EXTENDED ABSTRACT

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Effect of cyclodextrin and extraction method on extraction of phenolic compounds extraction from red wine pomace

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Abstract:

Extraction of phenolic compounds has always been conditioned by the use of organic solvents. Cyclodextrins (CDs) could be used as an alternative approach to reducing the use of organic solvents for the extraction of phenolic compounds. This work aims to determine the effect of CDs and extraction method on the extraction of phenolic compounds. Phenolic extractions were made from Monastrel red variety pomace. Extractions were made by ultrasound and microwave at 50ºC during 10 min with aqueous solutions of α-, β-, γ-, HP-α-, HP-β-, HP-γ-CD 50 mM, water and ethanol/water (1:1 v/v). Total phenolic compounds, procyanidins, flavanols, favonols and antioxidant capacity were measured. The final extraction of total phenolic compounds and antioxidant capacity obtained using cyclodextrins were higher than those obtained using water, but lower than those obtained using ethanol/water. Microwave assisted extraction recovered 75% more total phenolics than ultrasound. Extraction of flavanols and flavonols with CDs was similar to those obtained using ethanol/water. The type of CDs also had great impact on favonols extraction. CDs could be used as an alternative to organic solvents for the extraction of specific phenolic compounds from pomace.

List of abbreviations: Cyclodextrins (CDs). Hydroxypropyl (HP)

Short title: Extraction of grape pomace phenols with cyclodextrins

INTRODUCTION

Wine marc is the main byproduct in wine production and is mainly composed of grape skins and seeds (Barba et al, 2016). Phenolic compounds, particularly flavonoids, have been the subject of numerous studies due to its antioxidant potential and therefore its health benefits (Arvanitoyannis et al., 2006). Extraction of phenolic compounds using organic solvents is the most often used technique for recovery of bioactive compounds from plant materials. Despite its effectiveness, it implies huge energetic and economic outlay, it is polluting and can result in degradation of the bioactive compounds (Barba et al. 2016). Using microwave (MW) assisted extraction and ultrasounds (US) assisted extraction together with CDs represents a green alternative to the use of organic solvents (Bittar et al 2013, Baiano, 2014). CDs offer a wide range of possibilities for the

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formation of inclusion complexes with hydrophobic molecules. Its effectiveness has been proven in different industrial sectors and they are currently being used in pharmaceutical and food products (Martin del Valle, 2003; Astray et al., 2009).

MATERIALS AND METHODS

Chemicals and reagents

α-, β-, γ-, HP-α-, HP-β-, HP-γ-CD were purchased from Winplus International Limited (Zhejiang, China). Ethanol and Gallic Acid were purchased from Scharlab (Barcelona, Spain). HPLC Water and Methanol were purchased from J.T. Baker (Center Valley, PA, USA). Hexano, Fluorescein, AAPH, Trolox and Folin–Ciocalteu reagent were purchased from Sigma Aldrich (San Luis, MI, USA). Sodium carbonate were ACS reagent grade.

Plant materials

Pomace samples were provided by Bodegas San Isidro (Jumilla, Spain). The grape variety used is Monastrel and samples were collected in September 2014. The samples were stored in amounts of 500 g in sealed plastic bags and froze at -80ºC until use.

Methods

Wine pomace was defrosted, milled (30 sec) defatted with hexane twice for 15 min each time (1/10 (w / v)) and dried in an oven for 24 hours at 50ºC. Dried and degreased pomace was stored in a sealed plastic bag and refrigerated until use. MW assisted extraction samples were prepared in proportion 1/9 (w / v), the extraction time was 10 min at 850W in 30 sec cycles. Between cycles the sample was cooled to maintain the temperature of the process between 30-70 °C. US assisted extraction samples were prepared in proportion 1/9 (w/v), the extraction time was 10 min at 50ºC. Methods for Antioxidant capacity (ORAC method) and HPLC analysis were described by Lopez-Miranda et al. (2016).

RESULTS AND DISCUSSION

Results given in Figure 1 show that MW total phenols extraction was higher than the US extraction. Greater recovery was obtained with ethanol-water (29.7 mg/g), although the addition of CDs has improved the extraction rate by 20% for native CDs, and 80% for modified CDs compared to the extraction with water. In relation to the antioxidant capacity (Figure 2), a direct relationship between the amount of total phenolic compounds extracted and the antioxidant capacity of the extracts is observed.

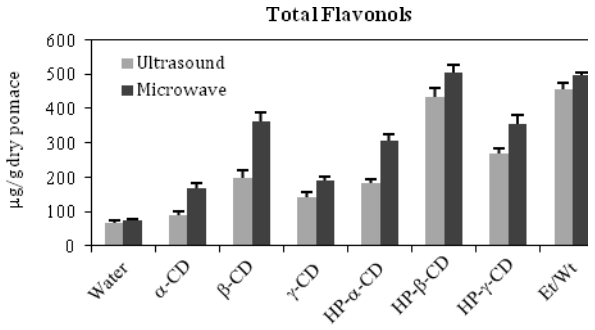

Figure 1. Total phenolic compounds (mg/g) extracted by Ultrasounds and Microwave with different extractants.

In order to determine the effectivity of the extraction on the different types of phenolic compounds the phenolic profile was analyzed by HPLC. Figure 3 illustrates the effect on the recovery of flavonols when running MW assisted extraction and using water (A), HP-β-CD (B) and ethanol-water (C) as extraction agents. For other types of CDs and phenols, the effect was the same differing only in the level of recovery reached for each compound.

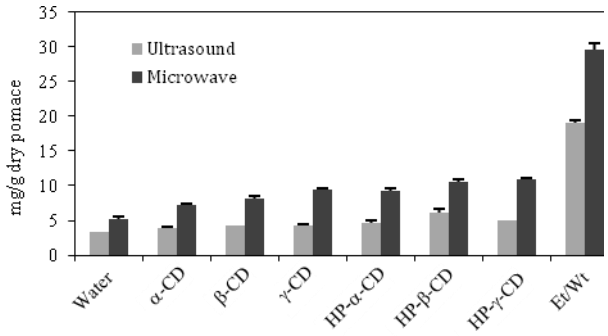

Figure 2. Antioxidant capacity (mM Trolox) of the extracts obtained using Ultrasounds and Microwave with different extractants.

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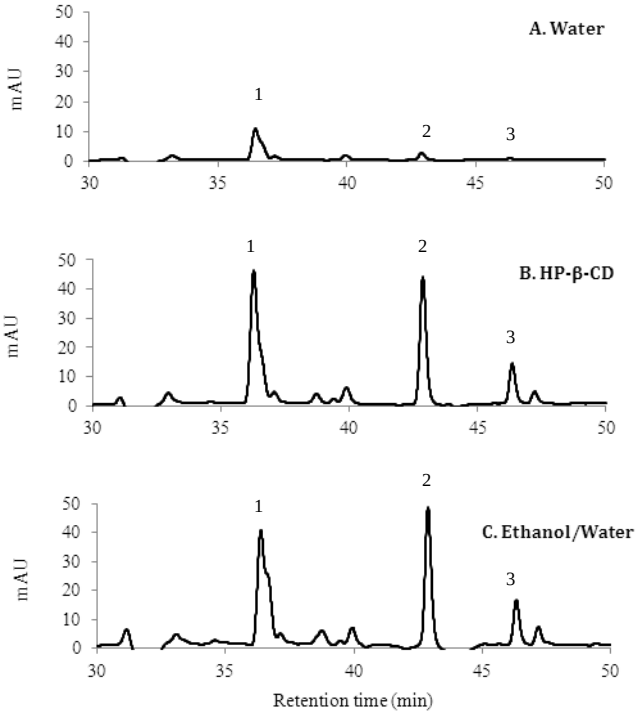

Figure 3. Chromatogram at 370 nm of wavelength corresponding pomace extracts obtained using MW with A. A Water, B. Ethanol/Water and C. HP-β-CD. 1. Quercetin 3-β-glycoside, 2. Quercetin, 3. Kaempferol.

Water has obtained very little recovery of quercetin 3-β-glycoside and quercetin and an almost nonexistent recovery of kaempferol. On the other hand adding HP-β-CD has allowed the recovery of a greater amount of such compounds reaching very similar figures to those obtained with ethanol-water. This is due to the tendency of these compounds to enter into the hydrophobic cavity of cyclodextrins increasing their water solubility and, thus allowing their recovery.

Figure 4 shows the extraction values for total procyanidins, flavanols and flavonols depending on the type of extraction and extraction agent used. As it was observed for total phenol extraction, microwave extraction remains the most effective for extracting each group of compounds. The effect of the CDs in the extraction has also been apparent. Firstly, it stands out the fact that procyanidins are the compounds that have been less influenced by the use of different types of CDs, while the extraction of total flavonols has been conditioned to the use different CDs.

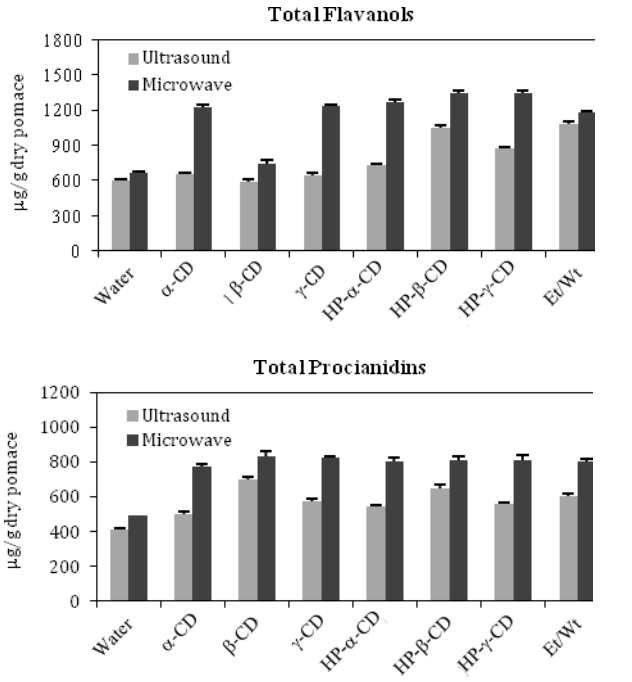

Figure 4. Total Flavanols, Procianidins and Flavonols (µg/g dry pomace) of extractions at 50ºC using Ultrasound and Microwave for 10 minutes.

β-CD and HP-β-CD have been the best performing CDs when it comes to favonols extraction. The high affinity of kaempferol and quercetin to form inclusion complexes with HP-β-CD had previously been observed by Lucas-Abellán et al. (2008) and Mercader-Ros et al. (2013). These compounds have been associated with high antioxidant potential, which is enhanced after encapsulation with HP-β-CD due to the protection offered by the CD towards free radicals (Mercader-Ros et al. 2010).

In conclusion, the use of CDs could be an interesting alternative to organic solvents for the extraction of specific phenolic compounds.

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