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Valorization of grape pomace: Extraction of bioactive phenolics with antioxidant properties

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ABSTRACT

Grape pomace can be regarded as an excellent and affordable source of polyphenolic compounds. Hence, the main objective of this work was to conduct a comparative study of different Portuguese grape varieties, using an extraction methodology with possible applications in sustainable agriculture and pest management. Scavenging capacity against DPPH•, oxygen radical absorbance capacity (ORAC), iron(II) chelating ability (ICA) and Folin–Ciocalteu assays were performed in order to evaluate the antioxidant capacity profile and total phenolic content (TPC) in ethanol/water extracts and aqueous grape pomace suspensions. Strong significant correlation between TPC and DPPH• ($R = 0.944$), and a low correlation between ORAC and the other assays was obtained ($R \leq 0.632$). ICA was not correlated with any of the other assays ($R \leq 0.263$). All grape pomace extracts have presented high antioxidant properties (ORAC) and chelating ability, ranging from 906 to 2337 μmol TE g^{-1} residue and from 55 to 104 % inhib. mg^{-1} residue, respectively. Results from HPLC analysis showed the presence of gallic acid, caffeic acid, syringic acid, (+)-catechin and (−)-epicatechin being syringic acid and (+)-catechin the major compounds. Although further studies are required, "*Touriga Nacional*" was the most promising grape cultivar regarding its highest values for TPC (142.4 ± 1.1 mg GAE g^{-1} dry residue), DPPH• (1.12 ± 0.04 mmol TE g^{-1} dry residue) and ORAC (1579 ± 244 μmol TE g^{-1} dry residue) assays. Since Portugal is a major wine producer, utilization of pomace generated during the wine elaboration steps opens a new trend toward a simple and relatively easy compounds extraction with high antioxidant activity in order to contribute to emerging industrial applications and sustainable agriculture.

1. Introduction

Portugal is the eighteenth largest global grape producer, with a production close to 839,000 million tons and the eleventh largest wine producer (FAOSTAT, 2012). Winemaking process generates significant amount of wastes (steams, seeds, peels and marcs). It is estimated that for each 6 L of wine, 1 kg of grape pomace is produced which is mainly destined to animal feed and for compost elaboration (Mendes et al., 2013). Nevertheless, large amounts of the residual quantities of bioactive substances are maintained into the vegetable tissues (Lapornik et al., 2005). Phenolic acids, several flavonoides, monomers (catechin, epicatechin and gallocatechin) and superior phenolic (proanthocyanidins or condensed tannins) were described among the main constituents (Cejudo-Bastante et al., 2011; Gonzaílez-Centeno et al., 2013; Iora et al., 2014; Yilmaz and Toledo, 2004). Due to this chemical composition, effluent treatment considerably increases the chemical oxygen demand (COD) and the biochemical oxygen demand (BOD_5). Considering the above, for the industrial sector this material represents a low cost source of usable polyphenolic compounds for the production of tannin adhesive due its high content of condensed tannins (Ping et al., 2012). Furthermore, considering the current governmental and legislative pressures, this fact tends to eliminate or reduce the costs associated with effluent treatments.

In this context, phenolics present in grape pomace represent bioactive substances with many applications also related to healthy benefits: scavenging activity against free radicals, anti-inflammatory properties (Terra et al., 2007), anti-proliferation and

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cancer therapy (Nandakumar et al., 2008). A literature search revealed that the number of publications on the key words: "antioxidant" and "grape pomace" has strongly increased in the last ten years emphasizing the growing interest in the topic. Currently the available information on the polyphenolic compounds from Portuguese grape pomace is limited. For Portuguese grapes; most of the published work focus on composition and antioxidant activity (whole or parts of the grape) (Cosme et al., 2009; Dopico-García et al., 2008; Matias et al., 2010; Paixao et al., 2007); on pomace chemical composition (Mendes et al., 2013; Prozil et al., 2012); and mainly within a wine matrix (Baptista et al., 2001; Jordao et al., 2010; Monagas et al., 2003).

Grape pomace extracts display widespread uses in pharmaceutical, cosmetic, and most recently regarding a new class of "phytosanitary bioproducts" able to control the incidence of crop diseases (Benouaret et al., 2014). Thus, data regarding the polyphenolic content and antioxidant capacity of Portuguese grape pomaces are still scarce, hindering their valorization. Hence, the present study aims to determine the antioxidant profile and the total phenolic content of Portuguese grape pomace extracts, made from the three most relevant red varieties, through two antioxidant assays (DPPH• and ORAC). The chelating ability (ICA assay) of grape pomace extracts was also performed.

For grape pomace, ethanol has been used as an extractive solvent due to its natural presence in wines (Spigno et al., 2007), its safety behavior (Shi et al., 2005), and its environmentally friendly behavior (Corrales et al., 2009), when compared to other organic solvents, namely methanol. Additionally, physicochemical characteristics (low boiling point) turn the solvent recovery operations easier and with a lower energy cost. Moreover, the medium in which the polyphenols are finally resuspended has implications on their destinies and applications, therefore, a resuspension of the bioactive extract in water increases their industrial applications.

The final antioxidant properties in ethanol/water extracts and aqueous re-suspensions of their dry residue, in order to enhance the approach toward the grape pomace emerging applications were also compared. It is also intended to select the Portuguese major red grape cultivar that would be most suitable for future polyphenolic extraction processing toward bioactive products recovery. To our knowledge, this paper represents one of the few attempts to assess the polyphenolic content and the antioxidant profile of the grape pomace coming from the most representative red varieties from the Douro region, Portugal.

2. Material and methods

2.1. Chemicals

All chemicals used were of analytical reagent grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH•), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4,4-disulfonic acid sodium salt (ferrozine) and 2,2-azobis(2-methylpropionamide) dihydrochloride (AAPH) were purchased for Aldrich (Milwaukee, WI). Folin–Ciocalteu (F–C) reagent and fluorescein sodium salt were obtained from Sigma (St. Louis, MO), while iron(II) chloride tetrahydrate, gallic acid, and (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were obtained from Fluka (Buchs, Switzerland). HPLC standards Gallic, *p*-hydroxybenzoic, caffeic, syringic, *p*-coumaric and o-coumaric, sinapic, ferulic acids employed for Phenolic Acids (*PA method*), whilst (+)-Catechin, (−)-Epicatechin, (−)-Epicatechin gallate, *trans*-resveratrol, quercetin, kaempferol and chlorogenic acid, used for Anthoxanthins and Stilbenes (*AX method*), were purchased from Sigma. Water from Sartorius AG system (resistivity >18 MΩ cm) (Göttingen, Deutschland) and absolute ethanol p.a. (Panreac Química, Spain) were used in the preparation of all solutions.

2.2. Solutions

For assessment of total phenolic content (TPC), the commercial F-C reagent was diluted 3:10 (v/v) in water. A solution of $Na_2CO_3{\cdot}10H_2O$ 24.3% (w/v) was prepared, corresponding to 9% (w/v) of sodium carbonate, and also gallic acid standard solutions ($1.0 - 15.0\ mg\ L^{-1}$) for calibration purposes. For the DPPH• assay, a stock solution of DPPH• in ethanol (600 μM) was prepared and kept in dark at room temperature. Three dilutions from the stock DPPH• solution (between 75 and 225 μM) were also prepared. For DPPH• assay, all Trolox standard solutions (5.0 – 50.0 μM) were prepared in ethanolic solution 50% (v/v). For iron(II) chelating ability (ICA) assay, all iron(II) solutions were freshly prepared including the stock solution (6 mM) at pH 3.0 and the iron(II) solution (0.12 mM) added to microplate. The ferrozine solution (0.6 mM) and a solution of acetate buffer (50 mM) were also prepared. For oxygen radical absorbance capacity (ORAC) assay, AAPH (40 mM) and fluorescein stock solutions (0.5 mM) were prepared at pH 7.4. Stock standard solutions for HPLC analysis, were prepared at $1000\ mg\ L^{-1}$. Working solutions (5 and $2.5\ mg\ L^{-1}$; 15 standards mixture) were also prepared. HPLC grade acetic acid and acetonitrile (Aldrich, Milwaukee, WI) were used. All solutions were filtered through a 0.45 μm membrane and degassed in ultrasound.

2.3. Red grape pomace samples

Douro′sregion is located in the northeast area of Portugal and classified by UNESCO as World Heritage. Red grape pomace was collected in a wine farm situated at average altitude of 150 m, coordinates 41° 10′ 10″ North latitude and 7° 38′ 14″ West longitude. Grapes were harvested upon ripening in 2012 vintage. The present research includes three autochthonous red grape cultivars (*Vitis vinifera* L. grape variety): (1) "*Touriga Nacional*" (TNac); (2) "*Touriga Franca*" (TF); (3) "*Tinta Roriz*" (TR) (Syn. "*Tempranillo*" in Spain; Syn. "*Aragonéz*" in South of Portugal). A mixture (Mix) composed of 1:1:1 proportion of each cultivar was also analyzed.

2.4. Preparation of the grape pomace extracts (GPE)

Seeds and skins of red grape and a given amount of stems (5–6% of the whole bunch that comprised the pomace) from the above grape varieties were obtained after the last alcoholic fermentation step, packaged into a dark-polyethylene-bag, labeled, frozen immediately and transported to the laboratory. Samples were defrosted at room temperature prior efficiently mixture to guarantee a representative proportion of seeds and skins. Most of the stems were removed manually. Then, a portion of each cultivar (500 g) was placed on a tray and dried in an oven (Thermo Scientific™, Pittsburgh, PA). Oven operating conditions were 55 °C with no forced air.

The final point of the drying was assessed by sampling and evaluating the moisture content by weighing differences till reaching less than 5% (w/w) (in triplicate). Finally, dried material was stored in dark-packaged polyethylene at −18 °C and grinding in the following day, was performed. A grinder for grains (food processor, KenWood, New Lane, UK) was applied to provide a particle size of 2–3 mm within intervals of a few seconds to prevent thermal stress of the material. The entire procedure was performed protecting the material from the light. The finely ground material was vacuum packaged in an oxygen barrier bag (Vacuum Packaging Machine, Sammic, Guipúzcoa Spain) covered with foil and stored at −18 °C until further use.