



Effect of artificial ageing using different wood chips on the antioxidant activity, resveratrol and catechin concentration, sensory properties and colour of two Greek red wines



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ABSTRACT

Two Greek red wines (Syrah and Cabernet) were artificially aged with different wood chips (white oak, red oak, Turkey oak, chestnut, Bosnian pine, cherry, common juniper, common walnut, white mulberry, black locust and apricot). The influence of each wood species was tested for up to 20 days. The optimum duration for the extraction of total polyphenols was 20 days (Syrah) or 10 days (Cabernet) when chips of white oak, chestnut, cherry, white mulberry, black locust and apricot were used. Resveratrol and catechin concentrations ranged within the limits previously reported in literature. A high antioxidant activity was established after 10 days of artificial ageing. The sensory evaluation showed that the best results were produced by the apricot chips after 5 days (Syrah) or black locust and apricot after 5 days (Cabernet). Colour was seen to increase with both time of ageing and number of wood chips added.

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1. Introduction

Wine polyphenols have been extensively studied in relation to their protective action in humans against cardiovascular and degenerative diseases (Villaño, Fernández-Pachón, Troncoso, & García-Parrilla, 2006). Additionally, they possess anti-inflammatory properties, growth-inhibitory effects in cancer cells, and the ability to reduce platelet aggregation. They can also activate the eicosanoid metabolism, and modulate nitric oxide production (which promotes vascular relaxation) (Villaño et al., 2006; Wei, Yu-Cai, & Wie, 2012).

Ageing of wine in wood barrels promotes changes in colour, structure, and especially aroma, since different reactions occur among phenolic compounds, while several compounds are extracted from wood, increasing wine complexity and stability (Fernández de Simón, Esteruelas, Muñoz, Cadahía, & Sanz, 2009; Rosso, Panighel, Vedona, Stella, & Flamini, 2009). The structural characteristics of wood (grain, porosity, permeability) and its chemical composition (polyphenols, tannins, volatile compounds) can influence the complex physical, chemical and biochemical processes that take place during the oxidative ageing of wine in barrels, affecting their composition and organoleptic properties, and contributing to their stability (Garde-Cerdán et al., 2010; Puech,

Feuillat, & Mosedale, 1999). The barrel is usually chosen depending on wood origin and the processes used in its manufacturing, e.g. seasoning and toasting (Chatonnet, Cutzach, Pons, & Dubourdieu, 1999; Doussot, De Jéso, Quideau, & Pardon, 2002).

In recent years, several new techniques have been introduced in winemaking. One of these involves putting new pieces of wood (oak chips or inner staves) into inert containers (Arapitsas, Antonopoulos, Stefanou, & Dourtoglou, 2004; Gómez García-Carpintero, Gómez Gallego, Sánchez-Palomo, & González Viñas, 2012; Álamo & Nevares, 2006). This technique offers some distinct and previously unfound flavour advantages, as well as new options in wine handling. Since wood is being put into wine and not wine into wood, the entire wood surface area is usable (while only about 40% of the total area is available in the case of barrels). The result is a compelling application that has been adopted by many researchers (Arapitsas et al., 2004; Koussissi et al., 2009).

Since, to our knowledge, there are very few reports concerning the artificial ageing of red wine using wood chips of various origin (except for oak tree) this study was carried out in order to investigate the effects induced in two red wines (Cabernet and Syrah) treated with a variety of chips originating from eleven different wood species. To the best of our knowledge, some of these species were used for the first time (i.e. Bosnian pine and apricot). The main interest was focused on some perspectives not previously considered, such as determination of total polyphenols, evaluation of the antioxidant activity, influence of each wood material during

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ageing on the colour parameters and, finally, optimization of the sensory properties of the red wines. Our main purpose was to produce an improved final wine both with the best sensory characteristics and an increased positive effect on consumer's health (high content of antioxidant compounds, especially resveratrol and catechin).

2. Materials and methods

2.1. Wine and wood materials

Young wines made from two red single-variety grapes (Cabernet and Syrah) belonging to a Greek appellation of Messenikolas area (Karditsa county, Greece) were used.

The wood materials used originated from eleven forest- and fruit-tree species, namely, white oak (*Quercus alba* L.), red oak (*Quercus rubra* L.), Turkey oak (*Quercus cerris* L. var. *cerris*), chestnut (*Castanea sativa* L.), Bosnian pine (*Pinus heldreichii* Christ. var. *leucodermis*), cherry (*Prunus avium* L.), common juniper (*Juniperus communis* L.), common walnut (*Juglans regia* L.), white mulberry (*Morus alba* L.), black locust (*Robinia pseudoacacia* L.) and apricot (*Prunus armeniaca* L.). Most wood materials originated from Greece; mainly from the Karditsa county except for white mulberry (Drama county, Greece) and Bosnian pine (Grevena county, Greece). The white oak and red oak samples came from imported wood. After drying (in open air for three months), wood samples were cut into chips measuring approx. $1 \times 1 \times 1$ cm (cubes). Toasting of wood chips was not carried out. The artificial ageing systems used in this work are shown in Table 1.

Wood chips (1 or 2 cubes, about 1 or 2 g, respectively) were immersed in 750 ml of wine placed in new glass bottles. The bottles were stored under the same conditions (relative humidity of air and temperature were controlled at approx. 75–80% and 16–20 °C, respectively) for 20 days in total. The wine bottles were shaken for 3–5 min daily during the ageing period. The ageing was stopped after 10 and 20 days (by withdrawing the wood chips from the wine) apart from the samples submitted to sensory evaluation for which sampling was done at 5, 10 and 20 days of ageing. Wines were filtered and filtrates were stored at –4 °C prior to analysis.

During the experiments, samples of Cabernet and Syrah wines (of the same origin as the other samples) without any ageing process were used as reference for comparison.

2.2. Analysis of wine samples during artificial ageing

2.2.1. Determination of phenolic content

2.2.1.1. Total content of polyphenols. The total content of phenolic compounds of wines was determined using the Folin–Ciocalteu procedure as modified by Chatzilazarou et al. (2010), using a

Shimadzu UV-1700 Spectrophotometer (Shimadzu Co., Japan) set at 750 nm. Results are expressed as mg of gallic acid equivalents (GAE) per litre of wine (mg/l).

2.2.1.2. Determination of resveratrol (RSV) and catechins (CAT) content of wines by HPLC. The samples of wine that showed the highest total polyphenol content were used for the determination of RSV and CAT. The determination of these phenolic compounds was performed by HPLC according to a modification of the method reported by Rodríguez, Lage-Yusty, and López-Hernández (2009). Specifically, the analysis was carried out on a Shimadzu Prominence CBM-20A (Shimadzu Europa GmbH, Germany) liquid chromatograph equipped with a Shimadzu SIL-20AC auto sampler and a Shimadzu CTO-20AC column oven (set at 28 °C). The column used was a Phenomenex Luna C18(2), (100 Å, 5 µm, 4.6 × 250 mm) (Phenomenex, Inc., USA). Detection was carried out using a Shimadzu RF-10AXL fluorescence detector set at 278 nm (excitation) and 360 nm (emission) for the detection of (+)-catechin or 300 nm (excitation) and 392 nm (emission) for the detection of trans-resveratrol. The mobile phase consisted of A (water:acetonitrile:acetic acid, 67:32:1 v/v/v) and B (water:acetic acid, 99:1 v/v). The gradient elution conditions were: 0 min (20% A and 80% B); 4 min (30% A and 70% B); 8 min (40% A and 60% B); 12 min (65% A and 35% B); 16 min (80% A and 20% B); 20 min (95% A and 5% B); 21.8 min (97% A and 3% B); 24 min (100% A) and 30 min (100% A). The flow rate was set at 0.8 ml/min and the injection volume at 20 µl. Wine samples were filtered through a 0.50 µm PTFE membrane filter (Advantec MFS Inc., USA) before injection into the HPLC.

Calibration curves were prepared for each polyphenol using standards with concentrations of 10, 25, 50 and 10 µg/ml for RSV (Sigma–Aldrich, Hohenbrunn, Germany) and 0.01, 0.03, 0.06, 0.09 and 0.1 µg/ml for CAT (Sigma–Aldrich). The linear regression equation ($y = ax + b$), the R^2 and the limit of detection of the method used were determined.

2.2.2. Determination of antioxidant activity

2.2.2.1. Rancimat method. Initially, purified vegetable oil was prepared. Specifically, sunflower oil (Elais S.A., Greece) was purified from trace metals and other pro-oxidants via adsorption chromatography to yield sunflower oil triacylglycerol fractions according to the method described by Fuster, Lampi, Hopia, and Kamal-Eldin (1998).

The Rancimat method used was a modification of that reported by Gortzi, Lalas, Tsaknis, and Chinou (2007). Wines were accurately weighed at a concentration of 3000 mg/l in purified sunflower oil and their activity was determined using a Methohm Rancimat 743 (Metrohm Ltd., Switzerland), along with another sample of sunflower oil without antioxidant (wine) as control. The conditions were set at a temperature of 100 °C and an air flow of 15 l/h. The protection factor (PF) was calculated as: $PF = (\text{induction period})$

Table 1
Artificial ageing systems.

Type of artificial ageing in Syrah (S) or Cabernet (C) wines	Species of wood chips in each ageing system	
	Common name	Botanical name
S or C	–	–
S ₁ or C ₁	White oak	<i>Quercus alba</i>
S ₂ or C ₂	Red oak	<i>Quercus rubra</i>
S ₃ or C ₃	Turkey oak	<i>Quercus cerris</i> var. <i>cerris</i>
S ₄ or C ₄	Chestnut	<i>Castanea sativa</i>
S ₅ or C ₅	Bosnian pine	<i>Pinus heldreichii</i> var. <i>leucodermis</i>
S ₆ or C ₆	Cherry	<i>Prunus avium</i>
S ₇ or C ₇	Common juniper	<i>Juniperus communis</i>
S ₈ or C ₈	Common walnut	<i>Juglans regia</i>
S ₉ or C ₉	White mulberry	<i>Morus alba</i>
S ₁₀ or C ₁₀	Black locust	<i>Robinia pseudoacacia</i>
S ₁₁ or C ₁₁	Apricot	<i>Prunus armeniaca</i>

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