



2 step hydrolytic conversion of orange processing waste into C5-C6 sugars: the “Hemicellulose Ahead” protocol

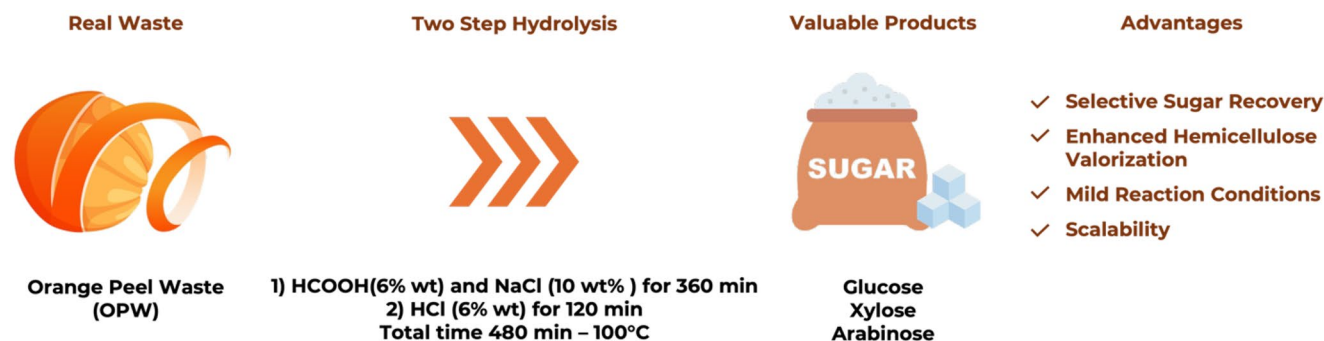
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Abstract

Practically viable chemical valorization of lignocellulosic biomass requires innovative and selective fractionation strategies. In this study, we present the “Hemicellulose Ahead” protocol: a novel two-step hydrolysis approach for converting industrial orange processing waste into valuable sugars. This strategy targets a first recovery of C5–C6 sugars from hemicellulose *via* acid hydrolysis with dilute formic acid, followed by glucose recovery from cellulose using dilute hydrochloric acid. The process is carried out in one pot under relatively mild conditions (100 °C, atmospheric pressure). Prior to hydrolysis, orange processing waste needs to be washed with pressurized 50:50 (wt:wt) ethanol:water mixture to remove free sugars, flavonoids and processing residues, which proved essential to prevent the formation of degradation products during the initial hemicellulose conversion. The sequential process was successfully scaled from 100 mL to 500 mL and 5 L batch reactors, with only minor variations in sugar yields across the different scales. The process provides a cost-effective pathway for valorizing citrus processing waste into C5 and C6 sugars. At the same time, the solid residue, rich in pectin, can be utilized for the recovery of this latter polysaccharide.

Graphical abstract



Keywords Bioeconomy · Biomass valorization · Hemicellulose · Citrus processing waste · Hemicellulose ahead

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Abbreviations

CPW	Citrus processing waste
OPW	Organic processing waste
FCOJ	Frozen concentrated orange juice
MCC	Microcrystalline cellulose
HCOOH	Formic acid
XRD	X-Ray diffraction
5-HMF	5-hydroxymethylfurfural
EtOH	Ethanol

1 Introduction

Economically viable biorefinery strategies have traditionally focused on the valorization of the cellulose and lignin fractions of lignocellulosic biomass. However, the pretreatment methods typically employed to break down these components often lead to the degradation of hemicellulose, thereby reducing the overall economic and material efficiency of the process (Galbe and Wallberg 2019; Chen et al. 2022). Hemicellulose, which can account for up to 40% of lignocellulosic biomass, is the second most abundant polysaccharide in nature after cellulose (Saha 2003; Rao et al. 2023). Despite its abundance, hemicellulose has historically received limited attention due to its complex and heterogeneous structure, variable degrees of polymerization after processing, the presence of side-chain substitutions, and its low solubility in both water and most organic solvents (Sousa et al., 2016). For these reasons, it is often undervalued or sacrificed in industrial processes such as conventional pulp and paper production or in biorefinery approaches that prioritize lignin valorization (Abu-Omar et al. 2021).

In recent years, research has increasingly recognized hemicellulose as a valuable and versatile component of lignocellulosic biomass. When properly recovered, it offers advantages in terms of cost-effectiveness, biodegradability, environmental sustainability, and mechanical properties of the resulting materials (Dulie et al. 2021; Josey et al. 2024; Nechita et al. 2021). To enhance the recovery and use of hemicellulose, recent strategies advocate the replacement of lignin-rich feedstocks like wood with low-lignin agricultural residues. These alternatives allow for easier deconstruction and facilitate the extraction of xylans, offering a more affordable and sustainable biomass supply chain (Rahmati et al. 2022).

Among these alternatives, citrus processing waste (CPW) stands out as a particularly promising biomass source. Unlike wood, which generally contains 20 to 30% lignin, CPW has a lignin content as low as 7 to 8%. This lower lignin content significantly reduces biomass recalcitrance, enabling milder and more efficient pretreatment processes. In this context, a tailored approach to biomass valorization

has been proposed, based on the comprehensive utilization of all fractions of lignocellulosic biomass. This strategy involves fractionating the biomass into its primary components, namely C5 and C6 sugars (derived from hemicellulose and cellulose, respectively) and aromatics (derived from lignin), under moderate process conditions in terms of temperature (150 to 250 °C) and pressure (50 to 70 bar) (Nechita et al. 2021; Rahmati et al. 2022).

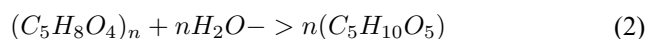
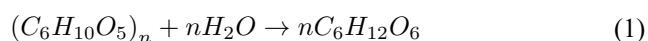
The effectiveness of this approach relies heavily on the pretreatment step, which is crucial in overcoming biomass recalcitrance. This intrinsic resistance of lignocellulosic biomass to chemical and biological degradation is attributed to several factors, including the crystalline structure of cellulose, the degree of lignification, and the structural complexity and heterogeneity of plant cell walls. A wide range of pretreatment technologies has been developed to address these challenges. These include physical methods such as milling, microwave irradiation, extrusion, and ultrasound; chemical methods such as acid or alkaline hydrolysis, organosolv processing, and the use of deep eutectic solvents; physico-chemical methods such as steam explosion, ammonia or carbon dioxide explosion, and liquid hot water; as well as biological treatments involving whole-cell systems or enzymatic hydrolysis (Baruah et al. 2018; Klemm et al. 2020; Dahadha et al. 2017; Klemm et al. 2005).

Additionally, citrus is among the most extensively cultivated fruit crops worldwide; global orange (*Citrus sinensis*) production reached approximately 48.8 million tonnes in the 2021–22 season and remained near 47.4 million tonnes in 2023–2024 (Statista 2025). The citrus processing industry generates huge amounts of residues, mainly in the form of pulp and peels. Indeed, around 40% of the annual orange harvest is used to produce frozen concentrated orange juice (FCOJ). This production chain is highly energy-intensive and generates approximately 13.7 million tonnes of orange processing waste (OPW), which accounts for about 45% of the original fruit mass (Neves et al. 2020). Often mistaken as “orange peel”, industrial OPW also includes crushed seeds and residual pulp with one kilogram containing about 233 g of dry matter (Siles López et al. 2010).

Over the last two decades, research has increasingly focused on developing technically and economically viable routes to extract high-value bioproducts from OPW (Siles López et al. 2010; Balu et al. 2012; Manakas et al. 2025; Siddiqui et al. 2022), extending beyond traditional low-value uses, such as animal feed. For example, CPW and OPW are excellent potential sources of microcrystalline cellulose (MCC) and nanocellulose (Ciriminna et al. 2024; Bangar et al. 2023). Besides pectin, starch, terpenes, flavonoids, sugars, proteins, and organic acids, OPW contains a significant amount of cellulose (9.21 wt%) and hemicellulose (10.50 wt%), while lignin is present in a modest

amount (0.84 wt%) [17], making this biomass interesting for the valorization of hemicellulose and cellulose fraction into C5 and C6 sugars (Fig. 1).

One promising strategy for cellulose and hemicellulose valorization involves acid-catalyzed hydrolysis, which converts the carbohydrate fraction into fermentable sugars, such as glucose and xylose, respectively, from cellulose (Eq. 1) and hemicellulose (Eq. 2) (Wyman et al. 2005). Acid hydrolysis employs either concentrated or diluted mineral and organic acids (Shahbazi and Zhang 2010). While concentrated acid hydrolysis offers high sugar yields, it suffers from drawbacks such as equipment corrosion and the difficulty of acid recovery. On the other hand, dilute acid hydrolysis is more favorable for industrial applications due to its milder reaction conditions, resulting in longer equipment life and reduced costs. Hydrolysis reactions are typically conducted at temperatures ranging from 120 to 210 °C.



The hydrolysis of hemicellulose resulted in complex mixtures of pentoses (xylose and arabinose) and hexoses (mannose and galactose) that can be subsequently used for the biological or chemical conversion of these saccharides to biofuels and biobased chemicals (Abejón 2018).

In this study, we introduce a two-step hydrolysis approach, termed herein “Hemicellulose Ahead”, designed to selectively hydrolyze OPW hemicellulose under mild conditions using a dilute organic acid, such as formic acid, followed by cellulose hydrolysis under stronger acidic conditions. The use of formic acid effectively reduces the overall environmental footprint, as it can be readily derived from biomass, thus reinforcing the circularity of the process (Valentini et al. 2019; Chen et al. 2023).

The developed sequential strategy is particularly effective with OPW due to its low lignin content, which reduces interference during hemicellulose hydrolysis and enables

clearer pathway development for the total recovery of C5 and C6 sugars, while minimizing degradation of hemicellulose-derived sugars. All hydrolysis reactions were conducted at 100 °C using standard glass reactors, eliminating the need for pressurized systems or corrosion-prone steel reactors. Indeed, the hydrolysis was successfully scaled up from 100 mL to 500 mL and 5 L batches, with only minor variations in sugar yields observed at the increased volumes.

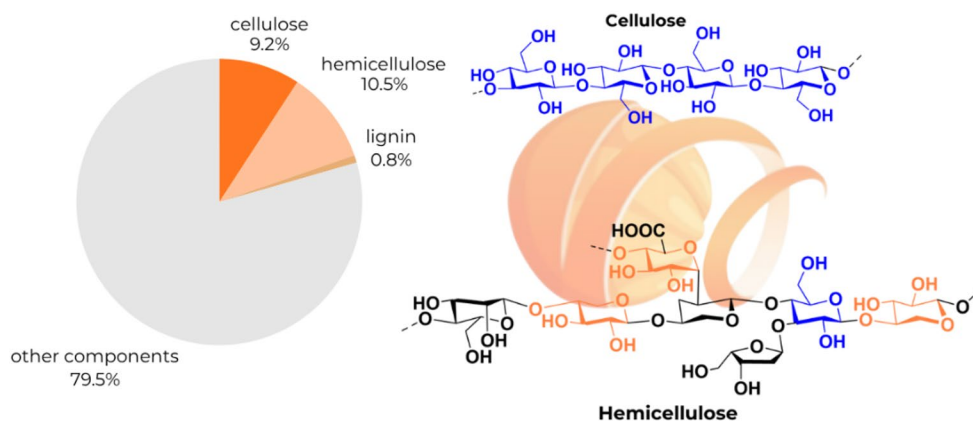
2 Experimental section

2.1 Materials and methods

All chemicals used were of analytical grade. Microcrystalline cellulose (MCC) (CAS: 9004-34-7), xylan (CAS: 9014-63-5), L-(+)-arabinose (CAS: 5328-37-0), H₂SO₄, HCl, HNO₃, AlCl₃ and HCOOH were all purchased from Merck Life Science (Milan, Italy). Ultrapure water (electric resistance 18.2 MΩ cm⁻¹) used in all hydrolysis experiments was purchased from Carlo Erba (Milan, Italy). Orange processing waste was purchased from a local orange juice factory (Amedeo Giovanni Srl, Reggio Calabria). XRD measurements were conducted using a Rigaku SmartLab 3 kW X-Ray diffractometer employing Cu Kα radiation λ=1.54 Å over the 10° to 80° diffraction angle range at a scan rate of 2°/min.

The moisture content of OPW was determined by the gravimetric method. A sample (5 g) of OPW was put in a pre-weighed container and dried in an oven at 105 °C for 24 h. After cooling in a desiccator, the sample was weighed. The weight loss is reported as a percentage of the original sample weight. The ash content (%) was determined by the incineration and gravimetric method. The proximate composition of OPW was determined using the method reported by Sánchez Orozco and coworkers (Sánchez Orozco et al. 2014). OPW consisted mainly of cellulose (30.5%), followed by hemicellulose (10.5%), and a smaller amount of lignin (5.0%), based on dry weight.

Fig. 1 Schematic representation of the major components of OPW



The liquid samples collected from each reaction were analyzed using an Agilent Technologies HPLC 1290 chromatograph equipped with a Bio-Rad Aminex HPX-87 H column. The analysis was carried out at 75 °C with a flow rate of 1 mL/min using 5 mM sulfuric acid as the mobile phase. For the detection of sugar monomers and other products, both Refractive Index and Diode Array detectors were used. The products were identified by matching their retention times to those of pure sugar employed as HPLC standards, as reported in previous work (Gumina et al. 2019). For the determination of the components' concentration, on the other hand, a calibration curve was constructed for each component.

2.2 Pretreatment of microcrystalline cellulose

Prior to catalyst screening for MCC hydrolysis, it was mechanically pretreated *via* ball milling. For this process, a sample (10 g) of dried MCC in a stainless-steel container (25 mL) was ball-milled for 3 h at 560 rpm using a Fritsch planetary ball mill. To investigate the influence of ball milling time on the structure of MCC, ball milling time was increased to 9 h while keeping the other parameters constant.

2.3 Acid catalyst screening

The hydrolysis reactions were carried out in a 100 mL three-neck round-bottom flask equipped with a reflux condenser. For each run, 30 mL of distilled water was added to the reactor, along with 1, 3, or 6 wt% of acid (either formic and/or hydrochloric acid) and, if required, additives such as mineral salts (NaCl), depending on the reaction. The three-neck glass reaction round-bottom flask with a reflux condenser, placed in a silicone oil bath, was then brought to 100 °C while being stirred continuously at 350 rpm using a magnetic stirrer (either a rod or a cross-shaped form). An amount of substrate $m_i = 0.1 \times m_{\text{total}}$ ($i = \text{MCC}$ or xylan, with m_{total} expressed in g) was then added at the reaction temperature of 100 °C. The reaction was carried out for 5 h. At 60-minute intervals, a 1.5 mL reaction sample was retrieved for analysis, filtered through a 0.45 μm paper filter, and cooled to an ice bath. In the case of MCC, the polysaccharide remaining in the filter was recharged into the reactor. After 5 h, the hydrolysis solution of MCC was washed 4 times with distilled water and filtered under vacuum using a Büchner funnel. Afterwards, MCC was dried in an oven at 100 °C for 24 h.

To determine the conversion of cellulose into glucose, under the assumption that the weight loss in cellulose results solely from its hydrolysis into monomers, the conversion was calculated according to Eq. 3:

$$X_{\text{cellulose}} = \Delta m_{\text{cellulose}} / m_{\text{cellulose_initial}} \cdot 100\% \quad (3)$$

The effects of various factors, including ball milling, reaction time, reaction temperature, and acid catalyst type and concentration, were examined.

2.4 Two-step hydrolysis of xylan-cellulose and OPW

Before hydrolysis, OPW was dried in an oven at 100 °C for 24 h. If necessary, dried OPW was ball-milled for 3 h at 560 rpm in a Planetary Mono Mill Pulverisette 6. Hydrolysis of OPW was performed in a Syrris (Royston, Great Britain) fully automated, jacketed, glass batch reactor for advanced temperature control. Before the catalytic tests on OPW hydrolysis, a preliminary screening experiment was conducted on xylan and MCC individually, used as reference substrates. The purpose of these tests was to obtain a well-defined reference for the hemicellulose (xylan) and cellulose (MCC) fractions present in OPW, in order to validate the sequential hydrolysis strategy, first applying formic acid and then hydrochloric acid, under controlled conditions. The substrate was added to the reactor with 63 g of distilled water and 7 g of NaCl (10 wt%) and was heated to the target reaction temperature. At TR=100 °C, 6 wt% formic acid (3.44 mL) was introduced. After 5 h, an aliquot (9.90 mL) of aqueous 36% (wt) of HCl (6 wt%) was added. For the hydrolysis of OPW, the procedure was the same as described above for the 1:1 xylan-cellulose mixture (ball milled).

3 Results and discussion

At the beginning of our study, a systematic investigation was conducted to identify two distinct homogeneous acid catalysts capable of enabling a consecutive hydrolysis process, initially targeting hemicellulose and subsequently cellulose. To establish this two-step approach, we began by systematically screening a range of homogeneous acid catalysts and concentrations on xylan, used as a model compound for hemicellulose, and MCC. This dual-substrate screening allowed us to evaluate catalyst reactivity and selectivity, and to optimize conditions for a sequential hydrolysis strategy targeting first hemicellulose and then cellulose. Variables such as acid type, concentration, and, in the case of MCC, the impact of pretreatment (*via* ball milling) were thoroughly evaluated. All reactions were conducted at 100 °C, which is significantly lower than the typical temperature range of 120–210 °C commonly used for the hydrolysis of hemicellulose and cellulose (Wyman et al. 2005).

These relatively mild conditions minimize the formation of sugar degradation by-products. Importantly, unlike

typical biomass hydrolysis processes that rely on pressurized stainless-steel reactors, our experiments were initially conducted in round-bottom glass reactors and later scaled up using a batch glass reactor operating under atmospheric pressure. The use of glass reactors not only allowed clear observation of the reaction medium but also avoided long-term corrosion issues often associated with strong acids and metallic reactors under harsh hydrothermal conditions.

3.1 Hydrolysis of Xylan and microcrystalline cellulose

Using xylan as a model for hemicellulose, among the mineral acids screened, HCl emerged as the most effective catalyst, achieving 85% conversion to xylose at 6 wt%, significantly outperforming other acids. Highlighting potential for selective hydrolysis, formic acid (HCOOH) exhibited a nearly linear increase in conversion with concentration, achieving about 68% conversion at 6 wt% (a 50% improvement over 3 wt%). Solid Lewis acid AlCl_3 showed limited efficiency, plateauing at ~52% conversion using a 3 wt% amount and above, indicating competing polysaccharide degradation mechanisms (Fig. 2a).

In the case of MCC hydrolysis (pretreated by ball milling), a different reactivity trend emerged. Strong inorganic acids promoted the highest conversions, with H_2SO_4 achieving slightly higher conversion than HCl (~42%) at 6 wt%. HNO_3 showed a clear concentration-dependent trend, with conversion rising steadily from ~18% at 1 wt% to nearly 40% at 6 wt%. The catalytic activity of AlCl_3 also increased with acid concentration, whereas HCOOH was significantly less effective, improving by only 2.5% across the full range of concentrations investigated (Fig. 2b).

The impact of physical pretreatment on hydrolysis efficiency was also assessed by comparing untreated and ball-milled MCC. The experiments confirmed that the mechanical disruption of the crystalline structure enhances the accessibility of glycosidic bonds. Using 6 wt% HCl, the conversion of MCC increased by 8% in ball-milled samples compared to a modest 4% improvement in untreated ones (Figure S1). Optimized conditions resulted in a 40% glucose yield, representing a 16% increase over untreated MCC. This enhancement correlates with structural changes observed by XRD (Figure S2), which revealed a transformation from crystalline to amorphous cellulose after 3 h of milling.

Considering the results obtained with xylan and ball-milled MCC, and to further evaluate the hemicellulose ahead strategy, other bio-based acids, such as maleic and oxalic acids, were tested prior to HCl addition, yielding results comparable to those obtained with formic acid. Future developments will focus on extending the process to a broader range of bio-based acids and on exploring heterogeneous catalysts, while also addressing the challenge of their recovery and reuse. Indeed, it enables 68% conversion of xylan to xylose while leaving cellulose virtually unaffected, as evidenced by a minimal glucose yield of 0.06% under the same reaction conditions.

At this point, the optimization of HCOOH concentration and reaction time for xylan treatment was performed, showing that xylose release increased with both parameters, confirming their crucial role in governing the extent of hemicellulose hydrolysis. The highest yield, exceeding 70 g/L, was achieved using 6 wt% HCOOH after 240 min of reaction. Due to insufficient proton availability for the effective cleavage of glycosidic bonds, xylose production

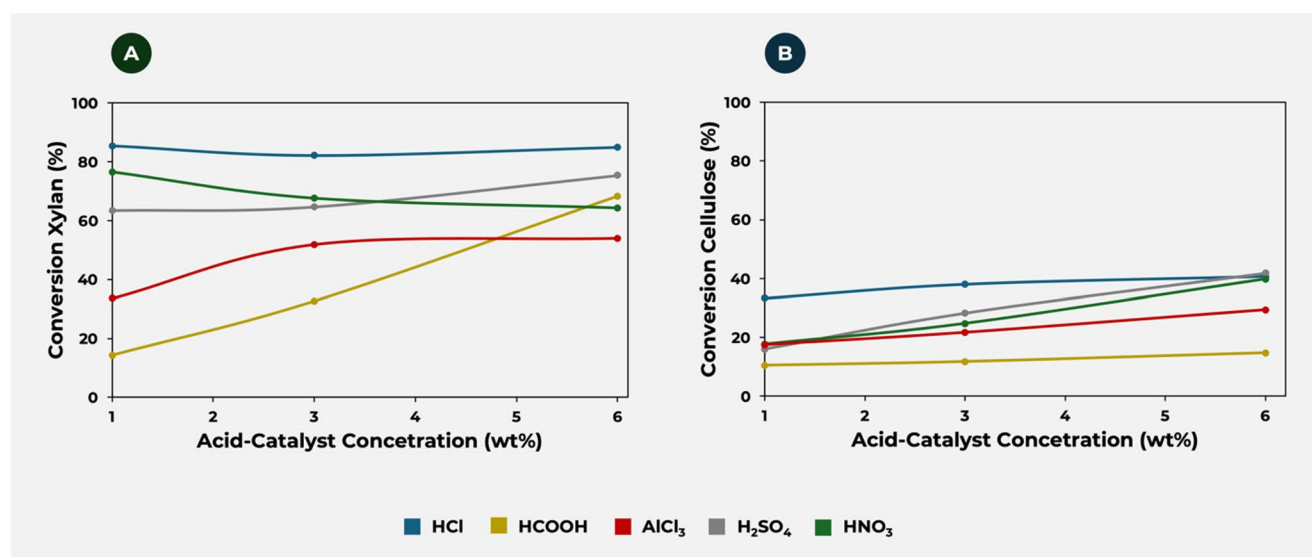


Fig. 2 (a) Conversion of xylan into xylose catalyzed by a variable concentration of different acids at 100 °C. (b) Conversion of ball-milled MCC promoted by a variable concentration of different acids at 100 °C

remained modest at a low HCOOH concentration (1 wt%), never exceeding 30 g/L, and exhibited a slow, nearly linear trend. In contrast, 3 wt% HCOOH resulted in a more pronounced release of xylose, with concentrations increasing significantly between 60 and 180 min. The highest xylose yield was observed with 6 wt% HCOOH, which not only exhibited the fastest sugar release but also maintained high productivity throughout the 4-hour reaction window. The sharpest increase in xylose concentration occurred between 120 and 180 min, suggesting that this time interval corresponded to the highest kinetic activity of the system, likely due to the rapid hydrolysis of the most accessible xylan regions. Beyond 180 min, the rate of xylose accumulation began to level off gradually, particularly at lower acid concentrations. This plateau may reflect substrate depletion, diffusion limitations, or the onset of monosaccharide degradation into byproducts such as furfural under prolonged exposure to acidic conditions. Despite this, at 6 wt%, the system maintained an upward trend in xylose release even at 240 min, indicating that higher acid concentrations can delay degradation and extend the effective hydrolysis window (Fig. 3).

Overall, these data confirm that both reaction time and acidity must be carefully optimized to maximize hemicellulose conversion while minimizing xylose loss *via* degradation.

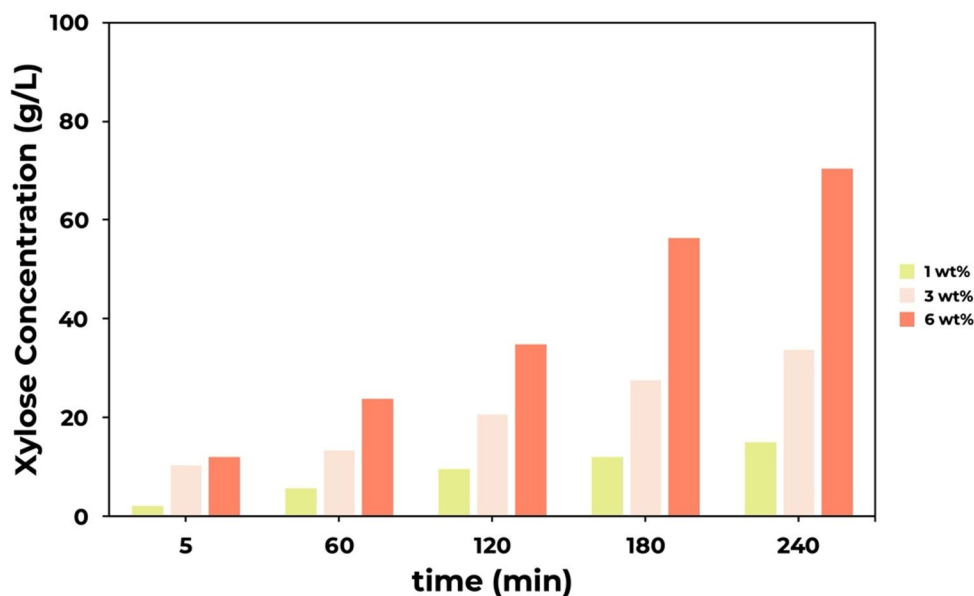
The addition of aqueous NaCl (10 wt%) in xylan hydrolysis with 6 wt% HCOOH resulted in a clear improvement in xylose yield, increasing by approximately 10%, likely due to increased ionic strength that facilitates proton mobility and glycosidic bond cleavage. Higher salt concentrations, such as 15 and 20 wt% NaCl, resulted in attenuated positive effects, with only marginal gains in xylose concentration observed up to 180 min. Beyond this time, yields started

to decline gradually. The most pronounced negative effect was observed at 20 wt% NaCl, where xylose concentration peaked at 180 min but then declined sharply, dropping from ~70 g/L to ~60 g/L by the end of the 300 min reaction. This behavior suggests that while a low concentration of NaCl enhances hydrolysis, a higher concentration of salt promotes degradation pathways, possibly by accelerating the dehydration of xylose to furfural and the potential condensation into humins, as indicated by the progressive darkening of the reaction mixture. Notably, at higher NaCl concentrations, xylose began to degrade earlier and more rapidly, indicating that reaction selectivity was compromised. These findings underscore the importance of fine-tuning salt concentration in acid-catalyzed hydrolysis systems, as moderate NaCl levels enhance yield. In contrast, excessive levels promote sugar loss and reduce process efficiency (Figure S3).

For MCC hydrolysis, a similar trend was observed (Figure S4) in response to increasing NaCl concentrations. Glucose production improved progressively, likely due to enhanced proton activity in the presence of chloride ions and a more favorable environment for glycosidic bond cleavage. In detail, glucose concentration increased steadily from around 20 g/L in the absence of NaCl to over 50 g/L at 30 wt% NaCl, demonstrating the strong positive effect of salt on MCC depolymerization under acidic conditions. This enhancement can be attributed to several effects, including increased ionic strength, which improves proton solvation and facilitates catalytic interactions with the cellulose matrix.

However, careful optimization of salt concentration is crucial to strike a balance between hydrolysis efficiency and product selectivity. In brief, while higher NaCl concentrations improved glucose yields, they also accelerate undesired degradation pathways, especially under extended

Fig. 3 Xylose yields in xylan hydrolysis. [Reaction conditions: substrate: xylan; T:100 °C; formic acid 6 wt%]



reaction times or high acid loadings. Indeed, chloride ions are known to promote glucose isomerization to fructose, which, while beneficial for hydrolysis kinetics, also opens up pathways for unselective acid-catalyzed C–C bond-forming reactions that lead to the production of humins [28]. Accordingly, visual inspection of the reaction mixtures (Figure S5) revealed a drawback to this enhancement. As NaCl concentration increased, so did the formation of dark-colored humins, indicative of condensation reactions involving glucose-derived 5-hydroxymethylfurfural (5-HMF) (Sanpitakseree et al. 2022).

3.2 Two-step hydrolysis of OPW

At this stage, the optimal reaction conditions, determined from preliminary studies using model xylan and ball-milled MCC, were applied to ball-milled OPW to optimize a one-pot two-step sequential hydrolysis protocol aimed at maximizing yields of xylose, arabinose, and glucose. The evolution of sugar concentrations over time was monitored after the addition of 6 wt% formic acid in the presence of 10 wt% NaCl, followed by 6 wt% HCl at 100 °C (Fig. 4).

Before catalyst addition, residual soluble sugars (mainly glucose, 6 g/L, and xylose, 2.8 g/L) originating from pulp, seeds, and peel remaining after juice extraction were observed. Upon the introduction of the catalyst, these sugars began to degrade progressively. Initially, glucose and xylose concentrations decreased over time, whereas arabinose appeared after 1 h, indicating its delayed release from the OPW matrix. After 300 min, glucose concentration peaked following HCl addition at 360 min, whereas xylose continued to decline, especially after the HCl addition, confirming its instability under harsh acidic conditions.

When 6 wt% HCl was added, glucose concentration increased from 5 g/L to 6.2 g/L within the first 60 min, indicating the onset of cellulose hydrolysis. In contrast, xylose concentration did not increase further, suggesting that its degradation under these acidic conditions outpaced its release from hemicellulose. As the reaction progressed, glucose concentration also began to decline at 480 min, indicating secondary reactions such as dehydration to 5-HMF and subsequent humin formation (Figure S6).

XRD analysis confirmed that both short (5 min) and prolonged (180 min) ball milling treatments of OPW effectively disrupted the crystalline structure of cellulose in OPW (Figure S7), producing an amorphous matrix more accessible to acid-catalyzed hydrolysis. However, despite this structural change, the hydrolysis performance remained similar for both treatments, as indicated by the sugar release profiles (Figure S8). This behavior can be attributed to the fact that the OPW used in this study had already undergone mechanical processing during orange-juice production.

At this stage, we investigated the impact of Cl ions. We observed that in the presence of 10 wt % NaCl during OPW hydrolysis, glucose isomerization to fructose was promoted, leading to its subsequent dehydration into 5-HMF, even before HCOOH addition. The early decline in glucose and xylose levels upon NaCl addition suggests that chloride ions accelerate sugar-degradation pathways, diminish sugar stability, and drive secondary reactions, even in the absence of an acid catalyst (Fig. 5).

To mitigate early sugar degradation, a pressurized water treatment was performed prior to the hydrolysis step. OPW was enclosed in a membrane bag and subjected to pressurized extraction in a chamber containing distilled water (OPW:H₂O = 1:5). Three successive cycles, two with water alone and one with a 50:50 EtOH:H₂O mixture, were

Fig. 4 Monomeric sugars yield by hydrolyzing 3 h ball-milled OPW. [Reaction conditions: substrate: 3 h ball-milled OPW; T:100 °C; 6wt% formic acid in the presence of 10 wt% NaCl, followed by the addition of 6 wt% HCl at 360 min]

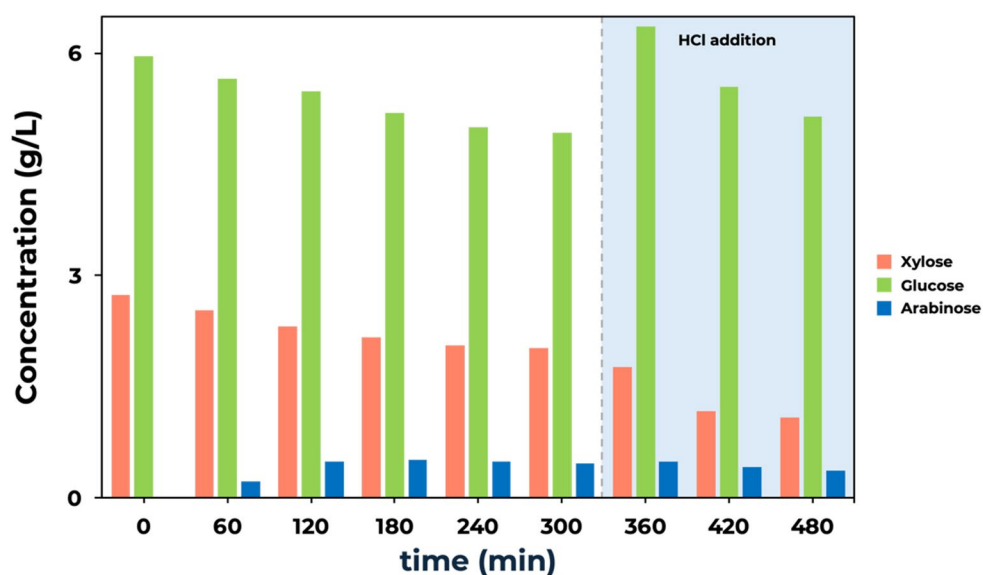


Fig. 5 Glucose and xylose yield following hydrolysis of OPW. [Reaction conditions: substrate: 3-h ball milled OPW; T:100 °C; mediated; solvent: formic acid 6 wt% and HCl 6 wt%, with (10 wt%) and without (0 wt%) of NaCl]

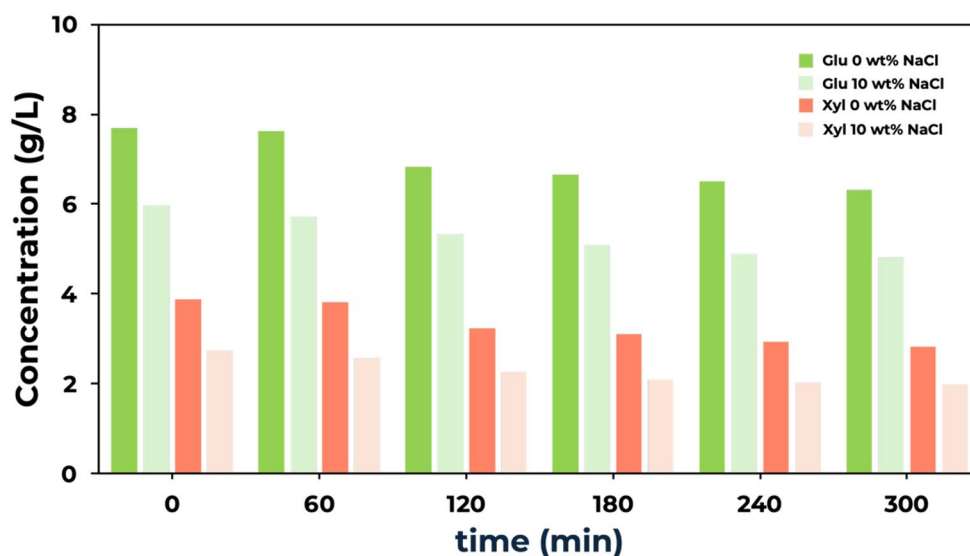
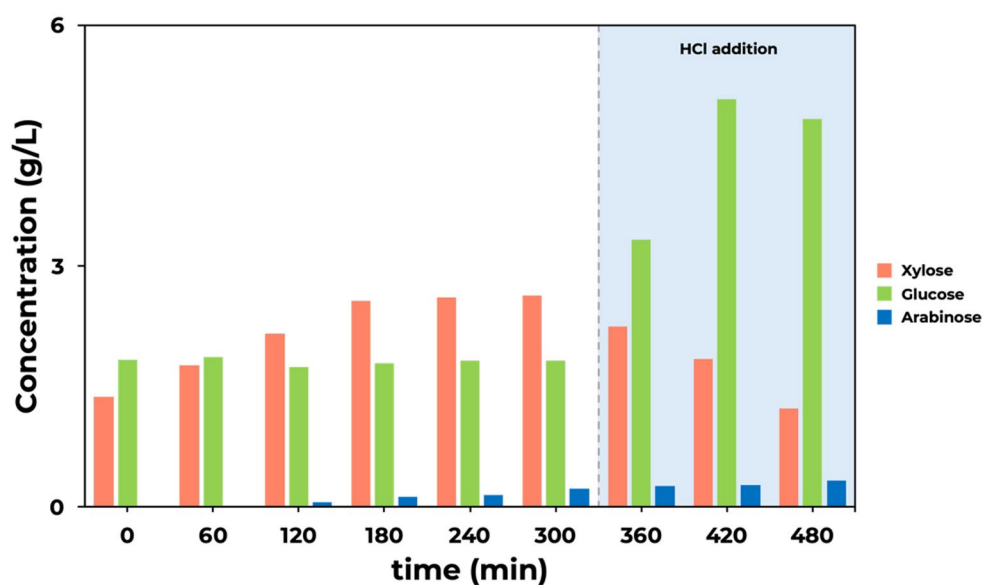


Fig. 6 The hydrolysis performance of pressure-extracted OPW. [Reaction conditions: substrate: pressure-extracted OPW; T:100 °C; formic acid 6 wt% in the presence of 10 wt% NaCl followed by the addition of HCl 6 wt% at 360 min]



sufficient to remove most soluble sugars, flavonoids, and volatile compounds. Consequently, the initial glucose concentration decreased from 5.7 to 1.83 g L⁻¹ at time 0 (Fig. 4), while xylose decreased from 2.57 to 1.36 g L⁻¹ (Fig. 6). These results suggest that the observed decrease reflects a balance between the degradation of residual sugars originating from the orange juice and the formation of sugars through hydrolysis. Indeed, the pressurized extraction of OPW enabled the partial removal of sugars remaining from orange-juice processing.

Following the addition of formic acid, as expected, the xylose concentration gradually increased throughout the entire first stage of hydrolysis, reaching a peak at 300 min, while glucose remained stable. Again, the release of arabinose from hemicellulose in OPW began after 120 min, also peaking at 300 min. In contrast, xylose concentration

dropped rapidly after HCl addition, reaching 1.2 g/L by the end of the process, confirming its sensitivity to acid-catalyzed degradation under harsher conditions. Additional control experiments using HCl as the sole acid from the beginning further confirmed this behavior, showing a strong degradative effect on pressure-extracted OPW and leading to humin formation and markedly lower sugar yields (Figure S9).

Importantly, no degradation products, such as furfural or 5-HMF, were detected during this first stage, indicating that the washing treatment effectively removed the soluble sugars originally present in the OPW. This key finding confirms that the sugars released during hydrolysis originated exclusively from the polysaccharides (cellulose and hemicellulose), rather than from residual sugars in the orange juice that are prone to rapid degradation.

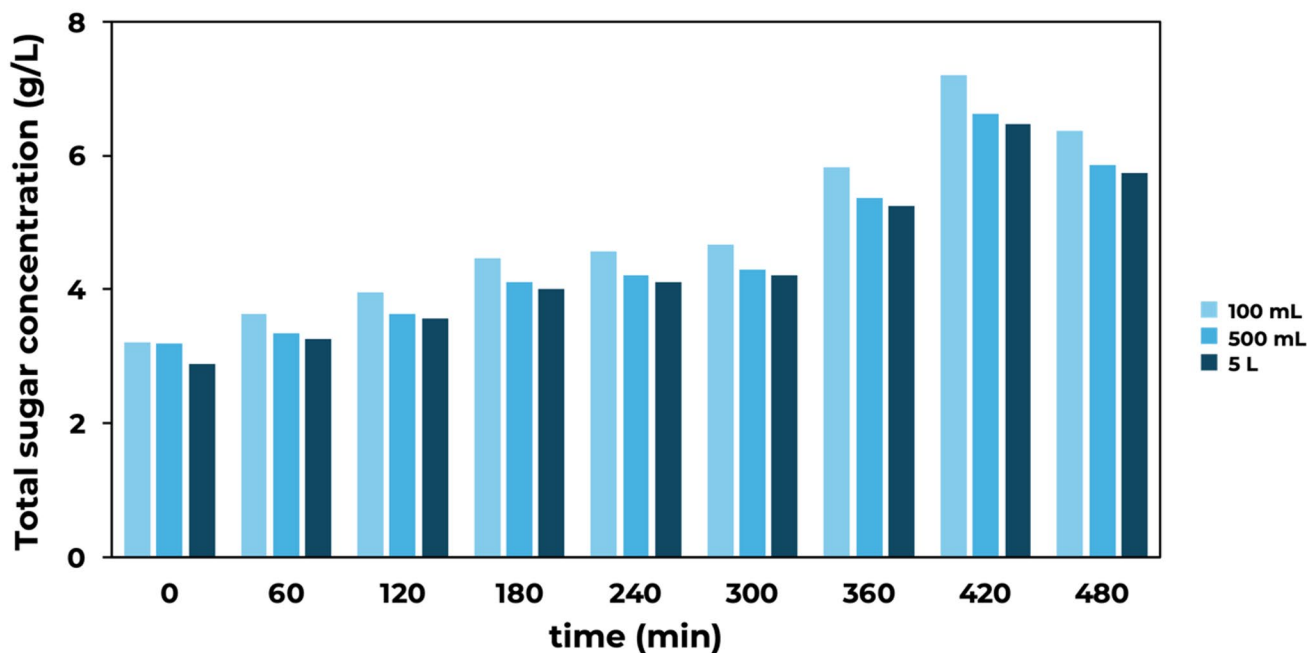


Fig. 7 Scale-up two stage hydrolysis of pre-washed OPW in 0.5 and 5 L jacketed glass reactors. [Reaction conditions: substrate: pressure-extracted OPW; T:100 °C; formic acid 6 wt% in the presence of 10 wt% NaCl followed by the addition of HCl 6 wt%]

In contrast to previous studies, the present work prioritizes the valorization of the hemicellulose fraction. As a result, the glucose yields obtained are lower than those typically reported for inorganic-acid hydrolysis (e.g., H_2SO_4 , yielding 21.887 g L^{-1} glucose and 9.286 g L^{-1} fructose) conducted at comparable or higher temperatures (100–125 °C) (Ayala et al. 2021). The presented Hemicellulose Ahead strategy enables efficient xylose recovery while minimizing hemicellulose caramelization. It is also important to note that substantial variability among orange cultivars can significantly influence both the biomass composition and the resulting sugar yields. In addition, the treatments applied to oranges can markedly affect the efficiency of sugar recovery.

3.3 Scale-up experiments

Scale-up experiments of the OPW two-step sequential hydrolysis were performed in a double-jacketed glass batch reactor system, which offers versatility and automated control. This system has a volume of 5 L and features blade geometries that ensure proper mixing and uniform heat distribution. Temperature regulation is achieved *via* a Huber circulator, with an internal sensor monitoring temperature near the stirring blade for real-time adjustments.

Sequential hydrolysis of extracted OPW was carried out for 8 h using the optimized conditions of the two-stage hydrolytic process: namely stage 1 at 100 °C with 6 wt% formic acid and 10 wt% of NaCl and stage 2 (at 360 min)

by adding 6 wt% HCl. Reactions were conducted both in 0.5 and 5 L glass reactors, maintaining a 10 wt% solid-to-liquid ratio.

Sugar yield data collected in both scaled-up experiments (Fig. 7) showed only modest reductions in sugar concentrations upon scaling from 100 mL to 0.5 L. The individual concentrations of xylose, glucose, and arabinose at each sampling time and reactor scale are reported in Table S1. Notably, the 500 mL reactor retained the yields observed in the 100 mL scale experiment, particularly in the first hydrolysis stage, where xylose release remained consistently high under optimal conditions. Slight decreases may be attributed to scale-dependent factors such as a slight decrease in mixing efficiency and heat transfer.

4 Conclusion

In conclusion, we have developed a viable process, namely “Hemicellulose Ahead,” for converting OPW into xylose, arabinose, and glucose. The approach valorizes both the labile hemicellulose and cellulose fractions *via* a two-step sequential acid hydrolysis protocol: first using 6 wt % HCOOH , then 6 wt% HCl. Remarkably, the hydrolysis operates effectively at just 100 °C, well below the typical 120–210 °C required in many biomass treatments, resulting in substantial reductions in energy consumption while maintaining high sugar yields (Manakas et al. 2025). The

optimized protocols minimize the loss of valuable sugar monomers *via* isomerization reactions, followed by the formation of humins.

We also found that washing OPW with pressurized water and aqueous ethanol is necessary to remove most soluble sugars (and free flavonoids and terpenes) abundant in OPW, thereby preventing the formation of degradation products such as furfural and 5-HMF in the early stages of the process. This cascade process not only prevents early sugar degradation but also maximizes sugar recovery from orange juice while enhancing the hydrolysis of hemicellulose and cellulose.

It is worth noting that HCOOH, used in the first hydrolysis step, is a biobased, non-volatile organic acid ideally suited for biomass processing (Qiao et al. 2023; Zhong et al. 2021; Ciriminna et al. 2016). Its use indeed does not introduce inorganic salt residues, while the acid can be efficiently recovered *via* thermal evaporation.

Notably, the entire process was easily scalable, as indicated by comparable yields of glucose and xylose across batch sizes ranging from 100 mL to 5 L, when using a jacketed, fully automated reactor system.

Finally, this approach is complementary to pectin extraction from fresh or dried citrus processing waste using hot mineral acid at pH 2 (Zhong et al. 2021). Pectin indeed is highly resistant to acid hydrolysis under the mild conditions of the “Hemicellulose Ahead” process, and no galacturonic acid is released during hydrolysis of OPW with dilute formic and hydrochloric acid. Hence, the latter process could be ideally applied to the pectin-rich fraction remaining after the extraction of hemicellulose and cellulose.

Looking ahead, further development of the process will focus on assessing a broader set of bio-based acids and on exploring heterogeneous catalysts, with particular attention to the practical challenges associated with their recovery and reuse. Moreover, this process, now validated for orange processing waste, will also be interesting to evaluate on lignocellulosic biomasses and wastes that contain higher amounts of lignin. This will allow an assessment of whether the protocol can be applied to recover the hemicellulose fraction prior to other catalytic conversion steps aimed at obtaining cellulose or lignin (for example, lignin-first approaches) (Paone et al. 2020). In this way, the process could enable the full recovery and valorization of all three major fractions.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no conflict of interest.

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