



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



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ARTICLE INFO

Article history:

Received 1 September 2015
Received in revised form 14 December 2015
Accepted 13 February 2016
Available online 15 February 2016

Keywords:

Catechin
Epicatechin
Grape pomace
Cyclodextrin

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.

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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, Saucier, & 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, Storch, & 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, Gabaldón, & Núñez-Delgado, 2008; Mercader-Ros, Lucas-Abellán, Fortea, Gabaldón, & Núñez-Delgado, 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–Ciocalteu phenol reagent were purchased from Sigma Chemical Co. (Great Britain). β - and HP- β -CDs were purchased from Winplus International Limited (China). APPH were purchased 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 extraction 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 $10,000g$ for 1 min and filtered using $0.45\ \mu\text{m}$ 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–Ciocalteu reagent according to a modification of the Kidron, Harel, and

Mayer (1978) method. Folin–Ciocalteu reaction was made mixing 100 μL of diluted grape pomace extract (1/10 v/v extract/water), 150 μL of Folin Ciocalteu 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 $\mu\text{g}/\text{mL}$ 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-Tek Instruments, 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, version 3.4, 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 ($5\ \mu\text{m}$) 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 ($\mu\text{g}/g_{\text{db}}$) 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

et al., 2009) and structural information derived from experimental data. The structure of β -CDs was extracted from the crystal structure of the Protein Data Bank (PDB) with code 3CGT. Molecular docking calculations were carried out using default parameters in AutoDockVina (Trott & Olson, 2010). Graphical representations of the docking results were prepared using PyMOL (Molecular Graphics System, version 1.3, Schrödinger, LLC).

2.8. Statistical analysis

Data were analysed by using one way analysis of variance (ANOVA) of the statistical analysis software SPSS (v.21). Significant differences between means were analysed by using the Tukey test.

3. Results and discussion

3.1. Total phenolic compounds (TPC) and antioxidant activity

The TPC extracted and the antioxidant activity of the extracts obtained using different solvents as extract agents, as well as two extraction energy sources, stirring and ultrasound, both at 25 °C, are shown in Table 1.

The ethanol/water mixture provided the highest extraction yield of TPC and antioxidant activity. The TPC values obtained were 21.2 mg/g_{db} (dry base) and 23.5 mg/g_{db} for stirring and ultrasound extraction, respectively (Table 1). These values were similar to those observed by Louli et al. (2004) (26 mg/g) and lower than those obtained by Lafka et al. (2007) (35 mg/g) and Negro, Tommasi, and Miceli (2003) (41 mg/g). With respect to antioxidant activity, the ethanol/water extracts presented 42.1 and 48.1 mM Trolox for the stirring and ultrasound extraction methods, respectively.

The extraction energy applied (stirring or ultrasound) affected TPC extraction. As an average of all the extractions, the TPC and antioxidant activity of the extracts were, respectively, 14% and 21% higher in those obtained by using ultrasound than when using the stirring method (Table 1). Although this tendency was observed in all solvents studied, differences between stirring and ultrasound methods were only statistical significant in 6 of the 14 cases studied. The fact that the improvement in antioxidant activity was higher (21%) than the improvement in TPC extraction (14%) indicates that ultrasound extraction improves the extraction of phenolic compounds with a greater relative antioxidant activity.

Removing the organic solvents from the extraction agent had a negative effect on phenolic extraction. When water was used as extraction agent, the TPC extracted was 3.67 mg/g_{db} and 4.18 mg/g_{db} for stirring and ultrasound, respectively, 82% lower than the values obtained with ethanol/water extraction (Table 1). These values were slightly higher than those observed by Bustamante et al.

(2008) for aqueous extractions. The antioxidant activity of aqueous extracts was 82% lower than that presented in ethanol/water extracts for both the stirring and ultrasound methods.

In an attempt to increase the levels of TPC extracted from grape pomace in the absence of organic solvents, aqueous solutions of β - or HP- β -CDs were used. When CDs were added to the extraction water, an increase in the TPC extracted was observed, as well as in antioxidant activity of the extracts (Table 1). This effect was previously described by Ratnasooriya and Rupasinghe (2012). As regards the extractions obtained by using ultrasound, the addition of 13 mM β - or HP- β -CDs increased the value TPC up to 5.23 mg/g_{db} and 5.54 mg/g_{db}, respectively, representing an increase with respect to water extraction of 25% and 32%, with an improvement in the antioxidant activity of the extracts of 33% and 75%, respectively. It was observed that the increase in antioxidant activity of the extracts was not proportional to the increase in concentration of TPC extracted. The use of CDs not only improved the extraction of total phenolics with respect to water, but also the antioxidant activity of the phenolic compounds. In previous studies with pure phenolic compounds, some authors concluded that complexation with CDs increases the antioxidant capacity of encapsulated phenolic compounds (Lucas-Abellán et al., 2008; Mercader-Ros, Lucas-Abellán, Fortea, et al., 2010).

TPC and antioxidant activity of the extracts also increased with CDs concentration. Increasing β -CD concentration from 8 to 13 mM had little effect on TPC and antioxidant activity, with statistical significant differences only between TPC of 8 and 13 mM of β -CD with ultrasound extraction. Increasing the concentration of HP- β -CDs from 13 to 50 mM the TPC extracted and antioxidant activity increased by around 30%, with statistical significant differences between 13 and 50 mM of HP- β -CD in both stirring and ultrasound extractions.

Although the extraction of TPC and antioxidant activity using CDs was lower than that obtained by ethanol/water extraction, the addition of cyclic sugars improved TPC extraction and antioxidant activity compared with water extraction.

3.2. HPLC identification and molecular model

In order to determine the effect of CDs on the extraction of phenolic compounds, extracts were characterized by HPLC analysis. Fig. 1 shows four representative chromatograms of extracts obtained using ethanol/water (Fig. 1A), water (Fig. 1B), β -CDs (13 mM) (Fig. 1C) and HP- β -CDs (13 mM) (Fig. 1D).

Identification of phenolic compounds by HPLC analysis showed the effect of CDs on the extraction of the main phenolic compounds gallic acid (1), catechin (2) and epicatechin (3). HPLC analysis has permitted to identify clearly catechin and epicatechin showing biggest signals for both compounds (Fig. 1A), while water led to

Table 1

Total phenolic compounds (TPC) in mg/g_{db} (dry base) and antioxidant activity (AA) in equivalent of mM of Trolox (mM Tr) of extracts obtained at 25 °C after 40 min of extraction time and using different solvents: ethanol/water (Et/Wt), water (Wt), β -cyclodextrins (β -CDs) and HP- β -cyclodextrins (HP- β -CDs), and extraction methods: stirring or ultrasound.

Solvent	TPC mg/g _{db}			AA mM Tr		
	Stirring	Ultrasound	S. S.	Stirring	Ultrasound	S. S.
Et/Wt	21.2 ± 0.80 ^d	23.5 ± 0.40 ^d	*	42.1 ± 1.56 ^d	48.1 ± 3.53 ^e	ns
Wt	3.67 ± 0.24 ^a	4.18 ± 0.14 ^a	ns	7.70 ± 1.04 ^a	8.9 ± 0.19 ^a	ns
β -CDs (mM)	8	4.34 ± 0.10 ^{ab}	*	9.80 ± 0.15 ^a	11.2 ± 0.58 ^{ab}	*
	13	4.45 ± 0.33 ^{ab}	ns	9.20 ± 0.33 ^{ab}	11.9 ± 1.75 ^{abc}	ns
HP- β -CDs (mM)	13	4.64 ± 0.50 ^{ab}	ns	12.1 ± 1.52 ^b	15.6 ± 0.38 ^{bcd}	ns
	25	5.09 ± 0.30 ^b	ns	12.3 ± 0.17 ^b	15.8 ± 1.23 ^{cd}	*
	50	6.41 ± 0.24 ^c	7.39 ± 0.55 ^c	*	16.1 ± 0.51 ^c	19.3 ± 1.13 ^d

Values represent means of triplicate extraction (\pm SD).

In each column, statistical difference between means is shown ($p < 0.05$) (a–e).

Statistical significance (S.S.) between stirring and ultrasound for TFC and AA: (**) $p < 0.01$; (*) $p < 0.05$; (ns) no significant.

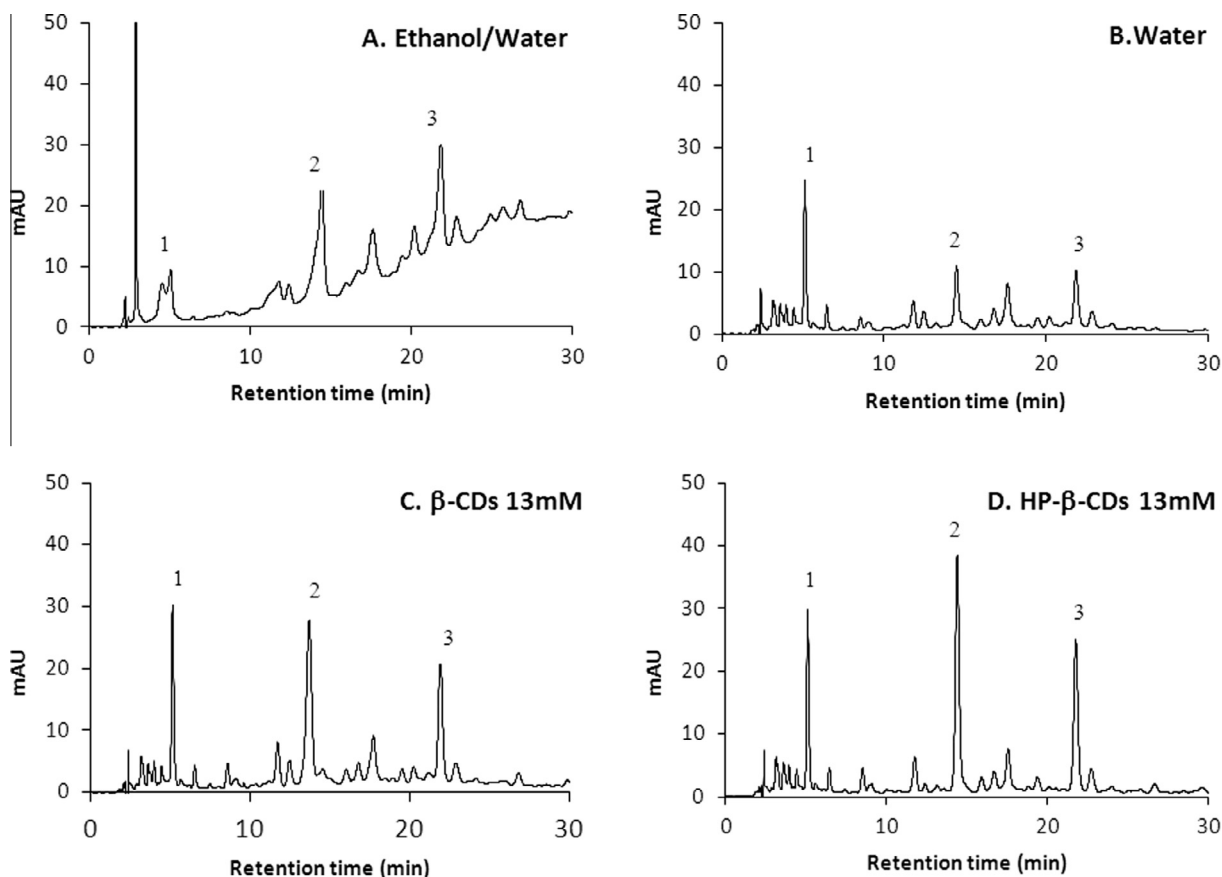


Fig. 1. HPLC profiles of extracts obtained at 25 °C after 40 min of extraction using ethanol/water (A), Water (B), β -CDs 13 mM (C) and HP- β -CDs 13 mM (D). 1. Gallic acid, 2. Catechin. 3. Epicatechin.

a significantly lower extraction, especially of compounds as catechin and epicatechin (Fig. 1B). However, the addition of CDs to the extraction water considerably increased catechin and epicatechin extraction (Fig. 1C and D). With respect to the signal detected by the HPLC, the area corresponding to catechin extracted in the presence of β -CD or HP- β -CDs was 4-fold that obtained by using water without CDs. In the case of epicatechin, the area detected by the HPLC was more than twice as large as water for both β - or HP- β -CDs. These results show that catechin and epicatechin, due to their polarity and chemical structure, could be complexed in the hydrophobic cavity of CDs, forming soluble inclusion complexes that allow their selective extraction in aqueous medium.

In order to understand the interaction between catechin or epicatechin with β -CDs, docking simulations were carried out. The structural information about the non-covalent binding process obtained by docking for catechin and epicatechin is shown in Fig. 2. Both guest molecules fit well into the β -CDs hydrophobic cavity, where their hydrophobicity is more similar to that of the core. In addition, some of the polar groups of these molecules interact with β -CD due mainly to electrostatic and hydrogen bond interactions.

3.3. Catechin and epicatechin extraction

To analyze the specific impact of CDs on catechin and epicatechin extraction, the type and concentration of CDs, extraction time, temperature and method (stirring and ultrasound) were studied.

Fig. 3A and B show the evolution of catechin and epicatechin extraction at 25 °C using the stirring method, when ethanol/water, water, β -CDs 13 mM or HP- β -CDs 13 mM were used as extractant agents.

Catechin and epicatechin concentrations increased with extraction time for all the extracting agents used (Fig. 3A and B). The highest values were obtained at 90 min extraction time, reaching values around 1000 $\mu\text{g}/\text{g}_{\text{db}}$ for catechin (Fig. 3A) and 700–900 $\mu\text{g}/\text{g}_{\text{db}}$ for epicatechin (Fig. 3B). These data were similar to those obtained by Iacopini et al. (2008) from grape skins, and lower than those observed by Maier et al. (2009) obtained from grape seed. Extraction was very fast during the first 10 min in the case of both compounds but then slowed down from 10 to 90 min of the extraction process (Fig. 3). This extraction dynamic was also described by Sánchez et al. (2008) and Bucic-Kojik et al. (2013). Water showed a very low extraction capacity for catechin and epicatechin compared with ethanol/water, providing values of 90 $\mu\text{g}/\text{g}_{\text{db}}$ and 108 $\mu\text{g}/\text{g}_{\text{db}}$ for catechin and epicatechin, respectively, after 90 min of extraction. These values are higher than those obtained by Ratnasooriya and Rupasinghe (2012).

With respect to catechin extraction, statistical significant differences between CDs solutions (β - or HP- β -CDs) and ethanol/water were only observed at 10 min of extraction (Fig. 3A). Ethanol/water led to slightly higher catechin extraction values than those obtained using β - or HP- β -CDs, but greater than 16% and 14%, respectively, at 40 min (Fig. 3A). At 90 min of extraction, no statistical significant differences between ethanol/water and CDs extractions were observed.

In the case of epicatechin, the best extracting agent was HP- β -CDs, followed by ethanol/water and β -CDs (Fig. 3B). By 90 min, the HP- β -CDs solution had extracted 13% more epicatechin than ethanol/water, and no statistical significant differences between ethanol/water and β -CDs extraction were observed (Fig. 3B).

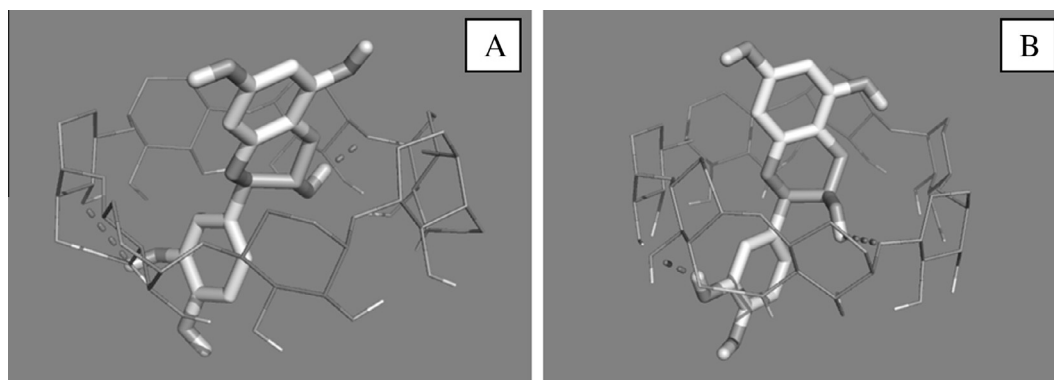


Fig. 2. Docking results obtained for the complexation of catechin (A) and epicatechin (B) with β -CDs. Cyclodextrin core is represented with thin lines and hydrogen bonds with yellow dashes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

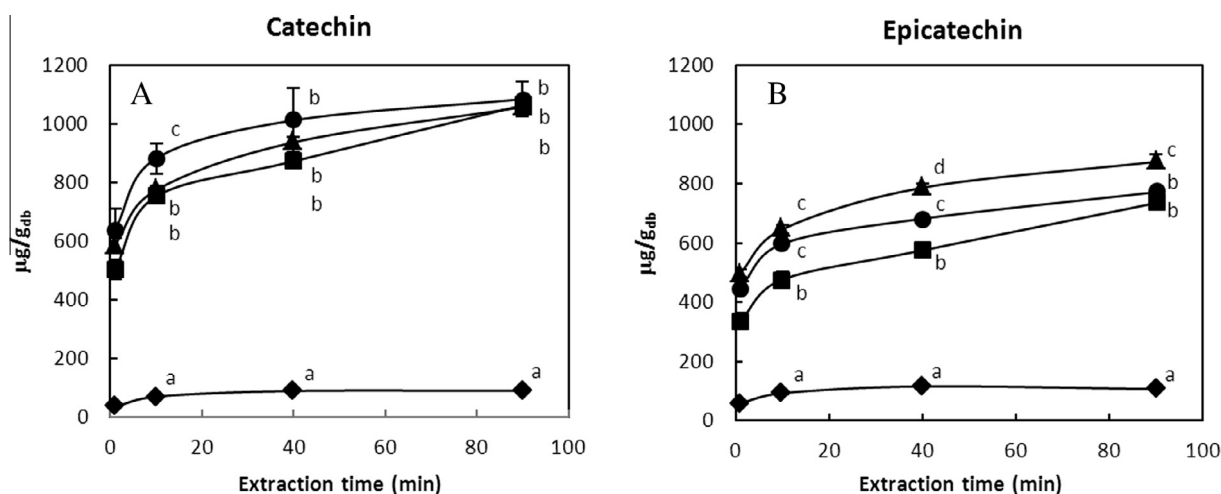


Fig. 3. Evolution of catechin (A) and epicatechin (B) extracted ($\mu\text{g}/\text{g}_{\text{db}}$) at 25 °C depending on extraction time (min) and extracting solvents ethanol/water (●), water (◆), β -CDs (■) and HP- β -CDs (▲). Values represent means of triplicate extraction. For extraction times 10, 40 and 90 min, statistical difference between means are shown ($p < 0.05$) (a–d).

The temperature was also an important factor during the solid–liquid extraction process. Only moderate temperatures are suitable in order to prevent damage or negatively effect on phenolic compounds and their biological activity (Bucic-Kojic et al., 2013). Extractions of catechin and epicatechin at 40 °C are shown in Fig. 4A and B.

Catechin and epicatechin extraction increased with the extraction time at 40 °C, being more intense during the first 10 min, after which the extraction rate became more moderate (Fig. 4). At 40 °C, there were significant differences in the amount of catechin and epicatechin extracted between water and CDs solutions.

Regarding to the extraction of catechin, there were no statistical significant differences between β - and HP- β -CDs. Extraction with β -CDs at 40 °C reached a maximum of 1348 $\mu\text{g}/\text{g}_{\text{db}}$, representing a 26% increase compared with extraction at 25 °C. Extraction with HP- β -CDs reached a maximum of 1467 $\mu\text{g}/\text{g}_{\text{db}}$, which represents 39% of increase in relation to extraction at 25 °C.

In the case of epicatechin, maximum extraction were 896 $\mu\text{g}/\text{g}_{\text{db}}$ for β -CDs and 1112 $\mu\text{g}/\text{g}_{\text{db}}$ for HP- β -CDs, which represents an increase of 22% and 27%, respectively, in relation with extraction observed at 25 °C. It could be observed statistical significant differences between β - and HP- β -CDs from 10 to 90 min of extraction time.

These results demonstrated that catechin and epicatechin could be extracted from grape pomace by using CDs aqueous solutions,

reaching similar level of extraction than using ethanol/water as extraction solvent.

Finally, the effect of the type of energy applied, stirring or ultrasound, to extract catechin and epicatechin from grape pomace was studied. Fig. 5A and B show the extraction of catechin and epicatechin in the presence of β - or HP- β -CDs, as well as water or ethanol/water, using stirring or ultrasound as energy source, at 25 °C for 40 min.

As can be seen in Fig. 5A and B, with respect to the extraction method, the trend was similar to that observed for TPC (Table 1). The yield obtained with ultrasound (Fig. 5, dark gray bars) was slightly higher than with stirring (Fig. 5, light gray bars). On average, the improvement in catechin and epicatechin extraction obtained by using ultrasound was around 12% for all the extraction agents except for water, in which case, the increase was 119% and 109% for catechin and epicatechin, respectively.

An increase in CDs concentration also improved catechin and epicatechin extraction in both methods, as was observed in the case of TPC (Table 1).

4. Conclusions

It can be concluded that CDs can be used as an alternative to organic solvents for the extraction of phenolic compounds, espe-

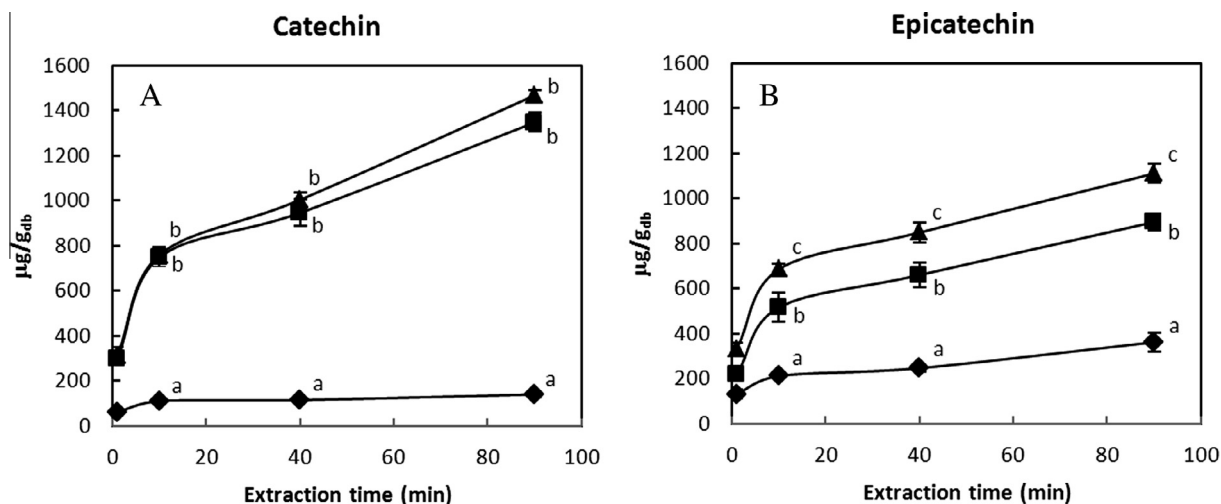


Fig. 4. Evolution of catechin (A) and epicatechin (B) extracted ($\mu\text{g}/\text{g}_{\text{db}}$) at $40\text{ }^{\circ}\text{C}$ depending on extraction time (min) and extracting solvents water (\blacklozenge), β -CDs (\blacksquare) and HP- β -CDs (\blacktriangle). Values represent means of triplicate extraction. For extraction times 10, 40 and 90 min, statistical difference between means are shown ($p < 0.05$) (a–c).

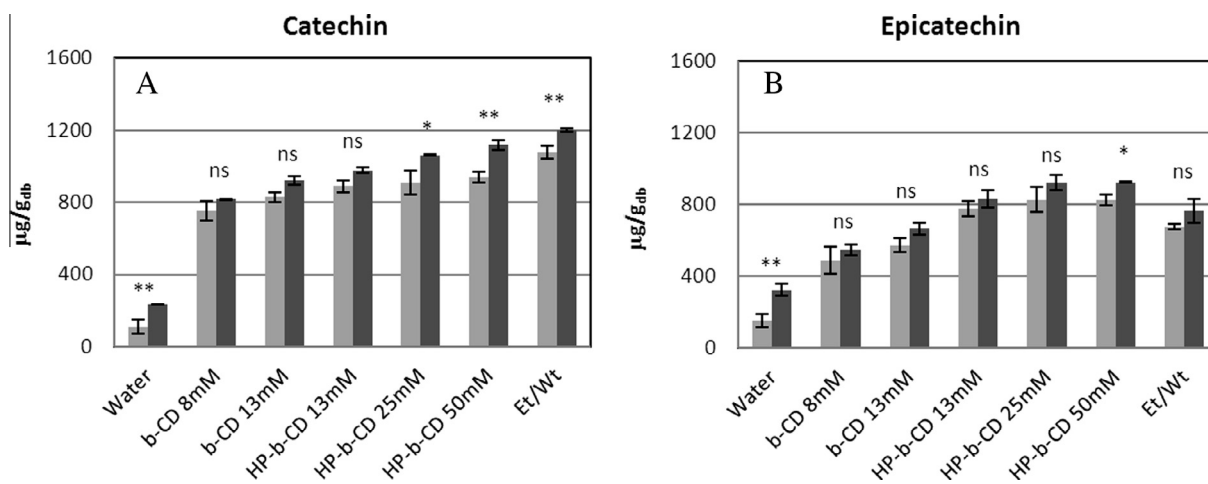


Fig. 5. Catechin and epicatechin extracted ($\mu\text{g}/\text{g}_{\text{db}}$) by stirring (light gray) and ultrasound (dark gray) at $25\text{ }^{\circ}\text{C}$ during 40 min using different solvents: Water, β -cyclodextrins (b-CD 8 and 13 mM), HP- β -cyclodextrins (HP-b-CD 13, 25 and 50 mM) and ethanol/water (Et/Wt). Values represent means of triplicate extraction. Statistical significance between stirring and ultrasound: ($**$) $p < 0.01$; ($*$) $p < 0.05$; (ns) no significant.

cially catechin and epicatechin. The extraction yield of catechin and epicatechin using aqueous solutions of CDs was similar to that obtained using organic solvents such as ethanol. So, CDs could be considered a viable solution for the selective extraction of some interesting phenolic compounds from grape pomace.

Acknowledgments

This research was supported by the Agencia de Ciencia y Tecnología de la Región de Murcia under the project PFE-seneca/06/10 and Bodegas San Isidro in Jumilla (Spain).

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