Biorefinery concept for the valorization of grapevine shoots: Study case for the Austrian variety Grüner Veltliner

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ABSTRACT

Identifying feedstocks and their integral valorization is essential for the sustainable production of chemicals, materials, fuels, and energy. Waste materials from different agricultural activities can be a potential source. Specifically, winemaking and grape crops are a source of both the large amounts and variety of residues. The grapevine shoots are the primary residue resulting from the pruning of grape crops, consisting of the stems and leaves. More than 46 thousand hectares in Austria are dedicated to grape crops, and almost half of this area corresponds to the variety *Grüner Veltliner*. Italian, Portuguese, and Spanish grapevine residues have been studied to produce bioactive compounds; however, the most important Austrian grape variety has not been thoroughly studied for this purpose. The grapevine shoots offer different lignocellulosic platforms and valorization strategies if the leaves and stem are evaluated separately. This work evaluated the extraction of bioactive compounds and the production of hemicellulosic sugars, lignin, and a cellulose-enriched pulp from Grüner Veltliner's grapevine shoots under a biorefinery concept. Pressurized Liquid Extraction was the selected technology for extracting bioactive compounds, and Liquid Hot Water and Organosolv were chosen for hemicellulose and lignin hydrolysis. Quercetin was the flavonoid found in higher concentrations in the leaves (10.6 mg/g of dry feedstock) and resveratrol in the stems (1.9 mg/g of dry feedstock); both components were found in higher yields than other grape varieties reported in the literature. In addition, hemicellulose and lignin hydrolysis reached yields (0.18 and 0.08 g/g of dry feedstock, respectively) in the same order of magnitude as other feedstock used for hemicellulose and lignin valorization (e.g., wheat straw). These results clearly show the potential of this under-valorized feedstock and encourage further study of the downstreaming of the intermediate products and a deeper study of the production cycle of the shoots to determine the actual available amount to be used.

1. Introduction

Being the world's most abundant biomass with complex chemical compositions, lignocellulosic feedstocks are of particular interest as it provides the potential to produce a wide range of value-added products. The sustainable production of chemicals, materials, and fuels from biomass is thus essential in an integrated biorefinery to reduce the dependence on finite fossil fuels. Therefore, there is a vital need for scientific and technological advancements to develop economically viable biorefinery systems (Maity, 2015). Therefore, identifying specific feedstock and their integral valorization, meaning using all of its constituents as much as possible, is a relevant task. In the effort to transition to a bio-based circular economy, the valorization of several residues and waste materials from different agricultural activities has gained interest. Specifically, exploiting the waste materials from the winemaking and grape (*Vitis vinifera*) production industries could be highly beneficial because of the large amounts and variety of residues they generate (Contreras et al., 2022).

In Austria, over 46,000 ha are dedicated to grape crops, and 331,428 tons of grapes were produced in 2017, with the top grape variety cultivated being *Grüner Veltliner* (15,015 ha planted, corresponding to 47.3 % of the total white wine area in Austria) (Statistics Austria, 2021). The organic leftovers ("waste") generated during cultivation and harvesting has been estimated to be 5 tons per hectare of land per year (Zacharof, 2017), with the primary residues being grapevine shoots (comprising the primary growth structure of the grapevine (stems) and the leaves). Generally, this residue is left in the fields after the pruning stage and is either left in the ground for re-fertilization, or collected for composting or burning (Çetin et al., 2011; Loupit et al., 2020). Pruning can take place twice/thrice during the year (e.g., end of winter/beginning of spring, in summer during the formation of the fruit, or in fall after the harvesting) (Torregrosa et al., 2021). Multiple authors have evaluated the potential of this residue for the extraction of bioactive compounds, mainly for Italian and Spanish wine varieties (Delgado-Torre et al., 2012; Kalli et al., 2018; Moreira et al., 2018; Pintać et al., 2018; Zacharof, 2017). However, two specific unresolved questions were identified. First, the specific *Grüner veltliner* variety, Austria's most cultivated white wine variety, has not been characterized for extractive compounds. Second, during the pruning of the vineyards, parts of the stem and leaves are cut. Hence, vine shoots are a mixture of leaves and a woody stem, both parts offering different lignocellulosic platforms and valorization strategies. Winter and early summer pruning generates mostly stems as the cold conditions make the grapevine lose the leaves, whereas fall pruning contain both leaves and stems. However, until the collection takes place, a part of the leaves may fall/degrade.

This scientific study evaluates the valorization potential of Grüner Veltliner's grapevine shoots as a biorefinery concept. Grapevine shoots were collected, separated into leaves and stems, air-dried, and both parts were evaluated to extract bioactive compounds as well as the production of hemicellulosic sugars, lignin, and a cellulose-enriched pulp. The aim of this study is to determine the valorization potential of both parts of the shoots, leaves and stems. Therefore, this is a prospective study assuming both parts being available and collected. We used Pressurized Liquid Extraction (PLE) to extract bioactive compounds, as the high pressure increases solubility and decreases viscosity and surface tension, which leads to higher mass transfer (Hendriks and Zeeman, 2009; Serna-Loaiza et al., 2019). For the PLE, ethanol was selected as extractant, and different temperatures and solvent concentration were assessed. Then, we selected Liquid Hot Water (LHW) and Organosolv (OS) as pretreatments for hemicellulose and lignin hydrolysis. LHW only uses water as a reactant and the hydrolysis reaction is auto-catalyzed by the released acetic acid from the hemicellulose backbone. OS solubilizes part of the hemicellulose and removes the majority of lignin in a sulfur-free form, which could be further used for pharmaceutical and cosmetic applications (Beisl et al., 2018; Huijgen et al., 2010). Ultimately, a mass balance was performed with the results obtained during the bioactive and lignocellulosic extractions stages to gather information about the distribution of the targeted components.

2. Materials and methods

2.1. Raw material

Grapevine shoots of the variety *Grüner Veltliner* were collected after the grape harvesting season at the end of summer/beginning of autumn in September 2020 in Hollabrunn (Austria), as shown in Fig. 1. The shoots consisted of leaves and stems. The moisture content of the leaves and stems was 62.3 % and 52.5 %wt, respectively. The samples were air-dried until a moisture content below 10%wt, and the particle size was reduced in a blade mill equipped with a 2 mm mesh and stored under dry conditions. The samples were separated into leaves and stems. Both plant parts were investigated on the content of arabinan, galactan, glucan, xylan, mannan, lignin, extractives, ash, and moisture. The characterization was performed according to the National Renewable Energy Laboratory (NREL) NREL/TP-510–42618, NREL/TP-510–42622, and NREL/TP-510–42619 (Sluiter et al., 2012, 2008a, 2005).

2.2. Reagents

Ultra-pure water (18 MΩ/cm) was used for the pretreatments. Ethanol, abs. 100 % a.r. (> 99.8 vol% C_2H_5OH) was purchased

Fig. 1 – Grapevine shoots samples from the variety Grüner Veltliner used in this study. (a) Collected samples. (b) Example of a single grapevine shoot branch.

from Chem-Lab NV (Zedelgem, Belgium). Standards for carbohydrates (arabinose, galactose, glucose, xylose, and mannose), acetic acid (99.7 %), 2-furaldehyde (furfural, 99%), hydroxymethylfurfural (HMF, 99 %), and sulfuric acid (98 %) were purchased from Merck (Darmstadt, Germany).

2.3. Biorefinery concept

2.3.1. General description of the biorefinery concept

The proposed biorefinery concept consists of two main sections: an 'extraction' section focused on bioactive compounds and a 'platform-valorization' section focused on valorizing the lignocellulosic fractions, as shown in Fig. 2.

Hemicellulosic sugars, lignin, and a cellulose-enriched pulp are the obtained products from this primary biorefining strategy, meaning that these are intermediate products that require further processing to be sold in the market. Hemicellulosic sugars could be used as substrate for fermentations, the extracted lignin for the production of colloidal lignin particles, and the cellulose-enriched pulp could be used for cardboard making (Adamcyk et al., 2021; Romero-García et al., 2022; Scapini et al., 2021). The first step consisted of separating the grapevine shoots into leaves and stem and characterizing both fractions. Then, each separate fraction was evaluated for extracting bioactive compounds, and depending on the composition, the valorization of lignocellulosic was performed. The leaves were used for the production of hemicellulosic sugars and the stems for both hemicellulosic sugars and lignin production.

2.3.2. Scope, assumptions, and limitations of the biorefinery concept

As mentioned previously in the Introduction, grapevine shoots can consist of a mixture of leaves and stems, and the proportion between both parts depend on the pruning season and on the collection time after the pruning. At the current state of handling/management of shoots in the vineyards, after the pruning takes places, the collection can happen after a time and a part of the leaves may degrade/fall. To find a middle point in this situation, meaning, if the leaves are actually usable parts or not, we decided to work with air-dried (and not freshly collected) shoots, which could be closer to a prospective scenario where the shoots (leaves and stems) could be collected and still used as a whole part. this is a prospective study assuming both parts being available and collected. We understand this is an idealization of the current state of availability of the feedstock and would implicate changes in the logistic and activities at the crop management level.

2.3.3. Valorization of the extractives: bioactive compounds

Flavonoids, stilbenoids, and condensed tannins were identified and selected as targeted bioactive compounds. Consequently, PLEs for both, the leaves and stem was performed in a 1 L stainless-steel high pressurize autoclave (Zirbus, HAD 9/16, Bad Grund, Germany) at different temperatures (50, 75, and 100 °C) and water ethanol ratio (1:0, 1:1 and 0:1, in volume, referred as 0 vol%, 50 vol% and 100 vol% EtOH, respectively) for a holding time of 30 min. Additionally,
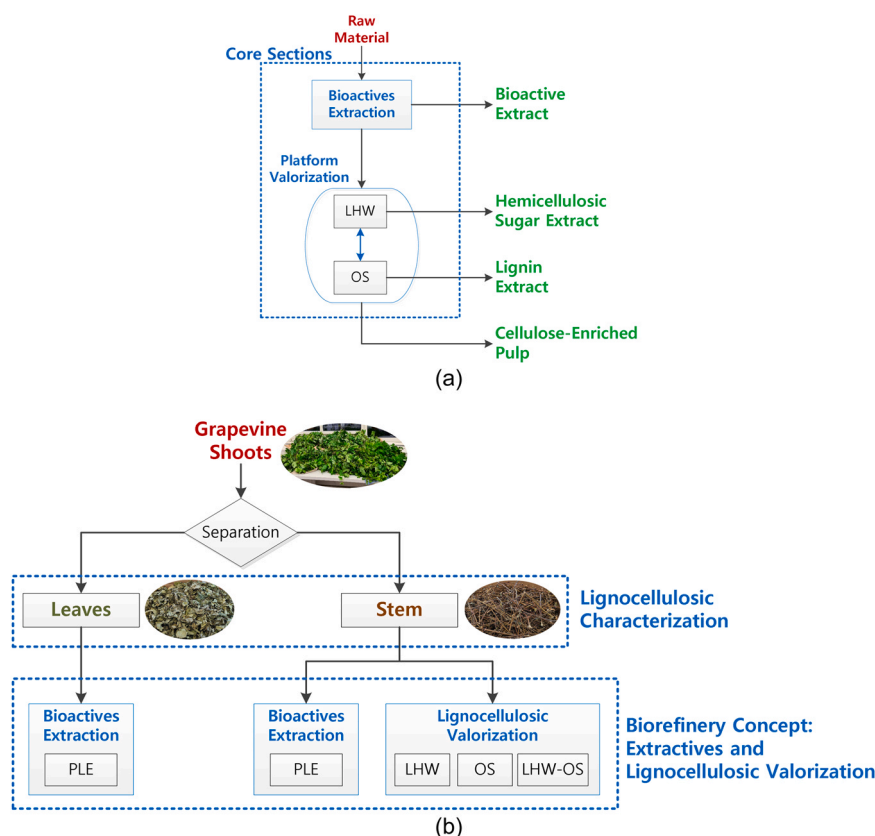

Fig. 2 – Proposed biorefinery concept for the valorization of grapevine shoots. (a) General scheme of the developed biorefinery concept. (b) Stages carried out to evaluate the valorization of grapevine shoots. PLE: Pressurized Liquid Extraction, LHW: Liquid Hot Water, OS: Organosolv, LHW-OS: Sequential LHW followed by OS.

the selected bioactive compounds were quantified and the ferric reducing antioxidant power (FRAP) was determined.

2.3.4. Valorization of the lignocellulosic platforms

The standalone evaluation of sugar production with LHW (160 °C, 90 min holding time; and, 180 °C, 30 min holding time) and lignin extraction (180 °C, 60 min total operation time, according to (Beisl et al., 2018)) with OS were studied for the platform valorization stage. The extractions were carried out for the leaves, the stems, and the leaves after extracting bioactive compounds. As stems have a higher share of lignin due to their wood stem structure, a similar strategy to the one presented by (Serna-Loaiza et al., 2022, 2020) was followed in addition to the standalone LHW and OS: performing a sequential LHW-OS and characterizing the sugar and lignin extracts obtained thereof. The general procedure was feeding the raw material to the reactor and carrying out the LHW extraction. Then, the mixture was separated (pressing), the extract was collected (LHW extract), and the solid was washed with water at 50 °C. The solid was pressed again, the washing was collected (LHW Washing), and the solid was used to carry out the second pretreatment (Organosolv). In this phase, after the LHW stage and respective washing, the moisture content and mass of the solid were determined, and this same solid was used for the subsequent OS stage. The pressing and washing process was repeated with 60%wt aqueous ethanol at 50 °C.

Both LHW and OS were carried out in a stainless steel high pressurize autoclave (Zirbus, HAD 9/16, Bad Grund, Germany), stirring at 200 rpm. The initial dry mass of wheat straw used for the LHW was approximately 35 g (38.58 g wet mass), with a solid/liquid ratio of 1 g of dry solid per 11 g of solvent. The moisture content of the solid was subtracted from the prepared solvent. The reactor was heated to 160 °C and cooled down after the 90 min holding time. Subsequently, the solid and liquid fractions were separated using a hydraulic press (Hapa, HPH 2.5) at 200 bar and a centrifuge (Sorvall, RC 6 +) at 24,104 g for 20 min. The extract's density was determined using a density meter (DE45 DeltaRange, Mettler Toledo, Columbus, United States). The supernatant was stored at 5 °C until further analysis. The solid pressed fraction was submerged in water at 50 °C and manually disintegrated in the water for 5 min. The amount of water used for washing corresponded to the same amount used for the solvent without correcting the moisture content (385 g). Then, the solid was pressed, the wash was collected and stored at 5 °C for analysis, and the solid was used for the OS stage, which was carried out using 60%wt aqueous ethanol at 180 °C. The dry matter content of the solid was calculated, and a solid/liquid ratio of 1 g of dry solid per 11 g of solvent was used, subtracting the moisture content. The total operation time was fixed at 60 min (heating time approximately 45 min, and holding time of 15 min). After the extraction, the separation of liquid/solid fractions (press and centrifuge), solid washing and pressing, and storage were done as described for the LHW stage. The washing, in this case, was performed using 60%wt aqueous ethanol at 50 °C.

2.4. Characterization of bioactive compounds

2.4.1. Preparatory enzymatic hydrolysis

The first step for quantifying bioactive compounds in the grapevine shoot extracts was enzymatic hydrolysis with a snailase. The method was adapted from (Kornpointner et al., 2022). Briefly, 20 µL of sample were mixed with 80 µL McIlvaine buffer (pH 5.5; 0.1 M incl. 20 µM sodium ascorbate) and 5 mg snailase enzyme (Abbexa, UK) were dissolved in 100 µL buffer. Then, 100 µL snailase solution was added to the diluted sample and vortexed. The sample was heated at 37 °C for 25 min, and the hydrolysis was stopped with 30 µL formic acid. Subsequently, 200 µL of diluted samples were combined with 100 µL of acetonitrile (0.1% formic acid). After 1 h, the sample was centrifuged for 10 min, filtered through a 0.22 µm filter, and 50 µL were transferred into an HPLC vial.

2.4.2. Flavonoid quantification by HPLC analysis

The quantification of flavonoids was carried out using a Dionex UltiMate© RSLC System connected to a DAD-3000RS Photodiode Array Detector (Thermo Scientific, Germany) with a Dionex Acclaim™ RSLC 120 C18 column (2.2 µm, 120 Å, 2.1 × 150 mm, Bonded Silica Products: No. 01425071). The injection volume was 4 µL with a 0.2 mL/min flow rate and an oven temperature of 25 °C. The mobile phases were the same as for the UPLC analysis with the following gradient program: 15 min from 20 to 53 vol% (B), 5 min to 95 vol% (B), 10 min at 95 vol% (B), 1 min to 20 vol% (B) and post-run 10 min 20 vol% (B). The method was modified according to (Kornpointner et al., 2022). All solvents were HPLC-grade, and commercial quercetin standards (≥99%, Extrasynthese, France) were used to quantify. Calibration between 0.1 and 100 µg/mL ($y = 0.580x$, $r^2 = 0.999$). Isorhamnetin, kaempferol, myricetin and resveratrol are expressed as quercetin equivalents. All analysis was performed at 340 nm.

2.4.3. Condensed tannin estimation

Condensed tannins were determined with vanillin assay adapted and modified from Broadhurst and Jones, 1978 (Broadhurst and Jones, 1978). A 4% vanillin solution in methanol was prepared and 30 µL sample, or 15 µL sample with 15 µL EtOH, were mixed with 300 µL vanillin solution and vortexed. Then, 150 µL of concentrated HCl was added, vortexed, and samples were put in dark. After 15 min, 250 µL of the mixture was placed in a 96-well microplate, and the absorbance was recorded at 500 nm with a SPECTROstar Nano absorbance microplate reader (BMG LABTECH, Ortenberg, Germany). Water was used as blank, and an external calibration was carried out with catechin-hydrate ≥ 98% (Sigma Adlrich). Standard dilutions between 0.10 and 0.75 mg/mL ($y = 22.3x + 0.256$; $r^2 = 0.999$) were prepared and 50 µL were mixed with 450 µL vanillin solution for calibration and measured as described before All samples were measured once without vanillin (300 µL of pure methanol instead of the 4% vanillin solution). Sample absorbance was calculated as the difference between the absorbance at 500 nm of the sample with and without vanillin. Results were calculated as mg catechin equivalents per g of dry feedstock.

2.4.4. Determination of ferric reducing antioxidant potential (FRAP)

The antioxidant potential of the extracts was measured with the FRAP assay using the method described in literature (Benzie and Strain, 1996; Kornpointner et al., 2021). Solutions were prepared mixing 300 mM acetate buffer pH 3.6, 20 mM FeCl$_3$.6 H$_2$O and 10 mM 2,4,6-tripyridyl-s-triazine in 40 mM HCl in a proportion of 10:1:1. Briefly, 1 µL sample was mixed with 1800 µL FRAP working solution, and after 30 min, 250 µL

of the sample was transferred to a 96 well microplate, and the absorbance at 593 nm was recorded by the same photometer as mentioned in chapter 2.4.3. Water was used as blank, and as standard was Na-ascorbate dissolved in water. The dilutions for the calibrations were done with water between 2 and 100 µg/mL. Calibration ($y = 0.268x + 0.00897$; $r^2 = 0.999$) was performed by adding 50 µL of standard solution to 950 µL FRAP working solution and measuring the absorbance as mentioned before. Results are expressed as mg of ascorbic acid (AA) equivalents per g of dry feedstock.

2.4.5. Determination of the extraction yield

The extraction yield of each bioactive compound was calculated using Eq. (1). The calculation considers the initial mass of sample put into the reactor, the measured concentration of each bioactive compound, the total mass and density of the extract, divided by the total amount of dry feedstock. $C_{BAC, \ i}$ is the concentration of the bioactive compound i measured by HPLC in (µg/mL). $M_{Extract}$ and $\rho_{Extract}$ are the total mass and the density of the extract in (g) and (g/mL), respectively. $M_{Feedstock}$ corresponds to the initial dry matter of feedstock in (g). $Y_{BAC, \ i}$ corresponds to the extraction yield (mg of bioactive compound i per g of dry feedstock) of the bioactive compound i with respect to the added dry matter of feedstock.

$$Y_{BAC, \ i} = \frac{C_{BAC, \ i} {}^* M_{Extract}}{\rho_{Extract} {}^* 10^3 {}^* M_{Feedstock}} \tag{1}$$

2.5. Determination of carbohydrates, lignin, and degradation products

Sugars and degradation products were characterized according to the NREL/TP-510–42623 (Sluiter et al., 2008b). Monomeric sugars were analyzed using HPAEC-PAD (ICS-5000, Thermo Scientific, USA) with deionized water as eluent. Oligomeric sugars were hydrolyzed (diluted sulfuric acid) at 120 °C and analyzed as monomers. A sugar recovery standard was used to account for losses. Furfural, HMF, and acetic acid were determined using HPLC (LC-20A HPLC system, Shimadzu, Japan) by UV and RI detection with a Shodex SH1011 analytic column at 40 °C with 0.005 M H_2SO_4 as mobile phase. The lignin concentration was measured as acid-soluble lignin (ASL) and acid-insoluble lignin (AIL). The extract was dried, and the solid was submitted to the protocol established in the NREL/TP-510–42618 (Sluiter et al., 2012). The AIL was determined by a gravimetric method and ASL by UV/VIS absorption at 205 nm using a Shimadzu UV-1800 spectrophotometer. Extraction yields were calculated based on the measured concentrations, the solid-liquid ratio, the density of the extract, and is referred as grams per gram of dry feedstock using Eq. (2). Y_i is the extraction yield of component i per added feedstock in dry basis (g/g), $Conc_i$ is the concentration of the measured component in [mg/L], SL_{ratio} is the solid-liquid ratio (1 g of dry feedstock per 11 g of solvent), and $\rho_{Extract}$ is the density of the respective extract in [g/mL].

$$Y_i = \frac{Conc_i {}^* SL_{ratio} {}^* 100}{\rho_{Extract} {}^* 10^6} \tag{2}$$

| Table 1 – Lignocellulosic characterization of the grapevine shoots (leaves and stem) ($n = 3$, SD).					
Component	Weight percentage – Dry basis (%wt)				
	Leaves			Stem	
Arabinan	0.34	±	0.02	0.37	±	0.02
Galactan	1.63	±	0.13	1.30	±	0.09
Glucan	12.73	±	0.50	22.91	±	1.40
Xylan	2.63	±	0.09	10.46	±	0.61
Mannan	0.47	±	0.03	1.11	±	0.07
Lignin	17.71	±	0.72	17.58	±	1.18
Extractives	52.42	±	1.48	36.65	±	0.45
Ash	3.46	±	0.08	1.63	±	0.11
TOTAL - Dry Weight Basis	91.39	±	1.73	92.00	±	1.99
Moisture	11.38	±	0.37	12.83	±	0.82

## 3. Results and discussion

3.1. Raw material characterization

The first step to determine the valorization potential of grapevine shoots is the lignocellulosic characterization of the stems and the leaves. Table 1 shows the lignocellulosic characterization on a dry basis. As observed, the leaves have ~16%wt more content of extractives compared with the stem, which corroborates the potential for the extraction of bioactive compounds. Regarding the possible valorization of other platforms, the stem has a significant glucan content (12.73%wt for the leaves, compared to 22.91%wt in the stem), and assuming that all glucan represents cellulose, the available hemicellulose is 5.54% and 14.39%wt, for a total sum of carbohydrates of 18.27%wt and 37.30%wt for the leaves and stems, respectively. Apart from that, both parts have similar concentrations of lignin (~18%wt) as found in grasses as wheat straw (Serna-Loaiza et al., 2020), in the lower range of hardwoods (18–25%wt) (Rowell et al., 2012), and below the content of softwoods (25–35%wt), and nutshells (30–40%wt) (Mendu et al., 2011). Based on the composition, stems have a better overall distribution of structural carbohydrates and lignin, at the level of grasses and softwood, which makes it more interesting for the valorization of lignocellulosic platforms. On the other hand, leaves have a high content of bioactive components, lignin is on a similar level as the stem, but carbohydrates are low.

3.2. Bioactive extraction stage

Herein, the potential of grapevine shoots from *Grüner veltliner* to extract bioactive compounds is evaluated. The targeted components of interest were the flavonols, myricetin, quercetin, kaempferol as well as isorhamnetin, the stilbene resveratrol as well as the content of condensed tannins. Those flavonoids have been reported before to be accumulated in grapevine shoots as well as high contents of different condensed tannins (Cebrián et al., 2017; Luque-Rodríguez et al., 2006; Moreira et al., 2018). Fig. 3 shows the PLE extraction yields for the investigated grapevine shoot leaves and stems. Quercetin was the predominant flavonoid in leaves. For all bioactive compounds, the highest extraction yields were obtained by extracting with 50 vol% ethanol, yielding
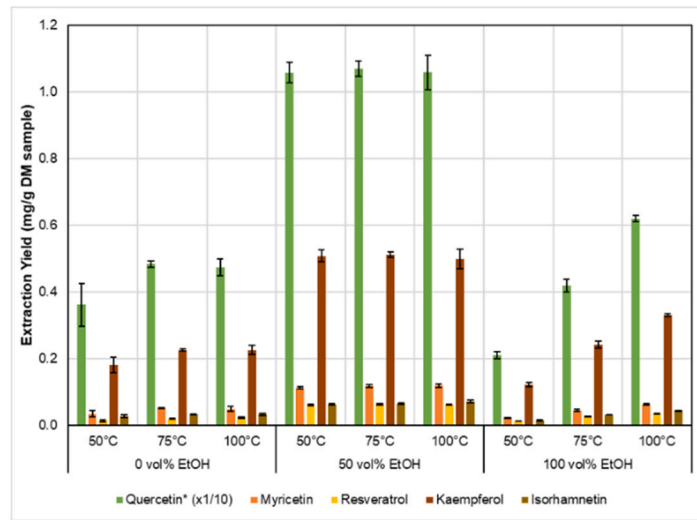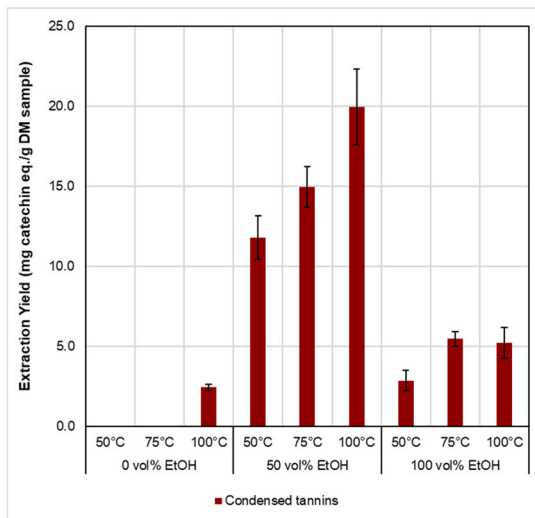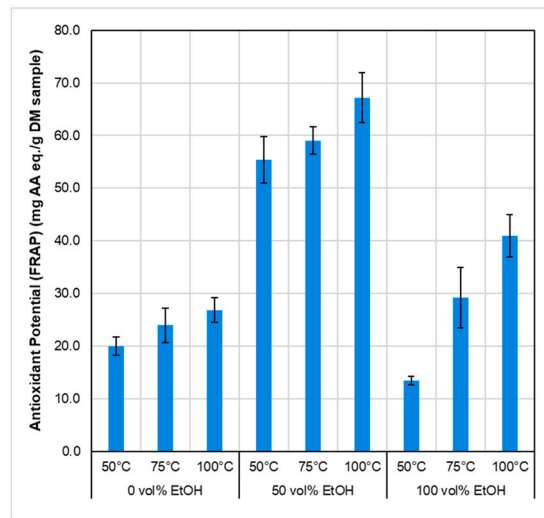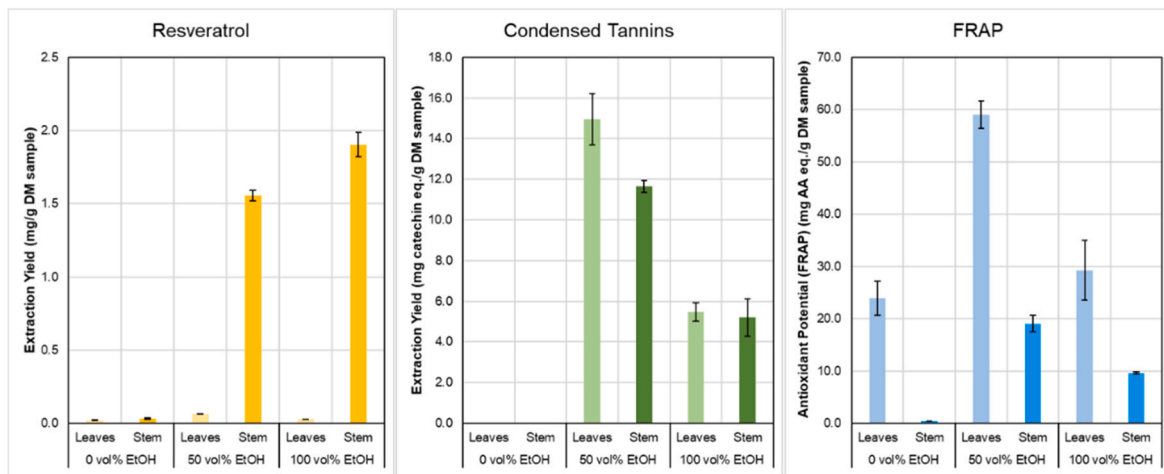
Fig. 3 – PLE extraction yields for the grapevine shoot leaves and stems. (a) Extraction yield of quercetin, myricetin, resveratrol, kaempferol, and isorhamnetin for the leaves. *Quercetin was divided by a factor of 10 for scaling purposes. (b) Extraction yield of condensed tannins for the leaves. (c) Antioxidant potential of the grapevine shoot leaves extracts. (d) Comparison of the PLE extractions at 75 °C of the leaves and stems for resveratrol, condensed tannins and FRAP. (n = 3, SD).

Table 2 – Comparison of the bioactive compounds obtained in this work with other studies reported in the literature.

Description of the used grapevine shoots

	This work							Ref. 1	Ref. 2[a] White Airén	Ref. 2[a] Red Tempranillo	Ref. 3
Grape type	White							Red	White	Red	White
Variety	Grüner veltliner							Touriga Nacional Tinta Roriz[b]	Airén	Tempranillo	Airén
Part	Leaves				Stem			Whole vineshoots[c]			
Extraction conditions											
Temp. (°C)	100	50	75	100	75	75	75	55	75	75	100
Time (min)	30							120	12	12	15–60
Solid Load	1:11 (w/w)							1:40 (w/v)	1:5 (w/w)		1:5 (w/v)
Solvent	Water	50 vol% EtOH					% EtOH	50 vol % EtOH / 12.5 wt% EtOH	50 vol	% EtOH	Water
	50 vol% EtOH			100 vol% EtOH	50 vol						
Bioactive Compounds											
Extraction Yield (mg/g of dry matter feedstock)											
Quercetin	4.74	10.57	10.69	10.58	ND	ND	ND	0.26	0.003	0.003	NR
Myricetin	0.05	0.11	0.12	0.12	ND	ND	ND	0.50	NR	NR	NR
Resveratrol	0.02	0.06	0.06	0.06	0.03	1.56	1.90	1.01	0.15	0.17	0.02
Kaempferol	0.23	0.51	0.51	0.50	ND	ND	ND	0.38	NR	NR	NR
Isorhamnetin	0.03	0.06	0.07	0.07	ND	ND	ND	NR	NR	NR	NR

ND: Not Detected; NR: Not Reported; EtOH: Ethanol

Ref. 1: (Moreira et al., 2018); Ref. 2: (Cebrián et al., 2017); Ref. 3: (Sánchez-Gómez et al., 2014).

[a] The extraction technology of this work is not directly comparable as it used a microwave-assisted temperature extraction.

[b] Average value reported for both varieties. The values were compared with the Conventional Extraction reported by the cited work. The extraction yields were taken as the average between the reported values for the samples

[c] Not reported if a subsequent separation of the leaves and stems of the shoots was done

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between 30 up to 80% more of the investigated compounds than the other extraction conditions. Apart from that, the influence of temperature was analyzed. An increase in temperature led to higher extraction yields for condensed tannins at 50 vol% EtOH and for flavonoids at 100 vol% EtOH.

In addition, the antioxidant capacity of the extracts obtained from the leaves was measured. Extraction with 50 vol% ethanol led to a 32 % and 78 % higher overall antioxidant potential, when compared with other extraction conditions. Furthermore, higher temperature additionally increases the antioxidant potential of the extracts. Hence, it can be assumed that high temperature leads to the co-extraction of additional secondary metabolites contributing potentially to the extract's radical scavenging capacity. On the other hand, PLE extractions were carried out at 75 °C with varying ethanol concentrations for the valorization of the stem´s bioactive compounds. The extracts were characterized for the same components identified in the stem. However, in contrary to the leaves extracts, high amounts of resveratrol were detected and no flavonols. Yields of resveratrol were 30% up to 99% higher than those of the leaves. Extraction with total ethanol rendered the highest extraction yields. Condensed tannins and antioxidant capacity were the highest, when extracting with 50 vol% ethanol, which corresponds to the behavior observed for the leaf extracts.

Table 2 shows a comparison of other reported bioactive compounds in extracts from grapevine shoots. Both red and white grape types and different grape varieties are used for comparison. To the author's best knowledge, it was not possible to find studies on the specific bioactive compounds extracted from the variety *Grüner veltliner*. In addition, only studies reporting extraction technologies comparable with PLE were selected. Among the detected compounds, *Grüner veltliner* extracts with PLE showed higher extraction yields for quercetin (from the leaves) and resveratrol (from the stem).

Table 3 – Extraction yields obtained for the leaves in the standalone LHW (160 °C and 90 min) and the LHW (160 °C and 90 min) after PLE (75 °C and 50 vol% EtOH). ($n = 2$).

Component		LHW	LHW after PLE
		Extraction Yield (g/g DM FS)	
Monomeric	Hemicellulosic	3.99E-04	4.23E-04
	Cellulosic	2.00E-04	6.15E-04
Total	Hemicellulosic	0.026	0.019
	Cellulosic	0.022	0.038
	Summed[a]	0.048	0.057

[a] Summed total sugars correspond to the sum of total hemicellulosic and cellulosic sugars.

These results corroborate the potential of this feedstock for the extraction of bioactive compounds.

3.2.1. Lignocellulosic valorization of the GVS leaves after the PLE

After studying the extraction of bioactive compounds, 75 °C and 50 vol% EtOH were chosen and the solid after the extraction was used to evaluate the sugar production through LHW. The selected conditions were the same as the conditions selected for the stems (160 °C for 90 min). Additionally, a standalone LHW at the same conditions was carried out to identify if performing the PLE first improves the sugar production. Table 3 shows the extraction yields obtained for the leaves for the standalone LHW and the LHW after PLE. The yields were not considerably high with values below 0.03 g of sugars per g of dry feedstock. However, the performing the PLE first increased the yields. This can be explained because in the PLE extraction, extractives are solubilized, which makes the solid more accessible to hydrolysis in the subsequent LHW stage. A similar trend was observed by (Scopel and Rezende, 2021) while processing elephant grass first with

(a)

(b)

(c)
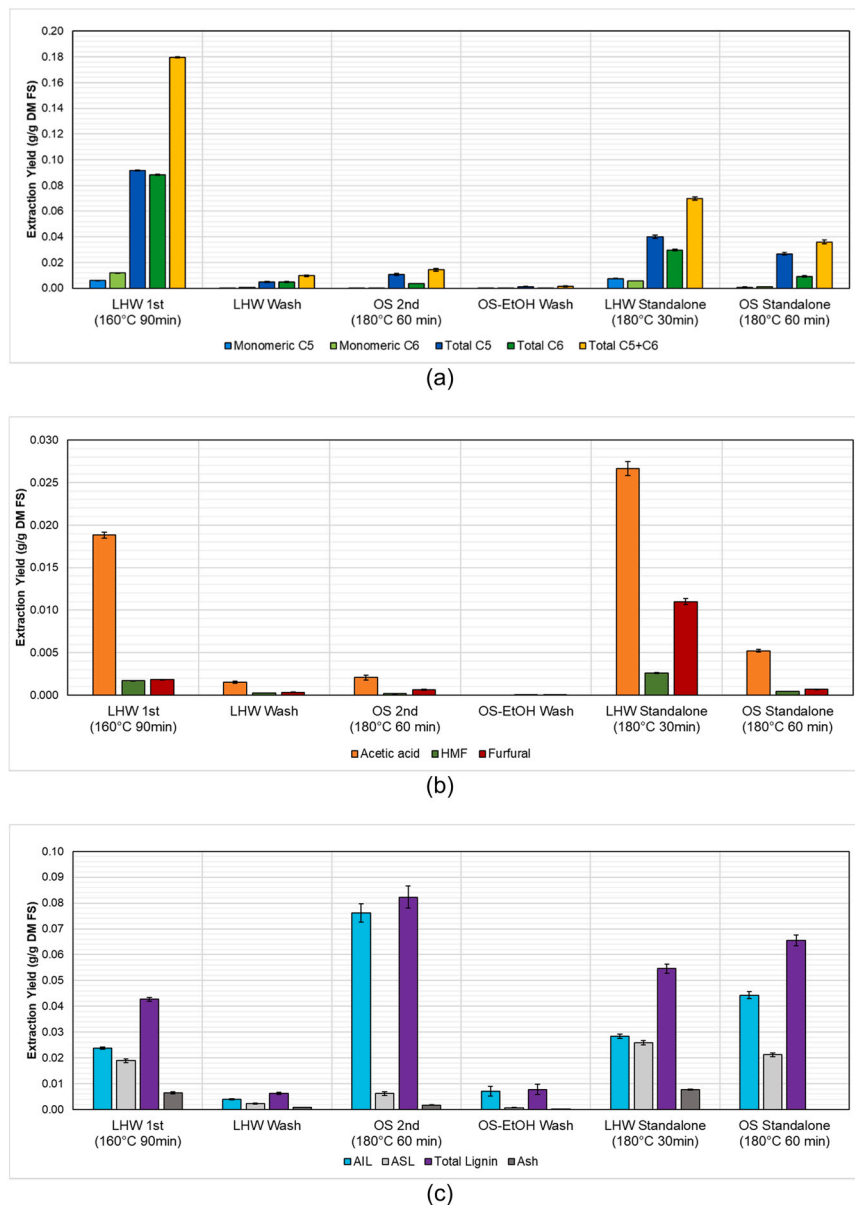
Fig. 4 – Extraction yields of the LHW and OS stages in terms of sugars (a), degradation products (b), and lignin (c) for the standalone and sequential LHW-OS of the GVS stems. DM FS: Dry matter feedstock.

PLE followed by an alkaline pretreatment, and (Kitrytė et al., 2018) in a sequential extraction of cannabinoids followed by an enzymatic hydrolysis. In both cases, performing PLE improved the yields in the subsequent stage.

3.3.   *Lignocellulosic valorization stage of the GVS stems*

This section focuses on the valorization of the stem platforms. Due to the similar lignocellulos composition when compared with wheat straw, a sequential LHW-OS pretreatment was performed (Serna-Loaiza et al., 2020). After the sequential LHW-OS pretreatment, sugar and lignin extracts as well as a cellulose-enriched solid were obtained. For comparison purposes, two additional extractions were carried out: a standalone OS at 180 °C and 60 min to corroborate if the sequential pretreatment improved lignin extraction, and a standalone LHW at 180 °C and 30 min holding time to compare the conditions chosen for the sequential pretreatment. Fig. 4(c) shows the characterization of the fractions extracts obtained during the sequential combination LHW-OS.

The LHW stage has a higher sugar concentration (16 g/L of total sugars for the sugar extract compared with 1.3 g/L for the lignin extract), and the ratio between pentoses and hexoses is almost 50 %. When comparing the LHW-1st extract and respective wash (LHW Wash), sugar concentrations were below 7 % and between 8 % and 19 % for the other components in the wash. In addition, when compared with the standalone LHW, it can be observed that the selected conditions rendered doubled the sugar extraction yields. Regarding the degradation products, the LHW stage has the higher concentration as it is the stage where most hemicellulose is hydrolyzed. When compared the LHW-1st with the standalone LHW, a higher concentration is observed for the standalone process, which means more hydrolyzed sugars were further degraded to HMF and furfural. For the OS stage, the lignin extraction yield was approximately two times higher than the yield reached in the LHW stage, which is favorable for the process as more lignin is available in the OS extract for further valorization. In addition, compared with the standalone OS, the lignin extraction yield increased 20.3 %, which corroborates that the sequential LHW-OS
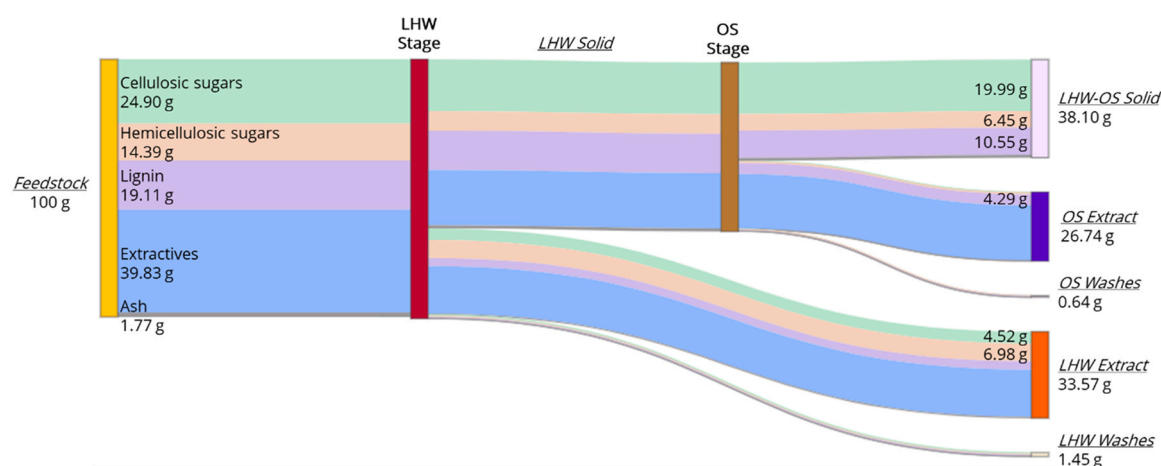
Fig. 5 – Distribution of cellulose, hemicellulose, lignin, extractives, and ash through the different fractions of the LHW-OS sequential pretreatment of the GVS stems. Hemicellulosic and cellulosic sugars in the OS Extract are 0.82 g and 0.08 g, respectively. Lignin in the LHW Extract is 3.2 g.

pretreatment improved the integral valorization of the feedstock. When comparing the OS-2nd extract with the OS-EtOH wash, all components in the wash are between 8 % and 12 % of the values reached in the extract.

3.4. Mass balance of the sequential LHW-OS of the GVS stem

Subsequently, the composition of the final solid was calculated based on the liquid fractions, hence identifying the distribution of components along the stages. Fig. 5 shows the distribution of cellulose, hemicellulose, lignin, extractives, and ash, along with the different fractions of the sequential pretreatment. Degradation products were backward calculated to sugars, and the total amount of sugars were converted into the respective oligomer and, therefore, determined the solids' composition in the different stages. It can be assumed that glucan corresponds to cellulose and the other carbohydrates (arabinan, galactan, xylan, and mannan) as hemicellulose, and the values were scaled up to 100 g of solid feedstock. An extraction yield of 19.7 %, 55.2 %, 44.8 %, 100.0 %, and 37.5 % was achieved for cellulose, hemicellulose, lignin, extractives, and ash. These values were calculated as the fraction in the final solid (LHW-OS solid) compared with the mass in the feedstock.

The final solid has a composition of 52.4 %, 16.93 %, 27.7 %, 0.0 %, and 1.1 %wt of cellulose, hemicellulose, lignin, extractives, and ash, respectively, representing 38.1 % of the initial mass of the feedstock. The LHW extract solubilized and hydrolyzed 28.04 % of the initial hemicellulosic sugars and 12.85 % of the lignin contained in the feedstock. On the other hand, the OS extract contains 17.24 % of the lignin from the feedstock. These results indicate that even when the sequential pretreatment increases the extraction yields, the level of delignification of the feedstock did not reach 50 %, leaving room to improve the pretreatment stage. In addition, a significant amount of glucose was produced, and it should be investigated if the cellulosic fibers were hydrolyzed or if the hemicellulose heteropolymer contains a significant concentration of glucose, as this would determine the possible use of the final solid for pulp and paper applications. Fig. 6 presents the mass balance for the grapevine shoots up to the calculated valorization of quercetin (using the highest obtained yield for the PLE extraction at 75 °C with 50 vol%

EtOH), other flavonoids (summing the yields for the other characterized bioactive compounds), condensed tannins, and the yields for the production of total sugars and lignin. In total, the valorization of the leaves and stem into extracts, sugars, and lignin represented 4 % and 62 % of the solid, respectively (as shown in Fig. 6(a)).

However, there are certain possibilities to improve the usage of the feedstock, especially the leaves. As shown in Fig. 6(b), using the leaves remnant after the PLE extraction for sugar production increases the absolute sugars output in ∼50%, and the overall valorization of the leaves increases from 4 % to 69 %. In addition, the biomass parts that cannot be used to produce materials could supply the process with energy. Therefore, the theoretical methane production was determined using the calculated composition of the remaining solids after the extraction and platform-valorization stages. It should be noted that this study does not optimize the energy supply of the developed biorefinery concept; however, having an idea of the potential energy production is a relevant factor for the sustainability of the process. Considering that the exact chemical composition of a pretreated feedstock is rarely known, the theoretical methane yield can be calculated by degrading the Chemical Oxygen Demand (COD) of a substrate while biogas is formed (Bischofsberger et al., 2009; Linke et al., 2000). For this purpose, Eq. (3) was used to calculate the theoretical amount of methane obtained from a given feedstock (Wang et al., 2010).

$$Mass(CH_4) = 0.25^*Mass(Feedstock)^*COD_{Mix} \quad (3)$$

COD_{Mix} is the COD of a mixture calculated using AspenPlus V10. The software calculates this parameter for a mixture (kg O_2/kg feedstock) based on the chemical composition of a substance $C_CH_HCl_{Cl}N_NNa_{Na}O_OP_PS_S$, calculated at 25 °C. The factor 0.25 corresponds to the stoichiometric factor from the oxygen demand of methane (Wang et al., 2010). The physical properties of the lignocellulosic components (cellulose, hemicellulose, lignin, and sugars) were taken from the Aspen Plus® NREL database for biofuel components (Wooley and Putsche, 1996) and based on the calculated compositions. The remaining solid from the leaves showed a slightly higher theoretical amount of produced methane than the stems, and the composition of both remaining solids rendered the highest theoretical methane production (0.27 g of methane per g of remaining solid, 410.9 mL CH_4/g remaining solid).

(a)

(b)
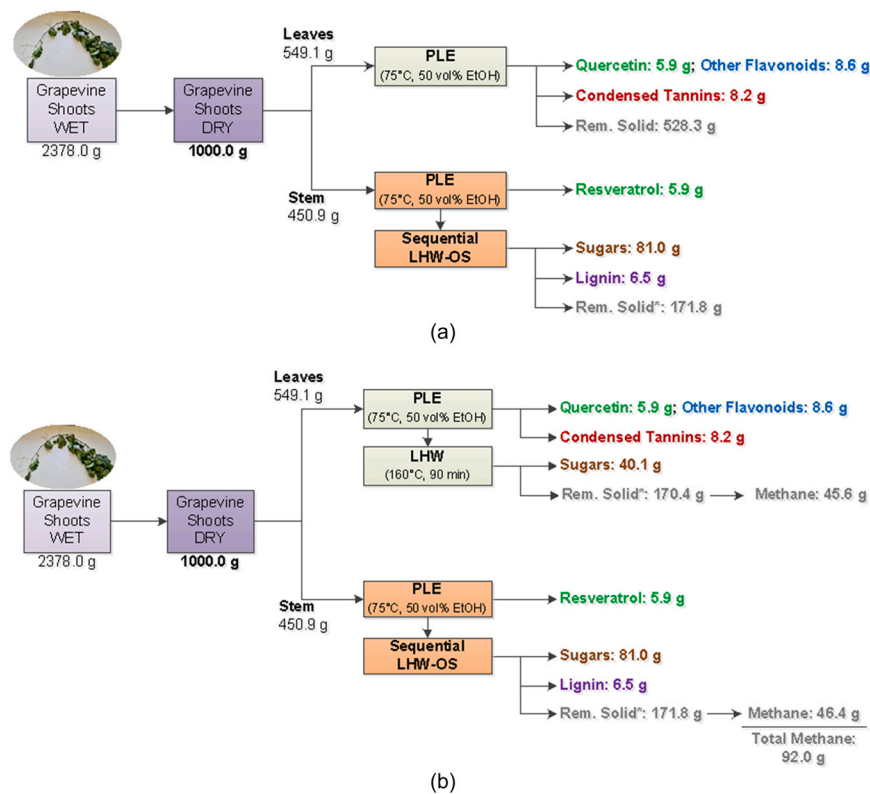
Fig. 6 – Schematic mass balance of the valorization of the grapevine shoots into bioactive compounds, sugars, lignin and the remaining pulp. (a) Current scheme including the production of bioactive compounds, sugars, lignin and the remaining pulp. (b) Prospective valorization including using the leaves remnant after the PLE extraction for sugar production and theoretical production of methane from the remaining solids. *Remaining Solids (Rem. Solid) corresponds to the solid after the pretreatment, including the solubilization of extractives, ash, and hemicellulose.

This theoretical value is in similar range for other feedstocks as maize (349.5 mL CH_4/g dry feedstock), maize silage (393.3 mL CH_4/g dry feedstock), sorghum (341–378 mL CH_4/g dry feedstock), sugar beet (350–400 mL CH_4/g dry feedstock), or wheat (270–330 mL CH_4/g dry feedstock) (Martínez-Gutiérrez, 2018). It is important to note that the referenced results correspond to experimental tests, and not theoretical calculations. The experimental value for the remaining solids will be lower, and even closer to the values reported for comparison.

4. Conclusions

This work evaluated the valorization potential of Grüner Veltliner's grapevine shoots under a biorefinery concept. The specific composition and potential bioactive compounds of the variety *Grüner veltliner* were determined. The leaves and the stems as separated fractions offer a different profile of bioactive components with potential applications, namely, quercetin from the leaves and resveratrol from the stem. Both components were found in concentrations higher than other reported grape varieties. On the other hand, the stems showed an interesting lignocellulosic composition similar to other feedstocks typically used for hemicellulosic sugars production and lignin extraction (e.g., wheat straw). In addition, the final solid after the pretreatment has a decreased content of hemicellulose, lignin, and extractives, meaning a cellulose-enriched solid.

Therefore, subsequent studies should focus on characterizing the chemical properties of the extracted lignin

(e.g., molar mass distribution, S-G-H ratio, among others), as this characterization determines the possible applications of the lignin. For the cellulose-enriched pulp, it would be necessary to determine the fiber length of the GVS stems and the pulp after the pretreatment, evaluate if the usage as pulp for cardboard making is feasible, or evaluate experimentally the biogas production potential, and evaluate the usage for the energy supply of the process. Another topic that should be further studied is the integration and valorization of the grapevine shoots as a whole or as separate fractions, meaning to determine if two separate process lines should be performed to valorize the leaves and stems separately, or processing them together to increase the available solid to be processed. As mentioned at the beginning of this study, the actual availability and usability of the leaves and stems at the crop level, meaning during the pruning stage, can vary significantly among producer. Therefore, understanding the necessary changes and adaptations at the crop stage will determine the actual availability and the state (e.g., fresh, air-dried, with/without leaves) of the shoots.

These findings open a new field of interest and research questions for the valorization of this specific "under-valorized residue" (feedstock) produced in Austrian vineyards: the downstreaming for the purification or use as a mixture of the extracted bioactive compounds, studying the seasonal variability in the composition along the crop stages, and evaluation the supply-chain and production cycle of the shoots to determine the actual available amount to be used, among others.

Declaration of Competing Interest

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

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