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Bioactive compounds of red grapes from Dão region (Portugal): Evaluation of phenolic and organic profile

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ABSTRACT

Objective: To improve the knowledge on the metabolite profile of five red grapes from Dão region (Portugal), concerning to the phenolic characteristics (coloured and non-coloured phenolics) and organic acid composition.

Methods: Five red grapes collected from Dão region were studied. The profiles of phenolic compounds and organic acids were estimated by high-performance liquid chromatography with diode-array detection and high-performance liquid chromatography with UV detector, respectively.

Results: Totally 24 phenolic compounds were identified, and distributed by several classes: 8 anthocyanins, 1 hydroxybenzoic acid, 4 hydroxycinnamic acids, 1 stilbene, 4 flavan-3-ols, 6 flavonols. Additionally, 10 organic acids were detected in all samples. Total contents of each phenolic class and organic acids amounts varied significantly among the different grape cultivars investigated. The principal components analysis differentiates the Touriga Nacional from the other varieties due to their high contents in anthocyanins, non-coloured phenolics and organic acids. Touriga Nacional is an important red grape cultivar, highly esteemed in Dão region for its ability to produce high quality wines.

Conclusions: The results suggest that the red grapes from Dão region present a good composition in bioactive compounds, being important for the production of wines with superior quality.

1. Introduction

The tradition of wine production in Portugal dates back to centuries of history, with enviable potential for producing high quality wines. This country is responsible for producing of the best wines in the world, being the first to have a demarcated region (region of Douro, where it is produced the Port wine), which ensures the production of genuine wines originated in a particular region. Dão is a unique region with important viticulture traditions, located in north central Portugal, from which the excellent edapho-climatic conditions are turned to advantage for vineyard culture, which corresponds to 20 000 ha. The Dão region presents a temperate climate, although cold and rainy in winter and frequently is very hot in summer. Dão wines with Denomination d'Origine Contrôlée arise from vineyards established in granite land, between 400 and 500 m altitude.

Grapes from *Vitis vinifera* L. belong to the world's largest fruit crops, being a variety known for the best quality wines [1]. Grapes and products derived from them constitute an important

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factor worldwide. Viticulture is one of the activities with the highest impact in the Portuguese economy, representing about 15% of all agricultural production, and placing Portugal among the largest producers of wine worldwide.

Grape ripening is a physiological period that influences the composition of the grapes and determines varietal characteristics, which have influence on the future of wine quality. Grapes undergo many changes during the ripening process which involves a number of physical and biochemical modifications, like weight, volume, rigidity, sugar, acidity, colour and aroma [2]. The optimum level of harvesting can be determined by the level of soluble solids, berry weight, titratable acidity, as well as full flavour characteristics [2]. To harvest the grapes at ideal maturity, it is necessary to investigate their profile and composition in phenolics and organic acids in the field throughout maturation.

Chemical composition is one of the most quality criteria for fruit products. The grapes content in phenolic compounds and organic acids is of great importance for the organoleptic characteristics of grapes and wines, being related with the degree of grape ripening [2,3].

Grapes are a rich source of phenolic compounds (mainly in skin and seed), which play an important role in oenology due to their influence on some important sensory properties of grapes and wines, such as colour, stability, bitterness and astringency [1,4]. Due to their antioxidant and anti-inflammatory properties, phenolic compounds are associated with several beneficial physiological effects that are derived from moderate wine consumption [5]. The study of phenolic composition of grapes may allow the establishment of one or more biomarkers specific for a particularly type of grapes, allowing to assess their chemical evolution during growth and maturation [1,5–7].

The most abundant non-coloured phenolics in skin are flavonols, while flavan-3-ols monomer such as (+)-catechin and (−)-epicatechin, as well as dimers, trimmers and polymeric forms, also called procyanidins (2–10 units), are present mainly in grape seeds. These compounds may contain subunits of gallic acid, epigallocatechin of epicatechin gallate linked by an interflavan bond [4,5,8–10].

Anthocyanins are the main pigments of red grapes located in skins and appeared manly during the ripening, which are mainly responsible for the colour of red wine [1,10]. The major anthocyanins found in grapes are derived from cyanidin, peonidin, delphinidin, petunidin and malvidin, and they generally occur as glycosides and acylglycosides; malvidin-3-*O*-glucoside is the most abundant [5,6,10,11].

The organic acids composition of grapes and wines is important, because they have influence on the organoleptic properties (flavor, colour and aroma) and on the stability and microbiological control of the products. Tartaric and malic acids are the predominant organic acids in grapes juices, on the other hand, succinic and citric acids are present in minor proportion [6,12].

Therefore, in this work we aimed to contribute to the knowledge of the metabolite profile of the main red grapes from Dão region (Portugal), and determine simultaneously their non-coloured and coloured phenolics and organic acids contents. The phenolic compounds were determined by high-performance liquid chromatography with diode-array detection (HPLC/DAD) and organic acids by high-performance liquid chromatography with ultraviolet detection (HPLC/UV). Then, the principal components analysis (PCA) was used to analyze the results previously obtained.

2. Materials and methods

2.1. Standards and reagents

All chemicals used were of analytical grade. The standard compounds were purchased from various suppliers: oxalic, aconitic, citric, ketoglutaric, tartaric, malic, quinic, succinic, shikimic, fumaric, cafeic, *p*-coumaric and ferulic acids were from Sigma–Aldrich (St. Louis, MO, USA). Delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, malvidin-3-*O*-glucoside were from Extrasynthèse (Genay, France). *trans*-Caffeoyltartaric acid (*t*-CAFTA) and *trans*-*p*-coumaroyltartaric acid (*t*-COUTA) were kindly supplied by Dr. C. Garcia-Viguera (CEBAS-CSIC, Murcia, Spain). Epigallocatechin, catechin, epicatechin, epigallocatechin gallate, epicatechin gallate, resveratrol-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, laricitrin-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside and syringetin-3-*O*-glucoside were from Extrasynthèse (Genay, France); gallic acid was from Fluka (Buchs, Switzerland). Water was deionized using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Grape samples

Five samples of five red grapes varieties from Dão region (Portugal) were harvested during September of 2012, in Quinta das Camélias, located in Sabugosa: Tondela (Portugal). The varieties under study were: “Jaen”, “Touriga Nacional”, “Alfrocheiro”, “Tinta Roriz” and “Syrah”. After harvested, the grapes were preserved at −20 °C and dried in a lyophilizer apparatus (Labconco 4.5, Kansas City, MO, USA).

2.3. Phenolic compounds

2.3.1. Extraction

The non-coloured and coloured phenolic compounds were extracted according to the procedure described by Oszmianski and Lee [13]. Aliquots of 5 g of powder sample were weighed and extracted with 100 mL of MeOH (80%) along 2 h under stirring after flushing with nitrogen to avoid oxidations. Then, the extract was centrifuged for 10 min at 4000 r/min. Continuing the material was again extracted during 15 min with 100 mL of MeOH (80%). The both supernatants were evaporated to dryness under reduced pressure at 30 °C. The resultant extract was dissolved with 50 mL of deionised water and placed into the column. The solid-phase extraction cartridge was preconditioned with 20 mL of ethyl acetate, 20 mL of methanol and 20 mL of 0.01 mol/L HCl. After passage of the sample, the column was washed with 3 mL of 0.01 mol/L HCl. Then, the fraction I, designed by non-coloured phenolics was eluted with 20 mL of ethyl acetate. The fraction II, designed by anthocyanins was eluted with 40 mL of methanol containing 0.1% HCl. The fractions I and II were evaporated under reduced pressure, and the dried extracts obtained were re-dissolved with 1 mL of methanol (non-coloured phenolics) and in 20 mL of acidified water, pH 3.0 (anthocyanins), using a membrane-filtered (0.45 μm).