



## Short communication

## Selection of grapevine leaf varieties for culinary process based on phytochemical composition and antioxidant properties

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## ARTICLE INFO

## Article history:

Received 4 February 2016

Received in revised form 22 May 2016

Accepted 30 May 2016

Available online 31 May 2016

## Keywords:

*Vitis vinifera* L.

Grapevine leaves

Variety

Antioxidant

Phytochemicals

## ABSTRACT

Grapevine leaves are an abundant sub-product of vineyards which is devalued in many regions. The objective of this work is to study the antioxidant activity and phytochemical composition of ten grapevine leaf varieties (four red varieties: Tinta Amarela, Tinta Roriz, Touriga Franca, and Touriga Nacional; and six white varieties: Côdega do Larinho, Fernão Pires, Gouveio, Malvasia Fina, Rabigato, and Viosinho) to select varieties to be used as food ingredients.

White grapevine leaves revealed higher antioxidant potential. Malvasia Fina reported better antioxidant properties contrasting with Touriga Franca. Phenolic content varied between 112 and 150 mg GAE g<sup>-1</sup> of extract (gallic acid equivalents), hydroxycinnamic acid derivatives and flavonols varied between 76 and 108 mg CAE g<sup>-1</sup> of extract (caffeic acid equivalents) and 39 and 54 mg QE g<sup>-1</sup> of extract (quercetin equivalents).

Malvasia Fina is a good candidate for culinary treatment due to its antioxidant properties and composition in bioactive compounds.

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## 1. Introduction

*Vitis vinifera* L. is one of the most widely cultivated crop worldwide, with an estimated grape production over 77 million tonnes in 2013 (FAOSTAT, 2015). The wine making industry and the grapevine itself lead to the production of high volumes of by-products. One of those by-products is the grapevine leaves. Grapevine leaves are considered a delicacy in many countries of the Mediterranean Basin (Harb, Alseekh, Tohge, & Fernie, 2015), while in others are considered a waste and strategies to valorize them are inexistent. According to recent studies (Andelković, Radovanović, Andelković, & Radovanović, 2015; Farhadi, Esmailzadeh, Hatami, Forough, & Molaie, 2016; Katalinić et al., 2013), grapevine leaves are an excellent source of bioactive compounds, mainly phenolic compounds, that exert several beneficial properties. Grapevine leaves, according to Aguilar et al. (2015), are good natural products for the treatment of a series of health problems, like hypertension, diarrhea, hemorrhage, inflammatory disorder, hypoglycemia and chronic venous insufficiency. Therefore, the inclusion of grapevine leaves in the human diet is a good strategy to valorize this vine by-product, increasing as well the intake of important minor

components (Monagas, Hernández-Ledesma, Gómez-Cordovés, & Bartolomé, 2006), with nutritional and healthy properties.

Grapevine leaves inclusion in human diet has to overcome culinary processes, since in their raw form they are not edible. Therefore, in an initial phase, the study of different grapevine leaf varieties is essential to identify those with higher properties and phytochemicals, to be then submitted to culinary processes, in order to give to consumers highest levels bioactive components. Therefore, in the present study, the main objective was to perform a screening on the antioxidant properties and phytochemical composition of ten grapevine leaves from white and red varieties, in order to select the most suitable varieties to be submitted for culinary processes.

## 2. Material and methods

## 2.1. Plant material

The selection of grapevine leaf varieties for culinary process was based in ten varieties, being the most cultivated in the Planalto Mirandês (Trás-os-Montes region, in the Northeast region of Portugal).

Six white grapevine leaf (Côdega do Larinho, Fernão Pires, Gouveio, Malvasia Fina, Rabigato, and Viosinho) and four red grapevine

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leaf varieties (Tinta Amarela, Tinta Roriz, Touriga Franca, and Touriga Nacional) were selected, as illustrated in Fig. 1. The ten varieties were collected within the same edaphoclimatic conditions and agronomic practices. The harvest was made in July 2015 and three independent samples (approximately 500 g of leaves) per variety were collected. Once in laboratory, leaves were visually inspected and those with signs of pests and diseases as well as mechanical damages or with birds excrements were rejected for analysis, being all the debris removed and leaves cleaned when necessary with distilled water. Dust was removed with a slightly humidified scientific paper, while persistent and dried earth was removed with a jet of distilled water to avoid mechanical damages and cells disruption in the leaves. All samples were then frozen.

## 2.2. Samples preparation and extraction conditions

All samples were freeze-dried and then grinded. The aqueous extraction was performed according to Malheiro et al. (2012) with small modifications as briefly described: 5 g of freeze-dried leaves were extracted with 250 mL boiling water during 45 min, and then the extract was filtered through Whatman No. 4 paper. The aqueous extract was frozen, freeze-dried and dissolved in water to a final concentration of 50 mg mL<sup>-1</sup>. For each parameter assessed in this study, per extract, two replicates were performed.

## 2.3. Antioxidant activity determination

### 2.3.1. Scavenging effect on DPPH radicals

The capacity to scavenge the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the method of Hatano, Kagawa, Yasuhara, and Okuda (1988). The extract solution (0.3 mL) was mixed with 2.7 mL of a methanol solution containing DPPH radicals ( $6 \times 10^{-5}$  mol/L). The mixture was shaken vigorously and left to stand for 60 min at room temperature in the dark (until stable absorbance values were obtained). The reduction of the DPPH radical was measured by continuous monitoring of the absorption decrease at 517 nm (Genesys 10UV, Thermo Electron Corporation). DPPH scavenging effect was calculated as the percentage of DPPH discoloration using the following equation: % scavenging effect =  $[(ADPPH - AS)/ADPPH] \times 100$ , where AS is the absorbance of the solution when the sample extract has been added, and ADPPH is the absorbance of the DPPH solution. The extract concentration providing 50% inhibition (EC<sub>50</sub>) was calculated from the graph of scavenging effect percentage against extract concentration in the solution.

### 2.3.2. Scavenging effect on ABTS radicals

The ABTS method was applied according to that described by Karaçelil et al. (2015), based on the capacity of a sample to inhibit the ABTS radical. The ABTS radical was generated by chemical reaction with potassium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>). To 25 mL of ABTS (7 mmol/L) were added 440 mL of K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (140 mmol/L), being the solution kept in darkness during 12–16 h at room temperature in order to form the radical. An accurate volume of the previous solution was diluted in ethanol until an absorbance of  $0.70 \pm 0.02$  at  $\lambda = 734$  nm (Genesys 10UV, Thermo Electron Corporation). Once the radical was formed 2 mL of the ABTS radical solution were mixed with 100 mL of aqueous extract of grapevine leaf at different concentrations (0.01–2 mg mL<sup>-1</sup>) and the absorbance measured at  $\lambda = 734$  nm. The ABTS scavenging effect and EC<sub>50</sub> values were calculated according to the previously mentioned for the DPPH method.

### 2.3.3. Reducing power

The reducing power was determined according to a described procedure (Berker, Güçlü, Tor, & Apak, 2007). The extract solution (1 mL from 0.1 to 2 mg mL<sup>-1</sup>) was mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After cooling, 2.5 mL of 10% trichloroacetic acid (w/v) were added and the mixture was centrifuged at 1000 rpm for 8 min (Centorion K24OR-2003 refrigerated centrifuge). The upper layer (2.5 mL) was mixed with 2.5 mL of deionised water and 0.5 mL of 0.1% ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm (higher absorbance readings indicate higher reducing power). Extract concentration providing 0.5 of absorbance (EC<sub>50</sub>) was calculated from the graph of absorbance at 700 nm (Genesys 10UV, Thermo Electron Corporation) against extract concentration in the solution.

### 2.3.4. Total reducing capacity

Total reducing capacity was performed according to Singleton and Rossi (1965), with some modifications. Thus, 1 mL (at 0.5 mg mL<sup>-1</sup>) of the extract solution was mixed with 1 mL of Folin–Ciocalteu's phenol reagent. The mixture was shaken vigorously and left to stand for 3 min. After that, 1 mL of a saturated solution of sodium carbonate was added and the total volume was adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after that the absorbance was read at 725 nm in a Thermo Electron Corporation Genesys 10UV spectrometer. Gallic acid was used as standard, being the results expressed in mg of gallic acid equivalents (GAE) g<sup>-1</sup> of extract.

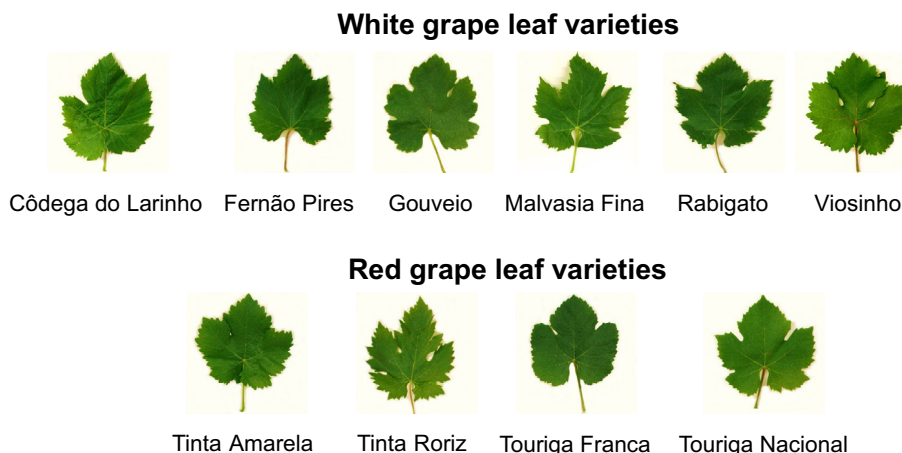


Fig. 1. White and red grapevine leaf varieties in study.

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