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Valorization of three varieties of grape

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ABSTRACT

In the present investigation, seed methanolic extracts of three *Vitis vinifera* (Muscat d'Italie, Syrah and Carignan) were assayed for their antioxidant activities. Results showed that there are strong variations in the contents of total phenols (440.97–121.94 mg GAE g^{-1} DW), flavonoids (48.07–16.81 mg EC g^{-1} DW) and tanins (37.15–14.9 mg EC g^{-1} DW) from the studied seeds. The phenolic composition of these extracts was determined by RP–HPLC after acid hydrolysis. The main phenolic compound was quercetin with 27.2% in Muscat d'Italie, 48.8% in Syrah and 28.4% in Carignan. Besides, all seed extracts showed remarkable DPPH radical scavenging activity with IC_{50} values ranged from 1.8 to 30 μg ml^{-1}. EC_{50} values of reducing power activity ranged from 100 to 120 μg ml^{-1}. The high phenolic content and the considerable antioxidant activities of *vitis* seed extracts could potentially be considered as an expensive source of natural antioxidants.

1. Introduction

Wine is widely cropped in the Mediterranean basin. It produces important amounts of by-products whose composition is not well known and is quite varied, which brings about their mediocre utilization in this region. However, Tunisian winemaking grape cultivation covers a surface of 18,000 ha and the yearly production is 6000 t (APIA, 2005). Recent investigations have stressed the importance of vinification by-products as plant materials particularly rich in a wide range of polyphenols (Bonilla et al., 1999). Marcs, stems and dregs (sludgy residual deposits at the bottom of fermentation vats) represent sources of antioxidants that have been relatively unexploited to date, but are of increasing industrial interest. If stalks are stripped from grape prior to crushing, winery marc consists of approximately 30% seeds and 70% skin and pulp. However, studies on grape seeds are rather limited, despite their richness in polyphenolic substances, mainly monomeric and oligomeric flavanols. The Hellenic vineyard is composed principally of native *Vitis vinifera* species, many of them being occasionally studied, and there have been no reports so far of the polyphenols in the grape seeds.

Nowadays, antioxidants have gained more importance because of their positive involvement as health promoters in conditions such as cardiovascular problems, atherosclerosis, treatment of many forms of cancer, and the ageing process (Packer, 1999). Many antioxidant compounds, naturally occurring in plant sources have been identified as free radical scavengers (Duh, 1998). Recent years, interest has considerably increased in finding naturally occurring antioxidants for use in food or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenicity (Zheng and Wang, 2001). A number of synthetic antioxidants, such as 2- and 3-*tert*-butyl-4-methoxyphenol (butylated such as 2- and 3-*tert*-butyl-4-methoxyphenol (butylated hydroxyanisole, BHA), and *tert*-butylhydroquinone (TBHQ) have been added to foodstuffs but because of toxicity issues, their use is being questioned (Valentao et al., 2002). Therefore, attention has been directed towards the development and isolation of natural antioxidants from plant sources. Crude extracts of spices, herbs, and other plant materials rich in polyphenolics are increasingly of interest to the food industry because they have the capacity to retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Amarowicz et al., 2004).

The composition and properties of grape have been extensively investigated, and it was reported that grape contain large amounts of phenolic compounds (Ricardo da Silva et al., 1990). The by-products of the wine and juice industries such as grape seed

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and pomace, have also been used as natural antioxidants (Ahn et al., 2002; Jayaprakasha et al., 2001) due to their large quantities of monomeric phenolic compounds such as (+)–catechins, (−)–epicatechin and (−)–epicatechin-3-*O*-gallate, and dimeric, trimeric and tetrameric procyanidins (Saito et al., 1998).

In the present investigation, phenolic content and composition of seed methanolic extracts of *V. vinifera* (Carignan, Syrah and Muscat d'Italie) were determined by spectrophotometry and RP–HPLC. Antioxidant activity against DPPH radical and reducing power of these extracts were assayed too.

2. Materials and methods

2.1. Chemicals

Butylated hydroxytoluene (BHT) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma. Folin–Ciocalteu reagent and sodium azide were purchased from Aldrich. Authentic standards of phenolic compounds were purchased from Sigma and Fluka. Stock solutions of these compounds were prepared in HPLC-grade methanol. These solutions were wrapped in aluminum foil and stored at 4 °C. All other chemicals used were of analytical grade.

2.2. Plant material

Our study carried on rape seeds of grape of three varieties (*V. vinifera* L.): Carignan (red grape), Syrah (red grape) and Muscat d'Italie (white grape). 1 kg of samples have been harvested, lasting September 2007, from Takelsa (Temime (North-Eastern Tunisia; latitude 36,78°(N); longitude 10,63°(E), altitude 96 m). Seeds were manually separated and dried at ambient temperature in dark until used.

2.3. Polyphenol extraction

The air-dried seeds were finely ground with a blade–carbide gringing (IKA-WERK. Type: A:10). 1 g of this ground material was extracted by stirring with 10 ml of pure methanol for 30 min. The extracts were then kept for 24 h at 4 °C, filtered through a Whatman N°4 filter paper, and evaporated under vacuum to dryness and stored at 4 °C until analyzed (Mau et al., 2001).

2.4. Total phenolic content

Total phenolic contents were assayed using the Folin–Ciocalteu reagent, following Singleton's method slightly modified by Dewanto et al. (2002). An aliquot (0.125 ml) of a suitable diluted methanolic seed extract (0.25 mg ml^{-1}) was added to 0.5 ml of deionized water and 0.125 ml of the Folin–Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min, before adding 1.25 ml of 7% Na_2CO_3 solution. The solution was then adjusted with deionized water to a final volume of 3 ml and mixed thoroughly. After incubation for 90 min at 23 °C, the absorbance versus prepared blank was read at 760 nm. Total phenolic content of seeds (three replicates per treatment) was expressed as mg gallic acid equivalents (GAE) per gram of dry weight through the calibration curve with gallic acid. The calibration curve range was 50–400 mg ml^{-1} (R^2 = 0.99).

2.5. Determination of total flavonoids

Total flavonoid contents were measured according to Dewanto et al. (2002). 250 μl of the methanolic seed extract appropriately diluted was mixed with 75 μl $NaNO_2$ (5%). After 6 min, we added 150 μl of 10% aluminum chloride and 5 min later, 500 μl of NaOH (1 M) was added to the mixture. Finally, the mixture was adjusted to 2.5 ml with distilled water. The absorbance versus prepared blank was read at 510 nm. Total flavonoid content of seeds (three replicates per treatment) was expressed as mg catechin equivalents (CE) per gram of dry weight through the calibration curve with catechin. The calibration curve range was 50–500 mg ml^{-1}.

2.6. Determination of condensed tanins

In presence of concentrated H_2SO_4, condensed tanins were transformed by the reaction with vanillin to anthocyanidols (Sun et al., 1998). 50 μl of the methanolic seed extract appropriately dilute was mixed with 3 ml of 4% methanol vanillin solution and 1.5 ml of H_2SO_4. After 15 min, the absorbance was measured at 500 nm. Condensed tannin contents of seeds (three replicates per treatment) were expressed as mg catechin equivalents (CE) per gram of dry weight through the calibration curve with catechin. The calibration curve range was 50–600 mg ml^{-1}.

2.7. Hydrolysis and identification of phenolic compounds using RP–HPLC

Dried samples from seeds were hydrolyzed according to the method of Proestos et al. (2006), slightly modified. 20 ml of methanol containing BHT (1 g l^{-1}) were added to 0.5 g of a dried sample. Then 10 ml of 1 M HCl were added. The mixture was stirred carefully and sonicated for 15 min and refluxed in a water bath at 90 °C for 2 h. The obtained mixture was injected to HPLC. The phenolic compounds' analysis was carried out using an Agilent Technologies 1100 series liquid chromatograph (RP–HPLC) coupled with an UV–vis multiwavelength detector. The separation was carried out on a 250 × 4.6-mm, 4-μm Hypersil ODS C_{18} reversed phase column at ambient temperature. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% sulphuric acid (solvent B). The flow rate was kept at 0.5 ml min^{-1}. The gradient program was as follows: 15% A/85% B 0–12 min, 40% A/60% B 12–14 min, 60% A/40% B 14–18 min, 80% A/20% B 18–20 min, 90% A/10% B 20–24 min, 100% A 24–28 min (Bourgou et al., 2008). The injection volume was 20 μl, and peaks were monitored at 280 nm. Samples were filtered through a 0.45 μm membrane filter before injection. Peaks were identified by congruent retention times compared with standards. Analyses were performed in triplicate.

2.8. DPPH assay

The electron donation ability of the obtained methanol extracts was measured by bleaching of the purple-colored solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato et al. (1988). 2 ml of methanolic extracts at different concentrations (Carignan seed extracts: 1, 5 and 10 μg ml^{-1}; Syrah seed extracts: 1, 5, 10 and 50 μg ml^{-1}; Muscat d'Italie seed extracts: 1, 4 and 5 μg ml^{-1}) were added to 0.5 ml of a 0.2 mmol l^{-1} DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min. The absorbance of the resulting solution was then measured at 517 nm after 30 min. The antiradical activity (three replicates per treatment) was expressed as IC_{50} (mg ml^{-1}), the concentration required to cause a 50% DPPH inhibition. A lower IC_{50} value corresponds to a higher antioxidant activity of seed extract (Patro et al., 2005). The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect}(\%) = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100$$

where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample at 30 min. BHT was used as a positive control. Samples were analyzed in triplicate.