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Valorization of hop (*Humulus lupulus* L.) pruning to produce valuable compounds using two biorefinery strategies: Conventional processing and microwave-assisted autohydrolysis

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ABSTRACT

The beer industry produces a significant quantity of residues, including hop pruning from the *Humulus lupulus* L. plant. In this work, two alternative schemes of biorefinery were evaluated for the first time for valorization of this residue. A conventional processing, involving the water extraction of compounds (110 °C for 30–60 min), was proposed to obtain phenolic compounds, followed by weak acid treatment to optimize the hemicelluloses solubilization. Alternatively, innovative processing, based on autohydrolysis assisted by microwave was also evaluated for the co-extraction of antioxidants and oligosaccharides. Results obtained from these biorefineries showed that after 30 min of aqueous extraction phenols (33.86 mg GAE/g raw material) and flavonoids (42.50 mg RE/g raw material) were successfully solubilized with an antioxidant activity of 6.09, 43.56, and 29.79 mg TE/g raw material using the DPPH, ABTS, and FRAP methods, respectively. The second stage of conventional process (123.5 °C; 1.69 % HCl; 59.6 min) yielded the highest values of xylooligosaccharides and xylose (16.38 g/L) and glucan content (53.25 %). Alternatively, 5.50 g/L of xylooligosaccharides and xylose were obtained along with antioxidant phenolics measuring 31.74 mg GAE/g raw material and 61.06 mg RE/g raw material, using microwave-assisted autohydrolysis (200 °C for 5 min). The antioxidant activity of these bioactive compounds was 20.80, 29.82, and 44.01 mg TE/g raw material for the DPPH, ABTS, and FRAP assays, respectively. Overall, this study shows the feasibility of hop pruning processing under two biorefinery schemes, in which between 10.32 and 17.11 g of phenolic compounds and xylan derivatives per 100 g of raw material can be obtained, with high potential to be used in the pharmaceutical, food or chemical industries.

1. Introduction

Hop is a plant mainly used as ingredient to produce beer (responsible for its aroma and bitterness) and, to a lesser extent, for pharmaceutical and cosmetic purposes. The useful parts of the plant for this target are the female flowers due to the presence of great amounts of bioactive components (Astray et al., 2020; Bocquet et al., 2018; Karabín et al., 2016). In this sense, and like happens with other products from the agri-food industry, only a part of the hop plant is used for food or pharma-cosmetic uses, while the rest of the plant (accounting for a high percentage of the plants' weight) is considered waste and used for incineration or composting (Kopeć et al., 2022). However, the utilization of the remaining parts of the biomass that are not employed nor consumed is a key point in a system of finite resources and with a high population increase, since they content a significant number of compounds of high added-value susceptible of utilization (Fafal et al., 2022; Fierascu et al., 2019).

The production of hop has been increasing globally in the past 10 years (around a 41 % of rise) reaching up to 16 million tons of hop cones in 2022. This growth in the hop production also implied an analogous increase in the harvested area (36 %) reaching up to 103,000 ha (FAOSTAT, 2024). The main producing countries are the United States, Germany, the Czech Republic, China, and Poland (Bocquet et al., 2018). In this context, the residual biomass after the harvest of hop cones (hop

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pruning) would be considered as waste, although presenting an interesting range of polymers and bioactive compounds that could be valorized. Nonetheless, the feasibility of hop pruning as a potential source to produce bioproducts has not been attempted so far, to the extent that the authors are aware of it.

In order to assess an appropriate valorization of the selected biomass, the choice of pretreatment as a first step of a lignocellulosic biorefinery system is key (Kumar et al., 2020). This pretreatment must respond to a series of objectives such as: (i) a large recuperation of the isolated polymers and other compounds of the lignocellulosic biomass, (ii) the production of noxious or inhibitory components must be limited in order to reduce the danger of undesirable effects, (iii) it must have a controlled severity for degradation, desired or not, of sugars in other compounds, (iv) the energy required must be as little as possible, even implementing the energy integration between the pretreatment stage, etc. (Galbe and Wallberg, 2019). Among the different pretreatments, they can be classified in conventional (or traditional) and innovative technologies. The conventional are considered well-known methods that may present some drawbacks such as lower extraction yield, longer reaction time, higher energy consumption, less eco-friendly or not cost-effective, whereas the innovative ones may surpass some of these drawbacks providing higher yields, lower cost or being environmentally sustainable, *inter alia* (Usman et al., 2022).

In this sense, non-structural components (such as phenolic compounds) and/or hemicellulose of lignocellulosic biomass can be solubilized using mild conditions of aqueous extraction or dilute acid treatment. In this context, the extraction with water allows the recovery of antioxidant compounds (Gil-Martín et al., 2022) while the pretreatment with dilute acid favors the nearly quantitative hydrolysis of the hemicelluloses, as well as the partial solubilization of the lignin, and the enhancement of the enzymatic digestibility of the cellulose, being able to obtain almost quantitative conversions (Solarte-Toro et al., 2019; Zhu et al., 2019). Throughout the process, the acid can break the polysaccharide-lignin bonds, which results in the recovery of most of the sugars in monomeric form (Rezania et al., 2020) the benefits of this method are its high performance, easiness, and low cost (Chen et al., 2017; Padilla-Rascón et al., 2020). The main disadvantages are the production of inhibitory products or the need to neutralize (Niju et al., 2019). On the other hand, when that acid is diluted, it needs higher times and temperatures than if it were concentrated, and the use of acid can corrode the equipment used in the process and shows environmental problems (Zhu et al., 2019).

On the other hand, microwave-assisted autohydrolysis is also a suitable alternative that can enable the extraction of phenolic compounds and other bioproducts such as xylose and xylooligosaccharides. Microwaves are considered an ecological and effective technology for the extraction of these compounds (del Río et al., 2021). This procedure is grounded on the effect of microwave energy, transformed into heat through ion conduction and dipole rotation, which rises the pressure and temperature within the cell matrix, causing the breakdown of the cell structure, thus enhancing the extraction of compounds (Gullón et al., 2020). This heating provokes the self-ionization of water, improving the liberation of acid groups (such as acetyl groups or uronic acids) which act as catalysts of the reaction and enable the solubilization of water-soluble polysaccharides and other non-structural compounds (Del-Castillo-Llamosas et al., 2023). The main benefits of this method are high yield, short times, low temperatures, and minimal degradation of bioactive compounds (Lopes et al., 2020; Montenegro et al., 2021). As disadvantages, it has a high cost of equipment due to being a less known technology.

Taking into account all of this, herein we propose for the first time a process for both the extraction of antioxidant phenolics and hemicelluloses (in the form of xylose and xylooligosaccharides) from unexplored hop pruning following two biorefinery strategies, employing either conventional or innovative extraction technologies, evaluating the amount of compounds obtained and the different phytochemical profiles.

The first part of the study, based on conventional treatments, has the objective of prior solubilizing phenolic compounds through a mild aqueous extraction followed by dilute acid pretreatment, while the second part of the work, based on innovative treatments, as microwave-assisted autohydrolysis allows the extraction of both compounds simultaneously. Additionally, the phytochemical profile of phenolic compounds from hop pruning after both strategies was evaluated by HPLC-MS. Therefore, this research represents a first attempt to valorize the hop pruning waste to obtain added-value compounds within a biorefinery frame.

2. Materials and methods

2.1. Reagents used

Acetic acid, ethanol (96 %), ferric chloride (III) hexahydrate, formic acid, glucose, methanol, potassium persulfate, sodium hydroxide (97 %), sodium nitrite (97 %), sulfuric acid (98 %) and xylose were acquired from Carlo Erba Reagents (Sabadell, Barcelona, Spain). 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) (98 %), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (97 %) and 2,4,6-tripyridyl-s-triazine (TPTZ) (98 %) were supplied by Alfa Aesar Chemicals (Ward Hill, Massachusetts, USA). Aluminum chloride, hydrochloric acid (37 %) and sodium carbonate were obtained from Scharlau (Sentmenat, Barcelona, Spain). Folin Ciocalteu and sodium acetate were acquired from VWR International (Sentmenat, Barcelona, Spain). Gallic acid (98 %) and Rutin were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). 5-Hydroxymethylfurfural (HMF) (98 %), Furan-2-Metanal, levulinic acid (98 %) and Trolox (97 %) was purchased from Acros Organics (Gell-Belgium). Arabinose was obtained from the commercial house ITV Reagents (Spain). Reagents are pure if not specified.

2.2. Feedstock and chemical characterization

Hop pruning (HP) (*Humulus lupulus* L.) studied, of *nugget* variety, was supplied by a local producer (LUTEGA-Lúpulo Tecnología de Galicia, Sociedad Cooperativa Gallega) in Mabegondo (A Coruña, NW Spain) in 2020. HP was dried at room temperature to reach a moisture content lower than 10 %, crushed to a diameter $\leq$ 1 mm, and then stored in dry and dark place.

Chemical characterization was carried out, where the moisture and ashes of the raw material were studied by the following procedures (Sluiter et al., 2008a, 2008b). The raw material was submitted to Soxhlet extraction to determine the ethanol extractives (Sluiter et al., 2008d). The solid obtained was analyzed by a quantitative acid hydrolysis to determine its polysaccharide content (Sluiter et al., 2008c). This method is divided into a first stage of hydrolysis with H_2SO_4 at 72 % (30 °C for 1 h), which breaks down polysaccharides to oligomers, and a second stage with the same acid at 4 % (121 °C for 1 h), which breaks down oligomers to monomers. The remaining solid was determined as Klason lignin, while the liquid phase was further analyzed by liquid chromatography (Agilent 1200 series with a Rezex ROA-Organic acid H^+ column (Phenomenex) at 60 °C, using a mobile phase with H_2SO_4 at 0.03 M, at a flow rate of 0.6 mL/min and a refractive index detector at 40 °C). The uronic acids (determined as equivalents in galacturonic acid) were quantified by colorimetric process for the liquid phase after quantitative acid hydrolysis (Blumenkrantz and Asboe-Hansen, 1973).

In addition, protein content of HP was obtained by Kjeldahl method and calculated based on the nitrogen content converted according to the lignocellulosic materials factor (6.25) (A. Sluiter et al., 2010). The assays were performed in triplicate.

2.3. Hop pruning processing

2.3.1. Strategy based on mild water extraction followed by diluted acid pretreatment

For sequential fractionation of HP, raw material was firstly subjected to water extraction at 110 °C for the solubilization of non-structural components (such as phenolic compounds), using a solid-liquid ratio of 15 (g/g), and residence time of 30 or 60 min in an autoclave based on previous experiments (data not shown). Solid and liquid fractions were separated by filtration, and solid fraction was washed with water until neutral pH. This fraction was dried at room temperature to determine the solid yield, polysaccharides content and for further treatment. Liquid phase was analyzed for the quantification of oligomers, monomers (see Section 2.5), phenolic compounds, flavonoids (see Section 2.6) and antioxidant capacity (see Section 2.7).

Afterwards, a diluted acid treatment was applied on the HP solid from the previous water extraction. For that, the phenolic-free HP solid was mixed with a desired hydrochloric acid concentration and heated up to a desired temperature for a set residence time. After cooled down, solid and liquid fractions were separated by filtration, washing the solid thoroughly with deionized water until neutral pH. The solid was analyzed for polysaccharides determination (see Section 2.2) and the liquid fraction for monomer/oligomers determination (see Section 2.5).

Optimization of dilute acid pretreatment was carried out by a Box-Behnken design was developed, evaluating temperature (110–130 °C), time (15–60 min) and hydrochloric acid concentration (0.25–1.75 % w/w) as independent variables, based on previous experiments (data not shown). These variables were studied by response surface methodology (RSM) with a triplicate in the central point, and fitted to a second-order polynomial:

$$y_j = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum\sum_{i<j=1}^{3} \beta_{ij} x_i x_j + \sum_{i=1}^{3} \beta_{ii} x_i^2$$

Where y_j reflects the dependent variables (j=1–2), β_0, β_i, β_{ij}, and β_{ii} reflects the regression coefficients obtained from the experimental results by means of the least-squares methodology, and x_i and x_j reflect the independent variables (dimensionless and normalized), that can range −1 to 1. Microsoft Excel's Data Analysis Add-In (USA) was used for the fitting of experimental data by a regression analysis. The appropriateness of the model was assessed by means of the absence of fit, the coefficient of determination (R^2) and the F-test value evaluated by the analysis of variance.

To reach the maximum values of the response variables concurrently, a multi-response surface optimization was evaluated, obtaining the optimized conditions by means of the software STATGRAPHICS Centurion XVI (version 16.1.11.). Model validation was carried out via performing the experiments at those optimized conditions (experimental results) and comparing them with the predicted results.

2.3.2. Strategy based on microwave-autohydrolysis

This strategy, based on the use of microwave-assisted autohydrolysis, allows the simultaneous solubilization of phenolic compounds and hemicelluloses. HP and water were introduced in G30 reaction vials in a ratio of 15 g water/g HP in G30 for microwave (Monowave 450 from Anton Paar, Graz, Austria). The effect of temperature and time in the solubilization of phenolics compounds and hemicelluloses was evaluated. The temperatures employed were 170 °C (5–20 min), 180 °C (5–20 min), 190 °C (5–12 min), and 200 °C (2–10 min), based on previous experiments (data not shown). For all assays, the stirring speed was fixed at 900 rpm, the target temperature was reached in 5 min (being the power of the equipment internally selected to satisfy this requirement, but never surpassing 300 W) and the cooling rate was set in 5 min.

After treatment was ended, the solid and liquid fractions from HP were recovered by filtration. Solid and liquid phases were analyzed for polysaccharides' determination (see Section 2.2) and oligomers/monomers (see Section 2.5), phenolics, flavonoids (see Section 2.6) and antioxidant capacity (see Section 2.7), respectively.

2.4. Chemical characterization of the liquid fractions resulting from the hop pruning processing

Liquid phases obtained after the pretreatments were analyzed for monomers quantification by direct injection in HPLC, and for oligomers quantification after an acid posthydrolysis (H_2SO_4 at 4 % w/w, 121 °C for 20 min) and subsequent injection in HPLC. The oligomers quantifications were assayed in three replicates.

2.5. Total phenolic (TPC) and total flavonoids content (TFC)

The liquid fractions obtained from the mild water extraction (described in Section 2.3.1) and after microwave treatments were submitted to analysis for the quantitation of phenolic compounds and flavonoids.

The colorimetric method based on Singleton et al. (1999) was employed to determine the total phenolic content (TPC). For this purpose, Folin Ciocalteu reagent diluted 1:10 (v/v) was added over the liquid phase in a ratio of 500 μL of liquid phase to 2500 μL of reagent, vortexed before adding 2000 μL of Na_2CO_3 (concentration of 75 mg/mL). Samples were incubated in dark for 1 h and measured for absorbance at 760 nm. The TPC results were expressed in mg gallic acid equivalents (GAE)/g raw HP on dry basis.

The method of Zhishen et al. (1999) was used to measure the total flavonoids content (TFC). To 1000 μL of the liquid phase, 300 μL of 5 % sodium nitrite was added, vortexed and incubated in the dark for 5 min, 300 μL of 10 % aluminum chloride was added, again, vortexed and incubated for 6 min in the dark, and the reaction was neutralized by adding 2000 μL of 1 N sodium hydroxide solution. After stirred and incubated for 5 min, the absorbance was determined at 510 nm. The TFC results were expressed in mg rutin equivalents (RE)/g raw HP on dry basis. TPC and TFC assays were carried out in three replicates.

2.6. Antioxidant capacity assays

The liquid fractions obtained from the mild water extraction (described in Section 2.3.1) and after microwave treatments were subjected to analysis for antioxidant capacity. Antioxidant capacity was determined by three colorimetric methods described by Gullón et al. (2017). The reduction of the radical form of 2,2-diphenyl-1-picrylhydrazyl (DPPH), was performed following the method by Brand-Williams et al. (1995) and is based on the reduction, by means of an antioxidant, to a non-radical form of DPPH, which makes the compound colorless. Reduction of the radical form of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), the method by Re et al. (1999), is based on the discoloration of said compound, and ferric ion antioxidant reducing power (FRAP), this method evaluates the reducing power of the extracts based on the transformation of the ferric tripyridyltriazine complex (FeIII-TPTZ) present in the FRAP solution to its ferrous variety (Benzie and Strain, 1999). For three methods, trolox was selected as standard and results were expressed in mg Trolox equivalents (TE)/g raw HP on dry basis. The assays were carried out in triplicate.

2.7. Profile of phenolic compounds from hop pruning by HPLC-ESI

A liquid:liquid extraction was used to separate phenolic compounds from HP extracts after mild water extraction (Section 2.3) and microwave-assisted autohydrolysis (Section 2.4), in ratio 1:1 (v/v) with ethyl acetate. This mixture was poured in decanting flask to separate after agitation at room temperature for 15 min. The ethyl acetate part was recuperated, and the aqueous fraction was decantated twice more with ethyl acetate. The ethyl acetate was completely removed by a rotatory evaporator set at 40 °C.

Samples were resuspended in methanol and injected in an Agilent 1260 series HPLC (Palo Alto, CA, USA) with AB SCIEX Triple Quad 3500 detector (AB Sciex, Foster City, CA, USA) and equipped with an electrospray source of ionization (ESI) to determine he phenolic compounds. The volume of injection was of 5 μL, with two mobile phases formed by formic acid 0.1 % and acetonitrile with formic acid 0.1 % and with a flow of 0.3 mL/min in a Luna C18 column (Phenomenex). To convert the samples components to ions, a positive/negative source of ionization was used (turbo V™) in combination with nitrogen (used as nebulizer and collision gas). Additionally, the data was obtained by multiple reaction monitoring (MRM).

2.8. Statistical analysis

TPC, TFC, DPPH, ABTS, FRAP and xylooligosaccharides data (measured as mean value ± standard deviation), were subjected to statistical analysis by the software R (version 4.1.0). In addition, a one-way ANOVA with a posterior Tukey's test was made to determine the statistical differences among samples, this difference was considered significant when $p < 0.05$.

3. Results and discussion

3.1. Raw material characterization

Hop pruning (HP) was chemically characterized and the results are as follows (expressed in g per 100 g of dry hop pruning): glucan, 35.02 ± 0.18 xylan, 11.49 ± 0.11; arabinan, 1.22 ± 0.06; acetyl groups, 2.18 ± 0.03; Klason lignin, 17.42 ± 0.50; ethanolic extractives, 14.95 ± 0.52; uronic acids (measured as galacturonic acid equivalents), 8.11 ± 1.85; ashes, 3.62 ± 0.13; proteins, 3.32 ± 0.79, others (by difference), 2.67.

Almost the 97 % of components from HP were identified, highlighting the presence of polysaccharides (close to a 50 % of the weight), Klason lignin (17 %) and uronic acids (8 %), whereas the ashes and proteins appeared in lower proportions. The obtained results are analogous to those obtained for similar agro-industrial wastes such as rape straw with 29.1, 12.6 and 18.2 % of glucan, xylan, and lignin, respectively (Li et al., 2023) or wheat straw with 57.7 and 18.6 % of polysaccharides and lignin, respectively (Labauze et al., 2022).

3.2. Mild water extraction followed by diluted acid pretreatment

3.2.1. Mild water extraction

The first strategy to valorize hop pruning evaluated the recovery of phenolic compounds using mild water extraction and a subsequent dilute acid pretreatment to solubilize the hemicelluloses, retaining the glucan in the solid phase. The mild water extraction was performed at 110 °C for 30 or 60 min based on previous experiments (data not shown) and bibliography (Gómez-Cruz et al., 2022), and the main results can be consulted in Table 1. When looking at the solid yield (which can give information regarding the solubilized and non-solubilized fractions), not much difference can be observed. In that sense, the solubilization was very similar on both extractions. Similar values were also observed regarding the glucan, hemicelluloses, and lignin (retaining more than 90 % in all cases). On the other hand, small amounts of carbohydrates were found in the liquid phase, highlighting glucan and xylan derivatives, with an average of 1.69 g of glucose+glucooligosaccharides/L and 1.62 g of xylose+xylooligosaccharides/L.

Regarding the phenolic content and antioxidant activity, analogous values of TPC, TFC, DPPH, ABTS and FRAP were found for both conditions.

The obtained results can be positively compared to others in the bibliography. For instance, Afonso et al. (2022) reached a TPC up to 20 mg GAE/g extract when mixing the grounded hop cone with boiling distilled water for 5 min, and comparatively around 179 mg GAE/g extract in the current work was extracted at 110 °C for 30 min. Additionally, Fischer et al. (2023) applied subcritical CO_2 extraction of hop, reaching TPC value of up to 82.13 mg GAE/g extract and TFC values of up to 54.24 mg quercetin equivalent/g extract, whereas the mild water extraction applied to hop pruning (in the current study) enabled the obtainment of around 220 mg RE/g extract. On the other hand, Maietti et al. (2017) studied the antioxidant activity of young shoots of wild hop extracted by methanol, obtaining up to 0.50 mg TE/g measured by DPPH radical scavenging, whereas mild water extraction selected in this work enable to reach about 6–7 mg TE/g.

Table 1
Chemical composition of solid and liquid phases after mild water extraction of hop pruning.

T (°C)	110	110
t (min)	30	60
Solid yield (%)	79.87	80.18
SOLID PHASE COMPOSITION (g/100 g pretreated solid)		
Glucan	40.3 ± 0.57	37.27 ± 1.51
Hemicelluloses	18.25 ± 0.63	18.29 ± 0.45
Lignin	23.18 ± 0.55	24.74 ± 0.56
LIQUID PHASE COMPOSITION		
Chemical composition (g/L)		
Glucose and gluocooligosaccharides	1.72 ± 0.01	1.65 ± 0.03
Xylose and xylooligosaccharides	1.60 ± 0.04	1.64 ± 0.07
Arabinose and arabinooligosaccharides	0.25 ± 0.01	0.27 ± 0.04
Acetic acid and acetyl groups	0.18 ± 0.01	0.21 ± 0.00
Furans	0.00 ± 0.00	0.00 ± 0.00
Phenolics content		
TPC (mg GAE/g initial HP)	33.86 ± 1.15	29.98 ± 0.57
TFC (mg RE/g initial HP)	42.50 ± 1.75	41.17 ± 1.98
Antioxidant activity		
DPPH (mg TE/g initial HP)	6.09 ± 1.34	6.91 ± 0.21
ABTS (mg TE/g initial HP)	43.56 ± 2.85	38.79 ± 1.02
FRAP (mg TE/g initial HP)	29.79 ± 1.68	29.2 ± 0.79

GAE-gallic acid equivalents, RE-rutin equivalents, TE-Trolox equivalents.

Taking into account the abovementioned, any of the conditions employed would be enough to solubilize high amounts of antioxidant phenolics, hence, 110 °C and 30 min was the condition selected as first step of this strategy for hop pruning valorization.

3.2.2. Dilute acid pretreatment: Box Behnken design

Hop pruning extracted by mild water extraction at 110 °C for 30 min was submitted to dilute acid pretreatment varying the acid concentration of hydrochloric acid (0.25–1.75 % w/w), temperature (110–130 °C) and time (15–60 min) using a Box-Behnken design. The chosen independent variables and their ranges were selected from preliminary studies (data not shown). The experimental conditions and main results (glucan content of the processed solid and xylose+xylooligosaccharides in the liquid phase) can be consulted in Table 2, while the complete characterization of solid and liquid fractions is exhibited in Supplementary material. Additionally, Fig. 1 shows the response surface of both glucan and xylose+xylooligosaccharides regarding acid concentration and time.

As can be observed, the glucan content was increased when employing harsher conditions of pretreatment, i.e., higher acid concentration and larger residence time, up to a maximum value of 54.94 g glucan/100 g of processed HP. This can be related to the solubilization of other components of the HP, especially hemicelluloses, increasing the glucan content. However, harsher conditions also were related to a lower glucan recovery (regarding initial value of glucan), coming from 95 % for the experiment 1–76 % for experiment 10.

A similar trend was observed for the solubilization of xylose and xylooligosaccharides (see Table 2). Close to 17 g/L, especially in the form of xylose, were obtained at experiment 11 (close to 85 % of recovery regarding xylan from the raw material), whereas only 2.41 g/L, mostly in the form of xylooligosaccharides, were found at the mildest condition (experiment 1, around 14 % of recovery regarding xylan from

Table 2
Experimental conditions (expressed as dimensional and dimensionless (in brackets) independent variables) assessed, and results obtained for dependent variables y_1 (g glucan/100 g of processed HP) and y_2 (g xylose+xylooligosaccharides /L). Standard deviation was lower than 5 %.

Run	Independent Variables			Dependent Variables	
	HCl (% w/w)-x_1	T (°C)-x_2	t (min)-x_3	Gn (g/100 g)-y_1	X+XO (g/L)-y_2
1	0.25 (-1)	110 (-1)	37.5 (0)	43.99	2.41
2	0.25 (-1)	120 (0)	15 (-1)	43.45	2.77
3	0.25 (-1)	120 (0)	60 (1)	43.53	5.42
4	0.25 (-1)	130 (1)	37.5 (0)	43.95	6.58
5	1.00 (0)	110 (-1)	15 (-1)	45.46	8.10
6	1.00 (0)	110 (-1)	60 (1)	47.35	11.71
7	1.00 (0)	120 (0)	37.5 (0)	49.64	14.74
8	1.00 (0)	120 (0)	37.5 (0)	47.61	14.16
9	1.00 (0)	120 (0)	37.5 (0)	49.16	14.24
10	1.00 (0)	130 (1)	15 (-1)	49.27	14.74
11	1.75 (1)	130 (1)	60 (1)	54.24	16.98
12	1.75 (1)	110 (-1)	37.5 (0)	50.54	13.39
13	1.75 (1)	120 (0)	15 (-1)	48.10	12.18
14	1.75 (1)	120 (0)	60 (1)	53.96	16.10
15	1.75 (1)	130 (1)	37.5 (0)	54.94	15.65

the raw material). This effect can also be observed in Fig. 1**b**, with an increase of concentration at higher acid concentration and larger residence time. Similar observations were realized by Padilla-Rascón et al. (2020) when studying the solubilization of carbohydrates of olive stones varying temperature (120–130 °C), sulfuric acid concentration (7.5–17.5 g acid/100 g olive stones) and solid loading (20–60 % w/v), or by Deshavath et al. (2017) when studying the dilute sulfuric acid (0.01–0.4 M) pretreatment on sorghum stalks.

Table 3 displays the regression coefficients obtained for each model considering a second-degree polynomial, besides the statistical significance and correlation parameters. In both cases, the significance level was higher than 99.5 %, the R^2 varied between 0.962 and 0.988, and the values of F were high. In this context, all these parameters implied a good adequacy and fitting of the data to the model. Moreover, both dependent variables studied reflected a positive influence by acid concentration (b_1), temperature (b_2) and time (b_3). Concerning the glucan, it was also significantly affected by the correlation between acid concentration and time (b_1b_3), whereas the quadratic effect of acid concentration (b_1b_1) affected negatively to the concentration of xylose+xylooligosaccharides.

With the main aim of maximizing the recuperation of components from HP, a multiple response optimization was performed to estimate the conditions that would maximize both the recovery of glucan in the spent solid and the obtainment of xylose+xylooligosaccharides in the liquid phase. Those conditions were 1.69 % (w/w) of hydrochloric acid, at 123.5 °C for 59.6 min, with predicted results of 55.47 g glucan/100 g pretreated solid and 17.05 g xylose+xylooligosaccharides/L. Three experiments using these conditions were conducted to verify the suitability, attaining the following experimental values: 53.25 ± 0.79 47 g glucan/100 g pretreated solid and 16.38 ±0.57 17.05 g xylose+xylooligosaccharides/L (the complete chemical characterization of solid and liquid phases can be consulted in Supplementary material). The good correlation between both, empirical and predicted, values is verified, with an error of around 4 % for both variables studied. In this sense, the obtained results indicated a good validation of the model to optimize the recovery of glucan and xylose+xylooligosaccharides from HP.

3.3. *Microwave-assisted autohydrolysis*

As an alternative to the sequential extraction of phenolic compounds by mild water extraction and carbohydrates by dilute acid pretreatment, microwave-assisted autohydrolysis of HP was carried out.

Temperatures between 170 and 200 °C and residence times between 2 and 20 min were evaluated, and the results for total phenolic and flavonoid content (TPC, TFC) and the antioxidant capacity measured by DPPH, ABTS and FRAP are presented in Fig. 2. Firstly, TPC values (see Fig. 2**a**) varied in a narrow range of 24.66–31.74 mg GAE/g initial HP. The conditions of 170 °C for 10 min and 200 °C for 5 min presented the

Table 3
Regression coefficients and statistical parameters determining the correlation and significance of the model.

Coefficients	Gn- y_1	X+XO- y_2
b_0	48.81[a]	14.38[a]
b_1	4.08[a]	5.02[a]
b_2	1.88[a]	2.29[a]
b_3	1.60[b]	1.55[a]
b_1b_2	1.11	-0.48
b_1b_3	1.44[c]	0.31
b_2b_3	0.77	-0.34
b_1b_1	-1.14	-4.31[a]
b_2b_2	0.69	-0.55
b_3b_3	-0.41	-0.94[c]
R^2	0.962	0.988
F	14.01	46.37
Significance level (%)	99.52	99.97

[a] Significant coefficients at the 99 % confidence level.
[b] Significant coefficients at the 95 % confidence level.
[c] Significant coefficients at the 90 % confidence level.

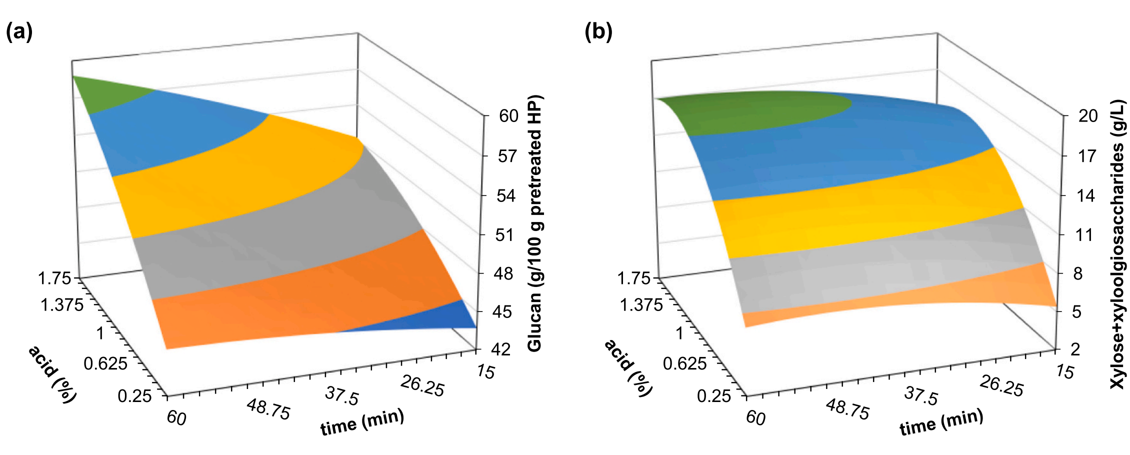

Fig. 1. Response surfaces for (a) glucan (g/100 g pretreated HP), and (b) xylose+xylooligosaccharides (g/L) as a function of acid concentration (%) and time (min), fixing the variable temperature at 130 °C.

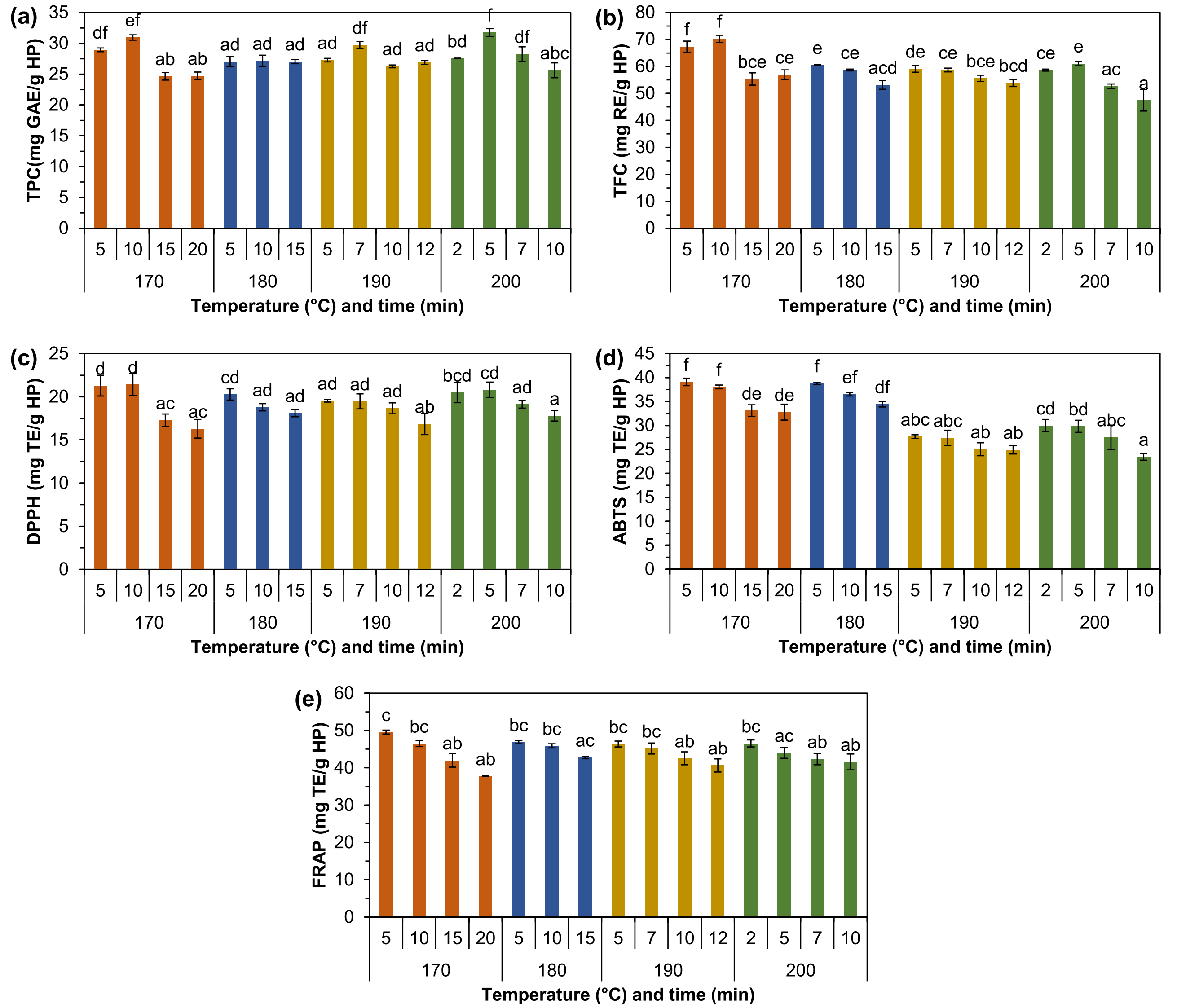

Fig. 2. Results regarding (a) TPC (mg GAE/g HP), (b) TFC (mg RE/g HP), (c) DPPH, (d) ABTS, and FRAP (mg TE/g HP) of the liquid phase after microwave-assisted autohydrolysis of hop pruning. Different letters represent significant differences with $p<0.05$.

highest value, being significantly different when comparing to the other conditions. These values are in the range of those obtained by Del-Castillo-Llamosas et al. (2023) when pretreating avocado seed by microwave-assisted autohydrolysis between 170 and 200 °C for 5 min, reaching between 13.23 and 25.96 mg GAE/g avocado seed. However, higher TPC was observed after ethanolic microwave extraction of avocado seed, reaching up to 89.39 mg GAE/g (Weremfo et al., 2020).

Regarding the TFC (see Fig. 2**b**), the results showed a decreasing tendency when employing harsher conditions, i.e., higher temperatures and larger residence times. In this sense, the mildest conditions (170 °C during 5 and 10 min) were found to enable significantly higher TFC values, up to 70.24 mg RE/g initial HP, whereas the harshest conditions (200 °C during 7 and 10 min) the TFC value was as low as 47.49 mg RE/g initial HP. This tendency may be caused owing to the more labile nature of flavonoids to high temperatures and is confirmed by other authors such as Del-Castillo-Llamosas et al. (2023). Moreover, the obtained data can be positively compared to those obtained after low transition temperature mixture extraction with glycerol and ammonium acetate (3:1) on spent filter coffee or eggplant peels (12.48–24.69 mg RE/g) (Manousaki et al., 2016).

Regarding the antioxidant capacity of the liquid phase (see Figs. 2**c**, 2**d** and 2**e**), it was evaluated by three different complementary methods. DPPH values varied between 16.00 and 21.41 mg TE/g HP, ABTS values between 23.46 and 39.08 mg TE/g HP, and FRAP values between 37.74-y 49.57 mg TE/g HP. The highest value measured by DPPH scavenging assay was for the liquid phase extracted at 170 °C for 10 min, although not being significantly different ($p>0.05$) when compared to other extracts obtained at higher temperatures but short residence times. Moreover, the liquid phase obtained at the lowest temperature and short residence time resulted in higher ABTS values, although being significantly similar to the values obtained at 180 °C at residence times from 5 to 15 min. Nevertheless, higher temperatures of reaction significantly decreased the antioxidant capacity measured by the ABTS scavenging assay. Finally, FRAP tendency is similar to that obtained for DPPH assay, reaching significantly similar values for liquid phases obtained at any temperature but at short residence times. The results can be positively compared to the ABTS results obtained by Bassani et al. (2020) when subjecting wheat straw to autohydrolysis at 190 °C for 15 min, reaching 23.50 mg TE/g, whereas in the current work, the highest value reached 38.77 mg TE/g. Comparatively, FRAP value obtained from avocado seed extract using methanolic and ethanolic (50 % v/v) solutions for 24 h at 4 °C resulted in 79.24–109.85 mg TE/g, that were higher than the obtained in the current work (Segovia et al., 2018).

Additionally, the monomeric and oligomeric composition of the liquid phase was also analyzed, and the results are displayed in Table 4.

Regardless the conditions employed, the main component in the liquid phase was represented by xylooligosaccharides, varying from 1.10 to 4.75 g/L. The concentration of this oligomers increased at harsher reaction conditions, i.e., higher temperatures and larger residence times, although triggering the formation of xylose at harsher conditions as well. In this context, 200 °C for 5 min was the condition conducing to the highest xylooligosaccharides concentration being significantly different ($p<0.05$). Moreover, the condition led to a xylose+xylooligosaccharides concentration of 5.50 g/L, corresponding to a recovery of 63 % regarding initial xylan. Similarly, Pino et al. (2019) selected 190 °C for 10 min as optimal condition to obtain the highest xylooligosaccharides content from agave bagasse, up to 8.37 g xylooligosaccharides/L, whilst harsher conditions led to its degradation. Dávila et al. (2021) arrived with a similar conclusion, reporting that shorter experimental times at higher temperatures (180 °C for 20 min) were necessary to obtain a higher oligosaccharide content when using microwave-assisted autohydrolysis on vine shoots.

The other oligomeric species varied in a narrow range: 0.26–0.67 g glucooligosaccharides/L, 0.00–0.69 g arabinooligosaccharides/L and 0.25–1.00 g acetyl groups linked to oligomers/L. Conversely, the glucose and arabinose presented a decreasing tendency when augmenting the temperature and residence time (probably causing the formation of degradation products such as hydroxymethylfurfural or furfural) whereas xylose and acetic acid tended to increase, reaching their maximal value at the harshest condition of 200 °C for 10 min (1.13 g xylose/L and 1.02 g acetic acid/L). A similar trend was observed by Jesus et al. (2017) when pretreating vine pruning by autohydrolysis at temperature between 180 and 200 °C and residence time from 10 to 90 min.

Table 5
Phytochemical profile of phenolic compounds from mild water extraction (at 110 °C for 30 min, LSR=15 g/g, conventional strategy) and (microwave-assisted autohydrolysis (at 200 °C for 5 min, LSR=15 g/g, alternative strategy). Standard deviation was lower than 5 %.

Compounds	Type	Conventional strategy (μg/g initial HP)	Alternative strategy (μg/g initial HP)
Catechin	Flavonoid	141.73	32.82
Epicatechin	Flavonoid	32.05	7.75
Luteolin	Flavonoid	0.30	0.14
Quercetin	Flavonoid	0.24	0.67
Rutin	Flavonoid	65.12	22.79
3,4-dihydroxibenzoic acid	Phenolic acid	11.51	95.25
4-hydroxybenzoic acid	Phenolic acid	5.30	10.49
Ferulic acid	Phenolic acid	2.60	1.53
Gallic acid	Phenolic acid	1.66	38.30
p-coumaric acid	Phenolic acid	38.87	68.96
Salicylic acid	Phenolic acid	0.07	2.01
Syringic acid	Phenolic acid	1.58	3.95
Vanillic acid	Phenolic acid	1.19	3.00
Syringaldehyde	Phenolic aldehyde	1.59	6.48
Vanillin	Phenolic aldehyde	7.93	20.43

3.4. Phytochemical profile of phenolic compounds from hop pruning processing

The phenolic compounds recovered after the mild water extraction and microwave-assisted autohydrolysis from HP, were thoroughly identified and quantified by HPLC-ESI, and the results are summed up in Table 5.

A total of 5 flavonoids, 8 phenolic acids and 2 phenolic aldehydes were identified. A similar quantity of phenolic compounds was found after both processing strategies (311.74 *vs* 314.52 μg/g HP). However, as may be expected due to its more labile nature, flavonoids tended to appear in higher amounts in the liquid phase of water extraction due to employing mildest conditions, representing the 75 % of the phenolic compounds identified. Moreover, the content in catechin and rutin can be highlighted, comprising the 46 and 21 % of all the phenolic compounds found in that liquid phase, respectively. Conversely, the harsher conditions employed in the microwave-assisted autohydrolysis provoked a higher concentration of phenolic acids and aldehydes, representing the formers around 71 % of the total of phenolics identified, and highlighting the presence of 3,4-dihydroxibenzoic acid and *p*-coumaric acid, comprising 30 and 22 % of all the phenolics in these extracts.

Other authors have found in hop and hop-derived products (such as

Table 4
Chemical characterization of the liquid phase after microwave-assisted autohydrolysis of hop pruning. Different letters represent significant differences with $p<0.05$ amongst same species of oligomers (GO, XO, ArO and AcO).

Runs	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Temperature (°C)	170	170	170	170	180	180	180	190	190	190	190	200	200	200	200
time (min)	5	10	15	20	5	10	15	5	7	10	12	2	5	7	10
LIQUID PHASE (g/L)															
Glucose	0.50	0.68	0.80	0.85	0.69	0.68	0.64	0.65	0.59	0.46	0.49	0.53	0.38	0.26	0.20
Xylose	0.58	0.56	0.62	0.71	0.61	0.54	0.60	0.58	0.67	0.62	0.77	0.58	0.75	0.71	1.13
Arabinose	0.58	0.76	0.90	0.96	0.81	0.75	0.66	0.80	0.80	0.63	0.66	0.64	0.54	0.39	0.00
Acetic acid	0.18	0.31	0.42	0.39	0.34	0.47	0.57	0.49	0.60	0.61	0.84	0.50	0.79	0.77	1.02
GO	0.67 ± 0.00^{h}	0.63 ± 0.02^{gh}	0.38 ± 0.02^{bcd}	0.41 ± 0.05^{cd}	0.62 ± 0.04^{gh}	0.33 ± 0.02^{ac}	0.26 ± 0.00^{a}	0.56 ± 0.02^{fg}	0.34 ± 0.02^{ac}	0.33 ± 0.02^{ac}	0.30 ± 0.02^{ab}	0.40 ± 0.02^{cd}	0.43 ± 0.02^{de}	0.46 ± 0.02^{de}	0.51 ± 0.01^{ef}
XO	1.10 ± 0.02^{a}	1.79 ± 0.01^{b}	1.92 ± 0.03^{b}	1.93 ± 0.05^{b}	2.52 ± 0.11^{c}	2.75 ± 0.05^{d}	3.16 ± 0.05^{e}	3.61 ± 0.03^{f}	3.75 ± 0.04^{fg}	3.98 ± 0.03^{h}	4.33 ± 0.02^{i}	3.86 ± 0.03^{gh}	4.75 ± 0.05^{j}	4.44 ± 0.01^{i}	4.37 ± 0.00^{i}
ArO	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.15 ± 0.13^{abd}	0.01 ± 0.06^{a}	0.08 ± 0.12^{ab}	0.10 ± 0.01^{abc}	0.06 ± 0.01^{ab}	0.18 ± 0.02^{abe}	0.29 ± 0.02^{cde}	0.25 ± 0.02^{e}	0.34 ± 0.00^{de}	0.34 ± 0.01^{f}	0.69 ± 0.02^{be}
AcO	0.25 ± 0.01^{a}	0.37 ± 0.00^{ad}	0.35 ± 0.19^{abc}	0.33 ± 0.05^{ab}	0.55 ± 0.04^{bd}	0.56 ± 0.02^{cde}	0.58 ± 0.00^{df}	0.77 ± 0.04^{efg}	0.80 ± 0.03^{fh}	0.77 ± 0.01^{efg}	0.77 ± 0.03^{efg}	0.80 ± 0.01^{fh}	1.00 ± 0.00^{h}	0.83 ± 0.02^{gh}	0.88 ± 0.01^{gh}

AcO-acetyl groups linked to oligomers, ArO-arabinooligosaccharides, GO-glucooligosaccharides, XO-xylooligosaccharides.

beer) the presence of flavonoids (Gribkova et al., 2022), such as catechin, epicatechin, (Di Domenico et al., 2020; McLaughlin et al., 2008), quercetin, and rutin (Di Domenico et al., 2020), or phenolic acids such as gallic acid, vanillic acid, ferulic acid, *p*-coumaric acid and syringic acid (Zhao et al., 2010).

The phenolic profile found for hop pruning, was also observed for other agro-industrial wastes such as wheat straw liquors after autohydrolysis at 190 °C for 0–180 min, with the presence of ferulic acid, *p*-coumaric acid, vanillic acid, syringaldehyde and vanillin (Bassani et al., 2020). Other example would be the phenolic compounds identified in olive wastes, comprising rutin, luteolin and quercetin especially in the leaves and olive mill wastewater (Ladhari et al., 2021). Conversely, 17 phenolic compounds were detected in brewery waste stream, and among them catechin, epicatechin, quercetin, gallic acid, catechin, 4-hydroxybenzoic acid, *p*-coumaric acid, or ferulic acid (Barbosa-Pereira et al., 2013).

3.5. Overall mass balance and strategies comparison

Mass flow schemes regarding the strategies carried out in this study are exhibited in Fig. 3. Strategy based on conventional methods consisted of a first mild water extraction, which enabled the recovery of 3.39 kg of phenolics, whereas up to 4.76 kg of monosaccharides and oligosaccharides and small amounts of acetic acid and acetyl groups linked to oligomers (0.21 kg) were also obtained in this process. After that first stage, the spent solid, still maintaining great part of the glucan, hemicelluloses, and lignin, was subjected to a dilute acid pretreatment using a solution of 1.69 % of HCl at 123.5 °C for 59.6 min (optimized conditions), obtaining 11.61 kg of xylan derivatives, mostly in oligomeric form (9.27 kg), also recovering other monomers and oligomers in lower proportions. Around 53 kg of HP were obtained after this biorefinery scheme using sequential treatments, mainly composed of cellulose (28.24 kg), lignin (19.27 kg) and a small proportion of hemicelluloses (2.66 kg).

Alternatively, the biorefinery strategy grounded on a microwave-assisted autohydrolysis single-step process enabled the recovery of a similar quantity of phenolics (3.17 kg) when compared to the process from other biorefinery scheme, obtaining in the same stream 7.15 kg of xylan derivatives (especially in oligomeric form, 6.18 kg), acetic acid and acetyl groups (2.03 kg), besides other monosaccharides and oligosaccharides (2.22 kg). After this process, around 86 kg of HP were recovered, showing higher glucan and hemicelluloses content and slightly lower values of lignin in comparison to other strategy.

These results indicate a lower solubilization than strategy based on mild water extraction followed by dilute acid pretreatment. Furthermore, a lesser formation of pseudo-lignin (that is formed via the blending of carbohydrates and lignin degradation products) was also observed. This provokes an increase in the weight of Klason lignin after the process (Sannigrahi et al., 2011), especially during the dilute acid pretreatment.

In this sense, this work entailed the evaluation of unexplored hop pruning potential to obtain xylan derivatives and antioxidant phenolics within two biorefinery schemes. Although as a general trend, the obtained results may seem similar, the phytochemical profile and the xylan derivatives amounts were very different, when using conventional or innovative treatments. Besides, although the energy consumption of the microwave heating may be lower (Dávila et al., 2021) when compared to conventionally heated reactors, the industrial scalability of the alternative technology is still a task to be surpassed. In addition, the assessment of environmental impact and techno-economic studies would validate the pros and cons of both strategies, and their viability in an industrial biorefinery scheme.

4. Conclusions

In this study, HP was evaluated as potential source of bioproducts using two biorefinery strategies: a conventional aqueous extraction followed by dilute acid treatment and an alternative microwave-assisted autohydrolysis. From 100 kg of HP, the recovery of phenolic compounds was similar regardless of the strategy (3.39 kg vs 3.17 kg). However, the

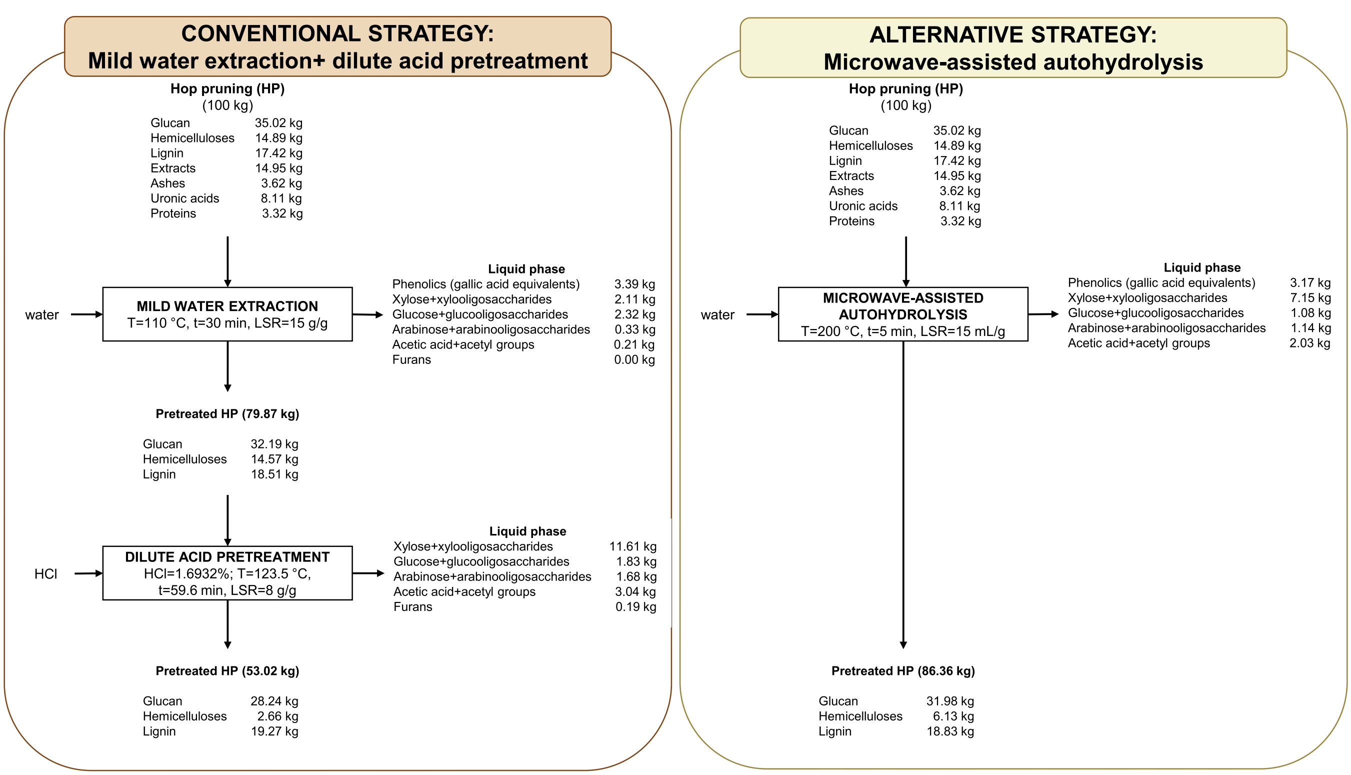

Fig. 3. Overall mass balance concerning both strategies outlined in this work for the valorization of 100 kg of hop pruning. Processing based on (a) mild water extraction followed by diluted acid pretreatment and (b) microwave-assisted autohydrolysis.

phytochemical profile (by HPLC-ESI) showed that the conventional aqueous extract presented higher content of flavonoids, while the extract obtained from microwave extraction had higher content of phenolic acids and aldehydes. Concerning the hemicellulose-derived saccharide recovery, the dilute acid pretreatment was more effective in obtaining higher amounts of these compounds (11.61 kg vs 7.15 kg) being xylooligosaccharides the main obtained component (9.27 kg vs 6.18 kg). Furthermore, both strategies also resulted in solids enriched in glucan and lignin, that me be used to produce several biobased products, with the aim of its integral valorization by the biorefineries philosophy.

CRediT authorship contribution statement

Aloia Romaní: Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization. **Beatriz Gullón:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization. **Fernando Rodríguez-Rebelo:** Writing – review & editing, Investigation, Formal analysis. **Pablo G. Del-Río:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Alexandre Rubira:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis.

Declaration of Competing Interest

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

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2024.119174.

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