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Enzyme-assisted extraction of phenolics from winemaking by-products: Antioxidant potential and inhibition of alpha-glucosidase and lipase activities

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ABSTRACT

Phenolics in food and agricultural processing by-products exist in the soluble and insoluble-bound forms. The ability of selected enzymes in improving the extraction of insoluble-bound phenolics from the starting material (experiment I) or the residues containing insoluble-bound phenolics (experiment II) were evaluated. Pronase and Viscozyme improved the extraction of insoluble-bound phenolics as evaluated by total phenolic content, antioxidant potential as determined by ABTS and DPPH assays, and hydroxyl radical scavenging capacity, reducing power as well as evaluation of inhibition of alpha-glucosidase and lipase activities. Viscozyme released higher amounts of gallic acid, catechin, and prodelphinidin dimer A compared to Pronase treatment. Furthermore, *p*-coumaric and caffeic acids, as well as procyanidin dimer B, were extracted with Viscozyme but not with Pronase treatment. Solubility plays an important role in the bioavailability of phenolic compounds, hence this study may assist in better exploitation of phenolics from winemaking by-products as functional food ingredients and/or supplements.

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1. Introduction

Grapes and their derived beverages are important sources of food phenolics (da Silva et al., 2016; Tao et al., 2016). However, winemaking generates a large amount of by-products (e.g. skins and seeds). These by-products serve as rich sources of phenolics belonging to several classes of compounds such as phenolic acids, flavonoids, including anthocyanins, as well as proanthocyanidins (Cheng, Bekhit, McConnell, Mros, & Zhao, 2012; de Camargo, Regitano-d'Arce, Biasoto, & Shahidi, 2014).

Phenolic and/or polyphenolic compounds have attracted much attention due to their wide range of potential health benefits, as substantiated by both *in vitro* and *in vivo* studies (de Camargo et al., 2014; Vicente, Ishimoto, & Torres, 2014). The role of food phenolics in preventing degenerative, vascular and heart disease and as anti-inflammatory and antimicrobial agents have also been reported (Alasalvar & Bolling, 2015; Shahidi & Ambigaipalan, 2015). Additionally, phenolic compounds may play an important role in amiliorating certain types of cancer, including colorectal cancer (Shahidi & Ambigaipalan, 2015). The chemical structures of these molecules are as important as their detection and concentration, which may reflect in a different correlation between a particular molecule and its activity.

Phenolic compounds are present in the soluble (free and esterified) and insoluble-bound forms, the proportion of each one depends not only on the starting material but also on their cultivar and an eventual processing to which they are subjected. For example, the esterified phenolics from lentils were generally in higher amount, but some cultivars also showed higher content in the fraction containing insoluble-bound phenolics (Alshikh, de Camargo, & Shahidi, 2015), whereas berry seed meals had higher content of insoluble-bound phenolics (Ayoub, de Camargo, & Shahidi, 2016). Peanut skin submitted to gamma-irradiation had increased free and insoluble-bound phenolic contents upon processing (de Camargo, Regitano-d'Arce, Gallo, & Shahidi, 2015). As antioxidants, phenolic compounds may counteract oxidative reactions in food subjected to treatments such as gamma irradiation and pasteurization as well as during long-term storage, which may affect its shelf-life and sensory characteristics (da Silva et al., 2014; de Camargo at

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al., 2012a). Furthermore, biologically relevant molecules such as lipids, proteins, lipoproteins and DNA may also be protected from oxidatively reactive compounds.

In a previous study at this department (de Camargo et al., 2014), it has been demonstrated that, regardless of the process (juice or winemaking), insoluble-bound phenolics were major fractions in grape processing by-products. The same study also provided evidence about the dominant benefits of insoluble-bound phenolics of grape by-products in inhibiting copper-induced human LDL-cholesterol oxidation and peroxyl radical-induced DNA strand breakage. These results demonstrated the potential of the insoluble-bound phenolics from winemaking by-products as their major source of bioactive compounds.

Enzyme-assisted extraction has been regarded as an alternative method for improved extraction of food phenolics (Montella et al., 2013; Papillo, Vitaglione, Graziani, Gokmen, & Fogliano, 2014), especially the insoluble-bound phenolics, which are linked to carbohydrates and proteins of cell wall matrices. However, to the best of the authors' knowledge, there is no literature providing the effect of enzyme-assisted extraction on the ratio of soluble to insoluble-bound phenolics from winemaking by-products although this has been reported for germinating lentils by Yeo and Shahidi (2015). Thus, in the present study, winemaking by-products (cv. Tempranillo) were treated with Pronase and Viscozyme to improve the solubility of phenolics present in the sample. The effects were studied based on the change in the distribution pattern of soluble/insoluble-bound phenolics as well as their chemical profile, antioxidant properties (antiradical activity) and reducing power. The resultant products were also evaluated for their effect in deactivating alpha-glucosidase and lipase, which have a key role in the prevention and management of diabetes and obesity, respectively.

2. Material and methods

Winemaking by-products (cv. Tempranillo) were kindly provided by Santa Maria Winery (Lagoa Grande, Pernambuco State, Brazil). Hexane, acetone, diethyl ether, ethyl acetate, methanol, acetonitrile, formic acid, hydrochloric acid, sodium hydroxide, potassium persulfate, sodium chloride, trichloroacetic acid, sodium carbonate, dimethyl sulphoxide and Tris base were purchased from Fisher Scientific Ltd. (Ottawa, ON, Canada). Pronase, Viscozyme, alpha-glucosidase from *Saccharomyces cerevisiae*, and type II crude porcine pancreatic lipase, catalogue numbers P5147, V2010, G5003, and L3126, respectively, as well as Folin Ciocalteau's phenol reagent, DPPH, ABTS, mono- and dibasic potassium phosphates, hydrogen peroxide, DMPO (5,5-dimethyl-1-pyrroline-*N*-oxide), ferrous sulphate, potassium ferricyanide, ferric chloride, Trolox, *p*-nitrophenyl β-D-glucopyranoside, *p*-nitrophenyl octanoate, caffeic, gallic, and *p*-coumaric acids, catechin and epicatechin were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada).

2.1. Effect of enzyme treatment on the starting material (Experiment I)

The first experiment was designed to study the effect of pretreatment of selected enzymes on the soluble/insoluble-bound phenolic ratio and *in vitro* bioactivity. The sample (50 g) was freeze dried at −48 °C and 30×10^{-3} mbar (Freezone 6, model 77530, Labconco Co., Kansas City, MO), ground with a coffee bean grinder (Model CBG5 series, Black & Decker, Canada Inc., Brockville, ON, Canada) and the powder was passed through a mesh 16 (sieve opening 1 mm, Tyler test sieve, Mentor, OH) sieve. The powder so obtained was defatted three times with hexane (solid/solvent, 1:5, w/v) using a Warring blender (Model 33BL73, Warring Products Division Dynamics Co. of America, New Hartford, CT). Defatted samples were recovered by vacuum filtration and stored at −20 °C (de Camargo et al., 2014). Defatted samples (10 g) were suspended in 100 mL of Viscozyme solution (2% in 0.1 M phosphate buffer, pH 4) and stirred for 12 h at 37 °C in a gyratory water bath shaker (Model. G76, New Brunswick Scientific Co. Inc., New Brunswick, NJ) or 100 mL of Pronase solution (1 mg/mL in 0.1 M phosphate buffer, pH 8) and stirred for 1 h. Controls containing each respective buffer (devoid of enzyme) were prepared at the same time and under the same conditions. Treated samples and respective controls were freeze dried to obtain a dry powder and further used for extraction of soluble and insoluble-bound phenolics (within one week). The extraction of soluble phenolics was carried out with 70% (v/v) acetone (2.5%, w/v) in a gyratory water bath shaker at 30 °C for 20 min. After centrifugation at 4000*g* (IEC Centra MP4, International Equipment Co., Needham Heights, MA), the upper layer was collected and the extraction was repeated twice. The combined supernatants were evaporated under vacuum at 40 °CC (Buchi, Flawil, Switzerland) to remove the organic solvent. The extract so obtained (soluble phenolics) was stored at −20 °C until used for further analysis within three months. To the dry residue remaining after the extraction of soluble phenolics, 4 M NaOH was added, and hydrolyzed, while stirring under nitrogen for 4 h at room temperature (23–25 °C). The resulting slurry was acidified to pH 2 with 6 M HCl. Phenolics released from their insoluble-bound form were then extracted with diethyl ether and ethyl acetate (1:1, v/v), and reconstituted in HPLC-grade methanol (de Camargo et al., 2014).

2.2. Effect of enzyme treatment on the residue remaining after extraction of soluble phenolics (Experiment II)

The second experiment was carried out to evaluate the effect of enzyme treatment on the yield and identity of phenolics remaining after extraction of its soluble counterpart. The effect of enzyme treatment on the *in vitro* bioactivity was also evaluated. In short, the extraction of soluble phenolics was carried out with 70% (v/v) acetone as described above, and only the dry residue remaining after this extraction was treated with Viscozyme or Pronase, using the aforementioned conditions. After enzyme treatment the resulting slurry was acidified to pH 2 with 6 M HCl. Phenolics released from their insoluble-bound form upon enzyme treatment were then extracted with diethyl ether and ethyl acetate (1:1, v/v), and reconstituted in HPLC-grade methanol. To compare results, an alkali hydrolysis was carried out as described above. Thus, three different extracts were obtained in this experiment (phenolics released from their insoluble-bound form upon Viscozyme, Pronase, and NaOH treatment).

2.3. Total phenolic content (TPC)

The TPC (Swain & Hillis, 1959) was evaluated using the same procedure and equipment as described elsewhere (de Camargo, Vidal, Canniatti-Brazaca, & Shahidi, 2014). The results were expressed as milligram gallic acid equivalents (GAE) per gram of defatted samples.

2.4. HPLC-DAD-ESI-MS^n analysis

HPLC-DAD-ESI-MS^n analyses were conducted to investigate the effect of Viscozyme and Pronase treatments on the residue remaining after extraction of soluble phenolics. This allowed for the positive, or tentative, identification and quantification of major phenolics as affected by each treatment. The extract obtained using alkali extraction was also evaluated. This approach was chosen to examine the effects of enzyme treatment on the fraction containing insoluble-bound phenolics and individual components present,

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