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CHEMICAL PROFILE AND ANTIOXIDANT POTENTIAL OF FOUR TABLE GRAPE (*Vitis vinifera*) CULTIVARS GROWN IN DOURO REGION, PORTUGAL

PERFIL QUÍMICO E POTENCIAL ANTIOXIDANTE DE QUATRO CULTIVARES DE UVAS DE MESA (*Vitis vinifera*) PRODUZIDAS NA REGIÃO DO DOURO, PORTUGAL

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SUMMARY

The aim of this work was to improve the knowledge about the potential of Douro region (North of Portugal) to produce table grapes of superior quality. Skin, pulp, and seeds of four table grapes 'Alphonse Lavallée', 'Cardinal', 'Dona Maria' and 'Muscat Hamburgo' produced in Dour region, collected at commercial maturity stage, were evaluated. Phenolic compounds, organic acids and sugars were determined in each cultivar and in the different parts of the grape by HPLC-DAD, while the antioxidant properties were evaluated by DPPH, Cuprac and lipid peroxidation assays. Phenolics from different classes were found: four anthocyanins (delphynidin, cyanidin, petunidin and malvidin), two hydroxibenzoic acids (gallic acid and protocatechuic acid), three hydroxycinnamic acids (caffeic, chlorogenic and coumaric acids), one flavonol (rutin), three flavan-3-ols ((+)-catechin, (-)-epicatechin, and (-)-epicagallocatechin)), one oligomer (procyanidin B2) and one stilbene (resveratrol). In addition, three organic acids (tartaric, malic and citric acids) and two types of sugars (glucose and fructose) were detected in all samples. Differences were found between cultivars ($p<0.001$) and sample material type ($p<0.001$). Skins presented the higher content in anthocyanins, seeds in flavan-3-ols and pulps in phenolic acids, organic acids and free-sugars. The cultivar 'Cardinal' showed the highest content in phytochemicals, while 'Dona Maria' was the cultivar with the lowest content. Nonetheless, the content of flavan-3-ols and organic acids in 'Dona Maria', associated with its higher antioxidant capacity, makes it an interesting choice as table grape from healthier point of view. According to our results, Douro region have proper environmental conditions to produce these table grapes.

RESUMO

Com este trabalho pretendeu-se aprofundar o conhecimento sobre o potencial da região do Douro (norte de Portugal) para a produção de uvas de mesa de qualidade superior. Foram avaliadas diferentes partes dos bagos (película, polpa e grainhas) de uva de mesa de quatro cultivares distintas, 'Alphonse Lavallée', 'Cardinal', 'Dona Maria' e 'Moscatel de Hamburgo' produzidas na região Douro e colhidas em plena fase de maturação comercial. Em cada cultivar e nas diferentes partes dos bagos procedeu-se à avaliação dos teores em composto fenólicos, ácidos orgânicos e açúcares por HPLC-DAD. As propriedades antioxidantes foram avaliadas pelos métodos de DPPH, Cuprac e peroxidação lipídica. Foram encontradas diferenças significativas entre cultivares ($p<0,001$) e tipo de material analisado ($p<0,001$). Os principais compostos fenólicos encontrados foram: quatro antocianinas (delfinidina, cianidina, petunidina e malvidina), dois ácidos hidroxibenzóicos (ácidos gálico e protocatecuico), três ácidos hidroxicinâmicos (ácidos cafeico, clorogénico e cumárico), um flavonol (rutina), três flavanóis ((+)-catequina, (-)-epicatequina e (-)-epicagalhocatequina)), um oligómero (procianidina B2) e um estilbeno (resveratrol). Acrescem ainda, três ácidos orgânicos (ácido tartárico, málico e cítrico) e dois tipos de açúcares (glucose e frutose). De acordo com os resultados, as películas apresentaram teores mais elevados em antocianinas, as grainhas em flavanóis e as polpas em ácidos fenólicos, ácidos orgânicos e açúcares livres. De entre as cultivares avaliadas, destaca-se a cultivar 'Cardinal' por apresentar um maior teor médio em fitoquímicos e a cultivar 'Dona Maria' por apresentar um elevado teor em ácidos orgânicos e flavanóis o que, associada à sua elevada capacidade antioxidante, a torna uma cultivar interessante como uma uva de mesa bastante saudável. Baseado nos resultados obtidos, podemos afirmar a região do Douro tem condições ambientais adequadas para a produção destas uvas de mesa.

Key words: table grapes, Douro region, chemical composition, bioactive compounds, quality.
Palavras-chave: uvas de mesa, Douro, composição química, compostos bioativos, qualidade.

INTRODUCTION

Grapes are one of the most popular and widely cultivated groups of berry fruits in the world. There are about more than 50 species of grapes (Remaily, 1987) and most of them are found in temperate zones, between latitudes of 40° and 50°N in northern hemisphere and between latitudes of 30° and 40°S in the southern hemisphere (Kok, 2014). However, in recent times, the production of grapes extended to tropical and subtropical countries such as Bolivia, Brazil, Colombia, Peru, Guatemala (in South America), Madagascar, Namibia, Tanzania (in Africa) and Vietnam, China and India (in Asia) (Jogaiah *et al*., 2013). In Portugal, the viticulture activities are one of the most relevant socio-economic activities, with an average of 195 thousands of hectares of planted area in 2016 (OIV, 2017) mainly destined for wine production. Portugal is currently the 11th wine producer with 6.600 million of hectoliters (Aurand, 2017), and the 9th exporter in the world with a total amount of 727 million of Euros in 2016 (OIV, 2017). Portugal is at the top level of the European wine hierarchy, with 31 DOCs (Denominação de Origem Controlada - meaning Controlled Denomination of Origin). Each of these regions has its own strictly defined geographical boundaries. In the north, the Douro and Porto region, former "Douro Demarcated Region", is famous for "Port wine" production and it was responsible for 21% of total Portuguese wine production in 2016 (IVV, 2017a). In the Douro region as well in other regions of Portugal a large number of grape biotypes and cultivars are present, representing an important source of biodiversity (IVV, 2017b). Nonetheless, the large majority of vineyards are destined for wine production and only few to production of table grapes. Moreover, a consistent information about chemical profile of table grapes produced in the Douro region and the potential of this region to produce table grapes with superior quality is very limited or even null.

Different studies have shown that grapes contain large amounts of antioxidant phytochemicals, including phenolics, flavonoids, anthocyanins, resveratrol and carotenes (Mendes-Pinto *et al.*, 2004; Castillo-Muñoz *et al.*, 2007; Yang *et al.*, 2009; Bunea *et al.*, 2012; Flamini *et al.*, 2013) and other related beneficial compounds for human health. Based on their richness of bioactive compounds, the intake of grapes have been associated with many positive health effects such as cardioprotective, anti-inflammatory, anti-carcinogenic, antiviral, and antibacterial (Xia *et al.*, 2010; Ras *et al.*, 2013; Oliveira *et al.*, 2013; Georgiev *et al.*, 2014; Vaisman and Niv, 2015; Rasines-Perea and Teissedre., 2017). Although the information about chemical properties of wine grapes are widely known, the same information about table grapes is still scarce, particularly with table grapes produced in Portugal and in particularly in the Douro region. International cultivars such as 'Alphonse Lavallée', 'Cardinal', 'Muscat Hamburg, and other table grapes are important for the future of the table grape industry and several studies have shown that they have potential to be grown in different geographies (Mitić *et al.*, 2012; Topalovic *et al.*, 2012; Rolle *et al.*, 2013; Isci *et al.*, 2015; Kok, 2016). However, less information is available about their potential to be produced in Portugal. Similar situation occurs with the cultivar 'Dona Maria'. 'Dona Maria' is a Portuguese cultivar created in 1950 in the research center "Estação Agronómica Nacional", Oeiras, Portugal (Marreiros, 2013) but the physicochemical characterization of their grapes is until now very limited and it is mainly focused on their biometrical properties, such as size, width and length, and chemicals such as soluble solids, acidity and total sugars. No consistent information is available about their content in phenolics, stilbenes, anthocyanins or organic acids. In addition, information about their antioxidant potential is limited. Therefore, we set a study in which we compare the phytochemical profile and antioxidant potential of four different cultivars of table grapes grown in the Douro region ('Alphonse Lavallée', 'Cardinal', 'Dona Maria' and 'Muscat Hamburg'), in order to assess their differences or similarities, and to understand if the Douro region has potential to grown table grapes as it does successfully with wine grapes. Polyphenols, organic acids and sugars were determined at commercial maturity stage using high-performance liquid chromatographic (HPLC) methods, while antioxidant activities were determined by colorimetric methods.

MATERIAL AND METHODS

Standards and reagents

All chemicals used in this study were of analytical grade. Citric acid, tartaric acid, malic acid, naringin, glucose and fructose standards were purchased from Sigma–Aldrich (Tauferkichen, Germany). Gallic acid, protocatechuic acid, (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, procyanidin B2, resveratrol, chlorogenic acid, coumaric acid, caffeic acid, rutin, cyanidin-3-O-glucoside, delphinidin-3-*O*-glucoside malvidin-3-O-glucoside, petunidin-3-O-glucoside standards were purchased from Extrasynthèse (Genay, France). Methanol and acetonitrile were HPLC gradient and purchased from Panreac chemistry (Lisbon, Portugal) and Sigma-Aldrich

(Taufkirchen, Germany), respectively. 2,2,-diphenyl-1-picryl-hydrazyl, ammonium acetate (NH4Ac), copper(II)chloride ($CuCl_2$), neocuproine, ethanol, benzoyl, pyridine, diethyl ether, iron sulphate (FeSO4), orthophosphoric acid, potassium dihydrogen phosphate, trifluoroacetic acid (TFA), trichloroacetic acid (TCA), tiobarbituric acid (TBA) and trolox were purchased from Sigma-Aldrich (Taufkirchen, Germany). The aqueous solution were prepared using ultra-pure water (Isomanttle UL 5L, Isopad, Gemini BV Laboratory, Apeldoorn Netherlands)

Grape samples

Four table grapes cultivars, 'Alphonse Lavallée', 'Cardinal', 'Muscat Hamburg' (red grapes) and 'Dona Maria' (white grape) were grown in 1976-17C rootstocks, at the same altitude (470 m) and in the same type of soils, in Quinta de Prados, Vila Real, Douro region, North of Portugal. 250 g of grapes from each cultivar and replicate (three replicates per cultivar) were harvested at commercial maturation stage during September 2016. After harvest, the samples were transported to laboratory, where they were divided in skins, pulps and seeds and then freeze-dried (Scanvac Coolsafe 554 Pro, Labogene^{Tm}, Bjarkesvej 5, DK-3450 Allerød Danmark). Each dried part was then milled in a commercial blender (Taurus, Mod. Aromatic, 150 W, Taurus, Spain) until reach a fine powder. The samples were then stored at -20 ºC until analytical determinations. Moisture contents of grape skins, pulps and seeds were determined to express the results of analytical experiments in fresh weight (FW) basis. The moisture content was calculated by placing the fresh material in a freeze-dryer under vacuum for 72 hours. The difference between the initial and final weight was used to calculate the moisture content.

Polyphenols

Extraction

The extraction of polyphenols from the different parts of the grapes was done using the method adopted by Aires *et al*. (2017). Briefly, dried powder of each sample (0.040 g) was extracted in triplicate with 950 µL 70% methanol and 50 µL of internal standard compound (naringin, Sigma-aldrich, Tauferkichen, Germanay) in a warm-bath at 70 º C for 30 minutes with intermittent agitation. The extracts were centrifuged at 15286 g during 15 minutes at 4ºC (Centrifuge 5804R, Eppendorf, Hamburg, Germany), and the respective supernatants were filtered through PTFE 0.2 µm, 13 mm (Teknokroma, Spain) to amber HPLC vials (Chromabond 2-SVW(A) ST-CPK, Sigma-Aldrich, Tauferkichen, Germany) to avoid light degradation. The extracts were then stored under refrigeration (-20 ºC) prior to the HPLC-DAD analysis.

HPLC-DAD analysis

The identification and quantification of polyphenols were performed through HPLC-DAD, according to the procedure adopted by Aires *et al*. (2017). A HPLC (Gilson) system equipped with one mixture chamber (Gilson, model 811A), two pumps (Gilson, model 305 and 306), automatic injector (Gilson, model 231X), oven (Jones chromatography) and a diode array detector (DAD) (Thermo, Finnigan Surveyor detector) was used to identify the polyphenols present in the different parts of the grapes. The mobile phase was composed by water with 0.1% of TFA (solvent A) and acetonitrile with 0.1% TFA (solvent B). 10 µL of each extract was injected into a C18 column (250 mm × 4.6 mm; 5 µm particle size, ACE, Advanced Chromatography Technologies, Aberdeen, United Kingdom). The elution was performed at a flow rate of 1 mL/min with the following gradient: 0 min 100% A, 5 min 100% A, 15 min 80% A, 30 min 50% A, 45 min 0% A, 50 min 0% A, 55 min 100% A, and 60 min 100% A. The detection was made at 280, 320, and 370 nm for phenolics in general, and 520 nm for anthocyanins, in particular. Phytochemicals were identified through peak retention time, UV spectra and UV maxima absorbance bands, along with comparison with those found for commercial external standards. The quantification was performed using the internal standard method and the results (mean ± standard deviation (SD)) were expressed as µg/g fresh weight (FW) of three replicates.

Organic acids

Extraction

The extraction of organic acids was based on the method performed by Philips *et al*. (2010). Briefly, one-gram dw was extracted by 10 mL ultra-pure water in a sonicator (Sonorex Digitec DT 100, Bandelin, Germany) for 5 minutes. Then the extracts were centrifuged at 1960 g for 15 minutes (Centrico 250, UniEquip Laborgerätebau- und Vertriebs, Germany), filtered through a 0.20 μm PTFE, 13 mm (Teknokroma, Spain) to amber HPLC vials, and kept in -20 °C until analytical determination by HPLC.

HPLC-DAD analysis

The content of organic acids in extracts was determined through HPLC-DAD, according to the method of Philips *et al*. (2010), using a HPLC (Gilson) system equipped with one mixture chamber (Gilson, model 811A), two pumps (Gilson, model 305

and 306), automatic injector (Gilson, model 231X), oven (Jones chromatography) and a diode array detector (DAD) (Thermo, Finnigan Surveyor detector), with a C18 column (250 mm × 4.6 mm; 5 µm particle size, ACE, Advanced Chromatography Technologies, Aberdeen, United Kingdom), with a mobile phase of potassium dihydrogen phosphate (6.8 g L^{-1}) and 85% orthophosphoric acid (pH 2.1), under isocratic conditions, with a flow rate of 0.8 mL/min and an injection volume of 20 μL. The detection was made at 210 nm. The identification of organic acids was made comparing with commercial pure external standards, their peak retention time, UV spectra and UV maxima absorbance bands. The results were expressed as mg/g FW.

Individual Sugars

Extraction

For extraction of soluble-free sugars the method of Daniel *et al.* (1981) was followed, in which 100 mg dw of each sample was homogenized with 80% aqueous ethanol (5 mL) at 20 °C for 2 h, centrifuged at 1960 g during 10 minutes at 4 °C (Centrico 250, UniEquip Laborgerätebau- und Vertriebs, Germany). Afterwards, 100 μL of each supernatant was completed evaporated under nitrogen atmosphere, followed by addition of 500 μL of derivatization reagent (10% benzoyl in pyridine). The mixtures were heated at 37 °C for16 h and then 1 mL of diethyl ether was added, followed by a vigorous agitation in a vortex. The extracts were then centrifuged at 15286 g (Centrifuge 5804R, Eppendorf, Hamburg, Germany) for 20 minutes at 4 ºC. Then, 750 μL of each supernatant was transferred to another vial and dried until complete evaporation under nitrogen atmosphere. After that, each residue was diluted with 750 μL of 100% methanol and kept in -20 °C until determination of sugars by HPLC.

HPLC-DAD analysis

Adopting the same procedure of Daniel *et al.* (1981) but with small modifications, the individual sugars were determined through HPLC-DAD. A HPLC (Gilson) system equipped with one mixture chamber (Gilson, model 811A), two pumps (Gilson, model 305 and 306), automatic injector (Gilson, model 231X), oven (Jones chromatography) and a diode array detector (DAD) (Thermo, Finnigan Surveyor detector) was used. The mobile phase was composed by water with 0.1% of TFA (solvent A) and acetonitrile with 0.1% TFA (solvent B). The elution was performed using a flow rate of 1mL/min, with the following gradient: 20% A (0-10 minutes), 7% A (7-10 min), 10 % A (10-12 min), 0% A (12-20 min), 0% A (20-25 min), 20% A (25-30 min), 20% A (30-35 min). The injection volume was 20 μL and the detection was made at 270 nm. The separation and identification of individual sugars was done by comparing with commercial pure external standards, their retention time, UV spectra and UV max absorbance bands. The results were expressed as mg/g FW.

Antioxidant activities

Extraction

To determine the antioxidant activity of each grape cultivar an additional extraction was done using the same procedure adopted for the polyphenol extraction but without internal standard.

DPPH radical scavenging activity

The DPPH radical scavenging activity of each extract was determined using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay (Siddhraju and Becker, 2003) conducted in a 96-microplate wells. A freshly 100 µM DPPH solution (4 mg of 2.2-diphenyl-1-picrylhydrazyl radical in 100 mL of 95% of aqueous ethanol) was prepared and added to each microplate well (285 µL of DPPH solution), followed by the addition of 15 µL extract. A blank sample (all reagents and extraction solvent instead of sample) was considered in the first well of microplate. After that, the microplates were incubated in the dark, at room temperature for 30 minutes. After this period, the absorbance values were recorded at 517 nm wavelength in a microplate reader (Multiskan™ FC Microplate Photometer, USA), and the results were expressed as % DPPH radical scavenging capacity, using the following formula: % DPPH scavenging capacity= $(Abs_{blank} - Abs_{sample}/Abs_{blank}) \times 100$

Cupric ion reducing antioxidant capacity (CUPRAC)

The CUPRAC assay was performed using the classical method of Apak *et al*. (2004) with small modifications and conducted in a 96 microplate wells. Briefly, to each well of microplate was added sequentially 50 µL of $CuCl_2$ (10 mM in water), 50 µL of neocuproine (Sigma-Aldrich, Tauferkichen, Germany) at 7.5 mM in 96% ethanol, and 50 µL of NH4Ac buffer (1mM in water, pH 7.0), 25 µL of sample and 25 µL of double distilled water. The microplates were then incubated in the dark at room temperature for 30 minutes and after the values of absorbance were recorded at 450 nm against blank (all reagents except $CuCl_2$) in a microplate reader (Multiskan™ FC Microplate Photometer, USA). A calibration curve was made using the commercial compound trolox as standard and the results were expressed as µM of trolox equivalents per g of fresh sample (µM TE/g FW).

Inhibition of lipid peroxidation bioassay

The inhibition of lipid peroxidation assay was used to measure the lipid peroxide formed using egg yolk homogenates as lipid-rich media, as described by Dakera *et al.* (2008). Briefly, in a microplate of 96 wells, 20 µL of substrate (homogenate of egg yolk at 10% in 0.1 M phosphate buffer, pH 7.4) was added followed d by addition of 5 µL of FeSO4 (1mM in water), 20 µL of plant extract and 65 µL of double distilled water. The microplates were then incubated at 37 ºC for 15 minutes. After this period, 50 µL of tricloroacetic acid (50% in water) and 100 µL of tiobarbituric acid (TBA) (0.8% in phosphate buffer) were added sequentially to each microplate followed by a new incubation at 95 ºC for 15 minutes until a pink colour appearance. Then, the absorbance values were recorded at 532 nm in a microplate reader (Multiskan™ FC Microplate Photometer, USA). A complete oxidized extract (egg yolk + $FeSO_4$, without extract) was used as control. The results were expressed as % lipid peroxidation inhibition using the following formula: (%) lipid peroxidation inhibition = ($Abs_{control}$ - Abs_{sample}/$Abs_{control}$) x 100.

Statistical analysis

All data were expressed as mean ± standard deviation (SD) of triplicate determinations. The mean values were compared using ANOVA, and the Duncan test was used to determine differences between cultivars and between grape parts with statistical significance. Statistical analysis was carried out using SPSS version 17 Software (SPSS-IBM, Orchard Road-Armonk, New York, USA). A principal component analysis (PCA) using JMP version 13.2.1 Software (SAS Institute, Inc., Cary, USA) in order to identify which variables determine similarities and differences between grape cultivars.

RESULTS AND DISCUSSION

The content of phenolic acids, flavonoids, anthocyanins, stilbenes (resveratrol), organic acids and free sugars are presented in Tables I to III, and their levels varied significantly with cultivar ($p<0.001$) and with berry fraction evaluated ($p<0.001$).

TABLE I

Average levels of phenolic acids, flavonoids and stilbenes (µg/g FW) found in the four table grapes by HPLC-DAD at different wavelenght

Teores médios em ácidos fenólicos, flavonóides e estilbenos (µg/g peso fresco) nas quatro cultivares de uva de mesa por HPLC-DAD a diferentes comprimentos de onda

Cultivar	Sample part	250 nm		280 nm				306 nm	320 nm			370 nm
		Gall	Protocat	Cat	Epicat	Procyan	Epigall	Resv	Chlor	Coum	Caf	Rut
'Alponse Lavallée'	Seeds	7.7±0.8	12.0±0.4	20.7±1.1	36.3±2.4	21.1±1.3	26.9±1.3	n.d.	n.d.	n.d.	n.d.	n.d.
	Skin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	73.3±8.7
	Pulp	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.7±0.3	0.7±0.2	1.3±0.7	n.d.
'Cardinal'	Seeds	29.7±7.6	n.d.	109.9±3.1	130.4±12.2	59.7±12.4	27.8±8.6	n.d.	n.d.	n.d.	n.d.	n.d.
	Skin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	21.5±4.3	n.d.	n.d.	n.d.	49.9±5.0
	Pulp	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.0±0.3	n.d.	n.d.	n.d.
'Dona Maria'	Seeds	n.d.	5.9±0.4	16.6±1.2	27.6±1.0	9.4±1.2	12.59±0.5	n.d.	n.d.	n.d.	n.d.	n.d.
	Skin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	19.0±1.6	2.2±0.3	n.d.	26.1±1.3
	Pulp	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
'Muscat Hamburg'	Seeds	18.3±1.2	20.3±1.0	38.3±3.4	60.9±10.0	36.7±0.4	44.5±4.7	n.d.	n.d.	n.d.	n.d.	n.d.
	Skin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	129.1±3.2
	Pulp	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.5±0.5	n.d.	n.d.	n.d.
Source of variation												
Cultivar (C)		***	***	***	***	***	***	***	***	***	***	***
Sample part (S)		***	***	***	***	***	***	***	***	***	***	***
C x S		***	***	***	***	***	***	***	***	***	***	***

Gall – Gallic acid; Protocat - Protocatechuic acid; Cat – (+)-Catechin; Epicat – (-)-Epicatechin; Procyan – Procyanidin B2; Epigall – (-)-Epigallocatechin; Resv – Resveratrol; Chlor – Chlorogenic acid; Coum – Coumaric acid; Caf – Caffeic acid; Rut – Rutin. Values are expressed as mean ± standard deviation of three replicates. The symbols means: not detected (n.d.); highly significant (***, at $p<0.001$).

The 'Cardinal' presented the highest mean of total polyphenols (sum of individual phenolics from the different berry parts) (850.4 µg/g FW) followed by 'Muscat Hamburg' (753.1 µg/g FW), 'Alphonse Lavallée' (582.1 µg/g FW) and 'Dona Maria' (125.2 µg/g FW). As expected, red grapes showed highest content of polyphenols (Tables I and II) and the fractions of seeds and skins have significantly higher concentration of polyphenols than those determined for pulps.

TABLE II

Average levels of anthocyanins (μg/g FW) found in the four table grapes by HPLC-DAD at 520 nm wavelenght[3]

Teores médios de antocianinas (μg/g peso fresco) nas quatro cultivares de uva de mesa determinadas por HPLC-DAD a u comprimento de onda de 520 nm

Cultivar	Sample part	Delfinidin-3-*O*-glucoside	Cyanidin-3-*O*-glucoside	Petunidin-3-*O*-glucoside	Malvidin-3-*O*-glucoside
'Alponse Lavallée'	Seeds	n.d.	0.07±0.0 a	n.d.	1.0±0.0 a
	Skin	n.d.	22.70±1.5 c	n.d.	348.7±6.9 b
	Pulp	0.15±0.0	1.60±0.1 b	n.d.	n.d.
'Cardinal'	Seeds	n.d.	0.21±0.0 a	n.d.	3.0±0.2 a
	Skin	21.90±2.0 b	48.10±1.7 c	82.1±1.3	256.7±6.0 b
	Pulp	0.70±0.0 a	2.20±0.3 b	n.d.	n.d.
'Dona Maria'	Seeds	n.d.	0.09±0.0 a	n.d.	0.94±0.0 a
	Skin	n.d.	0.27±0.0 b	n.d.	4.37±0.2 b
	Pulp	n.d.	n.d.	n.d.	n.d.
'Muscat Hamburg'	Seeds	n.d.	0.23±0.0 a	n.d.	2.9±0.39 a
	Skin	n.d.	38.1±1.8 c	n.d.	360.5±5.6 b
	Pulp	0.74±0.1	0.67±0.0 b	n.d.	n.d.
Source of variation					
Cultivar (C)		***	***	***	***
Sample part (S)		***	***	***	***
C x S		***	***	***	***

Values are expressed as mean ± standard deviation of three replicates. Mean values followed by different letters in a column within the same cultivar are significantly different at $p<0.05$ by Duncan test. The symbols means: not detected (n.d.); highly significant (***, at $p<0.001$).

The group of anthocyanins prevails in the skins of different cultivars, while the group of catechins was dominant in the seeds. Instead, the profile of phenolic acid distribution was highly dependent of cultivar. Gallic acid, a hydroxybenzoic acid, prevails in seeds of all cultivars, but chlorogenic acid and coumaric acid, two phenolic acids from hydroxycinnamic acid group, were found in the pulps of 'Alphonse Lavallée', 'Cardinal' and Muscat Hamburg', while in 'Dona Maria' grapes they were found in skins. The flavonol rutin, was found in the skins of all studied cultivars. All cultivars presented the same profile of anthocyanins in seeds, when detected, namely malvidin-3-*O*-glucoside > petunidin-3-*O*-glucoside > cyanidin-3-*O*-glucoside > delfinidin-3-*O*-glucoside (Table II). In all cultivars the highest content of phenolics were found in skins followed by seeds and pulps, which is accordance with Rockenbach *et al.* (2011) that reported higher content of phenolics in skins of grapes comparatively to seeds and pulps. Also Yilmaz *et al.* (2015) reported a higher content of phenolics in skins of 22 grapes cultivars (red and white), which is consistently according to our results. Although several authors have reported that pulps of grapes usually do not have any type of anthocyanins, we detected the presence of dephinidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside in the pulps of 'Alphonse Lavallée', 'Cardinal' and Muscat Hamburg', which is consistent with the findings of He *et al*. (2010, 2017), who found the same types anthocyanins in similar cultivars of red grapes.

Literature consistently reports that red grapes are beneficial for human health because they have high amounts of polyphenols and they act as protective agents against inflammatory, mutagenic and degenerative processes (Kumar and Pandey, 2013; Sosa *et al.*, 2013; Hussain *et al.*, 2016). Our findings show that white grapes from 'Dona Maria' could have similar benefits due to their content in catechins, chlorogenic acid and caffeic acid, and rutin. Catechins and respective derivatives have been considered effective scavengers of reactive oxygen species *in vitro* and may act indirectly as antioxidants through their effects on transcription factors and enzyme activities (Higdon and Frei, 2003). Chlorogenic and caffeic acids, two of the most representative hydroxycinnamic acids have also been associated with the reduction of oxidative and inflammatory stress conditions (Liang and Kitts, 2016). Rutin, a typical flavonol largely abundant in plants and known as quercetin-3-rutinoside, rutoside or vitamin P, has been explored for a number of biological effects such antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective activities (Ganeshpurkar and Saluja, 2017). Therefore, foods (in this case, grapes) with high content of such compounds are thus highly

beneficial for human health and must be included in the human diet on a daily basis, if possible.

Another important finding of our work is the amount of each polyphenol detected. The average contents for the different parts of the grapes were higher than the values found by other authors for wine and table grapes (Cantos *et al.*, 2002; Topalovic and Mikulic-Petkovsek, 2010; Ferrandino *et al.*, 2012; Topalovic *et al.*, 2012; Nile *et al.*, 2015), meaning that the Douro region has suitable conditions to produce these table grapes. The significant differences between the values presented in this study and the values from those authors are a consequence of the influence of geographical location, cultural practices, in addition to the varietal differences.

The Table III shown the average content of organic acids, ranging from 0.9 to 43.6 mg/g FW, and significant differences ($0.001<p<0.05$) were found between cultivars. Tartaric acid was predominant in all cultivars and its average levels varied between 31.10 mg/g FW in 'Alphonse Lavallée' and 37.38 mg/g FW in 'Dona Maria. Average levels of malic acid were found to be significantly higher in 'Cardinal' (8.3 mg/g FW) whilst citric acid was higher in 'Moscatel Hamburg' (4.1 mg/g FW).

TABLE III

Average content of organic acids and sugars in the four table grapes studied

Teor médio em ácidos orgânicos e açúcares nas quatro cultivares de uva de mesa estudadas

Cultivar	Sample part	Organic acids (mg/g FW)			Sugars (mg/g FW)	
		Tartaric acid	Malic acid	Citric acid	Glucose	Fructose
'Alponse Lavallée'	Seeds	1.7±0.2 a	1.8±0.1 a	1.4±0.3 a	7.9±0.7 a	8.1±0.0 a
	Skin	33.3±1.0 b	5.2±0.8 b	1.7±0.2 a	167.6±7.8 b	165.5±5.4 b
	Pulp	33.7±0.7 b	7.1±0.8 c	2.3±0.3 b	210.9±4.7 c	299.4±9.6 c
'Cardinal'	Seeds	4.0±0.3 a	4.9±0.6 a	4.0±0.2 b	99.8±4.3 a	94.0±4.0 a
	Skin	33.9±1.0 b	6.4±0.8 b	2.6±0.4 a	123.2±19.6 b	116.5±4.3 b
	Pulp	35.9±2.1 b	6.2±0.5 b	n.d.	269.7±10.8 c	342.7±5.9 c
'Dona Maria'	Seeds	6.7±0.2 a	0.6±0.0 a	n.d.	18.1±1.2 a	18.5±1.8 a
	Skin	28.0±1.6 b	6.1±0.7 b	0.9±0.2	141.6±4.2 b	138.5±2.1 b
	Pulp	43.6±2.6	8.8±0.7 c	n.d.	238.7±24.3 c	313.4±8.0 c
'Muscat Hamburg'	Seeds	2.3±0.5 a	2.4±0.2 a	1.6±0.2 b	9.7±1.0 a	11.9±0.3 a
	Skin	26.4±0.4 b	5.6±0.2 b	2.0±0.2 b	141.6±21.7 b	149.7±5.0 b
	Pulp	39.9±1.7 c	7.0±0.4 c	4.2±0.3 c	229.9±20.6 c	293.2±14.4 c
Source of variation						
Cultivar (C)		***	*	***	***	**
Sample part (S)		***	***	n.s.	***	***
C x S		***	***	***	***	***

Values are expressed as mean ± standard deviation of three replicates. Mean values followed by different letters in a column within the same cultivar are significantly different at $p<0.05$ by Duncan test. [3]The symbols means: not detected (n.d.); not significant (n.s., at $p>0.05$) significant (*, at $p<0.05$), very significant (**, $p<0.01$); highly significant (***, at $p<0.001$).

As also shown in Table III, the two main sugars identified were fructose (ranging from 35.0 to 360.4 mg/g FW) followed by glucose (ranging from 28.7 to 295.2 mg/g FW). The highest levels of fructose and glucose were mainly found in berries pulps, followed by skins and seeds.

The levels for organic acids and free sugars in this study for 'Alphonse Lavallée', 'Cardinal' and 'Muscat Hamburg' are in agreement with those reported for tables grapes (Topalovic and Mikulic-Petkovsek, 2010; Aubert and Chalot, 2018). For 'Dona Maria' grapes, we believe that this is the first time that values for organic acids are reported. The higher contents of tartaric and malic acids and the very low content of citric acid associated with considerable amounts of fructose in pulp and skins, seems to justify why these grapes in Portugal are often reported as one of the most sweetness white table grapes.

The Figure 1 illustrates the average values of antioxidant properties assayed with the DPPH, Cuprac and lipid peroxidation methods. As shown, the antioxidant properties assayed were significantly affected by cultivar and berry part assayed. The highest values were achieved for seeds, followed by skins and pulps, following the same trend observed for the phenolics content. Similar observation was made by Yilmaz *et al* (2015), which reported high antioxidant activity values in skins and seeds of table grapes extracts and linking them with the high content

of phenolics in these berry fractions, particularly with anthocyanins. In the current work, the results were consistent in the three methods assayed (Figure 1), which confirms that in the grape cultivars studied the seeds are the part of berry grapes with high antioxidant potential. This trend could be related with its high content of polyphenols, particularly the group of phenolic acids or catechins and procyanidins that are prominent in this part of berries (Table I). Like in phenolics, the highest antioxidant activities were found for 'Cardinal' cultivar in all the three methods assayed, which can be associated with high content of polyphenols in this cultivar (Tables I and II). However, surprisingly, seeds of 'Dona Maria' showed similar antioxidant levels to other three cultivars, probably due to the presence of protocatechuic acid, catechins and anthocyanins (Tables I and II). It means that from the health point of view, 'Dona Maria' grapes have potential due to the richness of antioxidant compounds in its seeds.

According to many authors, antioxidant activity of fruits, vegetables and foods in general, results mainly from the presence of phenolics. The Figure 2 shown the PCA analysis results and it was possible to understand that the group of catechins is critical for the antioxidant activities exhibited by all cultivars studied, particularly by the seeds. The cumulative variation (component 1 + component 2) found in all cultivars (Figure 2) means that variation of antioxidant activity found in 'Cardinal', 'Muscat Hamburg', 'Alphonse Lavallée' and 'Dona Maria' was positively associated with the variation of this group of polyphenols in 99.4, 96.5, 97.5 and 99.1 %, respectively. According to the PCA analysis, higher values of antioxidant activity exhibited by seeds of 'Cardinal' cultivar were highly related with the contents of (+)-catechin, (-)-epicatechin and procyanidin B2, while in 'Muscat Hamburg' and 'Alphonse Lavallée' the antioxidant activity properties were more dependent on the variations in (-)-epicatechin and (-)-epigallocatechin. In 'Dona Maria', the antioxidant activity of its seeds was highly related with the content of (+)-catechin and (-)-epicatechin. In the case of skins, the antioxidant activity was more related with the variation of flavanol and phenolic acids, particularly rutin and chlorogenic acid, respectively (Table I, Figures 1 and 2). In the case of pulps, only the presence of some anthocyanins, particularly in Cardinal', 'Muscat Hamburg' and 'Alphonse Lavallée' cultivars seems to justify the moderate values of antioxidant activity found for these cultivars (Table II, Figures 1 and 2).In 'Dona Maria' grapes, the absence of any type of polyphenols seems to justify the zero values found for the antioxidant activity.

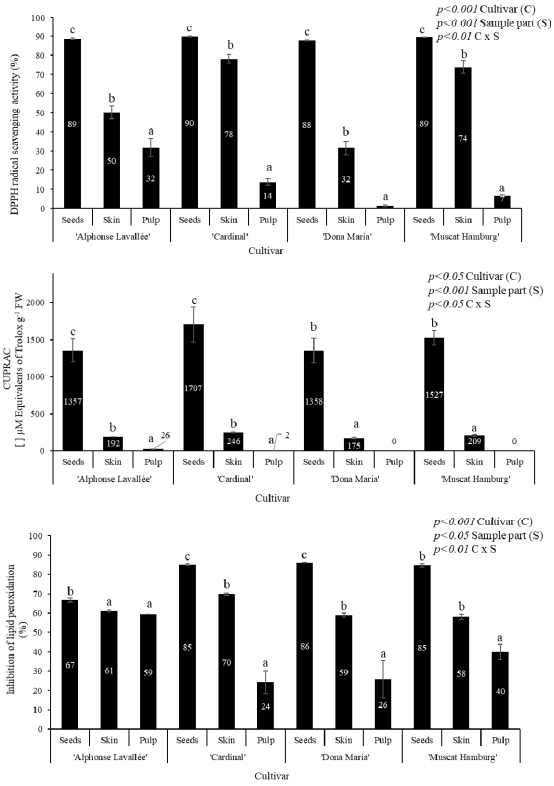

Figure 1. Antioxidant activity of the table grapes studied in the current work. Mean values with different letters for in the same cultivar are significantly different from another at p<0.05 by Duncan test.

Atividade antioxidante nas quatro cultivares de uva de mesa estudadas no presente trabalho. Valores médios com diferentes letras na mesma cultivar são estatisticamente diferentes a p<0,05 pelo teste de Duncan.

In addition, the Pearson´s correlation coefficients presented in Table IV summarize the most significant positive correlation coefficients between the various parameters evaluated in this study. The highest significant correlations, as we expected after PCA analysis, were found between the group of catechins and antioxidant activities, which seems to justify why the seeds had the highest antioxidant activities (Figure 1). These data clearly show that the antioxidant capacity is dependent on both the level and type of phenolic compounds present. Anthocyanins like delfinidin and cyanidin, and other phenolics such as chlorogenic acid, caffeic acid, rutin and resveratrol are present in lower amounts and thus having lower significance for the antioxidant activity values. Nonetheless, their presence in the studied grapes must not be neglected because increase their quality value, since these compounds have been

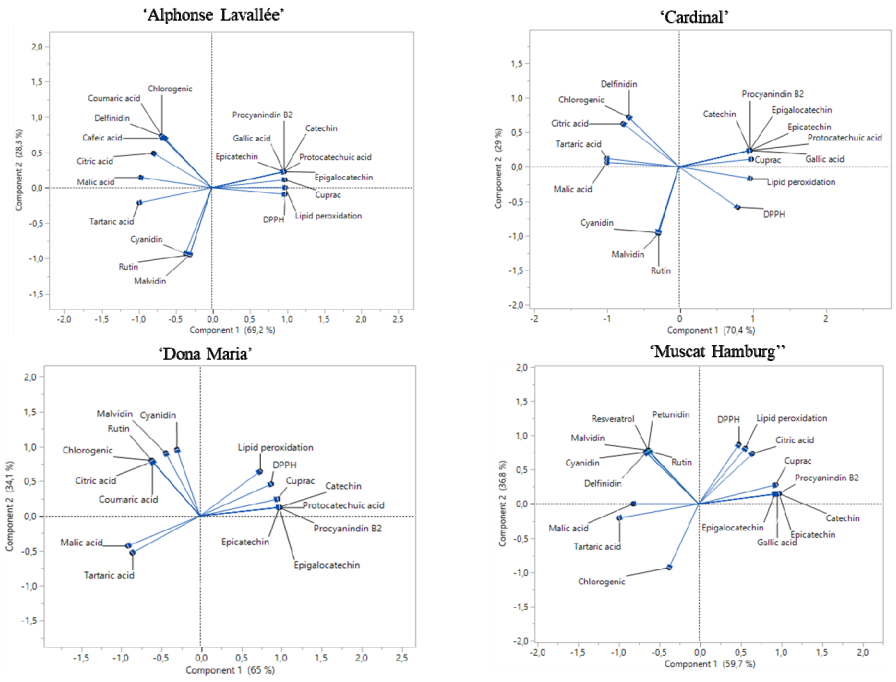

Figure 2. Principal component analysis results.

Resultados da análise em componentes principais.

Table IV

Correlation coefficients of Pearson´s between all polyphenols and organic acids and antioxidant capacity in the four table grapes

Coeficientes de correlação de Pearson entre os teores em polifenóis, ácidos orgânicos e capacidade antioxidante nas quatro cultivares de uva de mesa

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	Gallic acid	1	0.41*	0.94**	0.97**	0.95**	0.77**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.69**	-0.33*	0.41*	0.53**	0.75**	0.54**
2	Protocatechuic acid		1	n.s.	0.47**	n.s.	0.85**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.72**	-0.70**	n.s.	0.54**	0.67**	0.48**
3	(+)-Catechin			1	0.97**	0.99**	0.69*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.68**	n.s.	0.42*	0.53**	0.78**	0.57**
4	Procyanidin B2				1	0.98**	0.84**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.78**	-0.45*	0.36*	0.61**	0.86**	0.61**
5	(-)-Epicatechin					1	0.74**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.74**	-0.41*	0.38*	0.58**	0.83**	0.60**
6	(-)-Epigallocatechin						1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.87**	n.s.	n.s.	0.66**	0.90**	0.63**
7	Resveratrol							1	n.s.	n.s.	n.s.	n.s.	0.98**	0.70**	0.99**	0.37*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
8	Chlorogenic acid								1	0.90**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.48**	-0.40*	n.s.
9	Coumaric acid									1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
10	Caffeic acid										1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
11	Rutin											1		0.82**	n.s.	0.93**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
12	Delfinidin-3-O-glucoside												1	0.70**	0.99**	0.36	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
13	Cyanidin-3-O-glucoside													1	0.71**	0.89**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
14	Petunidin-3-O-glucoside														1	0.38	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
15	Malvidin-3-O-glucoside															1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
16	Tartaric acid																1	0.87**	n.s.	-0.84**	-0.96**	-0.79**
17	Malic acid																	1	n.s.	-0.76**	-0.79**	-0.70**
18	Citric acid																		1	n.s.	n.s.	n.s.
19	DPPH																			1	0.80**	0.89**
20	Cuprac																				1	0.78**
21	Lipid peroxidation																					1

The symbols mean: *correlation is significant at p<0.05, **correlation is significant at p<0.01, n.s. correlation is not significant (p>0.05). The numbers 1 to 21 correspond to the compounds in the first column.

associated with positive effects in human health (Xia *et al*., 2010; Mitić *et al.,* 2012). Minors correlations found in this study suggest that scavenging of reactive species does not only depend on phenolics. Other compounds such as vitamins, minerals, carotenes, and their synergisms may contribute to the antioxidant activity as observed by Bunea *et al*. (2012). Based in our results, catechins are the major determinants for the antioxidant activities, which is in agreement with the results of Dani *et al.* (2007) and Radovanović *et al.* (2010), who, in previous studies with white and red grapes found that catechins were the most determinant the polyphenols for the antioxidant activities. They suggest that they can be easily used as biomarkers for the authentication of red grapes cultivars. We found similar antioxidant values and moderate levels of catechins in the white grapes of 'Dona Maria' cultivar, suggesting that catechins can be present in white grape cultivars, even if their level is low or moderate.

CONCLUSIONS

The results from this study shown that four table grapes grown in Douro region and its respective fractions of seeds and skins have high content of phytochemicals and high antioxidant activities, allowing to conclude that Douro region has suitable environmental conditions to produce the studied table grapes. Although the red grape cultivars presented the highest antioxidant activities linked with the high content of anthocyanins and catechins, the white cultivar 'Dona Maria' might be a very promising cultivar due to its diversity of polyphenols and high antioxidant activity; therefore, its production should be incremented.

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