Antioxidant and antimicrobial activities of extracts obtained from the refining of autohydrolysis liquors of vine shoots

Beatriz Gullón[1], Gemma Eibes[*1], Maria Teresa Moreira[1], Izaskun Dávila[2], Jalel Labidi[2], Patricia Gullón[2]

[1]Department of Chemical Engineering, Institute of Technology, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain

[2]Chemical and Environmental Engineering Department, University of Basque Country, 20018 San Sebastián, Spain

Abstract

Vine shoots, considered a residue from winery operations, possess valuable antioxidant and antimicrobial activities that can be potentially obtained under the scheme of biorefinery. In this framework, we evaluated the autohydrolysis of vine shoots combined with a further stage of extraction with ethyl acetate as viable valorization process. The extraction yield ranged from 0.95 to 3.80 g extract/100 g vine shoots. Moderately high temperature (215 ºC) was required for the maximum recovery of phenolics, flavonoids and antioxidant activities. The major phenolic compounds identified were derived from lignin: vanillin, acetovanillone, guaiacylacetone, syringaldehyde and acetosyringone. The ethyl acetate extract from the liquors obtained at 200 ºC was assayed for antimicrobial activity against Gram positive and negative bacteria showing values of MIC and MBC in the range of 5-20 mg/mL. This work showed that the antioxidant extracts could be used as cheap source of natural compounds, with potential applications in the food and pharmaceutical industries.

Keywords: antioxidant, vine shoots, autohydrolysis, antimicrobial, biorefinery, ethyl acetate

* Corresponding author at: Institute of Technology, Universidade de Santiago de Compostela
E-mail address: gemma.eibes@usc.es (Gemma Eibes)

1.Introduction

The concept of biorefinery is defined, according to the International Energy Agency (IEA) Bioenergy Task 42, as "the sustainable processing of biomass into a spectrum of marketable biobased products and bioenergy" (Rombaut et al., 2014). The multi-purpose cascading biorefinery takes advantage of the various components in biomass and their intermediates in a series of technical and economically viable processes, compatible with reduced environmental impacts (Xiu et al., 2011).

An approach to achieve the fractionation of biomass framed within the biorefinery concept is the hydrothermal processing or autohydrolysis that is based on the use of hot and compressed water as extraction method for several raw materials such as hardwood, softwood, forest, agricultural and industrial residues (Hernández et al., 2014). Autohydrolysis treatment is an environmental friendly technology because aside from water and the lignocellulosic material, no chemicals are used (Vegas et al., 2005). The rationale behind this process is based on the autoionization of water and the endogenous production of organic acids from the lignocellulosic material which causes the breakdown of xylan chains, yielding a solution containing mainly oligosaccharides (Vegas et al., 2005).

However, as reported elsewhere, additional side processes can take place simultaneously with the hydrolytic degradation of xylans, including extractive removal, solubilization of acid-soluble lignin, acetic acid generation from acetyl groups, sugar dehydration to furfural and hydroxymethylfurfural ,ash neutralization and Maillard reactions (Vegas et al., 2005). As consequence of all these effects, undesired non-saccharide

compounds, such as phenolic or sugar derived compounds are present in the aqueous phase from the hydrothermal processing, which need to be removed in a further stage leading to final isolates with increased contents of oligosaccharides (Vegas et al., 2005).

Several approaches have been proposed to carry out the refining of hydrolysates. In particular, solvent extraction was proposed as a suitable alternative to isolate non-saccharide fractions with antioxidant properties from liquors obtained by hydrothermal processes (Vegas et al., 2005). This strategy allows an integrated benefit such as obtaining a refined aqueous phase enriched in oligosaccharides with potential application in the food sector and the recovery of a fraction soluble in the organic phase mainly made up of phenolics and extractive-derived compounds with remarkable antioxidant activity (Conde et al., 2011). Likewise, antimicrobial properties have been reported for antioxidant extracts from lignocellulosic materials, being potentially useful as a source of food additives aimed at increasing the shelf life of food (Moreira et al., 2016). In fact, the rising awareness of consumers by the health-food binomial has promoted the research on natural sources of antioxidant and antimicrobial compounds that can substitute their synthetic versions in the food and cosmetic sectors (Moreira et al., 2016).

Grape crop is a very important economic activity that generates vast amounts of agricultural residues (particularly vine shoots and leaves). According to Peralbo-Molina and Luque de Castro (2013), vine shoots are produced in an estimated total of 11.2–16 million tons each year worldwide. Taking into account these data and the scarce literature about their hydrothermal processing, Dávila et al. (2016) carried out the non-

isothermal processing of vine shoots at different severity conditions in aqueous media (autohydrolysis reaction) to obtain solutions containing valuable chemicals (oligosaccharides) as well as extractive and acid-soluble fractions of lignin that need to be removed in additional purification treatments. Here we attempt to go one step further than Dávila et al. (2016), and we propose a strategy which permits both the refining of the saccharide fraction and the recovery of an antioxidant rich fraction. In this sense, solvent extraction with ethyl acetate has been reported by its high selectivity in the removal of extractive and lignin derived compounds such as resins, fatty acids, alcohols, esters, waxes and low-molecular weight phenolics (Vegas et al., 2005) while yielding a refined aqueous phase with increased content of saccharide-derived compounds.

To our knowledge, the use of the liquid stream from the autohydrolysis treatment of vine shoots to obtain two separated fractions containing added value compounds with several activities and properties, has not been explored yet.

The main objective of this work is to assess a solvent extraction stage to separate ethyl acetate soluble compounds (EASCs) with antioxidant activity with the aim of refining the oligosaccharide fraction obtained from the autohydrolysis liquors of vine shoots. The flow diagram depicted in Figure 1 shows a possible multiproduct process for the different streams obtained from the autohydrolysis of vine shoots. This strategy is part of a holistic biorefinery outline for the valorization of vine shoots into a huge variety of chemicals and added-value products.

2. Materials and Methods

2.1. Sample conditioning

Vine shoots (*Vitis vinifera* variety Hondarribi Zuri) were locally collected (Hoiarztun, Guipúzcoa, Basque Country, Spain), milled using a laboratory cutter mill to a particle size smaller than 0.4 mm, homogenized in a single lot to avoid compositional differences, dried at room temperature until constant moisture and stored in darkness at room temperature until use.

2.2. Autohydrolysis treatment and solvent extraction of liquors

Vine shoots were mixed with water at a liquid-solid ratio of 8 g/g (db) and heated in a 1.5 L stainless steel 5100 Parr reactor under non-isothermal conditions with standard heating temperature profile between 180 and 215 ºC. After cooling, the liquor was separated from the solid phase by filtration under vacuum using filter paper No.41 MN640W. The liquor was extracted with ethyl acetate at a liquor:solvent ratio of 1:3 (v/v) at room temperature under stirring for 15 min in a single extraction stage. Both immiscible phases were separated by decantation. The ethyl acetate soluble phase was vacuum evaporated at 40 ºC for the recovery of the solvent and the removal of volatile dissolved compounds.

2.3. Characterization of the autohydrolysis liquors, extracts and refined liquors

The streams used in this work were: the autohydrolysis liquors obtained in the temperature range from 180 to 215 ºC, the ethyl acetate soluble compounds obtained by extraction from these liquors and the refined liquors

after the solvent extraction. The analyses described below were carried out in triplicate.

2.3.1. Sugar composition and NVC content of autohydrolysis liquors and refined liquors

The autohydrolysis liquors and refined liquors were analyzed to determine its composition into the target compounds such as oligosaccharides and other non-volatile compounds (ONVC) following the methodology described by Dávila et al. (2016). An aliquot of autohydrolysis liquors and refined liquors was neutralized with $BaCO_3$, filtered through 0.22 μm nylon membranes and analyzed by HPLC for the determination of mannose using a Jasco LC Net II/ADC chromatograph equipped with a refractive index detector operated at 30 ºC. The column used for this quantification was a 300 x 7.8 mm CARBOSep CHO-682 column (Transgenomic) operating at 80 ºC and eluting 40 μL of the sample at 0.4 mL/min using distilled water as mobile phase. The determination of other monosaccharides (glucose, xylose and arabinose), galacturonic acid, acetic acid, hydroxymethylfurfural and furfural was carried out by HPLC using a 300 x 7.8mm Aminex HPX-87H column (Bio-Rad Laboratories) eluting 20 μL of the sample with 0.6 mL/min of 0.005 M H_2SO_4 at 50 ºC. Other aliquots of the autohydrolysis liquors and refined liquors were subjected to a quantitative post-hydrolysis (treatment with 4% H_2SO_4 at 121 ºC for 30 min). The monosaccharides and acetic acid present in the resulting samples were quantified by HPLC as aforementioned. The increase in the concentrations of monosaccharides, acetic acid and galacturonic acid observed during the post-hydrolysis provided a measure of the oligomer concentration and their degree of

substitution by galacturonic and acetyl groups. The oligosaccharides were expressed as monosaccharide equivalents.

The content of non-volatile compounds (NVC) in the autohydrolysis liquors and refined liquors was measured by oven-drying at 105 ºC until constant weight. The content of impurities (called other non-volatile compounds, denoted as ONVC) was calculated by the difference between the NVC and the saccharide content (including monosaccharides, oligosaccharides and oligosaccharide substituents) determined by HPLC as described above.

2.3.2. Extraction yield

The yield of the solvent extraction process was calculated on the basis of the EASC content. The extraction yield was measured by the content of non-volatile solids in extracts by oven-drying at 105 ºC until constant weight and calculated as g of non-volatile solids per 100 g of vine shoots (% w/w vine shoots, db).

2.3.3. Total phenolic content (TPC) and Total flavonoid content (TFC) determination

The autohydrolysis liquors and the EASCs were evaluated for the total phenolic content, expressed as mg of gallic acid equivalents (GAE)/g dried vine shoots, according to the Folin-Ciocalteau spectrophotometric method (Singleton and Rossi, 1965). The total flavonoid content of the different streams was determined following the method described by Blasa et al. (2005). Rutin was used as standard and the results were expressed in mg of rutin equivalents (RE)/g dried vine shoots.

2.3.4. Antioxidant activity

The measurement of antioxidant activity was carried out only for EASCs. Three complementary methods were used as explained hereunder. For all antioxidant activity assays, Trolox was used as standard and results were expressed as mg of Trolox equivalents (TE)/g vine shoots db as mean of three replicates.

The DPPH (α,α-Diphenyl-β-picrylhydrazyl) assay was performed following the methodology described by von Gadow et al. (1997). Two milliliters of a methanolic solution of DPPH with a concentration of $6 \cdot 10^{-5}$ M was added to 50 μL of an ethanolic solution of each sample (crude and refined liquors and extracts), and the decrease in absorbance at 515 nm after 16 min was recorded in a Shimadzu UV-1800 spectrophotometer.

The Trolox equivalent antioxidant capacity (TEAC) was measured using the ABTS assays according to Re et al. (1999). This method is based on the scavenging of ABTS radical 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid). ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate (final concentration). The mixture was allowed to stand in darkness at room temperature for 12-16 h before use and then diluted with phosphate buffer saline (PBS) (pH 7.4) to an absorbance of 0.70 at 734 nm and equilibrated at 25 ºC. After the addition of 2 mL of diluted $ABTS^{\bullet+}$ solution to 20 μL of diluted samples or Trolox standards in ethanol or PBS, the absorbance readings were recorded for 6 min.

The ferric reducing antioxidant power (FRAP) test is based on the reduction by the antioxidant compound of Fe^{3+} to Fe^{+2} which forms a colored complex (593 nm) with 2,4,6-tripyridyl-s-triazine (Fe^{+2}-TPTZ) in

acetate buffer at pH 3.6 (Benzie and Strain, 1996). Briefly, the reactive solution was freshly prepared with 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM 2,4,6-tripyridyl-s-triazine in 40 mM HCl and 2.5 mL 20 mM $FeCl_3 \cdot 6H_2O$ in distilled water. Samples (0.1 mL) were mixed with 3 mL of the FRAP reactive solution.

ABTS and DPPH assays are based on the antioxidant ability to react with the free radicals generated in the assay systems, whereas the FRAP assay measures the reduction of Fe^{3+} (ferric iron) to Fe^{2+} (ferrous iron) in the presence of antioxidants. Hence, the FRAP assay is a direct method which measures antioxidants or reductants in a sample, whereas the other assays are more indirect because they measure the inhibition of reactive species generated in the reaction mixture (Singh and Singh, 2008).

2.3.5. Qualitative analysis of the extracts by HPLC

The ethyl acetate soluble phases were dissolved in the mobile phase (aqueous solution of acetonitrile/water in a ratio 1:8 (v/v) with 1 % (v/v) of acetic acid) and further analysed by HPLC using a Jasco LC Net II/ADC chromatograph equipped with a photoarray detector (MD-2018) with a Teknocroma Mediterranean sea18 TR-010006 column (25 cm x 0.46 cm), eluting 40 µL of the sample at 40 ºC with a flow of 0.5 mL/min. The compounds used as standards for the qualitative analysis were furfural, vanillin (Panreac, Spain), 5-hydroxymethylfurfural (Acros Organics, USA), acetovanillone (SAFC, USA), gallic acid (Scharlau, Spain), acetosyringone vanillic acid, syringaldehyde, synaptic acid, syringic acid, guaiacol, syringol, phenol, coumaric acid, guaiacylacetone, catechol, 4-hydrobenzoic acid,

quercitin, 4-hydroxybenzaldehyde (Sigma-Aldrich Co., Germany), considering what it was found in literature (Conde et al., 2011).

2.3.6. Gas Chromatography- Mass Spectrometry (GC-MS) analysis

The ethyl acetate soluble phases from the extraction of the autohydrolysis liquors obtained at different temperatures were analyzed by GC-MS. The extracts were dissolved in ethyl acetate at a concentration of 0.02% (w/v) and analyzed in an Agilent Technologies 7890A gas chromatograph (GC) coupled to an Agilent Technologies 5975C mass spectrometer (MS) using He as gas carrier with a 1 mL/min flow. One µL of the samples was introduced in the GC in split mode. The separation was carried out using a 30 m x 0.25 mm x 0.25µm film thickness HP-5MS (5% phenyl-methylpolysiloxane) column. Temperature was increased from 50 ºC to 120 ºC (5 min) at 10 ºC/min, then to 280 ºC (8 min) at 10 ºC/min and finally to 300 ºC (2 min) at 10 ºC/min. The mass spectrometer was in electron ionization (EI) mode with electron energy of 69.9 eV. The compounds were identified by the analysis of retention time and mass spectra of the compounds compiled in the National Institute of Standards Library (NIST) and other reports elsewhere (Nunes et al., 2010).

2.4. *In vitro* assessment of the antimicrobial activity of the EASCs

2.4.1. Bacterial strains and culture conditions

The ethyl acetate soluble compounds from the autohydrolysis liquors obtained at 200 ºC under non-isothermal conditions was tested for antibacterial activity against six bacterial strains: *Listeria innocua* (NCTC 10528), *Staphylococcus aureus* (isolated from food sample, accession number 18N, collection from CINATE), *Escherichia coli* (ATCC 25922),

Bacillus cereus (DSM 4313), *Pseudomonas aeruginosa* and *Salmonella* sp. (isolated from food sources, internal collection from CINATE). Strains were mixed with glycerol at 15% (v/v) and stored in criovials at -80 ºC until use. The frozen stock cultures were reactivated by inoculation in sterile Mueller-Hinton broth (MHB) at 37 ºC for 10-12 h under aerobic conditions without shaking, and then an aliquot from each culture was transferred to fresh MHB and cultured overnight at 37 ºC in tubes. The variable concentrations of the EASCs were inoculated at 2% (v/v) with each tested bacteria resulting in a final population of 10^6 CFU/mL.

2.4.2. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of EASCs

The minimal inhibitory concentration (MIC) is defined as the lowest concentration of extract necessary to inhibit microbial growth after 24 h of incubation at 37 ºC. The minimal bactericidal concentration (MBC) is considered as the lowest concentration of extract capable to inhibit bacterial growth and, in addition, the initial viability of the microorganisms is reduced by 99.9% after 24 h of incubation at 37 ºC (Moreira et al., 2016).

Antimicrobial activity of EASCs was assessed using a microdilution assay according to Moreira et al. (2016). The tested EASCs concentrations ranged from 5 to 20 mg/mL. Negative and positive controls were carried out with the aim of checking potential contaminations and for comparative purposes. The negative control was an abiotic control with MHB supplemented with DMSO (5% v/v) and EASCs at variable concentrations; the positive controls were prepared with inoculated MHB supplemented with

DMSO (5% v/v) without EASCs. The microplates were incubated for 24 h at 37 ºC.

Bacterial growth reductions were evaluated by comparing viable cell counts between the positive control and the trials with the EASCs at 0 h and 24 h. For this, 100 µL of each culture media was diluted using peptone water through serial decimal dilutions and 20 µL of each dilution was placed on MH agar. Plates were counted after incubation at 37 ºC for approximately 48 h. All assays were performed in triplicate to confirm the reproducibility of the results.

2.5. Statistical analysis

Statistical analysis was performed using SPSS for Windows version 23.0 (IBM SPSS, Chicago, IL). Significant differences among the results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test considering a significance level of $p<0.05$.

3.Results and discussion

3.1. Sugar composition and NVC content of autohydrolysis liquors and refined liquors

The autohydrolysis liquors and refined liquors were constituted by volatile (VC) and non-volatile compounds (NVC). The composition of the NVC fraction included monosaccharides (glucose, arabinose, xylose and mannose) and galacturonic acid, oligosaccharides obtained from the hydrolysis of glucan, arabinan, xylan and mannan and also acetyl and galacturonyl substituents (as part of oligosaccharides). However, apart from the saccharides, the NVC fraction also contained compounds derived from extractives, acid-soluble lignin and proteins, which could be present due to

additional side processes, such as extractive removal, solubilization of acid-soluble lignin or ash neutralization, which could occur simultaneously to hemicellulose hydrolysis (Vegas et al., 2005). The latter compounds were denoted as other non-volatile components (ONVC). Table 1 shows the composition of the NVC fraction of the autohydrolysis liquors and refined liquors obtained at different temperatures. Autohydrolysis at 200 ºC resulted in the liquor with the highest content of oligosaccharides. Figure 2 shows the mass balances of the NVC fraction of the various streams involved on the autohydrolysis and refining stages. The NVC content of autohydrolysis liquors obtained at 200 ºC was 27.7 kg/100 kg dry vine shoots, of which 23.84 kg corresponded to substituted oligosaccharides (given by the joint contribution of glucooligosaccharides, xylooligosaccharides, arabinooligosaccharides, mannooligosaccharides, galactooligosaccharides, acetyl and galacturonyl groups linked to oligomers), and 2.13 kg/100 kg dry vine shoots were ONVC. These results are in line with the reported by Caparrós et al. (2007) who carried out the autohydrolysis of *Pawlonia Fortunei L.* wood trimmings at 200 ºC obtaining 20.40 kg NVC/100 kg dry raw material.

In order to reduce the content of compounds to be discarded, a refining step was performed. This separation method with ethyl acetate resulted in a decrease of the ONVC content in the refined liquors as shown in Table 1. The removal of the undesired compounds was variable with percentages between 11.7 to 50.6% of the ONVC. The total content of substituted oligosaccharides increased by 2.6% to 14.5%, in agreement with the results reported by Vázquez et al. (2006). Regarding the aqueous phase, this fraction contained 22.86 kg of total oligosaccharides/100 kg dry

vine shoots and 0.98 kg ONVC/100 kg dry vine shoots, which represented a slight decrease of the oligosaccharides content and a marked reduction of ONVC. Therefore, the purity of the substituted oligosaccharides obtained after the purification step is suitable for food-related applications (usually with purity in the range 75–95%). Additionally, 1.75 kg of antioxidant extract/100 kg of dry vine shoots was also obtained. These results confirm the suitability of the purification step with ethyl acetate to obtain two separated streams containing value added products.

3.2. Extract yield, phenolic and flavonoid content

Total phenolic (TPC) and total flavonoid (TFC) contents in the autohydrolysis liquor of vine shoots and in the ethyl acetate extracts as well as the yield of ethyl acetate extraction are summarized in Table 2. The extraction yield ranged from 0.95 to 3.80 g extract/100 g dry vine shoots. The highest extraction yield corresponded to the highest temperature tested. This is in agreement with the findings reported by Conde et al. (2011) that also observed an increase in the yield with the severity of treatment for various lignocellulosic materials.

Increasing both phenolic and flavonoid content with temperature was observed in the autohydrolysis liquor and in the ethyl acetate phase after solvent extraction ($P < 0.05$). This increase was more evident for the phenolic content, since the gallic acid equivalents of the ethyl acetate extracts were 4.2 times higher at 215 ºC compared to those at 180 ºC, whereas the rutin equivalents were only 1.7 times higher.

The extraction at higher temperature also influenced the yields of phenolics, that ranged from 44% (at 180 ºC) to 80% (at 210 ºC) as well as

flavonoids, with a recovery range between 68% (at 180 ºC) to 85% (at 210 ºC). The antioxidants present in the vine shoots are amphiphilic and it seems that those present at the higher autohydrolysis temperatures are more soluble in the organic phase than in water, which increased recovery yields (Garrote et al., 2008).

The maximum phenolic content in the ethyl acetate extract (1.62 g GAE/100 g of dry matter at 215 ºC) was significantly higher than that reported by Rajha et al. (2015) for the extraction of polyphenols from vine shoots. In this report, the optimal TPC after 48 h of β-cyclodextrin (β-CD)-assisted extraction was 0.58 g of GAE/100 g of dry matter. In another work, the intensification of the polyphenol extraction of vine shoots was performed using high-voltage electrical discharges as a pretreatment, obtaining 3.45 g of GAE/100 g of dry matter (Rajha et al., 2014). The authors concluded that the pretreatment induced cellular and structural damage in vine shoot tissues with product fragmentation. In the present research, the high content of phenolics in the autohydrolysis liquor (0.88-2.25 g GAE/100 g dry matter) is in line with the values reported for both liquid hot water and steam explosion treatment of olive tree pruning (Conde et al., 2009). These results suggest that partial lignin depolymerization may take place, at least under the harsher autohydrolysis conditions. On the other hand, it has also been reported that high autohydrolysis temperatures promote the release of phenolic compounds linked to the oligosaccharides solubilised and cause the partial depolymerization of lignin yielding small molecular fragments with phenolic nature (Table 3) (Leschinsky et al., 2008).

3.3. Antioxidant activity

The results obtained showed that antioxidant properties of extract are dependent on temperature ($P < 0.05$). Increasing the autohydrolysis temperature of vine shoots led to higher antioxidant activities of the ethyl acetate extracts using three different methods (Table 3). The ethyl acetate extracts with most active radical scavenger capacity were obtained at 210-215 ºC, both against ABTS and DPPH radicals. In addition, the highest reducing power was achieved at 215 ºC, as shown by FRAP values of the extracts.

Hence, optimal autohydrolysis temperature depends on the target product. The highest concentration of oligosaccharides was achieved at temperatures close to 200 ºC. Beyond this value, higher temperatures led to sugar decomposition. On the other hand, the highest content of phenolics, flavonoids and antioxidant activity of the ethyl acetate extracts was obtained at the maximum temperature autohydrolysis, 215 ºC. Nevertheless, the extracts from the autohydrolysis at 200 ºC still showed high activity, in the range of 74-80% of the maximum antioxidant capacity. Interestingly, at this temperature the specific antioxidant activity, i. e. the value of trolox equivalents per gram of extract (db) reached its maximum (2.03 and 1.14 g TE/g extract as measured by ABTS and FRAP assays, respectively). Conde et al (2011) analyzed the antioxidant activity of ethyl acetate extracts from the autohydrolysis liquors of different lignocellulosic wastes. The TE measured by the ABTS assay were in the range of 0.53-0.85 g TE/g extract, being the maximum for chestnut bur samples. In the present research, vine shoot extracts showed higher antioxidant capacity (1.58-4.45 g TE/g), even higher than the one reported for the synthetic

antioxidant BHA (3-tert-butyl-4-hydroxyanisole) (1.80 g TE/g) using ABTS assay (Conde et al., 2011).

3.4. Identification of phenolic compounds by HPLC-UV/PAD

In the ethyl acetate extracts obtained in the autohydrolysis conditions that lead to the maximum concentration of oligosaccharides (200 ºC in non-isothermal conditions), the major phenolic compounds identified by HPLC were gallic acid, 5-hydroxymethylfurfural (5-HMF), synaptic acid, furfural, catechol, 4-hydroxybenzoic acid, vanillic acid and syringic acid (Figure 3). These findings are in agreement with the ones reported by Garrote el al. (2008), which found the abovementioned compounds in autohydrolysis liquors of barley husks. Rivas et al. (2013) identified gallic acid, vanillic acid and syringic acid among others in autoydrolysis liquors of *Eucalyptus globulus*.

3.5. Identification and quantification of lignin-derived compounds and sugar-derived compounds by GC-MS

In order to get a deeper knowledge on the composition of ethyl acetate extracts, GC-MS analyses were carried out. The identified compounds were classified in two categories: sugar derived compounds and lignin derived compounds (Table 4).

On the basis of the data collected in Table 4, it has been inferred that the temperature of the autohydrolysis treatment exhibited an important influence both on the composition and on the percentage of each compound in the extract. The sugar derived compounds (furfural and hydroxymethylfurfural) were not detected in the extracts from the liquors obtained at temperatures lower than 195 ºC, but at higher temperatures

the quantity of these compounds in the extract increased from 4.3% at 195 ºC to 15.3% at 210 ºC. This outcome was in line with other research works that reported that a sequence of secondary reactions takes place at high temperatures causing the dehydratation of pentoses and hexoses to furfural and hydroxymethylfurfural (Conde et al., 2009; Dávila et al., 2016). In this sense, the highest temperature led to a 5-HMF percentage in the extract of 21.2%. The presence of this compound in the extract could be interesting, since in recent years 5-HMF has been reported to present some pharmacological effects, such as antioxidant, antiischemic, and antityrosine enzyme effects, improving blood rheology, and affecting the role of glycyrrhizin metabolism (Li et al., 2011). Zhao et al. (2013) demonstrated that 5-HMF exhibited novel antioxidant activity by scavenging the ABTS and DPPH free radicals in a dose-dependent manner. Furthermore, 5-HMF displayed higher antiproliferative activity on human melanoma A375 cells than other cell lines. They suggested that 5-HMF could be developed as a novel natural antioxidant with potential applications in cancer chemoprevention.

The increase of the diversity of lignin derived compounds at higher autohydrolysis temperature could be due to the increased concentration of acetic acid in the medium, which would produce the cleavage of lignin-carbohydrates bonds (El Hage et al., 2010) and the depolymerization of the lignin into small molecular fragments (Leschinsky et al., 2008). The major compounds found among the lignin derived compounds were vanillin, acetovanillone, syringaldehyde and acetosyringone. Garrote et al. (2007) found a wide variety of phenolic compounds in the dicloromethane extracts of autohydrolysis liquors of corn cobs being vanillin, syringaldehyde, 4-

vinylguaiacol and 4-vinylphenol the major components. The first two compounds were also found as the major components of steam explosion liquors of *Olea europea* wood (Castro et al., 2008).

It is important to highlight that the maximum concentration of lignin derived compounds determined by GC-MS was observed at 190 ºC (28.5 mg/100 g extract) while it decreased at higher temperature. This rationale behind this can be explained on the balance between solubilization of lignin and sequential repolymerization (Garrote et al., 2008), which is not related to the progressive increase of TPC (measured by the Folin-Ciocalteau method) and antioxidant activity of the extracts with increasing temperature (Tables 2 and 3). In fact, the enhanced antioxidant capacity could be attributed to the gradual generation of HMF and other sugar derived compounds. As mentioned above, HMF displayed scavenging activity against ABTS and DPPH radicals at concentrations above 25 μg/mL (Zhao et al., 2013). Furthermore, Chen et al. (2014) also found that HMF displayed a dose-dependent response to ORAC at low concentrations (≥1.563 μg/mL) and to TPC and FRAP at high concentrations (≥625 μg/mL).

3.6. Antimicrobial activity of the EASCs

Due to the potential toxicity and carcinogenicity of synthetic antioxidants and the potential persistence of microorganisms resistant to the available antibiotics, the use of extracts rich in antioxidants with antibacterial properties has been proposed (Moreira et al., 2016; Khaled-Khodja et al., 2014). To best of our knowledge there are no studies on the antimicrobial properties of ethyl acetate extracts from the vine shoots

liquors. In this research, considering a possible application of this extract as food preservative, several microorganisms associated with spoilage of food products were tested. The antimicrobial activity of the EASCs from the liquors at 200 ºC were evaluated by determining the values of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using the microdilution assay. Table 5 summarizes the results of the antimicrobial activity (MIC and MBC) of the EASC from the liquors at 200 ºC tested. Overall, the results indicated that EASC showed antimicrobial activity against the selected set of bacteria.

Regarding antimicrobial effect for each specific strain, *S. aureus* and *L. innocua* were the most sensitive strains with MIC and MBC value of 5 and 10 mg/mL respectively. Dong et al. (2011) have reported MIC and MBC values in the range of 1.25-5.625 and 1.5-10 mg/mL respectively, for lignin residue of corn stover obtained in the ethanol production for *S. aureus*. More recently, Moreira et al. (2016) also found MBC values ranging from 20-40 mg/mL for extracts obtained of liquors of lignocellulosic materials against *S. aureus*. On the other hand, *E. coli* was the strain more resistant, showing the highest MIC and MBC values (15 and 20 mg/mL respectively). In the present study, *P. aeruginosa*, *Salmonella* spp and *B. cereus* showed similar values of MIC and MBC of 10 and 15 mg/mL, respectively. From a qualitative standpoint, the findings of our study are in line with related literature.

Several authors have reported that Gram negative bacteria are more resistant than Gram positive bacteria due to the presence of hydrophobic lipopolysaccharide in the outer membrane that blocks the penetration of

antimicrobial agents reducing the sensitivity of these bacteria to these compounds (Moreira et al., 2016).

The antibacterial activity of these extracts could be attributed to the presence of diverse molecules including 5-HMF, furfural, syringaldehyde, vanillin, among others. In fact, these compounds have been reported in the literature for their important antibacterial properties (Chai et al., 2013; Rivero-Cruz et al., 2008). For instance, Cava-Roda et al. (2012) evaluated the antibacterial activity of vanillin against several food-related bacteria, namely *Escherichia coli* O157:H7 and *Listeria monocytogenes*. In this context, vanillin is added in a wide range of food products as flavouring agent and by its bioactive properties including antimicrobial and antioxidant properties, being considered as a nutraceutical molecule (Mourtzinos et al., 2009).

Chai et al. (2013) reported the capacity of three furan compounds for prevent growth of *Bacillus subtilis* and *Salmonella* bacteria. Rivero-Cruz et al. (2008) evaluated antimicrobial activity of different compounds isolated of Thompson seedless raisins including 5-HMF, against two oral pathogens, *Streptococcus mutans* and *Porphyromonas gingivalis*, and they suggested that these compounds may benefit periodontal health.

On the other hand, many of these compounds which are related with the degradation of lignin are present in the hydrolysates from a variety of lignocellulosic raw materials used for biofuels or chemicals production (Mussatto et al., 2004). In this context, the role of vanillin or syringaldehyde to inhibit *Candida guilliermondii* growth on xylose-to-xylitol bioconversion has been studied (Cortez and Roberto, 2010).

Taking into account that our extracts are a complex mixture of several bioactive molecules, it is not possible to attribute their antimicrobial effects to a particular constituent. In fact, several authors have reported that the antibacterial activity of different extracts is due to a synergistic effect between various constituents that are present in their composition (Moreira et al., 2016). Various mechanisms of action have been reported to explain the antimicrobial activity of antioxidant compounds, with a detrimental impact on the pathogens physiology such as damage on the cell membrane, coagulation of the cell content, inhibition of cellular functions and enzyme synthesis, among others (Aleksic and Knezevic, 2014).

Conclusions

The autohydrolysis liquors were extracted with ethyl acetate to obtain organic phases with antioxidant and antimicrobial activities and aqueous phases enriched in oligosaccharides. The operation at higher temperature resulted in a rise of the release of phenolic compounds and low molecular fragments from lignin to the aqueous phases, increasing the content of soluble compounds in ethyl acetate. The extraction yield, the TPC and the TFC increased gradually with temperature achieving maximum values at 215 ºC (3.80 g extract/100 g vine shoots, 1.62 g GAE/100 g vine shoots, 0.92 g RE/100 g vine shoots, respectively). The antioxidant capacity of ethyl acetate extract in terms of DPPH, ABTS and FRAP presented the same tendency that the one observed for the previous parameters. The extracts from the liquors obtained at 200 ºC showed inhibitory and bactericidal effects on both Gram positive and negative bacteria, indicating that could be potentially used as food additives aimed at increasing the shelf life of

food. The major phenolic compounds identified on the ethyl acetate extracts were derived from lignin such as vanillin, acetovanillone, guaiacylacetone, syringaldehyde and acetosyringone. The recovery of these phenolic compounds is a key point within an integral fractionation process by allowing the utilization of purified oligosaccharides contained in the aqueous phase and by endowing high added value to the organic fraction.

ACKNOWLEDGEMENTS

This work was funded by the Spanish Ministry of Economy and Competitiveness (MINECO, CTQ2014-58879-JIN). The authors B. Gullón, G. Eibes and M.T. Moreira belong to the Galician Competitive Research Group GRC 2013–032 and to the CRETUS Strategic Partnership (AGRUP2015/02). All these programmes are co-funded by FEDER (EU). The authors I. Davila, J. Labidi and P. Gullón belong to the Basque Competitive Research Group IT1008-16 funded by the Department of Education of the Basque Government. B. Gullón and P. Gullón would like to express her gratitude to MINECO for financial support (Grant references FPDI-2013-17341 and FPDI-2013-18748, respectively). I. Dávila would like to thank the Agriculture, Fishing and Food Department of the Basque Government (scholarship of young researchers training).

Notes

The authors declare no competing financial interest.

References

Aleksic, V., Knezevic, P., 2014. Antimicrobial and antioxidative activity of extracts and essential oils of *Myrtus communis* L. Microbiol. Res. 169, 240-254.

Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239 (1), 70-76.

Blasa, M., Candiracci, M., Accorsi, A., Piacentini, P.M., Albertini, M.C., Piatti, E., 2005. Raw *Millefiori* honey is packed full of antioxidants. Food Chem. 97 (2), 217-222.

Caparrós, S., Ariza, J., Garrote, G., López, F., Díaz, M.j., 2007. Optimization of *Paulownia Fortunei L.* autohydrolysis-organosolv pulping as a source of xylooligomers and cellulose pulp. Ind. Eng. Chem. Res. 46, 623-631.

Castro E., Conde E., Moure A., Falqué E., Cara C., Ruiz E., Domínguez H., 2008. Antioxidant activity of liquors from steam explosion of *Olea europea* Wood. Wood Sci. Technol. 42 (7), 579-592.

Cava-Roda, R.M., Taboada-Rodríguez, A., Valverde-Franco, M.T., Marín-Iniesta, F., 2012. Antimicrobial activity of vanillin and mixtures with cinnamon and clove essential oils in controlling *Listeria monocytogenes* and *Escherichia coli* O157:H7 in Milk. Food Bioprocess Tech. 5, 2120-2131.

Chai, W.M., Liu, X., Hu, Y.H., Feng, H.L., Jia, Y.L., Guo, Y. J., Zhou, H.T., Chen, Q. X., 2013. Antityrosinase and antimicrobial activities of furfuryl alcohol, furfural and furoic acid. Int. J. Biol. Macromol. 57, 151-155.

Conde, E., Cara, C., Moure, A., Ruiz, E., Castro, E., Domínguez, H., 2009. Antioxidant activity of the phenolic compounds released by hydrothermal treatments of olive tree pruning. Food Chem. 114, 806-812.

Conde, E., Moure, A., Domínguez, H., Parajó, J.C., 2011. Production of antioxidants by non-isothermal autohydrolysis of lignocellulosic wastes. LWT - Food Sci. Tech. 44 (2), 436-442.

Cortez, D. V., Roberto, I.C., 2010. Individual and interaction effects of vanillin and syringaldehyde on the xylitol formation by *Candida guilliermondii*. Bioresour. Technol. 101 (6), 1858-1865.

Dávila, D., Gordobil, O., Labidi, J., Gullón, P., 2016. Assessment of suitability of vine shoots for hemicellulosic oligosaccharides production through aqueous processing. Bioresour. Technol. 211, 636-644.

Dong, X., Dong, M., Lu, Y., Turley, A., Jin, T., Wu, Ch., 2011. Antimicrobial and antioxidant activities of lignin from residue of corn stover to ethanol production. Ind. Crop. Prod. 34, 1629-1634.

El Hage, R., Chrusciel, L., Desharnis, L., Brosse N., 2010. Effect of autohydrolysis of *Miscanthus x giganteus* on lignin structure and organosolv delignification. Bioresour. Technol. 101 (23), 9321-9329.

Garrote, G., Falqué, E., Domínguez, H., Parajó, J.C., 2007. Autohydrolysis of agricultural residues: Study of reaction byproducts. Bioresour. Technol. 98 (10), 1951-1957.

Garrote, G., Cruz, J.M., Domínguez, H., Parajó, J.C., 2008. Non-isothermal autohydrolysis of barley husks: Product distribution and antioxidant activity of ethyl acetate soluble fractions. J. Food Eng. 84 (4), 544-552.

Hernández, V., Romero-García, J.M., Dávila, J.A., Castro, E., Cardona, C.A., 2014. Techno-economic and environmental assessment of an olive stone based biorefinery. Resour. Conserv. Recycl. 92, 145-150.

Khaled-Khodja, N., Boulekbache-Makhlouf, L., Madani, K., 2014. Phytochemical screening of antioxidant and antibacterial activities of methanolic extracts of some Lamiaceae. Ind. Crop. Prod. 61, 41-48.

Leschinsky, M., Zuckerstätter, G., Weber, H.K., Patt, R., Sixta, H., 2008. Effect of autohydrolysis of *Eucalyptus globulus* wood on lignin structure. Part 1: Comparison of different lignin fractions formed during water prehydrolysis. Holzforschung 62 (6), 645-652.

Li, M.M., Wu, L. Y., Zhao, T., Xiong, L., Huang, X., Liu, Z.H., Fan, X.L., Xiao, Ch.R., Gao, Y., Ma, Y.B., Chen, J.J., Zhu, L.L. Fan, M., 2011. The protective role of 5-HMF against hypoxic injury. Cell Stress Chaperon. 16 (3), 267-273.

Moreira, D., Gullón, B., Gullón, P., Gomes, A., Tavaria, F., 2016. Bioactive packaging using antioxidant extracts for the prevention of microbial food-spoilage. Food Funct. 7 (7), 3273-3282.

Mourtzinos, I., Konteles, S., Kalogeropoulos, N., Karathanos, V.T., 2009. Thermal oxidation of vanillin affects its antioxidant and antimicrobial properties. Food Chem. 114, 791-797.

Mussatto, S.I., Roberto, I.C., 2004. Alternatives for detoxification of diluted-acid lignocellulosic hydrolyzates for use in fermentative processes: a review. Bioresour. Technol. 93 (1), 1-10.

Nunes, C.A., Lima, C., Barbosa, L.C.A., Colodette, J.L., Gouveia, A.F.G., Silvério, F.O., 2010. Determination of *Eucalyptus* spp lignin S/G

ratio: A comparison between methods. Bioresour. Technol. 101 (11), 4056-4061.

Peralbo-Molina, A., Luque de Castro, M.D., 2013. Potential of residues from the Mediterranean agriculture and agrifood industry. Trends Food Sci. Tech. 32 (1), 16-24

Rajha, H.N., Boussetta, N., Louka, N., Maroun, R.G., Vorobiev, E., 2014. A comparative study of physical pretreatments for the extraction of polyphenols and proteins from vine shoots. Food Res. Int. 65, 462-468.

Rajha, H.N., Chacar, S., Afif, Ch., Vorobiev, E., Louka, N., Maroun, R.G., 2015. β-Cyclodextrin-assisted extraction of polyphenols from vine shoot cultivars. J. Agr. Food Chem. 63 (13), 3387-3393.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26 (9-10), 1231-1237.

Rivas, S., Conde, E., Moure, A., Domínguez, H., Parajó, J.C., 2013. Characterization, refining and antioxidant activity of saccharides derived from hemicelluloses of wood and rice husks. Food Chem. 141 (1), 495-502.

Rivero-Cruz, J.F., Zhu, M., Kinghorn, A.D., Wu, C.D., 2008. Antimicrobial constituents of Thompson seedless raisins (*Vitis vinifera*) against selected oral pathogens. Phytochem. Lett. 1, 151-154.

Rombaut, N., Tixier, A.S., Bily, A., Chemat F., 2014. Green extraction processes of natural products as tools for biorefinery. Biofuels Bioprod. Bior. 8, 530-544.

Singh, S., Singh, R.P., 2008. *In vitro* methods of assay of antioxidants: An overview. Food Rev. Internat. 24 (4), 392-415.

Singleton, V.L., Rossi, J.A., 1965. Colorimetric of total phenolics with phosphomolybdic – Phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144-158.

Vázquez, M.J., Alonso, J.L., Domínguez, H., Parajó, J.C., 2006. Enhancing the potential of oligosaccharides from corncob autohydrolysis as prebiotic food ingredients. Ind. Crop. Prod. 24, 152-159.

Vegas, R., Alonso, J.L., Domínguez, H., Parajó, J.C., 2005. Manufacture and refining of oligosaccharides from industrial solid wastes. Ind. Eng. Chem. Res. 44 (3), 614-620.

von Gadow, A., Joubert, E., Hansmann, C.F., 1997. Comparison of the antioxidant activity of aspalathin with that of other plant phenols of Rooibos tea (*Aspalathus linearis*), α-tocopherol, BHT and BHA. J. Agr. Food Chem. 45 (3), 632-637.

Xiu, S., Zhang, B., Shahbazi, A., 2011. Biorefinery processes for biomass conversion to liquid fuel. Chapter 8 pages: 167-190, in "Biofuel's Engineering Process Technology" Edited by Dr. Marco Aurelio Dos Santos Bernardes ISBN 978-953-307-480-1.

Zhao, L., Chen, J., Su, J., Li, L., Hu, S., Li, B., Zhang, X., Xu, Z., Chen, T., 2013. *In vitro* antioxidant and antiproliferative activities of 5-hydroxymethylfurfural. J. Agr. Food Chem. 61 (44), 10604-10611.

FIGURE CAPTIONS

Figure 1. Flow diagram of multiproduct process for the different streams obtained from the autohydrolysis of vine shoots.

Figure 2. Material balances of the streams involved on the autohydrolysis of vine shoots at 200 ºC (S_0 = 4.01) and refining stages.

Figure 3. HPLC UV-PAD chromatogram of the ethyl acetate extracts from the autohydrolysis liquors of vine shoots at 200 ºC.

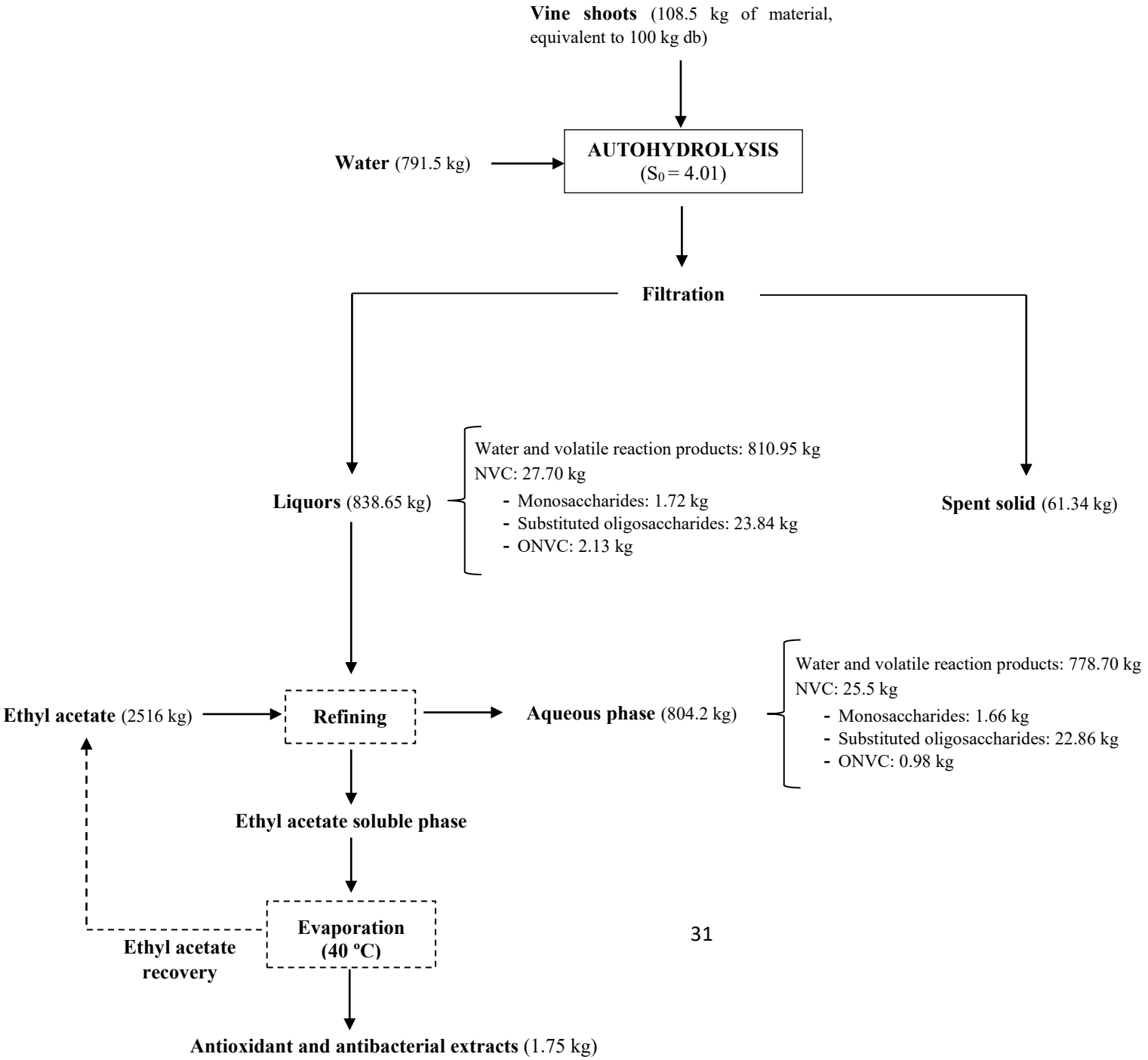
Vine shoots (108.5 kg of material, equivalent to 100 kg db)
Water (791.5 kg)
AUTOHYDROLYSIS
(S_0 = 4.01)
Filtration
Liquors (838.65 kg)
Water and volatile reaction products: 810.95 kg
NVC: 27.70 kg
- Monosaccharides: 1.72 kg
- Substituted oligosaccharides: 23.84 kg
- ONVC: 2.13 kg
Spent solid (61.34 kg)
Ethyl acetate (2516 kg)
Refining
Aqueous phase (804.2 kg)
Water and volatile reaction products: 778.70 kg
NVC: 25.5 kg
- Monosaccharides: 1.66 kg
- Substituted oligosaccharides: 22.86 kg
- ONVC: 0.98 kg
Ethyl acetate soluble phase
Evaporation (40 ºC)
Ethyl acetate recovery
Antioxidant and antibacterial extracts (1.75 kg)

Figure 2

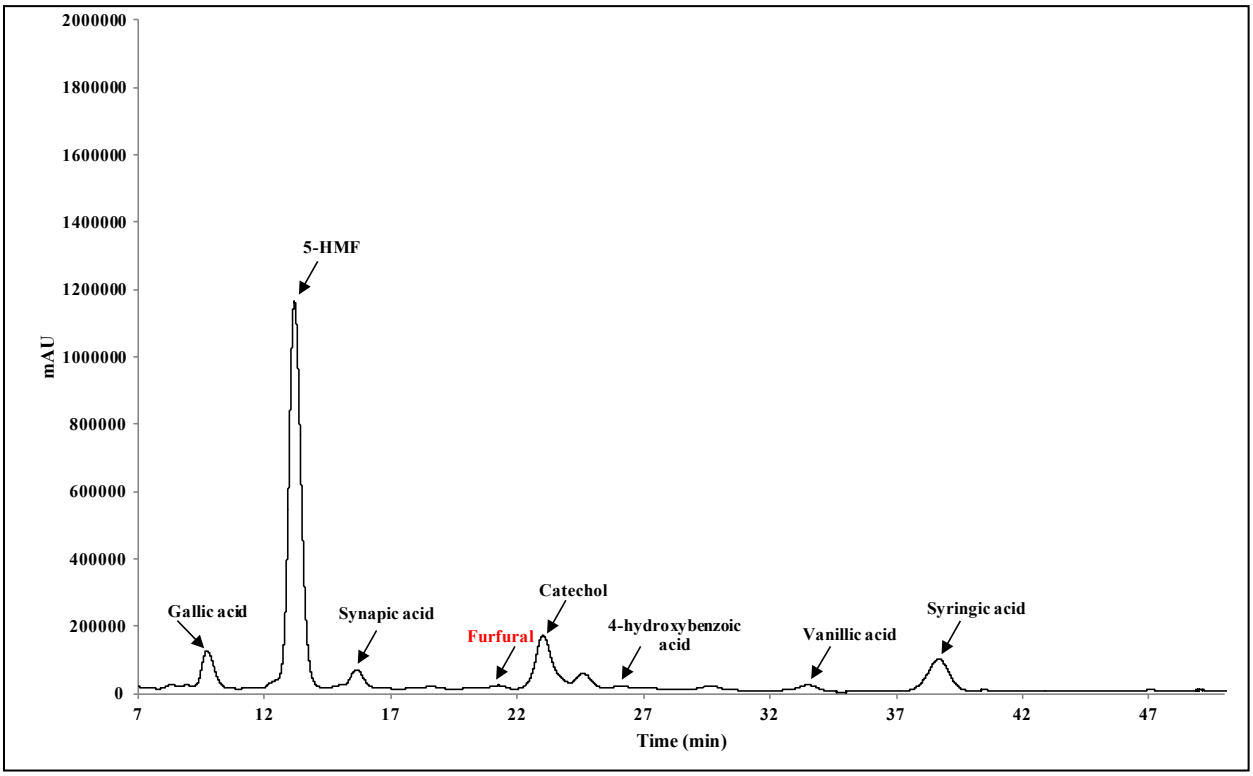
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
mAU
5-HMF
Gallic acid
Synapic acid
Furfural
Catechol
4-hydroxybenzoic acid
Vanillic acid
Syringic acid
7
12
17
22
27
32
37
42
47
Time (min)

Figure 3

Table 1. Sugar and NVC content of the autohydrolysis liquors (a) and the refined liquors (b) (expressed as g/g of NVC) at maximum temperatures assayed.

(a) autohydrolysis liquors

Fraction	Temperature (ºC)							
	180	185	190	195	200	205	210	215
Glucose	0.018	0.016	0.011	0.009	0.010	0.012	0.022	0.042
Xylose	0.003	0.003	0.002	0.004	0.009	0.020	0.037	0.041
Arabinose	0.021	0.020	0.015	0.012	0.011	0.010	0.007	0.007
Galactose	0.012	0.011	0.009	0.009	0.008	0.009	0.013	0.014
Mannose	0.005	0.004	0.004	0.003	0.003	0.005	0.009	0.014
Acetic acid	0.031	0.033	0.040	0.051	0.060	0.094	0.145	0.218
Galacturonic acid	0.029	0.027	0.024	0.024	0.022	0.022	0.015	0.012
GOS	0.299	0.283	0.243	0.256	0.262	0.258	0.210	0.144
XOS	0.205	0.262	0.284	0.341	0.370	0.371	0.217	0.110
ArOS	0.031	0.031	0.026	0.026	0.022	0.011	0.011	0.014
GaOS	0.036	0.032	0.041	0.040	0.038	0.024	0.009	0.000
MaOS	0.024	0.023	0.025	0.031	0.037	0.031	0.041	0.061
AcOS	0.058	0.068	0.117	0.113	0.100	0.100	0.072	0.063
GalactOS	0.060	0.060	0.054	0.043	0.032	0.029	0.030	0.031
Total oligosaccharides	0.714	0.760	0.790	0.849	0.861	0.824	0.589	0.423
ONVC (by difference)	0.199	0.158	0.145	0.090	0.077	0.098	0.307	0.447

(b) refined liquors

Fraction	Temperature (ºC)							
	180	185	190	195	200	205	210	215
Glucose	0.018	0.017	0.012	0.009	0.010	0.013	0.024	0.048
Xylose	0.003	0.003	0.002	0.004	0.009	0.020	0.042	0.047
Arabinose	0.021	0.021	0.015	0.013	0.012	0.010	0.008	0.008
Galactose	0.012	0.012	0.009	0.009	0.008	0.009	0.015	0.016
Mannose	0.005	0.004	0.004	0.003	0.004	0.005	0.010	0.016
Galacturonic acid	0.030	0.028	0.026	0.024	0.023	0.022	0.016	0.013
GOS	0.308	0.295	0.257	0.263	0.273	0.265	0.232	0.165
XOS	0.211	0.272	0.300	0.350	0.385	0.381	0.241	0.126
ArOS	0.032	0.032	0.027	0.027	0.023	0.011	0.012	0.016
GaOS	0.037	0.034	0.044	0.041	0.040	0.025	0.010	0.000
MaOS	0.025	0.024	0.026	0.032	0.038	0.032	0.045	0.070
AcOS	0.060	0.071	0.124	0.116	0.104	0.103	0.080	0.072
GalactOS	0.062	0.063	0.057	0.044	0.033	0.030	0.033	0.035
Total oligosaccharides	0.735	0.791	0.835	0.871	0.897	0.847	0.654	0.484
ONVC (by difference)	0.176	0.123	0.096	0.067	0.038	0.074	0.232	0.367

GOS: glucooligosaccharides; XOS: xylooligosaccharides; ArOS: arabinooligosaccharides; GaOS: galactooligosaccharides; MaOS: mannan-oligosaccharides; AcOS: acetyl groups linked to oligomers; GalactOS: galacturonyl groups linked to oligomers; ONVC: other non-volatile compounds.

Table 2. Effect of temperature during hydrothermal processing of vine shoots on the total phenolic content (TPC), total flavonoid content (TFC) of autohydrolysis liquors and ethyl acetate extracts.

Temperature (ºC)	Autohydrolysis liquors		Ethyl acetate extracts		
	TPC (g GAE/100 g vine shoots, db)	TFC (g RE/100 g vine shoots, db)	Extraction yield (g extract/100 g vine shoots, db)	TPC (g GAE/100 g vine shoots, db)	TFC (g RE/100 g vine shoots, db)
180	0.88 ± 0.053^{a}	0.78 ± 0.033^{a}	0.95 ± 0.174^{a}	0.39 ± 0.020^{a}	0.53 ± 0.057^{a}
185	1.10 ± 0.034^{b}	0.84 ± 0.005^{ab}	1.17 ± 0.164^{a}	0.52 ± 0.031^{a}	0.60 ± 0.048^{ab}
190	1.22 ± 0.013^{bc}	0.87 ± 0.015^{abc}	1.25 ± 0.178^{a}	0.55 ± 0.083^{a}	0.66 ± 0.053^{abc}
195	1.30 ± 0.032^{c}	0.85 ± 0.006^{ab}	1.30 ± 0.121^{a}	0.59 ± 0.041^{a}	0.61 ± 0.011^{abc}
200	1.53 ± 0.029^{d}	0.82 ± 0.004^{a}	1.75 ± 0.214^{ab}	0.84 ± 0.039^{b}	0.69 ± 0.046^{abc}
205	1.89 ± 0.024^{e}	0.99 ± 0.006^{bcd}	2.80 ± 0.241^{bc}	1.46 ± 0.016^{c}	0.83 ± 0.067^{bc}
210	2.01 ± 0.044^{e}	1.07 ± 0.002^{cd}	3.50 ± 0.214^{c}	1.60 ± 0.032^{c}	0.91 ± 0.067^{c}
215	2.25 ± 0.040^{f}	1.10 ± 0.007^{d}	3.80 ± 0.185^{c}	1.62 ± 0.025^{c}	0.92 ± 0.055^{c}

In each column different letters indicate significant differences ($p \leq 0.05$)
GAE: gallic acid equivalents; RE rutin equivalents

Table 3. Effect of temperature on the antioxidant activity (analyzed by the DPPH, ABTS and FRAP methods) of the ethyl acetate extracts from the vine shoots autohydrolysis liquors.

Temperature (ºC)	DPPH (g TE/100 g vine shoots, db)	ABTS (g TE/100 g vine shoots, db)	FRAP (g TE/100 g vine shoots, db)
180	0.58 ± 0.025^{a}	1.58 ± 0.065^{a}	0.72 ± 0.072^{a}
185	0.66 ± 0.017^{a}	2.01 ± 0.215^{a}	0.91 ± 0.073^{a}
190	0.67 ± 0.012^{ab}	2.08 ± 0.085^{a}	0.89 ± 0.081^{a}
195	0.73 ± 0.021^{ab}	2.35 ± 0.113^{a}	0.97 ± 0.065^{a}
200	0.82 ± 0.029^{b}	3.56 ± 0.190^{b}	1.99 ± 0.122^{b}
205	0.99 ± 0.034^{c}	4.25 ± 0.219^{bc}	2.29 ± 0.195^{bc}
210	1.01 ± 0.016^{c}	4.45 ± 0.086^{c}	2.57 ± 0.139^{bc}
215	1.05 ± 0.048^{c}	4.39 ± 0.098^{c}	2.68 ± 0.148^{c}

In each column different letters indicate significant differences ($p \leq 0.05$)
TE: trolox equivalents.

Table 4. Effect of the temperature during the autohydrolysis of vine shoots on the composition (mg compound/100 g of extract).

Nº	Retention time (min)	Name	Main fragments (m/z)	Temperature (ºC)							
				180	185	190	195	200	205	210	215
Sugar derived compounds											
1	3.7	Furfural	96, 39, 29	ND	ND	ND	ND	ND	1.9	4.6	0.9
2	5.4	5-methyl furfural	110, 53, 27	ND	ND	ND	ND	ND	1.2	1.0	ND
3	9.9	5-hydroxymethyl furfural	97, 126, 41	ND	ND	ND	4.3	10.3	14.9	15.3	21.2
Lignin derived compounds											
4	5.6	Phenol	94, 66, 39	ND	ND	ND	ND	ND	0.6	ND	ND
5	7.3	Guaiacol	109, 124, 81	ND	ND	ND	1.9	2.5	3.3	2.7	2.3
6	9.2	Catechol	110, 64, 63	ND	ND	ND	0.9	1.5	1.6	1.8	1.5
7	13.7	Syringol	154, 139, 96	ND	ND	ND	1.0	ND	1.1	ND	ND
8	13.9	4-hydroxy benzaldehyde	121, 122, 93	ND	ND	ND	ND	ND	0.6	ND	ND
9	14.9	Vanillin	151, 152, 109	ND	2.7	3.5	3.2	3.2	3.2	2.9	2.3
10	16.6	Acetovanillone	151, 166, 123	ND	2.3	2.2	1.0	1.0	0.8	1.2	0.8
11	17.4	Guaiacylacetone	137, 180, 122	ND	3.1	1.9	1.2	1.4	1.1	0.9	0.7
12	19.4	Syringaldehyde	182, 181, 167	4.9	5.5	8.0	5.2	5.1	4.4	5.3	3.7
13	20.3	Acetosyringone	181, 196, 153	5.2	8.3	12.9	5.1	4.1	3.0	3.1	2.7

ND: not detected

84 **Table 5.** Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations
85 (MBC) (mg/mL) of the ethyl acetate extracts from the vine shoots autohydrolysis liquors
86 obtained at 200 ºC. All assays were done in triplicate.
87

Microorganism	**MIC**	**MBC**
Gram (-)		
E. coli	15	20
P. aeruginosa	10	15
Salmonella sp	10	15
Gram (+)		
B. cereus	10	15
S. aureus	5	10
L. innocua	5	10

88
89
90
91