Carbohydrate Polymers 83 (2011) 1895–1900

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Determination of Na, K, Ca and Mg in xanthan gum: Sample treatment by acid digestion

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ARTICLE INFO

Article history:
Received 9 September 2010
Received in revised form 15 October 2010
Accepted 22 October 2010
Available online 30 October 2010

Keywords:
Xanthan
Sodium
Potassium
Calcium and magnesium
Acid digestion

ABSTRACT

A simple alternative decomposition procedure for the determination of Na, K, Ca, and Mg in xanthan gum was proposed. The samples were treated in the presence of HNO_3 and $HClO_4$, ensuring complete digestion. After decomposition, Na and K content were determined by flame atomic emission spectrometry and Ca and Mg content by flame atomic absorption spectrometry. The accuracy of the method was verified by comparing the results with those obtained from the conventional decomposition by calcination method, and there were no significant differences between the results at the 95% confidence level. The limits of detection in the sample were 0.011, 0.004, 0.45 and 0.001 mg g^{-1} for Na, K, Ca and Mg, respectively. The relative standard deviations were lower than 10%. The method proposed is simple, fast, reproducible, and requires less sample than calcinations method.

1. Introduction

Xanthan gum, an extracellular microbial polysaccharide produced by bacteria of the genus *Xanthomonas* (Galindo, 1994; Jansson, Kenne, & Lindberg, 1975; Ross-Murphy, Morris, & Morris, 1983) has a wide range of applications in food and non-food industries as a stabilizer, emulsifier and suspending agent (Katzbauer, 1998; Vendruscolo, Rodrigues, Pereira, Redies, & Vendruscolo, 2006) due to its rheological properties. Xanthan gum produces highly viscous solutions, even at low concentrations (Born, Langendorff, & Boulenguer, 2002; Galindo, 1994; García-Ochoa, Santos, Casas, & Gómez, 2000; McNeely & Kang, 1973) because of its high molecular weight and its secondary structure (Born et al., 2002; Katzbauer, 1998; Rinaudo, 2001), but the viscosity decreases with decreasing molecular weight (Borges, Paula, de Feitosa, & Vendruscolo, 2009; Viturawong, Achayuthakan, & Suphantharika, 2008).

Xanthan gum is used in the food industry mainly because of its compatibility with many food ingredients and additives (Challen, 1994), such as proteins, lipids and other polysaccharides, such as starch and pectin (Sutherland, 1998). Xanthan gum is also compatible with various salts (ionic strength), even at high concentrations. It is stable over a wide pH range and its solutions are very resistant to high temperatures (Born et al., 2002; Challen, 1994; Galindo, 1994; McNeely & Kang, 1973).

Xanthan is an anionic heteropolysaccharide (Ross-Murphy et al., 1983) whose primary structure is based on repeated pentasaccharide units. The backbone chain is composed of two glucose units linearly linked by β1 → 4 linkages, and the trisaccharide side chain consists of two D-mannose units alternating D-glucuronic acid; the internal mannose unit is acetylated and about half of the terminal D-mannose units (external) contain pyruvic acid residues (Cadmus et al., 1976; Galindo, 1994; Jansson et al., 1975; Sloneker & Jeanes, 1962). In these acidic residues, cations, such as Na, K, Ca and Mg, are linked in different proportions, where the cations originate from the salts used in production media or added after fermentation.

Xanthan properties are influenced by the concentration and nature of added salts (Rinaudo, 2001; Ross-Murphy et al., 1983). The presence of electrolytes in xanthan solutions influences rheological behavior, especially viscosity (Borges, Vendruscolo, Martins, & Lomba, 2009; García-Ochoa et al., 2000), and the influence of the salts on viscosity is dependent on xanthan concentration (Cadmus et al., 1976; Carrington, Odell, Fisher, Mitchell, & Hartley, 1996; Pelletier, Viebke, Meadows, & Williams, 2001; Rinaudo, 2001).

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0144-8617/$ – see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.carbpol.2010.10.059

The ordered conformation of xanthan gum is stabilized by salt (Katzbauer, 1998; Born et al., 2002) and is responsible for its remarkable rheological properties (Capron, Brigand, & Muller, 1997) and by the extraordinary stability of the polymer. Therefore, the presence of salt is necessary for optimal functionality (Katzbauer, 1998) because it increases the thermal stability of xanthan solutions (Borges, Paula et al., 2009; Kierulf & Sutherland, 1988; Xie & Lecourtier, 1992).

Because the salt content affects xanthan behavior, it is necessary to have an easy, appropriate, and reliable method for its determination. In the literature, calcination is used to prepare samples of xanthan (Borges, Paula et al., 2009; Borges, Vendruscolo et al., 2009) and other polysaccharides (Freitas et al., 2009) for quantitative determination of Na, K, Ca and Mg by photometric and spectrometric methods. While drying in muffle furnace, the sample decomposes via burning of the organic fraction to yield an inorganic ash residue, soluble in dilute acid. Depending on the sample's composition, this method can be time consuming and result in loss by volatilization. The sample can react with the crucible material and create loss or contaminate the analytes, depending of the temperature, the crucible material and sample composition (Kebbekus, 2003; Magalhães, Flores, Krug, Barin, & Mesko, 2008). Because of systematic errors, some authors recommend avoiding decomposition through drying in muffle furnace (Magalhães et al., 2008).

In this study, was proposed a new and very simple method of preparing samples of xanthan by acid digestion for Na and K determination by flame photometry and Ca and Mg determination by atomic absorption spectrometry. To develop the method, five different xanthan samples were used and the results were compared with the currently used calcination method to check its accuracy.

2. Materials and methods

2.1. Materials

2.1.1. Bacterial strain

Xanthomonas arboricola pv pruni strain 101 was used in this study. The bacterial strain was maintained on a SPA agar (Hayward, 1964) stored at 4 °C and subcultured monthly.

2.1.2. Xanthans

In this study, both commercial xanthan samples and samples produced in our laboratory by fermentation were used, as described in Section 2.2. Three samples of xanthan obtained from commercial sources were used: xanthan gum packaged and distributed by Farmaquímica Industrial Ltda (Part Number: 200708A-N05, Brazil), xanthan produced by Sigma–Aldrich (Part Number: 056K007, United States) and xanthan packaged and distributed by Aksy Comercial Ltda (Part Number: 200805BG02, Brazil), here codified as Xc-A, Xc-B and Xc-C, respectively.

2.1.3. Reagents

Analytical reagent grade materials were used in all of the experiments. The ultrapure water used to prepare all solutions was obtained in a Direct-Q 3 Water Purification System (Millipore Corporation, Bedford, MA, USA), with a resistivity of 18.3 MΩ cm. All glass apparatus was washed and subsequently immersed in 10% (v/v) HNO_3 for 48 h and then rinsed with ultrapure water prior to use. Working reference solutions of Na, K, Ca and Mg were prepared in acid, as the samples were, by serial dilution of a stock solution containing 1000 mg L^{-1} (Fluka, Buchs, Germany). The following reagents were used for the digestion and calcination of samples: nitric acid, hydrochloric acid (Synth, Brazil) and perchloric acid (Vetec, Brazil). The nitric acid and the hydrochloric acid used in this work were further purified by distillation in a sub-boiling quartz system (MA-075 from Marconi, Piracicaba, SP, Brazil). Potassium chloride (Synth, Brazil) and cesium chloride (Sigma–Aldrich, Germany) were used as ionization buffers.

2.1.4. Apparatus and instrumental parameters

A heated digestor block (MA-4025 from Marconi, Piracicaba, SP, Brazil) was used for acid digestion of the sample. All samples were weighed using an Ohaus Adventurer analytical balance (Model AR 2140, Pine Brook, NJ, USA) with a resolution of 0.1 mg and tare maximum of 210 g.

2.1.4.1. Flame photometer. A flame photometer (Model B462 Micronal, São Paulo, SP, Brazil) was used for sodium and potassium determination. The analyses by flame atomic emission spectrometry (FAES) were performed under the following conditions: 5 mL min^{-1} sample volume, 8 s settling time of reading, air (9 L min^{-1}) at a pressure of 1 kgf cm^{-2} and butane gas flame (liquefied petroleum gas).

2.1.4.2. Atomic absorption spectrophotometer. Calcium and magnesium were determined in xanthan samples using an atomic absorption spectrophotometer (Model AA-6300 Shimadzu, Japan) equipped with a deuterium arc background correction by flame atomic absorption spectrometry (FAAS). An air-acetylene flame was used for all determinations. The spectrometer was operated under the following conditions: flame mode with a Ca and Mg hollow cathode lamp source, wavelengths of 422.7 nm for Ca and 285.2 nm for Mg, a spectral band pass of 0.7 nm for both elements. The lamp current used was 10 mA for Ca and 8 mA for Mg.

2.2. Methods

2.2.1. Xanthan production

The xanthan was produced by fermentation in a submerged culture. The process was performed in a bioreactor (BioStat® B Braun Biotech International) with a 7 L production medium (Universidade Federal de Pelotas. WO/2006/047845, 2005) under two pH conditions: uncontrolled pH and pH 9 controlled by addition of 2 mol L^{-1} NaOH.

The resulting fermented broths were thermally treated at 121 °C for 15 min, and the polysaccharides were dried at 56 °C after being recovered by precipitation with 96% ethanol. The xanthan samples were dried until they reached constant weight and then were powdered to particle size using 60-150 mesh and will be referred to as Xp-pH uncontrolled and Xp-pH 9.

2.2.2. Sample treatment

2.2.2.1. Calcination. To validate the proposed procedure, all samples were prepared by the method in current use, and the results were compared by statistical analysis. Two hundred milligrams of xanthan was calcinated in a muffle furnace at 550 °C to form ash. The duration varied from 5 to 8 h. The ashes formed were treated with 6 mL of aqua regia (1HNO_3:3HCl, v/v), and resulting salts were diluted in 50 mL of 1% HCl solution (ASTM D1428-64, 1981).

Two calcination procedures were performed in order to verify the accuracy of the results and identify possible sources of error: calcination in a Pyrex® beaker and calcination in a Chiarotti® porcelain crucible. To this end, Na and K were determined.

2.2.2.2. Acid digestion. Two procedures were performed to decompose the samples using acid digestion. In the first procedure, two xanthan samples (Xc-B and Xc-C) were treated only in the presence of HNO_3. Approximately 100 mg of each sample was weighed into glass digester flasks, and 5 mL of concentrated HNO_3 was added. The mixture was heated in a digester block at 100 °C for about 3 h. After cooling at room temperature, the samples were diluted with

ultrapure water to a final volume of 50 mL using a volumetric flask, with subsequent dilutions appropriate for each analyte for subsequent analysis when necessary. In the second procedure, all the samples were treated as previously described. However, after 2 h of heating in the presence of HNO_3 (at which point only the material that is most difficult to oxidize remains), the mixture was cooled at room temperature, and 2 mL of $HClO_4$ was added carefully to promote more efficient oxidation of organic material. The mixture was again heated at the 100 °C for more 1 h, and the complete digestion was observed with total removal of the organic material. The samples were diluted with ultrapure water to a final volume of 50 mL using a volumetric flask after cooling at room temperature, and were diluted to appropriate levels for each analyte for subsequent analysis.

2.2.3. Procedure

Calibration curves for Na and K determination were used with concentration ranges of 0.5–10 mg L^{-1} and 0.2–0.8 mg L^{-1}, respectively. To minimize ionization interference, 0.1% (m/v) cesium chloride was added to the calibration solutions and samples for K determination, second manufacturer's recommendations. Concentration ranges of 0.5–3 mg L^{-1} and 0.1–0.6 mg L^{-1} was used to obtain calibration curves of Ca and Mg, respectively. Potassium chloride (0.1%, m/v) was added to all samples and calibration solutions to minimize ionization interference, according to manufacturer's recommendations. Calibration curves were obtained for all analytes used in this work, and all solutions used to generate these curves were in the same media later used in sample decomposition.

2.2.4. Statistical analysis

All experiments were performed in triplicate with three readings each, and the results were submitted to statistical paired *t*-tests (*t*-tests with paired data) with a 95% limit of confidence and also to variance analysis, with a comparison of means using the Tukey test at the 5% significance level.

3. Results and discussion

3.1. Preliminary studies

3.1.1. Evaluation of conventional procedure for xanthan sample preparation

In the initial stages of this work, the accuracy of the conventional calcination procedure was verified and possible sources of error were identified. The calcination times for different samples varied; this is probably due to differences in the chemical composition of the xanthan because the stability of xanthan depends on the molecular conformation, the rigidity of the cellulosic chain and the composition of the side chain (Born et al., 2002). The results and summary statistics (Table 1) show a significant difference between the results obtained for calcination in a porcelain crucible and calcination in a beaker (Pyrex®). The samples of xanthan calcinated in porcelain crucibles had higher concentrations of Na and K compared to samples calcinated in beakers with the exception of sample Xc-B, which had statistically similar values of K concentration. This exception can be explained by the high standard deviation in the measured potassium concentrations for this sample.

To judge the accuracy of the results, a comparison of the methods was performed through paired *t*-tests with a confidence level of 95%, and the Na concentrations of the xanthan samples yielded a value of $t > t_{crit}$. This result verifies that the two procedures provide different results. The K determination showed a value of $t < t_{crit.}$ within a 95% confidence, indicating statistically similar results. However, comparing the results with the mean comparison (Tukey, $p < 0.05$), there is a significant difference.

Table 1
Analytical results for Na and K determination by flame atomic emission spectrometry after calcination.

Analyte	Sample	mg g^{-1}	
		Porcelain crucible	Beaker
Na	Xc-A	34.2 ± 0.24[a]	31.2 ± 1.49[b]
	Xc-B	55.8 ± 0.08[a]	50.5 ± 1.11[b]
	Xc-C	34,8 ± 0.45[a]	29,6 ± 0.41[b]
	Xp-pH 9	74.4 ± 0.42[a]	65.0 ± 0.47[b]
	Xp-pH uncontrolled	3.05 ± 0.16[a]	1.99 ± 0.02[b]
K	Xc-A	2.03 ± 0.13[a]	1.41 ± 0.15[b]
	Xc-B	56.4 ± 1.91[a]	54.8 ± 0.52[a]
	Xc-C	3,07 ± 0.12[a]	2.46 ± 0.02[b]
	Xp-pH 9	29.2 ± 0.47[a]	26.3 ± 0.41[b]
	Xp-pH uncontrolled	54.3 ± 0.97[a]	42.4 ± 0.51[b]

[*] Values = means ± SD; $n = 9$.
[**] Lines with different letters are significantly different by Tukey test ($p < 0.05$).

The differences between the two sample preparations may be a result of interaction between the sample and the crucible material (Kebbekus, 2003; Magalhães et al., 2008). Porcelain is made from clay, which contains the elements of interest (Na and K) (Kamseu et al., 2007) and overestimation of these elements in the samples is certainly the result of contamination with the porcelain crucibles during the calcination process. Porcelain is a porous material and can form active sites through interaction with acid during the decontamination process, making it difficult to clean between calcination steps and resulting in a memory effect (Kebbekus, 2003). Therefore, it is recommended that the conventional calcination procedure of xanthan samples is carried out in high-quality Pyrex® glass beakers.

3.1.2. Optimization of digestion in acid

In order to develop a methodology for the sample preparation by acid digestion, preliminary studies were performed with the aim of completely decomposing the samples. It was attempted to digest the samples Xc-B and Xc-C using nitric acid only and then compared the results for Na and K determination with the conventional calcination decomposition method performed in a beaker.

The results presented in Table 2 were submitted to statistical analysis, which verified that the different procedures gave significantly different results. The acid digestion yielded lower values for both analytes because the cellulosic main chain is highly resistant to hydrolysis and xanthan samples presented thermal stability against hydrolysis under certain conditions, which may be related to its chemical composition; specifically, the presence of salts, side chain size and composition (Born et al., 2002; Challen, 1994; Katzbauer, 1998). The thermal stability of xanthan solutions is superior to many other water-soluble polysaccharides and polymers in general (Katzbauer, 1998).

Chemical degradation of xanthan can be achieved using strong oxidants at high temperature (Born et al., 2002). Differences in chemical composition of the xanthan samples influence the stability of the molecule. The stability of xanthan is increased when the molecule is in an ordered conformation (Born et al., 2002; Katzbauer, 1998; Pelletier et al., 2001; Smith, Symes, Lawson, & Morris, 1981), and the double helix confers resistance against degradation by acid (Born et al., 2002) because it impedes depolymerization (Katzbauer, 1998). Some authors suggest that the side chain protects the linkages β-1,4 on the main chain against hydrolysis and subsequent loss of viscosity (Challen, 1994). Acetyl groups tend stabilize the ordered form, while pyruvate groups tend to favor the disordered form (Pelletier et al., 2001; Smith et al., 1981). Therefore, xanthan samples with different amounts of these substituents have different resistances against degradation.

Table 2
Analytical results for xanthan samples after treatment with acid digestion in nitric acid and calcination by flame atomic emission spectrometry.

Analyte	Sample	Acid digestion		Calcination in beaker	
		mg g^{-1}	RSD (%)	mg g^{-1}	RSD (%)
Na	Xc-B	30.7 ± 1.16	3.8	50.5 ± 1.07	2.1
	Xc-C	19.1 ± 0.29	1.5	29.6 ± 0.41	1.4
K	Xc-B	33.3 ± 0.82	2.4	54.8 ± 0.52	1.0
	Xc-C	1.77 ± 0.01	0.3	2.46 ± 0.02	0.8

*Values = means ± SD; n = 9; RSD = relative standard deviation.

According to Kebbekus (2003) and Flores, Krug, Barin, and Arruda (2008), some substances are difficult to degrade with a single acid. Therefore, a combination of two or more acids may be used in order to take advantage of interactions in the acid mixture that generate chemically unstable intermediates that considerably accelerate decomposition. It is very difficult to obtain complete oxidation using only nitric acid because of its very low boiling point (120 °C). This low boiling point limits its oxidative efficiency because high temperatures are required to break carbon–carbon linkages. Hydrochloric acid also was evaluated together with nitric acid, but the results do not showed improvement. Perchloric acid has high oxidizing power but, when used alone, it presents an explosion risk and should be used in combination with other acids for safety reasons. Thus, perchloric acid is generally used after the addition of nitric acid (Flores et al., 2008) as proposed in the procedure developed for xanthan sample mineralization, the results of which were described below.

3.2. Sodium and potassium determination

The calibration curves for these analytes studied show good linear correlation coefficients (R > 0.99) independent of the method used for sample preparation. The limits of detection (LDs) were calculated as 3 times the standard deviation of 10 measurements of the blank on the sensitivity curve and were calculated for each method (Table 3).

A comparison of the acid digestion and calcination methods was performed to obtain information about the accuracy of the results. As shown in Table 4, similar results were achieved using the two approaches. The results were analyzed using statistical paired t-tests and the concentration measured for both elements using acid digestion compared with calcination showed no significant differences ($t < t_{crit}$, accepting the null hypothesis) between the results at the 95% confidence level, indicating that acid digestion is an appropriate sample preparation method.

As shown above, the difference between the measured analyte concentrations between the acid digestion and calcination methods was less than 5%, indicating good agreement between results obtained using these different sample preparation procedures. The relative standard deviations (RSD) were lower than 5% for all measurements except for the calcinated Xc-A concentration of K, which was high because of the low K concentrations in the sample.

The functionality of the xanthan gum is a direct consequence of its unique chemical structure (Born et al., 2002; Challen, 1994; Rinaudo, 2001). The rigid ordered conformation is responsible for the rheological properties of the molecule (Capron et al., 1997), and the organized state is stabilized by salts (Born et al., 2002; Katzbauer, 1998; Rinaudo, 2001); therefore, the presence of salts is necessary. According to García-Ochoa et al. (2000), commercial xanthan is about 3.6–14.3% (m/m) monovalent salt. Our results show that the commercial samples Xc-A and Xc-C are not within the specified limits, with values below the estimated 3.18 and 3.12% (m/m), respectively. However, these values are close to the expected minimum. The other samples had values within the limits specified by the literature: Xc-B has 10.18%, Xp-pH 9 8.96% and Xp-pH uncontrolled 4.50% (m/m).

Both commercial xanthan and that produced by *X. arboricola* pv pruni strain 101 showed significantly different results for Na and K content as assessed by the Tukey test at the 5% significance level with the exception of commercial samples Xc-A and Xc-C, which yielded similar results for Na content as seen in Table 3. This is a consequence of operational conditions, such as fermentation medium composition and pH control. Xanthan Xp-pH 9 presents a higher content of Na; this may be due to the large volume of NaOH added to maintain this pH condition during fermentation. As expected, also was observed that the xanthan produced at uncontrolled pH has the lowest concentration of Na. Our results agree with the findings of Borges, Paula et al. (2009a). These authors found that the Na content in xanthan produced by strain 106 of *X. arboricola* pv pruni varied with the volume of NaOH used to maintain the pH and with the stirrer speed. Specifically, Na content increased with increased stirrer speed and pH.

3.3. Calcium and magnesium analysis

The detection limits (LDs) were calculated as described above and are presented in Table 5. Each method presents different limits of detection as a result of different values of the blank sample. Good linearity (R > 0.99) was obtained for the calibration curves of Ca and Mg from both sample preparation methods (acid digestion and calcination).

Table 6 shows the analytical results obtained for the two analytes in xanthan from both methods of sample treatment. Applying the statistical paired t-tests at a confidence level of 95%, the sample preparation procedures of acid digestion and calcination did not present significant differences in determination of the analytes studied here. In other words, the two procedures provide similar results for Ca and Mg content in xanthan. Therefore, xanthan sam-

Table 3
Figures of merit for the determination of Na and K in xanthan by flame atomic emission spectrometry after treatment with acid digestion and calcination.

Analyte	Range (mg L^{-1})	Acid digestion			Calcination		
		as (mg L^{-1})	LD(mg g^{-1})	R	as (mg L^{-1})	LD(mg g^{-1})	R
Na	0.5–10	0.1402	0.011	0.9974	0.1322	0.011	0.9982
K	0.2–0.8	1.3300	0.004	0.9984	0.9750	0.004	0.9998

*Range: concentration range of the calibration solutions; a: slope of the calibration curve; LD: limit of detection in the measuring solution; R: correlation coefficient of the calibration curve.

Table 4
Analytical results for xanthan samples, obtained with different sample preparation methods, by flame atomic emission spectrometry.

Analyte	Sample	Acid digestion		Calcination	
		mg g^{-1}	RSD (%)	mg g^{-1}	RSD (%)
Na	Xc-A	30.5 ± 0.14^{c}	0.5	31.2 ± 1.49^{c}	4.8
	Xc-B	48.1 ± 1.55^{b}	3.2	50.5 ± 1.07^{b}	2.1
	Xc-C	28.7 ± 0.25^{c}	0.9	29.6 ± 0.41^{c}	1.4
	Xp-pH 9	64.2 ± 0.78^{a}	1.2	65.0 ± 0.47^{a}	0.7
	Xp-pH uncontrolled	1.93 ± 0.03^{d}	1.5	1.99 ± 0.02^{d}	1.0
K	Xc-A	1.35 ± 0.05^{e}	3.8	1.41 ± 0.15^{e}	10.4
	Xc-B	53.7 ± 0.30^{a}	0.6	54.8 ± 0.52^{a}	1.0
	Xc-C	2.49 ± 0.02^{d}	0.8	2.46 ± 0.02^{d}	0.8
	Xp-pH 9	25.4 ± 0.24^{c}	0.9	26.3 ± 0.41^{c}	1.6
	Xp-pH uncontrolled	43.1 ± 0.13^{b}	0.3	42.4 ± 0.51^{b}	1.2

*Values = means ± SD; $n = 9$.
**Columns with different letters to each element are significantly different by Tukey test ($p < 0.05$).

Table 5
Figures of merit for the determination of Ca and Mg in xanthan by flame atomic absorption spectrometry after treatment with acid digestion and calcination.

Analyte	Range (mg L^{-1})	Acid digestion			Calcination		
		as (mg L^{-1})	LD(mg g^{-1})	*R*	*as* (mg L^{-1})	LD(mg g^{-1})	*R*
Ca	0.5–3	0.0808	0.45	0.9995	0.0850	0.11	0.9977
Mg	0.1–0.6	0.9817	0.0010	0.9986	1.9050	0.0004	0.9998

*Range = concentration range of the calibration solutions; *a* = slope of the calibration curve; LD = limit of detection in the measuring solution; *R* = correlation coefficient of the calibration curve.

ples can be treated by acid digestion for analyte determination and this procedure is simpler, easier, and requires less time than the conventional calcination method.

As demonstrated above, good rates of agreement were obtained for the results between the different sample preparation procedures, with differences of less than 10% for both analytes between values obtained by acid digestion and calcination. This difference is a result of the low concentrations of the analytes studied in the xanthan samples. For all samples and for both sample preparation procedures, the relative standard deviations (RSD) were below 5%, being considered a good RSD level.

The xanthan samples differed significantly in Mg content; however, only Xc-A and Xc-C showed values of Ca content higher than the detection limits, and there was a significant difference between the results. The total content of divalent salts for xanthan have been estimated to be in the range of 0.085 to 0.17% (m/m) (García-Ochoa et al., 2000), and both commercial samples and those produced by *X. arboricola* pv pruni presented different values from the literature. Xc-B has a total content of divalent salts below the expected value (0.024%, m/m) and in the other samples, the values found were above the expected values: Xc-A 1.16%, Xc-C 1.047%, Xp-pH 9 0.212% and Xp-pH uncontrolled 0.297% (m/m).

Xanthan is an anionic polysaccharide whose properties are influenced by the nature and concentration of salts (Cadmus et al., 1976; Carrington et al., 1996; Rinaudo, 2001). The presence of ionic material stabilizes the three-dimensional network of xanthan gum, thus promoting additionally stabilizing viscosity (Challen, 1994; Kierulf & Sutherland, 1988; Xie & Lecourtier, 1992) because the order–disorder temperature transition of the xanthan molecule is strongly dependent on the presence of electrolytes (Rinaudo, 2001; Xie & Lecourtier, 1992), such that the transition temperature is higher after the addition of salt. According Ross-Murphy et al. (1983) and Xie & Lecourtier (1992), divalent salts are more effective. On the other hand, a high salt concentration can cause a conformational change in the xanthan molecule, reducing the hydrodynamic volume and consequently decreasing the viscosity (Carrington et al., 1996; Pelletier et al., 2001).

The salt content can stem from the production media (residual), alkali added to maintain pH, or salts added after fermentation (Borges, Paula et al., 2009). Therefore, the high salt content in xanthan can be the result of an inadequate recovery process. The salt content is a very important parameter for controlling polymer quality, and this is why the development of a reliable and easy to

Table 6
Analytical results for xanthan samples, obtained different sample preparation methods, by flame atomic absorption spectrometry.

Analyte	Sample	Acid digestion		Calcination	
		mg g^{-1}	RSD (%)	mg g^{-1}	RSD (%)
Ca	Xc-A	10.9 ± 0.15^{a}	1.4	10.8 ± 0.06^{a}	0.5
	Xc-B	<LD		<LD	
	Xc-C	9.59 ± 0.16^{b}	1.7	10.0 ± 0.24^{b}	2.4
	Xp-pH 9	<LD		<LD	
	Xp-pH uncontrolled	<LD		<LD	
Mg	Xc-A	0.69 ± 0.02^{d}	2.9	0.67 ± 0.01^{d}	1.5
	Xc-B	0.24 ± 0.01^{e}	2.4	0.22 ± 0.01^{e}	2.6
	Xc-C	0.88 ± 0.01^{c}	0.7	0.89 ± 0.01^{c}	0.6
	Xp-pH 9	2.12 ± 0.03^{b}	4.0	2.01 ± 0.02^{b}	0.9
	Xp-pH uncontrolled	2.97 ± 0.08^{a}	2.9	2.91 ± 0.05^{a}	1.8

*Values = means ± SD; $n = 9$; <LD = below the detection limit.
**Columns with different letters to each element are significantly different by Tukey test ($p < 0.05$).

perform sample preparation method, as proposed in this work, is so important.

4. Conclusions

Considering the results obtained in our preliminary studies on the conventional calcination procedure, it is recommended that xanthan samples be prepared in high quality glass beakers. The procedure proposed for sample treatment by acid digestion using nitric acid and perchloric acid compares well with the more conventional calcination decomposition method for the purpose determining Na, K, Ca and Mg in xanthan. This is a fast, easy, simple, and reproducible method and requires half the amount of sample, avoids loss of volatile analytes and is less susceptible to contamination. This method for sample preparation by acid digestion is thus adequate for routine analysis of analytes studied in xanthan samples, resulting in precise and accurate values.

Acknowledgements

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for their research scholarship and to the Ministério da Agricultura, Pecuária, e Abastecimento (Mapa) and CNPq for their financial support.

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