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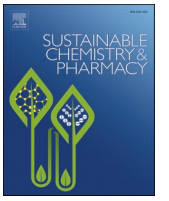

Bioconversion of vine shoots into renewable products using ohmic heating extraction and autohydrolysis

Diego Cardoza [a,b], Joana S. Gomes-Dias [c], Sara G. Pereira [c], Inmaculada Romero [a,b], Eulogio Castro [a,b,*], Cristina M.R. Rocha [c,d,**]

[a] Department of Chemical, Environmental and Materials Engineering, University of Jaén, 23071, Jaén, Spain
[b] Institute of Biorefineries Research (I3B), University of Jaén, 23071, Jaén, Spain
[c] CEB - Centre of Biological Engineering, University of Minho, Campus Gualtar, 4710-057, Braga, Portugal
[d] LABBELS - Associate Laboratory, Braga, Guimarães, Portugal

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ABSTRACT

The valorization of vine shoots (VS), a lignocellulosic waste from the viticulture industry, was evaluated using different alternative and sustainable extraction processes. An innovative biorefinery approach, sequentially combining ohmic heating extraction (OHE) and autohydrolysis (AH), was selected for the extraction of nutraceutical compounds and bioethanol production. Overall, the use of both technologies improved the extraction yield of target compounds, modulated their profiles (due to the affinity of each solvent used), and improved the saccharification of the residual biomass when compared to non-sequential processing, reinforcing the advantages of the novel approach proposed. An optimal combination of OHE (80 °C for 60 min) followed by AH at 200 °C for 30 min showed the highest recovery of phenolic compounds (31.3 mg GAE/g VS) and antioxidant activity (48.5 mg TE/g VS with FRAP methodology). Simultaneously, the recovery of glucose in the solid fraction (96.3 %) was promoted, making it suitable for simultaneous saccharification and fermentation, with ethanol yields of up to 61.26 % (achieving 20.57 g/L after 72 h). The novel cascading valorization process applied in this work maximizes the extraction of value-added products and aligns with circular economy principles by promoting the efficient use of this agro-industrial waste, reinforcing the importance of environmentally-friendly processing in the production of nutraceuticals, biofuels and bioproducts.

1. Introduction

The utilization of agro-industrial waste has gained relevance due to the search for sustainable alternatives for energy production and value-added products. In this context, the circular economy plays a crucial role as it promotes the reuse of waste within a continuous cycle, transforming them into new resources and minimizing waste generation (Merli et al., 2018). Among these, vine shoots (VS) stand out as a plentiful lignocellulosic source, especially in wine-producing regions. According to Food and Agriculture Organization of the United Nations, the world vineyard area was about 7.2 million hectares in 2023 (FAOSTAT, 2024). Agricultural

* Corresponding author. Department of Chemical, Environmental and Materials Engineering, University of Jaén, 23071, Jaén, Spain.
** Corresponding author. CEB - Centre of Biological Engineering, University of Minho, Campus Gualtar, 4710-057, Braga, Portugal.
E-mail addresses: ecastro@ujaen.es (E. Castro), cmrocha@ceb.uminho.pt (C.M.R. Rocha).

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practices derived from viticulture generate various types of waste, including VS. It is estimated that approximately 2 tons of VS are produced per hectare annually (David et al., 2020; Kokkinomagoulos et al., 2024; Muñoz-Realpe et al., 2025) which means a production of 14.4 million tons of VS in 2023. Therefore, their valorization is crucial for the circular economy and agro-industrial sustainability. Vine shoots, rich in cellulose, hemicellulose, and lignin, have significant potential for the extraction of phenolic compounds with antioxidant properties (Duarte et al., 2024) and the production of bioethanol (Pachón et al., 2020). Additionally, vine shoots are gaining attention as a versatile raw material for both biochemical and thermochemical valorization pathways. Their high holocellulose content—reaching up to 64 % depending on the grape variety—enables the recovery of fermentable sugars, while their lignin content supports their use in energy-dense solid biofuels such as pellets (Senila et al., 2020b). In fact, the structural composition of VS, which resembles that of hardwood, makes them suitable for a wide range of processes—from antioxidant extraction to the production of platform molecules like furfural, HMF, and bioethanol (Senila et al., 2020a).

For the extraction of phenolic compounds from raw materials such as VS, one innovative method explored is ohmic heating extraction (OHE). This process involves applying a direct electric current to the materials, generating uniform internal heating that facilitates the release of bioactive compounds, such as antioxidants, without damaging their structure (Maciel et al., 2024). OHE utilizes an electric current to generate temperature, whereas conventional extraction (CE) heats the sample using circulating hot water, without resorting to an electric field (Ferreira-Santos et al., 2024). Compared to conventional methods, OHE is more energy-efficient, reduces extraction times, and better preserves the quality of antioxidants, making it a promising option for the food and pharmaceutical industries (Markhali and Teixeira, 2024). As it is a mild process, only easily extractable compounds will be recovered, and it is not expected that it will significantly damage the strong VS lignocellulosic structure. Therefore, the biomass can be subjected to further pretreatments to exploit its lignocellulosic components. Efficient transformation of these components requires pretreatments that facilitate the breakdown of the complex lignocellulose structure, enabling the release of compounds of interest (Castro et al., 2023). One such pretreatment is autohydrolysis (AH), a process that uses water under controlled high temperature and pressure conditions to decompose the structure of lignocellulosic biomass. During this process, water acts as a reactive medium, releasing weak acids (such as acetic acid) present in the biomass itself, which in turn catalyze the degradation of hemicelluloses and other polymers. This hydrothermal treatment facilitates the release of sugars and other soluble compounds, such as oligosaccharides (Domínguez et al., 2020). The sugars released from this pretreatment can be transformed into economically valuable products through various treatments. However, AH is less effective at solubilizing cellulose, which remains contained in the solid fraction resulting from this pretreatment. One of the treatments that can solubilize this cellulose into glucose is enzymatic hydrolysis with appropriate enzymatic cocktails (Ávila et al., 2020). During the hydrolysis, enzymes break down complex polymers such as cellulose into simple sugars, facilitating their subsequent fermentation (de Souza et al., 2019). Simultaneous saccharification and fermentation (SSF) is another treatment that can solubilize cellulose using enzymes (Afedzi and Parakulsuksatid, 2023). In this case, and as the name suggests, saccharification by enzymes and fermentation occur simultaneously. This treatment offers the advantage of achieving higher efficiency, as the sugars released during hydrolysis are immediately fermented by microorganisms. This configuration prevents the accumulation of sugars in the medium and thus the inhibition of enzymes, and reducing processing time (Kumar, R. & Prakash, 2023). However, it is important to mention that SSF generally requires operating at suboptimal temperatures for cellulase enzymes, which could potentially affect the efficiency of enzymatic hydrolysis under certain conditions.

This study presents a significant approach on the valorization of lignocellulosic residues, specifically VS, and aims to systematically evaluate the sequential integration of OHE and AH to maximize both the yield of high-value phenolic antioxidants and bioethanol production from the same biomass, optimizing key parameters such as temperature and treatment time. For the best of authors' knowledge, this is the first work that sequentially combines ohmic heating extraction with autohydrolysis to extract high-value antioxidant compounds, followed by the recovery of glucose, which can subsequently be used for biofuel production through fermentation. This cascading biorefinery approach not only maximizes the recovery of valuable compounds but also promotes a zero-waste process, favouring the development of sustainable processes within the circular economy, offering an efficient and sustainable solution that integrates multiple biomass valorization stages.

2. Material and methods

2.1. Raw material

After harvesting the grapes, the VS were collected. In the laboratory, the raw material was air-dried at room temperature until a final moisture content of 10.33 % ± 0.12 %. Subsequently, the material was milled using a laboratory mill (Retsch, SM 100, Fisher Scientific S. L., Madrid, Spain) to a particle size of approximately 1 cm, homogenized into a single batch, and stored at room temperature until use. The material characterization was carried out following the methodology established by the National Renewable Energy Laboratory (NREL), while the sugar measurement was performed using high-performance liquid chromatography (HPLC). In both cases, the procedures were detailed in section 2.7.3, yielding the following chemical composition (% w/w, dry basis): cellulose (as glucose), 37.3 %; hemicellulose, 18.5 % (xylose 18.0 %, galactose 1.5 %, arabinose 0.8 %, mannose 0.5 %); lignin, 23.9 %; ash, 3.0 %; acetyl groups, 3.4 %; extractives, 9.0 % (Silva Rabelo et al., 2023).

2.2. Ohmic and conventional extraction

Two different extraction methods were employed: ohmic heating extraction (OHE) and conventional extraction (CE). Both methods employed a solid-liquid ratio of 1 g VS per 40 mL of 45 % (v/v) ethanol-water solution with an electrical conductivity of 2.3 mS/cm,

adjusted with 20 % of NaCl (w/v) and monitored using a conductivity meter (HCO 304, VWR™, USA) at room temperature.

Extractions were conducted at a constant temperature of 80 °C for 60 min (Jesus et al., 2020). Glass vessels with double walls and a water jacket were used, containing two stainless steel electrodes placed at a constant distance of 7 cm. Voltage was generated using a function generator (RIGOL DG1022Z) with a sinusoidal wave at a frequency of 25 kHz, connected to an amplifier (Peavey CS3000, Meridian, MS, USA). Temperature was controlled using a type K thermocouple (with a temperature precision of ±1 °C, Omega, 709, USA) placed at the geometric center of the sample volume, connected to a data logger (National Instruments, USB-9161, USA) operating with PicoLog software. Both OHE and CE experiments were performed in triplicate and average values and standard deviation are shown.

During OHE, an electric field of 10 V/cm was applied during the heating phase, reducing to 3.4 V/cm once the desired temperature was reached, to replicate the heating profile of the CE. Conversely, conventional extractions (0 V/cm) were performed as negative controls for electrical effects, using a circulating water bath thermostat (Witeg WCR-P8). A magnetic stirrer (2.0 cm in length) was employed to promote solution homogenization and simultaneously enhance heat transfer (Jesus et al., 2020). After extraction experiments, the slurries were filtered to separate the liquid extracts and the solid fractions.

2.3. Autohydrolysis

The resulting solids from OHE and CE were subjected to an autohydrolysis stage at different conditions. A pressurized reactor (Parr model 4848) was employed for the AH pretreatment. This reactor had a maximum capacity of 1.9 L and was equipped with a pressurized vessel for the samples, along with two internal stirrers. It also featured a cooling system consisting of circulating water, and a heating system with a metal heating jacket surrounding the sample vessel. Different combinations of temperature (180, 200 °C) and retention times (60, 45, 30 min) were tested (Fig. 1), to replicate the conditions reported in the literature as optimal for oligosaccharides and phenolic compounds recovery in three different approaches: i) direct extraction at 180 °C, 60 min, to mimic the time and temperature binomial applied in a first-stage autohydrolysis; ii) 200 °C, 30 min, to mimic the time and temperature binomial applied in a first-stage autohydrolysis and, iii) 200 °C, 45 min to achieve the same severity factor has the one reported for a two-step autohydrolysis treatment (Jesus et al., 2017). All tests were conducted with a solid-liquid ratio of 2.5 % (w/v) and an agitation speed of 150 rpm. The cooling process was performed by removing the heating jacket and connecting the cooling system allowing the sample to cool to room temperature (Jesus et al., 2017). All AH experiments were performed in duplicate and the average results and standard deviation are shown. AH was applied both independently and as part of a two-stage valorization process, where autohydrolysis was performed after OHE and CE.

2.4. Enzymatic hydrolysis

The solid fractions obtained from both extractions (OHE and CE), both alone and combined with AH, and AH alone were subjected to enzymatic hydrolysis tests. Initially, the pretreated solids were suspended in a total volume of 35 mL of a 0.1 M acetic acid/sodium acetate buffer (pH 4.8), with a solid loading of 5 %, in 250 mL Erlenmeyer flasks. Cellic® Ctec2 enzyme was added at a concentration of

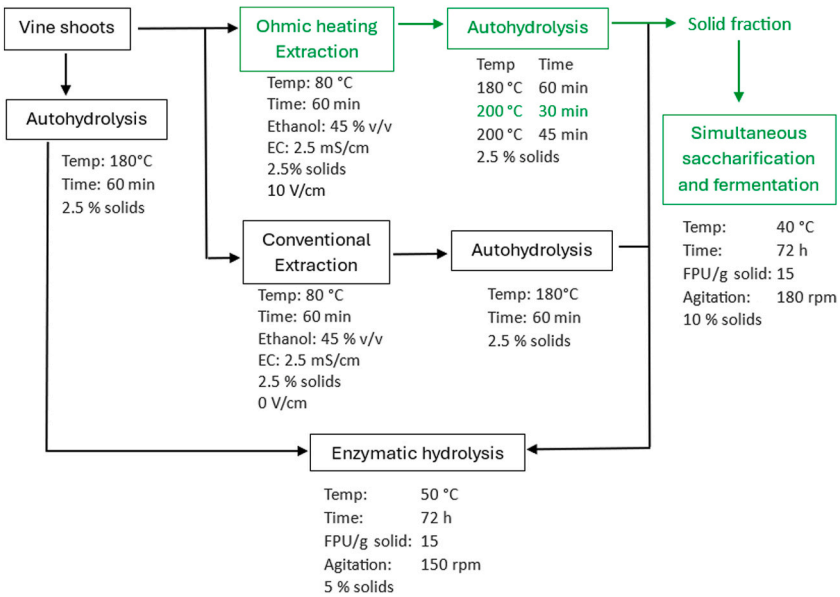

Fig. 1. Scheme summarizing the process configurations performed for the fractionation and valorization of vine shoots (the selected process configuration is highlighted in green)
EC: Electrical conductivity.

15 FPU/g solid, and the mixture was then incubated at 50 °C and 150 rpm for 72 h. The activity of Cellic® Ctec2 was determined to be 115 FPU/mL, as described by (Gomes et al., 2021). All experiments were carried out in triplicate, and the average results and standard deviation are shown.

The results are expressed as *Saccharification yield* (SY), calculated as g of glucose obtained by enzymatic hydrolysis per 100 g of glucose in the substrate; and *Enzymatic hydrolysis yield* (EHY), calculated as g of glucose obtained by enzymatic hydrolysis per 100 g of glucose in raw material.

2.5. Incubation of Saccharomyces cerevisiae

S. cerevisiae (Fermentis Ethanol Red, France) was employed in the SSF process of the solid material obtained from OHE followed by AH. The inoculum was prepared by cultivating lyophilized yeast in an orbital shaker at 30 °C and 150 rpm for 24 h. Inoculation took place in 100 mL Erlenmeyer flasks containing 25 mL of culture medium, with a needle inserted to facilitate the release of gases generated by the metabolic activity of the yeast. The culture medium comprised 30 g/L glucose, 5 g/L yeast extract, 2 g/L NH_4Cl, 1 g/L KH_2PO_4, and 0.3 g/L $MgSO_4{\cdot}7H_2O$. After 24 h, the yeast was re-inoculated into fresh Erlenmeyer flasks containing culture medium and incubated in an orbital shaker at 35 °C and 150 rpm for 16 h. Subsequently, the inoculum was ready for use in the SSF experiments.

2.6. Simultaneous saccharification and fermentation

The SSF tests were conducted under microaerobic conditions in 100 mL Erlenmeyer flasks with a total volume of 25 mL in citrate buffer (50 mM, pH 5.0), maintaining a solid-liquid ratio of 10 % (w/v). SSF assays were initiated by simultaneously introducing Cellic® CTec2 enzymes (15 FPU/g of dry substrate) and *S. cerevisiae* inoculum (4 % v/v), resulting in a cell addition of 0.25 g/L. These experiments were performed in an orbital shaker at 40 °C and 180 rpm for 72 h, with sampling conducted every 24 h. All SSF tests were performed in triplicate, and no additional salts or nutrients were incorporated into the culture medium. All equipment utilized was sterilized in an autoclave at 120 °C for 30 min. Inoculation and microbial handling were conducted within a laminar flow hood, previously sterilized by UV radiation for 10 min.

2.7. Analytical methods

2.7.1. Phenolic compounds' characterization

The total phenolic content (TPC) in liquid fractions was quantified using the Folin-Ciocalteu method employing 96-well plates. 20 μL of samples or standards were added, followed by 100 μL of Folin-Ciocalteu solution diluted in water (at a ratio of 1:10) and 80 μL of 7.5 % sodium carbonate. The reaction was incubated at 42 °C in the dark for 30 min, and the absorbance was measured at 750 nm using a microplate reader. The results were calculated using a standard curve and expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g) (Gomes et al., 2021).

The phenolic compounds' profile of the liquid fractions from both extractions and autohydrolysis were quantified using HPLC composed of a quaternary pump, an autosampler, and a diode array detector (DAD). Separation was carried out on an ACE 5C18 column (5 μm, 250 × 4.6 mm i.d.) at a flow rate of 1 mL min-1. The column temperature was maintained at 25 °C, with an injection volume of 20 μL. Mobile phase A consisted of water-formic acid (99.9:0.1; v/v), and mobile phase B of acetonitrile-formic acid (99.9:0.1; v/v). The DAD was set at wavelengths of 280, 310, and 520 nm while continuously collecting a UV/VIS spectrum from 200 to 600 nm. Chromatograms were analyzed using Shimadzu LabSolutions software (Gomes-Dias et al., 2024).

2.7.2. Determination of antioxidant activity

The antioxidant activity of liquid fractions generated at different process stages (OHE, CE, and AH) was determined. To perform the DPPH radical scavenging assay, 10 μL of the sample or Trolox standard were added to 190 μL of DPPH solution. The mixture was then incubated in darkness at room temperature for 30 min, after which the absorbance was measured at 520 nm using a microplate reader. The inhibition of DPPH free radicals was calculated using the following formula: % inhibition = (Abs blank - Abs sample)/Abs blank x 100 (Teixeira-Guedes et al., 2019). The antioxidant activity was calculated by interpolating the Trolox calibration curve, with results expressed in milligrams of Trolox equivalent per gram of dry weight (mg TE/g).

The determination of ABTS radical scavenging activity was conducted by adding 12 μL of sample or standard to the microplate, followed by 188 μL of ABTS solution. The plate was incubated for 30 min at room temperature, in the absence of light, and subsequently the absorbance was measured at 734 nm. The inhibition of ABTS radicals and the antioxidant activity were measured in the same manner as described earlier for DPPH (Domínguez-Perles et al., 2014).

The ferric reducing antioxidant power (FRAP) assay was conducted by adding 20 μL of sample followed by 280 μL of FRAP solution. The reaction was incubated at 37 °C in darkness for 30 min and then read at 593 nm (Teixeira-Guedes et al., 2019). Trolox was used as a standard, and the results were expressed in milligrams of Trolox equivalent per gram of dry weight (mg TE/g).

2.7.3. Other analytical determinations

The chemical composition of all solid fractions resulting from extraction and autohydrolysis was determined following the methodology of the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008). The sugars present in the liquid fractions from both extraction methods (OHE and CE), as well as those obtained from AH, were quantified using high-performance liquid chromatography (HPLC). For this purpose, an Agilent Technologies liquid chromatograph (model 1260) was used, equipped with a

refractive index detector and an ICSep ICE-COREGEL 87H3 column. The column was operated at 65 °C, using 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 mL/min. The concentration of glucose and xylose in the enzymatic hydrolyzates and the ethanol concentration produced during SSF were determined using the same methodology described for sugars in liquid fractions. All analytical determinations were performed in triplicate, and the average results are shown. The relative standard deviations were below 3 %.

2.8. Statistical analysis

All statistical analyses, including ANOVA, were conducted using R software (The R Project for Statistical Computing), version 4.3.2, with a significance level set at $p \leq 0.05$. Tukey's multiple comparisons test was also performed. Significant differences ($p < 0.05$) are indicated with superscript letters for each condition; means sharing the same letter do not differ significantly from each other.

3. Results and discussion

3.1. Recovery of antioxidant compounds from VS

The VS were initially subjected to a stage of extraction using two different heating systems (ohmic and conventional) followed by a second stage of pretreatment (autohydrolysis) to maximize their utilization. OHE was applied to the VS under controlled conditions of temperature (80 °C) and time (60 min), as indicated in Section 2.2. For comparison, a CE treatment was also performed using the same temperature and time settings. After the OHE and CE, a second pretreatment stage was carried out using AH at temperatures of 180–200 °C and times of 30, 45, and 60 min (Fig. 1). For comparative purposes, VS was subjected to AH pretreatment without previous extraction. The total phenolic content (TPC) of the liquid fractions obtained from OHE, CE, AH, and their combinations (extraction plus pretreatment) (Fig. 1) was measured to evaluate their impact on phenolic compounds' recovery. Additionally, FRAP, DPPH, and ABTS assays were performed to determine their antioxidant activity.

According to the statistical analysis, there were significant differences in the total phenolic compounds' recovery among the evaluated tests ($p \leq 0.05$) (Table 1). However, the test consisting of OHE followed by AH at 200 °C for 30 min showed the most significant highest antioxidant activity. Regarding FRAP assay, tests combining OHE with AH at 200 °C for 45 and 30 min respectively, yielded the highest and statistically significant mean values (52 ± 1.7 and 48.5 ± 3.4 mg TE/g VS). Additionally, the ABTS assay also exhibited statistical differences among the tests. The tests with the highest values, yet statistically equal, included OHE combined with AH at 200 °C for 45 min and OHE combined with AH at 180 °C for 60 min (38.8 ± 0.4 and 38.7 ± 0.4 mg TE/g VS), as well as AH at 180 °C for 60 min with prior CE (36.7 ± 2.5 mg TE/g VS).

The most significant differences in the DPPH assay were observed in tests combining OHE with AH and CE with AH (both AH conducted at 180 °C for 60 min) with values of 30.1 ± 0.2 and 27.9 ± 0.1 mg TE/g VS, respectively. It is evident that tests involving OHE followed by AH yielded the best results in all antioxidant-related analyses (TPC, FRAP, ABTS, and DPPH). Although in some cases there was no statistical difference between the tests with the highest values, it is crucial to consider energy efficiency and resource optimization. In this regard, the AH treatment for 30 min would be more advantageous in terms of energy efficiency, as it requires less time and provides statistically similar results to treatments of longer duration (45 and 60 min). Additionally, the combination of OHE with AH may present an advantage, as it can achieve higher phenolic compounds' concentrations. In a study using ultrasound-assisted extraction, TPC concentrations of 0.98 mg/g VS were achieved when methanol was used as solvent extraction, and 12 mg/g VS using levulinic acid (Duarte et al., 2024; Muñoz-Realpe et al., 2025). optimized ultrasound-assisted extraction of VS with ethanol (55 °C, 62 % amplitude, 6 min, 59 % ethanol) and reported 11 mg/g of TPC and 7.41 mg/g TE by FRAP. In this work, comparable results of TPC were obtained by OHE and CE, 7.8 mg/g VS and 9.5 mg/g VS, respectively. Likewise, an antioxidant activity of 12.6 and 7.7 mg TE/g VS was determined in these liquors, respectively. However, the OHE-AH combinations in this study achieved higher TPC concentrations (ranging from 24.3 to 31.3 mg/g VS), highlighting its efficiency in terms of extraction. Additionally, since OHE generates heat volumetrically, it rapidly reaches the desired temperature (10 min to achieve 80 °C), making it a more efficient option compared to CE which heats more slowly, as it relies on heat transfer from an external source to the interior of the material (30 min to reach 80 °C),

Table 1
Total phenolic content and antioxidant activity of liquid fractions obtained from different process configurations.

Extraction stage	T	t	Pretreatment stage	T	t	TPC	FRAP	ABTS	DPPH
	°C	min		°C	min	mg GAE/g VS	mg TE/g VS		
OHE	80	60	–	–	–	7.8 ± 0.1^{D}	12.6 ± 0.3^{D}	20.3 ± 0.1^{D}	16.5 ± 0.1^{D}
CE	80	60	–	–	–	9.5 ± 1.0^{D}	7.7 ± 0.1^{D}	26.6 ± 1.0^{C}	14.2 ± 1.4^{D}
CE	80	60	AH	180	60	23.8 ± 2.4^{BC}	24.3 ± 2.4^{C}	36.7 ± 2.5^{A}	27.9 ± 0.1^{AB}
OHE	80	60	AH	180	60	24.3 ± 0.3^{B}	34.0 ± 0.3^{B}	38.7 ± 0.4^{A}	30.1 ± 0.2^{A}
OHE	80	60	AH	200	30	31.3 ± 1.5^{A}	48.5 ± 3.4^{A}	36.1 ± 4.2^{A}	23.3 ± 1.4^{C}
OHE	80	60	AH	200	45	26.4 ± 0.1^{B}	52.0 ± 1.7^{A}	38.8 ± 0.4^{A}	25.8 ± 1.7^{BC}
–	–	–	AH	180	60	19.2 ± 1.1^{C}	6.3 ± 0.2^{D}	31.5 ± 0.0^{B}	24.6 ± 0.1^{BC}

T: temperature; t: time; OHE: ohmic heating extraction; CE: conventional extraction; AH: autohydrolysis; VS: vine shoots; means with a common letter are not significantly different ($p < 0.05$).

which also results in higher energy consumption (Pereira et al., 2021).

In the HPLC analysis, different phenolic acids were identified and evaluated, including gallic acid, protocatechuic acid, and p-coumaric acid, as well as important flavonoids such as catechin and taxifolin (Fig. 2). The most effective treatments were OHE followed by AH at 200 °C for 30 (OHE-AH2) and 45 min (OHE-AH3). Notably, OHE with AH at 200 °C for 30 min showed higher phenolic compounds' extraction yields and higher concentrations of phenolic acids and flavonoids in the extracts; for instance, gallic acid reached 0.78 mg/g VS (19.4 mg/L), surpassing OHE (0.21 mg/g VS; 5.2 mg/L) and OHE with AH at 200 °C for 45 min (0.40 mg/g VS; 9.9 mg/L), suggesting a high antioxidant capacity. In the case of flavonoids, applying AH after OHE favored their extraction. Notably, OHE with AH at 200 °C for 45 min (OHE-AH3) stood out with high extraction and high concentrations of catechin in the extracts (1.36 mg/g VS; 34.1 mg/L) and taxifolin (0.85 mg/g VS; 21.3 mg/L). Similarly, OHE with AH at 200 °C for 30 min (OHE-AH2) also achieved good results, 1.18 mg/g VS (29.6 mg/L) of catechin and 0.79 mg/g VS (19.9 mg/L) of taxifolin. In contrast, when VS was subjected to only an extraction step, regardless of whether it was OHE or CE, the presence of flavonoids was scarce. Thus, liquids from OHE and CE showed catechin concentrations lower than 2 mg/L, and the presence of taxifolin was not detected. This is consistent with a study that also used VS but employing a methanol-water mixture. After the AH process, compounds such as quercetin, kaempferol, and taxifolin were identified in the liquid fraction, which seems to indicate that this pretreatment favors the extraction of flavonoids (Difonzo et al., 2023).

Difonzo et al. (2023) also reported that higher concentrations of phenolic compounds' were directly related to higher antioxidant activity. In the study, VS extracts showed the highest total phenolic content (58.03 mg GAE/g) and the highest antioxidant activity measured by ABTS (16.43 mg TE/g) and DPPH (62.61 mg TE/g). Conversely, lower phenolic content in the shoots (28.76 mg GAE/g) led to lower antioxidant activity, with values of 5.96 mg TE/g for ABTS and 26.95 mg TE/g for DPPH. These results further emphasize the strong correlation between phenolic content and antioxidant capacity. In summary, OHE followed by AH at 200 °C for 30 and 45 min were the most effective process schemes in terms of phenolic compound' and flavonoid recovery. These results align with other studies suggesting that ohmic heating extraction enhances the yield of phenolic compound' extraction (Pereira et al., 2021).

The three different methods used differ in their fundamentals: metal (ferric) reducing power is the main factor FRAP, while organic radical scavenging is the main factor in ABTS (oxidation by peroxyl radicals) and DPPH (reduction by compounds); moreover, DPPH is known to underestimate the antioxidant capacity of aqueous extracts due to its low solubility and coagulation in this media, and can give falsely low readings for antioxidant capacity of samples containing eugenol and other similar phenols due to a reversible reaction; FRAP and ABTS have the disadvantage of not being proportionally correlated with the number of electrons a molecule can donate; FRAP and ABTS assessment of antioxidant activity of polyphenols such as caffeic acid, tannic acid, ferulic acid, ascorbic acid, and quercetin is slow and can take up to several hours, often leading to underestimation of the values ((Karadag et al., 2009)).

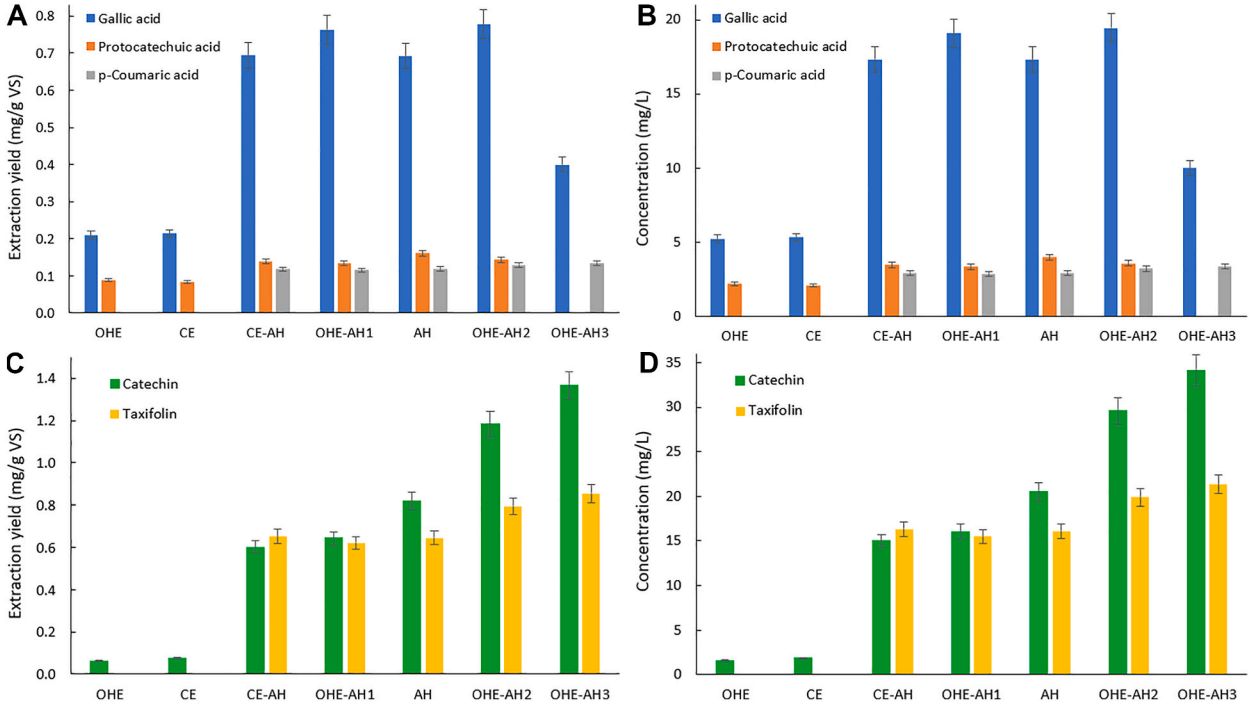

Fig. 2. Phenolic acids' extraction yield (a), concentration of phenolic acids (b), flavonoids extraction yield (c) and concentration of flavonoids (d), in the liquid fractions obtained from different extraction and pretreatment stages.
OHE: Ohmic heating extraction; CE: Conventional extraction; CE-AH: Conventional extraction with autohydrolysis at 180 °C for 60 min; OHE-AH1: Ohmic heating extraction with autohydrolysis at 180 °C for 60 min; AH: Autohydrolysis at 180 °C for 60 min; OHE-AH2: Ohmic heating extraction with autohydrolysis at 200 °C for 30 min; OHE-AH3: Ohmic heating extraction with autohydrolysis at 200 °C for 45 min.

These interferences with other fractions are a key parameter to have in consideration. Overall, it is observed that the extraction conditions with lower TPC yield are also the ones with lower antioxidant activity, regardless of the method used. However, on contrary to FRAP and ABTS, the highest DPPH antioxidant activity was not recorded in the fraction with the highest TPC yield. When it comes to the antioxidant activity of phenolic compounds by DPPH, an antagonist effect of the combination of kaempferol and resveratrol is reported, as well as an interference of resveratrol with other phenolics (Joshi et al., 2022). Allied to the information regarding the effect of eugenol, the authors believe that the phenolic profile of the samples can be the source of these differences.

3.2. Effect of extraction and autohydrolysis on sugar solubilization

Process configurations including only an extraction stage achieved a solid recovery higher than 90 % with both methods, which means that low biomass solubilization occurred due to the low severity of the extraction stage. However, when VS were pretreated with or without previous extraction, solid recovery ranged from 54 to 57 %. As for the glucose recovered in the solid fraction, the OHE followed by AH at 200 °C for 30 and 45 min, OHE and CE achieved the significantly highest recoveries ($p \leq 0.05$) among all tests evaluated (96.3 ± 2.1 %, 98.3 ± 2.5 %, 94.5 ± 2.4 % and 95.3 ± 2.8 %, respectively) (Table 2). AH without any prior extraction achieved a recovery of 85.6 ± 2.2 %. Regarding the liquid fraction, glucose recovery was 3.6 % with the OHE with AH at 200 °C for 30 min. Of the tests that achieved significantly higher recoveries ($p \leq 0.05$) were OHE with AH at 180 °C for 60 min and CE with AH at 180 °C for 60 min, with recoveries of 13.2 ± 0.5 % and 12.5 ± 0.6 % respectively (Table 3). This suggests that the combination of OHE with AH enables most of the glucan to remain in the solid fraction, which is beneficial because the cellulose present in the solid can later be utilized for the production of value-added products.

It is also observed that OHE alone was largely ineffective in solubilizing hemicellulose, with the majority remaining in the solid fraction (96.5 ± 2.9 %), and CE yielded similar results (93.6 ± 2.3 %) (Table 2). These results are consistent with the recoveries obtained for hemicellulosic sugars in the liquid fraction. The CE with AH at 180 °C for 60 min showed the highest efficiency, with a recovery of 44.4 ± 1.3, followed by OHE with AH at 180 °C for 60 min, with 42.1 ± 1.4 (Table 3). This suggests that methods involving AH are particularly effective for releasing hemicellulose into the liquid phase, enabling the recovery of sugars such as xylose and arabinose, which have multiple industrial applications. These sugars, key components of hemicellulose, are used in the production of biofuels, bioplastics, and high-value-added chemicals (Hero et al., 2018). This was to be expected, since cellulose has beta linkages, which are more difficult to break than the alpha linkages of hemicellulose, making it more difficult to solubilize. It is therefore normal for hemicellulose to have higher solubilization levels than cellulose. Similar results were obtained in another study where autohydrolysis was performed at temperatures ranging from 180 °C to 215 °C (Dávila et al., 2016). The study reports that at 200 °C, only 4.8 % of hemicellulosic sugars were solubilized. In contrast, in our study using OHE and autohydrolysis at 200 °C, 17.6 ± 0.9 % were solubilized at 30 min, and 11.1 ± 0.4 % at 45 min. In this study, only water was used to minimize environmental impact and promote more sustainable practices, as the use of catalysts like H_2SO_4 not only increases costs but also requires that the resulting waste liquids be properly managed and neutralized to prevent potential water contamination (Kumar, P. et al., 2009). Moreover, it should be considered that the VS have already been used in a previous stage to extract phenols, which means that it had undergone a first valorization, which makes these hemicellulose solubilization data quite positive as they would correspond to a second utilization of the same biomass. This is very beneficial in terms of valorization, as it allows the production of multiple products from a single raw material.

The two-stage valorization of VS proposed in this study yielded fractions that can be utilized for various purposes. In the first stage (OHE), the liquid fraction provides phenols, mainly phenolic acids, and other antioxidant compounds that are useful in pharmaceuticals, phenolic resins, cosmetics, and polymers (Cañas et al., 2024). Antioxidants are also used to extend the shelf life of food, protect the skin, and in plastics (Ji et al., 2024). In the second stage, the liquid fraction obtained through the AH pretreatment, phenols are still obtained (phenolic acids and flavonoids) but also hemicellulosic sugars, with the latter being employed as a thickening agent in foods,

Table 2
Characterization of solids and recovery of solids and sugars after different extraction and pretreatment stages.

Extraction stage	T	t	Pretreatment stage	T	t	Cel.	Hemi.	Lignin	Solid recovery	GRS	RHSS
	°C	min		°C	min	%					
OHE	80	60	–	–	–	24.7 ± 1.3^{C}	17.3 ± 0.8^{A}	35.6 ± 1.8^{BC}	92.6 ± 2.6^{A}	94.5 ± 2.4^{AB}	96.5 ± 2.9^{A}
CE	80	60	–	–	–	23.9 ± 0.7^{C}	16.8 ± 0.4^{A}	34.7 ± 1.7^{C}	92.7 ± 2.8^{A}	95.3 ± 2.8^{AB}	93.6 ± 2.3^{A}
CE	80	60	AH	180	60	36.3 ± 1.1^{B}	6.2 ± 0.3^{B}	36.2 ± 1.4^{BC}	57.3 ± 1.1^{B}	87.0 ± 1.7^{AB}	21.1 ± 1.7^{B}
OHE	80	60	AH	180	60	36.1 ± 1.7^{B}	6.6 ± 0.4^{B}	47.7 ± 1.9^{A}	57.8 ± 1.6^{B}	84.4 ± 1.9^{B}	22.0 ± 2.1^{B}
OHE	80	60	AH	200	30	43.2 ± 1.7^{AB}	BD^{C}	47.6 ± 2.2^{A}	55.1 ± 1.9^{B}	96.3 ± 2.1^{AB}	BD^{C}
OHE	80	60	AH	200	45	45.2 ± 2.3^{A}	BD^{C}	46.0 ± 2.3^{AB}	53.8 ± 2.1^{B}	98.3 ± 2.5^{A}	BD^{C}
–	–	–	AH	180	60	35.6 ± 1.5^{B}	6.7 ± 0.3^{B}	50.8 ± 2.1^{A}	55.6 ± 1.7^{B}	85.6 ± 2.2^{AB}	22.4 ± 1.5^{B}

T: temperature; t: time; Cel.: cellulose; Hemi.: hemicellulose; GRS: glucose recovery in the solid; RHSS: recovery of hemicellulosic sugars in the solid fraction; BD: below the detection limit; means with a common letter are not significantly different ($p < 0.05$).

Table 3
Composition of liquid fractions in sugars and sugar recovery after different extraction and pretreatment stages.

Extraction stage	T	t	Pretreatment stage	T	t	Glucose	Xylose	GRL	RHSL
	°C	min		°C	min	g/L		%	
OHE	80	60	–	–	–	0.07 ± 0.01^{C}	0.11 ± 0.01^{C}	2.4 ± 0.4^{C}	2.2 ± 0.5^{E}
CE	80	60	–	–	–	0.07 ± 0.01^{C}	0.13 ± 0.01^{C}	1.2 ± 0.7 ^{C}	3.1 ± 0.8^{E}
CE	80	60	AH	180	60	0.88 ± 0.04^{A}	2.0 ± 0.1 ^{A}	12.5 ± 0.6 ^{A}	44.4 ± 1.3 ^{A}
OHE	80	60	AH	180	60	0.83 ± 0.04 ^{A}	1.9 ± 0.1 ^{A}	13.2 ± 0.5 ^{A}	42.1 ± 1.4 ^{A}
OHE	80	60	AH	200	30	0.45 ± 0.02^{B}	0.76 ± 0.04^{B}	3.6 ± 0.3 ^{C}	17.6 ± 0.9^{C}
OHE	80	60	AH	200	45	0.41 ± 0.02^{B}	0.49 ± 0.02^{B}	1.7 ± 0.5 ^{C}	11.1 ± 0.4^{D}
–	–	–	AH	180	60	0.86 ± 0.04 ^{A}	1.75 ± 0.09 ^{A}	9.2 ± 0.4^{B}	33.7 ± 1.1^{B}

T: temperature; t: time; GRL: glucose recovery in liquid fraction; RHSL: recovery of hemicellulosic sugar recovery in the liquid fraction; means with a common letter are not significantly different ($p < 0.05$).

in paper manufacturing, biofuels, bioplastics, and pharmaceutical materials (Piñón-Muñiz et al., 2023). Finally, the solid fraction from AH contains glucose, which is used in food industry and as a substrate for fermentation in the production of ethanol and other industrial bioproducts (Hu et al., 2024).

3.3. Effect of extraction and autohydrolysis on enzymatic saccharification

Enzymatic hydrolysis was performed on the solid fractions obtained during the two stages of VS valorization. The tests that achieved the highest saccharification yields were OHE with AH at 200 °C for 30 and 45 min (41.1 ± 1.7 % and 36.1 ± 2.1 %, respectively). Both saccharification yields corresponded to enzymatic hydrolysis yields higher than 20 % (Table 4). As can be expected, when VS were subjected to an extraction stage without further AH, the lowest glucose solubilization levels by enzymes were determined (0.92 ± 0.12 % and 3.32 ± 0.17 %, respectively). This could be due to autohydrolysis causing alterations in the lignin, which tends to modify the average pore size of the pretreated solid. This modification in porosity may have facilitated enzyme accessibility to cellulose, thereby improving its efficiency (de Paiva Carvalho et al., 2024). These results compare favorably with those reported using the same raw material after an acid pretreatment (150 °C and 1.2 % w/v H_2SO_4), with a saccharification yield of 19.7 %. In this case, a second organosolv pretreatment was necessary to increase the enzymatic digestibility of biomass (Cardoza et al., 2024). Further, the preliminary extraction step performed with OHE seems to also contribute to this increased accessibility, when compared to the CE, with an increase in saccharification yield and in the enzymatic hydrolysis yield of ca. 20 % for the combined process, at the same AH conditions (180 °C, 60 min).

3.4. SSF of biomass after ohmic heating extraction and autohydrolysis

The process configuration including ohmic heating extraction and autohydrolysis at 200 °C for 30 min achieved a glucose recovery in solid of 96.3 % and yielded the highest antioxidant compound extraction in the shortest time, resulting in greater energy savings. Further, the solids resulting from this process had also no residual hemicellulose, being mainly composed of glucose and lignin. Therefore, this process scheme was selected and the solid obtained under these conditions was used as a substrate for SSF, as it provided the best results in terms of yield and time. Additionally, untreated biomass was used as a control. The control showed minimal ethanol production (less than 1 g/L), as expected when using untreated biomass, as reported for lignocellulosic biomass in other studies (Malik et al., 2022). In contrast, the solid treated with OHE and AH at 200 °C for 30 min yielded promising results, reaching up to 20.57 g/L of ethanol after 72 h (Fig. 3). Considering theoretical ethanol conversion limits, an ethanol yield of 61.26 % was achieved, which is favorable given that autohydrolysis was conducted solely with water, without acidic catalysts. In another study (Cardoza et al., 2024), theoretical ethanol yields of more than 98 % were obtained from VS, but in that case, the biomass was pretreated using sulfuric acid as a catalyst. In the present study, the process was conducted using only water. This approach offers resource savings and

Table 4
Results of enzymatic hydrolysis of the solid fractions generated in the different process configurations.

Extraction stage	T	t	Pretreatment stage	T	t	Glucose	Xylose	SY	EHY
	°C	min		°C	min	g/L		%	
OHE	80	60	–	–	–	0.13 ± 0.01	0.05 ± 0.01	0.92 ± 0.12	0.62 ± 0.12
CE	80	60	–	–	–	0.44 ± 0.01	0.12 ± 0.01	3.32 ± 0.17	2.2 ± 0.08
CE	80	60	AH	180	60	2.55 ± 0.12	0.63 ± 0.03	12.8 ± 0.63	7.3 ± 0.4
OHE	80	60	AH	180	60	3.10 ± 0.16	0.92 ± 0.04	15.6 ± 0.78	8.9 ± 0.4
OHE	80	60	AH	200	30	9.78 ± 0.49	0.82 ± 0.04	41.1 ± 1.7	26.2 ± 1.1
OHE	80	60	AH	200	45	8.94 ± 0.45	0.63 ± 0.02	36.1 ± 2.1	23.4 ± 0.9
–	–	–	AH	180	60	3.61 ± 0.18	0.86 ± 0.03	17.4 ± 1.3	10.8 ± 0.4

T: temperature; t: time; SY: saccharification yield (g glucose by enzymatic hydrolysis/g glucose in the substrate); EHY: enzymatic hydrolysis yield (g glucose by enzymatic hydrolysis/g glucose in raw material).

environmental benefits, avoiding the need for acids that require specific disposal measures to prevent acidification of sensitive ecosystems (Wang et al., 2024).

3.5. Mass balance of the sequential processes

Fig. 4 presents the global mass balance for the utilization of VS, using a two-stage valorization process that obtained the best results: OHE with AH at 200 °C for 30 min. Its overall performance was consistent and positive across all, making it the most efficient process globally. The diagram suggests a cascade utilization scheme within the context of a biorefinery. Initially, an OHE at 80 °C for 60 min is carried out, yielding two fractions. The hydroethanolic liquid fraction allows for the recovery of phenols. Next, the resulting solid fraction is subjected to pretreatment by AH at 200 °C for 30 min. This processing step produces two additional fractions: the liquid fraction allows for further recovery of phenols and hemicellulosic sugars, while the solid fraction can either undergo enzymatic saccharification to obtain glucose that can be used as a platform molecule. However, if the goal is to obtain ethanol, the best option would be to carry out an SSF, as it would yield a higher amount of ethanol (7.44 g/100 g biomass). On the other hand, with the glucose generated from enzymatic saccharification (9.53 g/100 g biomass), theoretically only 4.86 g of ethanol could be obtained.

The sequential process proposed in this work can be a source of several valuable products from a single residual biomass, using sustainable technologies. To answer the market demands for these compounds, higher solid loading should be revisited but while biomass concentrations above 15 % offer the advantages of lower energy consumption, lower capital costs, and reduced operational cycles, mass transfer limitations and non-uniformity problems also arise (Shiva et al., 2023). Thus, while these results provide a promising starting point for a cascading biorefinery of vine shoots, further work is still required to ensure the techno-economic viability of the process at scaled-up conditions.

4. Conclusions

Vine shoots have potential as a raw material for the extraction of phenolic compounds and bioethanol production through a cascading biorefinery approach. The innovative combination of ohmic heating extraction and autohydrolysis proved to be efficient in the extraction of phenolic compounds, with a synergetic effect of this combination being observed not only on individual phenolic compound recovery but also on the resulting antioxidant activity of the extracts; the optimized combination of treatments results in the recovery of approximately 32 mg GAE per gram of initial biomass, versus 7.8 $\pm$ 0.1 mg and 19.2 $\pm$ 1.1 mg when the technologies are not used sequentially. Specifically, an OHE treatment at 80 °C for 60 min followed by AH at 200 °C for 30 min achieved the highest total phenolic content (31.3 mg GAE/g VS) and FRAP antioxidant activity (48.5 mg TE/g VS), while enabling a glucose recovery in the solid fraction of 96.3 % and a subsequent ethanol yield of 61.3 % (20.57 g/L after 72 h of SSF). Additionally, the residual biomass was still enriched in cellulose, enabling further valorization through solid-state fermentation (SSF). This process scheme also demonstrated enhanced saccharification yields (41.1 %) compared to non-sequential treatments, indicating improved enzyme accessibility and process efficiency. Thus, the proposed process not only maximizes ethanol production and the recovery of high-value products but also aligns with the principles of a circular economy by efficiently valorizing lignocellulosic waste, offering a more efficient consumption and waste management strategy that could be scaled up in wine-producing regions.

CRediT authorship contribution statement

Diego Cardoza: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Joana S. Gomes-Dias:** Writing – review & editing, Investigation, Formal analysis. **Sara G. Pereira:** Writing – review & editing, Investigation, Formal analysis. **Inmaculada Romero:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Eulogio Castro:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Cristina M.R. Rocha:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization.

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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.

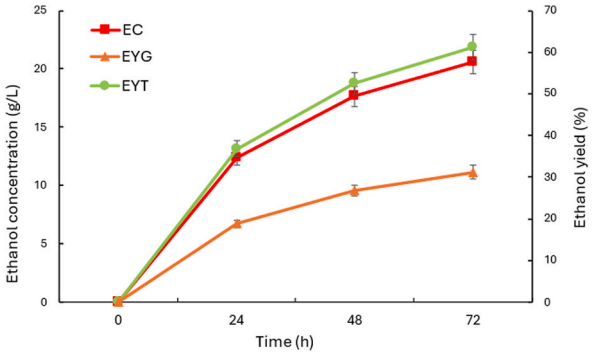

Fig. 3. Concentration and yield of ethanol obtained after SSF of the resulting solid from OHE with AH (200 °C, 30 min)
EC: ethanol concentration; EYG: ethanol yield referred to glucose in the substrate; EYT: ethanol yield referred to the theoretical yield.

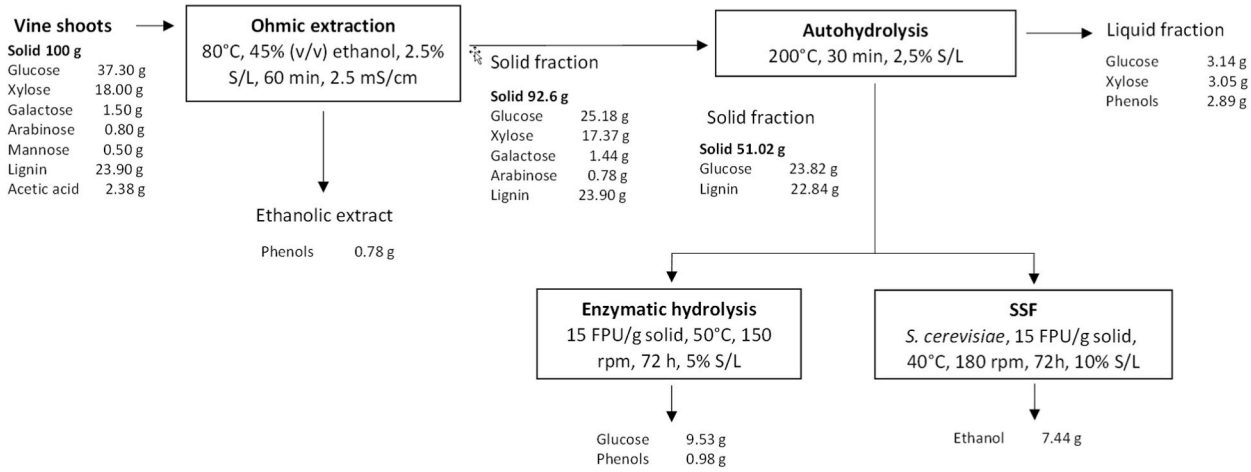

Fig. 4. Mass balance of the proposed process scheme for the valorization of vine shoots.

Data availability

Data will be made available on request.

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