

Chemical Deacetylation Natural Xanthan (Jungbunzlauer®)

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Abstract: With the aim of adapting a method for removal of acetyl groups from xanthan, reactions of chemical deacetylation were carried out with natural xanthan (Jungbunzlauer®) with variations on the following parameters: biopolymer and alkali concentration (sodium and potassium hydroxide). The deacetylation reaction was performed at 25 °C for three hours. The proposed methodology was efficient to promote the deacetylation reaction. The viscosity of xanthan increased when the alkali concentration was higher in the deacetylation reaction. Xanthan concentration in the solution used in the deacetylation reaction did not influence the solutions viscosity, as similar results in both tested biopolymer concentrations (0.5 and 1%) were obtained for all experiments in different shear rates. Deacetylation reactions at 25 °C for three hours with sodium and potassium hydroxide in 0.01 mol.L⁻¹ showed a viscosity of 410 and 420 mPa.s at 10 s⁻¹ and acetylation degree 1.3 e 1.4%, respectively.

Keywords: *Natural xanthan, deacetylation, reaction parameters.*

Introduction

Polysaccharides are the most abundant organic polymers obtained by biosynthesis and available worldwide from different sources and with a variety of structures. Bacterial biopolymers are very important polysaccharides and they are produced by fermentation in reproducible conditions. Bacteria of the genus *Xanthomonas* are responsible for the biosynthesis of the extracellular polysaccharide denominated xanthan. The industrial use of this polysaccharide is a result from their physicochemical properties^[1].

The main chain of xanthan consists of β -D-glucose units linked at 1 and 4 positions, which provide rigidity to the molecule. The chemical structure of the main chain is identical to that of cellulose. The lateral chain is composed of β -D-mannose-(1 \rightarrow 4)- β -D-glucuronic acid-(1 \rightarrow 2)- α -D-mannose, linked to carbon 3 of the main chain glucose residues. The terminal unit of β -D-mannose can bring out residues of pyruvic acid linked in the positions 4 and 6. The acetyl group can be linked in the position 6 of the unit of α -D-mannose^[2,3]. Some external mannoses can still contain a second acetyl group^[4]. These groups can be linked in variable proportions to the lateral chain depending on the strain, the conditions of bacterial growth and the parameters that are used in the process for the production of this biopolymer^[5]. The lateral chain provides solubility in aqueous medium^[6,7] also the pyruvyl and acetyl groups play a moderate role on xanthan conformational stability and rheology in aqueous solutions^[8].

In spite of the xanthan molecule complexity possesses it is possible to modify its structure and consequently its properties. This biopolymer different functional groups which are susceptible to chemical modification. Alterations in the acetyl levels and pyruvate groups can affect the xanthan rheological characteristics^[9], although there are divergences in relation to the influence of these components in the biopolymer viscosity. Some authors consider that these groups increase the solution viscosity^[10-14], while others say that the acetyl and pyruvate do not alter this viscosity^[6,15-17].

Therefore, research is needed to elucidate the true role of these groups in the xanthan rheological properties, in order to identify new applications for this biopolymer.

According to published studies, which set out different methodologies for the xanthan deacetylation, it would be interesting to carry out an evaluation of the parameters for this reaction, trying an optimization of the reaction conditions in such a way that it contributes to the characteristics desired and mainly obtain less cost product. Coviello and collaborates^[18] utilized for removed acetyl groups 2.5 \times 10⁻³ M NaOH solution at room temperature for 3 hours. Dentini^[19] used the same parameters for deacetylation reaction. Tako and Nakamura^[14,20,21] in his works reports the use of 10 mM potassium hydroxide for 10 hours for removal the acetyl groups and Houryieh^[22] used this same reagent in the concentration of 0.025 M for 2.5 hours at room temperature.

Aiming at adapting a method for acetyl groups removal from xanthan and considering the rheological characteristics of natural xanthan, reactions of chemical deacetylation were carried out with variations of the following parameters: biopolymer and alkalis concentrations (sodium and potassium hydroxide).

Experimental

Biopolymer

Fine particles (200 mesh) of natural xanthan (Jungbunzlauer®) were used for all experiments.

Biopolymer purification

The xanthan solutions, 2% (w/v), were dialyzed under agitation in deionized water (changing water three times a day) during 48 hours at 4 °C, in a semi permeable membrane with cut-off 12.000-16.000 D and porosity of 24 Å. The biopolymer solutions were dried in a stove at 56 °C and the obtained material

was milled in a disc mill (Fritsch model Pulverisette) to a particle size of 0.5 mm. After dialysis, purified xanthan was considered standard to be used in the experiments.

Chemical modification

The methodology used in this article was based on studies of the following authors: Coviello^[18], Dentini^[19] and Tako and Nakamura^[14,20,21].

The deacetylation reactions consisted of biopolymer hydrolysis in alkaline conditions. The reactions were carried out with xanthan solutions (0.5 and 1 %) and sodium hydroxide and potassium hydroxide, in the following concentrations: 0.0025; 0.005 and 0.01 mol.L⁻¹.

Erlenmeyer flasks (250 mL) containing 125 mL of each solution, respectively, were incubated in an orbital shaker (New Brunswick Scientific, model Innova 4230) at 300 rpm. For all reactions was pre-set a time of 3 hours and temperature of 25 °C.

For all experiments, the solutions were neutralized with hydrochloric acid (2 mol.L⁻¹). The biopolymers were recovered by precipitation with ethanol 96 °GL in the proportion of 1:4 (v/v), dried in an oven at 56 °C, until reaching a constant weight and then milled with a disc mill (Fritsch model Pulverisette) to a particle size of 0.5 mm.

The samples of the deacetylated xanthan with sodium and potassium hydroxide were also dialyzed following chemical deacetylation as it was previously described, in order to verify a possible salts influence on the deacetylated biopolymers final viscosities.

Sodium and potassium determination

The samples for analysis of sodium and potassium ions of natural and dialyzed xanthan were prepared according to AOAC^[23]. Ions were measured according to ASTM^[24], by spectroscopy of flame emission technique in Photometer of Flame Cole Parmer Model 2655-00.

Viscosity analysis

The aqueous solutions viscosity of deacetylated xanthan in different conditions of reaction was compared to the dialyzed and non dialyzed natural xanthan samples. This analysis was used as a previous study for the determination of the best deacetylation conditions.

For determination of rheological characteristics, xanthan solutions 1% (w/v) in deionized water were agitated for 2 hours at room temperature, heated at 60 °C for 20 minutes, and finally left at room temperature for 24 hours^[25].

The xanthan solutions viscosity was measured in reometer rotating mode (HAAKE model RS150), at 25 °C. System of coaxial cylinders was used, sensor DG 41 and shear rate 0.01-100 s⁻¹.

Acetylation degree

The quantitative determination of acetyl groups of the dialyzed, non dialyzed and chemically modified natural xanthan was performed by colorimetric analysis, based on the work of McComb and McCreedy^[26], with modifications.

For the analytical curve, 0.1089 g of β-D-glucose pentaacetate was used, which were dissolved by heating in 5 mL of ethyl alcohol and the volume checked for 100 mL with deionized water. From this solution, 1, 2, 3, 4, 5, and 7 mL were transferred to volumetric flasks of 50 mL, and they were filled up with distilled water. Five milliliter aliquots of these aqueous solutions represent 60, 120, 240, 300 and 420 µg of acetyl. In a volumetric flask of 25 mL, 1 mL of

the solution of hydroxylamine hydrochloride 37.5% (w/v), 1 mL of the solution of sodium hydroxide 94% (w/v), 5 mL of the standard solution contained in each volumetric flask of 50 mL previously prepared, and 5 mL of acid methanol solution 70.4% (w/v) were added. The final volume was checked with ferric perchlorate solution. The stock solution of ferric perchlorate was prepared using 1.93 g of ferric chloride, 5 mL of concentrated hydrochloric acid and 5 mL of perchloric acid 70%, which were evaporated in hot water bath almost to get drying. After evaporation, the remaining 6 mL were transferred to a volumetric flask of 100 mL containing deionized water. Sixty milliliter of the stock solution were transferred to a volumetric flask of 500 mL and the final volume was filled up with cold methanol. The sample control was prepared with 5 mL of deionized water. After 5 minutes, the measurements were determined in spectrophotometer (Pharmacia Biotech Ultrospec 2000) in the maximum wavelength at 520 nm.

For the samples preparation, 0.1 g xanthan was dissolved with vigorous agitation in 25 mL of hydroxylamine hydrochloride solution, 37.5% (w/v) and 25 mL of sodium hydroxide 94% (w/v); 2 mL of this solution were transferred to a volumetric flask of 25 mL, after that, 5 mL of deionized water and 5 mL of acid methanol solution 70.4% (w/v) were added, and the flask filled up with ferric perchlorate solution. After 5 minutes the measurements were determined in spectrophotometer at 520 nm.

Pyruvate degree

The quantitative determination of pyruvate groups of the dialyzed, non dialyzed and chemically modified natural xanthan was obtained by colorimetric analysis.

For the development of the standard curve analytical, the pyruvate of sodium solution 0.1 mol.L⁻¹, were applied by dilution of solutions in concentrations of 0.01; 0.025; 0.05; 0.1; 0.2; 0.3; 0.4 and 0.5 mmol.L⁻¹. From those concentrations, 1 mL was transferred to test tubes. The control sample was prepared with 1 mL of distilled water. In each tube 1 mL of the solution 2,4-dinitrophenylhydrazine and 1 mL of distilled water were added, which were agitated and conditioned in a water bath at 37 °C for 10 minutes and right after they were cooled off. Then 5 mL of ammonium hydroxide 0.6 mol.L⁻¹ were added in each tube. The readings were determined in spectrophotometer (Pharmacia Biotech Ultrospec 2000) at 420 nm.

The hydrolysis of the samples were performed using xanthan solution 1% in 2 mol.L⁻¹ hydrochloric acid, in a hot water bath at 80 °C for 16 hours, then 2 mL of the hydrolyzed material were transferred to a test tube and 18 mL of distilled water were added. The control sample solution was prepared with 2 mL of hydrochloric acid 2 mol.L⁻¹ and 18 mL of distilled water. Brackets of 1 mL from the previous solution were transferred to test tubes, performing three repetitions. To each tube 1 mL of 2,4-dinitrophenylhydrazine solution and 1 mL of distilled water were added and agitated and later conditioned in a hot water bath at 37 °C for 10 minutes and cooled off. Soon afterwards, 5 mL of ammonium hydroxide 0.6 mol.L⁻¹ were added to each tube. The readings were obtained in spectrophotometer at 420 nm.

Infrared analyses

The infrared spectra of natural dialyzed xanthan, deacetylated with 0.01 mol.L⁻¹ of sodium hydroxide (0.5% of the biopolymer), were obtained by grinding it with potassium bromide (KBr) powder, spectrophotometer degree, then pressed into a disk. It was used the range of the electromagnetic spectrum between 4000-400 cm⁻¹ in a spectrophotometer FTLA 2000 BOOMEN in order to observe the functional groups.

Statistic analyses

All experiments were run in triplicates. The statistics analyses of the acetyl degree and pyruvate groups were analyzed by the Kruskal-Wallis and Tukey test at 5% significance level.

Results and Discussion

Sodium and potassium content

In order to increase solubility and viscosity, natural xanthan contains higher concentrations of sodium and potassium salts^[27,28]. The natural xanthan presented 2.82 e 1.98% of the sodium and potassium content respectively. Therefore, the natural xanthan used in this experiment was submitted to a purification process. The dialysed xanthan presented 2.56% of sodium and 1.65% of potassium content.

Viscosity evaluation

The xanthan aqueous solutions showed pseudoplastic behavior, that is, the viscosity decreased as the shear rate increased^[29].

Bradshaw et al.^[15] reported that the commercial xanthan (Keltrol®) pseudoplasticity, remained unaffected after chemical modification.

By means of the rheological analysis it was observed that the natural dialyzed xanthan viscosity decreased (170 mPa.s to 10 s⁻¹) in relation to the non dialyzed sample (430 mPa.s to 10 s⁻¹). According to Whitcomb^[30], the viscosity of the purified polysaccharide is lower than the non dialyzed natural xanthan viscosity. Borges^[31], observed a similar behavior by evaluating samples of natural xanthan from the same origin as that used in the present experiment. In this study it was possible to observe that the salts content have influence on the biopolymer viscosity, in the conditions tested. It can be observed that after the dialysis procedure, the viscosity decreased for all shear rate studied. The natural xanthan already has an adding of salts, just to show an increase of viscosity. In order to verify only the effect of the deacetylation reaction on the xanthan viscosity, all samples after deacetylation were purified by dialysis to remove salts.

Table 1 showed the viscosity of natural, dialyzed and deacetylated xanthan.

The aqueous solutions viscosity of chemically modified xanthan increased with the increment on the alkali concentration used in the deacetylation reaction, except in the deacetylated biopolymer with potassium hydroxide (0.0025 and 0.005 mol.L⁻¹), in which

the viscosity practically did not change. Therefore, it was defined that the best alkali concentration for the deacetylation reaction was 0.01 mol.L⁻¹ regardless of the alkali used. Similar results were found by Bradshaw and collaborators^[15], who used potassium hydroxide (0.015 mol.L⁻¹) for 3 hours at room temperature under nitrogen for removing the acetyl groups from the commercial xanthan (Keltrol®) similar to the methodology used in this experiment.

According to Table 1, it could be noticed that the xanthan concentration in the solution used in the deacetylation reaction did not influence in the solutions viscosity, as similar results in both tested biopolymer concentrations (0.5 and 1%) were obtained for all experiments in different shear rates.

In most of the tested conditions, the deacetylated and dialyzed xanthan showed higher viscosity in relation to the samples only dialyzed, demonstrating that the deacetylation under certain conditions can increase the xanthan viscosity.

The samples submitted to the initial and final dialysis process were compared to the samples which were only initially purified, in order to verify a possible salts influence on the deacetylated biopolymers final viscosities (Table 2).

It was verified that the xanthan aqueous solutions viscosity was maintained, proving that the data obtained in this study are due to the reaction of chemical modification to which the biopolymer was submitted. However, the deacetylated samples that were not purified showed a higher viscosity than the other sample, thus proving the influence of the salts onto the viscosity of natural xanthan.

Based on these results, biopolymer (0.5%) and alkali concentration (0.01 mol.L⁻¹) parameters were selected for deacetylation reaction.

Acetylation and piruvatation degree

Table 3 shows the variation of the acetylation degree and pyruvate groups among the samples of natural xanthan, dialyzed and dialyzed deacetylated in the following concentrations: potassium hydroxide (0.01 mol.L⁻¹), sodium hydroxide (0.01 mol.L⁻¹) and biopolymer (0.5 and 1 %).

The analysis of acetyl content in the samples shows that this content varied in the range of 4.1 to 1.3 %. Slonecker and Jeanes^[10] found 4.6% content for acetyl groups for the biopolymer synthesized by the *Xanthomonas campestris* strain NRRL B-1459, which is used in the industrial xanthan production. The same acetylation degree was found in the sample of natural xanthan (Taiyo Kagaku Co. Ltd.)

Table 1. Viscosity (mPa.s) of natural xanthan measured in aqueous solutions 1% (w/v) at 25 °C, in different deacetylation conditions: sodium and potassium concentration (0.0025, 0.005 and 0.01 mol.L⁻¹) and biopolymer concentration (0.5 and 1%).*

Biopolymers	Viscosity							
	Shear Rate (s ⁻¹)							
	10		30		60		100	
	0.5%	1%	0.5%	1%	0.5%	1%	0.5%	1%
X	430.0 ± 7.07	430.0 ± 7.07	180.0 ± 0.00	180.0 ± 0.00	110.0 ± 0.00	110.0 ± 0.00	76.9 ± 0.99	76.9 ± 0.99
Xd	170.0 ± 5.77	170.0 ± 5.77	67.5 ± 6.52	67.5 ± 6.52	41.0 ± 4.85	41.0 ± 4.85	29.8 ± 0.10	29.8 ± 0.10
XdD H ₂ O	170.0 ± 0.00	170.0 ± 0.00	63.6 ± 2.76	63.6 ± 2.76	37.7 ± 2.33	37.7 ± 2.33	26.5 ± 2.33	26.5 ± 2.33
XdD KOH a	290.0 ± 4.14	250.0 ± 0.00	115.0 ± 7.07	110.0 ± 0.00	64.8 ± 2.76	57.0 ± 0.85	44.6 ± 0.28	36.7 ± 0.00
XdD KOH b	280.0 ± 0.00	300.0 ± 0.00	97.1 ± 0.00	93.9 ± 0.00	50.7 ± 0.78	49.4 ± 0.00	32.4 ± 1.63	33.0 ± 0.00
XdD KOH c	420.0 ± 7.07	480.0 ± 0.00	160.0 ± 0.00	180.0 ± 0.00	82.0 ± 2.05	97.4 ± 0.00	51.1 ± 0.64	61.3 ± 0.00
XdD NaOH a	200.0 ± 0.00	170.0 ± 7.07	72.8 ± 0.35	67.4 ± 7.07	40.6 ± 0.07	41.1 ± 4.45	27.6 ± 0.00	29.4 ± 0.00
XdD NaOH b	250.0 ± 0.00	230.0 ± 7.07	92.5 ± 0.85	78.8 ± 0.98	47.7 ± 0.35	42.5 ± 1.77	31.2 ± 0.00	28.5 ± 1.13
XdD NaOH c	410.0 ± 0.00	390.0 ± 0.00	170.0 ± 0.00	160.0 ± 7.07	100.0 ± 0.00	98.5 ± 5.87	70.6 ± 0.00	70.3 ± 4.03

a: 0.0025, b: 0.005, c: 0.01 mol.L⁻¹; X: natural xanthan; Xd: natural dialyzed xanthan; Xd_{H₂O}: natural dialyzed xanthan submitted to 300 rpm during 3 hours, in aqueous solution (control sample); XdD: natural dialyzed deacetylated xanthan. * Values are the average of three determinations. Average ± standard deviation.

Table 2. Viscosity (mPa.s) at 25 °C of aqueous solutions at 1% (w/v) of deacetylated xanthan; alkali and biopolymer concentrations 0.01 mol.L⁻¹ and 0.5%, respectively.*

Biopolymers	Viscosity							
	Shear Rate (s ⁻¹)							
	10		30		60		100	
X	430.0	± 7.07	180.0	± 0.00	110.0	± 0.00	76.9	± 0.99
Xd	170.0	± 5.77	67.5	± 6.52	41.0	± 4.85	29.8	± 0.10
XD KOH c	860.0	± 7.07	340.0	± 5.77	180.0	± 4.08	120.0	± 1.41
XdD KOH c	420.0	± 5.77	160.0	± 4.07	82.0	± 2.06	51.1	± 1.00
XdDd KOH c	420.0	± 0.00	170.0	± 0.00	97.2	± 0.00	68.5	± 0.00
XD NaOH c	710.0	± 6.02	270.0	± 5.77	140.0	± 3.86	89.1	± 2.09
XdD NaOH c	410.0	± 0.00	170.0	± 0.00	100.0	± 0.00	70.6	± 0.00
XdDd NaOH c	410.0	± 5.77	160.0	± 4.57	96.8	± 2.33	67.8	± 1.41

c: 0.01 mol.L⁻¹; X: natural xanthan; Xd: natural xanthan dialyzed; XD: natural xanthan deacetylated (without dialysis); XdD: natural xanthan dialyzed deacetylated; XdDd: natural xanthan dialyzed deacetylated and readialyzed on final process. * Values are the average of three determinations. Average ± standard deviation.

by Tako and Nakamura^[14]. Bradshaw et al.^[15] evaluating natural xanthan originated from Keltrol Kelco[®], found 4.1% of acetyl.

When using sodium hydroxide there was a 67.5% reduction in the acetyl content in relation to the natural dialyzed xanthan and when using potassium hydroxide there was a 65% reduction (Table 3), the viscosity of these biopolymers was 410 and 420 mPa.s to 10 s⁻¹, respectively (Table 2). Therefore the use of sodium hydroxide for the removal of acetyl groups from xanthan would be more advantageous, because the use this alkali gave a higher reduction of the acetyl groups of biopolymer chain apart from this compound provide a low cost to be applied in the industry. Since the viscosities were similar for both alkalis used, and thinking to apply this method in the industry, the sodium hydroxide would be the chosen compound because of its low cost and even to show a greater reduction of acetyl groups. If we compare the methodology used in this study the methods used by other authors, we verified that other works^[14,20,21] utilized a higher reactions time than the using of nitrogen atmosphere, which implies higher cost of proceeding in the industry. In another recent work is used although it is used a reaction time a little lower also uses nitrogen that involves process costs^[22].

The natural xanthan used in this experiment presented 3.6% of pyruvate groups (Table 3). Holzwarth and Ogletree^[32] found 3.1% pyruvate content to the Keltrol[®] xanthan; however, for this same biopolymer, Bradshaw et al.^[15] found 4.3%. Shatwell and Sutherland^[33] verified 4.4% of pyruvate in the synthesized xanthan by *Xanthomonas campestris* pv *campestris* strain 646.

By evaluating the pyruvate of deacetylated xanthan it was found that the degree of pyruvate groups were not significantly affected by the deacetylation reactions, as it was illustrated on Table 3.

Acetylation degree vs. viscosity

Figure 1 shows the comparison between acetylation degree and viscosity of the natural, dialyzed and deacetylated xanthan.

The deacetylated xanthan presented the viscosity values (410 mPa.s to 10 s⁻¹) and acetylation degree (1.3%) and (420 mPa.s to 10 s⁻¹) and acetylation degree (1.4%) with sodium hydroxide and potassium, respectively (Table 2 and 3). Tako and Nakamura^[14] concluded that side chains of xanthan become more flexible after the deacetylation. Consequently, the reduction of acetyl residue contributes to the reduction of intramolecular association with the backbone, favoring the intermolecular association.

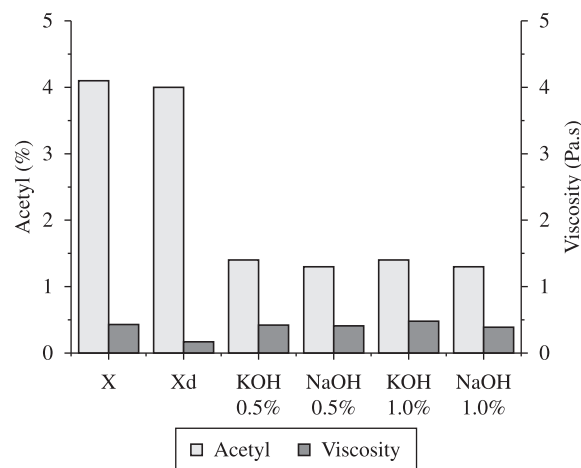
Infrared analyses

In Figure 2 illustrates the spectra of absorption of the natural xanthan, dialyzed and deacetylated with sodium hydroxide.

Table 3. Degree of acetyl and pyruvate groups (%) of natural xanthan, dialyzed and deacetylated in the concentrations: alkalis (0.01 mol.L⁻¹) and biopolymer (0.5 e 1 %).

Concentration biopolymers	Acetyl (%)	Pyruvate (%)
X	4.1 ± 0.06 a	3.6 ± 0.06 a, b, c, d
Xd	4.0 ± 0.11 a	3.7 ± 0.10 a
XCd KOH c 0.5%	1.4 ± 0.10 b	3.5 ± 0.06 b
XdD NaOH c 0.5%	1.3 ± 0.06 b	3.8 ± 0.10 c
XdD KOH c 1%	1.4 ± 0.11 b	3.7 ± 0.06 a
XdD NaOH c 1%	1.3 ± 0.06 b	3.5 ± 0.06 d

c: 0.01 mol L⁻¹; X: natural xanthan; Xd: natural xanthan dialyzed; XdD: natural xanthan dialyzed deacetylated. *Values following by same letters did not differ for the Tukey test in level of significance 5%.

**Figure 1.** Degree of acetylation (%) vs. viscosity (Pa.s) of the natural, dialyzed and deacetylated xanthan with sodium and potassium hydroxide (0.01 mol.L⁻¹) and biopolymer concentration (0.5 and 1.0%).

The technique of spectroscopic absorption vibration in the infrared was performed with the intention of proving the efficiency of the deacetylation reaction by the absence of absorption bands characteristic of certain functional groups.

The spectra of the xanthan samples present a strong and broad absorption band in 3416 cm⁻¹, considering the hydroxyl group (OH) in hydrogen bond and at 2922 cm⁻¹, in relation to the asymmetrical stretching of the group methylene (CH₂). The symmetrical stretching mode of the C-H links in the methyl (CH₃) group causes a band at 1373 cm⁻¹. However, at 1253 cm⁻¹ a band of symmetrical

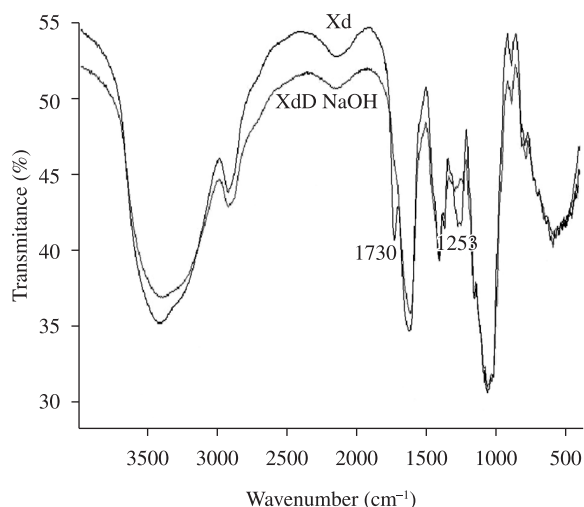


Figure 2. Infrared spectra, in KBr tablet: natural xanthan dialyzed (Xd) and dialyzed deacetylated with sodium hydroxide (0.01 mol.L^{-1}) (XdD NaOH) in the biopolymer concentration (0.5%).

stretching of the esters C-O bond was verified, and at 1618 cm^{-1} , for the asymmetrical stretching of the anion carboxylate.

The symmetrical stretching of the esters C=O bond is characterized by a band at 1730 cm^{-1} which is present in the spectra of the dialyzed xanthan. However, in the spectrum of the deacetylated xanthan with sodium hydroxide this band cannot be observed, proving the efficiency of the deacetylation reaction in the biopolymer. This also was verified in the chemical analysis of the deacetylated xanthan with sodium hydroxide where it was found the value 1.3% for the acetyl content. The methyl ester shows a band at 1250 cm^{-1} . This band can also be observed in the spectra of the dialyzed; however, the intensity is decreased in this deacetylated sample. The infrared spectroscopic provided the confirmation of the removal of the acetyl groups from the xanthan^[34], which was also detected through chemical analysis of the degree of acetyl groups (Table 3). The same behavior was observed when used the potassium hydroxide.

Conclusion

The proposed methodology was efficient in promoting the deacetylation reaction in comparing other with other literature methods. The deacetylation process changes the properties of the natural xanthan analyzed. The biopolymer viscosity and acetylation degree were influenced by alkali concentration and dialyses process. The treatment with NaOH and KOH 0.01 mol.L^{-1} at $25 \text{ }^\circ\text{C}$ for 3 hours and biopolymer concentration (0.5 and 1%) presented results similar. Using the concentration 0.01 mol.L^{-1} of the alkali was achieved the highest viscosity, 410 and 420 mPa.s at 10 s^{-1} , and deacetylation degree 1.3 and 1.4%, respectively, for the biopolymers without prior deacetylation. The infrared analyses corroborate for chemical analyses results.

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