has been found, however, to be nonrandom (Manners, 1989; Thompson, 2000). The proportion of amylose and amylopectin depends on the origin of the starch. Most starch contains 20–25% w/w of amylose, although amylose is practically nonexistent (<1% w/w) in some waxy maize starches. A blue complex is formed between linear regions of amylose chains and polyiodide ions in aqueous solutions allowing the determination of amylose content. Complexes between amylose and phospholipids can also be used to evaluate the amylose content (Mestres, Matencio, Pons, Myriam, & Fliedel, 1996). Starch in water at room temperature consists of granules suspensions which are birefringent (Buleon, Colonna, Planchot, & Ball, 1998) and crystalline to a certain extent (~30%). Two principal crystalline forms have been reported: monoclinic, found in most cereal starches, and hexagonal found in high amylose starches (Imberty & Perez, 1988; Wu & Sarko, 1978). Amylopectin is the predominant crystalline structure. Several studies have been carried out on the granular organization (distribution of A and B regions inside granules, helical

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or superhelical structure) which are summarized in recent reviews (Parker & Ring, 2001; Tester, Karkalas, & Qi, 2004).

The use of starch for industrial food applications has been described in the literature. Starches are commonly used to thicken liquid foods, such as soups and sauces. Most often the functional properties of starch are obtained by gelatinization, which consists of heating a suspension of granules above a characteristic temperature, causing the starch granule to swell irreversibly. The main reason for the swelling is the expansion of the amorphous regions in the granules leading to crystalline disruption, thereby enhancing the possible interaction of the granules with water, which results in swelling (Donovan, 1979). During the swelling, the linear amylose molecules diffuse out of the swollen granules and are preferentially solubilized. The final state can be viewed as a continuous phase of amylose in which more or less overlapped swollen granules, enriched with amylopectin, are suspended (Doublier, 1981; Hermansson & Svegmark, 1996; Morris, 1990). The solubilization of amylopectin is generally not observed but in some cases the solubilization of low molecular weight chains has been reported (Mizukami, Takeda, & Hizukuri, 1999).

The swelling of the granules leads to an increase of the starch granules volume fraction and consequently increases the viscosity of the medium. Above a certain swelling, “close packing” of the granules occurs (Evans & Haisman, 1979), leading to a rapid increase in, with a subsequent overshoot of, the storage and loss dynamic moduli (G' and G'', respectively). A further increase in the temperature leads to a slight decrease in both moduli before they reach steady values (Cervone & Harper, 1978). This complex behavior has been the subject of numerous rheological studies (Aguilera & Rojas, 1997; Doublier, 1981, 1987; Gonzalez-Reyes, Mendez-Montealvo, Solorza-Feria, Toro-Vazquez, & Bello-Perez, 2003; Kasapis, Sablani, & Biliaderis, 2000; Lagarrigue & Alvarez, 2001; Peressini, Bravin, Lapasin, Rizzotti, & Sensidoni, 2003; Rosalina & Bhattacharya, 2002) and has also been approached using other useful techniques like: small-angle X-ray scattering (SAXS), wide-angle X-ray scattering (WAXS), nuclear magnetic resonance spectroscopy (NMR) and differential scanning calorimetry (DSC) (Jenkins & Donald, 1998; Perry & Donald, 2002; Pielichowski, Tomasik, & Sikora, 1998; Tang, Brun, & Hills, 2001; Wootton & Bamunuarachchi, 1979).

The phenomenon of gelatinization is very complex and several models have been proposed to explain this behavior, which are summarized in Jenkins and Donald, 1998. A useful review on this aspect is very well documented in Parker and Ring (2001) and Tester et al. (2004).

Addition of different solutes, such as sugars, salts, acids and bases has been used to modify the gelatinization temperature (Chiotelli, Pilosio, & Le Meste, 2002; Roberts & Cameron, 2002; Sandstedt, Kempf, & Abbott, 1960; Wootton & Bamunuarachchi, 1979) that can be very useful regarding the baking process. Salts can be used, for instance, to increase the gelatinization temperature and preserve the granule structure, whereas alkalis are often used to decrease the swelling temperature in products for non-food application, such as adhesives.

Another phenomenon called “retrogradation” consists of a progressive aggregation of the amylose chains when the temperature is decreased after gelatinization. This phenomenon has an important impact on the rheological and textural properties of the paste and consequently has been the subject of numerous studies (Doublier & Choplin, 1989; Gidley, 1989; Miles, Morris, Orford, & Ring, 1985; Miles, Morris, & Ring, 1985).

Modification of starch has been employed in order to change the granular structure of starch and thus modify the final rheological properties of the paste after gelatinization (Bertolini, Mestres, Colonna, & Raffi, 2001; Lee, Kumar, Rozman, & Azemi, 2004). In these modification techniques, chemical cross-linking of starch is very often used, especially in food applications (Chatakanonda, Varavinit, & Chinachoti, 2000; Delville, Joly, Dole, & Bliard, 2002, 2003; Le Bail, Morin, & Marchessault, 1999; Nayouf, Loisel, & Doublier, 2003; Simkovic, 1996). In this respect, the cross-linking effect has been shown to limit the retrogradation process (Delville, Joly, Dole, & Bliard, 2003).

In this study, Brazilian cassava starch was investigated. One of the reasons for choosing this starch is its low cost, due to the large production in Brazil. Latin America produces an estimated 21% of the total world annual production of cassava (Balagopalan, Padmaja, Nanda, & Moorty, 1988). Therefore, there is a great interest to provide more information about the behavior of this starch, and compare its properties to other starches (potato, maize, and rice). Moreover, previous studies have shown that the films formed with the addition of a cross-linking agent present new properties, such as lower solubility and higher thermal stability (Chatakanonda et al., 2000; Delville et al., 2003; Yoneya, Ishibashi, Hironaka, & Yamamoto, 2003). In this study, we focus on the gelatinization of cassava starch in the presence of NaOH essentially through rheological experiments but also by DSC. Viscoelastic properties of gels formed in the presence of ethylene glycol diacrylate cross-linker were also studied. In addition, an attempt was made to evaluate molecular characteristics of the gelatinized starch: molecular weight (M_W), second virial coefficient (A_2), gyration (R_G), and hydrodynamic radius (R_H) using light scattering techniques (static and dynamic).

2. Materials and sample preparation

The cassava starch was kindly provided by POLIAMIDOS (Curitiba, Brazil). The moisture content was 13%. The proportions of amylose (16% w/w) and amylopectin (84% w/w) were determined by CIRAD (France), according to a method described in Mestres et al., 1996.

The NaOH was purchased from Aldrich, and was used in the preparation of the solution with distilled water in the starch dispersions. The starch dispersions were prepared in NaOH solution in a concentration range of 0.002–0.5 M. Depending on the concentration of starch used, the NaOH/starch ratio (percentage mass) ranged from 0.16% to 7.4% (w/w). The dispersions were prepared at room temperature by addition of dry powder to milliQ water or NaOH solution.

The cross-linking agent used, ethylene glycol diacrylate (EG), was purchased from Aldrich. The concentration was from 8.3×10^{-6} to 1×10^{-4} M, corresponding to a percentage of 0.56–5.6% (w/w) depending on the starch concentration. As an initiator, 4,4-azobis-(4-cyanovaleric acid) at 8.2×10^{-5} M was used, which was purchased from Aldrich. All the dispersions were prepared by moderate agitation (magnetic stirrer).

Distilled water, 0.4 M NaOH and DMSO/H_2O (90/10 v/v %) were used as solvents for the cassava starch. Two water treatments were carried out: 1 h at 80 °C, referred to as water-A, and 72 h at 80 °C, referred to as water-B. Both were heated with moderated stirring in an oil bath and after were stirred overnight at room temperature. A solution of 0.4 M NaOH was chosen because it was found that this concentration gave clear solutions. The solution was heated with stirring for 1 h at 80 °C, referred to as NaOH-A, in an oil bath and another solution was heated for 12 h with stirring (NaOH-B). For the 90% DMSO one solution was stirred for 12 h (DMSO-A) and another one for 72 h at 66 °C (DMSO-B).

Dilution series were made up at room temperature, yielding five concentrations in a range of 0.010–0.2 mg/ml. The 90% DMSO and 0.4 M NaOH solutions were filtered through 0.22 μm PTFE filters and 0.45 μm acetate filters, respectively.

3. Methods

3.1. Light scattering

The light scattering measurements were performed on a multi-angle laser light scattering ALV-5000 correlator. The experiments were performed at 25 °C within an angular range of 40°–140°, in steps of 10°, in homodyne mode, with a vertically polarized laser of wavelength 632.8 nm with toluene as the calibration standard. The data were collected and processed using the software ALV-5000 and the M_W, R_G, and A_2 then determined.

The refractive index increment for the cassava starch in 0.4 M NaOH solutions was determined experimentally as 0.116 ml/mg. For the water solutions, the refractive index increment was taken from the literature as 0.146 ml/mg (Bello-Perez, Colonna, Roger, & Parees-López, 1998). A refractive index increment of 0.074 ml/g was used for the 90% DMSO solutions (Han & Lim, 2004a).

The dynamic light scattering (DLS) measurements were made with a ALV-5000 correlator. The diffusion coefficient D was calculated from the first-order cumulant and contin analysis of the time decay of the correlation function measured at different scattering angles. The hydrodynamic radius was then calculated from the Stokes–Einstein relation (Eq. (1)), where kT is the Boltzman energy and η is the viscosity of the medium.

$$R_H = \frac{kT}{(6\pi\eta D)}. \tag{1}$$

3.2. Rheological measurements

Experiments were performed on a stress controlled rotational rheometer (Haake RS 300) equipped for most of the experiments with a 35 mm diameter, 2° angle cone and plate geometry. The temperature was controlled by water circulation via a Phoenix model cryostat. Stress sweep experiments at low frequencies (0.1 rad/s) were performed to determine the linear domain.

The viscoelastic properties of starch dispersions were analyzed at small amplitude oscillations as a function of temperature from 40 to 85 °C at a rate of 4 °C/min. A solvent trap was used to prevent evaporation and the edge of each sample was coated with a silicone oil of low viscosity. The frequency dependence analysis of storage (G') and loss (G'') moduli were measured at a temperature of 85 °C within the range 100.0–0.1 rad/s.

3.3. DSC measurements

The gelatinization temperatures were determined by DSC measurements in a Shimadzu 50 differential scanning calorimeter. Hermetic pans with 5% starch dispersion in pure water and in alkali media ([NaOH] = 0.01 M) were heated from room temperature to 100 °C at 10 °C min^{-1} under nitrogen atmosphere. The values reported are the mean of, at least, two determinations.

4. Results and discussion

4.1. Light scattering characterization

In this study, we evaluated the average mass molecular weight (M_W), A_2, R_G, and R_H of a gelatinized starch. As reported in a recent review (Parker & Ring, 2001), the literature has shown that the average mass molecular weights of amylose and amylopectin are around 10^5–10^6 and 10^8 g mol^{-1}, respectively. Accurate characterization of amylose and amylopectin in starch by light scattering is known to be extremely difficult. For this reason, starch granules need to be completely destructured without breaking the macromolecule structure (Roger, Bello-Perez, & Colonna, 1999). This is difficult, however, since the gelatinization process leads to incomplete solubilization of amylose and, to a greater extent, amylopectin. Moreover, the presence of large aggregates in the medium is common and makes scattering experiments difficult to carry out and interpret properly. The quality of the solvent and the effectiveness of the procedure for the starch granule dissolution have a great influence on the solubilization and consequently on the results obtained using light scattering. Depending on the preparation conditions for starch solutions, the rather stable native structures are often not fully broken up but still contain fragments of the supramolecular structure. Because of this complex granule structure, it is difficult to find the optimal dissolution conditions (Blennow, Bay-Smidt, & Bauer, 2001). Very strong alkaline media ($[\text{NaOH}] \geqslant 1$ M) (Blennow et al., 2001; Han & Lim, 2004b) as well as vortexing techniques (Han & Lim, 2004b) have been used to obtain almost completely solubilized starch. However, the alkaline system can hydrolyse the starch molecule, which can lead to incorrect interpretation of the results. Autoclaving has been used to solubilize several starches (Aberle, Burchard, Vorweg, & Radosta, 1994; Hanselmann, Ehrat, & Widmer, 1995; You & Lim, 2000), but under certain conditions an insoluble part still remains. DMSO (90/10) in water gives reproducible results (complete dissolution) but a boiling process and long dissolution time are required (Han & Lim, 2004a, 2004b). Using DMSO and microwave techniques, complete solubilization can be achieved (Bello-Perez et al., 1998; Bello-Perez, Roger, Baud, & Colonna, 1998; Roger et al., 1999) which allows researchers to evaluate the contribution of amylose and amylopectin to the light scattering (Roger et al., 1999).

Table 1 summarizes the results obtained in this study as well as others reported in the literature. It should be noted that the contents of solubilized amylopectin and amylose were not determined. It was found that the condition of treatment affected the results obtained in terms of the molecular parameters obtained.

The static light scattering showed that the average molecular weight, M_W, obtained using water as a solvent was lower than when 0.4 M NaOH and 90% DMSO were used as solvents. For the two systems using water (Water-A and Water-B) significant differences in the M_W values were not observed. This is surprising since NaOH and DMSO are better solvents than water. If aggregation in water occurs, a higher apparent molecular weight should be obtained. However, a decrease in the gyration radius, R_G, and hydrodynamic radius, R_H, values, obtained from DLS, was observed when the systems were kept at 80 °C for longer periods. This means that aggregates can be destroyed in this way. The M_W values increased for the NaOH-B treatment. On the other hand, the linear amylose chains, which are preferentially solubilized, can tend to associate with each other and form aggregates, and this association can result in an increase in the M_W values. Alkaline and aqueous solutions are reported in the literature as systems where starch solutions are not stable and structural changes can occur (Han & Lim, 2004a; Radosta, Haberer, & Vorwerg, 2001).

Experiments performed in Water, NaOH, or DMSO clearly confirmed the dispersion of results in terms of molecular parameters (R_G, M_W, or A_2) commonly observed in the literature, essentially due to incomplete solubilization. It would be interesting to try autoclaving or microwave techniques to achieve solubilization and see the difference in the results obtained.

4.2. Gelatinization process, effect of starch concentration

The structural change during the starch gelatinization which was monitored by the storage (G') and loss (G'') moduli behavior is illustrated in Fig. 1. For a better representation, only the loss modulus as a function of temperature is shown. The behavior of the storage modulus is exactly the same. The slight decrease in the moduli observed at the beginning is due to the decrease in viscosity of the medium when the temperature increases. Gelatinization then occurs, leading to a dramatic increase in the moduli, reaching a maximum before slightly decreasing when the temperature increases further. The temperature was taken at the inflexion point when the G' and G'' values increase very quickly until reaching the maximum. During the rapid increase of both moduli, G' becomes greater than G''. The gelatinization temperature decreases with starch concentration, in good agreement with the literature. The maximum values attained for G' and G'' also increase with the starch concentration. The gelatinization temperature shifts from 57 to 62 °C for dispersions of 15% and 2%, respectively. It has been proposed in the literature that a rapid increase in the moduli values is linked to the overlap of swollen granules, (close packing of the granule) increasing the elastic response and friction (Evans & Haisman, 1979). Therefore, by increasing the volume fraction and thus reducing the interparticle distance, the rheological signature is more rapidly detected, once the swelling of the granules has started. However, it should be mentioned that no significant influence of the concentration on the temperature of the moduli overshoot was detected, unlike the case of Roberts and Cameron, 2002.

The decrease in the storage and loss moduli values after the overshoot, is attributed to the progressive weakening of the starch granules and dynamic acceleration of the gelled amylose matrix (released from granules during gelatinization). We have tried to quantify this overshoot through the following expressions:

$$\Delta x = (G'_{\max} - G'_{80})/G'_{\max}, \tag{2}$$

$$\Delta x = (G''_{\max} - G''_{80})/G''_{\max}, \tag{3}$$

where $G'_{\max}$ and $G''_{\max}$ are, respectively, the overshoot values, and G'_{80} and G''_{80} are the values for the moduli at 80 °C. With such a normalization, the results show that the amplitude of the overshoot was the same across the whole concentration range studied.

4.3. Gelatinization process, effect of NaOH concentration

NaOH is an alkali that is commonly used to modify the gelatinization process of starch. Most studies in the literature have shown the gelatinization effect of NaOH in the

Table 1
Molecular parameters for cassava starch obtained in this study from light scattering techniques along with some results reported in the literature for several starch sources

Sample	Solvent	Solubilization extent (%)	M_W (g mol^{-1})	R_G (nm)	R_H (nm)	A_2 (mol cm^3 g^{-2})	Reference
Water A	Water		1.3×10^6	91	60	-2.5×10^{-3}	This study
Water B	Water		5.4×10^6	53	50	-7.0×10^{-5}	This study
Amylopectin[b]	Water	88.6	2.7×10^8	259	201		Roger et al. (1999)
Corn starch[b]	Water	94.7	2.5×10^8	267	201		Roger et al. (1999)
Corn starch (high amylose)[b]	Water	97.2	3.1×10^7	172	127		Roger et al. (1999)
Amylose–amylopectin (50–50)%	Water	97.5	2.3×10^7	187	139		Roger et al. (1999)
NaOH A	0.4 M NaOH		3.8×10^7	166	70	-3.6×10^{-3}	This study
NaOH A	0.4 M NaOH		2.7×10^8	176	86	-1.9×10^{-3}	This study
Cassava starch[a]	2 M NaOH			170–220			Blennow et al. (2001)
Amylopectin region	2 M NaOH		1.0×10^9				Blennow et al. (2001)
Amylose region	2 M NaOH		1.0×10^8				Blennow et al. (2001)
DMSO A	90% DMSO		7.5×10^7	172	139	-1.1×10^{-5}	This study
DMSO B	90% DMSO		1.1×10^7	114	77	-1.4×10^{-4}	This study
Waxy maize Amylopectin[b]	90% DMSO		$(564 \pm 20) \times 10^6$	349 ± 6	348		Millard, Wolf, Dintzis and Willett (1999)
Waxy maize Amylopectin[b]	90% DMSO		1.01×10^9	460 ± 20	348		Chen and Wyat (1999)
Corn starch[a]	90% DMSO	95.1 ± 0.5					Han and Lim (2004a)
Amylopectin region	90% DMSO		$(155 \pm 1.2) \times 10^6$	233 ± 0.3			Han and Lim (2004a)
Amylose region	90% DMSO		$(2.80 \pm 2.3) \times 10^6$	132 ± 1.8			Han and Lim (2004a)

[a] Molecular parameters obtained by size exclusion chromatography.

[b] Molecular parameters obtained by light scattering.

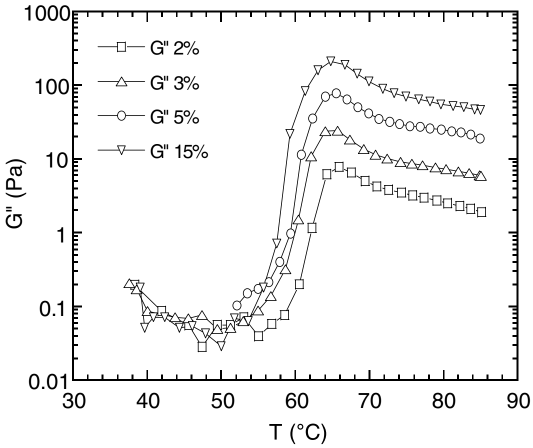

Fig. 1. Starch dispersions % (w/v) in distilled water. Evolution of loss modulus (G'') as a function of temperature.

starch dispersions for a concentration higher than 0.13 M, and a [NaOH]/[starch] ratio of around 5/95% in w/w (Ragheb, El-Thalouth, & Tawfik, 1995; Roberts & Cameron, 2002). Recently, it has been shown that the gelatinization of potato starch in the presence of NaOH is controlled by the [NaOH]/[starch] ratio in % w/w. In the chosen NaOH concentration range, a decrease in the gelatinization temperature was observed. This is explained by the disruption of intra- and inter-hydrogen bonding between amylose or amylopectin and NaOH (Ragheb et al., 1995; Roberts & Cameron, 2002; Wootton & Ho, 1989).

In this study, the NaOH concentration range was 2×10^{-3}–5×10^{-1} M corresponding to a NaOH/starch ratio of 0.2:99.8 or 7.4:92.6 depending on the starch concentration chosen. Gelatinization was studied through dynamic measurements and the results are illustrated in Fig. 2. For clarity, not all of the studied NaOH concentrations are shown. At high NaOH concentrations (i.e., $\geqslant$0.05 M) the classical behavior is observed, consisting of a lower gelatinization temperature and a decrease in the amplitude of the gelatinization peak, due to the swelling of the granules before the system is heated.

However, for [NaOH] $\leqslant$ 0.02 M, an increase in the gelatinization temperature is observed. The shift observed for 0.002 M is too weak to be meaningful, but this is not the case for other concentrations. The maximum shift obtained is about 5 °C. We have tried to quantify the effect of NaOH on the gelatinization temperature as well as the overshoot amplitude (Eqs. (1) and (2)) considering the NaOH/starch mass ratio described by Roberts and Cameron, 2002. The results are illustrated in Figs. 3 and 4. The increase in the gelatinization temperature reaches a maximum at around 1.5%. For a 15% starch concentration, the increase is slightly less pronounced and the decrease after the

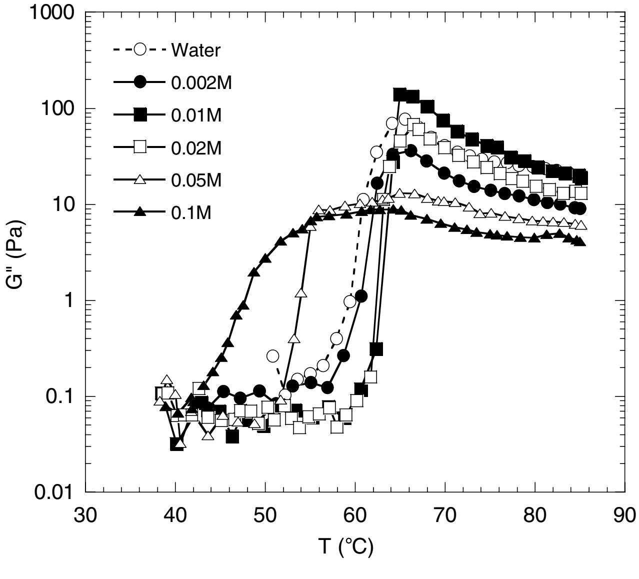

Fig. 2. Evolution of the loss moduli (G'') as a function of temperature at different NaOH concentrations in the 5% (w/w) dispersion of starch.

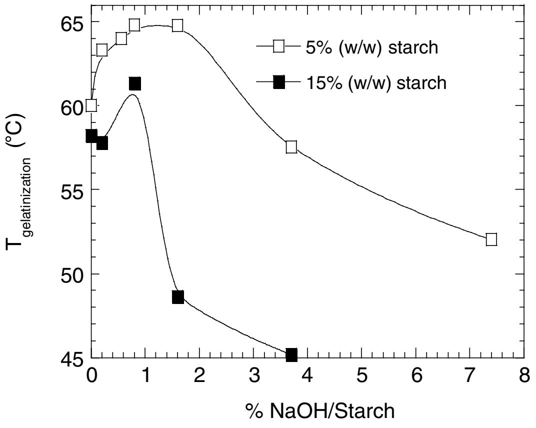

Fig. 3. Displacement of the gelatinization temperature for two starch dispersions versus NaOH/starch ratio.

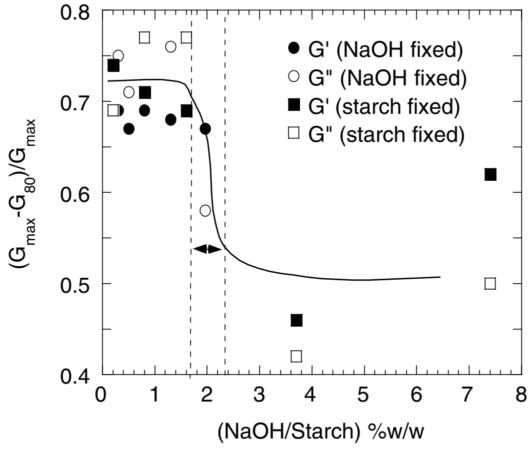

Fig. 4. Evolution of the overshoot of moduli during gelatinization with NaOH/starch ratio. Some of the results were obtained by varying the concentration of starch suspension while keeping [NaOH] at the same value (○,●), the others represents the opposite situation (□,■).

"maximum" zone is more pronounced. This is probably due to a higher pH (more [NaOH] needed) for the same NaOH/starch ratio, leading to hydrogen bonding disruption and swelling. A 2% w/w starch suspension was also studied in 0.01 M NaOH, corresponding to a NaOH/starch ratio of 4%, which resulted in an 8 °C higher gelatinization temperature. The overshoot of the moduli seems to be controlled also by the NaOH/starch ratio as shown in Fig. 4. Below a critical NaOH/starch ratio ~2%, the phenomenon of "close packing" of the granules followed by their weakening and disruption is not affected by NaOH. For ratios above 2%, swelling has already occurred to a certain extent before the heating procedure.

In the baking process, the modification of the gelatinization properties of starch by salt is commonly used and numerous studies have provided information on this aspect (Chiotelli et al., 2002; Ragheb et al., 1995; Sandstedt et al., 1960). An increase or decrease in the gelatinization temperature has been observed and interpreted in terms of "salting out" or "salting in", an effect which is related to the position of the salt in the Hofmeister Series (Kunz, Henle, & Ninham, 2004). However, it has been reported that NaCl increases the initial gelatinization temperature within a certain concentration range, with the opposite behavior being observed at higher concentrations (Chiotelli et al., 2002; Sandstedt et al., 1960; Wootton & Bamunuarachchi, 1979). In this study, we observed the same behavior for NaOH. It has been proposed that this complex behavior could be due to the antagonist effect of the salt on the water structure and on the starch (Chiotelli et al., 2002). Starch dispersions in the presence of 0.01 M NaCl present the same gelatinization temperature as the dispersion in the pure aqueous system (results not shown), which is in agreement with the literature for such low concentrations. However, using another alkali, such as KOH at [0.01 M], also leads to an increase in gelatinization temperature (4–5 °C) (not shown here).

In order to gain a greater insight into this phenomenon, we performed dialysis experiments. A starch dispersion at 5% in 0.01 M NaOH, having a pH close to 11.6, was dialyzed for 3 days, which resulted in a suspension at pH 6.0, slightly higher than the pH of water, 5.90. These samples showed the same behavior as those that were not dialyzed. In others words, the increase in gelatinization temperature was still present, although slightly less pronounced (3–4° in comparison to 4–5 °C). This could be an indication that specific interactions between Na$^+$ or K$^+$ and hydroxyl groups of the chains, are created initially and still persist after dialysis.

4.4. DSC measurements

To confirm those results and obtain more information about the shift in gelatinization temperature, DSC experiments were carried out, as described in the experimental part. The main thermal event registered was an endothermic peak at 64 °C with the onset at 57 °C (Fig. 5) for the 5% starch dispersion in pure water. For the alkali media ([NaOH] = 0.01 M), the results for the DSC measurements show a shift in the endothermic peak, it now being at 69 °C (two samples were studied and the values were 67.7 and 70.0 °C corresponding to an average value of 69 °C) with the onset at 61 °C. Moreover, it is clear that in this case the gelatinization process requires more energy in such an alkali media, as the enthalpy is reported to be −144.39 mJ/g in water and −249.08 mJ/g in 0.01 M NaOH.

Addition of alkali at very low starch concentrations increases the pH of the suspension (above 10). A subsequent increase in the amount of hydroxylate facilitates the starch interaction with the cations of the alkali used. Each cation interacts with the hydroxylate of different chains of amylose (or amylopectine) and may also alter the hydration of the glucose unit rendering the gelatinization more difficult to obtain, as shown in the rheological experiments (higher temperature) and DSC measurements (higher temperature and gelatinization enthalpy). Considering the results shown in Fig. 3, it seems that these interactions could be controlled via the NaOH/starch ratio. However, the amplitude of the shift is greatly affected by the initial starch suspension concentration. In other words, these results suggest that this phenomenon would not be observed for a high starch concentration and a NaOH concentration of [0.01 M]. However, the gelatinization peak, and thus the phenomenon of close packing of the granules, is controlled by the NaOH/starch ratio. The critical ratio is around 2%, which corresponds to the ratio where the contribution of NaOH to gelatinization becomes notable, when thermal gelatinization is necessary. For potato starch, a critical NaOH/starch ratio of 5/95, corresponding to the point of full gelatinization of starch by NaOH, has been reported (Roberts & Cameron, 2002).

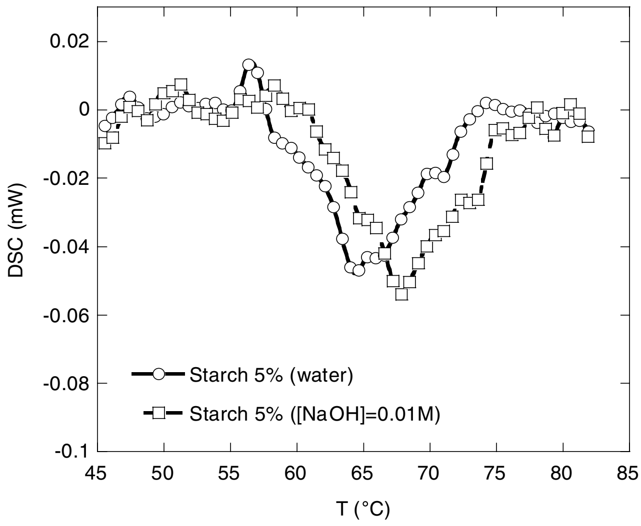

Fig. 5. DSC endotherm for cassava starch in pure water (○) and in the presence of 0.01 M NaOH (□).

4.5. Effect of ethylene glycol diacrylate as the cross-linking agent

It is known from the literature that the chemical modification of starch can improve its resistance to temperature or mechanical treatments. However, granules of native starch can be easily damaged during the baking process, thus having a negative impact on the rheological properties. Numerous studies have focused on the chemical modification of starch (Athawale & Lele, 1998; Delval, Crini, Janus, Vebrel, & Morcellet, 2001; Fang, Fowler, Sayers, & Williams, 2004; Kiatkamjornwong, Thakeow, & Sonsuk, 2001; Lawal, 2004; Mitchell & Hill, 1995; Simkovic, 1996; Wu, Wei, Lin, & Lin, 2003) and its effect on the starch properties. Starch cross-linking, in particular, is an approach which has been widely used (Chatakanonda et al., 2000; Delval et al., 2001; Le Bail et al., 1999; Lenaerts et al., 1998; Nayouf et al., 2003; Ravenelle, Marchessault, Legare, & Buschmann, 2002; Simkovic, Hricovini, Mendichi, & van Soest, 2004; Tecante & Doublier, 1999; Yoneya et al., 2003). Cross-linking can improve the gel state properties, and limit the retrogradation phenomenon. The mechanical resistance necessary for products with application in packaging, for example, can also be improved.

Today, research in this field is pushing towards reducing the unfavorable properties of polysaccharides and increasing their use in industrial applications (Arvanitoyannis, Psomiadou, & Nakayama, 1996; Debeaufort, Quezada-Gallo, & Voilley, 1998; Park, 1999; Petersen et al., 1999).

We have studied the effect of ethylene glycol diacrylate (EG) as a cross-linker, on the dynamic rheological properties of the gel formed by cassava starch. This cross-linker has not previously been used for cassava starch and good mechanical properties and biodegradability of the film formed from the gel obtained with this cross-linker have been obtained with this system. There is a great interest in obtaining information about the starch structure formed in solution. The behavior at 85 °C of gels formed from a 5% w/w dispersion of starch by increasing the concentration of EG is illustrated in Fig. 6.

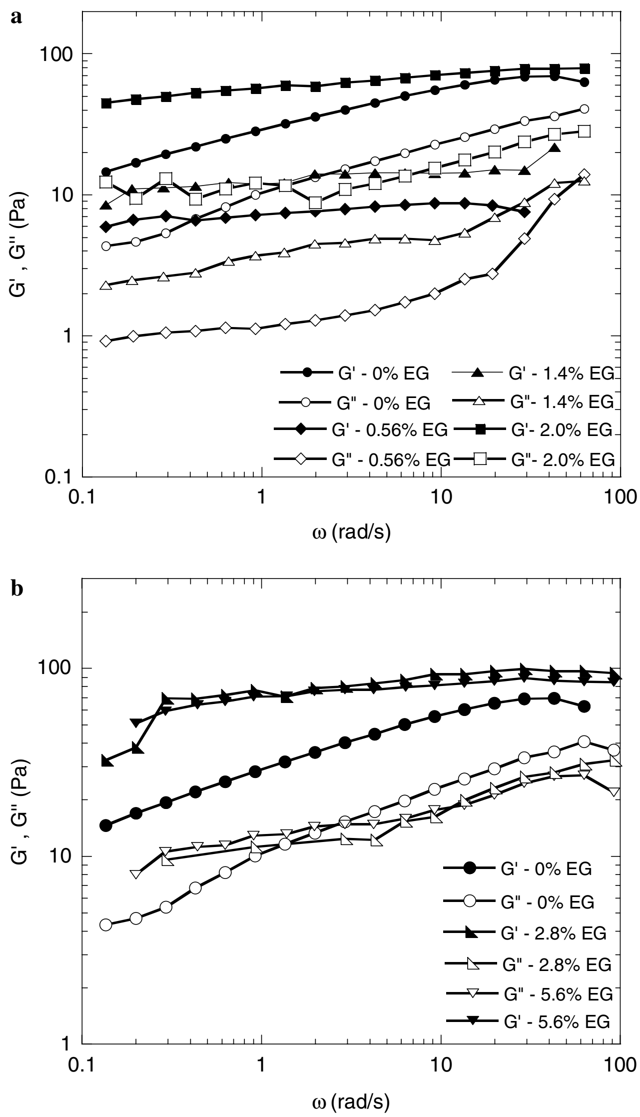

Fig. 6. (a) Storage (G') and loss (G'') moduli as a function of frequency for cross-linking agent EG concentrations of: 0%, 0.56%, 1.4%, and 2.0%; (b) Storage (G') and loss (G'') moduli as a function of frequency for cross-linking agent EG concentrations of: 0%, 2.8%, and 5.6%; both measurements were carried out at 85 °C with a 5% (w/v) dispersion of starch.

In pure water, one observes that G' is above G'' and both quantities present more or less the same frequency dependence, i.e., a power law $A\omega^n$, with $n \sim 0.3$ for G' and $n \sim 0.38$ for G''. The system is slightly above the critical gel point, where $G' = G''$ over a large frequency range (Winter & Chambon, 1986). At 0.56% of EG, both moduli decrease compared to the pure water system, however, the frequency dependence of G' is lower indicating that the dynamics of the system has been slowed down by chemical cross-linking. Moreover, the viscous character (G'') is far less pronounced in relation to the pure water system which reinforces the idea that covalent links have been created. At 1.4% w/w of cross-linker, the behavior observed is practically the same, however, both moduli are higher than those obtained for 0.56% w/w of EG, but still lower than water. At 2% w/w of cross-linker, the frequency dependence of G' is lower ($n < 0.1$) and values are higher than G' values in pure water. For concentrations of cross-linking agent higher than 2.0% w/w, a considerable difference regarding the values obtained for G' and G'' was not observed. It should be mentioned that for 2% of EG, the frequency dependence of G'' is quite complex, i.e., less marked at low frequencies and more pronounced at high frequencies. It is not clear whether or not the cross-linker concentration influences this transition. With such a process, it is not useful to add more EG to increase the extent of cross-linking. Such experiments have been similarly performed on 15% and 25% w/w starch suspensions. To summarize, the results with the 5% and 15% w/w starch suspension, which were chosen to represent the complex viscosity η^* versus frequency, are shown in Fig. 7(a and b).

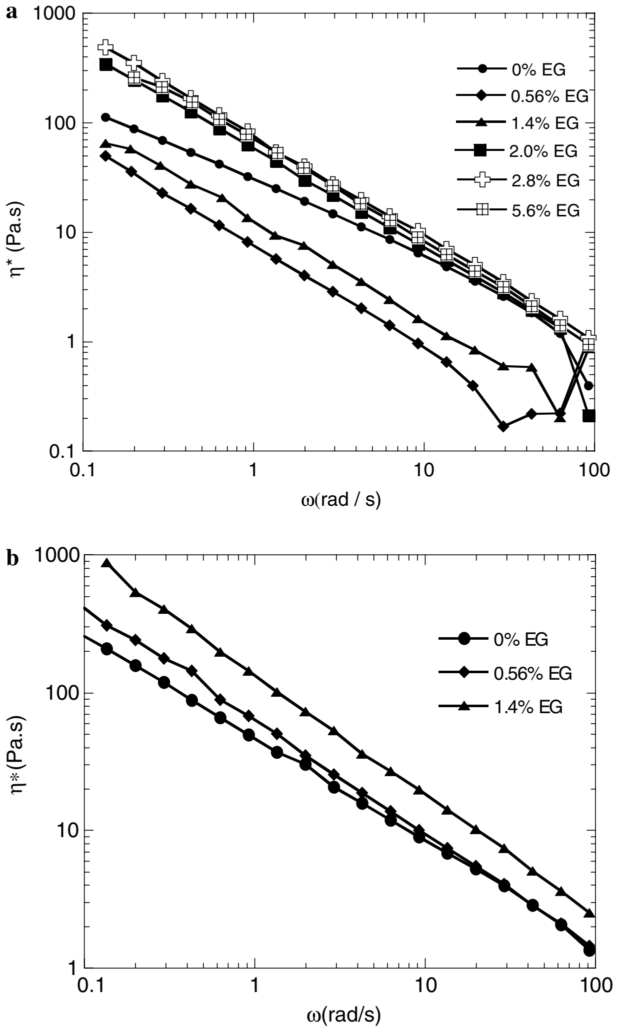

Fig. 7. Complex dynamic viscosity ($\eta*$) for several EG concentrations with a 5% dispersion (a) and a 15% dispersion (b) of starch, at 85 °C.

As G' follows a power law and is higher than G'', the complex viscosity should equally follow a power law $K\omega^n$. This agrees with our results, as shown in Fig. 7, in the frequency range studied. The results of the fitting by a power law are given in Table 2.

As shown previously, the consistency (illustrated by the parameter K) of the gel formed is affected in a complex manner. At low starch concentrations and low cross-linker content, the viscosity initially decreases with EG content (until 0.56%) and then increases again to a viscosity higher than that obtained using pure water. However, the elastic character (illustrated by n) is more or less the same whatever the EG content. The maximum improvement in the mechanical properties is obtained at 2.8% EG content. For the 15% starch suspension, an increase in the viscosity is observed for low cross-linker content (0.56%) as well as an increase in the elastic character of the gel formed with EG content. For the 25% starch suspension, the viscosity strongly increased at low cross-linker concentration along with the elastic character of the network. However, the improvement in the mechanical properties of the gel reaches a maximum within the EG content range studied.

The evolution of the dynamic rheological properties of the gel formed with the initial starch concentration and cross-linker content, is probably a consequence of a complex contribution of physical and chemical properties of the gel. In order to gain a greater insight into the phenomenon, we studied the retrogradation through dynamic measurements using the lower starch concentration (5%) and tried to evaluate the effect of the cross-linker. The results are given in Fig. 8 (a and b).

For starch in pure water (Fig. 8a), the data obtained just after cooling down to 25 °C (0 h) indicate that the system is essentially a viscous fluid ($G'' > G'$) and $G'' \sim \omega$. Moreover, the data for G' has got high noise level and the apparatus could not provide the G' value for $\omega > 2$ rad/s. After the system was left to cool to 25 °C, the "gel character" was lost. This result is different from that obtained by Aguilera and Rojas, 1997, where an increase in the viscoelastic properties was observed after gelatinization when the temperature decreased. Our results indicate that a physical gel was formed and the cohesion of this gel was due to the entanglement of expanded amylose chains and the overlap of remnants of the amylopectin granule. Our system, for the starch concentrations used and decreasing the temperature, induced a shrinkage of the amylose chain. Interaction and entanglement are thus limited. A progressive increase in the viscoelastic properties of the fluid is observed and a gel state was found after 72 h ($G' > G''$ and low frequency dependence) which is known as the retrogradation phenomenon.

In the presence of 2.8% EG at 0 h, G' is above G'' over a very large frequency range and both seem to reach a

Table 2
Parameters for the power law fitting (K,n) obtained for different starch suspensions and different EG content

EG content (%, w/w)	Suspensions (%, w/w)					
	5		15		25	
	K	n	K	n	K	n
0.00 (pure water)	29.8	0.69	46.9	0.72		0.74
0.56	7.5	0.92	63.4	0.82	299.0	0.88
1.40	12.7	0.92	137.0	0.88	287.0	0.88
2.00	58.0	0.93				
2.80	77.0	0.92				
5.60	69.0	0.88				

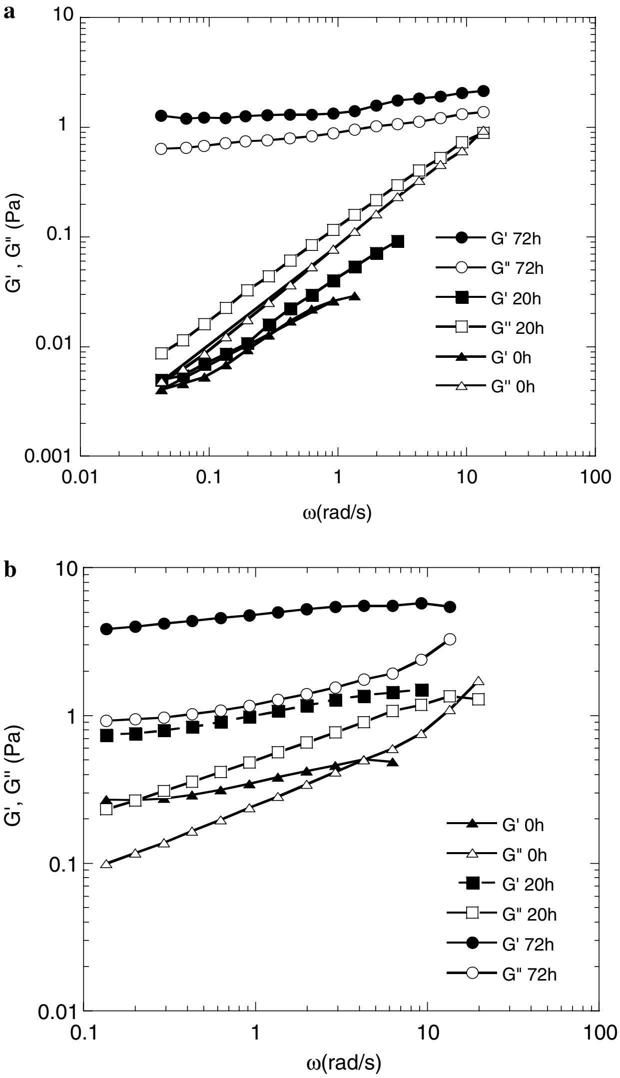

Fig. 8. (a) Viscoelastic properties of gel formed from a 5% starch suspension in water at 25 °C, just after cooling (0 h) then after 20 and 72 h: (b) same as (a) but in the presence of ethylene glycol diacrylate (EG) 2.8%.

plateau at low frequencies. Moreover, the value obtained for G' and G'' are above those obtained without cross-linker. Even if the values of G' and G'' are below those obtained at 85 °C, the signature of the chemical cross-linking is clear here. If part of amylose chain shrinks this can decrease the viscoelastic properties. The covalent bonds created are, of course, irreversible and a final "weak gel" state is observed. The retrogradation phenomenon is still there, as shown by the progressive increase in both moduli over time. However, it seems that the retrogradation is more "controlled". For example, at low frequencies, the differences between 0 and 72 h are lesser in the presence of EG than they are in the presence of pure water. Moreover, the increase in the moduli seems to be more progressive in the presence of EG.

It is clear from the measurements that chemical cross-linking during gelatinization occurs in the presence of EG. However, for the concentrations used, the contribution from the physical network and chemical cross-linking to the final gel structure and viscoelastic properties is subtle. At low starch concentration, the distance between the amylose or amylopectin chains would be large and, in the case of a low cross-linker content, intramolecular cross-linking would therefore be minimal. It could be that there is a shrinkage of the chains and a subsequent decrease in the probability of their interaction or entanglement with neighboring chains (which establishes the physical gel) and also a decrease in the complex viscosity. The angle of the complex viscosity slope given in Fig. 6 indicates that intermolecular cross-linking is present. When cross-linker content increases, the probability of intermolecular chemical cross-linking increases and the viscosity is greater than the viscosity of the physical gel (in pure water). When the starch concentration increases, the intermolecular distances between chains decreases, and the probability of creating intermolecular cross-linking at lower EG content increases. This explains the large increases in complex viscosity for a 15% starch suspension when a small amount of EG is added.

From a practical point of view, these experiments show that despite the lack of knowledge on the molecular characteristics of the starch (chain length etc.) an optimum for the EG content, depending on initial starch concentration, can be found.

5. Conclusion

Through this study, it has been established that the gelatinization temperature of cassava starch (16% amylose and 84% amylopectin) can be increased (4–5 °C), at high water content, using alkalis like NaOH or KOH at very low concentration (around 0.01 M or 1 at 2% alkali/starch). An explanation based on a "salting out" or "salting in" effect, as proposed in the literature for NaCl and other salts, is not plausible considering the concentration range used. Dynamic experiments performed before and after dialysis lead us to conclude that for such low alkali concentration, pH 10–11 is adequate to bring about specific interactions between the cations of the alkali and the hydroxylate present in the amylose and amylopectin backbones. These interactions are strong, as dialyzed suspensions also present a higher gelatinization temperature. DSC experiments confirm this hypothesis as the enthalpy of gelatinization increases in the presence of 0.01 M NaOH. We cannot confirm whether this behavior can be generalized for application to other starches with different botanical origins. Spectroscopic techniques may provide an understanding of how these interactions or "links" are geometrically arranged inside the starch chains.

Ethylene glycol diacrylate has been studied as a cross-linker and its effect on the dynamic rheological properties of the gel formed has been evaluated. A complex contribution from chemical and physical cross-linking has been enhanced by varying the starch concentration, as well as the cross-linker content.

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