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Enhancing *Acacia dealbata* valorization through microwave-assisted autohydrolysis: An energy-efficient approach to oligosaccharides and bioethanol production

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ABSTRACT

Microwave-assisted autohydrolysis (MAA) has gained attention as an alternative to conventional hydrothermal treatment (CHT) to solubilize hemicellulosic-derived compounds, such as oligosaccharides, within shorter residence times. MAA assays were conducted on *Acacia dealbata* wood, an invasive species, at severities (S_0) ranging from 3.63 to 4.64 to optimize xylooligosaccharides (XO) recovery and assess the enzymatic susceptibility of the spent solids for bioethanol production. S_0 between 3.77 and 4.15 yielded XO concentrations > 9.8 g/L corresponding to a recovery of > 80 % regarding initial xylan. Besides, at S_0= 3.77, a bioethanol yield of 71 % was attained (26.25 g/L). Furthermore, CHT was performed at S_0 values of 3.80 and 4.44 to compare the impact of both heating strategies under optimal conditions for (i) XO production and (ii) higher enzymatic susceptibility of the spent solid. MAA resulted in higher bioethanol yields and, particularly under harsher conditions, lower by-products formation and higher oligosaccharides content. Additionally, MAA consumed 2.60–2.75-fold less energy than CHT.

1. Introduction

The most recent United Nations report forecasts that the global human population is expected to reach 8.5 billion by 2030, and 9.7 billion in 2050, presenting significant challenges for sustainable development (United Nations, 2022). In this context, biorefineries and biomass sources have been recognized as promising alternatives to advance the fulfillment of the Sustainable Development Goals (SDGs) and to align with the aims outlined in the 2030 Agenda (Solarte-Toro and Cardona Alzate, 2021). It is projected that approximately 10–50 billion tons of dry lignocellulose are manufactured annually on a global scale (Li et al., 2022)

The spread of *Acacia dealbata*, an angiosperm tree, has led to structural alterations in the fire and hydrological regimes, biodiversity, and topsoil features within its non-native ecosystems (López-Hortas et al., 2021; Portela-Grandío et al., 2021; Vieites-Blanco and González-Prieto, 2020). Furthermore, climate change is likely to accelerate the proliferation of *Acacia dealbata* due to the heightened frequency and intensity of forest fires and alterations in hydrological dynamics (Cruz et al., 2021; Souza-Alonso et al., 2017). Recognizing the potential ecological threat, the European Union has classified *Acacia dealbata* as an invasive alien species. Consequently, its commercial use may be temporarily permitted only as part of management measures aimed at eradication, population control, or containment, subject to stringent justification and appropriate controls to prevent further proliferation (European Commission, 2014; Ministry of Agriculture, 2013). In this sense, the valorization of waste materials resulting from the management of invasive alien species has garnered growing interest owing to its potential to partially mitigate the expenses associated with large-scale eradication plans (Lorenzo and Morais, 2023). Several studies have explored the direct valorization of invasive Acacia spp. whole biomass as an energy source through pellet manufacture (Ferreira et al., 2023; Nunes et al., 2020) and pyrolysis (Ayaa et al., 2022; Charvet et al., 2021; Reza et al., 2019). Alternatively, the separate utilization of *Acacia dealbata* biomass has been assessed to obtain value-added products such as lupane-triterpenoids (Rodrigues et al., 2023) and tannins (Abilleira et al., 2021) from bark, bioactive compounds from leaves and twigs (Correia et al., 2022), and to recover the different lignocellulosic wood structure fractions (Almeida et al., 2022; da Costa et al., 2022; Magalhães et al., 2022; Portela-Grandío et al., 2021).

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Xylooligosaccharides (XO) are short-chain carbohydrates that can be produced through the hydrolysis of xylan, which is the main component of hemicellulose. Comprising xylose units linked by β-1,4-glycosidic bonds, XO are valued for their potential as prebiotics, antioxidants, and fortifying agents in food products (Michelin and Teixeira, 2020; Sharma et al., 2020). Recently, XO have been studied to modify the physico-chemical properties and functionality of different materials, such as alginate-gelatin hydrogels (Martins et al., 2021), hemicelluloses-chitosan films (Xu et al., 2019) or whey protein films (Xu et al., 2019).

Hydrothermal pretreatment is a well-established methodology for biomass fractionation, widely utilized for the selective recovery of the hemicellulose fraction. It is recognized as an environmentally friendly and cost-effective alternative and reduces the dependence on corrosive and expensive solvents (Ruiz et al., 2023; Sun et al., 2022). Despite the advantages, several challenges persist, including the high energy and water demands, as well as the production of fermentation inhibitors such as furfural and hydroxymethylfurfural (HMF), along with pseudo-lignin, particularly when operating under severe conditions (Rezania et al., 2020; Sun et al., 2022). In this context, microwave heating has garnered attention due to its ability to reduce reaction times, thereby decreasing energy consumption and minimizing the formation of byproducts (Singh et al., 2023; Yue et al., 2022). The present interest in developing more cost-effective and efficient multi-product bio-refineries is currently driving a wide range of research into microwave-assisted extraction processes, using both conventional and emerging solvents. Furthermore, there is an increased focus on the intensification of traditional biorefining processes, such as pyrolysis and acid hydrolysis (Lozano Pérez et al., 2024).

A number of studies exhibited that hydrothermal pretreatment enhances the enzymatic digestibility of the solid fraction, thereby improving bioethanol production in the context of second-generation biorefineries (Gomes et al., 2021; Sun et al., 2022). Efforts to enhance the cost-effectiveness of bioethanol production include the co-production of other value-added products, the choice of feedstock, and the optimization of pretreatment and fermentation technologies (Devi et al., 2022).

In this sense, this study evaluated for the first time the possible integration of an invasive species into a biorefinery scheme by producing oligosaccharides and bioethanol. Additionally, the study aimed to assess the effects of microwave-assisted autohydrolysis (MAA) and conventional hydrothermal treatment (CHT) on the composition of the obtained liquid and solid fractions, considering, as well, the energy consumption of the pretreatment.

2. Material and methods

2.1. Raw material

The *Acacia dealbata* Link wood employed within this work was sourced by the city council of Ourense (NW Spain). The biomass was air-dried and subsequently milled to a $\leq$ 3 mm-particles. Following this, it was kept in a cold dry dark location until analytical and experimental procedures were carried out.

2.2. Acacia dealbata characterization

The chemical composition of *Acacia dealbata* wood was analyzed using the standard procedures (similar to those published by the NREL) for moisture content (Sluiter et al., 2008a), ash (Sluiter et al., 2008b), extractives (Sluiter et al., 2008d) and quantitative acid hydrolysis for polysaccharide quantitation (Sluiter et al., 2008c). Following the two-step quantitative acid hydrolysis– (i)72 % H_2SO_4, 1 h and 30 °C, and (ii) 4 % H_2SO_4, 1 h, 121 °C– the liquid fraction recovered was filtered using a 0.45 μm membrane, and the concentration of monosaccharides and organic acids was quantified using high-performance liquid chromatography (HPLC) with an Agilent 1200 series model (CA, USA). The HPLC analysis employed a Rezex ROA-Organic Acid H^+ (8 %) column at 60 °C, a 3 mM H_2SO_4 mobile phase at 0.6 mL/min, and a refractive index detector at 40 °C. The solid residue remaining was dried at 105 °C for 24 h and weighed to determine Klason lignin (KL). Additionally, the uronic acids were quantified as equivalents in galacturonic acid using the colorimetric procedure on the liquid obtained after quantitative acid hydrolysis (Blumenkrantz and Asboe-Hansen, 1973). Each analytic procedure was conducted in triplicate to ensure accuracy and reproducibility.

The chemical composition of the *Acacia dealbata* wood, expressed in g per 100 g of solid on a dry basis ± standard deviation, was determined as follows: glucan 36.19 ± 0.55, xylan 17.10 ± 0.06, arabinan 0.94 ± 0.04, uronic acids 5.96 ± 0.19, acetyl groups 3.40 ± 0.08, KL 19.56 ± 0.95, ashes 1.01 ± 0.02, and extractives 8.07 ± 0.19.

2.3. Pretreatment of Acacia dealbata wood

In this study, two heating strategies for the autohydrolysis fractionation of *Acacia dealbata* wood were investigated. Both pretreatments were conducted at a consistency (C) of 6 g of solid per 100 g of total weight and the harshness, expressed as severity (S_0), was calculated using the Eq. (1):

$$\begin{aligned} S_0 &= \log(R_{0HEATING} + R_{0ISOTHERMAL} + R_{0COOLING}) \\ &= \log\left(\int_0^{t_H} \exp\left(\frac{T(t) - T_{REF}}{\omega}\right)\cdot dt + \exp\left(\frac{T_{ISOT} - T_{REF}}{\omega}\right)\cdot t_{ISOT} \right. \\ &\quad \left. + \int_{t_H + t_{ISOT}}^{t_H + t_{ISOT} + t_C} \exp\left(\frac{T'(t) - T_{REF}}{\omega}\right)\cdot dt\right) \end{aligned} \quad (1)$$

Where R_0 is the severity factor, T(t) and T'(t) are the heating and cooling temperature profiles, t_H (min) expresses the time needed to accomplish the temperature T_{ISOT} (°C), which is maintained for an established time t_{ISOT} (min), t_C (min) represents the time used in the cooling period, ω is the empirical parameter associated to the activation energy and T_{REF} is the reference temperature (according to literature: ω = 14.75 K and T_{REF} = 100 °C).

Following the pretreatments, the liquor fraction was collected using vacuum filtration, and the remaining solids were washed with distilled water until reaching a neutral pH. The liquid phase was preserved at 4 °C, while the solid fraction was air-dried at room temperature for 24 h before being subjected to gravimetric determination for the solid yield (SY).

2.3.1. Microwave-assisted autohydrolysis (MAA) of Acacia dealbata

The MAA pretreatment was carried out using a single-mode microwave reactor (Monowave 450 from Anton Paar GmBH) with 30 mL glass vials (Reaction Vial G30 from Anton Paar GmbH) under non-isothermal conditions. The mixture was stirred at 900 rpm, with a consistent heating time (t_H) of 5 min for all experiments until it reached a temperature of 230 °C. The residence times (t_{ISOT}) were from 0 to 6 min to achieve severities ranging from 3.63 to 4.64. Cooling was facilitated by an integrated air compressor, taking approximately 5–6 min.

2.3.2. Conventional hydrothermal treatment (CHT) of Acacia dealbata

The CHT was conducted in a 1.6 L high-pressure reactor (Büchi-glasuster® versoclave, Switzerland) under non-isothermal conditions. The reactor is equipped with a heating jacket and an agitator controlled by a PID system for temperature and stirring rate regulation. The stirring rate was maintained at approximately 500 rpm throughout the pretreatment, while the target temperature varied based on the desired severity. Specifically, maximum temperatures of 200 °C and 218 °C were selected to achieve severities of 3.77 and 4.44. These specific values closely align with the two preferred conditions identified in the MAA study for the production of XO and the enhancement of enzymatic susceptibility of the solid, respectively. The energy consumption during the

pretreatment was quantified using a power meter (Zaeel).

2.4. Chemical composition of the liquor fractions from the autohydrolysis treatments

The recovered liquors from the autohydrolysis process were subjected to triplicate analysis. The concentration of glucose, xylose, arabinose, acetic acid, and dehydration products (5-hydroxymethyl-2-furfural (HMF) and furfural) was determined by filtering an aliquot of the liquor and directly analyzing it using HPLC. Additionally, the total oligosaccharide content, including glucooligosaccharides (GO), xylooligosaccharides (XO), arabinooligosaccharides (ArO), and acetyl groups linked to oligomers (AcO), was quantified by subjecting another aliquot to quantitative post-hydrolysis (4 % H_2SO_4, 20 min, 121 °C), followed by filtration and injection into the HPLC (see Section 2.2 for analytical conditions). Non-volatile compounds (NVC) in the autohydrolysis liquors were analyzed in triplicate by oven-drying aliquots of the liquors for 24 h at 105 °C until constant weight.

2.5. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS)

According to Pérez-Pérez et al. (2023), an aliquot of the freeze-dried liquor was subjected to analysis using MALDI-TOF-MS on an autoflex TOF/TOF apparatus (Bruker, MA, USA). To achieve a concentration of 1 mg of lyophilized liquor/mL, 0.1 % trifluoroacetic acid was utilized, which was combined in a 1:1 ratio with a matrix of 50 % 2,5-dihydroxy-benzoic acid 0.1 % acetonitrile/trifluoroacetic acid. For the analysis, a positive refractor with a mass range of 500–3500 m/z and a nitrogen laser ($\lambda = 337$ nm) was employed.

2.6. High-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

The liquors obtained through autohydrolysis were analyzed using an HPAEC-PAD ICS3000 chromatographic system (Dionex, Sunnyvale, CA, USA) with a Carbo Pac PA guard column coupled to a CarboPac PA-1 column and an ISC3000 PAD detector. The method followed the procedure outlined by Gullón et al. (2014) and utilized XO (DP 2–6) from Megazyme (Megazyme International, Ireland) as standards to determine the degree of polymerization (DP) of the oligosaccharides in the liquors.

2.7. Enzymatic susceptibility of the solid fraction

The solid fractions obtained from the various MAA pretreatments were subjected to enzymatic hydrolysis (EH) to assess the impact of severity on the potential for glucose yield. The experiments were performed at the same consistency as the pretreatments (C = 6 %), employing a cellulase to substrate ratio (CSR) of 20 FPU/g solid, a cellulase ratio (CCR) of 5 IU/FPU, at a temperature of 50 °C with orbital agitation at 150 rpm, using a 0.05 N citric acid-sodium citrate buffer to maintain a pH of 5. The EH process was maintained for 72 h, and samples were collected at preset times. The tests were carried out in duplicate. Furthermore, EH was carried out as previously described on the solids obtained under selected conditions for both MAA and CHT, but with a higher consistency (C=11 %). This was performed to assess the potential valorization of these solids through PSSF (detailed in Section 2.9).

The commercial cellulase enzyme blends Cellic CTec2 kindly provided by Novozymes A/S (Copenhagen), was utilized in the study. The enzyme activity was quantified using the Filter Paper assay (Ghose, 1987), resulting in a value of 116 FPU/mL. The liquor was subjected to HPLC analysis, as detailed in section ↱2.2, to determine the glucose concentration (g/L). The glucan to glucose conversion (GCC) (%) was calculated using the following expression:

$$GCC = 100 \cdot \frac{G_t - G_{t=0}}{\frac{Gn}{100} \cdot \frac{180}{162} \cdot \frac{\rho}{\frac{1}{C} - \frac{KL}{100}}} \quad (2)$$

Where G_t is the glucose concentration (g/L) at time t and $G_{t=0}$ is the initial glucose concentration. The denominator represents the potential glucose concentration, which corresponds to the entire substrate conversion into glucose, where Gn represents the glucan content of pretreated acacia (g glucan per 100 g pretreated solid on an oven-dry basis), 180/162 is the stoichiometric factor for glucan hydration upon hydrolysis, ρ is the density of the reaction medium (with an average value of 1005 g/L), C represents the consistency, and KL signifies the Klason lignin content of pretreated acacia (g Klason lignin per 100 g pretreated solid on an oven-dry basis).

The GGC (%) was fitted to the hyperbolic empirical model described by Holtzapple et al. (1984):

$$GGC_t = GGC_{MAX} \cdot \frac{t}{t + t_{1/2}} \quad (3)$$

Where GGC_{MAX} is the maximum conversion and $t_{1/2}$ is the time to reach 50 % of GGC_{MAX}.

2.8. Yeast cultivation and inoculum preparation

The *Saccharomyces cerevisiae* Ethanol Red® strain (Lesaffre®, France) was cultivated at 30 °C and 200 rpm for 24 h in a medium composed of 20 g glucose/L, 20 g peptone/L and 10 g yeast extract/L in an orbital incubator (Incu-Shake series from SciQuip Ltd, UK). Following the growth phase, 10 mL of the culture was transferred to a fresh growth medium with the same composition and maintained for 15 h under the previously specified conditions. Subsequently, the cells were harvested via centrifugation, resuspended in a NaCl 0.9 % solution, and then inoculated to achieve an approximate concentration of 8 g fresh yeast/L (1.5 g/L on a dry weight basis).

2.9. Presaccharification and simultaneous saccharification and fermentation (PSSF) of the solid fraction

The solid fractions obtained from CHT and MAA at selected severities underwent presaccharification and simultaneous saccharification and fermentation (PSSF). These solids were sterilized with water for 15 min at 121 °C. The presaccharification process replicated the procedure described for EH (see section ↱2.6) except for using a higher consistency (C = 11 %) and adding 1 mL of sterilized nutrients (20 g peptone/L and 10 g yeast extract/L) to support yeast growth while omitting the buffer solution. Following the 72 h presaccharification stage, the inoculum was introduced to achieve a yeast concentration of 8 g/L in the medium (as described in Section 2.7). Subsequently, the fermentation process was then conducted in an orbital shaker for 9 h at 150 rpm and 35 °C. The assays were performed twice.

The PSSF samples underwent centrifugation, and the resulting supernatant was analyzed using HPLC according to the procedure specified for the liquors (section ↱2.4). However, owing to the presence of biomass in the mixture, the supernatant from the fermentation samples was filtered through 0.2 μm before being introduced to the HPLC. The ethanol yield (%) was determined using the equation below:

$$(\%)ethanol\ yield = \frac{[EtOH]_f - [EtOH]_0}{0.51 \cdot (f \cdot [Biomass] \cdot 1.111)} \cdot A \cdot 100 \quad (5)$$

Where $[EtOH]_f$ and $[EtOH]_0$ are the ethanol concentration (g/L) at final and initial times, 0.51 represents the conversion factor for glucose to ethanol based on the stoichiometry of glucose to ethanol conversion, f is glucan fraction of dry biomass (g/g), [Biomass] is initial dry biomass concentration (g/L), 1.111 is the stoichiometric factor that converts cellulose to equivalent glucose.

Table 1
Composition of the solid fraction recovered from MAA and CHT at different severities (expressed as g per 100 g *Acacia dealbata* o.d.b.), energy consumption (MJ/kg) and operation conditions. All analyses of solid phase composition and energy consumption were performed in triplicate.

Process	MAA	MAA	MAA	MAA	MAA	MAA	MAA	MAA	MAA	MAA	CHT	CHT
$T_{MÁX}$ (°C)	230	230	230	230	230	230	230	230	230	230	200	218
t_{ISOT} (min)	0	0.25	0.5	0.75	1	1.5	2	3	4	6	0	0
S_0	3.63	3.77	3.88	3.97	4.03	4.15	4.24	4.38	4.49	4.64	3.80	4.44
Energy consumed (MJ/kg)	20.19 ± 0.20	20.41 ± 0.56	20.47 ± 0.08	21.23 ± 0.78	21.69 ± 0.42	22.30 ± 0.18	22.34 ± 0.48	24.01 ± 0.48	25.56 ± 1.53	26.86 ± 0.39	53.07 ± 0.27	68.25 ± 0.31
Solid yield (%)	64.75	64.78	64.36	63.68	62.88	62.56	61.19	60.54	60.00	60.10	69.73	64.35
Solid phase composition (g per 100 g autohydrolyzed *Acacia dealbata*, o.d.b)												
Glucan	56.01 ± 1.05	57.01 ± 0.28	56.93 ± 1.31	58.85 ± 0.70	58.74 ± 1.23	59.27 ± 1.09	61.19 ± 0.87	61.69 ± 1.66	60.04 ± 0.99	59.20 ± 0.82	52.81 ± 0.15	56.82 ± 0.42
Xylan	8.00 ± 0.05	7.09 ± 0.00	5.85 ± 0.14	5.54 ± 0.07	5.33 ± 0.10	4.83 ± 0.12	4.08 ± 0.04	2.80 ± 0.05	2.58 ± 0.13	2.22 ± 0.13	6.44 ± 0.02	2.57 ± 0.12
Arabinan	0.24 ± 0.03	0.22 ± 0.03	0.22 ± 0.05	0.20 ± 0.08	0.19 ± 0.02	0.19 ± 0.04	0.20 ± 0.03	0.13 ± 0.01	0.10 ± 0.02	0.18 ± 0.08	0.39 ± 0.10	0.00 ± 0.00
Acetyl groups	1.30 ± 0.00	1.08 ± 0.06	0.89 ± 0.08	0.75 ± 0.02	0.77 ± 0.04	0.60 ± 0.05	0.59 ± 0.12	0.34 ± 0.03	0.35 ± 0.02	0.33 ± 0.22	1.18 ± 0.18	0.34 ± 0.02
Klason lignin	29.39 ± 0.95	29.57 ± 0.58	29.43 ± 0.41	29.14 ± 1.34	29.41 ± 1.04	30.84 ± 0.65	31.62 ± 1.18	32.33 ± 1.47	34.17 ± 1.41	33.92 ± 0.48	31.48 ± 0.23	34.06 ± 0.47

After completing the fermentation stage, the PSSF media underwent vacuum filtration and multiple washings with water to remove residual nutrients and metabolites generated during the experiments and to recover the spent solids. Subsequently, the solids were subjected to 24 h of air-drying and then gathered for analysis of moisture content and polymeric content using quantitative acid hydrolysis, following the procedure outlined for the raw material (Section 2.2).

2.10. Statistical analysis

Data from the PSSF assays were subjected to statistical analysis using R software (version 4.2.1). A one-way analysis of variance (ANOVA) followed by Tukey's test was used to assess differences between samples, with statistical differences considered at the 95 % level of significance.

3. Results and discussion

3.1. Effect of severity and autohydrolysis technology on the solid phase composition

CHT and MAA have been the subject of extensive research for selectively fractionate and recover the hemicellulosic fraction from hardwood feedstock in recent years. The reaction mechanism involves the release of oligomers and acetyl groups derived from hemicelluloses, which can be further degraded into the corresponding monomers and acetic acid, along with the generation of byproducts such as furfural and HMF depending on the severity of the process. Based on previous data on hardwood feedstock (del Río et al., 2022; Pérez-Pérez et al., 2023), S_0 was varied between 3.63 and 4.64 to maximize oligomers production at mild conditions and to reach a high enzymatic susceptibility of the glucan contained in the solid fraction under harsher conditions.

The degree of fractionation was assessed by measuring the recovered solid, expressed as solid yield (SY). It was observed that the SY decreased as severity increased, resulting in a range of 64.78–60.00 g of solid recovered per 100 g of feedstock oven-dry-basis for MAA, and 69.73–64.35 g per 100 g for CHT. These results are consistent with previous research on hardwood feedstock (Fang et al., 2022; Rigual et al., 2018). For example, Fang et al. (2022) demonstrated a negative linear correlation between S_0 and SY when birch sawdust was subjected to CHT, with S_0 ranging from 3.06 to 4.35 and SY ranging from 91.0 % to 66.3 %. Similarly, Rigual et al. (2018) observed a decrease in SY from 91.66 % to 66.58 % as S_0 increased from 2.95 to 4.90 during CHT using *Pinus radiata* sawdust.

Through mass balances, the solubilization of each biomass fraction can be assessed. The solubilization of hemicelluloses during MAA expressed as the sum of xylan, arabinan, and acetyl groups, increases from 71.26 % to 92.36 % as severity rises from 3.63 to 4.64, while only 1.87 % of glucan is solubilized at the highest severity (Table 1). The recovery values for KL exceeded 100 % when S_0 was higher than 4.24 in MAA. This phenomenon is attributed to the formation of pseudo-lignin with increasing S_0 and the presence of extracts that could be quantified as KL, as reported in previous works (Domínguez et al., 2020; Rigual et al., 2018). This increase in KL was more pronounced for CHT (Table 1), although the solubilization of hemicellulose at S_0 3.80 and 4.44 (74.00 % and 91.27 %) was comparable to that reported in MAA at similar severities, S_0 3.77 and 4.49 (74.68 % and 91.55 %).

These results are similar to those obtained in the literature using MAA in different hardwood feedstocks. For instance, 69.40 % and 93.79 % of hemicelluloses were removed from *Robinia pseudoacacia* wood at S_0 3.79 and 4.57 (Pérez-Pérez et al., 2023), and 88.40 % and 95.68 % were removed from *Paulownia elongata x fortunei* wood at S_0 3.77 and 4.42 (del Río et al., 2022). Similarly, Fang et al. (2022) demonstrated a positive correlation ($R^2 = 0.93$) between xylan removal and S_0, within the range of 3.06–4.35 in the CHT of birch sawdust. The xylan removal values ranged from 17.1 % to 73.8 % as S_0 increased.

No differences in glucan recovery were observed between the two technologies, as the yield in both cases was approximately 100 %. Consequently, glucan was the major component in the spent solids, with a variation in MAA between 56.01 ± 1.05 g per 100 g pretreated acacia at $S_0 = 3.63$ to a peak of 61.69 ± 1.66 g per 100 g pretreated acacia at $S_0 = 4.38$. A similar increase in the glucan content as severity rises was observed for CHT from 52.81 ± 0.15–56.82 ± 0.42 g per 100 g pretreated acacia at $S_0 = 3.80$ and 4.44, despite the values being lower due to the aforementioned higher formation of pseudo-lignin. del Río (2022) reported similar glucan composition and recovery in the spent solids from microwave-autohydrolyzed Paulownia wood at different severities. Despite the higher formation of pseudo-lignin (approximately 10 %) when the process is conducted as CHT, there are no noticeable differences between CHT and MAA in the composition of the obtained spent solids under the studied conditions.

One of the main benefits of MAA over CHT is its lower energy consumption (Singh et al., 2023). In the study, MAA required 2.60 and 2.75-fold less energy than CHT under mild ($S_0 = 3.77$–3.88) and harsher ($S_0 = 4.38$–4.49) conditions, respectively (Table 1). This reduction in energy consumption is attributed to the lower residence time required for hydrolyzing biomass fractions using microwave heating. Similarly, del Río et al. (2022) also reported that MAA consumed between 2.1 and 2.8 times less energy than CHT at the optimum conditions for producing XO ($S_0 = 3.98$) from Paulownia wood.

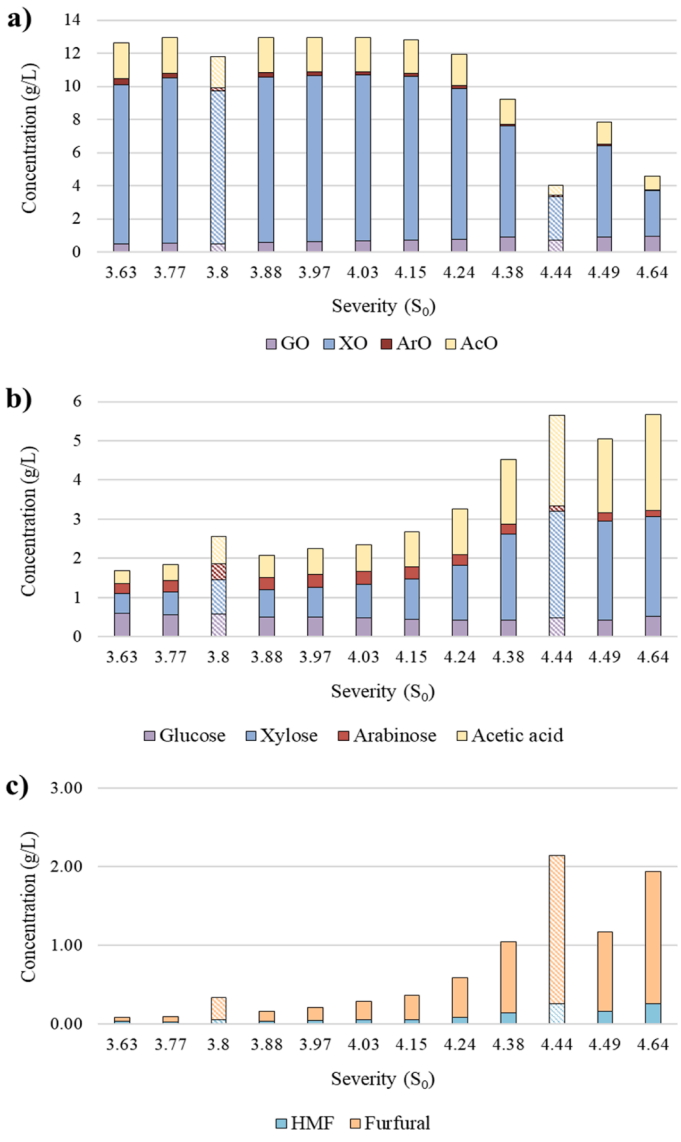

Fig. 1. Concentrations of oligomers (a) (GO: glucooligosaccharides; XO: xylooligosaccharides; ArO: arabinooligosaccharides; AcO: acetyl groups linked to oligomers), monosaccharides and acetic acid, (b) and furanic by-products (c) (HMF: hydroxymethylfurfural) in the liquors obtained through CHT (pattern fill) and MAA (solid fill). All analyses were performed in triplicate (standard deviation < 5 % in all cases).

Table 2
Experimental glucan to glucose conversion at 48 h and 72 h (GGC_t), and Holtzapple parameters obtained for the enzymatic hydrolysis (C=6 %) of *Acacia dealbata* wood after MAA and CHT at different severities ($R^2 > 0.9$).

MAA (S_0)	CHT (S_0)	$GGC_{48\ h}$ (%)	$GGC_{72\ h}$ (%)	GGC_{MAX} (%)	$t_{1/2}$ (h)
3.63	-	59.93	70.05	100.00	31.52
3.77	-	63.85	72.32	98.42	25.99
-	3.80	65.17	83.15	100	21.18
3.88	-	68.46	76.11	98.00	20.71
3.97	-	69.05	78.19	100.00	20.93
4.03	-	70.21	80.24	100.00	19.29
4.15	-	74.56	79.20	90.46	10.24
4.24	-	76.23	83.91	100.00	14.52
4.38	-	79.88	85.01	97.55	10.62
-	4.44	78.34	90.16	100	15.98
4.49	-	77.56	84.55	100.00	13.61
4.64	-	83.19	89.38	100.00	9.28

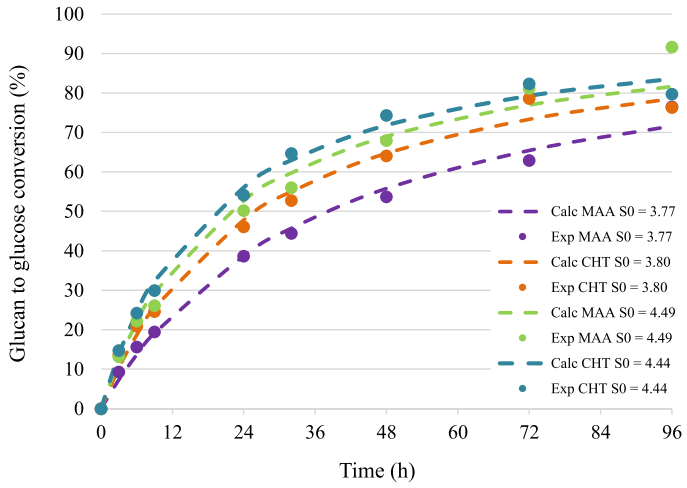

Fig. 2. Glucan to glucose conversion (GGC_t) from enzymatic hydrolysis of the autohydrolyzed *Acacia dealbata* through MAA (S_0 = 3.77 and 4.49) and CHT (S_0 = 3.80 and 4.44) at the selected conditions (C = 11 %). The dotted lines represent the Holtzapple adjustment (Calc) and dot the experimental data (Exp).

Table 3
Holztapple parameters calculated for adjusting the enzymatic hydrolysis of autohydrolyzed *Acacia dealbata* using MAA and CHT under the selected conditions (C = 11 %).

	MAA (S_0 = 3.77)	CHT (S_0 = 3.80)	MAA (S_0 = 4.49)	CHT (S_0 = 4.44)
T_{MAX} (°C)	230	200	230	218
GGC_{MAX} (%)	100.0	100.0	100.0	100.0
$t_{1/2}$ (h)	38.0	26.2	21.6	18.8
R^2	0.992	0.991	0.979	0.994

3.2. Effect of severity and autohydrolysis technology on the liquor chemical composition

The concentration of oligosaccharides, monomers, and by-products (furfural and HMF) in CHT and MAA liquors is depicted in Fig. 1. As expected, an increase in severity leads to greater monomer formation as oligomers break down after reaching the peak concentration of total oligosaccharides. At S_0 values ranging from 3.63 to 4.15 in MAA, the total oligosaccharides concentration exceeded 12 g/L, reaching a maximum of 12.97 g/L at 230 °C for 0.25 min (S_0 = 3.77). XO was the predominant component, constituting around 77 % of the total oligosaccharides under these conditions (9.97 g/L). In comparison, CHT liquor achieved a slightly lower XO concentration of 9.22 g/L at S_0 = 3.80. Similar maximum values have been reported in the literature using hardwoods as feedstock. For instance, MAA yielded a maximum concentration of XO of 9.45 g/L at S_0 = 3.98 (del Río et al., 2022) and 7.11 g/L at S_0 = 3.79 (Pérez-Pérez et al., 2023), using Paulownia and Robinia wood respectively. Similarly, CHT led to maximum XO recovery at severities 3.91 and 3.76 using birch (Fang et al., 2022) and poplar wood (Sun et al., 2021) respectively.

The concentration of XO in MAA liquors declines with increasing severity, reaching 2.76 g/L at the harshest condition studied (S_0 = 4.64). Similarly, with increasing severity in MAA, the concentrations of ArO and AcO decrease from 0.36 to 0.08 and 2.17–0.79 g/L, respectively. This reduction is attributed to the degradation of the oligomers into monomers under severe conditions. In contrast, within the range of severities studied, the concentration of GO increases with severity from 0.49 to 0.93 g/L (S_0 = 3.63–4.64), given the lower susceptibility of celluloses to hydrolysis reactions. The same trends were observed during

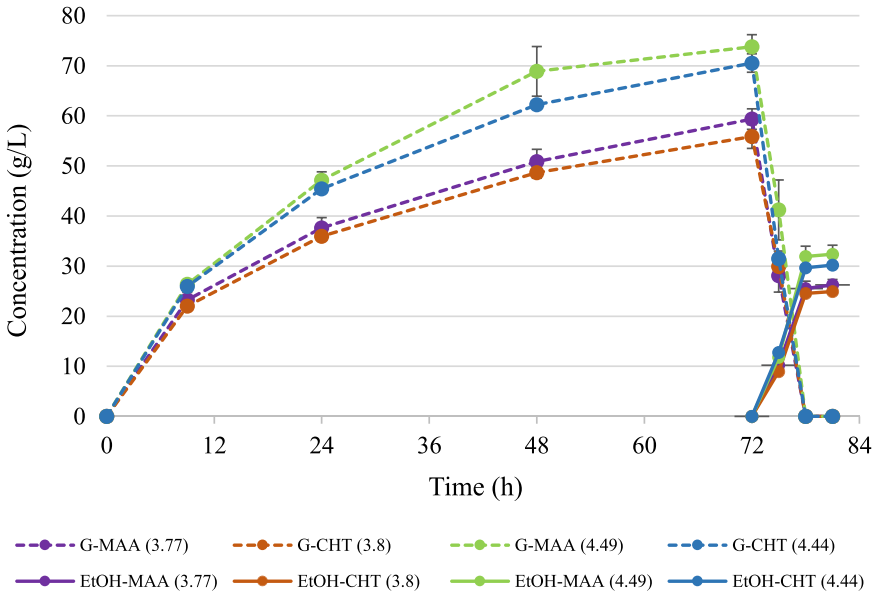

Fig. 3. Glucose (G) and ethanol (EtOH) concentrations during presaccharification and simultaneous saccharification and fermentation (PSSF) of the autohydrolyzed *Acacia dealbata* wood. All experiments were performed in duplicate.

Table 4
Results obtained from PSSF of autohydrolyzed *Acacia dealbata* ($GGC_{72\,h}$: glucan to glucose conversion after 72 h; $EtOH_{yield}$: ethanol yield; GEC: glucose to ethanol conversion). Different letters mean statistical differences at $p < 0.05$. All experiments were performed in duplicate.

Autohydrolysis	Severity (S_0)	$GGC_{72\,h}$ (%)	$EtOH_{yield}$ (%)	GEC (g/g)
MAA	3.77	81.5 ± 2.8^a	70.6 ± 2.9^a	0.406
CHT	3.80	82.4 ± 3.5^a	72.0 ± 1.5^a	0.407
MAA	4.49	96.0 ± 3.3^a	82.4 ± 4.6^a	0.409
CHT	4.44	96.6 ± 2.5^a	80.9 ± 0.8^a	0.398

CHT. The main difference is the lower concentrations of each oligomeric compound in CHT, which is more pronounced when comparing $S_0 = 4.44$ (4.04 g/L of total oligomers) to $S_0 = 4.24$ (9.24 g/L) and 4.38 (7.85 g/L) in MAA. In this sense, Domínguez et al. (2020) found that the oligomer content in the liquor was 10.46 times lower at $S_0 = 4.44$ compared to the maximum at $S_0 = 4.08$ when Paulownia wood was pretreated through CHT. In contrast, del Río et al. (2022) observed that the oligomer content was only 3.94 times lower at $S_0 = 4.65$ compared to the maximum at $S_0 = 3.98$ using MAA on the same feedstock. Although the studies utilized different consistencies in the assays, it is hypothesized that the longer residence time of CHT promotes a higher degradation of the oligomers at the same severities compared to MAA.

The concentration of total monosaccharides (glucose, arabinose, and xylose) in the liquors demonstrates a progressive increase with process severity, from 1.40 to 3.18 g/L in MAA and from 1.86 to 3.35 g/L in CHT, as shown in Fig. 1b. The concentration of monomeric sugars is higher for CHT, consistent with the results reported by del Río et al. (2022), which compared CHT with MAA in Paulownia wood and concluded that, for similar severities, CHT favors the hydrolysis of oligosaccharides into monomers due to longer residence times.

Xylose is the primary monosaccharide up to $S_0 = 3.77$, reaching maximum concentrations of 2.55 g/L in MAA ($S_0 = 4.64$) and 2.72 g/L in CHT ($S_0 = 4.44$). The concentration of glucose exhibits a slight decline as the severity of the MAA process increases, maintaining values between 0.60 and 0.42 g/L. Similarly, the concentration of arabinose remains relatively stable (0.33–0.15 g/L), with a slight decrease above $S_0 = 3.67$. CHT exhibits comparable glucose (0.57–0.40 g/L) and arabinose (0.41–0.14 g/L) levels.

During hydrothermal processes, acetic acid is formed through the hydrolysis of acetyl groups, while HMF and furfural are produced by the dehydration of monosaccharides (Ruiz et al., 2023). As depicted in Fig. 1b and Fig. 1c, the concentration of these compounds consistently increased with the severity, reaching 4.4 g/L in MAA. CHT resulted in higher levels of these by-products, with concentrations that are 45–64 % greater than those observed in MAA at similar severities. This difference has been previously reported in the literature and has been attributed to the longer residence times of CHT (del Río et al., 2022; Rigual et al., 2018; Rivas et al., 2020).

Finally, the NVC in the liquid phase exhibited an increase from 28.1 % ($S_0 = 3.63$) to a peak of 38.8 % at $S_0 = 3.88$, followed by a decrease to 21.27 % at the highest severity ($S_0 = 4.64$). Likewise, Domínguez et al. (2020) found that increasing severity in Paulownia wood CHT decreased NVC from 26.6 % to 23.2 % at $S_0 = 3.91$ and 4.08, respectively.

3.3. Structural characterization of the oligosaccharides (MALDI-TOF and HPAEC-PAD)

The liquid fractions collected at severities of 3.77 for MAA and 3.80 for CHT (conditions that allowed obtaining the maximum recovery of oligosaccharides) were submitted to analysis using MALDI-TOF-MS and HPAEC-PAD techniques to study the structural characteristics of the solubilized oligosaccharides. As far as the authors know, this work accomplishes the first comprehensive description of the structure of the oligosaccharides derived from acacia wood attained by hydrothermal pretreatment. Table S1 shows the tentative structure assignment of the solubilized oligosaccharides, which were identified as potassium or sodium adducts. Signals with the same *m/z* but different intensities were seen in the spectra of the two liquors, which could indicate variations in the amounts of each component in the liquid fractions.

The MALDI-TOF analysis suggested the presence of complex combinations of saccharides that consisted primarily of pentose chains (made up of xylose units according to composition results), highly replaced by acetyl and methylglucuronosyl groups reaching a DP in the range of 4–14. Furthermore, MALDI-TOF-MS spectra also revealed the presence of substituted hexose oligomers in the hydrolysates. The structural characteristics observed in this study agree with those published for other oligosaccharides obtained by hydrothermal treatment from several feedstocks (Pérez-Pérez et al., 2023; Rivas et al., 2020). Due to interferences with matrix peaks, MALDI-TOF analysis does not allow

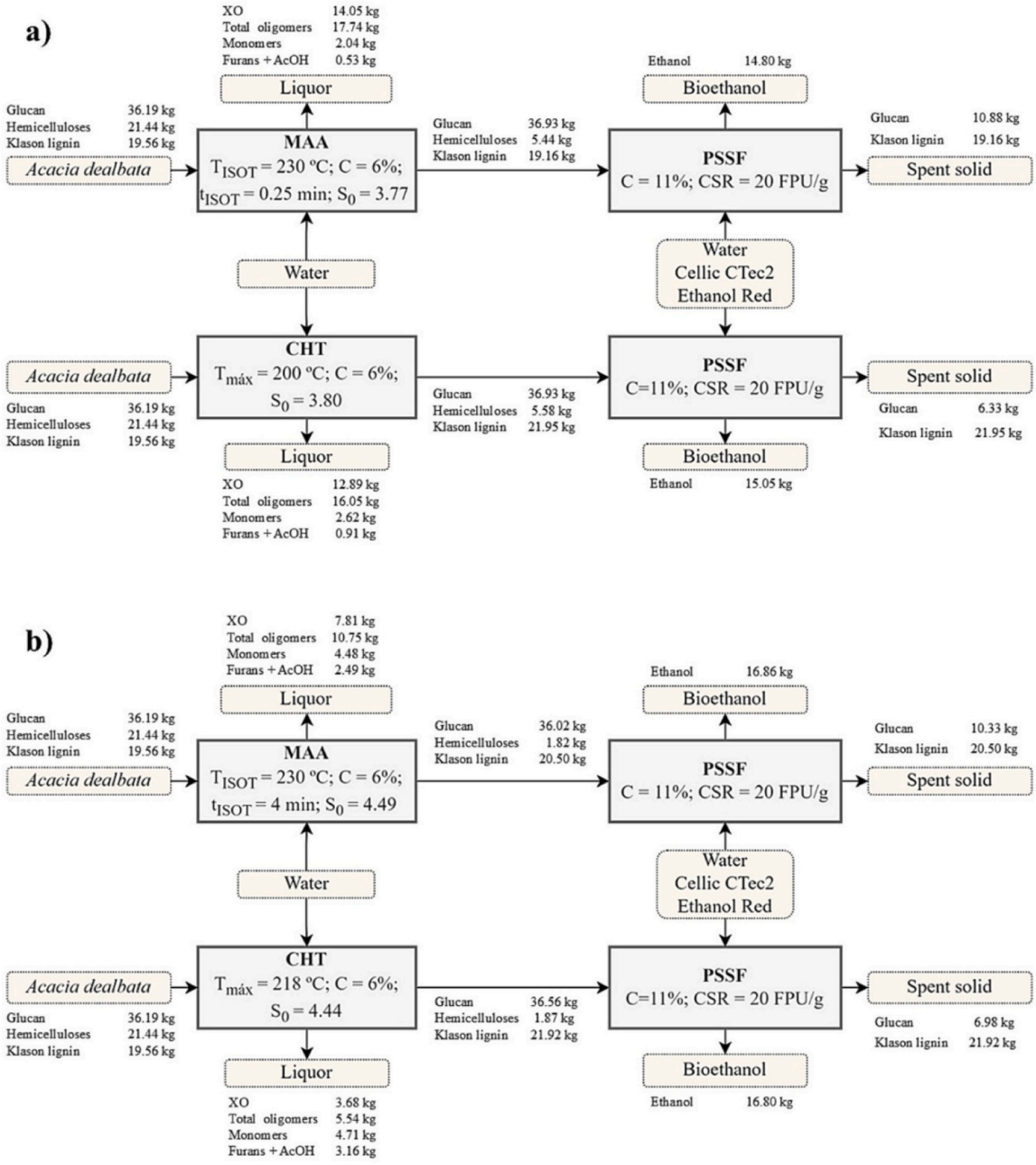

Fig. 4. Mass balances of the different autohydrolysis conditions selected (data expressed in kg/100 kg of *Acacia dealbata* wood, o.d.b.): a) autohydrolysis at S_0 = 3.77–3.80 and b) S_0 = 4.44–4.49 (AcOH: acetic acid).

the identification of compounds with *m/z* lower than 600. The presence of oligosaccharides in the range 2–6 was confirmed by HPAEC-PAD (data not shown).

3.4. Effect of severity and autohydrolysis technology on the enzymatic digestibility

Saccharification assays were performed on the spent solids obtained from the MAA process, following the procedures outlined in Section 2.6, to assess the impact of pretreatment severity on enzymatic susceptibility. The GGC over time was analyzed using the Holtzapple model to derive the $t_{1/2}$ and $GGC_{MÁX}$ parameters (Table 2). The GGC_{MAX} is close to 100 % in all experimental conditions tested. Conversely, the $t_{1/2}$ parameter exhibited a progressive decrease from 31.52 h (S_0 = 3.63) to 9.28 h (S_0 = 4.64) with increasing severity, indicating a higher reaction rate at elevated MAA severities. This trend has been observed in previous studies with other hardwoods as feedstock (Domínguez et al., 2020; Liu et al., 2020; Tan et al., 2020). For instance, Domínguez et al., (2020) reported a decrease $t_{1/2}$ parameter from 5.41 h (S_0 = 3.91) to 3.96 (S_0 = 4.44) as the severity of the autohydrolysis process increased using Paulownia wood. The results obtained for CHT at severities of 3.80 and 4.44 (see Table 2) exhibits similar GGC_{MAX} with equal or slightly higher $t_{1/2}$.

The impact of the heating strategy on enzymatic digestibility was investigated through saccharification experiments using spent solids obtained from MAA and CHT at mild (S_0 = 3.77–3.80) and harsher conditions (S_0 = 4.44–4.49) with a consistency of 11 % (condition that would be employed in the PSSF assay). The experimental data, depicted in Fig. 2, were fitted to the Holtzapple equation (Table 3), resulting in R^2 > 0.9. Notably, spent solids recovered from MAA required between 1.15

and 1.5-fold higher times to achieve half the conversion compared to those obtained from CHT at similar severities. Additionally, GGC_t values were slightly higher for the CHT spent solids compared to MAA at similar severities. For instance, 78–82 % of glucan was converted into glucose at 72 h for CHT solids at severities 3.80–4.44, while MAA solids achieved conversions of 63–81 % at the same time for severities 3.77–4.49 (Fig. 2). However, further investigation is necessary to ascertain the significance of these observed differences and to identify and discuss potential underlying causes.

3.5. Effect of severity on bioethanol production rate through PSSF

To assess the potential of the autohydrolyzed *Acacia dealbata* to produce bioethanol, PSSF experiments were conducted at high solid loadings (C = 11 %). Additionally, based on the EH results for MAA spent solids, the inoculum was introduced at 72 h because at that time up to 70 % of glucan was converted into glucose for severities above 3.77. Those experiments aimed to compare the effect of the heating strategy on bioethanol production and evaluate the difference between mild (S_0 = 3.77–3.80) and harsher conditions (S_0 = 4.44–4.49). The results depicted in Fig. 3 show the evolution of glucose and bioethanol concentrations during the PSSF assays.

The positive correlation between enzymatic glucose production and S_0 leads to higher ethanol concentrations under more severe conditions. However, there are no significant differences between the maximum ethanol concentration achieved with the MAA solid at S_0 = 3.77 and that obtained through CHT at S_0 = 4.44, as shown in Table 4. This suggests that employing MAA at lower severities results in similar ethanol concentrations without compromising the oligomer content of the liquor. On the other hand, the similar ethanol yields indicate that the fermentation stage is not affected by the heating technology selected within the studied operational conditions. The variations in the ethanol concentration obtained are associated with the different glucose concentrations at the time of inoculation. Similarly, Gomes et al. (2021) reported an ethanol yield of 78.10 % following PSSF of *Eucalyptus globulus* autohydrolyzed at S_0 = 4.04.

3.6. Mass balance of the selected conditions

To provide a more comprehensive understanding of the overall process, mass balances were calculated for 100 kg of *Acacia dealbata* wood for milder and harsher conditions for both heating strategies. The results are presented in Fig. 4a and Fig. 4b, respectively.

Under milder conditions, the yields of XO and bioethanol, the primary products in the proposed scheme, are comparable between the two heating strategies. This suggests that the criteria for selecting the optimal strategy are most likely the energy requirements and the initial investment costs. As previously indicated (see Section 3.1), MAA reduces energy consumption and processing time, though the scalability of the technology and the initial capital investment should also be considered.

For harsher conditions, the output of bioethanol produced rises by approximately 2 kg per 100 kg of wood in comparison to milder conditions. However, this is accompanied by a reduction in the recovery of oligosaccharides in liquors. This effect is more pronounced with the conventional heating strategy, where the total oligomer content drops to nearly half of that obtained through microwave heating, which makes MAA potentially more profitable. Nevertheless, a techno-economic analysis is required to ascertain whether the higher bioethanol production justifies the augmented energy demand and diminished oligomer recovery in comparison to milder conditions. Conversely, harsher conditions lead to approximately double the monomer concentration in the liquors, likely due to increased oligomer degradation and hemicellulose solubilization, suggesting further valorization opportunities for the liquor fraction, such as fermentation.

These results underscore the potential of MAA to develop a one-step multiproduct biorefinery platform that efficiently valorizes *Acacia dealbata*, a currently underutilized invasive species.

4. Conclusions

Microwave-assisted autohydrolysis (MAA) shows potential as a valuable approach for *Acacia dealbata* valorization in biorefinery applications. Under milder conditions (S_0 = 3.77–3.80), MAA achieves similar yields of oligosaccharide and bioethanol in comparison to conventional hydrothermal treatment (CHT). However, MAA presented advantages, particularly under harsher conditions, by retaining higher oligosaccharide concentrations and minimizing inhibitory by-products in the liquor. Moreover, MAA requires less energy and time compared to CHT. These findings suggest that MAA offers an efficient and sustainable method for the processing of *Acacia dealbata* wood, though further studies could explore the broader feasibility and optimization of MAA in biorefinery processes.

CRediT authorship contribution statement

Pablo G. Del-Río: Writing – review & editing, Visualization, Validation, Formal analysis, Conceptualization. **Beatriz Gullón:** Writing – review & editing, Visualization, Validation, Project administration, Methodology, Conceptualization. **Gil Garrote:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition. **Álvaro Lobato-Rodríguez:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psep.2025.107470.

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