Carbohydrate Polymers 75 (2009) 262–268

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

The influence of thermal treatment and operational conditions on xanthan produced by *X. arboricola* pv pruni strain 106

Caroline D. Borges [a,*], Regina C.M. de Paula [b], Judith P.A. Feitosa [b], Claire T. Vendruscolo [a]

[a] Centro de Biotecnologia, Universidade Federal de Pelotas, CP 354, CEP 96010-900, Pelotas, RS, Brazil
[b] Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, CP 6021, CEP 60455-760, Fortaleza, CE, Brazil

ARTICLE INFO

Article history:
Received 29 March 2008
Received in revised form 5 July 2008
Accepted 8 July 2008
Available online 13 July 2008

Keywords:
Xanthomonas arboricola pv pruni
Xanthan
Thermal treatment
Operational conditions

ABSTRACT

The influence of thermal treatment and operational conditions (pH and stirrer speed) used in the process of xanthan production by *Xanthomonas arboricola* pv pruni strain 106 were evaluated through yield of xanthan, aqueous solution and fermentation broth viscosity, sodium content, pyruvate and acetyl content and molar mass. Different conditions used during the fermentation affected the xanthan characteristics. Thermal treatment decreased the final yield and pyruvate and acetyl content, and increased the xanthan aqueous solution and fermentation broth viscosities, as well as molar mass. In this study the best combination of yield and viscosity was obtained with the use of pH 7 and 400 rpm during fermentation and post-fermentation thermal treatment. Aggregation of xanthan molecules promoted by heating and detected through an increase of molar mass was apparently affected by the sodium content. As a result, a correlation between molar mass and xanthan solution viscosity could be observed.

1. Introduction

Xanthan gum is an extracellular polysaccharide produced by pathovars of *Xanthomonas campestris* and by other *Xanthomonas* species (Sutherland, 1993). Due to its rheological properties, this polymer has been used in a broad range of industries including the production of foods, toiletries, cosmetics and water-based paints, as well as in oil recovery (Rosalam & England, 2006). The functionality of xanthan gum is a direct consequence of its unique chemical structure (Challen, 1994). As this structure is subject to changes, it has been widely studied.

Commercially, xanthan is produced from a pure bacterial culture and in an aerobic and submerged fermentation process. After fermentation, the broth is submitted to a thermal treatment to kill the microorganism which is a phytopathogen. This treatment also enhances xanthan removal from the cells, inactivates enzymes such as cellulases and can improve the xanthan rheological properties, leading to a transformation from a native to a renatured state (García-Ochoa, Santos, Casas, & Gómez, 2000; Rosalam & England, 2006; Smith & Pace, 1982). However, the thermal treatment can generate distinct rheological properties depending on the conditions used (pH, temperature, salinity, duration) and on the concentration of broths (Callet, Milas, & Rinaudo, 1989; Capron, Brigand, & Muller, 1998).

On the other hand, operational conditions used during fermentation can affect the xanthan characteristics (Casas, Santos, & García-Ochoa, 2000; López, Vargas-García, Suarez-Estrella, & Moreno, 2004; Papagianni et al., 2001). Culture conditions like temperature, pH, stirrer speed, air flow rate and medium composition are parameters that should be evaluated to optimize xanthan production and improve rheological properties of the gum, mainly when wild strains of *Xanthomonas* are studied.

Nevertheless, the effect of operational conditions has been studied separately from the effect of thermal treatment applied to the fermentation broth. Thus, this study aimed to evaluate the influence of thermal treatment on the characteristics of the xanthan produced by *Xanthomonas arboricola* pv pruni strain 106 under different conditions of pH and stirrer speed.

2. Materials and methods

2.1. Microorganism

Xanthomonas arboricola pv pruni strain 106, isolated from a peach tree at the Centro de Pesquisa Agropecuaria de Clima Temperado (CPACT-EMBRAPA, Pelotas, Brazil), was used in this study. The bacterial strain was maintained by lyophilization on SPA agar (Hayward, 1964) stored at 4 °C and subcultured at weekly intervals.

2.2. Xanthan production

The culture was grown in YM broth (Haynes, Wickerham, & Hesseltine, 1955), in an orbital shaker (B. Braun Biotech Interna-

* Corresponding author. Tel.: +55 53 3275 7585; fax: +55 53 3275 7350.
E-mail address: caroldellin@bol.com.br (C.D. Borges).

0144-8617/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2008.07.013

tional model Certomat BS-1). For larger volumes, it was used a 10 L fermentation vessel (B. Braun Biotech International model Biostat B) containing 7 L of production medium (Universidade Federal de Pelotas, WO/2006/047845).

Four conditions of pH were evaluated: uncontrolled pH and controlled at pH 5, 7 and 9 by addition of 2 M NaOH. In these runs the stirrer speed, air flow rate, temperature and fermentation time were maintained at 400 rpm, 1 vvm, 28 °C for 72 h, respectively.

After investigating the influence of pH, pH 7 was selected for the stirrer speed study, under the same conditions described above, using three stirrer speeds: 200, 400 and 600 rpm.

The fermentation broth resulting from different fermentations was submitted to two post-fermentation treatments: control (without thermal treatment) and sterilization at 121 °C for 15 min. Subsequently, the broth was centrifuged (Sorvall Instruments model RC 5C) at 4 °C, 16,000*g* for 30 min. Ethanol was added to the supernatant at a 4:1 ratio (v/v). Precipitated polymers were collected, dried in a stove at 56 °C until constant weight, and subsequently ground with a disc (Fritsch model Pulverisette) to a particle size of 0.5 μm. The xanthan yield was measured in grams of dry polymer per liter of fermented broth ($g\,L^{-1}$) by gravimetric methods. Xanthan partial hydrolysis (%) caused by the thermal treatment was determined through the difference in polymer yield between control treatment and thermal treatment.

2.3. Sodium content

Sodium content, resulting from NaOH added during fermentation to control pH, was determined in a flame photometer (Cole-Parmer model 2655-00). For this, 0.4 g of xanthan was calcined at 550 °C and the ashes were treated with 12 mL of aqua regia. The salts formed were diluted in 100 mL of 1% HCl solution (ASTM D1428-64, 1981).

2.4. Viscosity

Xanthan solutions (0.5% w/v) were prepared in deionized water, shaken for 2 h at room temperature, heated at 60 °C for 20 min and, finally, kept at room temperature for 24 h before performing the test (Xuewu et al., 1996). The xanthan aqueous solutions and fermentation broth viscosities were measured in a rheometer (HAAKE model RS150) in the rotary mode, at 25 °C. A coaxial cylinder system, DG 41 sensor, and shear rate of 0.01–100 s^{-1} for the fermentation broths and of 0.01–1000 s^{-1} for the 0.5% (w/v) xanthan solution were used.

2.5. Pyruvate and acetyl content

Pyruvic and acetic acid were measured colorimetrically by the 2,4-dinitrophenylhydrazone and by the hydroxamic acid methods according to Sloneker and Orentas (1962) and McComb and McCready (1957), respectively.

2.6. Molar mass

The xanthan molar mass was estimated by gel permeation chromatography (Shimadzu model LC-10AD) at room temperature using an Ultrahydrogel linear 7.8 × 300 mm column, 0.1 M $NaNO_3$ as the solvent and 0.5 mL min^{-1} flow rate (Silva, Paula, Feitosa, Brito, & Maciel, 2004).

2.7. Statistical analysis

All experiments were carried out in triplicate and the results were submitted to a variance analysis, with comparison of means using the Tukey test at 5% significance level.

3. Results and discussion

3.1. Xanthan yield

Xanthan production by *X. arboricola* pv pruni strain 106 was influenced by the pH and stirrer speed used, and the final polymer yield was influenced by the thermal treatment applied. Initially, the highest xanthan yield was achieved when pH 7 was used, in both post-fermentation treatments, followed by pH 9, 5 and uncontrolled pH (Fig. 1). The thermal treatment hydrolyzed partially the polymers in the pH range studied (Table 1), resulting in a decrease in recovery and, consequently, in xanthan yield. The highest degree of xanthan partial hydrolysis was observed at pH 9 (14%).

Table 1
Xanthan partial hydrolysis produced at different pH values and stirrer speeds during the thermal treatment*

Conditions	NaOH used for maintenance of pH during fermentation (mL)	Degradation (%)
pH		
Uncontrolled	–	8 ± 0.6^{b}
5	50	7.1 ± 0.3^{b}
7	220	6.5 ± 0.8^{b}
9	590	14 ± 1.2^{a}
Stirrer speeds (rpm)		
200	130	10.9 ± 0.7^{a}
400	220	6.5 ± 0.2^{b}
600	450	5.8 ± 0.2^{c}

* Means of three determinations ± standard deviation. Means with different letters are significantly different from each other ($p < 0.05$).

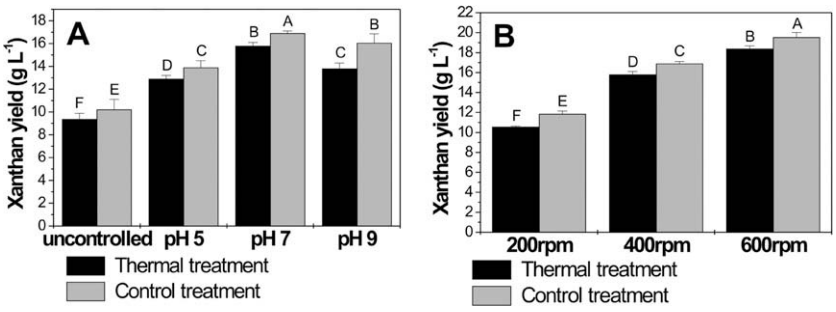

Fig. 1. Xanthan yield ($g\,L^{-1}$) at different pH values (A), stirrer speeds (B) and post-fermentation treatments. Means with different letters are significantly different from each other ($p < 0.05$).

The value of pH 7 was selected for the stirrer speed study, due to show the best results of xanthan production, and principally of viscosity. An increase in the stirrer speed favoured the xanthan production. At 600 rpm the highest yield was obtained, reaching 19.5 g L^{-1}. Hydrolysis caused by the thermal treatment was also observed during the stirrer speed study (Table 1) and was found to decrease with increasing stirrer speed, from 10.5% at 200 rpm to 5.8% at 600 rpm.

The effect of ionic strength on the xanthan characteristics is normally evaluated through salt addition to the polymer aqueous solution (Carrigton, Odell, & Fisher, 1996). In this study ionic strength was related to the sodium content present in the xanthan as a result of the NaOH added during fermentation. Sodium content in the xanthan varied according to the volume of NaOH used to maintain the pH and the stirrer speed used (Table 1). However, as expected, it was not dependent on the thermal treatment applied (Fig. 2). Sodium content increased with pH and with stirrer speed, due to the higher volume of NaOH added during fermentation under these conditions.

Thermal treatment of the fermentation broth can cause thermal degradation of the exopolysaccharides (Callet et al., 1989; Smith & Pace, 1982). According to Quinn (1999), xanthan degradation and partial hydrolysis can occur though acid (principally between pH 1–4) or base catalyzed hydrolysis. On the other hand, the thermal stability of xanthan is affected by the pH, salinity and temperature. The xanthan stability is significantly decreased above the transition temperature, or under conditions of low ionic strength. According to Holzwarth (1976) under conditions of low ionic strength, repulsion between the carboxylate groups tends to destabilize the structure; if salt is added, cations stabilize the ordered conformation against disruption by heat. This author showed that the temperature induced transition increased with Na^+ or Ca^{2+}.

The lower sodium content found in xanthan produced at uncontrolled pH and pH 5 increased the partial hydrolysis of the xanthan during the thermal treatment, but these results did not differ significantly from the results obtained at pH 7 (Table 1). At pH 9 the thermal treatment resulted in a high degree of degradation and darkening of the fermentation broth due to alkaline hydrolysis and, probably, caramelization reactions. In the second phase of this study, when the stirrer speed effect was evaluated, thermal hydrolysis could be correlated with sodium content. Increases in the sodium content decreased the partial hydrolysis of the xanthan (Table 1).

The results showed that pH control is an important tool in improving xanthan production. Some authors have studied xanthan production at uncontrolled pH (De Vuyst & Vermeire, 1994; Papagianni et al., 2001), but the majority report that controlled pH in the range of 6–8 is the optimum for this process (Casas et al., 2000; Esgalhado, Roseiro, & Collaço, 1995; Gupte & Kamat, 1997).

The results obtained for xanthan yield in the control treatment are in agreement with those obtained by Gupte and Kamat (1997), who evaluated xanthan production by *X. campestris* ICa-125 at different pH values. At pH 5 and 7 xanthan production reached 13.6 g L^{-1} and 17.5 g L^{-1}, respectively. Nevertheless, at pH 9 xanthan production obtained by these authors dropped dramatically to 3.1 g L^{-1}. In comparison, the production obtained in our study was slightly lower at pH 7, being 16.0 g L^{-1}. Liakopoulou-Kyriakides, Tzanakakis, Kiparissidis, Ekaterianiadou, and Kyriakidis (1997) showed that xanthan production increased with pH, reaching 17.3 g L^{-1} at pH 8.

According to the literature, high stirrer speed is necessary for xanthan production. Our results are in agreement with those of Peters et al. (1989) who evaluated xanthan production at distinct stirrer speeds (200, 400, 600 and 800 rpm). These authors did not observe a hydrodynamic effect with the increase in agitation, obtaining maximum production at 800 rpm (16.4 g L^{-1}). On the other hand, Casas et al. (2000) detected hydrodynamic stress with increasing stirrer speed (100, 300, 500 and 800 rpm). The production dropped when 800 rpm was applied, probably due to cellular damage, and at 100 rpm xanthan production was low due to oxygen transfer limitation. The highest xanthan production occurred at 500 rpm, reaching around 17.5 g L^{-1}.

3.2. Xanthan aqueous solution viscosity

The conditions applied during fermentation influenced the polymer quality, reflecting in the viscosity of the 0.5% (w/v) xanthan aqueous solutions. The viscosity values for the xanthan produced under uncontrolled pH and at pH 5 were similar and higher than the viscosity of the xanthan produced at pH 7 and 9, in the control treatment. For the same treatment, the increase in stirrer speed influenced positively the viscosity.

Thermal treatment of the fermentation broth showed a pronounced effect on the xanthan solution viscosity, independently of the pH or agitation condition evaluated during the fermentation. The viscosity values for the xanthan solutions for which the broth was submitted to thermal treatment were higher under all conditions tested, as showed in Fig. 3. The highest viscosity was reached when the xanthan was produced at pH 7 with the broth submitted to thermal treatment, followed by pH 9, 5 and uncontrolled pH. Regarding stirrer speed, the highest xanthan solution viscosity value was obtained at the intermediary stirrer speed studied.

Thermal treatment increased the xanthan solution viscosity produced at pH 7/400 rpm four-fold, which was considered the best combination of fermentation conditions in terms of yield and viscosity. Several reasons have been proposed to explain the increase in xanthan solution viscosity when the broth is thermally treated. According to Shatwell, Sutherland, Dea, and Ross-Murphy (1990a) the presence of salt and high temperature (110 °C) originates a conformational change in the molecules (a more expanded conformation) which, in turn, results in an increase in viscosity. Nevertheless, Born, Langendorff, and Boulenguer (2002) support

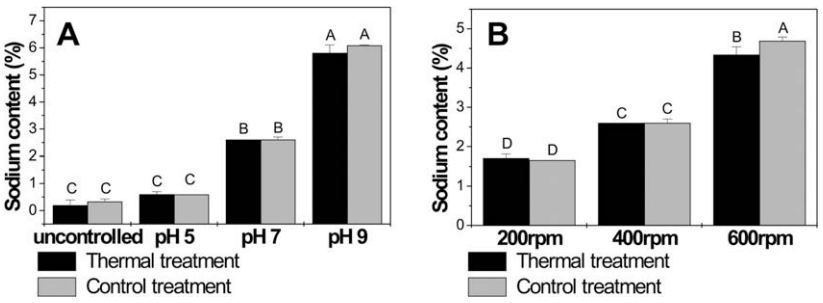

Fig. 2. Sodium content (%) of the xanthan produced at different pH values (A), stirrer speeds (B) and post-fermentation treatments. Means with different letters are significantly different from each other ($p < 0.05$).

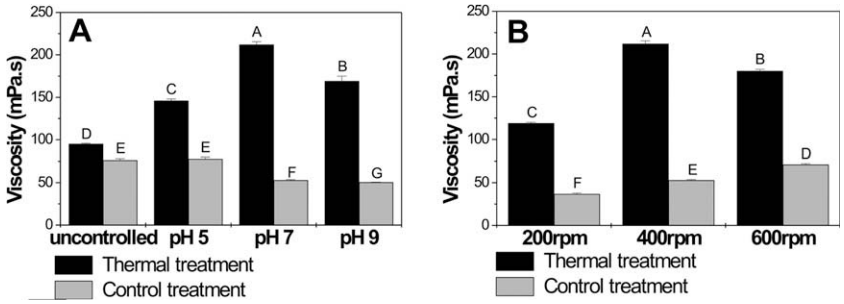

Fig. 3. Viscosity (mPa.s) at 25 °C at shear rate 19 s^{-1} of 0.5% (w/v) xanthan solution produced at different pH values (A), stirrer speeds (B) and post-fermentation treatments. Means with different letters are significantly different from each other ($p < 0.05$).

the hypothesis that single-stranded xanthan molecules associate during renaturation to form supramolecular structures.

The influence of thermal treatment on the xanthan solution viscosity could be correlated with the xanthan molar mass resulting from the different conditions applied during fermentation. These results will be discussed in Section 3.5.

In the control treatment, the adjusted pH did not improve the viscosity, unlike the results reported by Gupte and Kamat (1997) where the highest viscosity was reached at pH 6. This behavior may be dependent on the strain used. However, the increase in stirrer speed up to 600 rpm led to an increase in the viscosity of the polymer that was not thermally treated, since there was no oxygen limitation.

3.3. Fermentation broth viscosity

Similar to the results of the xanthan solution viscosity, thermal treatment increased the fermentation broth viscosity at all pH values and stirrer speeds evaluated. However, there was no correlation between the fermentation broth viscosity with the xanthan solution viscosity. This fact was also evidenced by Antunes, Moreira, Vendruscolo, and Vendruscolo (2000), who found that fermentation broth viscosity did not reflect in the quality of the polymer produced. High broth viscosities may be due to a high concentration of polymer with low quality.

In the control treatment there was a correlation between fermentation broth viscosity (Fig. 4) with xanthan yield. The viscosity increased up to pH 7, with a subsequent drop at pH 9, as well as with increasing speed stirrer, probably due to the amount of polymer accumulated.

The viscosity of the thermally treated fermentation broth could not be explained by the amount of polymer accumulated, since the xanthan yield decreased with the thermal treatment for all conditions evaluated and the fermentation broth viscosity increased. The results indicate that aggregation occurred between the molecules, however, the fermentation broth molar mass was not determined. Callet et al. (1989) also obtained an increase in the fermentation broth viscosity, when 1.5% $NaSO_4$ was added and thermal treatment applied. Their results suggest that the presence of external salt stabilized the molecule conformation and increased the broth viscosity due to conversion from the native ordered xanthan conformation to a renatured more viscous one, or to aggregation. According to these authors the pH had a pronounced effect on the viscosity of thermally treated broth, and the best stability was obtained at pH 7 or 8. Similarly, Esgalhado et al. (1995) evaluated the effect of pH on fermentation broth viscosity and xanthan yield, however, the broth was not submitted to thermal treatment. The optimum range was established between pH 7 and 8. Values above pH 8 were not evaluated by the authors.

Many studies on xanthan gum have analyzed only the fermentation broth viscosity (Callet et al., 1989; Esgalhado et al., 1995; López, Moreno, & Ramos-Comenzana, 2001). However, as in this study, the results can lead to mistaken conclusions, since the high viscosity of the thermally treated fermentation broth, obtained at pH 7/600 rpm and pH 9/400 rpm did not reflect an increase in the xanthan solution viscosity.

3.4. Pyruvate and acetyl content

Thermal treatment degraded the pyruvate substituent of the xanthan molecule under all conditions tested (Fig. 5). The highest pyruvate content was obtained at pH 7. Acid and basic pH hampered the synthesis of the pyruvate substituent. The acetyl substituent was degraded only in neutral and basic pH. The highest acetyl content was obtained under acid conditions, decreasing at pH 7 and 9, in both treatments (Fig. 6). An increase in the stirrer speed favored acetyl and pyruvate substituent synthesis. The highest pyruvate content was obtained at 600 rpm/pH 7. However, for the acetyl substituent the highest content was reached at 400 rpm/uncontrolled pH and pH 5.

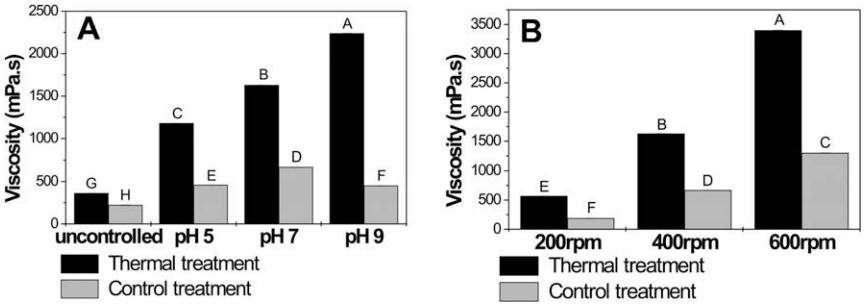

Fig. 4. Viscosity (mPa.s) at 25 °C at shear rate 10 s^{-1} of fermentation broth at different pH values (A), stirrer speeds (B) and post-fermentation treatments. Means with different letters are significantly different from each other ($p < 0.05$).

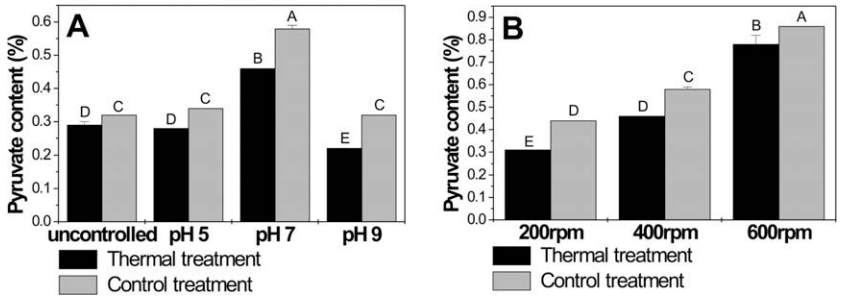

Fig. 5. Pyruvate content (%) of the xanthan molecule produced at different pH values (A), stirrer speeds (B) and post-fermentation treatments. Means with different letters are significantly different from each other ($p < 0.05$).

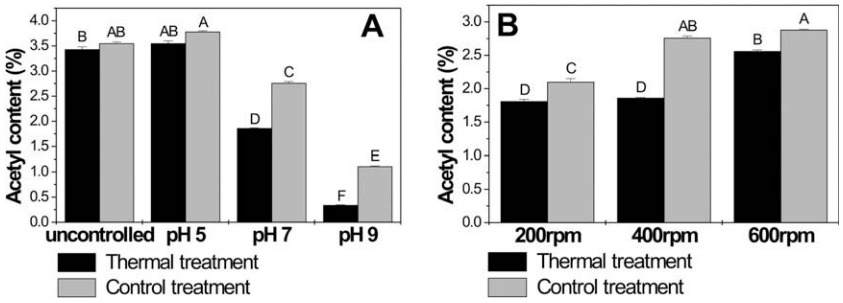

Fig. 6. Acetyl content (%) of the xanthan molecule produced at different pH values (A), stirrer speeds (B) and post-fermentation treatments. Means with different letters are significantly different from each other ($p < 0.05$).

It has been shown that the pyruvate content can be used as an indicator of the xanthan rheological quality. Xanthans with high pyruvate content (around 4%) are more viscous than those with low content (Sandford et al., 1977; Smith, Symes, Lawson, & Morris, 1981). However, in this study the improvement of the xanthan solution viscosity could not be correlated with an increase in pyruvate content, and similar results have been reported by other authors (Callet, Milas, & Rinaudo, 1987; Kennedy, Jones, & Barker, 1982; Torres, Brito, Galindo, & Choplin, 1993; Torrestiana, Fucikovsky, & Galindo, 1990). Similarly, the acetyl content can also influence the aqueous solution viscosity. Sloneker and Jeanes (1962) and Tako and Nakamura (1984) demonstrated that the xanthan solution viscosity could be increased by removing acetyl groups, nevertheless, as in this study Bradshaw, Nisbet, Kerr, and Sutherland (1983) and Callet et al. (1987) did not observe any effect on the rheological behavior on removing this group.

Variations in the pyruvate and acetyl contents with different operational conditions and strains occurs, according to Sutherland (1982), due to the presence of one or two acetylases and by irregular addition of pyruvate or by the presence of a mixture of pyruvylated and non-pyruvylated strands of polysaccharide. Values of up to 5% of acetyl and 8.1% of pyruvate in the xanthan molecule are possible (Shatwell et al., 1990a). The acetyl content reported in this study is in agreement with most results in the literature, ranging from 1.81% to 3.78%, except for xanthan produced at pH 9. However, the pyruvate content was lower than those reported in the literature, reaching a maximum of 0.86%. Other authors have also reported low concentrations of this substituent (Shatwell, Sutherland, & Ross-Murphy, 1990b; Sánchez, Ramírez, Torres, & Galindo, 1997).

The influence of thermal treatment and fermentation conditions in the xanthan chemical composition has been evaluated. The chemical composition of xanthan produced from broth with the pH adjusted before thermal treatment was evaluated by Callet et al. (1989). Similarly to our results, they observed that on heating at acid pH the pyruvates were hydrolyzed, but under basic pH the acetates were liberated. For uncontrolled pH (broth pH 5.7), hydrolysis of pyruvate was also observed but to a lower extent.

Distinct behaviors have been reported in the literature in relation to pyruvate and acetate contents of xanthan produced under different operational conditions. Casas et al. (2000) described that the acetate concentration was higher when stirrer speed was increased from 100 rpm (1.53%) to 500 rpm (4.44%) and the pyruvate concentration was not influenced by the stirrer speeds tested. However, a relation between degree of agitation and of pyruvilation of the xanthan was found by Papagianni et al. (2001). There was no significant difference in pyruvate content at 300, 400 and 600 rpm (3.00–3.49%), however, the effect became more pronounced when pyruvate content at 100 rpm was compared to that at 600 rpm (1.54 and 3.49%, respectively).

3.5. Molar mass

The xanthan molar mass was strongly influenced by thermal treatment for all conditions evaluated (Fig. 7). The results showed a correlation with the xanthan solution viscosity, except for the xanthan produced at different stirrer speeds submitted to the control treatment. The highest molar mass was obtained when the xanthan was produced at 400 rpm and pH 7 with thermal treatment.

The results suggest that the thermal treatment increased the xanthan molar mass by aggregation and, as a consequence, an increase in xanthan solution viscosity was observed. Thermal treatment leads to a molecular transformation from the native to renatured state (Capron, Brigand, & Muller, 1997). According to Young, Martino, Kienzle-Sterzer, and Torres (1994) an increase in the solution temperature or in the molecule electrostatic potential can disrupt the hydrogen bonds responsible for intermolecular association. The results at pH 9/400 rpm and pH 7/600 rpm suggest that renaturation in the presence of high ionic strength may have caused an increase in electrostatic interac-

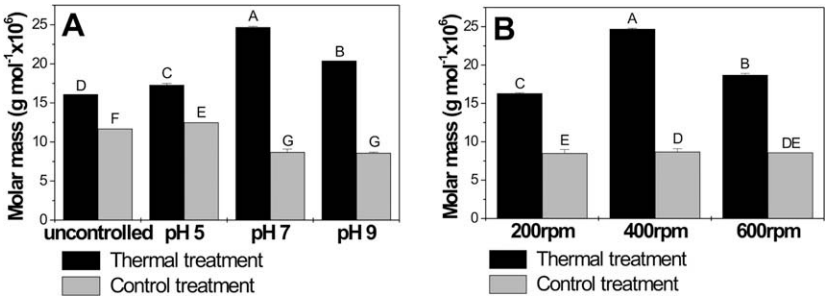

Fig. 7. Xanthan molar mass (g mol^{-1} × 10^6) produced at different pH values (A), stirrer speeds (B) and post-fermentation treatments. Means with different letters are significantly different from each other ($p < 0.05$).

tions, causing a decrease in the xanthan molar mass. Since sodium is a monovalent cation, it cannot be linked to more than one polysaccharide chain to promote interlinking of chains, as in the case of calcium. It only ionizes the polysaccharide promoting electrostatic interactions (Bueno & Garcia-Cruz, 2001; Tako & Nakamura, 1987). Other factors can also influence the extent of this intermolecular association, such as the presence of polymer contaminants and pyruvate and acetyl content (Capron et al., 1998; Lechner, Gehrke, & Nordmeier, 1996; Smith et al., 1981; Tako & Nakamura, 1984).

The intermolecular association may be enhanced by interaction between the pyruvate methyl groups and, thus, an increase in pyruvate content leads to an increase of solution viscosity (Smith et al., 1981). Xanthan molecule association is also related to an increase in pyruvate content, when high levels of the product are accumulated. Increases in the polymer concentration lead to the occurrence of polymer–solvent interactions rather than polymer–polymer interactions (Lechner et al., 1996). However, Tako and Nakamura (1984) observed that intensive intermolecular association can also occur with the deacetylated xanthan when in high concentration, resulting in high viscosity. Proteins can also reinforce the association between pyruvate groups, thereby creating hydrophobic microdomains located on the same main chain (intramolecular links) or between different chains (intermolecular links) (Capron et al., 1998).

Values reported for the xanthan molar mass range from 5×10^5 to 1.3×10^7 g mol^{-1} (Papagianni et al., 2001; Sánchez et al., 1997). This wide range results partly from the application of different techniques and also from a tendency for the polysaccharide strands to aggregate in solution (Sutherland, 1996).

Our results are in disagreement with Callet et al. (1989), Milas, Reed, and Printz (1996) and Capron et al. (1997). The former showed that thermal treatment always has a degrading effect on the polymer, causing a decrease in molar mass, even though, under some conditions, an increase in the broth viscosity is observed. The other authors did not observe a difference between the xanthan molar mass in native conformation and in renatured conformation.

Changes in the fermentation conditions are factors that can influence the xanthan molar mass (Casas et al., 2000; Kalogiannis, Iakovidou, Liakopoulou-Kyriakides, Kyriakidis, & Skaracis, 2003; Peters et al., 1989; Sánchez et al., 1997). The influence of the pH used during fermentation on xanthan molar mass has not been evaluated by other authors. Studies on the molar mass have shown different behaviors in relation to stirrer speed. According to Peters et al. (1989) an increase in the stirrer speed increased the xanthan molar mass from 6.9×10^6 g mol^{-1} at 200 rpm to 8.6×10^6 g mol^{-1} at 800 rpm. However, Papagianni et al. (2001) verified that the stirrer speed had almost no affect on the xanthan molar mass, obtaining a value of around 5×10^5 g mol^{-1}, similar to the control treatment for which the molar mass reached was around 8.6×10^6 g mol^{-1}.

4. Conclusions

The conditions used during fermentation affected the xanthan characteristics. The application of thermal treatment decreased the polymer yield, however, it increased the aqueous solution and fermentation broth viscosities, for all conditions evaluated. The best combination of yield and viscosity was obtained with the use of pH 7 and 400 rpm during fermentation and post-fermentation thermal treatment. The aggregation of xanthan molecules caused by the thermal treatment and detected through an increase in molar mass, was apparently affected by the sodium content. As a result, a correlation between molar mass and xanthan solution viscosity was observed.

Acknowledgments

We greatly appreciate the financial support of CNPq and CPACT-EMBRAPA for supplying the bacterial strain.

References

Antunes, A. E. C., Moreira, A. S., Vendruscolo, J. L., & Vendruscolo, C. T. (2000). Viscosidade aparente de biopolímeros produzidos por diversas cepas de *Xanthomonas campestris* pv pruni. *Ciência e Engenharia, 9*, 83–87.

ASTM D1428-64 (1981). Annual book of American standard testing methods, Philadelphia, USA.

Born, K., Langendorff, V., & Boulenguer, P. (2002). Xanthan. In A. Steinbüchel, E. J. Vandamme, & S. De Baets (Eds.). *Biopolymers* (Vol. 5, pp. 259–291). Weinheim: Wiley-VCH.

Bradshaw, I. J., Nisbet, B. A., Kerr, M. H., & Sutherland, I. W. (1983). Modified xanthan – its preparation and viscosity. *Carbohydrate Polymers, 3*, 23–38.

Bueno, S. M., & Garcia-Cruz, C. H. (2001). The influence of fermentation time and the presence of salts in the rheology of the fermentation broth of a polysaccharide-producing bacteria free of soil. *Journal of Food Engineering, 50*, 41–46.

Callet, F., Milas, M., & Rinaudo, M. (1987). Influence of acetyl and pyruvate contents on rheological properties of xanthan in dilute solution. *International Journal of Biological Macromolecules, 9*, 291–293.

Callet, F., Milas, M., & Rinaudo, M. (1989). On the role of thermal treatments on the properties of xanthan solutions. *Carbohydrate Polymers, 11*, 127–137.

Capron, I., Brigand, G., & Muller, G. (1997). About the native and renatured conformation of xanthan exopolysaccharide. *Polymer, 38*, 5289–5295.

Capron, I., Brigand, G., & Muller, G. (1998). Thermal denaturation and renaturation of a fermentation broth of xanthan: rheological consequences. *International Journal of Biological Macromolecules, 23*, 215–225.

Carrigton, S., Odell, J., & Fisher, L. (1996). Polyelectrolyte behaviour of dilute xanthan solutions: Sat effects on extensional rheology. *Polymer, 37*, 2871–2875.

Casas, J. A., Santos, V. E., & García-Ochoa, F. (2000). Xanthan gum production under several operational conditions: Molecular structure and rheological properties. *Enzyme and Microbiology Technology, 26*, 282–291.

Challen, I. A. (1994). Xanthan gum: A multifunctional stabiliser for food products. In K. Nishinari & E. Doi (Eds.), *Food hydrocolloids: Structures, properties and functions* (pp. 135–139). New York: Plenum Press.

De Vuyst, L., & Vermeire, A. (1994). Use of industrial medium components for xanthan production by *Xanthomonas campestris* NRRL B-1459. *Applied Microbiology and Biotechnology, 42*, 187–191.

Esgalhado, M. E., Roseiro, J. C., & Collaço, M. T. A. (1995). Interactive effects of pH and temperature on cell growth and polymer production by *Xanthomonas campestris*. *Process Biochemistry, 30*, 667–671.

García-Ochoa, F., Santos, V. E., Casas, J. A., & Gómez, E. (2000). Xanthan gum: Production, recovery and properties. *Biotechnology Advances, 18*, 549–579.

Gupte, M. D., & Kamat, M. Y. (1997). Isolation of wild *Xanthomonas* strains from agricultural produce, their characterization and potential related to polysaccharide production. *Folia Microbiologica, 42*, 621–628.

Haynes, W. C., Wickerham, L. J., & Hesseltine, C. W. (1955). Maintenance of cultures of industrially important microorganisms. *Applied Microbiology, 3*, 361–368.

Hayward, A. C. (1964). Bacteriophage sensitivity and biochemical type in *Xanthomonas malvacearum*. *Journal of General Microbiology, 33*, 287–298.

Holzwarth, G. (1976). Conformation of extracellular polysaccharide of *Xanthomonas campestris*. *Biochemistry, 15*, 4333–4339.

Kalogiannis, S., Iakovidou, G., Liakopoulou-Kyriakides, M., Kyriakidis, D. A., & Skaracis, G. N. (2003). Optimization of xanthan gum production by *Xanthomonas campestris* grown in molasses. *Process Biochemistry, 39*, 249–256.

Kennedy, J. F., Jones, P., & Barker, S. A. (1982). Factors affecting microbial growth and polysaccharide production during the fermentation of *Xanthomonas campestris* cultures. *Enzyme Microbiology Technology, 4*, 39–43.

Lechner, M. D., Gehrke, K., & Nordmeier, E. (1996). *Makromolekulare chemie*. Berlin: Birkhäuser.

Liakopoulou-Kyriakides, M., Tzanakakis, E. S., Kiparissidis, C., Ekaterianiadou, L. V., & Kyriakidis, D. A. (1997). Kinetics of xanthan gum production from whey by constructed strains of *Xanthomonas campestris* in bath fermentations. *Chemical Engineering & Technology, 20*, 354–360.

López, M. J., Moreno, J., & Ramos-Comenzana, A. (2001). *Xanthomonas campestris* strain selection for xanthan production from olive mill wastewaters. *Water Research, 35*, 1828–1830.

López, M. J., Vargas-García, M. C., Suarez-Estrella, F., & Moreno, J. (2004). Properties of xanthan obtained from agricultural wastes acid hydrolysates. *Journal of Food Engineering, 63*, 111–115.

McComb, E. A., & McCready, R. M. (1957). Determination of acetyl in pectin and in acetylated carbohydrate polymers. *Analytical Chemistry, 29*, 819–821.

Milas, M., Reed, W. F., & Printz, S. (1996). Conformations and flexibility of native and re-natured xanthan in aqueous solutions. *International Journal of Biology Macromolecules, 18*, 211–221.

Papagianni, M., Psomas, S. K., Batsilas, L., Paras, S. V., Kyriakidis, D. A., & Liakopoulou-Kyriakides, M. (2001). Xanthan production by *Xanthomonas campestris* in batch cultures. *Process Biochemistry, 37*, 73–80.

Peters, H., Herbst, H., Hesselink, P., Lünsdorf, H., Schumpe, A., & Deckwer, W. (1989). The influence of agitation rate on xanthan production by *Xanthomonas campestris*. *Biotechnology and Bioengineering, 34*, 1393–1397.

Quinn, X. F. (1999). Xanthan gum (Overview). In J. C. Salamone (Ed.), *Concise polymeric materials encyclopedia* (pp. 1652–1653). Boca Raton: CRC Press.

Rosalam, S., & England, R. (2006). Review of xanthan gum production from unmodified starches by *Xanthomonas campestris* sp. *Enzyme and Microbial Technology, 39*, 197–207.

Sánchez, A., Ramírez, M. E., Torres, L. G., & Galindo, E. (1997). Characterization of xanthans from selected *Xanthomonas* strains cultivated under constant dissolved oxygen. *World Journal of Microbiology & Biotechnology, 13*, 443–451.

Sandford, P. A., Pittsley, J. E., Knutson, C. A., Watson, P. R., Cadmus, M. C., & Jeanes, A. (1977). Variation in Xanthomonas campestris NRRL B – 1459: Characterization of xanthan products of differing pyruvic acid content. In P. A. Sandford & A. Laskin (Eds.), *Extracellular Microbial Polysaccharides* (pp. 192–210). Washington: American Chemical Society.

Shatwell, K. P., Sutherland, I. W., Dea, I. C. M., & Ross-Murphy, S. B. (1990a). The influence of acetyl and pyruvate substituents on the helix-coil transition behavior of xanthan. *Carbohydrate Research, 206*, 87–103.

Shatwell, K. P., Sutherland, I. W., & Ross-Murphy, S. B. (1990b). Influence of acetyl and pyruvate substituents on the solution properties of xanthan polysaccharide. *International Journal of Biological Macromolecules, 12*, 71–78.

Silva, D. A., Paula, R. C. M., Feitosa, J. P. A., Brito, A. C. F., & Maciel, J. S. (2004). Carboxymethylation of cashew tree exudate polysaccharide. *Carbohydrate Polymers, 58*, 163–171.

Sloneker, J. H., & Jeanes, A. (1962). Exocellular bacterial polysaccharide from *Xanthomonas campestris* NRRL B – 1459. *Canadian Journal of Chemistry, 40*, 2066–2071.

Sloneker, J. H., & Orentas, D. G. (1962). Pyruvic acid, a unique component of an exocellular bacterial polysaccharide. *Nature, 194*, 478–479.

Smith, I. H., & Pace, G. W. (1982). Recovery of microbial polysaccharides. *Journal of Chemical Technology and Biotechnology, 32*, 119–129.

Smith, I. H., Symes, K. C., Lawson, C. J., & Morris, E. R. (1981). Influence of the pyruvate of xanthan on macromolecular association in solution. *International Journal of Biological Macromolecules, 3*, 129–134.

Sutherland, I. W. (1982). Biosynthesis of microbial exopolysaccharides. In A. H. Rose & J. G. Morris (Eds.). *Advances in microbial physiology* (Vol. 23, pp. 80–142). New York: Academic Press.

Sutherland, I. W. (1993). Xanthan. In J. G. Swings & E. L. Civerolo (Eds.), *Xanthomonas* (pp. 363–388). London: Chapman & Hall.

Sutherland, I. W. (1996). Microbial biopolymers from agricultural products: Production and potential. *International Biodeterioration & Biodegradation, 38*, 249–261.

Tako, M., & Nakamura, S. (1984). Rheological properties of deacetylated xanthan in aqueous-media. *Agricultural and Biological Chemistry, 48*, 2987–2993.

Tako, M., & Nakamura, S. (1987). Rheological properties of Ca salt of xanthan aqueous media. *Agriculture and Biology Chemistry, 51*, 2919–2923.

Torres, L. G., Brito, E., Galindo, E., & Choplin, L. (1993). Viscous behavior of xanthan solutions from a variant strain of *Xanthomonas campestris*. *Journal of Fermentation and Bioengineering, 75*, 58–64.

Torrestiana, B., Fucikovsky, L., & Galindo, E. (1990). Xanthan production by some *Xanthomonas* isolates. *Letters in Applied Microbiology, 10*, 81–83.

Universidade Federal de Pelotas & Empresa Brasileira de Pesquisa Agropecuaria Embrapa Clima Temperado. Process for preparing a xanthan biopolymer. International Patent WO/2006/047845.

Xuewu, Z., Xin, L., Dexiang, G., Wei, Z., Tong, X., & Yonghong, M. (1996). Rheological models for xanthan gum. *Journal of Food Engineering, 27*, 203–209.

Young, S. L., Martino, M., Kienzle-Sterzer, C., & Torres, J. A. (1994). Potentiometric titration studies on xanthan solutions. *Journal of the Science of Food and Agriculture, 64*, 121–127.