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Use of cyclodextrins to recover catechin and epicatechin from red grape pomace

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ABSTRACT

The capacity of cyclodextrins (CDs) to extract phenolic compounds from grape pomace was evaluated and compared with that of ethanol/water or aqueous extraction. The extraction method (stirring and ultrasound), temperature and time were also studied. Total phenolic compounds (TPC) and antioxidant activity were measured, and HPLC analysis was used to identify the phenolic compounds. The extracts obtained using the ethanol/water mixture presented the highest TPC content and antioxidant activity, followed by those obtained using CD solutions. The addition of CDs to the extractant agent had a selective effect on the extraction of catechin and epicatechin. The yield of catechin and epicatechin by using aqueous solutions of CDs was similar to that obtained using organic solvents as ethanol.

1. Introduction

The interest in using or reusing wine by-products, particularly grape pomace, has resulted in the development of different applications including yeast production, seed oil extraction, energy production, decontamination of effluents with a high metal content, and compost production (Bustamante et al., 2008). In addition, grape pomace is a rich source of several high value added products, such as ethanol, citric acid, tartrates, oil seed, hydrocolloids, fiber dietary and phenolic compounds (Arvanitoyannis, Ladas, & Mavromatis, 2006).

Grape phenolic compounds are responsible for some of the most important wine properties, in particular color, astringency, flavor and body (Lafka, Sinanoglou, & Lazos, 2007). Wine phenolic compounds, particularly flavanols, have been intensely studied due to their relationship with the beneficial effects of moderate wine consumption on human health (Arvanitoyannis et al., 2006; Louli, Ragoussis, & Magoulas, 2004; Maier, Schieber, Kammerer, & Carle, 2009). Large quantities of these phenolic compounds are retained in the pomace after the elaboration of the wine (Makris, Boskou, & Andrikopoulos, 2007), which is why their recovery is of interest for the food, pharmaceutical and cosmetic industries.

Phenolic compounds with the greatest presence in grape pomace are the flavan-3-ols, catechin and epicatechin, and gallic acid (Lafka et al., 2007; Tsanova-Savova, Ribarova, & Gerova, 2005). Flavanols, which are mainly located in seeds (Kennedy, Sauicier, & Glories, 2005), are the phenolic compounds responsible for wine bitterness and astringency. Catechin and epicatechin can represent up to 60% of total phenolic compounds present in grape seed (Chedea, Braicu, & Socaciu, 2010). As regards antioxidant capacity, this could be greater than that of other minor compounds such as resveratrol or rutin (Iacopini, Baldi, Storchi, & Sebastiani, 2008). The role of flavanols in the prevention of cancer and cardiovascular diseases has received a lot of attention (Jang et al., 1997; Kuroda & Hara, 1999; Shrikhande, 2000).

The extraction of phenolic compounds is the first step to their use in industry. In general, solid–liquid extraction has been the most used extraction method, combining different types of organic solvents such as methanol, ethanol, acetone, ethyl acetate (Lafka et al., 2007). Other new extraction technologies studied include supercritical fluids (Da Porto, Natolino, & Decorti, 2014; Herrero, Cifuentes, & Ibañez, 2006), extraction assisted by electrical pulses (Bucic-Kojic, Sovová, Planinic, & Srecko, 2013; Sánchez, Sineriro, and Núñez, 2008) and polymeric adsorber resins (Kammerer & Kljusuric, 2005). However, such extraction techniques usually use organic solvents that are toxic for human, or the technology and equipment needed is too expensive or complex for use at an industrial level (Ratnasooriya & Rupasinghe, 2012). Replacing organic extraction solvents by exclusive aqueous extractions without affecting the extraction yield is one of the most pressing problems to be solved in the extraction of phenolic compounds from grape by-products.

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The use of CDs for this purpose has received comparatively little attention to date, although their ability to encapsulate compounds of different nature has been widely studied. Several studies have shown that CDs can increase the solubility of phenolic compounds in water (Lucas-Abellán, Fortea, Gabalón, & Núñez-Delicado, 2008; Mercader-Ros, Lucas-Abellán, Fortea, Gabaldón, & Núñez-Delicado, 2010; Mercader-Ros et al., 2010). It therefore seemed likely that the use of aqueous solutions of CDs could improve the extraction of phenolic compounds with a polarity and structure compatible with the CD cavity, enabling these compounds to be extracted without the assistance of organic solvents. Complexation with CDs also protects against oxidation and could improve the stability of the phenolic compounds complexed.

The aim of this work was to evaluate the capacity of CDs to contribute to the extraction of the phenolic compounds present in wine pomace, especially the main compounds catechin and epicatechin.

2. Material and methods

2.1. Samples preparation

Grape pomace from Monastrel variety (*Vitis vinifera* L.) was provided by Bodegas San Isidro located in Jumilla (Spain). Samples were collected immediately after wine pressing and stored at −80 °C until laboratory extraction and determination.

2.2. Reagents and standards

Methanol and water were HPLC grade and purchased from JT Baker (The Netherlands). Ethanol and hexane of HPLC grade, and Na_2CO_3 were purchased from Panreac (Germany). Phenolic standards (gallic acid, catechin and epicatechin) (purity of 98–99%), Folin–Ciocalteau phenol reagent were purchased from Sigma Chemical Co. (Great Britain). β- and HP-β-CDs were purchased form Winplus International Limited (China). APPH were purchase from Aldrich Chemistry (San Luis, USA).

2.3. Extraction of phenolic compounds

Prior to phenolic compound extraction, the fresh grape pomace was ground in a coffee grinder for 20 s, defatted by two-step extraction for 15 min with n-hexane at a ratio of 10:1 (v/w) in an orbital shaker Bunsen MC8 (Spain) at 2100 rpm and 25 °C, and dried in an oven at 50 °C for 24 h.

The extraction of the phenolic compounds was performed in a mixture of ethanol:water 1:1 (v/v), water or aqueous solutions of β - or HP-β-CDs (8 and 13 mM β-CDs; 13, 25 and 50 mM HP-β-CDs) for different extraction times (from 1 to 90 min), at two different extraction temperatures (25 or 40 °C), stirring in an orbital shaker (P-Selecta Mutimatic 9N, Spain) or by ultrasound (P-Selecta Ultrasons, Spain). The pH of all extractions solvents were adjusted to 1.5 using HCl. All extractions were made in a 9:1 (v/w) proportion of solvent volume to sample mass. The extracts were centrifuged in a microcentrifuge (Eppendorf Centrifuge S415D, Germany) at 10,000*g* for 1 min and filtered using 0.45 μm nylon filters (Chromafil, Germany). Extracts centrifuged and filtered were used for phenolic content and antioxidant activity determinations, and for HPLC analysis. All extractions, determinations and analysis were made in triplicate.

2.4. Phenol content determination

The total phenolic content (TPC) of grape pomace extracts was determined colorimetrically at 765 nm using the Folin–Ciocalteau reagent according to a modification of the Kidron, Harel, and Mayer (1978) method. Folin–Ciocalteau reaction was made mixing 100 μL of diluted grape pomace extract (1/10 v/v extract/water), 150 μL of Folin Ciocalteau reagent, 450 μL of 20% Na_2CO_3 and 2300 μL of distilled water. After 2 h of reaction, the absorbance of the sample was measured against a blank by using a spectrophotometer (Shimadzu model UV-1603, Japan). The calibration curve was made using gallic acid as standard, measuring the absorbance at 765 nm of 0, 2, 4, 6 and 8 μg/mL of gallic acid. TPC is expressed in mg of gallic acid equivalents per gram of dry pomace (mg/g_{db}) and determinations were made in triplicate.

2.5. Antioxidant activity (ORAC method)

The ORAC analyses were carried out on a Synergy HT multi-detection microplate reader, from Bio-TekInstruments, Inc. (USA), using 96-well polystyrene microplates with black sides and clear bottoms. Fluorescence was read through the clear bottom, with an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm. The plate reader was controlled by KC4, version3.4, software. The oxygen radical absorbance capacity was determined as described by Dávalvos with slight modifications (Dávalvos, Gómez-Cordovés, & Bartolomé, 2004). The reaction was carried out in 75 mM sodium phosphate buffer (pH 7.4), and the final reaction mixture was 200 μL. 100 μL fluorescein (3 nM, final concentration) and 70 μL of diluted grape pomace extract (1/100 v/v extract/water) were placed in the wells of the microplate. The mixture was preincubated for 30 min at 37 °C, before rapidly adding the AAPH solution (30 μL; 19 mM, final concentration) using a multichannel pipette. The microplate was immediately placed in the reader and the fluorescence recorded every 1.14 min for 120 min. The microplate was automatically shaken prior to each reading. A blank with fluorescein and AAPH using sodium phosphate buffer instead of the antioxidant solution and eight calibration solutions using Trolox C (6.25, 12.5, 15, 18.75, 21.25, 25, 27.5 and 31.25 μM) as antioxidant were also used in each assay. All reaction mixtures were prepared in triplicate and at least three independent assays were performed for each sample. In order to avoid a temperature effect, only the inner 60 wells were used for experimental purposes, while the outer wells were filled with 200 μL of distilled water. The results were expressed as relative fluorescence with respect to the initial reading. The net AUC corresponding to the sample was calculated by subtracting the AUC corresponding to the blank. Determinations of antioxidant activity were made for triplicate.

2.6. HPLC analysis

Identification and quantification of phenolic compounds of the grape pomace extracts was performed by HPLC analysis using an HPLC Agilent Technologies model 1200 equipped with a variable DAD detector set at 280 nm. 20 μL of centrifuged and filtered extract were injected. Separations were achieved on an endcapped (5 μm) HPLC Cartridge 250-4 LiChospher 100 RP-18. The flow rate was 1 mL/min. The mobile phase used was 0.5% formic acid in water (A) *versus* methanol (B) for a total running time of 60 min and the gradient changed as follows: solvent B started at 2%, then increased to 32% in 30 min, to 40% in 10 min, to 95% in 10 min, and returned to initial conditions in 10 min. The data were processed by Agilent ChemStation software. Catechin and epicatechin is expressed in μg per gram of dry pomace ($\mu g/g_{db}$) and determinations were made in triplicate.

2.7. Molecular model

The molecular structures for catechin and epicatechin used in this study were built manually using AutoDockTools (Morris