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ORIGINAL PAPER

Green Process for Xylooligosaccharides Production using an *Eucalyptus Kraft* Pulp

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Abstract

Xylooligosaccharides (XOS) are oligomers with recognized and important prebiotic properties, whose consumption is associated with several health benefits, including a positive impact on the immune system. In this work, XOS were produced through a green process of enzymatic hydrolysis performed directly on an intermediate product from a pulp and paper industry, *Eucalyptus* bleached *kraft* pulp. Focusing on an industrial, sustainable and more economical application, two goals were defined and validated: (i) no pretreatment of the substrate and (ii) the replacement of the commonly used buffer solution as reaction medium for only water. The influence of the most relevant operating conditions on the production of XOS as well as the respective yields obtained were very similar when using either buffer or water as the reaction medium. For the use of water, although the solution pH decreases during the enzymatic reaction, this change did not affect the production of XOS. For the optimized conditions, 80 °C and 100 U/g pulp, a maximum yield of 31.4 ± 2.6% per total xylan in the pulp was obtained, resulting in more than 50 kg of XOS per ton of pulp. The correspondent hydrolysate was mainly composed by xylobiose (66%) and xylotriose (29%), oligomers with the highest prebiotic effect.

Keywords Xylooligosaccharides · Prebiotics · Functional foods · Enzymatic hydrolysis · Bleached *Kraft* pulp · Pulp and paper industry

Introduction

The established link between the gut microbiome and the immune system combined with the rising awareness of a healthy nutrition has led, over the past decades, to an increasing interest in functional foods, including prebiotics [1]. More recently, the global covid-19 pandemic accentuated even more the consumers demand, with extensive research of their use as a prophylactic method to improve the immune system and minimize the inflammation caused by SARS-CoV-2 infection [2–4].

Xylooligosaccharides (XOS) are xylan derived oligomers, composed by xylose units, with great prebiotic potential [5, 6]. The regular intake of these non-digestible food ingredients that stimulate the growth and function of beneficial intestinal microorganisms (microflora), have reported several benefits, including the maintenance of gastrointestinal health, reduction of blood cholesterol, improvement of body weight, increase in mineral absorption, immune stimulation, glucose reducing ability, antioxidant and anticancer activity [1, 7].

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Lower degrees of polymerization (DP), like xylobiose (X2) and xylotriose (X3), are known to have the highest prebiotic effect as, due to their smaller DP, they are easily degraded by the host microflora [8, 9]. Also, X2 presents a relative sweetness similar to sucrose [10], which increases their spectrum in food applications. Regardless of the XOS production process, the final mixture contains other compounds that can be seen as impurities and need to be minimized as they affect XOS functionality [11]. The monomer xylose, for example, although it does not present any toxicity at dose levels equal or below 2.5% [12], it is not considered a prebiotic as it is mostly absorbed in the host small intestine and only a small part is utilized by the gut microflora [13]. Also, if present, it would contribute to an undesirable caloric content of the XOS mixture.

Due to the importance of these certified prebiotics [12, 14, 15] their production has been vastly studied from a variety of lignocellulosic biomass (LBC), including softwoods and hardwoods [16]. The presence of lignin and cellulose in these substrates can, however, inhibit xylan hydrolysis or lead to the formation of unwanted derivative compounds [11].

XOS can be produced from xylan contained in LCB by enzymatic treatments, chemical processes or autohydrolysis. The last two methods usually require high temperatures and pressures, corrosion resistant materials (e.g. acids for the chemical methods), high energy consumption and often result in the production of high amounts of unwanted by-products [5–7].

Enzymatic hydrolysis stands as the greenest alternative for producing XOS. It has been, in the recent past years, under the scope of the food industry for not requiring any special equipment (low temperatures and pressure) or strong chemical compounds, in contrast to the other common methods [7, 16]. Currently, there are certified and commercialized XOS produced by this process [12].

The careful choice of the most adequate enzyme turns enzymatic hydrolysis into a much more selective process, focusing only on the production of XOS, while minimizing the formation of unwanted by-products, the main products usually being X2 and X3 [5, 6, 17, 18]. To assure the enzyme best performance, the control of the solution pH is known to be a very important parameter [19]. For this reason, buffer solutions are recurrently used to maintain the activity and stability of the enzymes, improve the reaction yield and reduce enzyme consumption [7].

Despite all the advantages attributed to these enzymatic processes, namely those of being environmentally friendly; requiring low energy consumption and moderate operating conditions; resulting in high selectivity and low production of unwanted by-products, the large amount of buffer solution and high cost of the enzyme can be, however, major drawbacks for its industrial implementation [16]. The buffer use in large scale applications increases both the reagents and equipment costs as more complex downstream purification processes are required.

Many of the possible processes for XOS production are well documented, however, most of the published information concerns laboratory studies performed with an enormous variety of substrates, methods and equipment. There is, therefore, a lack of information concerning scale-up of these processes and how to enhance their efficiency with reduced costs and low waste.

In this work, a synergy is established between prebiotics increasing demand and pulp and paper industry, by producing xylooligosaccharides through enzymatic hydrolysis of bleached *kraft* pulp from *Eucalyptus* produced by a Portuguese pulp and paper company. This intermediate product, mainly composed by cellulose and hemicellulose, with 16–23% of xylan [20, 21], although very little exploited for XOS production, is widely produced and available in this industrial sector, representing a low-cost raw material. Furthermore, the post-hydrolysis pulp, although it suffers a decreasing bonding performance for having a smaller amount of xylan, if implemented in small portions in the fiber stream of the commercial pulp and/ or paper manufacturing processes does not affect the quality of the final products. Therefore, this is a truly waste-free production method.

For XOS production by enzymatic hydrolysis, the influence of the most relevant operating conditions was studied, including enzyme concentration, temperature, pH and time of the reaction. Also, due to the economic drawbacks of this process at industrial scale, a new approach is addressed aiming the use of water as the reaction medium instead of a buffer solution.

Materials and Methods

Materials

The bleached *kraft* pulp from *Eucalyptus* and the endo-xylanase used in this work were supplied by RAIZ (Forest and Paper Research Institute, Aveiro, Portugal). The main characteristics of the pulp are presented in Table 1.

Standard solutions of XOS with different degrees of polymerization, xylobiose (X2), xylotriose (X3), xylotetraose (X4), xylopentaose (X5) and xylohexaose (X6), were obtained from Megazyme, Ireland. All the other chemicals, including xylose (X1) > 99%, glacial acetic acid (CH_3COOH) 100%, sodium acetate ($C_2H_3NaO_2$) 99% and sulfuric acid (H_2SO_4) 96% were from Sigma-Aldrich Company, USA.

Table 1 Characteristics of the *kraft* pulp used in the experiments

Intrinsic viscosity (mg/L)	Polymeric degree	SR*	pH	Ashes (%m/m)	Moisture content (%)	Pentosan content (%)
960 ± 12	2837 ± 47	30^0	4.06 ± 0.05	0.25 ± 0.01	6.0 ± 0.3	21.4 ± 0.4

*Schopper-Riegler: Drainage capability

Enzymatic Hydrolysis

In this work, two different approaches were studied in what concerns the reaction medium: (i) enzymatic hydrolysis with an acetate buffer (EHB) and (ii) enzymatic hydrolysis with water (EHW). For all the experiments, the values considered were between 41.5 and 83.5 ºC for the temperature; 9.5–110.5 U/g pulp for the enzyme concentration and 3.7–5.3 for the solution pH, only when using buffer. As for the EHW, the solution pH was not adjusted. For both approaches, the experimental procedure was identical and is described below.

The enzyme used in the hydrolysis was previously assayed for its enzyme activity according to the endo-xylanase activity reported procedure [22]. One unit (U) is defined as the amount of enzyme that liberates 1 µmol of xylose per minute under the assay conditions.

Experimental Procedure

The reaction medium was first heated in a stirred jacketed reactor (200 mL) and, after reaching the defined temperature, a given amount of endo-xylanase was added and mixed during 2–3 min. The amount of pulp for a total consistency of 4% was then introduced. At this stage the hydrolysis begins, with the temperature and the stirring (100 rpm) remaining constant and the pH being monitored during all the reaction time. Samples were taken at predefined periods of 1, 2, 4, 6 and 8 h, heated in boiling water to deactivate the enzyme and analyzed by HPLC. Considering the HPLC results, the yields of XOS formation were defined by the following equations:

$$Yield\ per\ xylan(\%) = \frac{m_{XOS}(g)}{m_{total\ xylan\ in\ the\ pulp}(g)} \times 100 \quad (1)$$

$$Yield\ per\ pulp(\%) = \frac{m_{XOS}(g)}{m_{initial\ dry\ pulp}(g)} \times 100 \quad (2)$$

where m_{XOS} refers to the mixture of X2–X6.

At the end of the reaction, the suspension was submitted to vacuum filtration to separate the residual pulp from the filtrate containing XOS. The pulp was then dried at 60 ºC and further analyzed to determine the remaining pentosan content.

Design of Experiments

In order to maximize the production of XOS, the hydrolysis parameters were optimized by Response Surface Methodology (RSM) using Central Composite Design (CCD) for 3 factors and four replicates at the center point [23]. The chosen independent variables were temperature, a_1, enzyme concentration, a_2, and pH, a_3 (only for the buffer experiments, EHB), whose defined values and center point are listed in Tables 2 and 3 for buffer and water as reaction medium, respectively. For the experiments using water, EHW, only the temperature and the enzyme concentration require optimization. For these parameters, the same ranges as those considered for the buffer were used for comparison purposes (Table 3). With these assumptions, a total of 18 and 12 experiments were performed for EHB and EHW, respectively.

The response variables (Y) considered were the yields of XOS produced per xylan (Eq. 1), which are related to the

Table 2 Independent variables values for the use of buffer as reaction medium (EHB)

Experiment	Variables		
	Temperature (ºC) a_1	Enzyme concentration (U/g pulp) a_2	pH a_3
1	50.0	30.0	4.0
2	75.0	30.0	4.0
3	50.0	30.0	5.0
4	75.0	30.0	5.0
5	50.0	90.0	4.0
6	75.0	90.0	4.0
7	50.0	90.0	5.0
8	75.0	90.0	5.0
9	41.5	60.0	4.5
10	83.5	60.0	4.5
11	62.5	60.0	3.7
12	62.5	60.0	5.3
13	62.5	9.5	4.5
14	62.5	110.5	4.5
15	62.5	60.0	4.5
16	62.5	60.0	4.5
17	62.5	60.0	4.5
18	62.5	60.0	4.5

Table 3 Independent variables values for the use of water as reaction medium (EHW)

Experiment	Variables	
	Temperature (ºC) a_1	Enzyme concentration (U/g pulp) a_2
1	50.0	30.0
2	75.0	30.0
3	50.0	90.0
4	75.0	90.0
5	41.5	60.0
6	83.5	60.0
7	62.5	9.5
8	62.5	110.5
9	62.5	60.0
10	62.5	60.0
11	62.5	60.0
12	62.5	60.0

independent variables by the following generalized quadratic polynomial equation:

$$Y = x_0 + \sum x_i a_i + \sum x_{ii} a_i^2 + \sum x_{iii} a_i a_j \quad (3)$$

where a_i and a_j represent independent variables that influence the response variable, x_0 represents a constant term and x_i, x_{ii} and x_{iii} represent the coefficients of linear, quadratic and interaction parameters, respectively.

The statistical software Minitab® (version 17.1.0) was used for the regression analysis of the experimental data and the RSM plots.

Analytical Methods

Pentosans

Xylan quantification in the pulp was determined through pentosan content, from which 99% corresponds to xylan [24], using the Seaman hydrolysis method [25]. The pulp was soaked in 400 µL of 72% (m/m) H_2SO_4 at 25 ºC for 3 h and then diluted in 4.4 mL of distilled water at 100 ºC for 2.5 h. The amount of xylose produced was determined by HPLC and the result multiplied by 0.88 to give the pentosan content.

Moisture Content

The moisture content of the pulp was determined, in the course of the experiments, by the methodology described in [26].

High-Performance Liquid Chromatography (HPLC)

Prior to HPLC analysis, all the samples were filtered through a 0.2 µm syringe filter.

The enzymatic hydrolysis samples were eluted with 0.2 mL/min mili-Q water at 70 ºC using an Aminex HPX 42A column from Biorad® and analyzed through a refractive index (RI) detector model 133 Gilson (detection limit ≥ 5 ppm).

The samples resulting from pentosan content determination ("Pentosans" section) were eluted with 0.2 mL/min of H_2SO_4 5 mM at 50 ºC using an Aminex HPX 87H column from Biorad® and analyzed through a RI detector model 2142 LKB Bromma (detection limit ≥ 10 ppm).

Results and Discussion

Enzymatic Hydrolysis Experiments

Enzymatic hydrolysis is a mild and environmentally friendly method to produce xylooligosaccharides. The high cost of the enzyme, however, makes it a rather expensive process being therefore crucial to find approaches and conditions that maximize XOS production, as well as seek for possible modifications to turn it into a less expensive operation. The enzymatic treatment performed on a hardwood *kraft* pulp to produce XOS is a rarely explored procedure with, to our knowledge, very few reported works [27, 28]. This widely available raw material in pulp and paper industries is, however, an excellent substrate for producing XOS as it presents around 20% of xylan and no lignin. This absence of lignin is a huge advantage as, when present, it inhibits xylan hydrolysis [11].

The values of the operating conditions used experimentally, for the enzymatic hydrolysis, were defined by RSM-CCD, as described in the "Design of Experiments" section. For those conditions, the maximum XOS yields obtained per unit weight of total xylan present in the pulp (Eq. 1) stood in the range of 17–33% and 20–32%, which corresponds to the yields per initial pulp (Eq. 2) of 3.0–5.9% and 3.5–5.7%, for buffer and water as reaction medium, respectively. The hydrolysates were mostly composed by xylobiose and xylotriose, which are known to present higher prebiotic effect. The formation of xylotetraose was also observed, however, in lower quantities. Despite the different degrees of polymerization obtained throughout this study, they will be considered all together as XOS for simplification purposes.

Alongside XOS production, xylose, an unwanted by-product without prebiotic properties, was also formed in small amounts. Besides this, no other reaction products were detected, as it is common for this type of process, in contrast to what happens for acid hydrolysis or autohydrolysis [7].

At the end of the enzymatic treatment, an average pentosan content between 10 and 14% was determined for the hydrolyzed pulp.

Fitting Models

The enzymatic production of XOS usually depends on several operating conditions, including temperature, enzyme concentration, pH of the solution and time of the reaction. Each of these parameters, as well as their interaction, affect the hydrolysis rate and selectivity, making their optimization crucial. For this reason, the influence of the operating conditions was primarily optimized, by RSM, to achieve the highest XOS yield.

RSM uses a minimum set of experiments to determine the coefficients of a mathematical model (Eq. 3) as well as the optimum conditions for the previously defined independent process variables: temperature (a_1), enzyme concentration (a_2) and pH (a_3). The reaction time was not considered, at first, one of these variables for the design of the experiments represented in Tables 2 and 3. However, the monitoring of the evolution of XOS production at specific times during the enzymatic hydrolysis, as described in the "Experimental procedure" section, allows the simultaneous optimization of the time of reaction, a_4, without the need to perform additional experiments.

The yields of XOS as a function of the defined independent variables, calculated with the experimental results, are very well described by the regression equations obtained from the RSM presented in Table 4. For these, fittings with high values of R^2, between 0.946 and 0.961, were obtained, thus confirming the good agreement between the experimental and predicted results, being slightly better, however, for EHW. The model capacity to describe the experimental results can also be observed in Fig. 1, in which the average experimental and predicted yields, as a function of time, are compared for the center point operating conditions: 62.5ºC; 60 U/ g pulp (EHB and EHW) and pH of 4.5 (EHB).

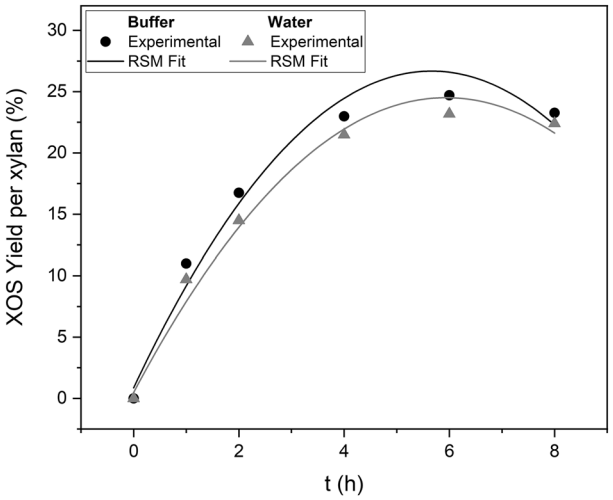

Fig. 1 Experimental and RSM predicted yields of XOS for the center point operating conditions for the use of buffer and water

Influence of the Operating Conditions

One of the main advantages of using RSM is the possibility to quickly understand how the reaction yields are affected by the various experimental conditions. For every case, the p-values of the studied variables, all lower than 0.05, show that each has a significant effect on the production of XOS. Enzyme concentration and reaction time are the most significant parameters, with a p-value always lower than 0.0001. This conclusion can be directly observed in Fig. 2, which shows the variation of the yields of XOS as a function of each variable under study for the use of buffer and water, considering the center point conditions: 62.5ºC; 60 U/g pulp; 4 h (EHB and EHW) and pH of 4.5 (EHB). It can be seen that the influence of the different experimental parameters is similar, regardless the reaction medium used. As previously stated, the enzyme dosage and reaction time have the highest impact on the yields, presenting steeper slopes. The use of a higher enzyme

Table 4 Regression equations obtained by RSM for XOS yields, using buffer and water, and corresponding fittings to the experimental results

Reaction medium	Equation	R^2
Buffer	$Y = 5.0 + 0.127a_1 + 0.0485a_2 - 4.61a_3 + 8.326a_4 - 0.000308a_1^2 - 0.000155a_2^2 + 0.666a_3^2 - 0.8052a_4^2 - 0.000001a_1a_2 - 0.0175a_1a_3 + 0.01855a_1a_4 - 0.0057a_2a_3 + 0.01413a_2a_4 - 0.270a_3a_4$	0.946
Water	$Y = 2.64 - 0.036a_1 - 0.0515a_2 + 6.876a_4 - 0.000146a_1^2 - 0.000054a_2^2 - 0.6809a_4^2 + 0.001053a_1a_2 + 0.00853a_1a_4 + 0.01134a_2a_4$	0.961

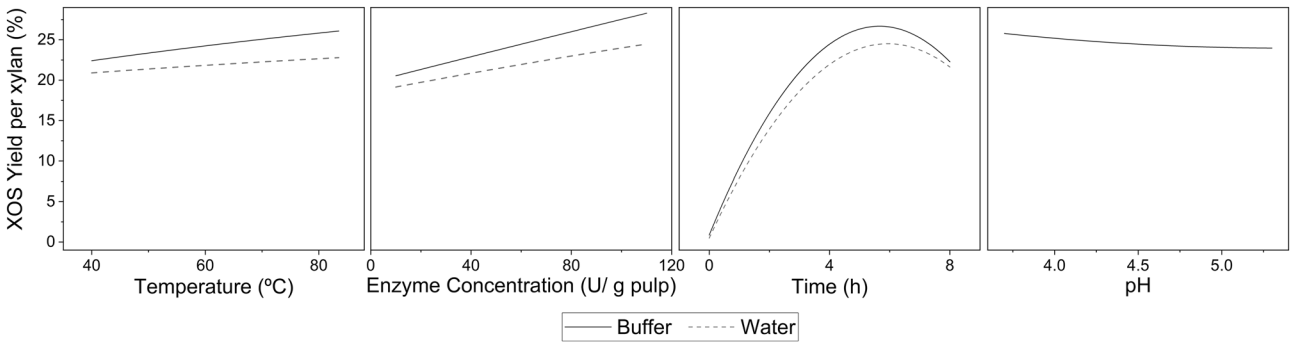

Fig. 2 Influence of the independent variables for the use of buffer and water (center point operating conditions)

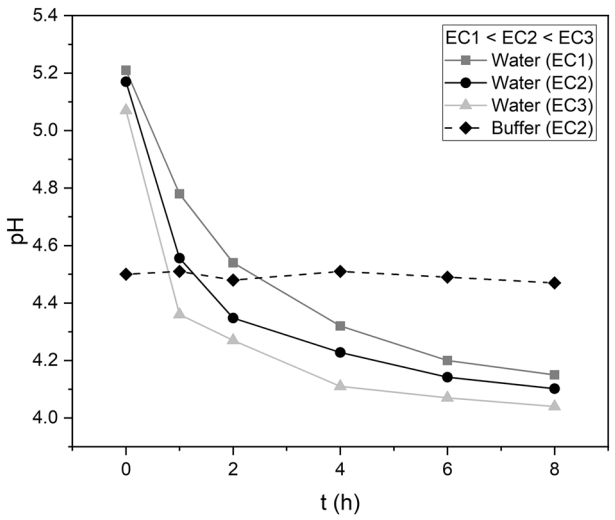

Fig. 3 pH variation during enzymatic hydrolysis for the use of buffer and water (with different enzyme concentrations - EC)

concentration leads to a greater production of XOS as reported in other studies [28–31].

As for the reaction time, XOS yields increase considerably until 4–5 h, after which, there is a trend for stabilization or even to a decrease. Chen et al. [30] and Wang et al. [28] also reported an increase in the production of XOS on the first hours of reaction, until 6 and 8 h, respectively, followed by a gradual decrease.

Although with lesser impact, high temperatures also favor the production of XOS as seen by Akpinar et al. [29] and Samanta et al. [32], who also found that the hydrolysis rate and yield improved with this parameter, but the reported increase was not significant.

Regarding the pH for EHB, its variation has the smallest effect on the yields. Nevertheless, lower pH values (< 4.0) seem to result in higher production of XOS. On the contrary, Akpinar et al. [29], Samanta et al. [32] and Singh et al. [31] noted a significant influence of the solution pH in XOS formation reaching optimum pH values of 5.4, 5.0 and 4.0, respectively. These differences on the optimum value are probably related to the characteristics and source of the enzyme used.

For EHW, as the solution pH cannot be controlled, the influence of this parameter was not studied. In contrast to EHB, in which the predefined pH remains constant through all the hydrolysis, for EHW the initial pH value of water rapidly decreases the moment the reaction starts, as represented in Fig. 3. A higher drop is observed for the first hours of reaction followed by a stabilization tendency. This reduction can be related to the formation of the enzyme–substrate transitional complex which changes the enzyme configuration and releases hydronium ions (H^+), resulting in the decrease of the solution pH as explained in Zheng et al. [33]. According to this, the stabilization tendency is therefore related to the enzyme dosage employed as confirmed in Fig. 3. The higher the enzyme concentration (EC3) the faster the pH decreases, tending towards lower values. Nevertheless, these low pH values that are achieved are not problematic as this parameter, for the studied range, has a low impact on the yields of the reaction. Also, although with a small influence, lower pH values favor the production of XOS, as seen in the parametric analysis related to buffer showed in Fig. 2.

In summary, from this analysis, it can be concluded that the optimum reaction conditions for the enzymatic production of XOS include high temperatures and enzyme concentrations, low pH values (for EHB) and reaction times between 4 and 6 h.

Optimum Conditions for XOS Production: Buffer (EHB) vs Water (EHW)

Buffer solutions, as stated before, are usually required to preserve the stability and activity of the enzyme. Its use on an industrial level is, however, associated to a significant increase in the production costs. So, if possible, the use of an alternative reaction medium such as water, for example, instead of the traditional buffer solution would be

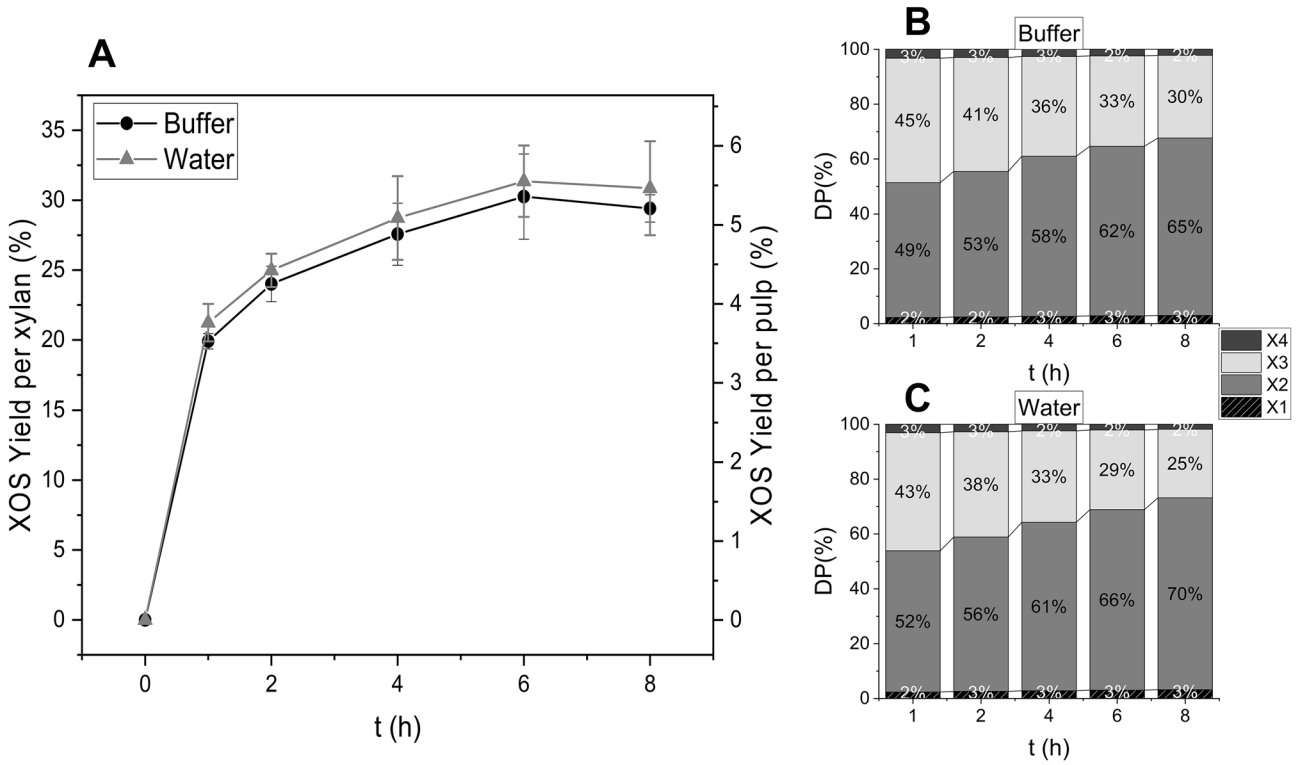

Fig. 4 Yield of XOS (**A**) and degrees of polymerization (**B** and **C**) as a function of time for the optimum conditions to maximize the production of XOS: buffer vs water

an important asset both for economic and environmental process perspectives.

As previously seen, the influence of the independent variables on the reaction yields was similar when using either buffer or water, thus leading to identical optimum operating conditions. The results of RSM optimization emphasized the need of using high enzyme concentrations of 100 ± 10 U/g pulp, high temperatures of 80 ± 2 ºC and a low pH value, when using buffer, of 3.7 ± 1. As temperature shows a small influence on the yield, a lower value could be used in order to reduce energy consumption. In this study, however, the value provided by RSM was adopted. These experiments were also monitored through time and their results are represented in Fig. 4.

In Fig. 4A, it can be seen that the profile of XOS yields throughout the reaction is practically identical for both reaction media. The maximum yields obtained for the optimized conditions are equivalent for buffer and water, these being $30.3 \pm 3.0\%$ and $31.4 \pm 2.6\%$, respectively, both at 6 h.

The distribution of polymerization degrees (Fig. 4B, C) is also similar for both situations. There is a gradual decrease in the amount of xylotriose due to its decomposition into smaller DP's, namely xylobiose, whose amount is increasing as the reaction proceeds. The main difference between EHB and EHW relies on both xylobiose and xylotriose distribution. Although the amount of X2 is always higher than X3 for the two reaction media, there is a slightly higher production of X3 when using buffer. At the maximum yield value (6 h), the enzymatic hydrolysate composition is 62% of X2 and 33% of X3 when using buffer and 66% of X2 and 29% of X3 for water.

Singh et al. [31] and Figueiredo et al. [34], with multiple step processes, reported maximum yields of XOS per xylan in the same order of magnitude of 35.2% and 27.1%, respectively, using xylan extracted from arecanut husk and from sugarcane bagasse, both soaked in buffer solutions.

On the other hand, Wang et al. [28], also with a buffer medium, obtained a XOS yield of 42.96% using a xylan-rich hemicellulose extracted from dissolving pulp, which is a substrate similar to the one used in this work. This higher yield might be due to the fact that the xylan was previously isolated and, therefore, there are no constrains on the enzyme action. However, despite the higher production of XOS, an additional step (xylan extraction from the pulp) is required, corresponding to an extra process cost.

In another study, where the primary goal was to optimize xylan extraction by a xylanase treatment of hardwood *kraft* pulp acidified with sulfuric acid, Hakala et al. [27] hydrolyzed up to 12% of xylan to xylooligosaccharides in 2 h. This value corresponds to half of the yield obtained for 2 h in the present work, for both EHB and EHW (Fig. 4).

So, from the results obtained for the optimized experimental conditions which maximize XOS production, it can be concluded that the yields of this single-step process, either using water or buffer, are comparable to others reported for different substrates and multi-step methodologies. Also, the reaction medium does not show a significant influence on XOS production from hardwood *kraft* pulp as no major differences on the yields or on the degrees of polymerization were observed. As to pH, a parameter to which enzymatic hydrolysis is typically very sensitive, it was found, in the present work, to have the smallest impact on the production of XOS (Fig. 2), meaning that pH variation during the reactions with water did not affect XOS yields.

This proven feasibility of replacing the often-used buffer solution in the enzymatic hydrolysis for water, without the loss of important products, is a major improvement to this process. Furthermore, it always results in a slightly higher amount of xylobiose in the reaction mixture, which is an additional advantage due to its higher degradability that results in a higher prebiotic effect [8, 9].

From all that was previously exposed, enzymatic hydrolysis can be performed directly on *Eucalyptus kraft* pulp using water, without the need of a pretreatment, resulting in more than 5 g of XOS/100 g of dry pulp (Fig. 4).

Conclusions

Enzymatic hydrolysis was already seen as the greenest option for XOS production. Now, according to the results obtained in this work, in which XOS were produced by directly performing an enzymatic treatment with water on *Eucalyptus bleached kraft* pulp, the process becomes even more ecofriendly by: (i) avoiding any previous treatment of the substrate and (ii) completely avoiding the use of chemical products by replacing the traditionally used buffer solution for water as reaction medium. With this process, similar yields to those reported using buffer on a variety of substrates were obtained, with the production of mostly xylobiose and xylotriose, oligosaccharides with the highest prebiotic effect.

Despite the several reported attempts to produce XOS through an enzymatic approach of diverse lignocellulosic biomasses, there is no report, to our knowledge, of the direct use of this hardwood *kraft* pulp. Choosing this substrate, very low waste is generated as the post-hydrolysis pulp can be further implemented in other processes. Also, by using only water, the downstream purification of XOS solution, if necessary, is also simplified (no salts), as well as any wastewater treatments.

Considering that one of the main arguments against enzymatic hydrolysis has always been its high enzyme and reagents cost, the fact that it is feasible to produce XOS using water as the reaction medium, allows to have a less expensive production methodology, being a major improvement for future industrial implementations.

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Author Contribution All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by PIAH, while under direct supervision of MLSS and AMFBA (project administrator). The first draft of the manuscript was written by PIAH and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability All data and materials presented in this manuscript comply with field standards.

Declarations

Competing interests The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval Ethical and professional conduct have been followed.

Consent for publication All authors consent the publication of this article.

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