http://dx.doi.org/10.5935/0103-5053.20150346

J. Braz. Chem. Soc., Vol. 27, No. 5, 919-924, 2016.
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0103 - 5053 $6.00+0.00

Article

Evaluation of the Use of a Reflux System for Sample Preparation of Xanthan Gum and Subsequent Determination of Ca, Cu, K, Mg, Na and Zn by Atomic Spectrometry Techniques

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The aim of this study was to evaluate the performance of a reflux system adapted in the digestion tubes in sample preparation of xanthan gum for subsequent determination of Ca, Cu, K, Mg, Na and Zn by using spectrometric techniques. Through the proposed method, the samples were digested with HNO_3 for 3 h in a digester block at 220 °C. The accuracy of the method was evaluated by comparing the results with another sample preparation method and by using recovery tests, which results vary between 83 and 103%. The efficiency of digestion was assessed and significant results were verified through residual carbon values, which were five times lower compared to conventional acid digestion in open system, with use of HNO_3 and $HClO_4$. The proposed methodology is a simple and accurate analytical strategy, which does not require the use of special equipment, neither a mixture of strong oxidizing acid in the sample preparation.

Keywords: xanthan gum, sample preparation, reflux system, atomic spectrometry techniques

Introduction

Xanthan gum is a heteropolysaccharide synthesized by several bacterial species of the genus *Xanthomonas* and has various applications in the food industry as well in the field of cosmetics and pharmaceuticals as a suspending agent, emulsifier and stabilizer due to its excellent rheological properties and non-toxic characteristic.[1-3]

When xanthan is added to aqueous solutions even in small quantities, it dramatically increases the viscosity of the medium and stabilizes it, which makes this polysaccharide economically viable. The industrial production of xanthan gum, as well as its commercialization and use, have progressively increased at an annual rate of 5-10%.[4]

As the salt content can affect the xanthan rheological properties, information about metals concentration (Ca, Fe, K, Mg, Na, Zn, etc.) are very important, and the appropriate and reliable method for the determination of metallic elements is necessary for a better characterization of xanthan gum samples.[5]

The methodology of sample preparation of xanthan and other polysaccharides for the elemental analysis by spectrometric techniques cited in the literature generally involves calcination, in order to prepare samples.[6-8] However, some authors recommend special attention in the sample preparation step of this procedure because of systematic errors, such as losses or analyte contamination, which are associated with the decomposition through drying in muffle furnace.[9,10]

According to the literature, an alternative decomposition procedure based on the sample treatment by acid digestion was developed and validated for determination of Na, K, Ca and Mg in different samples of xanthan gum, with accurate and precise results. This procedure involves heating the sample in the presence of mineral acids, i.e., combinations of nitric and perchloric acids for the complete digestion of the samples in an open system with conventional heating.[11] Although this method has advantages over the conventional method, the use of perchloric acid has some limitations. As this acid has

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oxidizing and explosive properties, it requires careful manipulation. Moreover, this reagent is now controlled by the Brazilian army, making its purchase more difficult and increasing the cost of analysis in Brazil.

Recently, the use of reflux systems in preparing the samples has been reported in the literature as a promising alternative to conventional methods of digestion acid. These systems allow the reflux and condensation of volatile species, avoiding the loss of analyte due to volatilization. Moreover, they combine the advantages of the wet-ashing technique, which are: sample masses and reagent volumes, minimal risk of explosion and application for mineralization of several types of samples.[12]

Therefore, Souza *et al.*[13] proposed a method based on the use of reflux system adapted in the digestion tubes for sample preparation of the xanthan gum and determination of volatile elements (Cd and Pb) by graphite furnace atomic absorption spectrometry (GF AAS). According to the authors, the samples were digested only with HNO_3 and the results for the analysis of respective analytes were satisfactory. Furthermore, this system has also been applied to other studies: determination of Hg in biological samples of fish;[14] Cd, Pb and Sn in meat samples;[15] As, Cd, Pb and Se,[16] Cu, Fe, Mn and Zn[17] in rice.

Based on the above considerations, the objective of this work is to evaluate the applicability of the method developed by Souza *et al.*[13] in sample preparation of xanthan gum for subsequent analysis of Ca, Cu, K, Mg, Na and Zn by atomic spectrometry techniques (flame atomic emission spectrometry, FAES; and flame atomic absorption spectrometry, FAAS). As these tecniques are based on the use of pneumatic nebulizer for introduction of samples and flame with atomizer, the complete digestion of the samples is an important parameter for an analysis without interferences (spectral and non-spectral), requiring particular attention in the sample preparation step. The analyses were performed in the presence of four different commercial xanthan samples and the results were validated through conventional acid digestion procedure.

Experimental

Apparatus and instrumental parameters

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. For the preparation of convencional acid digestion samples (HNO_3/$HClO_4$), a heated digestor block (MA-4025, Marconi, Piracicaba, SP, Brazil) was used, and for the preparation of acid digestion heating under reflux samples, a cold finger was introduced in each digester tube, which was adapted by Oreste *et al.*[14] in a recent study. Figure 1 shows the illustration of the reflux system used in this study. The system consists of a glass tube closed with a small glass tube (17 cm) and filled with cold water for the recirculation of the acids within the bigger tube (25 cm).

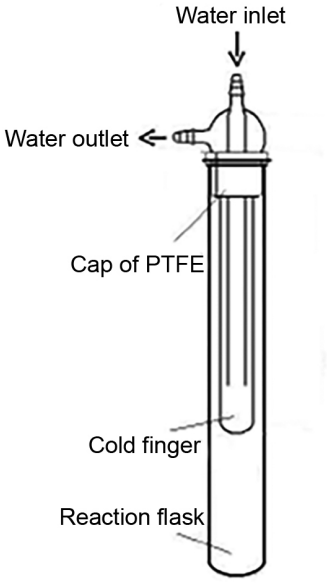

Figure 1. Schematic diagram of reflux system used in sample preparation procedure. Adapted by Oreste *et al.*[14]

For the determination of Na and K, a flame photometer (Model B462, Micronal, São Paulo, SP, Brazil) was used. The analyses by 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).

An atomic absorption spectrometer with flame model AA-6300 (Shimadzu, Kyoto, Japan) equipped with deuterium background correction was used for the determination of Ca, Cu, Mg and Zn in xanthan gum samples. An air/acetylene flame was used for all determinations. The spectrometer was operated using wavelengths of 422.7 nm for Ca, 324.8 nm for Cu, 285.2 nm for Mg and 213.9 nm for Zn. The lamp current used was 10 mA for Ca, 15 mA for Cu and Zn, and 8 mA for Mg.

A Shimadzu TOC-5000 total organic carbon analyzer (Shimadzu, Kyoto, Japan) was used for the determination of residual organic carbon content.

Reagents and samples

Analytical grade reagents were used throughout all procedures. The samples and standards were prepared

using high-purity water with a resistivity of 18.3 MΩ cm, which was obtained from a Direct-Q 3 water purification system (Millipore Inc., Bedford, MA, USA). Nitric acid (Synth, Diadema, SP, Brazil) was purified twice by sub-boiling in a MA-075 quartz system (Marconi, Piracicaba, SP, Brazil). All glassware was washed and subsequently soaked in 10% (v/v) HNO_3 for at least 48 h and then, rinsed three times with ultrapure water before use. Working solutions of Ca, Cu, K, Na, Mg and Zn were prepared daily by appropriate dilution of the stock solution containing 1000 mg L^{-1} (Fluka, Buchs, Switzerland) in ultrapure water. The following reagents were used for sample digestion: concentrated nitric acid (Synth, Diadema, SP, Brazil), 70% (v/v) perchloric acid (Vetec Química Fina, Duque de Caxias, RJ, Brazil) and 1% (m/v) cesium chloride (Sigma-Aldrich, St. Louis, MO, USA) were used as ionization buffer.

Four samples of xanthan gum obtained from commercial sources were used for the development of procedures of sample preparation and verification of the concentration of analytes: xanthan gum packaged and distributed by Farmaquímica Industrial Ltda (Part Number 200708A-N05, Porto Alegre, RS, Brazil); xanthan produced and distributed by CP Kelco U.S., Inc. (Kelgum 87, Part Number 010108N, Atlanta, GA, United States); xanthan packaged and distributed by Jungbunzlauer (Part Number 1645/11/06-FF250, Basel, Switzerland); and xanthan gum packaged and distributed by Sigma-Aldrich (Part Number 056K0007, St. Louis, MO, USA), here codified as Xc-A, Xc-B, Xc-C and Xc-D, respectively. In order to verify the applicability of the method in the analysis of the elements of interest, three xanthan pruni samples, provided by biopolimers laboratory (Universidade Federal de Pelotas, Capão do Leão, RS, Brasil) and produced by *Xanthomonas arboricola* pv. pruni strain 106 (pH 7.0) and 101 (pH 9.0 and 7.0) were used in this study, here codified as Xp-106-pH 7, Xp-101-pH 9 and Xp-101-pH 7, respectively. The fermentative process was carried out in a bioreactor (BioStat B, B. Braun Biotech International, Melsunger, Germany) at a 10 L vessel with 7 L of fermentation medium and the pH was controlled at 7 or 9 by addition of 2 mol L^{-1} NaOH.[18] The fermented broths were thermally treated at 121 ºC for 15 min and the polysaccharides were recovered by insolubilization with ethanol 96% (v/v), dried at 56 ºC until constant weight, and then powdered to particle size using 60-150 mesh. The xanthan pruni used in the experiments resulted from a mix of four fermentations performed. The polymers resulting from each fermentation were analyzed despite their production process and viscosity, and no significant difference at level of 5% was observed between them.

Sample preparation procedures

Acid digestion using reflux system

Based on the methodology described by Souza *et al.*,[13] xanthan gum samples were treated by acid digestion using an open system of the digester block with use of the reflux system (cold finger), which were cooled via refrigerated water attached to the tubes, the same schematic diagram presented in detail by Oreste *et al.*[14] in recent work. Approximately 100 mg of each sample was weighed directly into glass digester tubes and 5 mL of concentrated HNO_3 was added. The mixture was heated in a digester block at 220 °C under reflux for about 3 h. After cooling at room temperature, the solutions were filled up to 50 mL with ultrapure water for subsequent analysis. All the samples were digested in triplicate.

Conventional acid digestion

In order to evaluate the proposed procedure for the analysis of Ca, Cu, K, Mg, Na and Zn using flame with atomizer, all samples were treated by acid digestion based on the method described by Klaic *et al.*[11] and the results were compared by statistical tests. Approximately 100 mg of each sample was weighed into glass digester flasks, and 5 mL of concentrated HNO_3 was added. In this procedure, the mixture was kept in a digester block at 100 °C for about 2 h and after cooling at room temperature, 2 mL of 70% (v/v) $HClO_4$ was added to promote more efficient oxidation of organic material. Subsequently, the mixture was heated again at 100 °C for another 1 h. After cooling at room temperature, the solutions were filled up to 50 mL with ultrapure water for subsequent analysis. All the samples were digested in triplicate.

Procedure

Calibration curves for Ca, Cu, K, Na, Mg and Zn determination were used with concentration ranges of 0.5-2.0, 1.0-4.0, 0.2-1.4, 1.0-5.0, 0.1-0.4 and 0.5-3.0 mg L^{-1}, respectively. The standards were subsequently transferred to volumetric flasks and the volume completed with ultrapure water. To minimize ionization interference, cesium chloride (0.1%, m/v) was added to all xanthan samples and calibration solution for Ca and Mg determination, according to the manufacturer's recommendations. The analytical results were obtained by preparation of calibration curves using solutions in aqueous medium. The sample solutions were diluted with ultrapure water to be within linear calibration range. All experiments were performed in triplicate with three readings each, and the results were submitted to statistical paired *t*-test (*t*-test with paired data) at 95% of confidence level.

Results and Discussion

Evaluation of reflux system in sample preparation of xanthan gum

Previous studies have shown that only nitric acid was not efficient to obtain a complete decomposition of the xanthan gum samples in order to determine Na, K, Ca and Mg when the conventional digester block was used. However, it was possible when the mixture of nitric and percloric acid was employed, since chemical degradation of xanthan can be achieved using strong oxidants at high temperature.[11,19-21] Thus, a recent study showed satisfactory results for determination of Cd and Pb by GF AAS using the reflux system for sample preparation of xanthan gum in the presence of a single acid.[12] In this case, the author validated the methodology by recovery tests, which is sufficient because the GF AAS technique allows the elimination of the matrix during the pyrolysis step, not interfering in determining the analytes.

In this work, for evaluation of the use of a reflux system for sample preparation of xanthan gum and subsequent determination of Ca, Cu, K, Mg, Na and Zn using flame with atomizer, the methodology was validated by comparison of the digestion methods, as well as by recovery test. Furthermore, the efficiency of digestion samples was checked using a total organic carbon analyzer.

As shown in Table 1, similar results of concentrations for all elements were achieved and the two approaches of sample preparation methods reveal there was no significant difference with 95% confidence level for the analyte concentrations, using statistical paired tests ($t < t_{critical}$, accepting the null hypothesis), which indicates a good agreement between results obtained using these different sample preparation procedures. The relative standard deviations were lower than 9.2 and 8.5% for all measurements, using the conventional acid digestion and reflux system digestion, respectively. The results for the recovery test ranged from 83 to 103% for all analytes under study, showing a good efficiency of the method.

According to results shown in Table 2, the digester block coupled to a reflux system (cold finger), using only nitric acid as reaction medium, allowed a significant improvement in the oxidation of organic material when compared to the conventional system, with residual carbon values five times lower.

The efficiency associated with this method, allowing the chemical degradation of the samples with a single acid, was reached because this method involved the heating of the samples under reflux. Furthermore, it was possible to increase the temperature of the digester block (100 to 220 °C) above the boiling point of the acid medium, with this system.

In these processes, the acids used for digestion were also condensed by reflux, returning to the digestion flask,

Table 1. Analytical results for Ca, Cu, K, Mg, Na and Zn in xanthan gum samples after treatment using the conventional and reflux system by spectrometric techniques

Analyte	Sample	Conventional system / (mg g^{-1})	Reflux system / (mg g^{-1})
Ca	Xc-A[a]	9.4 ± 0.6 (6.4)	8.4 ± 0.3 (3.6)
	Xc-B[b]	0.46 ± 0.03 (6.5)	0.41 ± 0.01 (2.4)
	Xc-C[c]	0.24 ± 0.02 (8.3)	0.21 ± 0.01 (4.8)
	Xc-D[d]	0.21 ± 0.01 (4.8)	0.22 ± 0.01 (4.5)
Cu	Xc-A[a]	0.192 ± 0.004 (2.1)	0.208 ± 0.003 (1.4)
	Xc-B[b]	0.178 ± 0.001 (0.6)	0.176 ± 0.003 (1.7)
	Xc-C[c]	0.121 ± 0.005 (4.1)	0.130 ± 0.011 (8.5)
	Xc-D[d]	0.152 ± 0.002 (1.3)	0.155 ± 0.012 (7.7)
K	Xc-A[a]	1.4 ± 0.09 (6.4)	1.6 ± 0.02 (1.3)
	Xc-B[b]	14.4 ± 0.9 (6.3)	14.2 ± 0.5 (3.5)
	Xc-C[c]	27.9 ± 1.7 (6.1)	27.4 ± 0.9 (3.3)
	Xc-D[d]	44.2 ± 0.5 (1.1)	42.5 ± 1.6 (3.8)
Mg	Xc-A[a]	0.76 ± 0.03 (3.3)	0.79 ± 0.01 (1.1)
	Xc-B[b]	0.45 ± 0.02 (4.3)	0.45 ± 0.01 (1.1)
	Xc-C[c]	0.668 ± 0.003 (0.4)	0.656 ± 0.007 (1.1)
	Xc-D[d]	0.304 ± 0.004 (1.3)	0.298 ± 0.003 (1.0)
Na	Xc-A[a]	25.7 ± 0.5 (1.9)	26.7 ± 0.2 (0.7)
	Xc-B[b]	3.5 ± 0.2 (5.7)	3.6 ± 0.1 (2.7)
	Xc-C[c]	18.9 ± 0.1 (0.5)	18.4 ± 0.6 (3.3)
	Xc-D[d]	18.5 ± 1.7 (9.2)	18.1 ± 0.5 (2.8)
Zn	Xc-A[a]	0.177 ± 0.005 (2.8)	0.179 ± 0.007 (3.9)
	Xc-B[b]	< LOQ	< LOQ
	Xc-C[c]	< LOQ	< LOQ
	Xc-D[d]	0.100 ± 0.002 (2.0)	0.110 ± 0.005 (4.5)

Xanthan gum packaged and distributed by [a]Farmaquímica Industrial Ltda; [b]CP Kelco U.S., Inc.; [c]Jungbunzlauer and [d]Sigma-Aldrich. LOQ: limit of quantitation; average (n = 3) ± standard deviation; numbers in parenthesis are the relative standard deviation. LOQ for Zn is 0.004 and 0.003 mg g^{-1} for conventional and reflux system, respectively.

Table 2. Total content of residual carbon after acid digestion of samples of xanthan gum

Sample	Total residual carbon / %	
	Conventional system	Reflux system
Xc-A[a]	15.2 ± 0.4 (2.6)	2.9 ± 0.1 (3.4)
Xc-B[b]	18.2 ± 0.3 (1.6)	3.7 ± 0.1 (2.7)
Xc-C[c]	21.2 ± 0.5 (2.4)	4.7 ± 0.3 (6.4)

Xanthan gum packaged and distributed by [a]Farmaquímica Industrial Ltda; [b]CP Kelco U.S., Inc. and [c]Jungbunzlauer. Average (n = 3) ± standard deviation; numbers in parenthesis are the relative standard deviation.

Table 3. Figure of merit for the determination of Ca, Cu, K, Mg, Na and Zn in xanthan gum by using spectrometric techniques

Analyte	Range[a] / (mg L^{-1})	a[b] / (L mg^{-1})	R^{2c}	LOD[d] / (mg g^{-1})	LOQ[e] / (mg g^{-1})
Ca	0.5-2.0	0.042	0.999	0.016	0.052
Cu	1.0-4.0	0.094	0.999	0.001	0.004
K	0.2-1.4	0.375	0.998	0.017	0.056
Mg	0.1-0.4	1.069	0.999	0.008	0.025
Na	1.0-5.0	0.290	0.999	0.037	0.122
Zn	0.5-3.0	0.299	0.996	0.001	0.003

[a]Concentration range of the calibration solution; [b]slope of the calibration curve; [c]correlation coefficient of the calibration curve; [d]limit of detection in the measuring solution; [e]limit of quantification in the measuring solution. The linear function form is y = ax + b.

which increased its oxidizing power, eliminating the need for using perchloric acid.[12,15]

As shown below, the calibration curves in aqueous medium for all the species studied showed good linear correlation coefficients (R > 0.99). In this work, it was possible to use the external calibration, because the samples were prepared by acid digestion, resulting in a low residual carbon content, which minimizes any matrix effect on the sign of the analytes. The limits of detection (LOD) and quantification (LOQ) were calculated as being 3 and 10 times the standard deviation of ten measurements of the blank, divided by the slope of the calibration curve. Data are presented in Table 3.

Applications

Since the salt content is a very important parameter for controlling polymer quality, this method was applied to cations Ca, Cu, K, Mg, Na and Zn determinations on xanthan pruni samples produced under different conditions (Table 4).

Xanthan pruni samples were obtained under controlled pH, by adding 2 mol L^{-1} NaOH; therefore, the salt content had origin in the production medium and control pH solutions. The cations Ca, Mg and Zn were detected in low concentrations because salts with Ca and Zn were not used in the fermentation media and salts with Mg were used in low concentration. The higher sodium content in Xp-101-pH 9 than Xp-101-pH 7 and Xp-106-pH 7 is likely to be related to the NaOH solution used as pH controller. The higher the pH of the medium on the fermentative process, the greater the volume of NaOH solution used, and Mg is an important contaminant in this product.

Sodium content is mainly related to the control pH solution, whereas K is related to production media. Thus, as expected, Na contents found in Xp-106-pH 7 and Xp-101-pH 7 were similar, whereas higher sodium contents were found in Xp-101-pH 9. On the other hand, potassium content in this polymer was significantly reduced and changed by sodium, which is more electropositive.

Table 4. Analytical results for Ca, Cu, K, Mg, Na and Zn in xanthan gum samples after application of treatment using reflux system digestion by spectrometric techniques

Analyte	Sample	Reflux system digestion / (mg g^{-1})
Ca	Xp-106-pH 7[a]	0.35 ± 0.01 (2.9)
	Xp-101-pH 9[b]	0.36 ± 0.02 (5.6)
	Xp-101-pH 7[c]	0.30 ± 0.01 (3.3)
Cu	Xp-106-pH 7[a]	< LOQ
	Xp-101-pH 9[b]	< LOQ
	Xp-101-pH 7[c]	< LOQ
K	Xp-106-pH 7[a]	20.1 ± 0.6 (3.0)
	Xp-101-pH 9[b]	13.4 ± 0.6 (4.5)
	Xp-101-pH 7[c]	15.6 ± 0.4 (2.6)
Mg	Xp-106-pH 7[a]	1.19 ± 0.02 (1.7)
	Xp-101-pH 9[b]	2.19 ± 0.08 (3.7)
	Xp-101-pH 7[c]	1.88 ± 0.05 (2.7)
Na	Xp-106-pH 7[a]	20.2 ± 0.9 (4.5)
	Xp-101-pH 9[b]	44.7 ± 0.5 (1.1)
	Xp-101-pH 7[c]	27.9 ± 0.4 (1.4)
Zn	Xp-106-pH 7[a]	0.0060 ± 0.0001 (1.7)
	Xp-101-pH 9[b]	0.0131 ± 0.0004 (3.1)
	Xp-101-pH 7[c]	0.0061 ± 0.0001 (1.6)

Xanthan pruni samples provided by biopolimers laboratory (Laboratório de Biopolímeros, Universidade Federal de Pelotas, Capão do Leão, RS, Brasil) and produced by *Xanthomonas arboricola* pv. pruni strain: [a]106 (pH 7.0); [b]101 (pH 9.0); and [c]101 (pH 7.0). LOQ: limit of quantitation; average (n = 3) ± standard deviation; numbers in parenthesis are the relative standard deviation.

There is not an official preconization for salt content in commercial xanthans, but a content of monovalent ions (Na^+, K^+) has been found from 3.6 to 14.3% (m/m) and divalent ions (Ca^{2+}, Mg^{2+}) between 0.085 and 0.170% (m/m).[5,21] All xanthan pruni samples produced and analised had values according to the range verified on

the technical literature to monovalent salts for xanthans. However, only Xp-106-pH 7 is in accordance with the values of bivalent salts, perhaps because the commercial xanthan is commonly produced at pH 7, which needs lower amounts of pH controller solution.[5]

Conclusions

Results found in this work are very relevant because the difficulties usually encountered in the xanthan gum digestion have been overcome, allowing the complete degradation of the samples in the presence of a single acid. Our studies demonstrated the applicability of the use of the reflux system adapted to the digester block. This system enabled the heating of samples under reflux, not presenting loss of concentrated nitric acid due to volatilization in the digestion step, which usually occurs in conventional procedures with open system. Consequently, fewer reagents are consumed, reducing the risk of contamination. Moreover, the elimination of the use of percloric acid was also possible, resulting in greater safety at work. The results found for Ca, Cu, K, Mg, Na and Zn were accurate and precise. Thus, this methodology is appropriate for routine analysis of analytes studied in xanthan gum samples.

Acknowledgments

The authors are grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing scholarships, and CNPq/CAPES (Projeto Casadinho No. 552197/2011-4) for the financial support and scholarships.

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Submitted: October 13, 2015
Published online: December 7, 2015

FAPERGS/CAPES has sponsored the publication of this article.