



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, & Dubourdiou, 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>

with antioxidant)/(induction period without antioxidant). A PF greater than 1 indicates inhibition of lipid oxidation. The higher the value, the better the antioxidant activity (Lalas & Dourtoglou, 2003). The natural antioxidant α -tocopherol (α -toc) (Alfa Aesar GmbH & Co., Germany) (at a concentration of 100 mg/l) and wines without addition of wood chips were used for comparison.

2.2.2.2. DPPH[•] method. The method used was a modification of that described by Tsaknis and Lalas (2005). The antiradical activity of a sample was expressed as % disappearance of DPPH[•] (2,2-diphenyl-1-picrylhydrazyl) free radical 95% (Alfa Aesar GmbH & Co., Germany).

Methanolic DPPH[•] (1.0 ml, 0.1 mM) solution was added to 4.0 ml of sample solution. The mixture was shaken vigorously and left to stand for 30 min at 25 °C. The absorbance (A) of this solution was measured at 517 nm against a control comprising of 4.0 ml of methanol and 1.0 ml of 0.1 mM methanolic DPPH[•] solution. The absorbance at 517 nm of the sample solution (B) and the DPPH[•] solution (C) against 5 ml methanol was also measured.

The results were calculated using the following formula: % disappearance = $(A + B)/(C) \times 100$, where A is absorbance of DPPH[•] against sample + DPPH[•], B is absorbance of sample against methanol, and C is absorbance of DPPH[•] against methanol.

The antiradical activity of wine samples (each at a concentration of 3000 mg/l which was achieved with a volume of wine equal to 60 μ l) was determined and compared with those of the commonly used natural antioxidant α -toc (at a concentration of 100 mg/l) and wines without addition of wood chips.

2.2.3. Sensory analysis

The wines aged by wood species of white oak, chestnut, cherry, white mulberry, black locust and apricot were assessed for their visual, taste and aroma characteristics, as well as for their quality and general impression (in comparison to the reference wine samples) by two panellists highly experienced in the sensory analysis of red wine. Descriptive analysis was carried out according to the protocol of Thessaloniki International Wine Competition (Annex 3.1) (Fig. 9). The highest grade for each of the characteristics under evaluation was a score of 100 points. For each wine sample, an average overall assessment was presented. Thus, wine samples adding up 95–100 points were characterised as “great”, 90–95 points as “excellent”, 85–89 points as “very good”, 80–84 points as “good”, 75–79 points as “satisfactory”, 70–74 points as “medium”, below 69 points as “rejected” and, finally, below 59 points as “unsuitable” for consumption.

The sensory evaluation of samples was carried out after 5, 10 and 20 days of ageing.

2.2.4. Colour analysis

The colour of samples of wine that showed the highest total polyphenol content was analysed with a Lovibond CAM-System 500 Imaging Colourimeter (Tintometer Ltd., UK) which had the necessary software to calculate the CIE L^*a^*b parameters. The factor C^* for chroma was estimated according to the method reported by Álamo et al. (2010). The Chroma factor is given by the formula: $C^* = \sqrt{a^{*2} + b^{*2}}$. Brightness (L^*) and Chroma factor (which is calculated by a^* and b^* , together) determinations were carried out at the 10th and 20th day of artificial ageing in comparison to reference wine samples (no wood chips added) at 0 day.

3. Statistical analysis

Results are displayed as mean values of five simultaneous assays in all methods. Statistical significance (at $P < 0.05$) of the differences between mean values was assessed by Student's *t*-test.

All statistical analysis was performed using SPSS Version 16.0 (SPSS Inc., USA). Statistical analysis was not performed in the case of sensory analysis.

4. Results and discussion

4.1. Determination of total polyphenols using the Folin–Ciocalteu method

The results of total polyphenols content of Syrah and Cabernet wine samples (with and without artificial ageing after 0, 10 and 20 days) are shown in Figs. 1 and 2, respectively. Previous studies showed that average values of total polyphenol content (expressed as gallic acid equivalents in mg/l of wine) ranged from 260 to 3000 mg/l (Villaño et al., 2006; Šeruga, Novak, & Jakobek, 2011). The results of the present work are in line with those studies and, specifically, are classified at the highest level relating to the concentration of total phenolic content when two wood chips were used.

In both wines varieties, it was observed that total polyphenols varied for each type of wood chips used in this artificial ageing (Fig. 2). Significantly ($P < 0.05$) higher quantities of polyphenols (in comparison to the reference sample) were found when chips of white oak, chestnut, cherry, white mulberry, black locust and apricot were used. Additionally, it was proven that there was a greater (significant at $P < 0.05$) phenolic compounds extraction after 20 days of ageing when one wood chip was used. However, while Syrah samples appeared to follow the same trend when two wood chips were added (Fig. 1), those of Cabernet seemed to reach a significantly higher ($P < 0.05$) increase in phenolic compounds after 10 days of ageing followed by a significant ($P < 0.05$) reduction the next ten days (until 20th day). Probably, the biggest concentration of bioactive molecules extracted from two wood chips resulted in a promotion of reactions between them, which had a negative effect on the final concentration of phenolic compounds (Burin et al., 2010) in the case of Cabernet wine samples. It has been reported in related articles that polymerization of polyphenols and their oxidation resulted a decrease in the activity of their hydroxyl groups, which further reduced their reactivity with the reagent of Folin–Ciocalteu (Burin et al., 2010).

4.2. Determination of resveratrol (RSV) and catechin (CAT) by HPLC

The calibration curves (prepared for each polyphenol using standards) showed the following equations: $y = 116,764x - 70,251$ ($R^2 = 0.9995$) and $y = 207,981x + 129,481$ ($R^2 = 0.9824$) for RSV and CAT, respectively. The limit of detection was calculated as 1.0 μ g/ml for RSV and 0.1 μ g/ml for CAT.

The samples of wine that showed the highest total polyphenol content (white oak, chestnut, cherry, white mulberry, black locust and apricot) were used for the determination of their RSV (Fig. 3) and CAT (Fig. 4) concentration by HPLC. Concentrations ranged from 0.64 to 1.88 mg/l (Syrah samples) or 0.38 to 1.99 mg/l (Cabernet samples) for RSV, while for CAT were 13.5 to 23.4 mg/l (Syrah samples) or 12.9 to 20.9 mg/l (Cabernet samples). These results are within the limits set by previous studies (Proestos et al., 2005; Rodríguez et al., 2009; Vrček, Bojić, Žuntar, Mendaš, & Medić-Šarić, 2011; Šeruga et al., 2011) in red wines for RSV (0.04–4.10 mg/l) and CAT (7.80–60.00 mg/l).

The concentration of RSV in wine samples of Syrah, determined by HPLC, showed a significant ($P < 0.05$) increase during the time of artificial ageing. This increase was intensified when chestnut and mulberry wood chips were added. The concentration of RSV in Cabernet samples did not appear stable, and additionally it ranged among the different kinds of wood chips used. The only exception

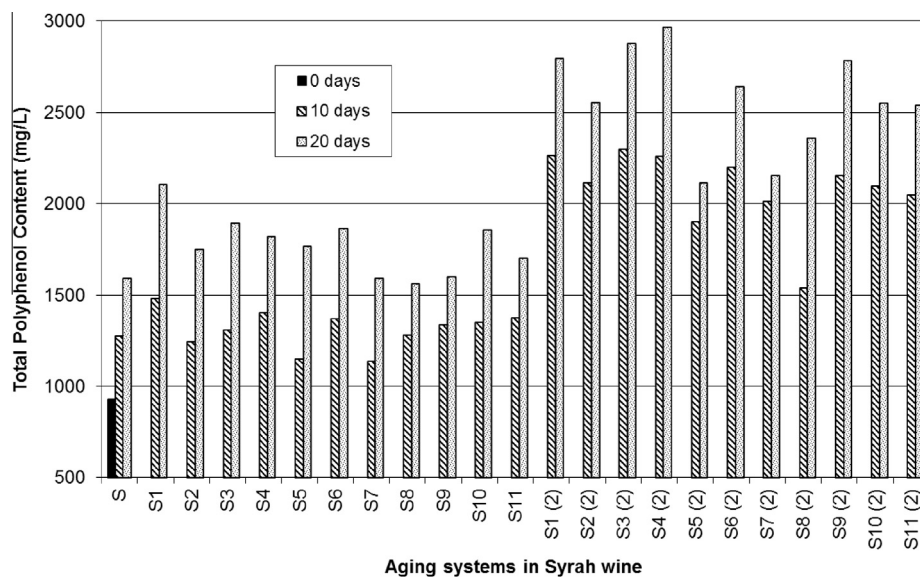


Fig. 1. Concentration of total polyphenol content (mg GAE/L) in samples of Syrah wine after at 0, 10 and 20 days of artificial ageing (note: S, control; S₁, S₂, etc. according to data of Table 1; notations of S(2), S₁(2), S₂(2), etc., stand for the same ageing systems in which two chips were added).

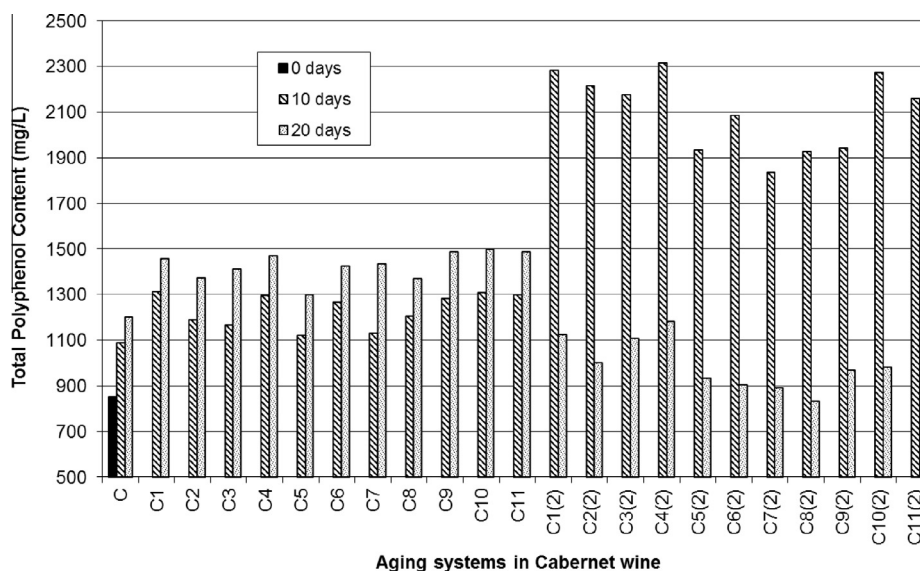


Fig. 2. Concentration of total polyphenol content (mg GAE/L) in samples of Cabernet wine after 0, 10 and 20 days of artificial ageing (note: C, control; C₁, C₂, etc. according to data of Table 1; notations of C(2), C₁(2), C₂(2), etc., stand for the same ageing systems in which two chips were added).

that is worth noticing was the significant increase ($P < 0.05$) in concentration on the 10th day. This concentration was doubled on the 20th day for the sample which was artificially aged with mulberry wood chips.

Regarding CAT, its concentration increased significantly ($P < 0.05$) under the influence of wood chips of chestnut, cherry, mulberry and apricot, after 10 days in Syrah, and decreased thereafter over time apart from the case of mulberry and apricot which caused a further increase in concentration until the 20th day. In the case of Cabernet, the type of wood chips had various effects on the concentration of CAT. While excluding the species of cherry and mulberry, all the other kind of wood chips showed a significant ($P < 0.05$) decrease in the concentration of CAT during time. Of course, most important of all was the significant increase in the concentration of CAT in all samples with wood chips after 10 days.

4.3. Determination of antioxidant activity using the Rancimat method

As indicated by the results (Fig. 5), after 10 days of artificial ageing a high antioxidant activity was established. All the samples of Syrah showed a significant ($P < 0.05$) increase in antioxidant activity during the time of artificial ageing (until 20th day). The highest increase was presented by the mulberry chips (PF = 1.74) which was even higher than that of α -toc (PF = 1.51) at the same concentration.

Concerning Cabernet wine, black locust and apricot wood chips showed the highest activity. Specifically, the black locust and apricot chips gave a PF of 1.87 and 1.84, respectively. However, Cabernet samples showed a significant ($P < 0.05$) decrease in antioxidant activity after the 10th day of ageing when two wood chips were added.

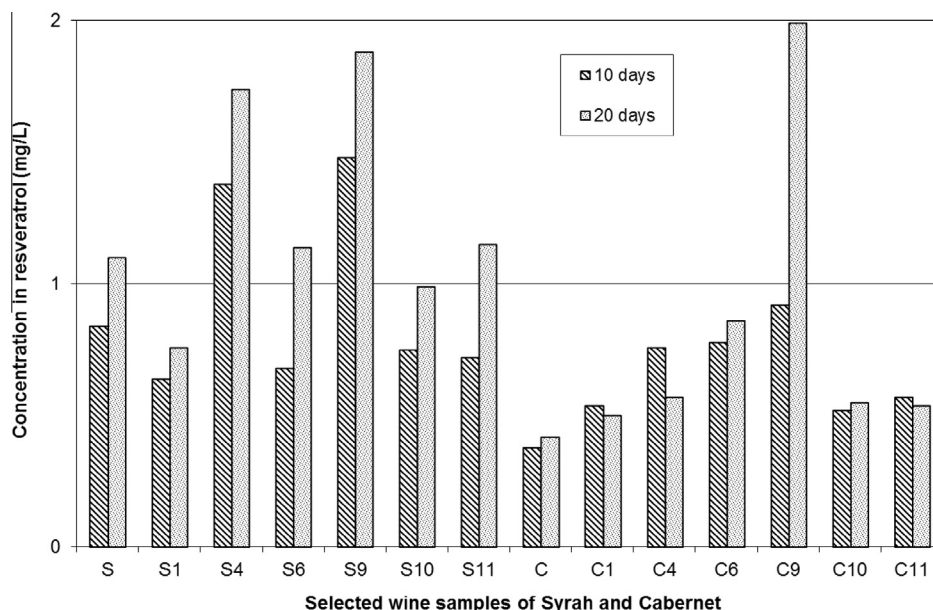


Fig. 3. Content of RSV (mg/l) in selected wine samples of Syrah and Cabernet after 10 and 20 days of artificial ageing.

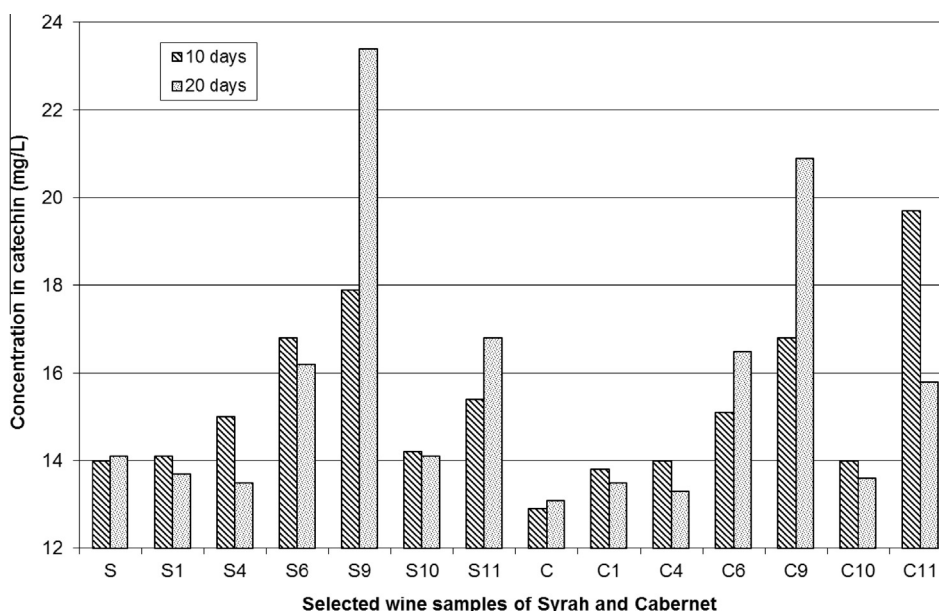


Fig. 4. Content of CAT (mg/l) in selected wine samples of Syrah and Cabernet after 10 and 20 days of artificial ageing.

It appears that the antioxidant activity (determined by the Rancimat method) is proportional to the total polyphenol content as it was observed earlier (Fig. 2). This is in agreement with literature (Šeruga et al., 2011) where it was indicated that the phenolic components extractable from wood chips have as a main mode of action the commitment of free radicals.

4.4. Determination of antioxidant activity by the DPPH[•] method

The concentration of 3000 mg/l (to be noted that the final concentration was achieved with a volume of wine equal to 60 μ l) presented a high antioxidant capacity regarding the capture of free radical DPPH[•]. However, the antioxidant activity was in all cases significantly ($P < 0.05$) lower than that of the natural antioxidant, α -toc.

The % of disappearance of DPPH[•] radicals ranged from 86.48% to 89.90% after 10 days of artificial ageing in the case of Syrah (87.94–92.13% after 20 days) (Fig. 6) while in the case of Cabernet from 84.27% to 90.12% after 10 days (87.78–92.13% after 20 days) (Fig. 7). The disappearance was increased (up to 92.80%), in the case of Syrah the 20th day when two wood chips were used. However, in the case of Cabernet the % disappearance was reduced after 20 days of ageing with the addition of two wood chips (Fig. 7). It appears that the antioxidant activity (determined by the DPPH[•] method) is proportional to the total polyphenol content as was observed earlier (Fig. 2). As indicated above (in the discussion of the results of the Rancimat method) this is in agreement with Šeruga et al. (2011) who reported that phenolic components (extracted from wood chips) show antioxidant action on free radicals.

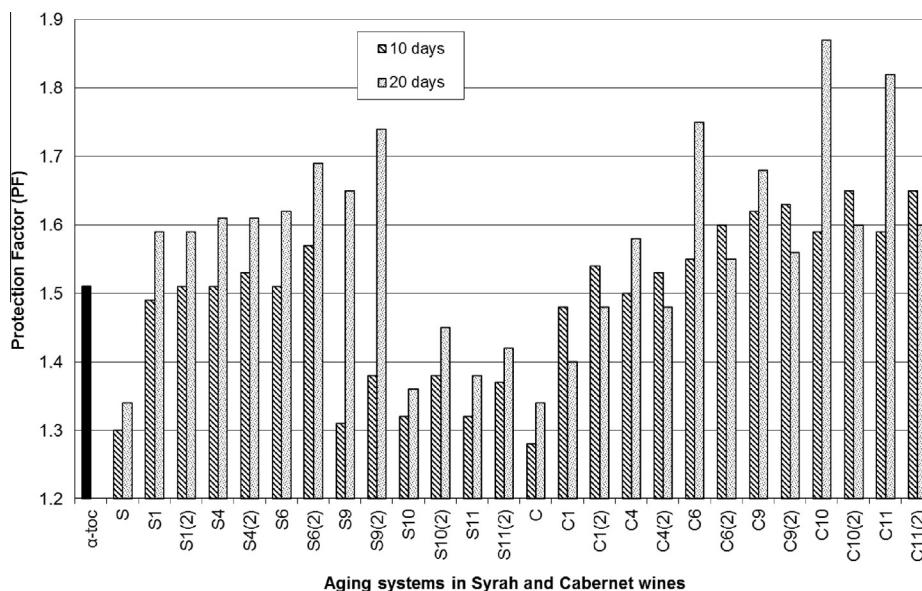


Fig. 5. Protection factor (PF) of Syrah and Cabernet wine samples (in concentrations 3000 mg/l), at 10 and 20 days of artificial ageing.

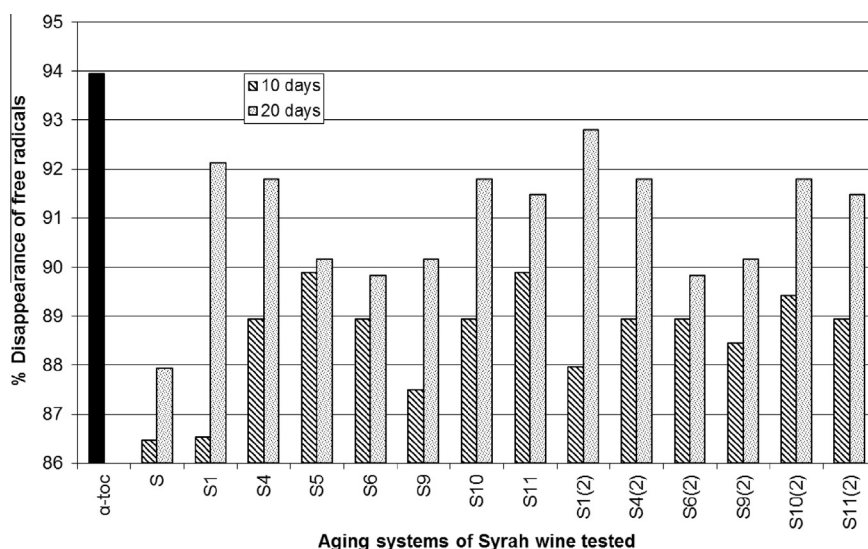


Fig. 6. Results of DPPH[•] experiments (% disappearance of free radicals) of Syrah samples.

4.5. Sensory testing

The wines (aged by wood species of white oak, chestnut, cherry, white mulberry, black locust and apricot) were tested for their organoleptic characteristics (visual, taste and aroma characteristics as well as for their quality and general impression in comparison to reference wine samples). The results are presented in Table 2.

Syrah was initially (before ageing) graded by the panellists as “satisfactory”. The addition of a wood chip of white oak and chestnut, cherry and black locust for 5 days improved the organoleptic characteristics (scores: 79.5, 84 and 80 and 81, respectively) of the wine at the level of “good”. The best results were produced from wood chips of apricot (score 88) which categorised the wine as “very good” by the two testers. However, the effect of the added wood chips was not the same at 10th and 20th day. Specifically, at the 10th day the wine showed a lower score. This was most intense on the 20th day. This phenomenon can be explained by the complicated physicochemical effects which cause polymerization of

bioactive molecules included (Rosso et al., 2009). This kinetics of polyphenol extraction does not seem to follow a linear increase or decrease (Karvela, Makris, Kefalas, & Moutounet, 2008).

Concerning Cabernet, the initial grading “good” was improved through artificial ageing to “very good” for all kind of wood chips during the testing time period (5 and 10 days). Additionally, a grade of “excellent” (score 90) was achieved (the 5th day) by the use of black locust and apricot wood chips. Again, as in the case of Syrah, the effect of the added wood chips was not the same at 10th and 20th day. The scores were lower the 10th day and even lower on the 20th day.

It should be noted that the extensive extraction of tannins (which react and polymerize with wine polyphenols) gave the “hard” and “sour” tannin characteristics reported by both testers. The increased volatile acidity of the samples can probably be explained by the existence of surplus oxygen in the initial storage containers of the samples which can be avoided in industrial scale by using an inactive gas or by filling the containers completely.

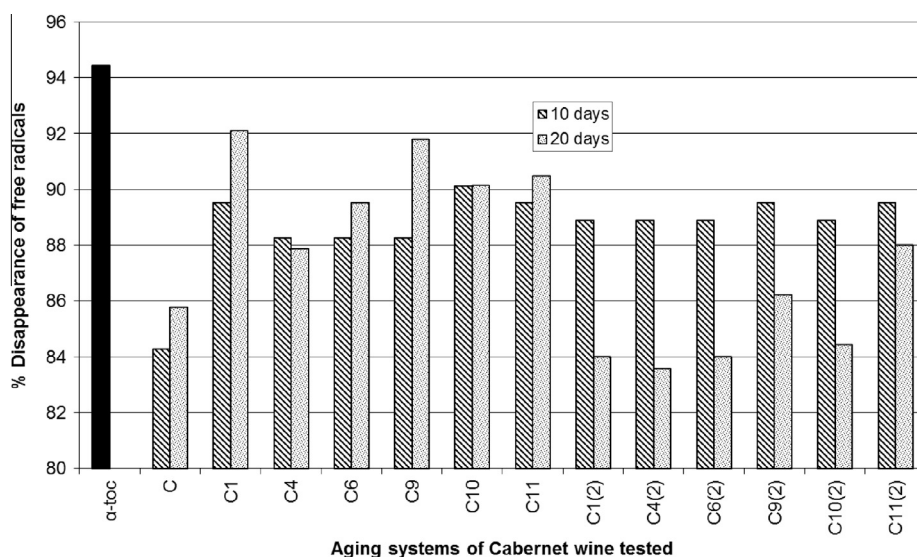


Fig. 7. Results of DPPH \cdot experiments (% disappearance of free radicals) of Cabernet samples.

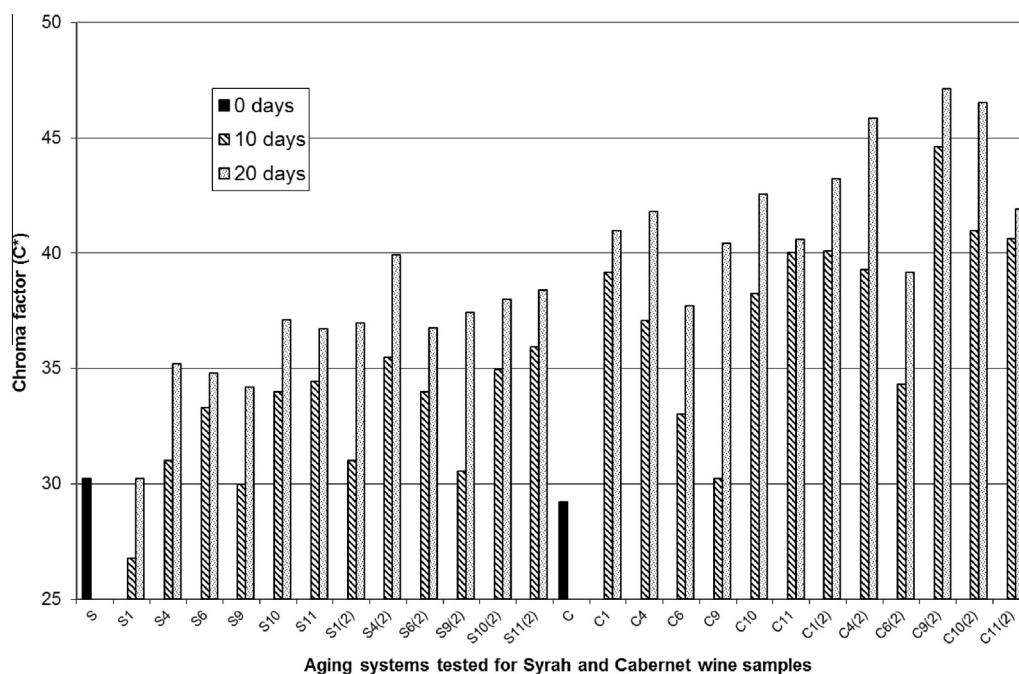


Fig. 8. Chroma factors (C^*) of Syrah and Cabernet wine samples, at 10 and 20 days of artificial ageing.

4.6. Determination of colour parameters

One of the primary sensory perceptions of a food or beverage is colour. It is considered that colour is correlated with aroma, taste and textural qualities of a wine. In addition colour can serve as an indicator of perceived quality. The colour of the samples presented a non-significant increase (results are not displayed) in brightness (L^*) of all wine samples with respect to the time of artificial ageing. The total colour changes, given by the ratio C^* (which relates the changes in factors a^* and b^* together), showed an increase during both time of ageing and number of wood chips added (Fig. 8).

The colour is directly related to the levels of anthocyanins (AC) of which concentration changes during ageing. When AC are involved in oxidation reactions or polymerization their concentration decreases, thus reducing the red colour. However,

derivatives of AC (that can be formed by ageing in oak barrels in the presence of phenolic compounds) have been reported and named as “new colours” can eventually enhance the colour of wines (Revilla & González-Sanjosé, 2001).

5. Conclusions

In both wines varieties, higher quantities of polyphenols were found when chips of white oak, chestnut, cherry, white mulberry, black locust and apricot were used. Determination of the concentrations of RSV and CAT ranged within the limits previously reported in literature. The concentration of RSV in wine samples of Syrah showed a significant increase during the time of artificial ageing, especially in the case were chestnut and mulberry wood chips were added. However, in the case of Cabernet samples RSV

Annex 3.1

SCORE SHEET		STILL WINES					
Jury	N°	Sample	N°	Category	N°		
		Excellent +				Inadequate -	Observations
Visual	Limpidity	<input type="checkbox"/> (5)	<input type="checkbox"/> (4)	<input type="checkbox"/> (3)	<input type="checkbox"/> (2)	<input type="checkbox"/> (1)	
	Aspect other than limpidity	<input type="checkbox"/> (10)	<input type="checkbox"/> (8)	<input type="checkbox"/> (6)	<input type="checkbox"/> (4)	<input type="checkbox"/> (2)	
Nose	Genuineness	<input type="checkbox"/> (6)	<input type="checkbox"/> (5)	<input type="checkbox"/> (4)	<input type="checkbox"/> (3)	<input type="checkbox"/> (2)	
	Positive intensity	<input type="checkbox"/> (8)	<input type="checkbox"/> (7)	<input type="checkbox"/> (6)	<input type="checkbox"/> (4)	<input type="checkbox"/> (2)	
	Quality	<input type="checkbox"/> (16)	<input type="checkbox"/> (14)	<input type="checkbox"/> (12)	<input type="checkbox"/> (10)	<input type="checkbox"/> (8)	
Taste	Genuineness	<input type="checkbox"/> (6)	<input type="checkbox"/> (5)	<input type="checkbox"/> (4)	<input type="checkbox"/> (3)	<input type="checkbox"/> (2)	
	Positive intensity	<input type="checkbox"/> (8)	<input type="checkbox"/> (7)	<input type="checkbox"/> (6)	<input type="checkbox"/> (4)	<input type="checkbox"/> (2)	
	Harmonious persistence	<input type="checkbox"/> (8)	<input type="checkbox"/> (7)	<input type="checkbox"/> (6)	<input type="checkbox"/> (5)	<input type="checkbox"/> (4)	
	Quality	<input type="checkbox"/> (22)	<input type="checkbox"/> (19)	<input type="checkbox"/> (16)	<input type="checkbox"/> (13)	<input type="checkbox"/> (10)	
Harmony – Overall judgement		<input type="checkbox"/> (11)	<input type="checkbox"/> (10)	<input type="checkbox"/> (9)	<input type="checkbox"/> (8)	<input type="checkbox"/> (7)	
Total		+	-	-	+	=	
Eliminated due to major defect							0
Signature of juror		Signature of President of the jury					

Fig. 9. Score sheet of the protocol of Thessaloniki International Wine Competition (Annex 3.1).

Table 2
Sensory rating (in the scale of 100 points) of wine samples (S₁, S₄, S₆, S₉, S₁₀, S₁₁, and C₁, C₄, C₆, C₉, C₁₀, C₁₁) by the two expert panellists at 5th (5d), 10th (10d) and 20th (20d) day of artificial ageing.

Wine sample	S	S ₁ (5d)	S ₁ (10d)	S ₁ (20d)	S ₄ (5d)	S ₄ (10d)	S ₄ (20d)	S ₆ (5d)	S ₆ (10d)	S ₆ (20d)
Points	78	79.5	71	<60	84	69.5	73.5	80	65	<60
Wine sample	S	S ₉ (5d)	S ₉ (10d)	S ₉ (20d)	S ₁₀ (5d)	S ₁₀ (10d)	S ₁₀ (20d)	S ₁₁ (5d)	S ₁₁ (10d)	S ₁₁ (20d)
Points	78	75.5	74.5	72.5	81	68	64	88	75.5	69.5
Wine sample	C	C ₁ (5d)	C ₁ (10d)	C ₁ (20d)	C ₄ (5d)	C ₄ (10d)	C ₄ (20d)	C ₆ (5d)	C ₆ (10d)	C ₆ (20d)
Points	80.5	85.5	81	73.5	85.5	79	78.5	87.5	80	68.5
Wine sample	C	C ₉ (5d)	C ₉ (10d)	C ₉ (20d)	C ₁₀ (5d)	C ₁₀ (10d)	C ₁₀ (20d)	C ₁₁ (5d)	C ₁₁ (10d)	C ₁₁ (20d)
Points	80.5	83.5	81.5	71	90	81.5	65.5	90	83	66.5

did not appear stable. The better results were produced by mulberry wood chips. The concentration of CAT increased by using wood chips of chestnut, cherry, mulberry and apricot, after 10 days in Syrah, and decreased thereafter, apart from the case of mulberry and apricot which showed a further increase in concentration until the 20th day. In the case of Cabernet, excluding the species of cherry and mulberry, all the other kind of wood chips caused a decrease in the concentration of CAT during time.

A high antioxidant activity (Rancimat method) was established after 10 days of artificial ageing. The highest increase was presented by the mulberry chips in Syrah and black locust and apricot chips in Cabernet. The samples presented a high activity regarding the capture of free radical DPPH.

The sensory test showed that the best results were produced by the apricot chips retained in wine for 5 days in the case of Syrah. Cabernet artificially aged by wood chips of black locust and apricot after 5 days showed the best scores.

Finally, the colour of the samples presented an increase during both time of ageing and number of wood chips added.

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