Extraction optimization for polyphenolic profiling and bioactive enrichment of extractives of non-pomace residue from grape processing

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1. Introduction

Grape and its by-products are sources of polyphenols that exert high antioxidant activity (Spigno and De Faveri, 2007). Grape juice is derivative product that contains phenolic compounds with bioactive properties. In the American continent, V. labrusca L. grapes are traditionally used for the production of grape juices, particularly the varieties Bordo and Isabel (Rizzon and Meneguzzo, 2007).

The production and global consumption of grapes increased significantly in the period of 2000–2014. The world grape production in 2014 reached almost 27 million tonnes (FAO, 2016). During grapes processing, some residues are generated, such as the pomace and non-pomace matrices. The pomace formed by grape skin and seeds is removed by pressing, and it is estimated that approximately 20% of the volume of grapes processed are converted into pomace residues (Spanghero et al., 2009). Following pressing, a centrifugation step is used for clarification, in order to separate suspended solids and reduce juice turbidity. The weight of this residual solid (non-pomace) is approximately 4–8% of the processed juice. The high generation of this colored non-pomace residue represents a great concern for industries, since the inadequate elimination of this fermentable matrix can lead to environmental impacts. The perishable characteristic of this plant material points out the necessity of chemical characterization in order to explore its bioactive potential and possible use by chemical and pharmaceutical industries (Haas et al., 2016).

Viticulture by-products contain high concentrations of phenolic compounds. The consumption of foods rich in polyphenols has been postulated to contribute to a reduced risk of chronic diseases, including cardiovascular, neurodegenerative, carcinogenic and Alzheimer disease (Vauzour et al., 2010; Haas et al., 2016; Soukup et al., 2017). It has been demonstrated that leaves and stems of grapevine and the grape pomace represent an ideal biomass to obtain phenolic compounds (Manca et al., 2016; Eftekhari et al., 2017). The bioactive constituents present in plant-based by-products contribute to their potential use in technological applications (Balasundram et al., 2006). Currently, efforts have been directed to obtain bioactive extractives of natural matrices...
using several extraction techniques (Goula et al., 2016, Haas et al., 2016, Bajoub et al., 2017). In addition to the extraction technique, the extraction efficiency is affected by several factors, among which the type of solvent has been considered one of the most important (Dorta et al., 2012). These combined factors are essential to explore residual matrices as the scientific and industrial importance of plant materials is reinforced by its chemical characterization and economical applications.

There is growing interest by fruit industries in the reutilization or economical applications of grape by-products, aimed at reducing processing waste and environmental impacts. These are mostly represented by grape pomace and its polyphenols-rich constituents such as seeds and peels that have found valuable applications in many research areas and pharmaceutical segments. In grape juice industries, the processing is focused on maintaining juice quality and accumulation of unutilized matrices is accelerated the more the grape juice is clarified, contributing to the increased disposal of solid waste. Therefore, the objectives of this work were to achieve polyphenol enrichment of the non-pomace residue obtained from red grape juice centrifugation through optimization of extractive techniques and optimize the extraction of anthocyanins of this highly-colored material.

2. Material and methods

2.1. Chemicals

Analytical standards of phenolic compounds were purchased from Sigma-Aldrich (St. Louis, USA) and Fluka (Steinheim, Germany). The reagents 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), 1,1-diphenyl-2-picrylhydrazyl and Folin–Ciocalteu were obtained from Sigma-Aldrich (St. Louis, USA). Acetone, ethanol, acetic acid, methanol, vanillin, ferric chloride, potassium chloride and sodium acetate were purchased from Vetec (Rio de Janeiro, Brazil). Chromatographic grade methanol and acetonitrile were obtained from Vetec (Rio de Janeiro, Brazil).

2.2. Residue material and non-pomace samples

The residue material was donated by a local grape juice industry located in São Marcos, South Brazil. The non-pomace residue samples were collected after the centrifugation step of the clarification process of grape juices. The centrifugation was performed at 100 rcf (g) for 10 min on a Pleralis decanter centrifuge with a processing capacity of 9000 L of juice per hour (Jesi, Italy). The highly-colored, wet non-pomace samples were obtained from processing of the grape varieties Bordo and Isabel (V. labrusca L.) and were then kept at −18 °C. The samples were frozen in an ultra-freezer (UFV 37 Terroni, Brazil) at −80 °C and lyophilized (countertop lyophilizer, Terroni, Brazil) for 24 h to a moisture content of 5% (w/w). The samples were then passed through a sieve of 20 mesh (0.9 mm) and stored at −20 °C (Haas et al., 2016).

2.3. Extraction of phenolic compounds

In preliminary experiments, three agitation methods were evaluated for the extraction of phenolic compounds using a hydroalcoholic solution of methanol (80% v/v) as solvent: ultrasound (Ultrasonic cleaner, USC 1400®, 40 kHz), orbital agitator (KMC-1300®) and plate agitator (B. Brain Biotech International, CERTOMAT™ MO, Melsungen, Germany). The time of extractions was set at 5, 30 and 120 min for orbital agitation, ultrasonic and plate agitation, respectively. The parameters of sample mass, solvent volume and solvent: water ratio were maintained constant for the extractions. After the agitation method was defined, the following solvents were evaluated for the preparation of the non-pomace extracts: methanol (80% v/v), acetone (80% v/v) and ethanol (80% v/v), with the other conditions kept constant. To verify the efficiency of each extraction method, the non-pomace samples were spiked with phenolic compounds (15 mg L⁻¹) and the individual polyphenols were determined by high performance liquid chromatography.

2.4. Experimental design

The polyphenol enrichment of the non-pomace extracts with anthocyanins was performed using a complete three-level factorial design (3³) containing three central points. Extraction time (X₁), concentration of solvent (X₂), and solid-liquid ratio (X₃) were the independent variables, and their coded levels were presented in Table 1. The levels of independent variables were based on the preliminary experimental results.

The design was constructed with 3 replications of the center point to estimate the experimental error, leading to 18 experiments carried out in random order. The preparation of extracts of the non-pomace residue was carried using methanol as solvent and extraction in orbital agitation, as selected from the preliminary experiments (Section 2.3). The observed response (y) was the sum of peak area of the individual anthocyanins obtained from the chromatographic analysis. In order to estimate the response, an empirical model composed of a second-order polynomial was constructed (Eq. (1)), where y is the predicted response, β₀ is the model constant, βᵢ is the coefficient of the linear effect, βᵢᵢ is the coefficient of the quadratic effect, βᵢⱼ is the coefficient of interaction between factors i and j, xᵢ and xⱼ are the independent coded variables, ε is the error, k is the number of variables, and i and j are the coded factors of the model.

\[ y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_i x_i^2 + \sum_{i=1}^{k} \sum_{j=i+1}^{k} \beta_{ij} x_i x_j + \epsilon \]  

(1)

2.5. Determination of the phenolic content and antioxidant activity

The extracts were prepared according to Jara-Palacios et al. (2014), Drosou et al. (2015) with modifications. The optimization of the extraction process contributed to the choice of the best conditions for extracting the phenolic compounds. The extraction method and solvent were determined according to Section 2.4. A sample ratio of 1:10 (w/v) and a hydroalcoholic solution of methanol: water (85:15 v/v) were used for the extractions. The procedure was carried out under mechanical agitation by orbital agitator for 5 min at a room temperature of 25 °C. The samples were centrifuged (Janetzki, K 24) at 1000 rcf...
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