



Influence of laccase pre-treatment and matrix composition on hydrothermal extraction of fibers from lignocellulosic agri-food residues

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Abstract

The valorization of agri-food residues increasingly relies on mild, water-based processes capable of recovering intact polysaccharides for food and biorefinery applications. However, the structural diversity of lignocellulosic by-products, and the dominant role of lignin in limiting accessibility, often restricts hemicellulose extraction under gentle conditions. Here, we evaluated a laccase-assisted approach to loosen biomass architecture and improve hydrothermal extraction of lignocellulosic residues, such as nutshells, leafy biomass, and fruit processing wastes, at 150 °C, the standard temperature for hemicellulose solubilization, and at a milder 125 °C, where extraction is usually limited. A comprehensive molecular characterization of insoluble dietary fiber revealed lignin contents ranging from 8 to 72% and hemicellulose proportions from 12 to 58%, which largely dictated extraction behaviour. At 150 °C, laccase pre-treatment had little impact on recalcitrant, lignin-rich matrices (e.g., hazelnut shells: $\leq 4\%$ sugar recovery). In contrast, at 125 °C, where conventional extraction is markedly limited, the enzymatic step significantly increased solubilization only in low-lignin, structurally open residues. The most notable case was orange waste, where extraction rose from 22 to 59% of total dietary fiber. However, the solubilized fractions were not hemicellulose-rich: arabinose- and galacturonic acid-containing polysaccharides represented up to 45–50% of released sugars, indicating a preferential mobilization of pectic domains. Altogether, the study delineates the realistic contribution of laccase in low-severity extraction schemes, highlighting where it can enhance polysaccharide recovery and providing practical guidance for designing targeted valorization strategies.

Keywords Hemicellulose · Laccase · Hydrothermal treatment · Agri-food residues · Dietary fiber · Valorization · Lignocellulosic biomass

Introduction

Lignocellulosic biomass represents the most abundant renewable resource on Earth, with its accumulation potentially leading to environmental issues [1]. It mainly derives from forestry, agricultural, agro-industrial, and food waste, which are widely available, renewable, and cost-effective resources. Its major structural constituents, namely cellulose, hemicellulose, and lignin, can be recycled and exploited for producing various value-added products, including ethanol, food additives, organic acids, and enzymes [2–4].

Hemicellulose has attracted increasing interest due to its abundance in lignocellulosic biomass and its potential valorization into food- and bio-based products [5, 6]. However, conventional extraction methods, namely acid hydrolysis, steam explosion, hydrothermal treatments (> 150 °C),

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alkaline extraction, and organosolv processes, usually involve harsh conditions [7] which promote the formation of degradation compounds and require extensive purification [8], making them unsuitable for food and pharmaceutical applications.

In this context, milder extraction strategies are required to selectively promote hemicellulose solubilization while preserving its polymeric or oligomeric structure, which is of particular interest for functional food applications such as xylo-oligosaccharides (XOS) production. In fact, although XOS originates from the partial depolymerization of hemicellulose during extraction, they should not be considered undesirable degradation products in this context. On the contrary, XOS are recognized as high-value functional ingredients due to their prebiotic properties and potential applications in food and nutraceutical formulations [9]. A distinction should therefore be made between hemicellulose depolymerization leading to valuable oligosaccharides, and excessive degradation occurring under high-severity conditions, which promotes the formation of undesirable compounds such as furfural, 5-hydroxymethylfurfural (HMF), organic acids, and phenolic derivatives that may compromise product purity and safety.

This study investigates a combined approach based on enzymatic pre-treatment with laccase followed by hydrothermal extraction, a water-based process carried out at elevated temperature and pressure, which is one of the most widely applied methods for solubilizing hemicellulose from lignocellulosic matrices. However, as anticipated, increasing hydrothermal severity promotes extensive hemicellulose hydrolysis beyond oligomer formation, leading to subsequent generation of undesired degradation compounds for food and nutraceutical applications. In this context, the enzymatic step was intended to promote matrix disaggregation and partial delignification, thereby enhancing hemicellulose accessibility and enabling its recovery under milder hydrothermal conditions. To the best of our knowledge, the combination of laccase pre-treatment with mild hydrothermal extraction as a strategy for recovering hemicellulose-derived fractions for food and nutraceutical applications has not been previously explored. Indeed, laccase-assisted treatments have primarily been applied to modify or remove lignin, thereby increasing cellulose accessibility for enzymatic hydrolysis in pulp and paper, textiles, and biofuel production [10]. In these processes, hemicelluloses are typically degraded or not recovered for food applications [11]. By contrast, applying laccases in food processes represents a promising and unexplored strategy to selectively recover it for potential use in food or nutraceutical products. The enzymatic step is intended to promote matrix disaggregation and partial delignification, thus enabling hemicellulose recovery under milder hydrothermal conditions. Laccases

(EC 1.10.3.2, para-benzenediol: dioxygen oxidoreductases) are multicopper enzymes renowned for their capacity to use molecular oxygen to catalyze the oxidation of a wide range of aromatic and non-aromatic substrates through a radical-mediated reaction mechanism. These enzymes are broadly distributed in nature and perform diverse biological functions, including, notably, delignification [12]. Multicopper oxidases reduce O_2 to H_2O without releasing harmful, partially reduced derivatives of O_2 called reactive oxygen species (ROS) [13].

Lignocellulosic biomasses were selected on the basis of their lignin/hemicellulose ratio and their high availability as industrial residues, namely, hazelnut and almond shells (nut by-product), hops biomass residue (sprigs, stems and leaves), pineapple, melon, orange and apple processing waste (mainly composed of peels, residual pulp, and, in the case of apples, the core along with the seeds) to assess whether variability in their (insoluble) fiber composition could affect enzyme activity and the extractability of the different fiber fractions.

The main objective of this research was to evaluate whether the enzymatic pre-treatment could enhance hemicellulose recovery from these by-products under milder hydrothermal conditions (125 °C), previously shown to generate limited amounts of polysaccharide degradation products in hazelnut shells [14] and further assessed in the present study through the quantification of the same degradation products (Table S1). This approach was compared with a more conventional extraction condition at 150 °C, where higher thermal severity was expected to promote increased formation of degradation products [14]. Moreover, the study investigated whether the variability in fiber composition among different agri-food matrices affected enzyme activity and the extractability of individual fiber fractions.

Materials and methods

Chemicals

D-fructose, D-glucose, D-arabinose, D-rhamnose, D-ribose, D-xylose, D-fucose, D-galactose, D-mannose, D-glucuronic acid, D-galacturonic acid, phenyl β -D-glucopyranoside, dimethylformamide (DMF), trifluoroacetic acid (TFA), ammonium hydroxide (NH_4OH), N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) with 1% of trimethylchlorosilane (TMCS), cetyltrimethylammonium bromide technical grade, disodium ethylenediaminetetraacetate, 2-ethoxyethanol, N-octanol, sodium lauryl sulfate, sodium phosphate monobasic, sodium phosphate dibasic, hydrochloric acid 37%, acetic acid, sodium acetate, tris(hydroxymethyl)

aminomethane (Tris base), laccase from *Aspergillus* sp. (EC 1.10.3.2; product code SAE0050, 1199 U/mL), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and 3-(trimethylsilyl)propionic acid (TSP) were acquired from Merck (Darmstadt, Germany). Bidistilled water was produced with a Milli-Q System (Millipore, Bedford, MA, USA). Methanol, ethanol absolute anhydrous, and sulfuric acid 95% (H₂SO₄) were bought from VWR International (Milan, Italy).

Samples

Samples from various agri-food by-products, namely hazelnut (Haz) and almond (Alm) shells (nut by-product), hops residue biomass (sprigs, stems, and leaves; Hop), and processing waste from pineapple (peels and core; Pin), melon (peels, seeds, and residual pulp; Mel), orange (peels, seeds, albedo, and residual pulp; Ora), and apple (seeds and core; App) were selected to cover a broad range of lignocellulosic compositions, including matrices with different combinations of lignin and hemicellulose content. The biomass samples were obtained from local agro-industrial companies and collected during the production process. After collection, samples were immediately frozen and stored under frozen conditions until delivery to the University laboratories, where they were subsequently freeze-dried prior to analyses and extraction procedures. Namely, food wastes matrices have been lyophilized in a LIO-5PDGT freeze-dryer (5Pa, Milano, Italy) to remove a major part of the moisture content from the samples, which were fresh and highly wet. A small portion of each lyophilized matrix (approximately 0.5 g) was put in an oven at 105 °C to determine its residual moisture content (see Section "Proximate composition, and insoluble fiber fractions isolation, quantification and characterization."). Then, each lyophilized food waste matrix has been ground (particle size $\leq 500 \mu\text{m}$) in an ultra-centrifugal mill Retsch ZM 300 (Retsch GmbH, Haan, Germany) to prepare the samples for the experiments.

Proximate composition, and insoluble fiber fractions isolation, quantification and characterization

The ground samples (see Section "Samples".) were characterized in terms of proximate composition according to the AOAC official methods of analysis [15]. Moisture content was determined by drying the sample in an oven at 105 °C, while ash content was measured through mineralization at 550 °C, in both cases until a constant weight was reached. Protein quantification was performed using a Kjeldahl system (DKL heating digester and UDK 139 semiautomatic distillation unit; Velp Scientifica, Usmate Velate, Italy),

(conversion factor of 6.25). Lipids were extracted with a Soxhlet system (SER 148/3; Velp Scientifica, Usmate Velate, Italy). Soluble and insoluble fiber were analyzed following the AOAC 991.43 method. The hemicellulose, cellulose, and lignin content of the raw materials were also quantified using the Van Soest method [16], a gravimetric approach widely employed for insoluble fiber fractionation, with the obtained values corrected for ash and protein contents.

The insoluble fiber (IDF) extracted using the AOAC standard method was subsequently subjected to a previously developed sequential fractionation protocol [17], which enables chemical characterization of fiber fractions. This protocol employs a two-step acid hydrolysis using trifluoroacetic acid (TFA) followed by sulfuric acid (H₂SO₄) to fractionate hemicellulose, cellulose, and lignin. The monosaccharide composition of the hemicellulose and cellulose fractions was determined by GC–MS analysis.

Laccase enzyme activity assay: pH, temperature and concentration effect

Laccase activity from *Aspergillus* sp. was determined spectrophotometrically following the method of More et al. [18], using ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) as substrate. All measurements were performed at 25 °C using an Agilent Cary 60 UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The oxidation of ABTS was monitored by measuring the increase in absorbance at 420 nm against a reaction blank.

The reaction mixture consisted of 3 mL of 0.5 mM ABTS prepared in a 100 mM buffer. Laccase was applied at different enzyme loadings (2, 4, 6, 10, 14 U/mL), corresponding to activity units reported by the supplier (1199 U/mL stock solution) and adjusted by appropriate dilution. To assess the effect of pH on enzymatic activity, the assay was performed using different buffer systems: sodium acetate (pH 3.5 and 5.0), sodium phosphate (pH 6.0 and 7.5), and Tris–HCl (pH 8.5). After identifying the optimal pH, the influence of temperature was tested at 30, 40, 50, and 60 °C.

Laccase activity was evaluated at different pH values and temperatures by monitoring ABTS oxidation over time. Reactions were performed under each condition and followed over short and extended time intervals to assess the evolution of enzymatic activity and identify optimal operating conditions.

Laccase enzymatic pre-treatment of lignocellulosic by-products

The experimental conditions for this step of the study were set based on the results obtained with the enzyme activity assays.

Approximately 2 g of each ground matrix (see Section "Samples".) was incubated with laccase from *Aspergillus* sp. at an activity of 50 U/g of matrix in 40 mL of 300 mM phosphate buffer (pH 7.5) at 30 °C for 24 h under continuous shaking using a heating dry bath (Thermo Fisher Scientific, Waltham, MA, USA). Control samples were also prepared by incubating the matrices under the same conditions (300 mM phosphate buffer, pH 7.5, 30 °C for 24 h) but without the addition of laccase. The suspension was subsequently heated at 95 °C for 10 min to inactivate the enzyme, and centrifuged in an Eppendorf 5810R centrifuge (Eppendorf, Hamburg, Germany) at 3214 ×g for 45 min at 4 °C, to separate the supernatant from the solid sample residue (pellet). Pellets were washed and stored at −18 °C for further analyses. The enzymatic reactions were performed in triplicate for each matrix.

Hydrothermal extraction of fiber from laccase-pretreated samples

Residual pellets obtained after the samples incubation with the enzyme laccase, together with non-pretreated samples (controls), were subjected to hydrothermal treatment. A Parr Mini Bench Reactor, type 4560 (Parr Instruments Co., Illinois, U.S.A.) equipped with a magnetic drive stirring system and a reaction vessel of 300 mL capacity, was used. A sample:bidistilled water ratio of 1:25 was selected with the aim of achieving a balance between adequate immersion of the biomass, promote uniform heat transfer, and facilitate efficient solubilization of hemicellulosic polysaccharides during the hydrothermal treatment [19]. The extraction was carried out both at 125° and 150 °C for 1 h. Then, the suspension was entirely recovered and centrifuged (3214 ×g; 45 min; 4 °C). Pellets were washed two times with 10 mL of bidistilled water, and the washings were combined with their corresponding supernatants, which were recovered and stored at −18 °C for subsequent analysis. Untreated controls were included to assess the specific contribution of the laccase pre-treatment.

Molecular characterization of the hydrothermal extracts

Monosaccharides composition (GC–MS)

The fiber extracts were dried in a Rotavapor® R-100 (Buchi, Switzerland) and subjected to acid hydrolysis (2N TFA) followed by trimethylsilyl derivatization (TMS) and gas chromatography–mass spectrometry analysis (GC–MS) to determine monosaccharide distribution, according to the protocol previously reported by Fuso et al. [14].

GC–MS analysis of monosaccharides was conducted using a 6890 N gas chromatograph coupled with a 5973 N mass selective detector (Agilent Technologies, Santa Clara, CA, USA). The separation was achieved on an SLB-5 ms column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Supelco, Bellefonte, PA, USA). Chromatographic data were acquired in scan mode (40–500 m/z), with the column temperature programmed from 60 °C to 270 °C. The initial temperature of 60 °C was maintained for 2 min, followed by a ramp to 160 °C at 10 °C/min, held isothermal for 5 min. Subsequently, the temperature was increased to 220 °C at 10 °C/min, held for 5 min, and then ramped to 270 °C at 20 °C/min, where it was maintained for an additional 5 min. Ionization was performed in electron impact (EI) mode at 70 eV. The ion source and interface temperatures were set at 230 °C and 250 °C, respectively. Quantification was performed using response factors based on the area and concentration ratios between the internal standard (phenyl β-d-glucopyranoside) and each monosaccharide in the standard solutions. The monosaccharides included d-glucose, d-fructose, d-arabinose, d-galactose, d-mannose, d-rhamnose, d-ribose, d-xylose, d-fucose, d-galacturonic acid, and d-glucuronic acid.

Total sugar content and degradation products analysis (¹H NMR)

Fiber extracts were further analyzed by ¹H NMR spectroscopy to determine total sugar content and the presence of degradation products derived from hydrothermal treatment, following the procedure described by Fuso et al. [20]. Briefly, 400 µL of each sample extract were directly transferred into an NMR tube, followed by the addition of 300 µL of D₂O and 100 µL of a TSP solution (2 mg/mL in D₂O). ¹H NMR spectra were recorded using a Bruker Avance III 400 MHz NMR Spectrometer (Bruker BioSpin, Rheinstetten, Karlsruhe, Germany) at 298 K.

Absolute sugar content quantified by ¹H NMR was used to calculate extraction yields. These yields were expressed as the percentage of total sugars in each extract relative to the total dietary fiber (TDF) content of the starting material. The same spectra were also evaluated for compounds associated with hydrothermal modification of plant polysaccharides, including free acetic acid, methanol, and formic acid. Different spectral regions were integrated for compound quantification, namely free acetic acid (1.99–2.05 ppm), free methanol (3.34–3.36 ppm), and formic acid (8.37–8.48 ppm). The calculated concentrations are reported in Table S1 (Supplementary Materials).

Statistical analysis

Extraction yields were analyzed separately for each matrix using two-way analysis of variance (ANOVA), considering extraction temperature and enzymatic pre-treatment as fixed factors, including their interaction. When significant differences were detected, pairwise comparisons among treatment combinations were performed using Tukey's HSD post hoc test. All other datasets were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test. Statistical analyses were performed with IBM SPSS software (Version 29.0.1.0; SPSS Inc., Chicago, IL, USA). Statistical significance was determined at a threshold of $p < 0.05$.

Results and discussions

Proximate and fiber composition of the lignocellulosic biomasses

The different agri-food residual biomasses were chosen as case-studies based on the different prevalence of strongly lignified plant cell-wall: nutshells (Haz and Alm), leafy material (Hop), "soft" fruit residues (App and Ora), and "hard" fruit residues (Pin and Mel). This intrinsic variability in cell-wall structure will allow to better understand the relationship between the effectiveness of laccase pre-treatment and the cell-wall composition. As a first step, the detailed composition of the residual biomasses and the specific distribution and molecular characteristics of the cell-wall components was determined, to provide an overview of

the selected biomasses as potential sources of hemicellulose and to compare the following hydrothermal extracts with the starting fiber composition. The proximate composition of each matrix is reported in Fig. 1.

Across all samples, the contribution of soluble dietary fiber (SDF) was consistently negligible, ranging from 1.5 to 9% of the dry matter respect to IDF representing a major component, particularly in nut shells, where hazelnut shells (Haz) and almond shells (Alm) contained 86% and 76% of IDF, respectively, with only minor contributions from proteins, lipids, ashes and residual fractions. In the remaining matrices, IDF accounted for 20–50% of the dry matter. Proteins were generally present at 10–15% except in nuts, where they represented only 2–4%. A prominent fraction corresponded to other compounds (22–54%), a category that mainly reflects digestible or soluble carbohydrates, normally calculated by difference [21, 22]. This finding is consistent with the nature of the materials: hops residue biomass (Hop), pineapple (Pin), melon (Mel), orange (Ora), and apple (App) by-products contained 45%, 32%, 22%, 54%, and 51% of digestible carbohydrates, respectively, values in line with previous reports on leaves [23] and fruit peels/pulp [21], which are known to be rich in free sugars and digestible/soluble carbohydrates.

As anticipated, the matrices were chosen according to their lignin–hemicellulose ratio, ranging from 0.5:1 to 6:1, a key parameter shaping the IDF fraction (Fig. 2). Considerable variability was observed. Hop and Haz displayed the highest lignin contents (72% and 46%, respectively), while Alm, Mel, and App showed intermediate values (25–30%). In contrast, Pin and Ora exhibited the lowest lignin proportions (8% and 14%). Hemicellulose content ranged from

Fig. 1 Proximate composition of lignocellulosic by-products, expressed as percentage on dry matter (%w/w). "Others" = obtained by difference, comprising digestible carbohydrates. Analyses have been performed in duplicate, and the results are expressed as mean SD

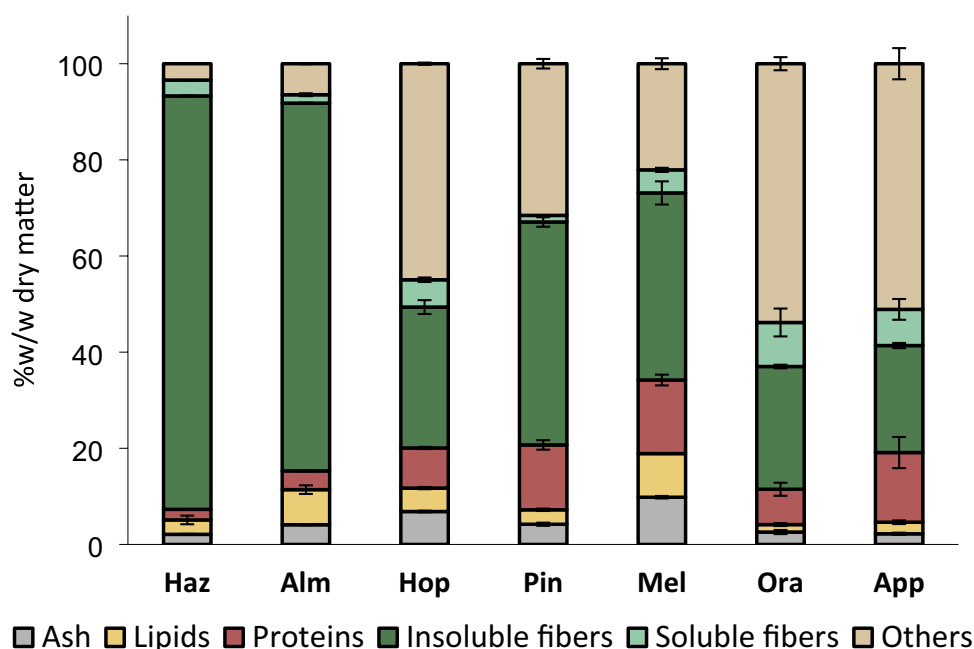


Fig. 2 Composition of the IDF fraction according to the Van Soest Method. Analyses have been performed in duplicate, and the results are expressed as mean SD

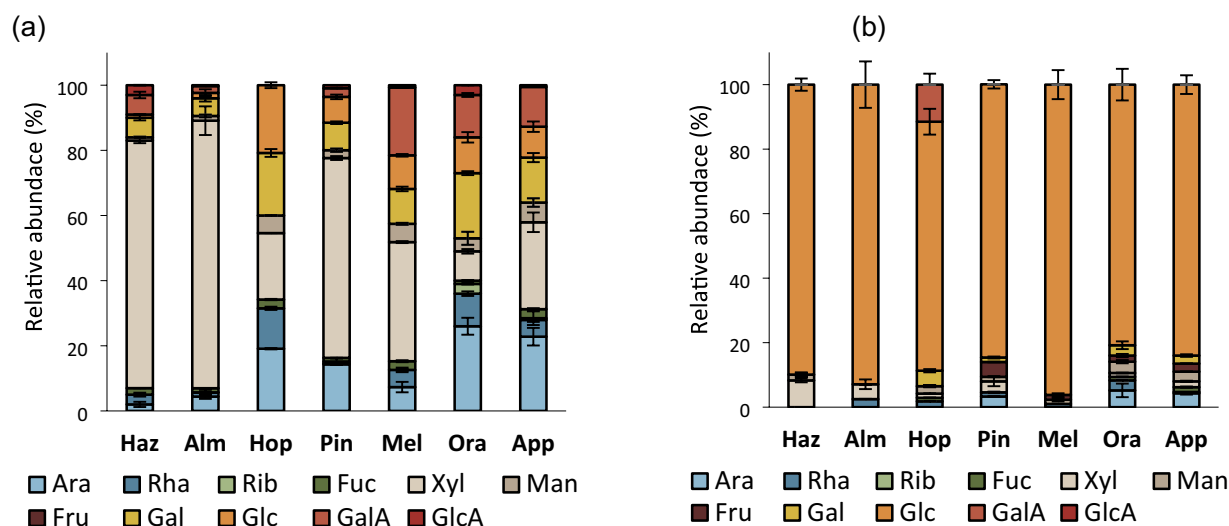
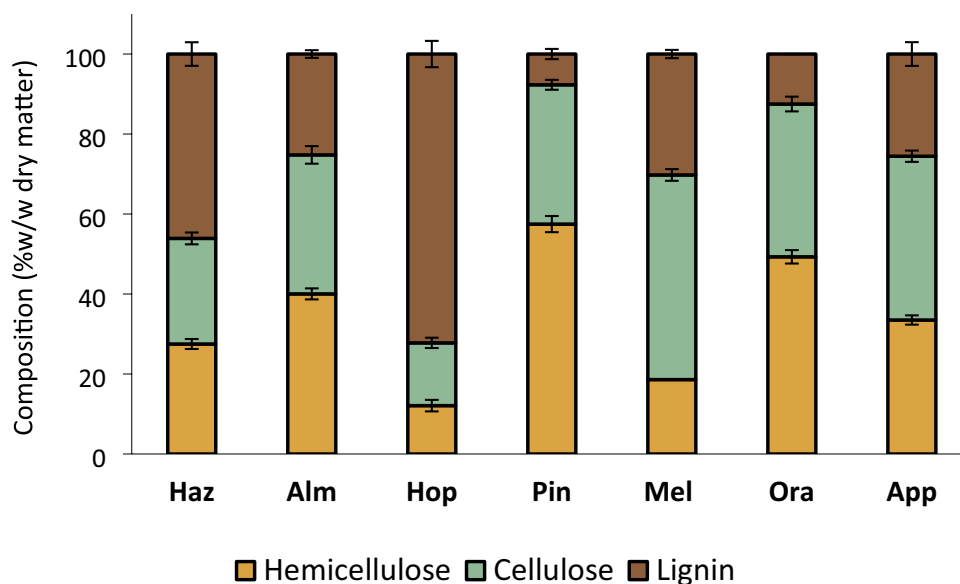


Fig. 3 Monosaccharide composition of hemicellulose (a) and cellulose (b) of IDF. Monosaccharides were analyzed by GC–MS. Analyses have been performed in duplicate, and the results are expressed as mean SD, and reported as % w/w of total monosaccharides

12% in Hop to 58% in Pin, with Haz (27%), Alm (40%), Mel (19%), App (34%), and Ora (50%) covering the intermediate range.

Overall, the proximate composition confirmed the marked heterogeneity of the selected agri-food by-products in terms of IDF content and lignin–hemicellulose ratio, allowing to study the effect of matrix on laccase effectiveness in improving the extractability of hemicellulose/fiber fractions. Notably, while most matrices appear promising for hemicellulose recovery, Hop stands out as particularly challenging due to their exceptionally high lignin content and low hemicellulose content.

To characterize the structural composition of the IDF fractions, a sequential acid hydrolysis was performed (see Section "Proximate composition, and insoluble fiber

fractions isolation, quantification and characterization."), with TFA targeting hemicellulosic sugars and subsequent H_2SO_4 hydrolysis releasing cellulose-derived glucose. This approach allowed discrimination between hemicellulose and cellulose within the IDF, complementing proximate and Van Soest analyses (Fig. 3).

Hemicellulose composition (Fig. 3a) varied substantially across the agri-food biomasses, reflecting both their botanical origin and plant tissue type.

In nutshells (Haz and Alm), xylose was predominant (76% and 82%, respectively), confirming their xylan-based hemicellulose, in line with previous reports [24]. Minor amounts of glucuronyl and galacturonyl residues were also detected, consistent with earlier findings [25].

Hop were characterized by a high content of arabinogalactans, with arabinose and galactose accounting for 19% and 19% of the total monosaccharides, respectively. Xylose represented 20%, indicating a moderate presence of xylans and xyloglucans [24]. Glucose constituted 21%, reflecting a glucan fraction [26]. Rhamnose was relatively low at 12%, while uronic acids were absent, confirming the lack of pectic polysaccharides [27]. Overall, Hop exhibited a typical foliar polysaccharide profile, dominated by arabinogalactans and hemicelluloses [28, 29].

The TFA hydrolyzed fraction of App contained 23% arabinose and 14% galactose, consistent with the presence of arabinogalactans with minor branching [30]. Xylose was 27%, indicating xylan content, while glucose accounted for 9% and rhamnose for 5%. Galacturonic acid was 12%, suggesting a minor contribution of pectic polysaccharides. Conversely, Ora showed higher arabinogalactan content with 26% arabinose and 20% galactose. Xylose and glucose were lower at 9% and 11%, respectively. Rhamnose reached 10% and galacturonic acid 13%, indicating the possible presence of small amounts of rhamnogalacturonan [27, 31]. Both fruit residues display abundant arabinogalactans, with App showing slightly higher xylan content and Ora richer in uronic acids and rhamnose, in agreement with previous findings [32].

Pin were heavily rich in xylose (61%), consistent with predominant xylan, while arabinose and galactose were lower at 14% and 8%, respectively. Glucose was 8%, and galacturonic acid and rhamnose were negligible (0% and 3%), reflecting a highly lignocellulosic structure with minimal pectic contribution. Mel showed 7% arabinose, 11% galactose, and 10% glucose, along with 37% xylose, indicating a mixed composition of arabinogalactans and heterogeneous xylans. Notably, galacturonic acid reached 21%, suggesting some residual pectic polysaccharides despite the insoluble fiber nature. These matrices are thus mainly structured by xylans and glucans, with variable contributions of pectic polysaccharides, depending on the specific residue [32], although Mel retained a substantial proportion of galacturonic acid, a feature that has also been described in earlier studies [33].

Moving to the monosaccharide composition of the fraction hydrolyzed in H_2SO_4 , all matrices exhibited the typical cellulose profile, with glucose accounting for 90%, 93%, 77%, 85%, 96%, 81%, and 84% of the total monosaccharides in Haz, Alm, Hop, Pin, Mel, Ora, and App, respectively. Minor amounts of xylose (1–8%) were detected in all samples, indicating hemicellulosic fractions resistant to TFA hydrolysis. Similarly, small quantities (0.1–5%) of arabinose, rhamnose, mannose, and galactose were observed. In Hop, 12% galacturonic acid was detected, which was absent in the TFA-soluble fraction, suggesting that a portion of

pectic polysaccharides remained associated with the cellulose-rich residue and was not released during the mild acid hydrolysis, as is often observed in plant tissues [34]. Such pectic residues typically require stronger acidic conditions (e.g., sulfuric acid hydrolysis) or thermo-hydrolytic treatments to be effectively solubilized [35, 36].

Laccase pre-treatment effect on hydrothermal extract composition

Each agri-food by-products fully characterized above was subjected to hydrothermal extraction to isolate valuable fiber fractions. Enzymatic pre-treatment by laccase was applied to evaluate its effect in a potential disaggregation of the lignocellulose, facilitating fiber extraction in milder conditions. Preliminary ABTS assays were performed to verify the oxidative activity of the commercial laccase preparation under the conditions selected for the pre-treatment. The assay was used as an operational screening tool rather than as a full kinetic characterization of the enzyme. Different laccase dosages were initially screened by monitoring the increase in absorbance at 420 nm over time. The dosage showing the highest oxidative response toward ABTS was selected for biomass pre-treatment and corresponded to 50 U/g of dry substrate (see Section "Laccase enzyme activity assay: pH, temperature and concentration effect"), calculated based on the supplier-reported activity of the commercial laccase preparation and the amount of dry biomass used. The absorbance profiles obtained under the tested conditions were used as a comparative screening tool to select the operational conditions for the subsequent biomass pre-treatment (see Fig. S1, Supplementary materials). Among the other tested conditions, 30 °C and pH 7.5 provided a suitable and reproducible oxidative response and were therefore selected for biomass pre-treatment (see Figure S2 and S3, Supplementary materials).

It should be noted that ABTS is a synthetic substrate and may lead to a different apparent pH optimum compared to more lignin-like phenolic substrates, due to differences in redox behavior [37]. To investigate the effectiveness of hemicellulose recovery and the specific contribution of laccase pre-treatment, water-soluble extracts from the lignocellulosic residues after hydrothermal extraction at 125 and 150 °C were analyzed in terms of total sugar content and monosaccharide composition. It should also be considered that commercial enzymatic preparations may contain additional enzyme activities derived from the production organism and purification process [38, 39]. Therefore, although laccase-mediated oxidation may contribute to increased accessibility of the lignocellulosic matrix, the possible presence of accessory enzymatic activities in the commercial preparation cannot be excluded. These activities may have

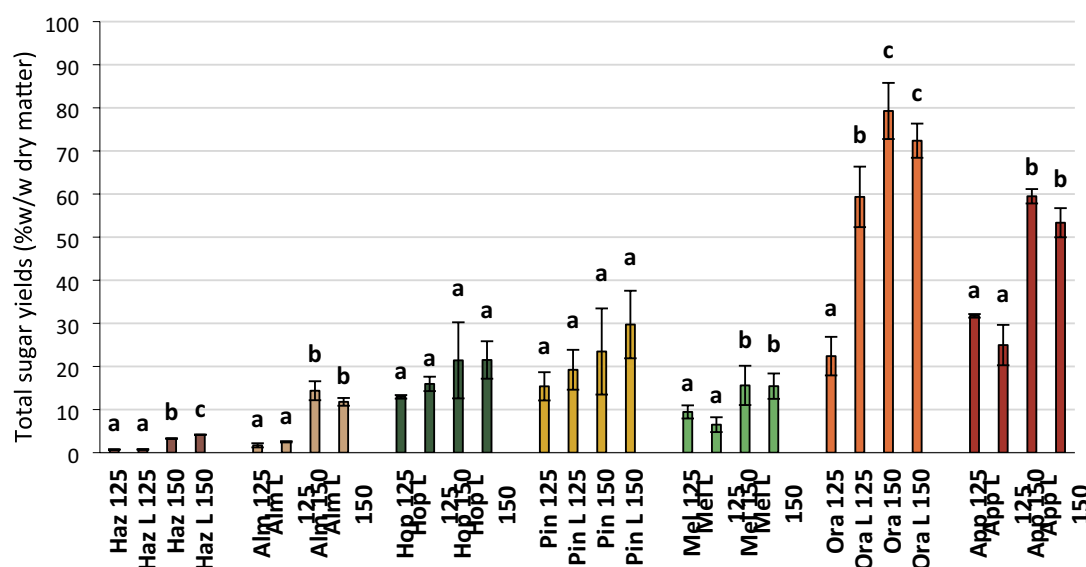
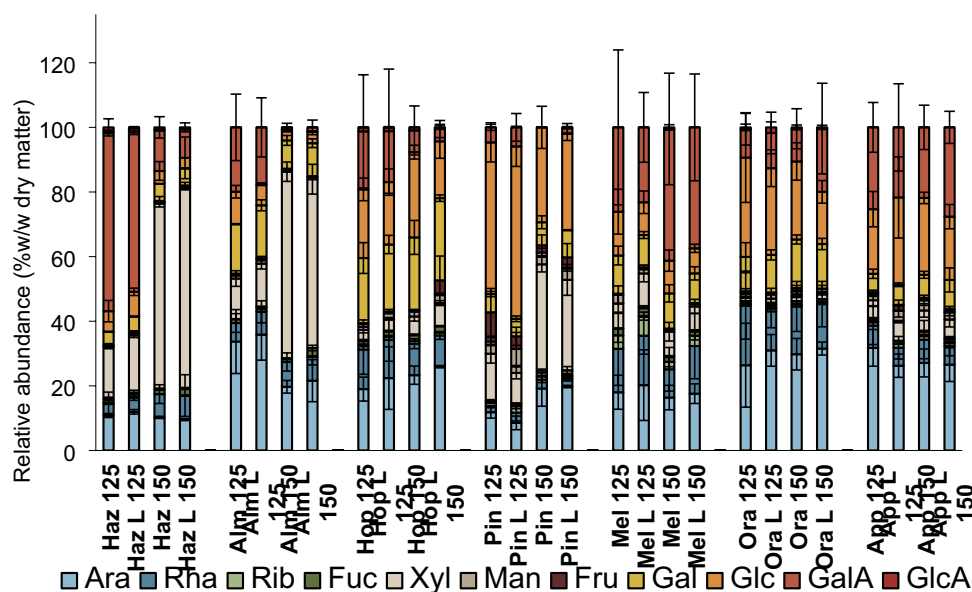


Fig. 4 Total sugar extraction yield, expressed as a percentage relative to the TDF content of the starting materials, as determined by ^1H NMR. Analyses were performed in triplicate, and results are presented as mean $\pm$ SD. Statistical analysis was performed by two-way ANOVA considering temperature and laccase pre-treatment as experimental

factors, followed by Tukey's HSD post-hoc test for multiple comparisons ($p < 0.05$). Different letters indicate statistically significant differences between groups, whereas groups sharing the same letter are not significantly different

Fig. 5 Monosaccharide composition of hydrothermally extracted samples, with or without laccase from *Aspergillus* sp. pre-treatment, determined by GC-MS. Analyses were performed in triplicate, and results are presented as mean $\pm$ SD



contributed to the overall modification of the cell wall architecture and, consequently, to the release of structural carbohydrate fractions. Therefore, the observed effects should not be interpreted as being exclusively attributable to laccase activity.

This approach allows the evaluation of hemicellulose distribution within the main solid-derived fraction obtained after each treatment step, which represents the primary focus of the process in terms of hemicellulose recovery.

Total sugar solubilized after HT were quantified by ^1H NMR (Fig. 4), providing a direct measure of extraction

efficiency, while GC-MS profiling allowed detailed characterization of the monosaccharide composition of the solubilized fractions (Fig. 5; Table S2).

Extraction yields (Fig. 4) were expressed relative to the total dietary fiber (TDF) content of the starting material. TDF was calculated as the sum of IDF and SDF fractions determined according to AOAC 991.43 (see Section "Proximate composition, and insoluble fiber fractions isolation, quantification and characterization"). A two-way ANOVA was performed separately for each matrix to evaluate the effects of temperature, enzymatic pre-treatment, and their

interaction on extraction yield. The significant interactions identified statistically are discussed in the corresponding matrix-specific sections. The complete results of the two-way ANOVA, including *p*-values for temperature, enzymatic pre-treatment, and their interaction, are reported in Table S3 (Supplementary Material).

This compositional parameter was determined on the untreated biomass and used as a fixed reference for yield normalization. In this context, TDF was considered a suitable baseline for all investigated matrices, as it reflects the total fiber content potentially available for hydrothermal solubilization in the native biomass. This approach allows extraction yields to be expressed relative to the initial biomass composition, ensuring comparability across matrices and processing conditions.

By comparing these data with the previously characterized IDF composition, it is possible to evaluate how fiber structure, lignin content, and matrix type influence polysaccharide solubilization. This integrated analysis not only highlights differences in extraction efficiency across matrices but also provides insights into the potential of the combined enzymatic-hydrothermal strategy to selectively recover hemicellulosic sugars under milder conditions. As the present study was specifically designed to evaluate hemicellulose recovery and compositional changes in the extracted fractions, parameters such as energy consumption and product purity were not directly quantified and were not included among the response variables considered in the experimental evaluation. A discussion of the results for each matrix is provided in the following sections.

Hazelnut shells

Haz represents a highly recalcitrant lignocellulosic matrix, characterized by a predominance of IDF and a xylan-rich hemicellulose fraction, as previously described (see Section "Proximate and fiber composition of the lignocellulosic biomasses"). Hydrothermal extraction at 125 °C resulted in extremely low yields ($\approx 1\%$ of total dietary fiber, TDF; Fig. 4), besides laccase pre-treatment. This indicates that mild hydrothermal conditions are insufficient to release significant amounts of polysaccharides from this lignin-rich substrate. The negligible effect of laccase under these conditions further confirms that the enzyme is unable to enhance fiber accessibility when the polysaccharides are embedded within such a rigid and lignified matrix, in agreement with the literature [40].

Increasing the extraction temperature to 150 °C led to moderately higher recoveries ($\approx 3\%$ of TDF for non-pretreated samples and $\approx 4\%$ for laccase-pretreated samples), with laccase providing a small but statistically significant improvement (Fig. 4). The two-way ANOVA confirmed a

significant interaction between temperature and enzymatic pre-treatment ($p < 0.05$), indicating that the effect of laccase was dependent on the extraction temperature. Nevertheless, the overall extraction remained limited, reflecting the intrinsic structural recalcitrance of Haz, which is consistent with its high lignin content ($\approx 46\%$).

The monosaccharide composition of the extracts further illustrates the temperature-dependent solubilization pattern (Fig. 5). At 125 °C, galacturonic acid predominated ($>50\%$), indicating that primarily pectic components were solubilized, whereas xylose accounted for only a minor fraction (16% in non-pretreated and 18% in laccase-pretreated samples), suggesting that xylan remained largely insoluble. At 150 °C, xylose became the predominant monosaccharide (58% in non-pretreated and 62% in laccase-pretreated samples), reflecting the enhanced solubilization of xylan from the hemicellulosic fraction [14] which constitutes approximately 28% of the IDF. Laccase pre-treatment did not significantly alter the overall monosaccharide profile, confirming its limited effectiveness in this lignin-dense matrix.

In summary, Haz exhibits extremely low polysaccharide recoveries under mild hydrothermal conditions, with substantial xylan solubilization occurring only at elevated temperatures. The high lignin content and the tightly packed hemicellulose network likely limit both enzymatic accessibility and hydrothermal extraction efficiency.

Almond shells

Alm, similarly to Haz, is a lignocellulosic matrix characterized by high IDF and low SDF and digestible carbohydrates, as described in Section "Proximate and fiber composition of the lignocellulosic biomasses". Differently from Haz, Alm's IDF is primarily composed of hemicellulose and cellulose, with a moderate lignin content ($\approx 25\%$), and the hemicellulose is predominantly xylan-based, consistent with previous findings [41].

Total sugar extraction yields, expressed relative to TDF (Fig. 4), indicated that laccase pre-treatment had no significant effect at 125 °C: yields were very low and essentially identical for non-pretreated ($\approx 2\%$) and laccase-pretreated samples ($\approx 3\%$). At 150 °C, extraction yields increased substantially, reaching $\approx 14\%$ for non-pretreated and $\approx 12\%$ for laccase-pretreated samples, with no statistically significant difference. These results indicate that extraction under mild hydrothermal conditions is limited, whereas harsher conditions allow partial solubilization of hemicellulose, consistent with its abundance in the IDF fraction.

The monosaccharide composition of the extracts (Fig. 5) further supports these findings. At 125 °C, xylose represented a minor fraction ($\approx 13\%$ in non-pretreated and $\approx 14\%$ in pretreated samples), whereas arabinose, galactose, and

uronic acids were relatively abundant, suggesting preferential solubilization of pectic- or arabinogalactan-like components. At 150 °C, xylose became the predominant sugar ($\approx 52\%$ in non-pretreated and $\approx 58\%$ in pretreated samples), reflecting selective extraction of the xylan-rich fraction under harsher hydrothermal conditions, as expected from the hemicellulose fraction ($\approx 40\%$ of IDF). Minor amounts of arabinose, galactose, and glucose were retained, while uronic acids decreased, indicating preferential solubilization of xylan.

As observed for Haz, laccase pre-treatment did not substantially alter the monosaccharide composition, confirming that enzymatic action is limited compared to the thermal effect, probably again due to the high lignified nature of the matrix. Total extraction yields, however, were higher than those of Haz, likely due to Alm's lower lignin content and higher hemicellulose fraction, which favor hydrothermal solubilization.

Hops residue biomass

Hop represents a lignocellulosic matrix characterized by a high lignin content and a relatively low hemicellulose fraction, as previously described (see Section "[Proximate and fiber composition of the lignocellulosic biomasses](#)"). This composition differs from Haz and Alm, indicating a matrix in which hemicellulose is even more embedded within a dense lignin-cellulose network, which is expected to influence both enzymatic accessibility and hydrothermal extractability [40].

Total sugar yields from hydrothermal extraction (Fig. 4) were 13% of TDF for non-pretreated and 16% for laccase-pretreated samples at 125 °C, with a slight but no statistically significant effect of enzymatic pre-treatment under mild conditions. Increasing the temperature to 150 °C enhanced the extraction yield to 21% for both non-pretreated and pretreated samples, confirming that thermal conditions dominate polysaccharide solubilization in this highly lignified matrix, without effect of laccase pre-treatment.

The monosaccharide composition (Fig. 5) of the extracts illustrates once again a more pronounced temperature-dependent trend than enzymic ones. At 125 °C, arabinose ($\approx 19\%$ in non-pretreated and $\approx 22\%$ in pretreated samples) and galactose ($\approx 20\%$ and $\approx 21\%$, respectively) were the most abundant sugars, whereas xylose ($\approx 3\%$) and mannose ($\approx 2\%$) were minor and essentially equivalent regardless of enzymatic pre-treatment. Uronic acids were present at moderate levels (GalA $\approx 16\%$ and 18% , GlcA $\approx 1\%$), reflecting partial solubilization of pectic domains. The comparison between extracts with and without laccase at this temperature shows only minor differences, suggesting limited

enzymatic influence on the monosaccharide composition under mild conditions.

At 150 °C, solubilization of hemicellulose- and cellulose-derived sugars increased and decreased, respectively. Xylose ($\approx 6\%$ in non-pretreated and $\approx 7\%$ in pretreated samples) and mannose ($\approx 2\%$ and $\approx 3\%$) increased slightly compared to 125 °C, while arabinose ($\approx 23\text{--}26\%$) and galactose ($\approx 23\text{--}25\%$) remained substantial. Glucose content showed greater variation, being $\approx 26\%$ in non-pretreated and $\approx 18\%$ in pretreated samples, and contextually arabinose and galactose increased (from 23% in non-pretreated to 26% in pre-treated sample, in both cases) reflecting partial co-extraction of arabinogalactans at the expense of cellulose following the laccase treatment. Uronic acids decreased compared to 125 °C (GalA $\approx 4\text{--}7\%$, GlcA $\approx 1\%$), consistent with partial degradation or solubilization. Laccase pre-treatment produced only minor compositional changes, with a slight decrease in glucose and a concomitant increase in arabinose and galactose at 150 °C, suggesting limited delignification, as expected, and partial co-extraction of arabinogalactans rather than enhanced cellulose hydrolysis. The overall extraction behavior of Hop was dominated by temperature effects, which governed polysaccharide solubilization and monosaccharide distribution.

Overall, Hop behaved similarly to Haz regarding temperature-driven polysaccharide release, although its hemicellulose is structurally distinct, displaying a more balanced distribution of arabinose, galactose, glucose, and minor xylose, rather than a xylan-dominated pattern. The high lignin content and limited hemicellulose likely restrict enzyme accessibility, rendering thermal extraction the primary factor controlling sugar release.

Pineapple processing waste

Pin, characterized by high IDF and low SDF, represents a matrix with a significant proportion of hemicellulose and cellulose and a minor contribution from lignin (see Section "[Proximate and fiber composition of the lignocellulosic biomasses](#)"). This compositional profile differs markedly from the previously discussed matrices, reflecting a material with mixed hemicellulosic characteristics, partially xylan-rich but containing relevant amounts of other carbohydrates. Digestible carbohydrates are non-negligible, accounting for approximately 32% of the dry matter, and should be considered when interpreting extraction outcomes.

Extraction yields (Fig. 4) indicated that the mild hydrothermal treatment at 125 °C was sufficient to solubilize a substantial fraction of the IDF, with no statistically significant differences between non-pretreated ($\approx 15\%$ of TDF) and laccase-pretreated samples ($\approx 19\%$). This suggests that, besides being poorly lignified, Pin contains polysaccharide

fractions that require harsher conditions for efficient extraction, as also reported by Banerjee et al. (2019), likely due to their tight association within the cell wall. Increasing the temperature to 150 °C led to slightly higher absolute yields ($\approx 23\%$ for non-pretreated and $\approx 29\%$ for pretreated samples), although these differences were not statistically significant, suggesting that 125 °C is already sufficient to achieve near maximal solubilization for this matrix.

Monosaccharide profiling (Fig. 5) of the extracts further elucidates the nature of the solubilized material. At 125 °C, the non-pretreated extract was composed primarily of glucose ($\approx 47\%$), with moderate amounts of xylose ($\approx 14\%$), mannose ($\approx 4\%$), fructose ($\approx 9\%$), and arabinose ($\approx 12\%$). The laccase-pretreated sample at the same temperature showed minor shifts: glucose increased slightly to $\approx 53\%$, xylose decreased to $\approx 10\%$, mannose increased to $\approx 7\%$, and arabinose decreased to $\approx 9\%$. These observations suggest that, unlike in previous cases, laccase may exert a subtle influence on monosaccharide composition at 125 °C, though overall patterns remain broadly comparable.

At 150 °C, the solubilized fractions displayed a notable increase in xylose content ($\approx 33\%$ in non-pretreated and $\approx 28\%$ in pretreated samples), accompanied by decreases in glucose ($\approx 29\%$ and $\approx 30\%$, respectively) and modest increases in arabinose ($\approx 19\text{--}20\%$). Mannose, fucose, galactose, and fructose remained relatively stable. These results indicate that higher temperatures enhance extraction of xylan-rich components, consistent with the partially xylan-dominated hemicellulose profile of Pin. Laccase pre-treatment at this temperature appears to have minimal effect, causing only slight adjustments in individual monosaccharide proportions.

Overall, Pin demonstrates distinctive extraction behavior compared to Haz, Alm, and Hop, likely due to the markedly diverse IDF composition. Its mixed hemicellulosic nature, together with substantial digestible carbohydrate content, results in early and efficient solubilization under moderate hydrothermal conditions, with limited impact from laccase supplementation. However, considering that the purpose of the treatment was to recover dietary fiber, the concurrent solubilization of digestible carbohydrates represents an undesired outcome, as it reduces the structural purity and physiological relevance of the extracted fiber fraction. These findings reinforce the importance of matrix-specific composition in determining both the extent and the nature of soluble fractions obtained under controlled hydrothermal treatments.

Melon processing waste

Mel was identified as a matrix characterized by a balanced distribution of structural polysaccharides, with cellulose

representing the main fraction, followed by lignin and hemicellulose (see Section "Proximate and fiber composition of the lignocellulosic biomasses"). The hemicellulose fraction displayed a heterogeneous monosaccharide composition, consistent with the presence of both xylan-type backbones and pectic domains.

Extraction yields (Fig. 4) remained limited at 125 °C, corresponding to $\approx 10\%$ of TDF for non-pretreated and $\approx 7\%$ for laccase-pretreated samples. The difference was not statistically significant, indicating that enzymatic treatment did not enhance solubilization under these conditions. At 150 °C, yields increased to $\approx 17\%$ for non-pretreated and $\approx 15\%$ for pretreated samples, again without significant differences between treatments. These results suggest that temperature had a more relevant role than enzymatic action in promoting polysaccharide release, and that the increase from 125 to 150 °C reflected a general improvement in extraction efficiency rather than a shift in substrate selectivity.

Monosaccharide composition (Fig. 5) further elucidated the nature of the solubilized fraction. At 125 °C, galacturonic acid was enriched, representing $\approx 26\%$ in non-pretreated and $\approx 23\%$ in pretreated samples, confirming that pectin-derived domains were preferentially mobilized at this lower temperature. Arabinose ($\approx 18\%$ and 20% , respectively) and rhamnose ($\approx 13\%$ and 15%) were also abundant, supporting the predominance of pectic structures. Glucose ($\approx 14\%$ in non-pretreated and $\approx 10\%$ in pretreated samples) and galactose ($\approx 12\%$ and $\approx 10\%$) contributed to a lesser extent, while minor sugars such as xylose and mannose remained below 10%.

At 150 °C, galacturonic acid further increased to $\approx 41\%$ for non-pretreated and $\approx 37\%$ for pretreated samples, accompanied by a decrease in glucose ($\approx 10\%$ and $\approx 8\%$). Arabinose ($\approx 16\text{--}18\%$) and rhamnose ($\approx 9\text{--}15\%$) levels remained comparable, indicating that higher temperature reinforced preferential solubilization of pectin-rich domains while limiting the contribution of other polysaccharide classes. Considering that the IDF profile of Ora consisted of approximately 50% hemicellulose (Fig. 2), the absence of hemicellulosic sugars in the extract and the concurrent release of pectic components appears unexpected. This behavior may be attributed to the presence of insoluble pectic domains tightly associated with the cell wall matrix, which undergo partial solubilization under the applied conditions.

Overall, Mel responded differently to hydrothermal treatment compared with Haz, Alm, and Hop, with pectic domains being the most extractable fraction. Neither enzymatic pre-treatment nor temperature altered this general trend, although the increase at 150 °C emphasized the susceptibility of uronic acid-rich polysaccharides to hydrothermal solubilization.

Orange processing waste

Ora, as described in Section "[Proximate and fiber composition of the lignocellulosic biomasses](#)", is a lignocellulosic matrix characterized by moderate IDF predominantly composed of hemicellulose and cellulose. Hydrothermal treatments performed at 125 °C and 150 °C, in the presence or absence of laccase pre-treatment, yielded distinct extraction profiles.

At 125 °C, laccase pre-treatment significantly enhanced the extraction yield (Fig. 4), increasing it from $\approx 22\%$ of TDF in non-pretreated samples to $\approx 59\%$ in pretreated samples, indicating a pronounced enzymatic contribution under mild thermal conditions. Monosaccharide analysis (Fig. 5) of the extracted material revealed that laccase treatment resulted in a slight enrichment of arabinose ($\approx 26\%$ in non-pretreated and $\approx 31\%$ in pretreated) and galacturonic acid ($\approx 9\%$ and $\approx 11\%$), accompanied by a modest reduction in glucose ($\approx 31\%$ and $\approx 27\%$). At 150 °C, extraction yields were substantially higher in both conditions ($\approx 79\%$ for non-pretreated and $\approx 72\%$ for pretreated samples), with no statistically significant difference, indicating that thermal effects dominate the extraction process. The different response of laccase pre-treatment at the two extraction temperatures was confirmed by two-way ANOVA, which revealed a significant interaction between temperature and enzymatic pre-treatment ($p < 0.05$), indicating that the effect of laccase depended on the extraction temperature. Nonetheless, laccase pre-treatment at this temperature still influenced the monosaccharide composition, with notable increases in galacturonic acid ($\approx 10\%$ vs $\approx 20\%$) and decreases in glucose ($\approx 24\%$ vs $\approx 16\%$), suggesting preferential solubilization of pectic polysaccharides. Galactose accounted for approximately 12–15% of the total monosaccharides across all samples.

Collectively, these results highlight a distinctive effect of laccase pre-treatment at 125 °C, markedly increasing extraction yield. This behavior is consistent with the lower lignin content of orange waste, which may favor enzyme accessibility compared with more recalcitrant matrices [40]. However, the comparison with Pin, which also exhibits low lignin content and a high hemicellulose proportion but showed limited response to laccase pre-treatment, suggests that lignin content alone is not sufficient to explain enzymatic responsiveness. Instead, differences in the composition of the accessible polysaccharide fraction may contribute to this contrasting behavior, as Pin is characterized by a more xylan-rich hemicellulose, whereas Ora contains a higher proportion of fractions characterized by arabinose, galactose and galacturonic acid, indicative of insoluble pectic and arabinogalactan structures. This indicates that, under mild conditions, enzymatic treatment shifts the extraction toward different polysaccharides, promoting selective

solubilization of pectic material. In contrast, at higher temperatures (150 °C), thermal solubilization predominates, and the specific contribution of laccase pre-treatment is largely masked, resulting in similar overall yields irrespective of enzymatic addition.

It should be noted, however, that orange processing waste, similarly to Hop, Pin, Mel and App, contains a relatively high proportion of digestible carbohydrates, which may also contribute to the total sugars quantified after hydrothermal extraction. Therefore, the extraction yield alone cannot unequivocally distinguish between the release of pre-existing soluble carbohydrates and the solubilization of structural polysaccharides. For this reason, extraction yields were interpreted together with the monosaccharide composition of the extracts, which in this case suggests that the observed extraction behavior is associated with the solubilization of structural carbohydrate components rather than being primarily driven by soluble compounds already present in the matrix.

Apple processing waste

App is a lignocellulosic residue with a moderate hemicellulose fraction and relatively high cellulose and lignin content in the IDF. It is primarily composed of xylans and pectic polysaccharides, while soluble fiber and digestible carbohydrates are present at notable levels (see Section "[Proximate and fiber composition of the lignocellulosic biomasses](#)").

At 125 °C, the effect of laccase pre-treatment did not result in a statistically significant increase in extraction yield (Fig. 4) compared to the thermal control, although yields were relatively high ($\approx 25\%$ for non-pretreated and $\approx 30\%$ for laccase-pretreated samples). The extracted fraction at this temperature was enriched in arabinose and galacturonic acid, consistent with preferential solubilization of pectic polysaccharides rather than xylan-rich hemicellulose (Fig. 5). At 150 °C, extraction yields increased due to thermal solubilization ($\approx 40\%$ for non-pretreated and $\approx 42\%$ for pretreated samples), and the addition of laccase did not produce significant differences, confirming that enzymatic effects are masked at elevated temperatures. Monosaccharide composition further supports this interpretation: the major solubilized sugars are arabinose, galactose, glucose, and galacturonic acid, reflecting selective release of pectic and other non-cellulosic polysaccharides, while xylan-derived sugars remained largely insoluble.

These findings suggest that, for App, laccase pre-treatment under mild conditions is not effective in promoting hemicellulose extraction. The enzyme's impact is limited to subtle alterations in the profile of solubilized polysaccharides at lower temperatures, whereas thermal effects dominate the solubilization process at 150 °C.

Conclusions

This study provides a systematic evaluation of hydrothermal extraction of polysaccharides from a range of lignocellulosic residues, including hazelnut shells, almond shells, hops leaves, pineapple, melon, orange, and apple processing wastes. The results demonstrate that extraction efficiency and monosaccharide composition of the solubilized fractions are strongly matrix-dependent, reflecting differences in insoluble dietary fiber composition, lignin content, and hemicellulose structure. Furthermore, this work also provides a clear and detailed view of the insoluble dietary fiber distribution and molecular composition in the different matrices studied, which is a highly valuable information for interpreting extraction outcomes and guiding further valorization strategies.

Overall, the results indicate that hydrothermal extraction efficiency is primarily determined by matrix composition and the structural organization of polysaccharides within the IDF fraction. Laccase pre-treatment exerted matrix- and temperature-dependent effects, with its impact being strongly influenced by the structural organization and composition of the accessible polysaccharide fractions. While lignin-rich and recalcitrant matrices showed limited response, some less lignified matrices displayed enhanced solubilization of specific polysaccharide domains under mild thermal conditions, as observed for orange and pineapple waste. The limited efficacy observed in highly lignified matrices is consistent with the expected mechanism of laccase-mediated oxidation, which mainly involves phenolic lignin structures and may be insufficient to substantially modify highly condensed lignin networks. Moreover, the different responses observed among matrices with comparable lignin contents suggest that lignin abundance alone does not fully determine enzyme effectiveness, and that the accessibility and chemical nature of lignin and associated polysaccharide domains also influence the outcome of the pre-treatment. Future strategies incorporating redox mediators could enhance enzymatic action by extending oxidation to non-phenolic lignin structures, potentially improving accessibility and polysaccharide recovery. Additionally, considering that enzyme cost, stability, and reusability represent important factors for the development of sustainable biocatalytic processes, future studies on the most responsive matrices identified could investigate strategies aimed at improving laccase efficiency, such as enzyme immobilization, to reduce enzyme consumption and enhance the feasibility of larger scale applications. The composition of the solubilized fraction reflects both the inherent polysaccharide distribution and the extraction conditions, highlighting the importance of tailoring hydrothermal treatments and enzymatic pre-treatments to the target matrix for efficient

recovery of functional carbohydrates. From a practical perspective, these insights can guide the optimization of biorefinery processes aimed at the valorization of agro-industrial by-products. Moreover, the detailed compositional characterization of insoluble dietary fiber across different residues provides a valuable reference for the selection of raw materials in tailor-made bioprocesses, depending on the desired polysaccharide profile or downstream application. The results thus support the development of integrated and sustainable valorization pathways for diverse agri-food wastes, contributing to circular bioeconomy models where each residue is processed according to its molecular potential.

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

Declarations

Conflict of interest The authors declare no competing interests.

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