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Extraction of phenolic compounds from grapes and their pomace using β-cyclodextrin

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ABSTRACT

Use of aqueous cyclodextrins (CD) for recovery of selected bioactive phenolic compounds from grapes and their pomace was evaluated. When α, β and γ forms of CD were compared, β-CD was the most effective in recovering stilbenes, flavonols, and flavan-3-ols from grape pomace. The maximum quantified phenolics were obtained from the powder and the slurry of grape pomace when extracted with 2.5% (w/v) aqueous β-CD solutions at 60 °C for 12–24 h. With β-CD, total quantified phenolics obtained from the dry powder were 123 mg/100 g (DW) while from the slurry, they were 35.8 mg/100 g (FW). Incorporation of β-CD to grape mash prior to pressing for juice enhanced the recovery of phenolics in juice. Incorporation of β-CD was more effective in recovering flavan-3-ols than flavonols. Aqueous CD can effectively be used in recovering phenolics from by-products of fruit processing and therefore for functional foods and nutraceutical applications.

1. Introduction

Cyclodextrins (CD) are cyclic oligosaccharides, where glucose molecules are linked by α (1 → 4) glycosidic bonds to form a cylindrical structure (Lucas-Abellán, Fortea, López-Nicolas, & Núñez-Delicado, 2007). The cylinder forms a truncated cone, which has a hydrophilic rim and a lipophilic interior (Romo, Penas, Isasi, Garcıa-Zubiri, & González-Gaitano, 2008). Due to this specific structure, CD possesses an ability to form host–guest inclusion complexes with a wide range of compounds, thus increasing the water solubility of hydrophobic compounds (Del Valle, 2004). The guest molecules interact with the cavity of the host molecule (CD) with non-covalent forces, such as van der Waals forces, hydrophobic interactions and hydrogen bonds (Loftsson & Brewster, 1996).

The inclusion complexes formed between β-CD and some of the phenolic compounds have been studied (Gornas, Neunert, Baczynski, & Polewski, 2009). Phenolic compounds such as *trans*-resveratrol (*t*-R), rutin, epigallocatechin and chlorogenic acid form inclusion complexes with β-CD in a 1:1 molecular ratio (Gornas et al., 2009; Li, Xu, Liu, Sun, & Li, 2010; López-Nicolas & García-Carmona, 2008; Xu, Tan, Janson, Kenne, & Sandstroem, 2007). Recent studies on the complex formation between aqueous β-CD and oleuropein of olive leaf (Mourtzinos, Salta, Yannakopoulou, Chiou, & Karathanos, 2007), chlorogenic and caffeic acids of coffee brew (Gornas et al., 2009), oleoresin of turmeric rhizome (Haiyee, Said, Illias, Mustapha, & Hassan, 2009), catechin, epicatechin and quercetin of St. John's wort (Kalogeropoulos, Yannakopoulou, Gioxari, Chiou, & Markis, 2010), and *t*-R of *Polygonum cuspidatum* (Mantegna et al., 2012) were reported.

Grape pomace consists of residual seeds, skins and stems left after pressing for juice. About 70% of grape phenolic compounds remain in the wine pomace (Mazza, 1995) and is a rich source of unique health promoting polyphenols, such as *trans*-resveratrol glucoside (*t*-RGlu, polydatin or piceid) (Pinelo, Rubilar, Jerez, Sineiro, & Nunez, 2005; Ratnasooriya, Rupasinghe, & Jamieson, 2010). Use of grape pomace as an inexpensive source for recovering biactive components has been explored (Luque-Rodríguez, Luque de Castro, & Pérez-Juan, 2007).

In conventional bioactive extraction techniques, organic solvents were commonly used to recover phenolic compounds from plant tissues (Bonilla, Mayen, Meria, & Medina, 1999). However, the use of organic solvents is associated with environmental pollution, toxicological and safety concerns. Therefore, alternative extraction methods such as supercritical fluid extraction, microwave-assisted extraction using a polar solvent, and superheated water have been investigated (Shi et al., 2005; Wang & Weller, 2006). However, these techniques need advanced equipment and are thus, costly. As far as we are aware, no research has been

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reported on use of CD in recovering *trans*-resveratrol and other major phenolics from grapes and their pomace. In food industry, β-CD has been used as a flavour carrier and protectant at a level of 2% in numerous food products and also listed as a Generally Recognized As Safe (GRAS) food additive since 1998 (Szente & Szejtli, 2004).

The current research was carried out with the objectives of studying: how the number of glucose residues in CDs effect on inclusion complex formation, how aqueous β-CD-based extraction conditions effect on specific phenolic compound recovery from grape pomace (dry powder and slurry) and the potential of incorporating β-CD to grape mash before pressing for juice as a pre-treatment to enhance recovery of specific phenolic compounds in grape juice.

2. Materials and methods

2.1. Plant materials and chemicals

Grape pomace of cultivar 'Lucie Kulhman' was obtained after pressing fresh grapes for juice using a commercial wine pressing ratchet (Musca Wine Pressing and Supplies Ltd., Ottawa, ON). The pomace was stored at −20 °C until used for analysis. 'Red Globe' grapes were purchased from the local market for pre-treatment experiments of juice processing.

α-, β- and γ-CD were obtained from Cyclodextrin Technologies Development, Inc. (High Springs, FL, USA). Liquid chromatography standards used in the study were purchased from: chlorogenic acid, ferulic acid and quercetin (Q) from Sigma–Aldrich (Orkville, ON, Canada); and catechin, epicatechin (EC), epigallocatechin (EGC), epigallocatechin gallate (EGCG), epicatechin gallate (ECG), Q-3-*O*-glucoside (Q3Glu), Q-3-*O*-galactoside (Q3Gal), Q-3-*O*-rhamnoside (Q3R), *t*-R and *t*-RGlu from ChromaDex (Santa Ana, CA, USA).

2.2. Grape sample preparation

Pomace was thawed for 5–6 h at room temperature prior to use. Previous studies reported that 60 °C was the optimum temperature for solvent extraction of phenolic compounds and exposure to temperatures higher than 70 °C can cause degradation of phenolic compounds (Romero-Peréz, Lamuela-Reventos, Andres-Lacueva, & de La Torre-Boronat, 2001). Therefore, the pomace was oven dried at 60 °C with a dry air flow (Model 28, GCA/Precision Scientific, Chicago, IL, USA) for 7 h. The dried pomace was ground into a fine powder using a coffee grinder (Model 6378-33, Sunbeam Products Inc., Boca Raton, FL, USA). Dry matter content of the powder was obtained after oven drying at 105 °C until a constant weight was achieved.

The slurry was prepared by blending the pomace with either deionized water (DI), 80% ethanol (v/v) or 2.5% (w/v) aqueous β-CD solution at a ratio of 1:10 (w/w) pomace to solvent, using a commercial blender (Model HBB908, Hamilton Beach Proctor-Silex, Inc., Washington, NC, USA). After removing stems, 'Red Globe' grapes were washed and drained. A sample of 200 g was cut into slices (2–4 mm) using a food processor (Model FP 1445, The Black and Decker Corporation, Towson, ML, USA) and pressed for juice using a stainless steel fruit squeezer (Norpro Inc., Everette, WA, USA). Extract was filtered using six layers of cheesecloth and the juice was collected.

2.3. CD solutions

Required concentrations of β-CD were prepared by adding a measured weight of β-CD to the required volume of double distilled (DI) water and kept in a shaking water bath (70 rpm) at 60 °C until β-CD was completely dissolved.

2.4. Recovery of phenolic compounds from dry grape pomace powder

Three concentrations of β-CD (1.0%, 2.5% and 5.0%, w/v) were prepared separately, using either DI water or 80% ethanol (v/v). Grape pomace powder of 0.5 g and a measured weight of β-CD were added to either total volume of 10 ml water or 10 ml 80% ethanol solutions (1:20 solid to solvent ratio was selected based on the preliminary experiments, data not presented). These samples were placed in amber coloured vials with tightly fitting lids and kept in a shaking water bath (70 rpm) (Model WS 17, Geneq Inc., Montreal, QC, Canada) at 60 °C for 48 h. Eighty percent ethanol and DI water without β-CD were used as solvents for comparison.

Effect of extraction time and temperature on recovery of phenolic compounds was studied by placing in Erlenmeyer flasks containing 0.5 g of powdered pomace and 10 ml of 2.5% β-CD solution in DI water (concentration of β-CD was selected based on the above experiment) in shaking water baths (70 rpm) (Model WS 17, Geneq Inc., Montreal, QC) at four temperatures (room temperature (22 ± 1 °C), 45, 60 and 75 °C) for six time periods (12, 24, 36, 48, 60 and 72 h). A solution of 80% ethanol in DI water (without β-CD) was used as the control.

2.5. Comparison of α-, β- and γ-CD in aqueous solutions

Impact of the number of glucose residues in CD (α, β and γ) forms on recovery of phenolic compounds were compared. Concentration of each solution was 0.025 mol/l (α-CD = 2.4%, β-CD = 2.8% and γ-CD = 3.25%, w/v). Samples were prepared as described above using α-, β- and γ-CD, and were kept in a shaking water bath (70 rpm) (Model WS 17, Geneq Inc., Montreal, QC, Canada) at 60 °C for 12 h.

2.6. Recovery of phenolic compounds from grape pomace slurry

Effect of β-CD on recovery of phenolic compounds from the slurry was evaluated by keeping blends in a shaking water bath at room temperature for 24 h. Slurries prepared with 80% ethanol and DI water was used as the control. Effect of time and temperature on recovery of phenolic compounds was studied by keeping 10 g of the slurry prepared with 2.5% β-CD solution, in shaking water baths (70 rpm) under the following extraction conditions: room temperature for 24 h, 45 °C for 6 and 12 h and at 60 °C for 0.5, 6, and 12 h.

2.7. Application of β-CD as a pre-treatment to enhance recovery of phenolics in grape juice

To evaluate the ability of β-CD to enhance recovery of phenolic compounds in juice, five β-CD concentrations (0.1%, 0.25%, 0.5%, 1% and 2%, w/w) were added separately to the grape mash and the samples were incubated at room temperature (22 ± 1 °C) for 12 h, before pressing for juice. Controls were prepared by pressing the mash kept for 12 h without β-CD at room temperature.

2.8. Determination of phenolic concentration

Samples were prepared and analyzed for chlorogenic acid, ferulic acid, catechin, EC, EGC, EGCG, ECG, Q, Q3Glu, Q3Gal, Q3R, *t*-R and *t*-RGlu, using high performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS) as described by Rupasinghe, Wang, Huber, and Pitts (2008) and Ratnasooriya et al. (2010). The mobile phase of HPLC consists of acetonitrile, which allows the release of phenolic compounds from the inclusion complexes of CD. The total quantified phenolics were expressed as follows: individual concentrations of chlorogenic acid and ferulic acid were combined as an expression of total quantified phenolic acids;