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RESEARCH PAPER

Development of a purification process via crystallization of xylitol produced for bioprocess using a hemicellulosic hydrolysate from the cashew apple bagasse as feedstock

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Abstract
Xylitol was biotechnologically produced by *Kluyveromyces marxianus* ATCC36907 using the hemicellulosic hydrolysate of the cashew apple bagasse (CABHH). Sequentially, the present study investigated the recovery and purification of xylitol evaluating different antisolvents [ethanol, isopropanol and the ionic liquid 2-hydroxyl-ethylammonium acetate (2-HEAA)], their proportion in the medium (10–90% v/v), and their cooling rate (V_C 0.25–0.50 °C/min). These processes were contrasted with the crystallization process of commercial xylitol. This study is the first to assess xylitol crystallization using a protic ionic liquid. The hydrolysate obtained from a mild treatment with sulfuric acid contained mainly glucose and xylose at concentrations of 15.7 g/L and 11.9 g/L, respectively. With this bioprocess, a maximum xylitol production of 4.5 g/L was achieved. The performance of the investigated antisolvents was similar in all conditions evaluated in the crystallization process of the commercial xylitol, with no significant difference in yields. For the crystallization processes of the produced xylitol, the best conditions were: 50% (v/v) isopropanol as antisolvent, cooling rate of 0.5 °C/min, with a secondary nucleation of yield and purity of 69.7% and 84.8%, respectively. Under the same linear cooling rate, using ethanol, isopropanol or the protic ionic liquid 2-hydroxyl-ethylammonium acetate (2-HEAA), crystallization did not occur, probably due to the presence of carbohydrates not metabolized by the yeast in the broth, which influences the solubility curve of xylitol. With the results of this work, a possible economical and environmentally friendly process of recovery and purification of xylitol from CABHH could be proposed.

Keywords Xylitol · Crystallization · Antisolvents · Ionic Liquid · Cashew apple bagasse

Introduction

Xylitol, an alcohol consisting of five carbon atoms and five hydroxyl groups, is known to possess a high sweetening power and it is one of the most valuable biomass-derived chemicals [1]. Several health benefits associated with this sweetener, such as its tooth decay preventive properties and low glycemic index, have encouraged its usage in numerous applications in different industrial segments, such as in the food, pharmaceutical and dental industries [2]. The global xylitol market is expected to reach USD 1.37 billion by 2025, according to a report by Grand View Research [3]. Recently, more medicinal applications of xylitol have been reported, such as its anticancer activity by selectively inducing carcinogenic cell death via the regulation of glutathione levels [4].

Xylitol is produced by two main routes: chemical and biotechnological. Chemical production of xylitol is effective but it has major disadvantages, since it requires high temperatures and pressures, the use of metal catalysts, and it shows low selectivity—factors that increase production costs [5, 6]. An alternative to reduce these shortcomings is the production via biotechnological routes, which are conducted at low temperatures and pressures, and the microorganisms employed in the process are much more selective. This can considerably reduce the production cost of xylitol [7].

One of the main feedstocks for xylitol production is lignocellulosic material. These are residues constituted of cellulose, hemicellulose and lignin, and whose acid or enzymatic

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hydrolysis yields xylose and fermentable sugars that are usually employed in xylitol production [8–13]. In this context, the use of agro-industrial waste enables the manufacture of high value-added products, as well as the reduction of environmental-related issues, such as pollution, caused by conventional industrial practices. Among these materials, the cashew apple bagasse, an abundant residue containing a high hemicellulose content (~ 18% of its composition), can be highlighted [12, 14], and this raw material is plentiful in the Northeast region of Brazil, South-Eastern Asia and Africa, exhibiting an outstanding social and economic role. Cashew apple bagasse is obtained from the processing of cashew apple juice and it is usually discarded or used as animal feed [15]. Then, the possibility of the application of this waste in the production of xylitol can add value to a residue and also reducing the impact on the environment.

In summary, for lignocellulosic materials to be applied in bioprocess, they are hydrolysed, releasing sugars to the medium, and the fermentation process is conducted by microorganisms capable of metabolizing xylose to xylitol. Finally, xylitol can be recovered and then purified [12].

The costs of recovering and purifying the xylitol obtained by fermentation processes are the greatest bottleneck in the process, due to low product concentrations, the complex composition of the fermentation broth, as well as the several stages in the recovery process, which makes the downstream process more expensive than the production stage itself [16–18]. With the rapid growth of the xylitol industry, more attention has been paid to xylitol purification. One potential method for purifying the polyol is crystallization [17, 18].

Crystallization provides a final product with many desired characteristics, such as good consumer-grade appearance, high purity and uniform crystal sizes [19]. To facilitate the process, it is important to know the environment in which the solution is inserted, as well as the antisolvents capable of reducing the solubility of the product and the conditions in which crystallization will occur. In this investigation, a protic ionic liquid was evaluated as an antisolvent. Ionic liquids (ILs) have evolved as a new type of non-aqueous solvents for separation processes and biocatalysis, mainly due to their unique and tuneable physical properties [20, 21]. The ILs can be divided into two broad categories, protic ionic liquids (PILs) and aprotic ionic liquids (AILs). PILs are produced through proton transfer from a Bronsted acid to a Bronsted base by a simple synthesis and at a low cost, an example being 2-hydroxyl-ethylammonium acetate (2-HEAA) [22].

Some research groups developed strategies to recover xylitol from various fermentation broths with lignocellulosic hydrolysates [16–18], but the processes still need to become technically and/or economically viable. Besides, there are few studies in the literature on the crystallization of bioprocess-derived xylitol.

In this context, the present work aims to develop a biotechnological process of xylitol production using the hemicellulosic hydrolysate of the cashew apple bagasse as a culture broth, with the subsequent recovery and purification of the produced xylitol. The focus of the work is to develop a crystallization process that deliver good yields and high purity. Also, it investigated the influence of impurities on the fermented medium, the type of antisolvents, their proportion, and their cooling rate.

Materials and methods

Cashew apple bagasse: preparation, acid hydrolysis and characterization

Cashew apple bagasse (CAB) was donated by Jandaia Sucos do Brasil S/A. The material was washed, dried, ground and standardized (particle size 0.177–0.841-mm). Then, an acid hydrolysis of the hemicellulosic fraction of CAB was performed with sulfuric acid for xylose release. The hydrolysis was conducted with a 0.6 M sulfuric acid solution and 20% (w/v) of CAB at 121 °C for 30 min [14].

Following hydrolysis, the solid and liquid fractions were separated by vacuum filtration, and calcium hydroxide was added to the liquid fraction [0.04 g $Ca(OH)_2$ per mL of hydrolysate] to adjust the pH to 6.0, which is the optimum pH for the growth of the xylitol-producing microorganism. A new filtration was performed and the hydrolysate was used as the culture medium (CABHH). The CABHH medium was sterilized at 110 °C for 10 min.

The CAB and the hydrolyzed solid fraction (CAB-H) were characterized according to the methodology proposed by Gouveia et al. [23], to quantify cellulose, hemicellulose and lignin. The content of extractable fats and waxes was determined following the NREL/TP-510-42619 protocol [24], and total solids and ash, with the NREL/TP-510-42621 protocol [25].

Microorganism

Xylitol was produced by the yeast *Kluyveromyces marxianus* ATCC36907. The yeast was kept in slant YEPD Agar medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L dextrose, and 20 g/L agar) at 4 °C. The activation of the microorganism was performed on YEPD agar and incubated at 30 °C for 48 h. Then, three colonies were transferred to a culture medium containing 20 g/L glucose, 0.4 g/L urea, 1.2 g/L KH_2PO_4, 0.18 g/L Na_2HPO_4, and 10 g/L yeast extract, at pH 4.5 for inoculum preparation. The cells were incubated at 30 °C and 180 rpm for 24 h on an orbital shaker (Tecnal—TE 422, Tecnal, Piracicaba, SP, Brazil). Finally, the cell optical density was measured at

600 nm and the cell concentration was determined from standard curves on a dry mass basis.

Xylitol production

The biotechnological production of xylitol was performed using the hemicellulosic hydrolysate of the cashew apple bagasse (CABHH) as the culture medium. The process was carried out on an orbital shaker (Tecnal—TE 422, Tecnal, Piracicaba, SP, Brazil) at 30 °C and 180 rpm for 96 h, using 10% (v/v) inoculum and approximately 0.5 g/L initial cell concentration [12]. Samples were taken every 24 h for analysis of cell growth, substrate consumption (glucose and xylose) and product formation (xylitol). At the end of the process, the cells were separated by centrifugation (4500 rpm for 15 min) and the xylitol-rich liquid phase was concentrated by evaporation in a rotary evaporator (600 mmHg, 50 °C and 30 rpm).

Xylitol crystallization process

Antisolvent

In this study, isopropanol, ethanol and the protic ionic liquid 2-hydroxyl-ethylammonium acetate (2-HEAA) were evaluated as antisolvents. The isopropanol and ethanol were purchased from Sigma Chemical Co. (St. Louis, MO, EUA), while the 2-HEAA was synthesized by an acid–base reaction using acetic acid and monoethanolamine under the operational conditions mentioned by Reis et al. [21]. The 2-HEAA was characterized by nuclear magnetic resonance (NMR); the sample was diluted in 600 µL deuterated dimethyl sulphoxide (DMSO) and ^{1}H and ^{13}C NMR spectra were obtained by an Agilent DD2 600 MHz spectrometer equipped with an inverse detection One Probe, according to Reis et al. [21].

Determination of solubility curves

Solubility curves of xylitol, glucose, and xylose were performed using different solvents: water, ethanol–water 50% (v/v), isopropanol-water 50% (v/v), and 2-HEAA-water 50% (w/v), at temperatures ranging between 5 °C and 70 °C, under a constant agitation of 750 rpm.

With the data obtained from the study of the solubility, the molar fraction (X) and the activity coefficient (γ) for each chemical substance were determined according to Eqs. 1 and 2, respectively, where ΔH_f is the melting enthalpy charge, T is the test temperature and T_m is the melting temperature [26].

$$X = \frac{n}{n_{TOTAL}} \tag{1}$$

$$\gamma = \frac{1}{X} \exp\left\{ -\frac{\Delta H_f}{RT}\left[1 - \frac{T}{T_m}\right]\right\} \tag{2}$$

Crystallization

The crystallization processes were performed in a 100 mL jacketed reactor coupled to a circulating thermostated bath (LAUDA E 25-S, Berlin, Germany) that allow temperature-chilling ramps to be performed, using 50 mL of the concentrated fermented medium (200 g/L initial xylitol concentration). Assays using the synthetic solution of xylitol (Sigma Chemical Co.) were also performed at the same initial concentration. The different antisolvents (ethanol, isopropanol and 2-HEAA) were investigated to optimize the crystallization process at proportions in the range of 50%–90% (v/v or w/v for 2-HEAA), and their linear cooling rates (V_C, 0.25 °C/min and 0.5 °C/min).

The crystallization processes were performed at an initial temperature of 5 °C for 24 h and, when the temperature of the solution reached its saturation temperature (determined by the solubility curves), approximately three xylitol crystals were weighed and added to the solutions, to favor the crystallization process.

After crystallization, the solutions were subjected to vacuum filtration through 0.45-µm filters to separate the xylitol crystals. Finally, they were dissolved in water for further analysis and calculations of process yield and crystal purity.

Characterization of the crystallized xylitol

The crystals were analyzed by Fourier Transform Infrared (FTIR) and X-ray diffraction. The FTIR spectra were measured using a Cary 630 Agilent Technologies equipment, between 4000 and 600 cm^{-1} with a spectral resolution of 1 cm^{-1}. X-ray powder diffraction patterns were measured using a PANalytical X'PertPromMPO diffractometer (Malvern, United) and data were collected in the range of 2θ from 10° to 100°, with $\Delta\theta$: 0.013°, at a speed of 2°/min. To determine appearance of the xylitol crystals, scanning electron microscopy (SEM) was performed using a scanning electron microscope Quanta FEG 450 (with 10 kV, low energy). The samples were placed on stabs and metalized for analysis.

Analytical methods

For microbial growth, the cell concentration (in g/L) was determined by the optical density at 600 nm and a calibration curve. The concentrations of glucose, xylose, arabinose, xylitol and ethanol were determined by high performance liquid chromatography (HPLC) using a system equipped with a Waters refractive index detector (model 2414) and an Aminex column HPX-87H (Bio-Rad, Hercules, CA, USA).

The mobile phase was 5 mM H_2SO_4 solution, with a flow rate of 0.5 mL/min at 65 °C, and injection volume of 20 µL [11].

Results and discussions

Characterization of cashew apple bagasse and its hemicellulosic hydrolysate

The cashew apple bagasse used in this study was composed of 19.2% w/w cellulose, 17.7% w/w hemicellulose, 33.4% w/w total lignin, 6.4% w/w extractables and 1.5% w/w ash (Table 1). The CAB shows a similar or higher hemicellulose content than other materials, for example, wheat straw (24.7% w/w hemicellulose) [27], rice husk (14.4% w/w hemicellulose) [28], sugarcane bagasse (17.0% w/w hemicellulose) [29] and *Eucalyptus grandis* (17.6% w/w hemicellulose) [30]. The cellulose content remained the same (approx. 19.3% w/w) in the treated solid and the lignin content increased (from 33.4% to 54.8% w/w). These results are expected, since sulfuric acid breaks the bonds of hemicellulose molecules and the amorphous part of cellulose [13].

The hemicellulosic hydrolysate of the cashew apple bagasse contained 15.7 g/L glucose, 11.9 g/L xylose, 1.5 g/L acetic acid, and 0.5 g/L formic acid. Furfural and hydroxymethylfurfural (5-HMF) were not detected in the hydrolysate. The pH adjustment using $Ca(OH)_2$ can reduce the concentration of furfurals, which is important to the process, as these compounds can be highly toxic to the microbial growth by damaging crucial biological membranes [12].

Finally, the total concentration of fermentable sugars obtained via acid hydrolysis was approximately 28 g/L, including glucose and xylose. Xylose was the second most abundant component, corresponding to 42.5% of the total sugars present in the hydrolysate. However, the xylose concentration obtained (11.9 g/L) was higher than that reported in the literature from other lignocellulosic materials, i.e., 10.3 g/L from acid hydrolysis of rapeseed straw [11], 7.4 g/L in the hemicellulosic hydrolysate of eucalyptus wood [30], and 2.7 g/L in the hydrolysate of olive pruning [31].

Xylitol production

Xylitol was produced by *K. marxianus* ATCC36907 in the hemicellulosic hydrolysate of the cashew apple bagasse (CABHH). The production profile is shown in Fig. 1. The highest xylitol concentration (4.5 g/L) was achieved at 96 h and cell concentration was 5.3 g/L. Ethanol also was produced and its concentration peaked at 24 h (4.8 g/L), but it decreased over time, indicating that it is likely that at least part of the ethanol evaporated. As shown in Fig. 1, there was no complete consumption of any of the sugars and, at the end of the fermentation process, there still was 21.12% of glucose and 9.34% of xylose. The probable reason for this was that ethanol affected the yeast's performance, due to the glucose present in the hydrolysate during xylitol production, which may have modified the metabolic pathway of the yeast.

Therefore, the main parameters in xylitol production were: xylose consumption (10.8 g/L), glucose consumption (12.4 g/L), xylitol concentration (4.5 g/L), cell concentration (5.3 g/L), xylitol productivity [0.05 g/(L h)] and xylitol yield ($Y_{P/S}$, 0.42 g/g). Table 2 summarizes the main results between xylitol production in this work and those from the literature obtained by the same yeast (*Kluyveromyces*). From

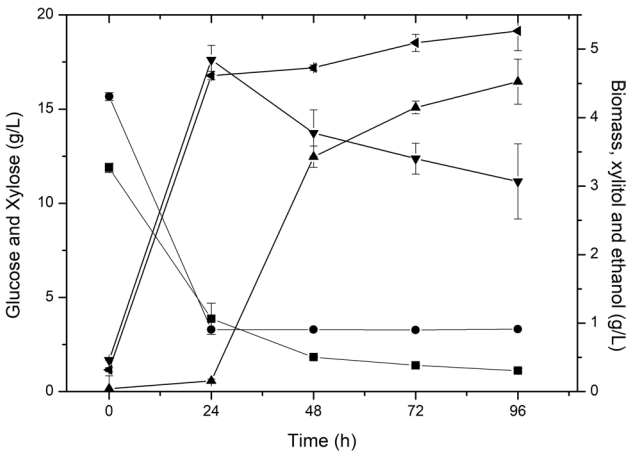

Fig. 1 Profile of the microbial process of xylitol production by *Kluyveromyces marxianus* ATCC36907 using the hemicellulosic hydrolysate of the cashew apple bagasse as a medium, at 30 °C and 180 rpm: glucose (black circle), xylose (black square), biomass (black left-pointing triangle), xylitol (black up-pointing triangle), ethanol (black down-pointing triangle)

Table 1 Chemical characterization of *in natura* and hydrolysed cashew apple bagasse. The acid hydrolysis was performed using 0.6 M H_2SO_4 at 121 °C for 30 min

Material	Cellulose (% w/w)	Hemicellulose (% w/w)	Lignin (% w/w)	Extractables (% w/w)	Ashes (% w/w)
In natura (CAB)	19.22 ± 0.25	17.73 ± 0.10	33.41 ± 1.66	6.41 ± 0.23	1.5 ± 0.01
Hydrolysed (CABH)	19.30 ± 0.09	4.53 ± 0.05	54.85 ± 0.12	22.54 ± 0.19	2.30 ± 0.00

Table 2 Comparison of concentration, productivity and yield of xylitol obtained by different *Kluyveromyces*

Microorganism	Concentration (g/L)	Productivity (g L/h)	Yield (g/g)	Reference
Kluyveromyces marxianus ATCC36907	4.52	0.05	0.42	Present study
K. marxianus CE 025	4.77	0.07	0.19	[32]
K. marxianus IIPE453	11.1	0.19	0.315	[10]
K. marxianus YZJ001	29.99	0.83	0.60	[33]
K. marxianus YZJ011	7.65	0.50	0.153	[33]
K. marxianus YZJ009	16.86	0.16	0.337	[33]
K. marxianus CCA 510	6.76	0.07	0.363	[12]

these data, it could be observed that the xylitol yield ($Y_{P/S}$ value) obtained in the present study was similar or higher. Nevertheless, there are some variations in productivities. Comparing the data obtained with the xylitol production by *Debaryomyces hansenii*, the $Y_{P/S}$ value was very similar [11].

The fermented hydrolysate was concentrated and the xylitol concentration obtained was 200 g/L, with the concentrations of unreacted xylose and glucose being 9.4 g/L and 26.4 g/L, respectively. This solution was then used in the crystallization studies.

Solubility curves

Figure 2 shows the behavior of the solubility curves for xylitol, glucose and xylose in different solvents at different temperatures: water, 50% (v/v) ethanol–water, 50% (v/v) isopropanol-water, and 50% (w/v) 2-HEAA-water. These curves are useful in the elucidation of the supersaturation behavior in the system and for understanding its different conditions.

In Fig. 2a, where only water was used as a solvent, xylitol was the most soluble substance at high and low temperatures. Its concentration decreased from 342.92 g/100 g to 73.6 g/100 g, when the temperature decreased from 70 to 5 °C. Glucose, in turn, showed an intermediate behavior with maximum solubility (225.42 g/100 g) at 70 °C, but at 5 °C, it was the least soluble, with a solubility of 19.7 g/100 g. Finally, xylose was the least soluble component at high temperatures (216.36 g/100 g at 70 °C) and, at low temperatures (5 °C), it presented an intermediate solubility, at 38.66 g/100 g.

In Fig. 2b, when using 50% (v/v) isopropanol-water as solvent, it was again observed that xylitol was the most soluble of the three solutes under the temperatures evaluated. It presented solubilities of 276.22 g/100 g and 3.6 g/100 g at 70 °C and 5 °C, respectively. Glucose

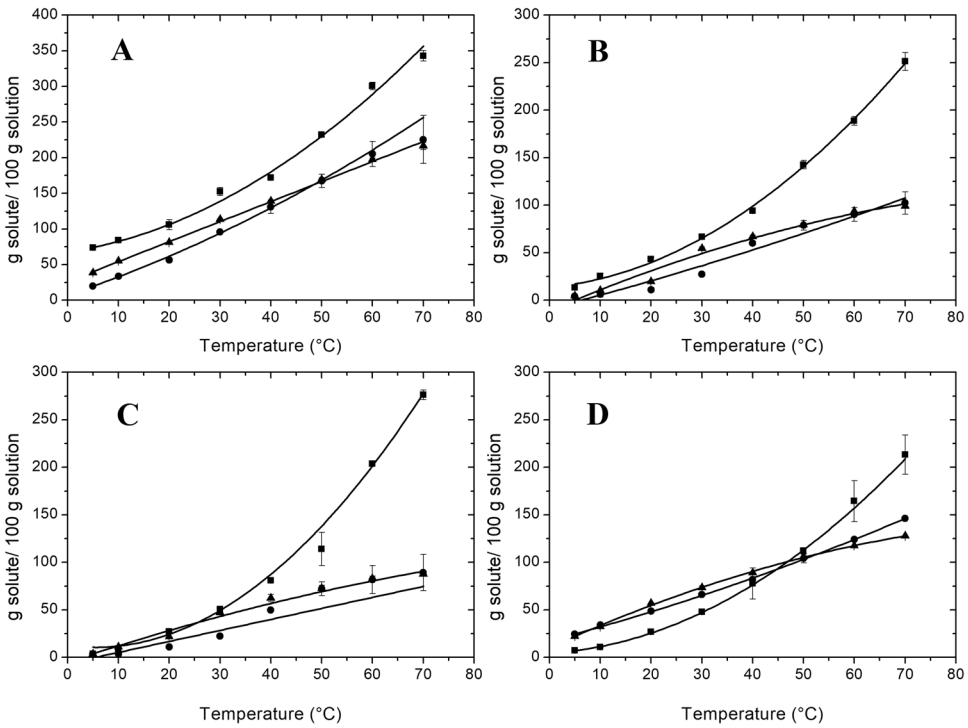

Fig. 2 Solubility curves of xylitol (black square), glucose (black circle) and xylose (black up-pointing triangle) in: water (**a**), 50% (v/v) isopropanol-water (**b**), 50% (w/v) 2-hydroxyethanolamine acetate-water (**c**), and 50% (v/v) ethanol–water (**d**)

presented a solubility of 89.12 g/100 g at 70 °C and 3.54 g/100 g at 5 °C, being the least soluble compound at the lowest temperature assessed. Xylose showed an intermediate solubility in the evaluated temperature range.

In the experiments conducted using 50% (w/v) 2-HEAA-water (Fig. 2c), the solubilities of xylitol, glucose and xylose at 70 °C were 213.25 g/100 g, 146.181 g/100 g and 127.746 g/100 g, respectively. And at low temperatures (5 °C), the solubilities of xylitol, glucose and xylose were 7.14 g/100 g, 24.6 g/100 g and 22.2 g/100 g, respectively.

The substances were more soluble using the 50% ethanol–water solution (Fig. 2d) under the evaluated temperatures. It was also observed that the alcohols showed a more significant antisolvent action than the protic ionic liquid 2-HEAA, since they provided less solubility at the same percentage of antisolvent.

When xylitol was compared to other carbohydrates (glucose and xylose), it showed the highest solubility at higher temperatures (70 °C) for the solvent used, but at low temperatures, the solubility of the three components was quite low and very similar to each other, indicating that the xylitol crystallization process can be influenced by these carbohydrates. However, when using isopropanol, the polyol presented a higher solubility at high temperatures and a lower solubility at low temperatures.

A few different operations can be used to create a supersaturation condition in a solution: cooling, evaporation, antisolvent addition or precipitation [34]. Due to the profile of the solubility curves for this process (see Fig. 2), crystallization was carried out by antisolvent addition and physical precipitation, from which medium-sized crystals were obtained and secondary nucleation was observed [34].

Therefore, to analyze the influence of the amount of antisolvent on the xylitol solubility, an experiment was carried out at a constant temperature of 15 °C, and varying the proportion of antisolvent, which ranged from 10 to 90%. In the experiments using ethanol and isopropanol, there were no noticeable differences in solubility between 10 and 50% (v/v), but for 70% and 90% (v/v), the results indicated a sharp drop in the solubility of xylitol (Fig. 3). In the assays using 2-HEAA, there was no significant difference in solubility when using 10% (w/v) and 30% (w/v) of antisolvent, but from 50 to 90% (w/v), there was, again, a noticeable solubility drop. However, this decrease in solubility at high antisolvent proportions was clearly more prominent when using alcohols than when using the protic ionic liquid (Fig. 3).

The proportion of antisolvent chosen for the crystallization experiments was 50% (v/v or w/v for 2-HEAA). The ratios of 10% and 30% were not used because they did not result in significant changes in solubility, and at 70% and 90%, it was very difficult to solubilize xylitol.

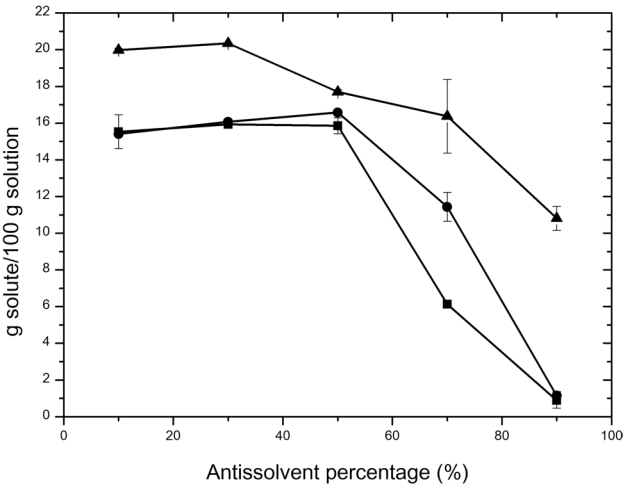

Fig. 3 Influence of the variation of antisolvent ratio on xylitol solubility at 15 °C. (black square) ethanol, (black circle) isopropanol and (black up-pointing triangle) protic ionic liquid 2-hydroxyethanolamine acetate (2-HEAA)

To better understand the thermodynamic behavior involving the solubility values, the activity coefficients were calculated, since they are a good indication of how the interactions between molecules deviates from the ideal behavior as a function of the molar fraction of each medium. According to Fig. 4, the initial values for the activity coefficients are higher, since, at that stage, they are the most distant from ideality and the interactions between solutes are minimal due to their low concentration in solution. As the molar fraction increases, so does the solute concentrations, which results in a decrease in the activity coefficients and an increase in the deviation from ideality.

The lowest values of the activity coefficients were found to be for the medium containing water, since it presented the highest solubility of xylitol and the largest molar fraction (Fig. 4).

Figure 5 shows the molar fraction of the xylitol as a function of temperature, for the different solutions evaluated. The solubility of xylitol increased with increasing temperature and the addition of antisolvents promoted a decrease in the solubility, as expected. The 50% (v/v) isopropanol solution presented greater solubility at high temperatures and lower solubility at low temperatures. These results are consistent with the data presented in Fig. 2. However, there is no significant difference in the solubility of xylitol in the different solutions evaluated at low temperatures. Thus being, all antisolvents were evaluated to verify the possible interference of the substances present in the hemicellulosic hydrolysate in the crystallization process.

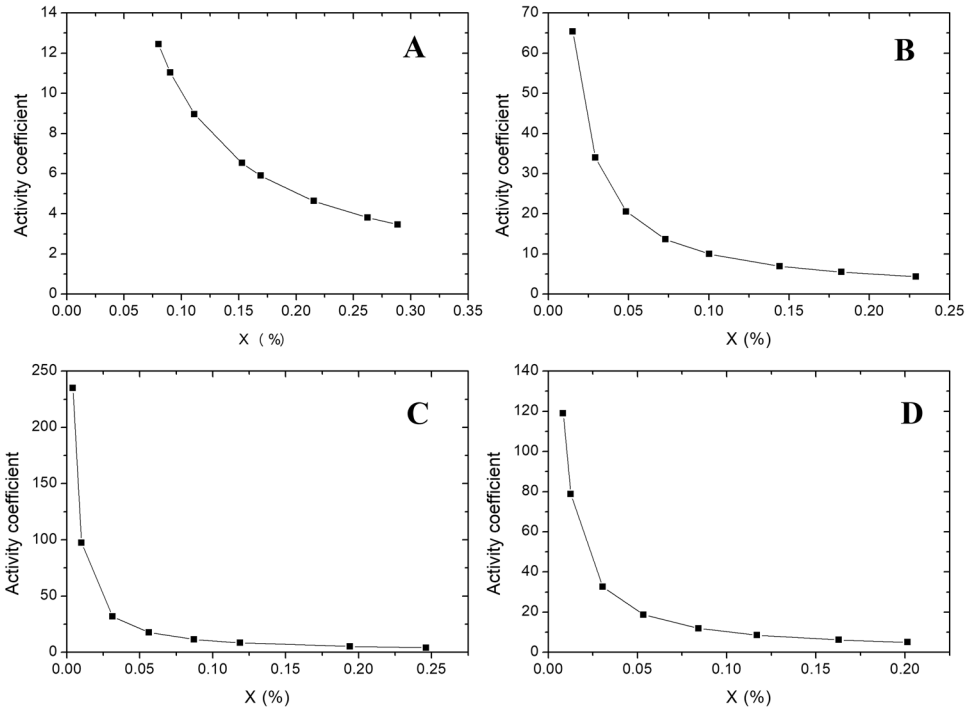

Fig. 4 Xylitol activity coefficients by mass fraction in different aqueous solutions: water (**a**), 50% (v/v) ethanol (**b**), 50% (v/v) isopropanol (**c**), and 50% (w/v) 2-hydroxyethanolamine acetate-water (**d**)

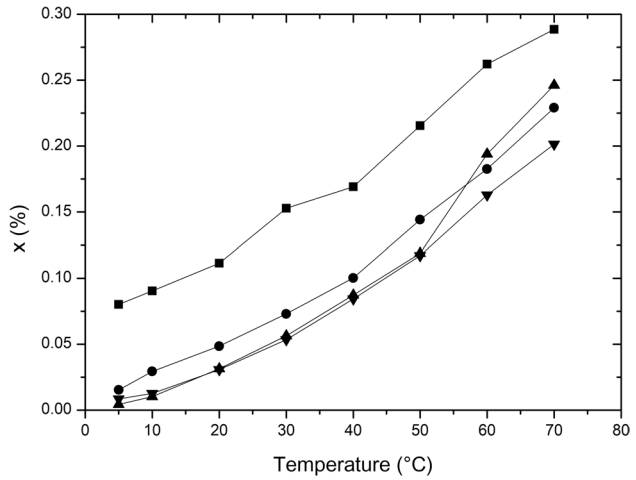

Fig. 5 Influence of the temperature on the molar fraction of xylitol in different aqueous solutions: pure water (black square); 50% v/v ethanol (black circle); 50% v/v isopropanol (black up-pointing triangle); and 50% (w/v) 2-hydroxyethanolamine acetate (black down-pointing triangle)

Xylitol crystallization

Table 3 shows the yields of the crystallization processes of xylitol, conducted at different antisolvent ratios and cooling rates (V_C), as well as a comparison of these processes to the commercial (synthetic) xylitol solution.

The process conducted is known as secondary crystallization, due to the crystallization taking place after the addition of crystals to the supersaturated solution (200 g/L xylitol). The studies started at a temperature of 10 °C above of the saturation temperature of each solution, to ensure that all xylitol present would be effectively dissolved. As the temperature decreased and approached saturation, crystals were added to induce its formation. In the experiments using commercial xylitol, the yields were similar for all tested antisolvents.

However, in the experiments using the hemicellulosic hydrolysate, it was observed that some conditions did not favor crystallization, especially those using the protic ionic liquid (2-HEAA). This was probably due to the liquid not being efficient as an antisolvent, since it was where xylitol presented the highest solubility, as shown in Fig. 2. According to Diniz et al. [35], the p*K*as of the molecules involved is an important factor for the formation of crystals. The acid character of 2-HEAA in conjunction with the pH of the xylitol solution (hydrolysate), which was also acid, did not favor the formation of crystals in these studies and crystallization did not occur.

In addition to the aforementioned, other facts that potentially interfered with the crystallization process was the xylitol concentration not being especially high at the beginning of the process, and the presence of other carbohydrates, unreacted xylose and glucose, as well as impurities, in the culture medium. High xylitol concentration favors its nucleation, increasing the efficiency of the crystallization process [18].

In the crystallization processes using ethanol as an antisolvent, the polyol did not crystallize at the cooling rate of

Table 3 Yields and purities obtained in the crystallization of xylitol produced using the hemicellulosic hydrolysate of the cashew apple bagasse as a culture medium, and yields obtained using the synthetic solution (commercial xylitol)

Antisolvent	$T_{initial}$ (°C)	T_{sat} (°C)	T_{last} (°C)	V_C (°C/min)	Synthetic solution	Hemicellulosic hydrolysate	
					Yield (%)	Yield (%)	Purity (%)
50% (v/v) ethanol	38	28.18	5	0.50	80.91 ± 1.29	NC	–
50% (v/v) ethanol	38	28.18	5	0.25	79.58 ± 0.59	58.65 ± 2.20	75.12 ± 0.03
50% (v/v) isopropanol	44	34.81	5	0.50	77.77 ± 3.15	69.70 ± 0.39	84.81 ± 0.02
50% (v/v) isopropanol	44	34.81	5	0.25	77.44 ± 3.61	69.27 ± 0.01	83.37 ± 0.07
50% (v/v) 2-HEAA	45	34.44	5	0.50	80.63 ± 0.90	NC	–
50% (v/v) 2-HEAA	45	34.44	5	0.25	81.56 ± 2.21	NC	–

0.50 °C/min, indicating that, probably, the crystallization temperature decreases at higher cooling rates [36].

The highest crystal purity (85%) and the highest crystallization yield (approx. 69%) were obtained using isopropanol 50% (v/v) as an antisolvent. Similar results were obtained by Wei et al. [17], who studied the crystallization of xylitol obtained by bioprocesses using the hemicellulosic hydrolysate of corncob. They reported that, from a concentrated solution with 750 g/L of xylitol, the final purity and yield was 95% and 60.2%, respectively. It should be noted that these authors used supersaturated solutions with a higher concentration than that of this study (750 g/L in the literature and 200 g/L in this work) and this factor increased the nucleation process [37]. Also, to increase the purity, a second crystallization or an impurity removal step, prior to the main crystallization process, could potentially be done.

Kresnowati et al. [16] reported the purification of xylitol from either the model solution or the oil palm empty fruit bunch hydrolysate fermented by *Debaromyces hansenii* via ultrafiltration and electrodeionization membranes. The process reports xylitol recoveries with high purity, but it presented low yields and high costs. Another important factor is that these authors [17] performed several steps to remove unreacted carbohydrates before crystallization, focusing on a higher purity. However, as a consequence, these steps increased the cost of the process.

In addition to the studies cited, the Table 4 shows recovery/purification methods reported in the literature and it was observed that the crystallization using isopropanol as antisolvent is more efficient than the precipitation method and other crystallization methods. The xylitol crystals obtained by membrane separation showed greater purity than the xylitol crystals obtained in this study, but it is a method that requires several process steps and it show high xylitol loss [38]. Isopropanol is a solvent widely used in industry and that needs to be handled correctly so as not to cause any accidents and after use, the effluents must be treated and disposed of correctly so as not to cause any environmental accident, being a common procedure with several chemical reagents.

The cooling rate did not influence the purity of xylitol in the assays performed using isopropanol. Using ethanol, crystallization did not take place at the high cooling rate (0.5 °C/min) and, at the low cooling rate, the process yielded 58.65% of xylitol with a purity of 75.12% (Table 3), results inferior to those obtained with isopropanol. This purity is linked to unreacted carbohydrates which, under these conditions, can precipitate or crystallize (see solubility curve in Fig. 2).

Table 4 Yield and purity of xylitol obtained by different separation and purification methods

Method	Yield (%)	Purity (%)	Reference
Crystallization using isopropanol as antisolvent	69.7	84.8	Present study
Activated charcoal treatment followed by vacuum concentration and crystallization method	43.4	NR	[37]
Crystallization using ethanol as antisolvent	60.2	95.0	[17]
Crystallization	42.0	89.0	[18]
Crystallization	29.0	92.0	[39]
Antisolvent precipitation	63.3	NR	[40]
Membrane separation using blend membrane, polyethersulfone—pluronic F127	NR	82–93%	[38]
Membrane separation	NR	90.3	[41]

NR data did not report

After crystallization, analyses were performed to determine the characteristics of the formed crystals. Figure 6 shows the FTIR spectra of crystals obtained using CABHH and the synthetic solution of xylitol. Figure 6a—assay A shows the crystallization of commercial xylitol, and Fig. 6b—assay B shows xylitol produced using the hemicellulosic hydrolysate of the cashew apple bagasse, under the following conditions of crystallization: 50% (v/v) ethanol at V_C of 0.25 °C/min; 50% (v/v) isopropanol at V_C of 0.25 °C/min (Fig. 6c, assay C); and 50% (v/v) isopropanol at V_C of 0.5 °C/min (Fig. 6d, assay D).

Xylitol is a five-carbon sugar-alcohol with five OH groups, one attached to each carbon. These groups are observed in Fig. 6a and c–d. The bands from 3500 to 3000 cm^{-1} and the bands 1470 cm^{-1} to 750 cm^{-1} are related to OH; the bands between 900 and 800 cm^{-1} correspond to CH_2; and finally, the bands located between 1000 and 1125 cm^{-1} represent CO. The FTIR spectra from the crystals obtained in the process using 50% (v/v) ethanol with a V_C of 0.25 °C/min (Fig. 6b), presented a different profile in the wavelength range from 1500 to 1000 cm^{-1}, and it also presented the most different visual characteristics to commercial xylitol. Yang et al. [42] obtained results for pure xylitol in which they found that the broad band around 3431 cm^{-1} corresponded to OH groups and water absorption, and the bands shown at 1416 cm^{-1} and 740 cm^{-1}, corresponded to OH groups. Also, CH_2 bonds appeared in bands between 859 and 920 cm^{-1} and the bands at 1000 cm^{-1}, 1055 cm^{-1}, 1086 cm^{-1} and 1116 cm^{-1} corresponded to CO bonds.

Crystals obtained of assays C and D, as described in the paragraph above, showed similar purity to crystals generated from the synthetic solution (assay A), being obtained under the conditions of 50% (v/v) isopropanol at 0.25 °C/min and 0.5 °C/min, respectively, and with purities of 83.37% and 84.8%, respectively. The crystals obtained by assay B (50% (v/v) ethanol at V_C of 0.25 °C/min) presented the lowest purity (75.12%).

The morphology of the xylitol particulates is of great importance since its appearance is an important aspect to consider in this product. Figure 7 shows microscopic images (15 × amplitude) and Fig. 8, scanning electron microscopy (SEM) images of the crystals obtained. The crystals from the process that used the synthetic xylitol solution (Fig. 7b) showed characteristics similar to the un-crystallized xylitol (Fig. 7a), and the crystals from the crystallization of the hemicellulosic hydrolysate show a brown color due to the components present in the fermented medium. In the process conducted using 50% (v/v) ethanol with V_C of 0.25 °C/min (Fig. 7c), the crystal is dark in appearance, indicating that part of the color of the hydrolysate attaches to the crystal

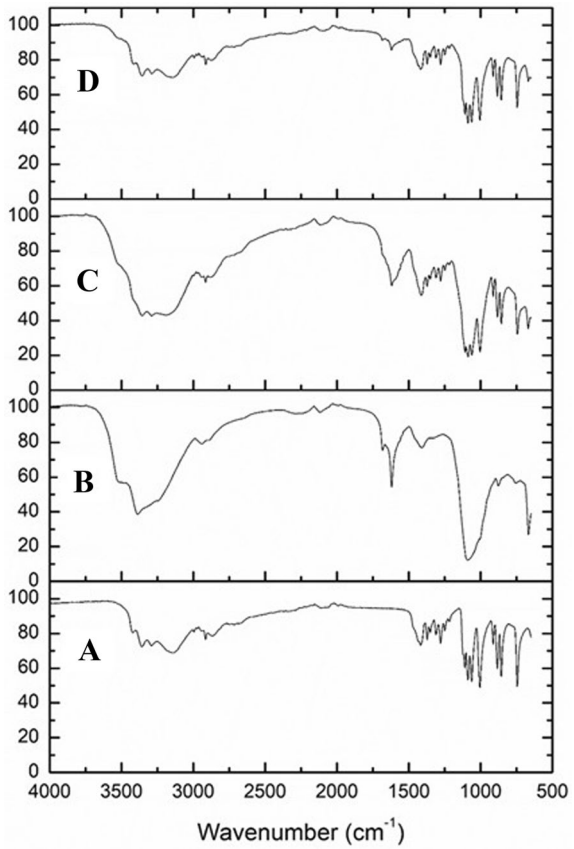

Fig. 6 FTIR of crystals obtained from the crystallization processes of commercial xylitol (**a**), and of xylitol produced using the hemicellulosic hydrolysate of the cashew apple bagasse, under the following conditions of crystallization: 50% (v/v) ethanol with V_C of 0.25 °C/min (**b**); 50% (v/v) isopropanol with V_C of 0.25 °C/min (**c**); and 50% (v/v) isopropanol with V_C of 0.5 °C/min (**d**)

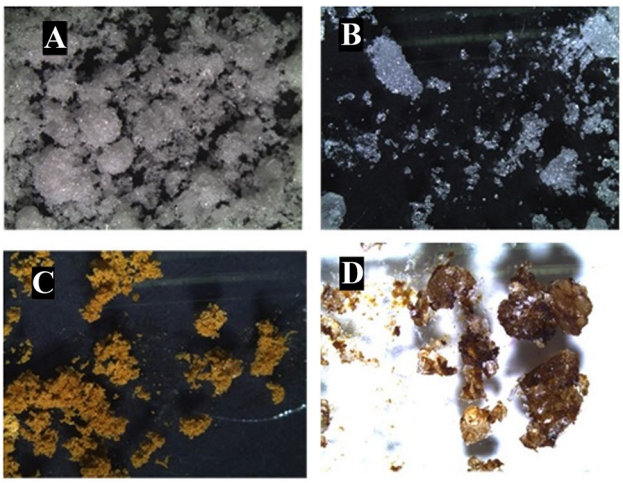

Fig. 7 Images of xylitol crystals obtained under ×15 magnification: commercial xylitol (**a**), xylitol obtained by crystallization of the synthetic solution at 50% (v/v) isopropanol with V_C at 0.5 °C/min (**b**), xylitol obtained by crystallization of the hemicellulosic hydrolysate in the process conducted using 50% (v/v) ethanol and V_C of 0.25 °C/min (**c**), xylitol obtained by crystallization of the hemicellulosic hydrolysate using 50% (v/v) isopropanol and V_C of 0.25 °C/min (**d**)

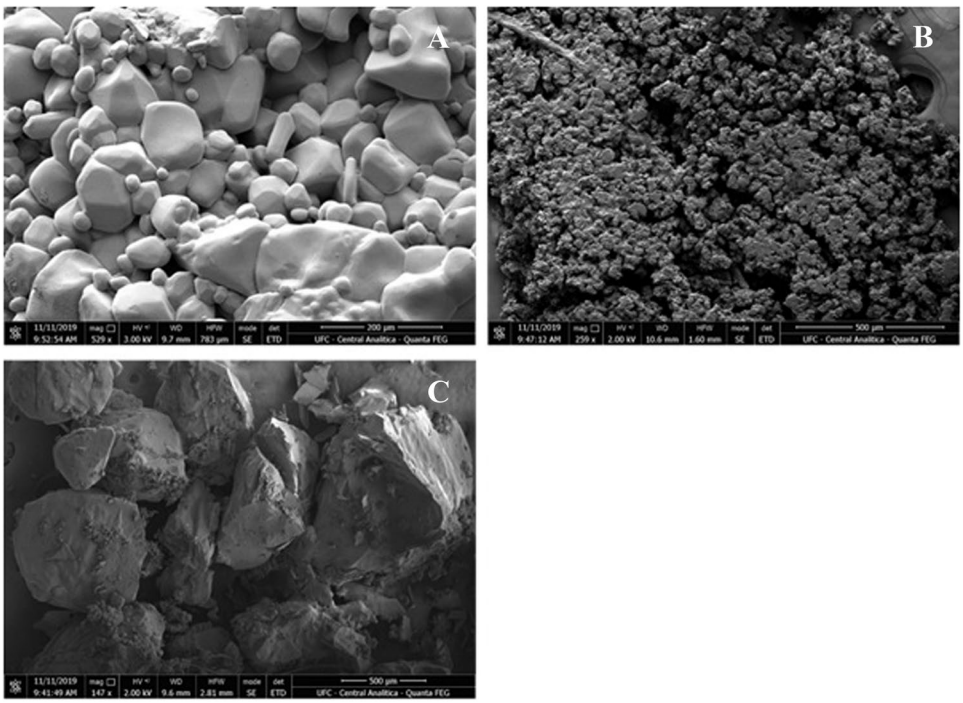

Fig. 8 Scanning electron microscopy (SEM) image of xylitol crystals obtained under the following conditions: xylitol obtained by crystallization of the synthetic solution at 50% (v/v) isopropanol with V_C of 0.5 °C/min (**a**); xylitol obtained by crystallization of the hemicellulosic hydrolysate in the process conducted using: 50% (v/v) ethanol at V_C of 0.25 °C/min (**b**); and 50% (v/v) isopropanol at V_C of 0.25 °C/min (**c**)

xylitol surface and also that the sugars present in the medium crystallized along with xylitol, these crystals being those that presented lower purity (75.12%). In the process using 50% (v/v) isopropanol at V_C of 0.25 °C/min (Fig. 7d), it is possible to obtain crystals lighter than those from the process using the 50% (v/v) ethanol at V_C of 0.25 °C/min (Fig. 7c). However, employing only the crystallization step was not enough for the obtainment of crystals with high purity.

From the SEM images, it can be observed that the size of the crystals obtained using ethanol (Fig. 8b) as an antisolvent are smaller than those obtained using isopropanol (Fig. 8c). Comparing the images of the xylitol obtained using the synthetic solution with that obtained from the hemicellulosic hydrolysate, a greater similarity is observed in the morphology of the xylitol obtained with isopropanol than when using ethanol as an antisolvent (Fig. 8). Therefore, the morphology and structure of xylitol seem to vary as a function of antisolvent.

An X-ray diffraction of the crystals was performed to determine their crystallinity (Fig. 9). It can be seen in Fig. 8 that assays b, c, d and e present noise. The highest crystallinity was obtained in the commercial xylitol, assay A (peaks 17.03°, 34.35° and 44.50°), what was not observed in other tests. The peaks of crystals obtained by assay A were used as a reference and the crystals obtained in other assays showed characteristic xylitol peaks. It was also observed that: in assay B, peaks similar to those in assay A were observed at 21.60°, 26.41°, 28.76°, 36.6°, 41.40° and 44.78°. The crystals obtained in assay C showed peaks similar to crystals obtained by assay A, at 26.41°, 35.41°, 36.60°, 41.40° and 47.82°. Assay D showed peaks similar to xylitol from assay A, at 26.41°, 28.76°, 35.41°, 36.60°, 41.40° and 47.82°. Finally, crystals obtained by assay E showed similarity to those in assay A, at 35.41°, 36.60°, 41.40° and 47.82°. The XRD results revealed amorphous peaks, indicating that the processes occurred without complete crystallization under the current experimental conditions, and the xylitol obtained using 50% (v/v) isopropanol with a V_C of 0.50 °C/min (Fig. 9e) show high crystallinity compared to the commercial xylitol (Fig. 9a).

Based on these results, the crystallization process using 50% (v/v) isopropanol with a cooling rate of 0.5 or 0.25 °C/min resulted in good recovery of xylitol with a level of purity of 85%. Also, in contrast with other methods of purification of xylitol produced by fermented media [16–18], this process has the advantage of being carried out in a single step and having a lower cost. Also, the process proposed makes use of non-toxic antisolvents, unlike the process reported by Hao et al. [43], who used methanol as an antisolvent.

Conclusion

The biotechnological process of xylitol production by *K. marxianus* ATCC36907 using the hemicellulosic hydrolysate of the cashew apple bagasse showed good yields ($Y_{P/S}$). Following production, an economical, environmentally friendly, single-stage process of recovery and purification of xylitol has been proposed. The crystallization using the protic ionic liquid 2-hydroxyl-ethylammonium

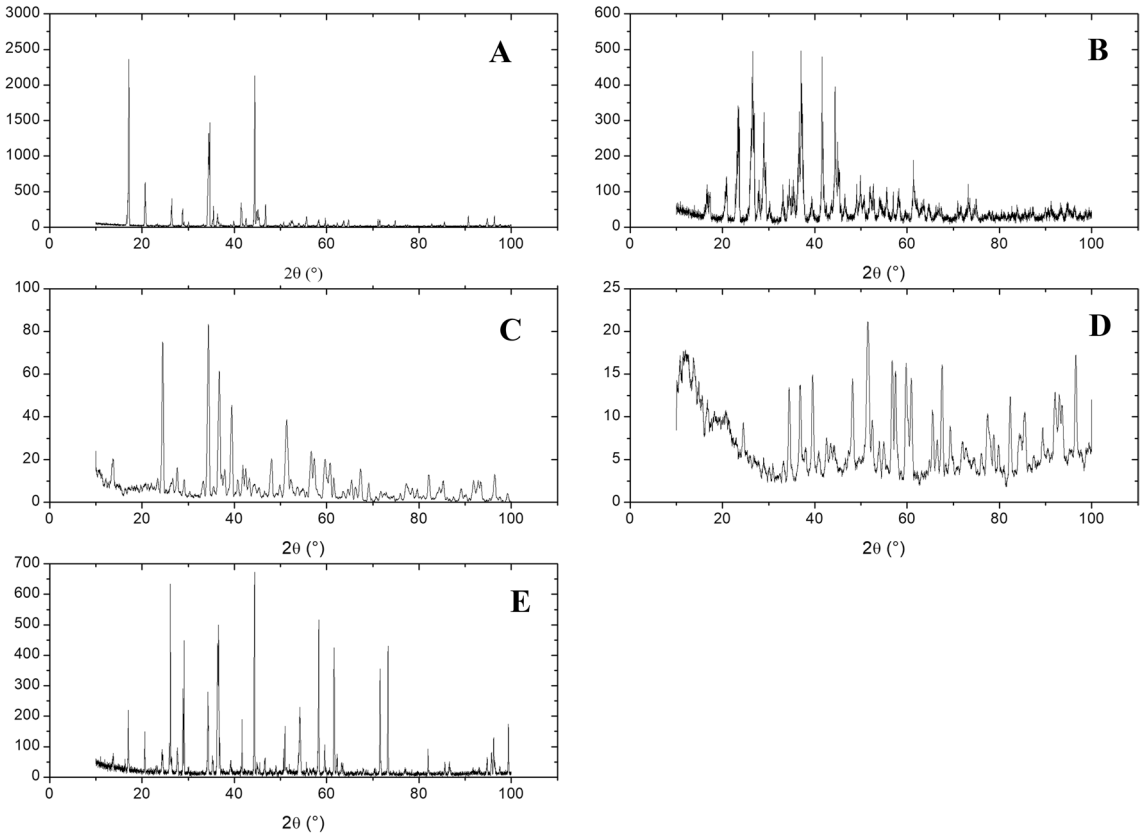

Fig. 9 X-ray diffraction of xylitol obtained under the following conditions: commercial xylitol (**a**), crystallized xylitol from the synthetic solution using 50% (v/v) isopropanol at V_C of 0.50 °C/min (**b**), xylitol obtained by crystallization of the hemicellulosic hydrolysate in the process using: 50% (v/v) ethanol with V_C 0.25 °C/min (**c**), 50% (v/v) isopropanol at V_C 0.25 °C/min (**d**); and 50% (v/v) isopropanol at V_C of 0.50 °C/min (**e**)

acetate as an antisolvent was not appropriate, and the best antisolvent was found to be isopropanol, with a crystal yield and purity of approximately 70% and 85%, respectively.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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