Corncob as Carbon Source in the Production of Xanthan Gum in Different Strains *Xanthomonas* sp.

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Abstract: Xanthan gum (XG) is a biopolymer obtained in fermentation and used as a rheology control agent in aqueous systems and in stabilizing emulsions and suspensions. XG, together with other polysaccharides, can form soft, cohesive composite gels. The carbon source in the fermentative process is responsible for one-third of the production costs, and the search for less expensive and sustainable alternatives is ongoing. The use of agricultural residues such as the corncob is highly suggestive due to their abundance. This study aims to evaluate the use of derived hemicellulose fractions from the alkaline extraction of corncob as a carbon source in the production of XG in trials using four strains of *Xanthomonas* sp. (629, 1,078, 254, and S6). The results indicate that strain 629 provides the higher yield (8.37 ± 5.75 g L^{-1}) while using a fermentation medium containing a carbon source of saccharose (1.25%), hemicellulose fractions (3.75%), and salts. In this same medium, the strain 629 produces gum in 3% aqueous solution, showing the higher apparent viscosity ($9,298 \pm 31$ mPa s^{-1}) at a shear rate of 10 s^{-1} at 25 °C. In conclusion, corncob is proven to be a promising sustainable alternative carbon source in the obtention of XG, improving the economic viability of the process within a biorefinery context. Saccharose must, however, also be included in the fermentation medium.

Keywords: *Xanthomonas campestris*; XG; pre-treatment; hemicellulose fraction; biopolymer; corncob

1. Introduction

Xanthan gum (XG) is a biopolymer of great biotechnological interest, used as a rheological control agent in aqueous systems and as a stabilizer, thickener, and emulsifier in suspensions [1]. It is safe to use in the food industry and is a food additive regulated in Europe [2]. It is highly pseudoplastic and promotes the sensory qualities of food [1]. It has interesting properties: being nontoxic, having a non-sensitizing effect, being an immunologic agent, thermally stable, and dietetic (low in calories), and providing a good substrate for cell growth [1]. It has an estimated worldwide production of 30,000 tons per year and an increasing production rate of 5–10% per year [1].

The XG is normally produced from biosynthesis in fermentation processes by Gram negative obligate aerobic chemoorganotrophic bacteria of the genera *Xanthomonas* [3]. It

has applications in several different sectors of industry, such as food, pharmacy/medicine, agriculture, wastewater treatment, corrosion inhibition, cosmetics, and petrochemical [1,4]. More recently, XG properties have also been recognized as interesting in bioremediation, in the treatment of effluents [3], and as encapsulation agent in the slow release of agrochemicals in agriculture. While in water, XG behaves as a hydrogel, containing hydrophilic properties, being capable of retaining water and other biologic fluids [5]. Being that XG considered a natural polymer, it has biocompatible and biodegradable properties that extend its applications [6].

Worldwide, the main carbon source used in XG production is corn starch, representing a relatively high cost (1/3 of the cost) when compared with extraction from organic residues [7]. Corn starch is priced between $590 and $650 per metric ton [8]. According to Leela and Sharma [9], the highest yield of XG by glucose is 14.7 g/L^{-1}, and by sucrose is 13.2 g L^{-1}. In addition to competing with human food, XG is considered a costly gum [10] and research has been produced in the search for alternative, less expensive, and sustainable sources. Agro-waste and biomass with a lignocellulosic content have been recognized as the most cost-effective subtracts in XG production [11]. Examples of these are tapioca pulp [12], sugarcane bagasse [13], orange peels [14], kitchen waste [15], rice bran [16], chicken feathers [17], coconut shell, cocoa husk [11], potato crop [18], and winery wastewater [19].

Maize is the second most produced crop worldwide, with 1.1 billion metric tons produced in 2021, representing 21% of the global crop production between 2000 and 2019 [20]. Based on data previously obtained [21], we have calculated the weight of the corncob in 5.6% of the weight of the grain harvested, and therefore, we calculate the production of cob as a residual in 61.6 million metric tons worldwide. Corncob is a low-cost substrate with high potential to provide fermentable sugars (glucan, Xylan, arabinan) [22,23]. The extraction of different fractions (cellulose, hemicellulose, and lignin) from corncob can be an alkaline one [24]. The hemicellulosic fraction extracted by alkali becomes cheaper than using corn starch. In comparison to the cost of starch, sodium hydroxide production costs, on average, around $150 per metric ton [25]. Therefore, sodium hydroxide being the main cost to produce the hemicellulosic fraction, the reduction in costs becomes clear.

The use of the cellulolytic and hemicellulosic fractions for the production of XG is suggestive due to the high usable carbon content [26]. The factors influencing the production of XG are the species and strains of *Xanthomonas* and the fermentation parameters (aeration, stirring, pH, temperature, concentration and source of carbon and nitrogen, and the micronutrients available in the medium) [4,27,28]. The parameters influencing XG production, apart from the carbon sources, are also the micronutrients available during fermentation (potassium, iron, and salts) and nitrogen. Moreover, salt concentration also affects the chemical configuration of the XG molecules, therefore directly influencing its function [9]. The carbon sources are provided in concentrations ranging between two and five percent, once bacterial growth is inhibited with higher percentages [29,30].

The use of isolated strains in XG production is paramount to avoid productivity and quality inconvenience [31]. Several strains of *Xanthomonas* sp. are used in XG production, such as *X.* sp. 1537; *X. campestris pv. mangiferaeindicae* 1230; *X. campestris pv. campestris* 254; *X. campestris pv. arracaciae* 1198; *X. axonopodis pv. manihotis* 1182; *X. campestris pv. campestris* 1078; *X. melonis* 68; *X. campestris pv. campestris* 729; *X. campestris pv. campestris* 607; *X. campestris pv. campestris* 1167 [27], but many others can be found in the literature. *Xanthomonas* sp. bacteria do not produce significant amounts of exopolysaccharides other than xanthan; therefore, it achieves high XG conversions [32,33]. These strains show different productivities depending on factors such as shaking, temperature, fermentation time, carbon source, production method (batch or continuous), and nutrients provided [34]. Therefore, strain adjustment for the specific fermentation environment is important.

The present study aims to evaluate the potential of the hemicellulosic fractions from corncob, extracted via alkaline hydrolysis and used as a carbon, macro, and micro-

nutrient source in the production of XG, using different strains of *Xanthomonas campestris*: 629, 1078, 254, and S6. Corncob has never been used as a carbon source in XG production, therefore showing the interest in studying different strains of *Xanthomonas campestris*.

2. Materials and Methods

2.1. Raw Materials

The bacteria *Xanthomonas campestris*, namely its strains 254, 1078, 629, and S6 have kindly been provided by UFRPE (Federal Rural University of Pernambuco) for the Culture Collection of the Biomaterials Laboratory of the Institute of Technology and Research (LBMat/ITP), and have been used as a fermentation agent in the trials for XG production. The strains have been reactivated and stored at 4 °C in a solid medium of Yeast Malt (YM), formed by (g L^{-1}): yeast extract 3.0, malt extract 3.0, peptone 5.0, saccharose 10.0, and agar 20.0. The bacteria have been inoculated in petri discs at 28 °C for 24 h and stored at 4 ± 1 °C. The strains have been reactivated every 4 weeks.

The corncob samples are obtained after harvesting and grain extraction, and are dried at room temperature (25 °C) for 120 h. These are then ground in a knife mill to a 40 mesh granulometry, following the procedure recommended by Ruzene and co-authors [24].

2.2. Pre-Treatment for Fractionation

Five-gram samples of dried and ground corncob are treated with a 100 mL solution of NaOH 0.75 mol L^{-1} at 55 °C for 120 min. The sample is filtered through a nylon membrane, washed with deionized water, neutralized to pH 7.0, and then dried at 50 °C for 24 h. The liquors obtained after extraction are filtered and stored frozen at -20 °C up to the moment of being used.

2.3. Quantification of Composition of the Corncob and Liquid Fractions Obtained after Alkaline Pre-Treatment

The corncob is analysed for quantitative acid hydrolysis with sulfuric acid 72% *(w/w)* following NREL protocols [35]. The quantification of sugars and acetic acid is performed after the hemicellulosic fractions obtained from the alkali extraction and corncob are not treated. Samples are filtered in solid phase extraction Sep-Pak C18 Plus Short cartridges (Waters Corporation, Milford, MA, USA) and are analysed in High Performance Liquid Chromatography (HPLC) equipped with a refractive index detector and a column Aminex HPX-87H, mobile phase consisting of 0.005 mol L^{-1} H_2SO_4 and a flow rate of 0.6 mL min^{-1} at 45 °C [36].

2.4. XG Production

For the inoculum preparation, 125 mL Erlenmeyer flasks, with 14 mL of medium YM, are incubated at 28 °C for 24 h, shaken at 150 rpm. After 24 h, the cellular concentration is determined in a spectrophotometer at 560 nm for inoculum standardization. The cellular concentration has given readings of 10^{11} CFU/mL.

For XG production, 14 mL of inoculum topped with 86 mL of fermentation medium containing: a carbon source (5.0 g L^{-1}) and a salt solution of $NH_4H_2PO_4$ (2.5 g L^{-1}), KH_2PO_4 (5.0 g L^{-1}), H_3BO_3 (0.006 g L^{-1}), $(NH_4)_2SO_4$ (2.0 g L^{-1}), $FeCl_3$ (0.0024 g L^{-1}), and $CaCl_2.2H_2O$ (0.002 g L^{-1}) is used. The trials have been conducted at 180 rpm, at 28 °C for 96 h, and then have been evaluated after the presence or absence of the salt solution and the sources of carbon, as described in Table 1.

Table 1. Composition of the fermentative medium for production of XG used in this study: saccharose (%, *w/w*), hemicellulosic fraction (%, *w/w*), and addition or not of salts.

Fermentation Medium	Saccharose (%, *w/w*)	Hemicellulosic Fraction[1] (%, *w/w*)	Supplementation with Salts [2]
Medium 1	5.0	0	yes
Medium 2	5.0	0	no
Medium 3	1.25	3.75	yes
Medium 4	1.25	3.75	no
Medium 5	0	5.0	yes
Medium 6	0	5.0	no

[1] Compounds obtained after the alkali extraction of the corncob. [2] Salt solution composition: $NH_4H_2PO_4$ (2.5 g L^{-1}), KH_2PO_4 (5.0 g L^{-1}), H_3BO_3 (0.006 g L^{-1}), $(NH_4)_2SO_4$ (2.0 g L^{-1}), $FeCl_3$ (0.0024 g L^{-1}), and $CaCl_2.2H_2O$ (0.002 g L^{-1}).

2.5. XG Recovery

The production is evaluated by freeze-dried XG (weight dry) in relation to the fermented sirup (g L^{-1}), adapting the previously described methodology [21]. After the fermentation in each of the trials, the samples are centrifuged at 16,000× *g*, at 4 °C for 20 min. Then, the supernatant liquid 92.8% ethylic alcohol is added in the proportion of 1:4 (*v/v*) for precipitation and recovery of the gum. The XG is then dried in an oven at 50 °C up to constant weight.

The biomass is recovered, resuspended in distilled water, and centrifuged again in 2 mL microtubes and dried in an oven at 50 °C to constant weight. The production is calculated applying Equation (1).

$$P = \frac{1000m}{v} \tag{1}$$

where:

P = Production
m = final mass
v = supernatant volume

2.6. Analysis of the Apparent Viscosity in the Aqueous Solutions of XG

The XG samples are initially subject to dialysis in water for 72 h at 4 °C, with water exchange every 8 h. The dialyzed material is lyophilized for 72 h (Freeze Drier LIOTOP model L101, Liobras, São Carlos, Brazil) at −50 °C, following the same protocol followed by Karken and co-authors [21].

For the analysis of the apparent viscosity, the lyophilized samples are diluted in 3% distilled water, shaken for 1 h and later left at rest for 12 h, and at 4 °C for rheometer analysis. The apparent viscosity is evaluated in a rheometer Anton Paar (model H-TD200, Graz, Austria) using a parallel plate system, under a shear rate between 10 and 100 s^{-1} at 25 °C. The results are projected in graphs of apparent viscosity (mPa s^{-1}), function of the shear rate (s^{-1}).

2.7. Characterization of XG by Fourier-Transform Infrared Spectroscopy (FTIR)

Xanthan gum samples are analysed by Fourier transform infrared spectroscopy (BOMEM MB-100 FTIR from Oxford Instruments, Abingdon, Oxfordshire, UK). The wavelengths range between 500 and 4000 cm^{-1} as described by Gunasekar and co-authors [12].

2.8. Statistical Analysis

Data are subjected to analysis of variance (ANOVA) using the Assistat-Statistical Assistant® software, version 7.7. The post-hoc test used is the Tukey's, and the ANOVA residuals are analysed for deviances to normality via the Kolmogorov-Smirnov test. The significance limits are set to $p < 0.05$.

3. Results

3.1. Chemical Composition of Corn Cob and Hemicellulosic Fraction (Liquid Phase)

The composition of the pre-treated and untreated (*in natura*) corncob can be seen in Table 2. The composition is as follows based on three replicate determinations (% ± standard deviation,): 26.3 ± 1.1 cellulose; 25.2 ± 1.7 of hemicellulose; 34.9 ± 1.2 total lignin; 11.8 ± 1.9 soluble lignin; and 23.1 ± 1.3 of Klason lignin with a total composition of 88.4 ± 2.2. These values agree with those obtained by other authors [37,38] for corncob.

Table 2. Chemical composition (%, *w/w*) of *in natura* corncob and hemicellulosic fractions (liquid phase) obtained after alkaline pre-treatment.

Chemical Composition (%, *w/w*)	Corncob *In Natura*	Hemicellulosic Fraction
Cellulose	26.3 ± 1.1	10.5 ± 0.9
Hydroxymethylfurfural	0.23 ± 0.11	0.18 ± 0.11
Hemicelluloses	25.2 ± 1.7	48.8 ± 1.3
Furfural	1.8 ± 1.1	0.6 ± 0.11
Total Lignin	34.9 ± 1.2	-
Soluble Lignin	11.8 ± 1.9	14.3 ± 1.1
Klason Lignin	23.1 ± 1.3	-
Total Composition	88.4 ± 2.2	74.4 ± 1.9

After alkaline extraction, the hemicellulose fraction obtained is analysed to evaluate its composition. Table 1 shows that this treatment allows for the recovery of 10.5 ± 0.9 of Glucan (cellulose), 48.8 ± 1.3 of hemicellulose, and 14.3 ± 1.1 of Lignin. These values suggest that alkaline pre-treatment is an extraction methodology with great potential in the recovery and fractionation of fermentable sugars and of great industrial interest.

Other authors [39–41] suggest the implementation of the biorefinery concept for the recovery of lignocellulosic residues obtained from the agri-food industries, and thus achieve sustainable development based on a circular bioeconomy. In this sense, considering the chemical composition of corncob *in natura* and the products obtained after its fractionation by means of alkaline treatment, it is suggested that the other fractions obtained in this study, namely the solid fraction, have a great potential for the conversion of chemicals compounds or derivatives following the biorefinery concept.

3.2. Yields of XG Using the Different Carbon Sources and the Different Strains of Xanthomonas campestris (629, S6, 254 e 1078)

The strain 254 is the most promising considering both the culture and the medium conditions, reaching mean productions of 11.58 g L^{-1} in a medium with salt supplementation. These values are similar to those obtained by Leela and Sharma [9] (13.2 g L^{-1}) using sucrose (Figure 1A). This strain in medium without salt addition reaches a production of 8.43 g L^{-1} (Figure 1B). The production values obtained by the strain 254, in the present trials, are above those obtained by Gunasekar and co-workers [12], using cassava pulp (7.1 g L^{-1}). They are also above the values obtained by Rottava and co-workers [27], reaching 7.99, 5.90, 8.93, 9.49, and 9.67 g L^{-1}, in a trial with five different strains of *Xanthomonas* sp. (*X. axonopodis* 1182 and *X. campestris* 1167, 1230, 254, and 1078), using a medium with 5% saccharose as a carbon source supplemented with salts.
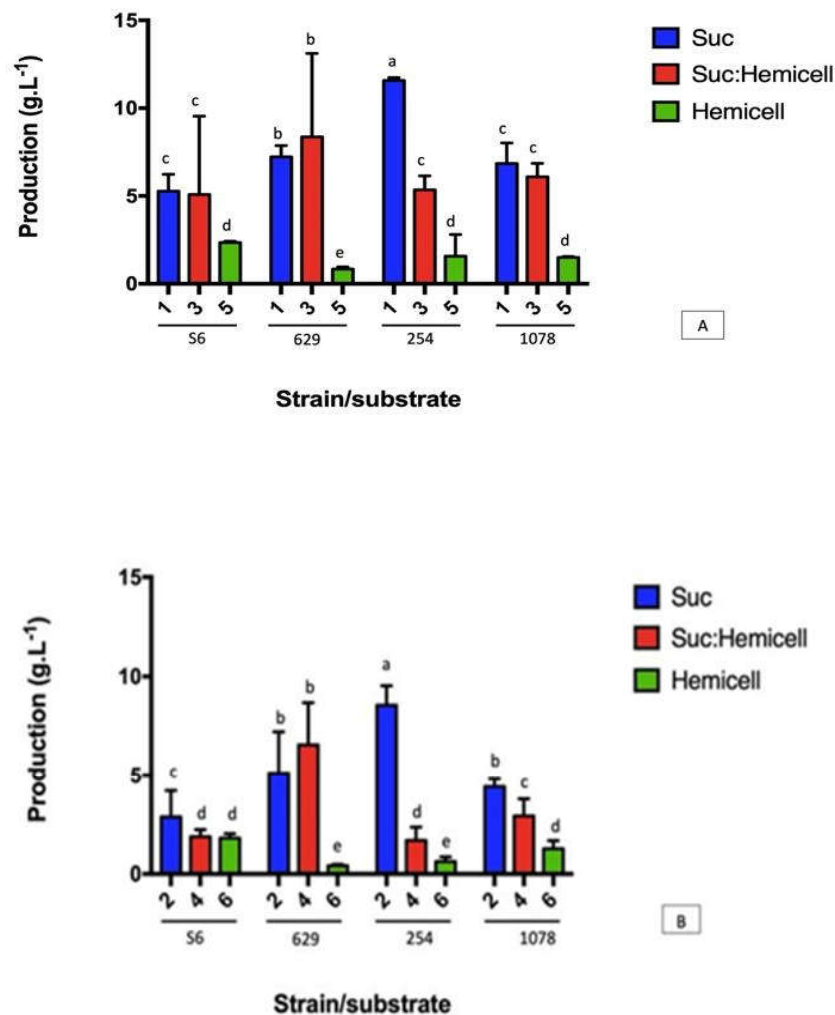
Figure 1. Yields of XG (g L^{-1}) obtained by fermentation, using four strains of *Xanthomonas* sp. (629, S6, 254 e 1078), and three different sources of carbon: 5% saccharose—Medium 1 and 2; hemicellulosic fraction (3.75%) with added saccharose (1.25%)—Medium 3 and 4; and hemicellulosic fraction only (5%)—Medium 5 and 6. (**A**) Salt added supplementation. (**B**) No salt supplementation. Different letters above the bars are indicative of significant differences ($p < 0.05$).

The most productive strain, considering a medium with hemicellulosic fractions, is the 629 with a mean yield of 8.37 g L^{-1} (medium 3 with 1.25% saccharose, 3.75% hemicellulosic fraction and added salt), followed by strain 1078 with 6.09 g L^{-1}. For medium 4 (same as 3 but no salt), the strain 629 reaches the best productive results (5.56 g L^{-1}). We can also infer that the salts addition has increased the XG bioproduction, independently of the strain used in the trial. Therefore, under the conditions of this study for production process optimization, the addition of salts is paramount. Similar results have been previously obtained by Luporini and Bretas [42].

According to Rottava and co-workers [27], the most promising culture medium for XG is saccharose supplemented with salts. However, the results from our study show viscosity values above those reported by them (maximum 1900 mPa s^{-1}), and similar ones for yield (9.67 g L^{-1}) with the *X. campestris* strain 1230, using saccharose as a carbon source

and adding salts. Such a fact highlights the potential of the corncob as a carbon source in the production of XG in bio fermentation processes.

The strain S6 is the most productive with a yield of 2.68 g L^{-1}, using medium 5 (5 % hemicellulosic fraction only supplemented with salts), which widens the possible sources of carbon in a biorefinery context to produce XG. The lower levels of production are also indicative of the need for some saccharose in the medium. Full yield results for the different combinations of strains and carbon source can be consulted in Table 3.

Table 3. Mean yields (g L^{-1}) of different Xanthan Gums produced in fermentation with different *Xanthomonas campestris* strains and carbon sources at 25 °C.

Strain	Medium (Carbon Source) [1]					
	1	2	3	4	5	6
S6	5.26 ± 1.14 [bcA]	2.88 ± 2.34 [cB]	5.08 ± 5.15 [bA]	1.88 ± 0.36 [cC]	2.34 ± 0.08 [aB]	1.81 ± 0,22 [aC]
629	7.23 ± 0.61 [bB]	5.02 ± 3.10 [cC]	8.37 ± 5.75 [aA]	6.56 ± 4.12 [bBC]	0.84 ± 0.11 [bC]	0.42 ± 0,067 [cD]
254	11.58 ± 0.16 [aA]	8.56 ± 1.18 [bB]	5.35 ± 0.93 [bC]	1.69 ± 0.67 [cD]	1.57 ± 1.23 [bD]	0.63 ± 1,23 [cD]
1078	6.84 ± 1.01 [bA]	4.42 ± 0.39 [cBC]	6.09 ± 0.76 [bAB]	2.93 ± 0.87 [cCD]	1.50 ± 0.05 [bD]	1.27 ± 0.421 [bD]

[1] Full composition of the medium (carbon source) can be found in Table 1. Means with different lowercase letters in columns and uppercase letters in rows are significantly different ($p < 0.05$).

The best yields in the absence of salts are those obtained with the strain 254 (8.56 g L^{-1}) using the medium with only saccharose as the carbon source (medium 2). These values are above those obtained by Gunasekar and co-workers [12] (4.5 g L^{-1}), using hydrolysed cassava pulp as the carbon source and using the *X. campestris* strain NCIM 2954.

The values (Table 3) are similar to those obtained by Brandão and co-workers [43] (7.23 g L^{-1}) using saccharose as the carbon source supplemented with salts. This fact justifies the predominance of mediums containing saccharose as the main carbon source in industrial processes, and at the same time, highlights the potential of the corncob as a sustainable alternative in bioprocesses for XG obtention.

The trials using minimum concentrations of saccharose (medium 4, 1.25% saccharose and 3.75% hemicellulosic fraction), have fair yields, especially in the strain 629 with a yield similar to that obtained in medium 2 (5% saccharose only and no added salt). The results obtained using the hemicellulosic fraction as the source of carbon, without added salt, are very satisfactory, having in mind exhaustive trials highlighting the need for saccharose as well as added salts in the biosynthesis of XG [44,45]. However, the yields obtained in this study are lower than those obtained by Mabrouk and co-workers [46] using sugar beet molasses as a carbon source (values between 11.5 g L^{-1} and 28 g L^{-1}).

3.3. Apparent Viscosity in XG Using Different Sources of Carbon and Different Strains of Xanthomonas sp. (629, S6, 254, and 1078)

As observed in Table 4, the higher levels of viscosity are obtained within medium 3 (1.25% saccharose, 3.75% hemicellulosic fraction and added salt), with exception for strain S6. The strain 629 achieves the highest viscosity (9298 mPa s^{-1}), closely followed by strains 254 and 1078 (both 9298 mPa s^{-1}). The lower viscosity levels are achieved using strain 629 in medium 2 (5% saccharose and no added salt). All the XG solutions obtained show pseudoplastic (non-Newtonian) behaviour, which has been recognized as a reason for its growing popularity [3].

Table 4. Means of the apparent viscosity (mPa s^{-1}) of aqueous solution of the different Xanthan Gums produced in fermentation with different *X. campestris* strains and carbon sources at 25 °C.

Strain	Medium (Carbon Source) [1]					
	1	2	3	4	5	6
S6	4869 ± 9 [aC]	2543 ± 11 [bE]	6599 ± 8 [cB]	4779 ± 6 [bC]	8790 ± 11 [aA]	3899 ± 12 [cD]
629	3292 ± 13 [dD]	1106 ± 4 [cE]	9298 ± 31 [aA]	7687 ± 13 [aC]	8710 ± 25 [abB]	8488 ± 8 [aB]
254	3711 ± 13 [cD]	3468 ±15 [aE]	9278 ± 13 [aA]	2870 ± 23 [cF]	8209 ± 20 [bB]	6572 ± 21 [bC]
1078	3711 ± 15 [bC]	3468 ± 13 [bE]	9278 ± 11 [bA]	2870 ± 15 [dF]	8209 ± 8 [cB]	3750 ± 14 [cD]

[1] Full composition of the medium (carbon source) can be found in Table 1. Means with different lowercase letters in columns and uppercase letters in rows are significantly different ($p < 0.05$).

The strain with the highest viscosity (8488 mPa s^{-1}) in the medium without addition of salt is 629 when only the 5% hemicellulosic fraction is used (medium 6), while in the case that only 5% sucrose is used for the fermentation process (medium 2), shows the lowest viscosity (1106 mPa s^{-1}). Such a fact reiterates the need for the optimization of physic-chemical processes, finding the right balance in bioprocesses to produce XG.

While comparing the viscosity achieved with and without added salt, for the same carbon source and strain, the viscosities achieved in the presence of salt is always higher. Carmona and co-workers [47] have also found these types of differences in viscosity while adding salt (NaCl) to the medium. The authors justify the differences with the electrostatic repulsion between the gum lateral chains, leaving it bounded with a very compact structure, resulting, therefore, in higher viscosity. In XG, the presence of salt promotes an ordered helix of its molecular structure, increasing the molar mass, and therefore the viscosity [7].

The viscosity differences obtained using the different strains may be explained based on the influence caused by the presence of salts in the medium and in the biochemical influences caused by the strain in use, impacting the molecular structure of the XG formed [3]. In this study, salt supplementation has had a decisive contribution in the optimization of production in all the strains used, due to the formation of strong ionic bonds [47]. The results obtained in this study have shown higher viscosity than those obtained by Rottava and co-workers [27], which has reported 260 mPa s^{-1} in 3% aqueous solutions, obtained in similar medium (5 % saccharose and added salts) and under the same shear rate. Under these same conditions, the different strains of *X. campestris* impact directly the production rates and the properties of the XG obtained. While using S6 strain, the higher viscosities are achieved (4869 mPa s^{-1}), but with lower production levels (7.23 g L^{-1}); while using 629 strain, lower levels of viscosity are obtained (3240 mPa s^{-1}).

The volatile fatty acids (acetic, butyric, and propionic) are main products of hemicellulose fermentation [48]. The production of XG using propionate salts (sodium, calcium, ammonium) shows the higher consistency index, followed by acetate and butyrate, respectively, in relation to the XG apparent viscosity obtained from other salts [49]. The higher consistency index promoted by the volatile fatty acids reduces the level of dissolved oxygen needed for the growth and cellular survivability in the medium after 30 h of fermentation time [49]. This explains the higher viscosity and the lower productivity once the fermentation processes occur in aerobiosis.

The rheological properties are an indicator of quality in XG [50,51]. The biotechnological potential of using hemicellulosic fractions associated with saccharose and salts are made evident in our study by the optimization of the XG viscosity. The potential use of the corncob is therefore emphasized as a carbon source in these fermentation processes.

3.4. Characterization of XG by Characterization of XG by Fourier-Transform Infrared Spectroscopy (FTIR)

The samples whose conditions and strains show better yield and viscosity results (strain 629 mediums 1, 2, 3 and S6 medium 3) have been subjected to FTIR analysis. The

results of these analysis are shown in Figure 2A,B. The spectra of the biopolymers produced in this show comparable characteristic peaks in the ranges between 500 cm^{-1} and 4000 cm^{-1}, with other reported FTIR spectrums of xanthan gum samples from previous studies [52,53].
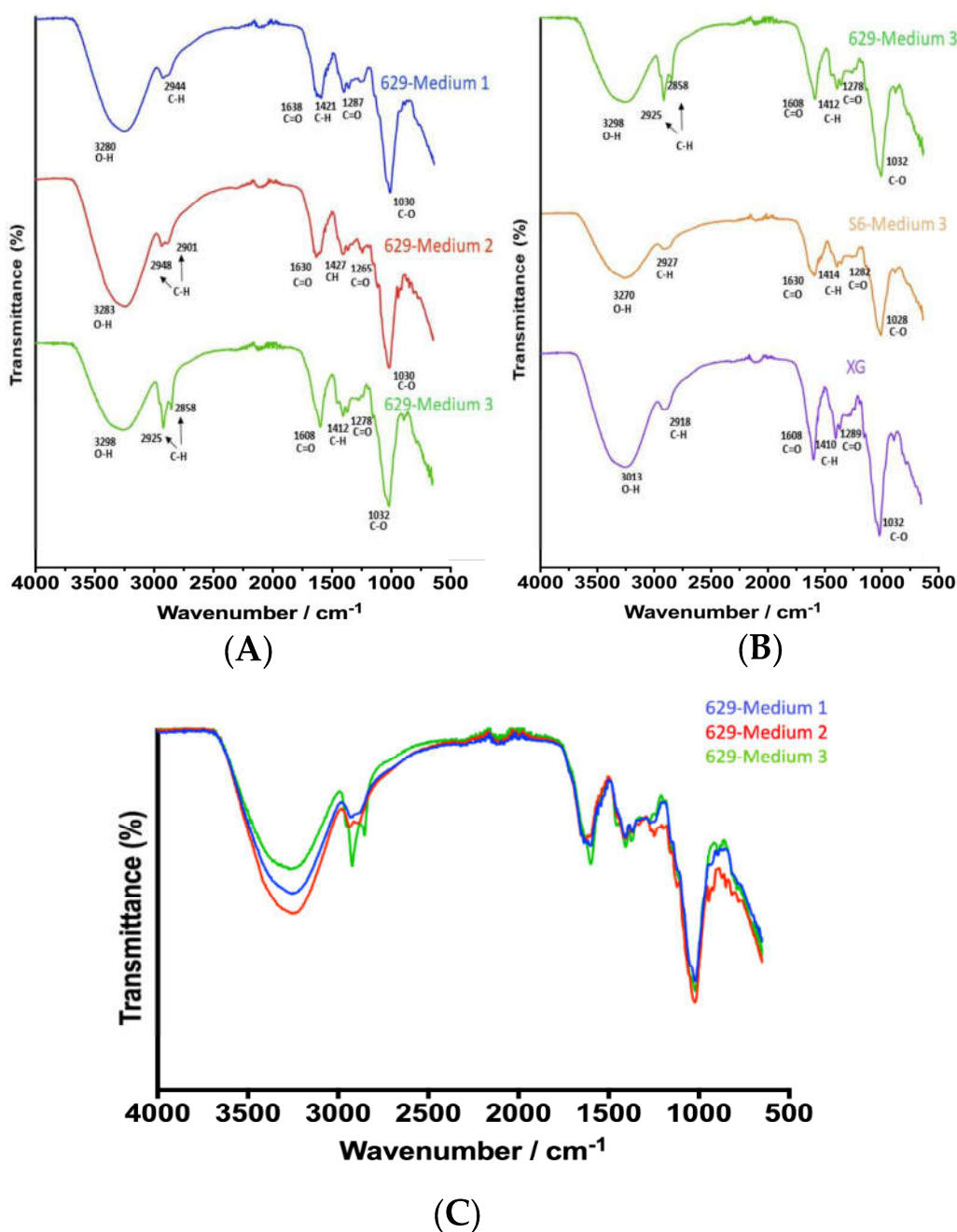

Figure 2. FTIR spectrum of the xanthan gum produced in this study showed higher yield and viscosity. (**A**) strain 629 medium 1, strain 629 medium 2 and strain 629 medium 3; (**B**) strain 629 medium 3, strain S6 medium 3 and, XG (commercial xanthan gum); (**C**) strain 629 medium 1, strain 629 medium 2 and strain 629 medium 3.

All samples analysed show a broad absorption peak between 3,270 cm^{-1} and 3,298 cm^{-1}, indicative of the elongation of the stretching vibration of the carbohydrate hydroxyl

group [54,55]. For the polysaccharides with higher viscosity (629-Medium 3 > 629-Medium 1 > 629-Medium 2), it can be observed that there is a decrease in the -OH signal along the increment of viscosity in Figure 2C. This can be explained by the presence of salts in Mediums 3 and 1, which have the property of crosslinking process by coordination of metals by -OH groups and consequently decreasing the hydroxyl signal. This gives a chemical stabilisation of the XG, thus resulting in higher viscosity. The influence of Fe (III) coordination by -OH groups from xanthan gum have been studied by other authors [52,53], and similar observations are obtained in the decrease in the -OH signal. In this work, xanthan gum produced from strain 629 medium 2 has no supplementation with salts, resulting in an observed more intense free -OH signal.

Samples produced by strains 629, S6, and commercial XG exhibit vibrations centred at 2,858 cm^{-1} to 2,948 cm^{-1}, which is assigned to the vibrational elongation mode of the C-H bond in methyl and methylene groups [56,57]. The peaks represented at 1,608 cm^{-1} to 1,638 cm^{-1} and 1,265 cm^{-1} to 1,289 cm^{-1} are attributed to the acetyl carbonyl (C=O) stretching [58]. As for the bands observed around 1,030 cm^{-1}, they are attributed to the vibrations of the C–O carrier stretches [59,60].

The main groups that participate in the formation of the XG synthesized in this work are OH, C-H, C=O and C-O groups. Therefore, it can be concluded that the changes in the FTIR spectrum of sample 629 are possibly due to the physicochemical interactions of the medium in the fermentation process, and that this change may also be related to the higher content of viscosity. Furthermore, FTIR spectra functional groups of commercial XG are identical to those of XG produced in this work.

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

4. Conclusions

The results of this trial have shown that strain 629 provides the higher yield while using a fermentation medium containing a carbon source of saccharose (1.25%), hemicellulose fractions (3.75%), and salts. In this same medium, the same strain produces XG in 3% aqueous solution, showing the higher apparent viscosity at a shear rate of 10 s^{-1} at 25 °C. The corncob has proven to be a promising alternative of a carbon source in the obtention of XG; however, saccharose must be included in the medium. The presence of salts is also of fundamental importance, not only in the nutrition of the fermenting bacteria, but also in the chemical stabilisation of the XG and resulting viscosity. The optimization of XG viscosity is crucial for the further development of xanthan synergistic mixtures with improved or induced gelation properties. In addition, regardless of production variables and bacteria, the *Xanthomonas* sp. bacterium is capable of bioconverting corn cob into XG with the same characteristics similar to commercial XG.

Future research may investigate the optimization of saccharose inclusion in the medium and any other potential strain of *Xanthomonas* sp. in alternative to strain 629. Corncob has been shown to have an immense potential to replace less cost-effective carbon sources, such as corn starch, in the fermentative processes for obtention of XG. To improve and reduce the production costs of XG, not only an alternative and promising carbon source is necessary, but also the genetic improvement of the strains of *Xanthomonas* sp. with the selection of mutations and genetic manipulation.

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