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Influence of Intermittent Heating during Maceration on the Antioxidant Capacity of some Grape Seeds and Skins

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Abstract

Ethanolic extracts from seeds and skins of three red grape varieties, namely 'Cabernet Sauvignon', 'Merlot' and 'Burgund' from a Romanian winery, were prepared by maceration using different temperature conditions. The stable free radicals DPPH (2, 2-diphenyl-1-picrylhydrazyl) and Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidin-N-oxyl) were used in order to evaluate the antioxidant capacity of the extracts. The variation in time of free radical concentration was followed by double integration of the EPR spectra of the samples obtained after maceration under different conditions (room temperature and intermittent heating). Results showed that the antioxidant capacity depends on the nature of analysed samples (either being seeds or skins) and grape variety. The results also show that the intermittent heating during maceration leads to a decrease of the antioxidant capacity of samples.

Keywords: antioxidant capacity, intermittent heating, maceration, EPR spectroscopy, grape seeds, grape skins

Introduction

Oxidative stress is closely related to aging, the pathogenesis and development of numerous human diseases including cardiovascular diseases, cancer, rheumatoid arthritis, Alzheimer's disease etc. Antioxidant defense supposes the activity of many enzymatic or non-enzymatic antioxidants of endogenous or dietary origin that destroy potential oxidants (e.g. free radicals) or prevent their generation (Fruhwirth *et al.*, 2006). The presence of micronutrient phytochemical compounds in food as antioxidants is recognized as playing an important role in the prevention of various diseases, since these compounds inhibit or delay the initiation or propagation of oxidizing chain reactions (Manach *et al.*, 2004).

Evidences continue to suggest that in addition to antioxidant vitamins and minerals, fruits and vegetables contain polyphenols, which are currently a subject of great interest. They are also important constituents of human diet. The most important polyphenols in food and beverages are flavonoids, which consist mainly of catechins, proanthocyanidins, flavonols, flavones, flavonones and their glycosides. Anthocyanidins are natural plant pigments and their concentration in fruit is the highest among all types of flavonoids (Stewart *et al.*, 2005). They are responsible for the red, purple and blue color of flowers and fruits and they actively contribute to the control of oxidative reactions providing protection *in vivo* and *in vitro* (Kennedy *et al.*, 2001; Howard *et al.*, 2002; Lapornik *et al.*, 2004; Lopez-Velez *et al.*, 2003).

Recovery of antioxidant compounds from plant materials is typically accomplished by different extraction techniques. Methanol and ethanol have been extensively used to extract antioxidant compounds from various fruits and vegetables. On the basis of extracting techniques, extraction under reflux was found more efficient, particularly since recovery of antioxidant components was higher with use of hot solvent systems under reflux (Shon *et al.*, 2004). Bushra *et al.* (2009) concluded that although higher extract yields were obtained by the refluxing extraction technique, higher total phenolic contents and antioxidant activity were found in the extracts prepared using a shaker.

Several methods (e.g. radical scavenging assay) have been developed for the measurement of antioxidant capacities of pure compounds, food extracts and body fluids. Radical scavenging assay method measures the ability of the sample components to react with radicals in the test system. Spectrophotometry is the commonly used method for evaluating the antioxidant activity. These methods involve a certain incubation period before recording the absorbance decrease of DPPH, the free radical usually added to the plant extract (Sanchez-Moreno *et al.*, 1998). Radical scavenging based methods have also been developed for the measurement of antioxidant capacities of pure compounds, food extracts and body fluids by evaluating the ability of the sample components to react with radicals

in the test system by means of spectroscopic methods (Antolovich, 2002; Miclaus *et al*., 2005).

The aim of this paper is to investigate the influence of the intermittent heating during the maceration on the antioxidant capacity of different varieties of grape seeds and skins, by electronic paramagnetic resonance (EPR). Grape and grape by-products contain large amounts of phenolic compounds that may act as antioxidants, although their concentration is affected by a number of factors, including grape variety, sun exposure, vinification techniques, and aging (Fernandez-Pachon *et al*., 2004; Halpern, 2008).

Materials and methods

Materials

The grape varieties, namely 'Cabernet Sauvignon', 'Merlot' and 'Burgund', from 2005 harvest, were purchased from a Romanian winegrowing region (Recas). The seeds and skins were obtained as by-products after pressing.

Reagents

Ethanol and methanol are analytical grade from Chimopar (Bucharest, Romania); DPPH (2,2-diphenyl-1-picrylhydrazyl) from Sigma and Tempol (2,2,6,6-tetramethyl-4-hydroxypiperidine-N-oxyl) from Fluka. The methanolic solutions of DPPH (5 mg/mL) and of Tempol (0.1 mg/mL) were prepared.

Maceration

Two samples (5 g) of each homogenized seed and skin powders were weighted and 50 ml of solvent (87% ethanol in distilled water) were added. Extraction was performed by maceration under different conditions: a) one batch of extracts were kept ten days at room temperature and b) another batch of extracts were incubated in a water bath at 45°C for one hour every day, during the complete maceration period. After ten days the mixtures were filtered and evaporated in a rotavapor (Bûchi, Flawil, Switzerland) at approximately 45°C. 100 mg of each residue was dissolved in 2 ml of 99.7% ethanol.

EPR measurement of antioxidant activity

The antioxidant capacity was evaluated using either Tempol or DPPH. In the case of Tempol 20 µl of each plant extract were injected with a Hamilton microsyringe into a quartz capillary (10 cm length and interior diameter 1 mm) and 5 µl of Tempol solution were rapidly added. When the antioxidant capacity was evaluated using DPPH, 15 µl of each plant extract were mixed with 10 µl of the DPPH solution.

EPR spectra were recorded at room temperature with a portable EPR Spectrometer "ADANI portable EPR Spectrometer PS8400", Resonance Instruments Inc., operating in X-band (9.1 GHz÷9.6 GHz) equipped with a computer acquisition system. The spectrometer settings were modulation frequency 100 KHz, sweep width 100 G; sweep time 30 s, receiver gain 2×101, except for 'Cabernet Sauvignon' seed extract using DPPH method when receiver gain was 1×101. The EPR spectra were accumulated in 2 minute intervals for 20 minutes, using the same parameters.

Results and discussion

The antioxidant activities of grape seed and skin extracts were evaluated by EPR spectroscopy. When grapes seeds and skins extracts react with DPPH or Tempol, the number of free radical molecules decreases in time, with different rates, depending on the concentration of antioxidant compounds in the extracts. Antioxidant capacity of extracts can be characterized by the decrease in time of EPR signal. The decrease in time of relative concentration of paramagnetic species (i.e. the number of free radical molecules) was obtained by double integration of the EPR signal (Fig. 1 a, b; Fig. 2 a, b). The abbreviations in the legends for all figures are: BP-'Burgund' skins; CP-'Cabernet Sauvignon' skins; MP-'Merlot' skins; BS-'Burgund' seeds; CS-'Cabernet Sauvignon' seeds; MS-'Merlot' seeds.

For all extracts the decrease of the relative concentration is an exponential function according to the formal second order kinetic model. The best fit was obtained using a second order exponential decay:

$$I(t) = I_0 + a_1 \cdot e^{(-k_1 \cdot t)} + a_2 \cdot e^{(-k_2 \cdot t)}$$

where I_0, a_1 and a_2 are parameters depending on experimental EPR conditions. The k_1 and k_2 are constant rate of DPPH resp. Tempol radical decay, depending on the quality of polyphenols content of the extracts.

The obtained experimental data showed that the antioxidant capacity depends on the grape varieties and it is also different for seeds and skins. Also, comparing Fig. 1 and Fig. 2, it could be observed that the antioxidant activity depends on the extraction conditions, as well and the best fit was obtained with DPPH. Intermittent heating of the extracts leads in most cases to an decrease of the extracted antioxidants. The experimental measurements also showed that no incubation period is necessary.

The trends of the decay rate of EPR signal show similar behavior regardless of the free radical (Tempol or DPPH) used for the antioxidant capacity determination. The comparison of antioxidant activities of the extracts leads to the conclusion that the effect of the intermittent heating during maceration significantly decreased the amount of antioxidant components and in this context the antioxidant activity. This behavior can be explained by degradation of antioxidant compounds with exposure to heat and the change in temperature during maceration. Consequently, it could be said that the present study showed that mild maceration conditions should be preferred for ensuring higher recovery of antioxidant compounds.

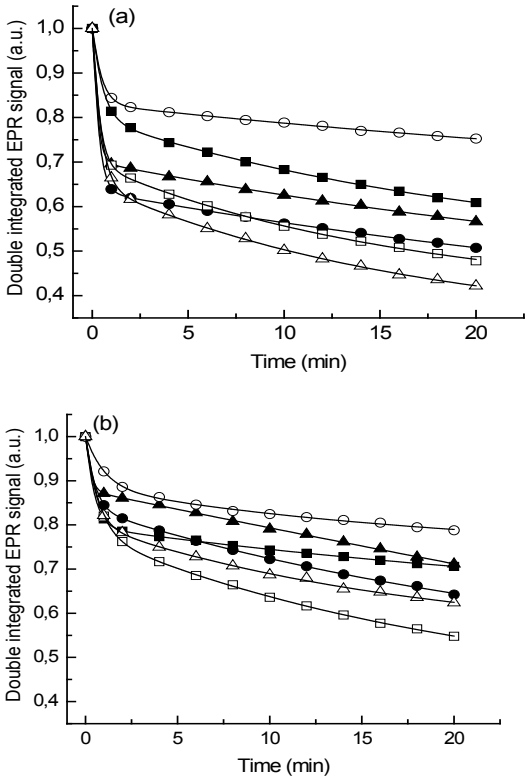

Fig. 1. Decay rates of EPR signal of extracts treated with Tempol: a-room temperature; b-intermittent heating (o-BP; •-CP; ▲- MP; □-BS; ▪-CS; Δ-MS)

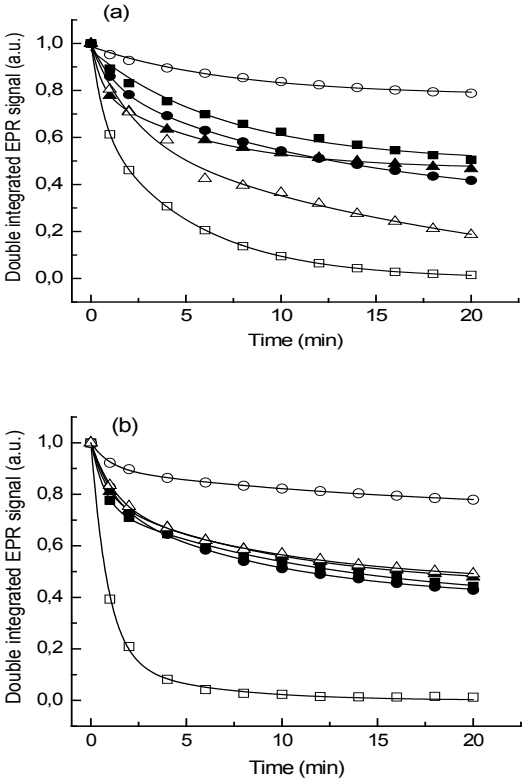

Fig. 2. Decay rates of EPR signal of extracts treated with DPPH: a-room temperature; b-intermittent heating (o-BP; •-CP; ▲ -MP; □-BS; ▪-CS; Δ-MS)

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