



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



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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 °C) 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 °C 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.

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

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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 Fig. 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 °C. 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 °C 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 °C, 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 BaCO₃, 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 °C. The column used for this quantification was a 300 × 7.8 mm CARBOsep CHO-682 column (Transgenomic) operating at 80 °C 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 × 7.8 mm Aminex HPX-87H

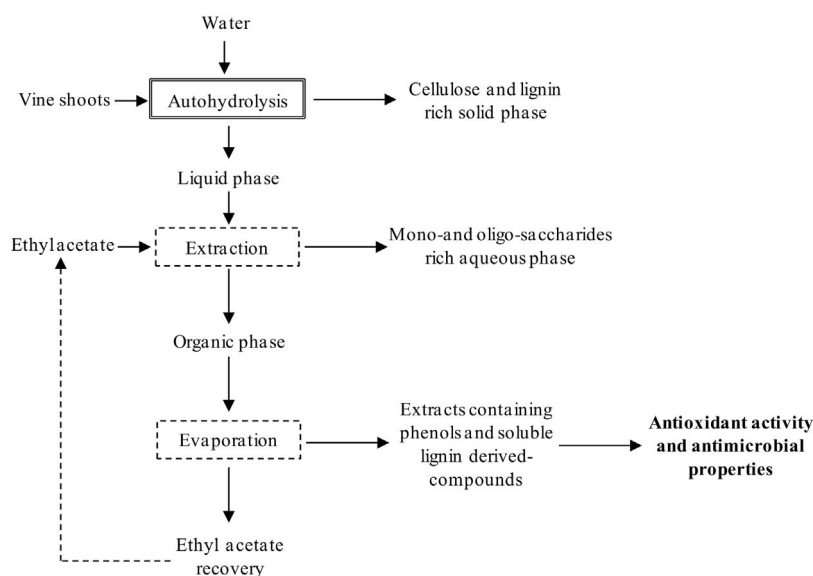


Fig. 1. Flow diagram of multiproduct process for the different streams obtained from the autohydrolysis of vine shoots.

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