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Chemical characterization of different granulometric fractions of grape stalks waste

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ABSTRACT

The chemical composition of grape stalks wastes from wine production was investigated after milling and separation in three different size material fractions. The chemical characterization included summative composition, total polyphenols, condensed tannins, lipophilic extractives ash composition, determination of acidic functional groups and FTIR analysis. Extractives are the main components of grape stalks, especially polar extractives soluble in hot water and in 1% NaOH. The milling and separation process results in an enrichment of extractives and ash in the finest fraction and conversely, an enrichment of structural components in the largest fractions.

The bands obtained by FTIR analysis confirm the presence of lignin, polysaccharides and polyphenolic compounds.

The results of this research show that fractioning of grape stalks waste is a way to enrich the material in target valuable components and to improve efficiency of resource utilization.

1. Introduction

Grape stalks are the grape lignocellulosic skeleton of grapes that are obtained from stripping operations during processing. Stalks are the main by-products of vineyards and represent between 2.5% and 7.5% of weight of grapes (Nerantzis and Tataridis, 2006; Garcia-Perez et al., 2010).

In the Mediterranean areas, these large amounts of grape stalks are obtained after the grape harvesting for wine production from August to October. An important wine producing region in the Mediterranean area is Catalonia, located in the north-east coast of Spain, where an average of 5000,000 HL of wine per year are produced from 400,000 tons of grapes resulting in the generation of more than 10,000 tons of grape stalks wastes (Escobar et al., 2012). Although these wastes are not intrinsically hazardous, they have to be landfill disposed, incinerated or biologically treated, therefore causing an economical and environmental problem.

The use of grape stalks and in general of lignocellulosic materials has attracted the attention of researchers not only for their re-use in agriculture but also as a bio-resource for the production of novel products after fractioning according to their chemical composition and properties. The use of lignocellulosic materials as a source of valuable products such as polyphenols, sugars, and lipids, is an interesting way to increase the value of this waste and to generate economic benefits.

Grape stalks waste has been investigated as a source of cellulose and hemicellulose (Spigno et al., 2008), natural antioxidants by the extraction of phenolic compounds (Garcia-Perez et al., 2010; Makris et al., 2007; Spigno and De Faveri, 2007), fermentable sugars via enzymatic treatment for biofuel production (Mazzaferro et al., 2011). Grape stalks were also used as carbon source to obtain activated carbon (Deiana et al., 2009) and as ionophore material to develop a sensor for the determination of Cr(VI) and Hg(II) in aqueous solutions (Fiol et al., 2007). Grape stalks mixed with WWTP sludge (Bertran et al., 2004) or olive mill sludge (Cayuela et al., 2006) were reported to produce compost with good agronomic value.

Grape stalks have been reported to be an effective sorbent for metal ions (Chubar et al., 2003; Fiol et al., 2004; Miralles et al., 2008) and organic compounds i.e. paracetamol (Villaescusa et al., 2011) and methylene blue (Olivella et al., 2012) removal from aqueous solutions. Most of the literature on grape stalks is devoted to investigate potential uses but studies on their complete chemical characterization are scarce. Grape stalks chemical composition has been reported to depend on several factors that include geographic origin, climate, time of harvest, and grape varieties (*Vitis vinifera* L). Recently, Spigno et al. (2013) have compared different cultivars

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and concluded that grape variety significantly influences their composition.

In general biomass wastes are heterogeneous materials that need to be reduced into small particles before processing. The existing milling techniques are not able to reduce biomass to homogeneous fractions and different particle sizes fraction can show different chemical characteristics. Miranda et al. (2013) found that extractives were preferably present in the finest fraction of milled birch and eucalypt barks while cellulose and hemicelluloses were enriched in the coarser fraction of eucalypt bark. The largest particle sizes of milled switchgrass and reed canary grass were reported to accumulate a higher proportion of lignin, cellulose and hemicelluloses (Bridgeman et al., 2007). Therefore, biomass fractioning could be used for enrichment of specific components by taking advantage of chemical and structural differences (Miranda et al., 2012).

In the case of grape stalks, the milling process performed in our laboratory resulted in particle size fractions with clear color, shape and textural differences. Up to our knowledge, chemical characterization of grape stalks fractions with different particle sizes have not been carried out. This paper studies the chemical composition of grape stalks fractions after milling and separation based on the different particle size, with the aim of evaluating their potential use as a natural bioresource. The chemical characterization includes the ash composition, summative chemical analysis, determination of total polyphenols, condensed tannins, and lipids in soluble extracts, and FTIR spectroscopy analysis.

2. Materials and methods

2.1. Grape stalks wastes

Grape stalks wastes were supplied by a wine manufacturing company from Empordà-Costa Brava (Catalonia) as generated during the complete grape de-stemming process. Grape stalks were milled using a mill (Retsch SM 2000) and sieved using a vibratory sieving apparatus (Retsch AS 200 basic). Three different particle size fractions were collected: $0.25 \leq \Phi \leq 0.424$; $1 \leq \Phi \leq 1.6$ and $1.6 \leq \Phi \leq 3.15$ mm. The different fractions of material were extensively washed with water to remove dust and minerals and dried at air-conditions. The resulted grape stalk fractions presented clear differences in color and shape: the larger fraction contained brown cylindrical structures with spherical peduncles, the medium fraction was predominantly formed by light brown fibrous material and the finest fraction contained dark brown rounded particles and a small fraction of short fibers.

2.2. Chemical summative composition

The chemical summative analysis of grape stalks included the determination of extractives soluble in solvents with different polarity, Klason and acid-soluble lignin, and monomeric composition of polysaccharides. Before chemical analysis, the $1 \leq \Phi \leq 1.6$ and $1.6 \leq \Phi \leq 3.15$ mm grape stalk fractions were ground to obtain particles lower than 0.424 mm.

The extractives were obtained by successive solvent extraction with dichloromethane, ethanol and water in a Soxtec extractor for 1.5 h with each solvent. The extractives solubilized by each solvent were determined using the mass difference between original and solid residue mass after drying at 105 °C. Results are reported as a percentage of the dry original samples.

An alkaline lixiviation with 1% NaOH of the extractive-free samples was performed in a stirred glass reactor with reflux using 1.0 g of material with a 1:50 solid:liquid ratio (g/mL), at 100 °C during 1 h. The material was filtered and washed with water, and the solubilized material determined by the mass difference of the solid residue after drying at 105 °C.

The Klason and acid-soluble lignin, and carbohydrates content were determined on the extracted materials after 1% NaOH extraction. The Klason lignin content was determined by the standard method TAPPI 13 m-54 and acid soluble lignin by TAPPI UM 250. Sulphuric acid (72%, 3.0 mL) was added to 0.35 g of the extracted sample and the mixture was placed in a water bath at 30 °C for 1 h. After this time, the sample was diluted to a concentration of 3% H_2SO_4 and hydrolysed for 1 h at 120 °C. The sample was vacuum filtered through a crucible and washed with boiling purified water. Klason lignin was determined by the mass residue after drying at 105 °C. Soluble lignin was determined on the combined filtrates by measuring the absorbance at 206 nm using a UV–vis spectrophotometer (Shimadzu UV-160A). Measurements of Klason and acid-soluble lignin were combined to give the total lignin content.

The polysaccharides were calculated based on the amount of the neutral sugar monomers released by total hydrolysis. The hydrolysed carbohydrates were derivatized to alditol acetates and separated by gas chromatography (GC) (HP5890A) equipped with a FID detector, using helium as carrier gas (1 mL/min) and a fused silica capillary column S2330 (30 m × 0.32 mm ID; 0.20 μm film thickness). The column program temperature was 225-250 °C, with 5 °C/min heating gradient, and the temperature of injector and detector was 250 °C. For quantitative analysis, the GC was calibrated with pure reference compounds and inositol was used as internal standard in each run (method adapted from TAPPI 249 cm-00).

2.3. Total polyphenol determination

The total polyphenol content (TPC) was determined in the solutions obtained by the extractions with ethanol, water and 1% NaOH. TPC was determined by spectrophotometry, using gallic acid as standard, according to the Folin–Ciocalteu assay. The calibration curve was obtained by preparing different standard concentrations of gallic acid within the range 0.1-0.6 mg L^{-1}. Briefly, a 100 μL aliquot of extracts, the gallic acid standard solutions (0.1-0.6 mg L^{-1}) and a blank (deionized water) were put in different tubes. Then, 4 mL of the Folin–Ciocalteu's phenol reagent diluted 1:10 were added to each tube, the tubes were shaken and allowed to react for 5 min. After this time, 4 mL of 7.5% Na_2CO_3 solution was added. After incubation in a thermostatic bath for 15 min at 45 °C, the absorbance against a blank was determined spectrophotometrically at 765 nm (Shimadzu UV-160A). Total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per 100 g dry mass.

2.4. Condensed tannins determination

The total tannins content was determined in the same extracts used for the total polyphenols determination. Condensed tannins were separated from the extract by precipitation with a 0.04% methyl cellulose solution in deionized water. To precipitate the condensed tannins, 1 mL of extract was put into contact with 1 ml of 0.04% methyl cellulose, 0.8 mL of saturated sodium ammonium solution and 2.5 mL deionized water. After 20 min, the solution was filtered and total polyphenol content was determined in the filtrate by following the same procedure as described in Section 2.3. The difference between total polyphenols content and polyphenols determined after precipitation with methyl cellulose corresponds to the condensed tannins.