



Research Paper

Impact of tannin addition on the antioxidant activity and sensory character of Malagousia white wine

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ABSTRACT

Enological tannins are assessed as promising alternative to SO₂ in order to control oxidative process during winemaking, due to allergic reactions incurred by sulfite sensitive individuals. In the present study, the commercial enological Tara tannin “Vitanil B” was added, as alternative to the addition of sulfites, at different concentrations (100–500 mg/L) in white wine from grapes of *Vitis vinifera* L. var. Malagousia in order to enhance antioxidant stability and sensory character of the wine. Considering photometric analyses and chromatic parameters results, tannin addition (300 mg/L) in Malagousia enhanced total phenolic content, antioxidant and antiradical activity and prevented color deterioration, for a storage period of 100 d, compared to control and sulfited wines. Moreover, aroma quality, body, after taste and overall acceptance of wine treated with 300 mg/L tannin, were highly appreciated and received the highest scores. The overall evaluation of tannin addition was performed by Principal Component Analysis, leading to discrimination of wines, according to photometric, color and sensory analysis parameters. Conclusively, tannin addition resulted in a considerable increase of total phenolic content, antioxidant and antiradical activity, compared to the control and sulfited wines, maintaining the sensory parameters and overall acceptance of Malagousia wine.

1. Introduction

Greek white wines account for approximately 67% of the annual wine production (Hellenic Ministry of Rural Development and Food, 2021) and, as reported by Tourtoglou et al. (2014), stand high (32.5% over 54.1% of red wine) among the preferences of the Greek consumers. *Vitis vinifera* L. var. Malagousia (ECP/GR, 2021) is a Greek white grape variety, thought to be extinct and known to very few in the 1970s. However, today, Malagousia is widely considered a world class grape, producing outstanding dry whites, medium pale to lemon green in color with an exceptional aromatic profile, showing hints of peaches, green bell pepper, basil and flowers (EDOAO, 2021). Moreover, according to Nanou et al. (2020), Malagousia wines were described by assessors as having lemon, grapefruit, and citrus blossom character, they also shared some descriptors with Assyrtiko wines, such as mushroom and earthy characters, and had some shared characters, like floral and citrus notes, with Moschofilero samples.

In oenology, the use of SO₂ is well known in the wine making areas of central Europe since Middle Ages, because of its antioxidant, antimicrobial and preservative action. Sulfur dioxide (SO₂) is widely used as additive during the vinification process (from must pressing to wine bottling).

In addition, this compound prevents the wine browning by inactivation of enzymes such as polyphenoloxidase (PPO), peroxidase (POD), and proteases, and also by inhibition of the Maillard reaction (Garde-Cerdán et al., 2008; Ribereau-Gayon et al., 2006). Moreover, the addition of sulfites protects wine aroma and reduces color loss usually observed during wine aging, by reducing the rate of phenolic polymerization (Oliveira et al., 2011).

Except for all the positive effects of SO₂, sulfites are included in the list of allergens of Regulation (EU) 2019/33 (European Commission 2019a); therefore, the negative effects on human health have been the subject of research for many years (Guerrero and Cantos-Villar, 2015; Vally and Thompson, 2003; Qin and Meng, 2009; OIV 2021a; OIV

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2021b). Consequently, the maximum concentration of SO₂ allowed by legislation in wines has been gradually reduced to 150 and 200 mg/L for dry red and white wines, respectively (Regulation (EU) 2019/934, European Commission 2019b).

Actually, there has been a growing interest in finding other preservatives and innovative technologies, replacing or at least complementing the action of SO₂ during the winemaking process. Several methods have been applied as alternatives to SO₂ use in wine production, including the following: a) the addition of chemical preservatives such as dimethyl decarbonate (Costa et al., 2008; Sonni et al., 2011), lysozyme (Azzolini et al., 2010; Sonni et al., 2009) and ascorbic acid (Barril et al., 2012); b) processes including high hydrostatic pressure (HHP) Briones-Labarca et al. (2017), pulsed electric field (PEF) (Delsart et al., 2015), ultraviolet irradiation (UV) (Fredericks et al., 2011), high power ultrasound (HPU) (Gracin et al., 2016) and low electric current (LEC) (Silva and van Wyk, 2021), and c) the use of phenolic compounds and plant extracts (Raposo et al., 2016; Sonni et al., 2009), killer toxins and bacteriocins or combined methods (Yildirim and Darici, 2020; Lisanti et al., 2019).

A number of plant materials containing phenolic substances have also been studied as wine additions for their antioxidant properties (Tzachristas et al., 2020; Proestos et al., 2015). Nevertheless, the proposed alternatives should ensure the protection against wine oxidation, the microbiological safety of wine and the maintenance as much as possible of wine sensory characteristics.

Concerning the substitution of SO₂ with natural plant extracts, tannins prevent the oxidative phenomena of musts and wines possibly as a consequence of a dual mechanism involving inhibition of enzymes and radical-scavenging activity. They have been used to facilitate the clarification of musts and wines, to contribute to wine structure and to improve the sensory impact of white wines (Sonni et al., 2009, 2011; Panero et al., 2015; Pascual et al., 2017). Hydrolysable tannins, such as gallotannins extracted from oak galls and ellagitannins from oak or chestnut, which are not naturally present in grapes, make up the most sold commercial tannins (Versari et al., 2013).

In the concept of SO₂ replacement with natural preservatives, the goal of the study was the application of wine tannins in white wines for their antioxidant stability and sensory improvement. To a further step, the present study aimed to assess the effect of the addition of “Vitanil B” tannin on Malagousia white wine overall quality, including antioxidant activity, color and sensory characteristics, through statistical evaluation.

2. Materials and Methods

2.1. Reagents and standards

The chemicals 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ), sodium persulfate, ferrous sulfate heptahydrate, iron(III) chloride hexahydrate, ABTS [2,2'-Azinobis (3 ethylbenzothiazoline-6-sulfonic acid)], Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid), gallic acid (3,4,5-trihydroxybenzoic acid), and Folin–Ciocalteu phenol reagent, as well as solvents of analytical grade were purchased from Mallinckrodt Chemical Works (St. Louis, MO, USA), Alfa Aesar GmbH (Karlsruhe, Germany), Tokyo Chemical Industry (Japan), and Sigma-Aldrich Chemie GmbH (Germany).

2.2. Process of vinification

A special vinification process for the production of white wine without added sulfites was performed with grapes of Malagousia variety at Roxanis Matsa estate in Kantza, Attica, as described below. Grape berries of Malagousia variety were harvested in plastic containers at optimum maturity stage, after the evaluation of detailed maturation data and organoleptic testing. Upon receipt, the grapes were carefully hand sorted and were kept at 10 °C until further use. Subsequently, the grapes were pressed under inert atmosphere and the resulting grape

pulp was gently pressed and left for 24 h. After the removal of wine mud, inoculation with selected yeast strains and addition of nutrients was performed in the must. The alcoholic fermentation was performed in a stainless-steel tank at controlled temperature, ranging from 14 to 18 °C. When the fermentation was completed, the wine lees were removed and the wine was transferred in a new stainless-steel tank. White wine was packed immediately after fermentation in 10 L plastic bags. The quality characteristics of Malagousia wine were measured according to the methods of analysis of the International Organization Of Vine And Wine (OIV, 2021c). Specifically, the corresponding values were: pH=3.33±0.05, alcohol=12.1±0.1%, density=0.9901±0.0004, total acidity expressed as tartaric acid=5.82±0.11 g/L, volatile acidity expressed as acetic acid=0.36±0.04 g/L, fructose=0.9±0.1 g/L, glucose=0.7±0.1 g/L and glycerol=4.8±0.2 g/L.

2.3. Wine and model wine samples preparation

“Vitanil B” (MARTIN VIALATTE, France) was the tannin chosen for the addition in Malagousia white wine. According to product specification, “Vitanil B” is a commercial enological Tara pod (bean originating from South America) clear tannin extracted from gallic alcohol, perfectly adapted to the fining of white wine. Its main application fields are the clarification of white wines, the protection of must from oxidation and the limitation of the development of taste reduction during fermentation. “Vitanil B” (MARTIN VIALATTE, France), was added at four different concentration levels (ranging from 100 to 500 mg/L) in multiple 100 mL aseptic well-sealed brown glass bottles, containing Malagousia white wine. Model Wine solutions were prepared by adding 6 g/L (+)-tartaric acid in hydroalcoholic mixture 12% vol, and pH was set at about 3.3 by adding 4M sodium hydroxide. Model wines were subjected to the same tannin addition level accordingly to the wine samples. White wine samples containing potassium metabisulphite (200 mg/L), corresponding to 108±4 mg/L of total sulfur dioxide and 43±3 mg/L of free sulfur dioxide, were also used for comparison purposes. The treatment and levels of tannin and sulfur dioxide addition in wine and model wine samples are given in Table 1. All the wine and model wine samples were stored at 4 °C until further analysis. All chemical analyses were performed in triplicate at regular intervals of 25 days for a total period of four months.

2.4. Determination of total phenolic content (TPC)

The total phenolic content (TPC) of wine samples was determined according to a modified micromethod of Folin–Ciocalteu’s colorimetric assay (Andreou et al., 2018). The absorbance was measured at 750 nm with a visible spectrophotometer (Spectro 23, Digital Spectrophotometer, Labomed Inc., USA). The results were expressed as mg Gallic acid equivalents (GAE) per L of wine, using a standard curve with a range of 25–2600 mg/L Gallic acid ($y = 0.0005x + 0.0783$, $R^2 = 0.9989$).

2.5. Scavenging activity on 2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS^{•+})

The antiradical activity of the wine samples was determined according to the method described by Lantzouraki et al. (2015). Absorbance was measured at 734 nm with a visible spectrophotometer (Spectro 23, Digital Spectrophotometer, Labomed Inc., USA). Trolox, a water soluble form of vitamin E, was used as a standard compound, and the antiradical activity of each sample was expressed as mg Trolox Equivalents (TE) per L of wine. A standard curve was prepared with a concentration range of 0.20–1.50 mM Trolox ($y = 0.2876x - 0.002$, $R^2 = 0.9995$).

2.6. Ferric reducing/antioxidant power assay (FRAP)

The Ferric Reducing/Antioxidant Power for each wine sample was

Table 1
Coding of wine and model wine samples, according to treatment.

		Wine Samples						
		W	WSO ₂	W1	W2	W3	W4	W5
Treatment	Control	Addition of potassium metabisulphite (200 mg/L)		Addition of Tannin (100 mg/L)	Addition of Tannin (200 mg/L)	Addition of Tannin (300 mg/L)	Addition of Tannin (400 mg/L)	Addition of Tannin (500 mg/L)
		Model Wines						
		MW0	MW1	MW2	MW3	MW4	MW5	
Treatment		6 g/L tartaric acid in hydroalcoholic mixture 12% vol	Addition of Tannin (100 mg/L)	Addition of Tannin (200 mg/L)	Addition of Tannin (300 mg/L)	Addition of Tannin (400 mg/L)	Addition of Tannin (500 mg/L)	

evaluated based on the reduction of Fe(III) in the form of ferric-2, 4,6-tripyridyl-s-triazine complex to Fe(II), as described by Lantzouraki et al. (2016). The absorbance was measured at 595 nm with a visible spectrophotometer (Spectro 23, Digital Spectrophotometer, Labomed Inc., USA). A standard curve ($y=0.0003x+0.0081$, $R^2=0.9969$) was prepared using various concentrations (50–1800 μM) of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ stock solutions. The results were expressed as mg Fe(II) per L of wine.

2.7. Color measurement

The chromatic characteristics of wine samples were defined by the colorimetric coordinates L^* (lightness), a^* (redness/greenness), b^* (yellowness/blueness), C (chroma), and h (hue angle in degrees). The above values were measured with a tristimulus chromatometer (model CR-400, Minolta, Tokyo, Japan) calibrated with a white standard plate (L^* : 97.83, a^* : -0.45, b^* : +1.88). Color parameters were evaluated at days 0, 25, 50, 75, 100, and 125. Three random readings per sample were taken and averaged.

2.8. Sensory analysis

The sensory analysis of the wine samples was evaluated using Quantitative Descriptive Analysis (QDA), developed by Tragon Corporation in 1974. A 12-member panel was trained in a number of preliminary sessions, using different wines that represent the range of characteristics that may be tested, in order to develop a common vocabulary for the description of the sensory attributes of wine samples and to familiarise them with scales and procedures. Ten descriptors were included in the analysis: color (intensity), odor (intensity), aroma (quality), oxidation, taste (intensity), taste (balance), body, bitterness, aftertaste and overall acceptance. The wine samples were randomly evaluated by assigning a score between 1.00 (absence of sensation) and 9.00 (extremely intense) in individual booths under incandescent white light, in a special room which met the requirements of ISO 8589:2007.

2.9. Statistical analysis

Spectrophotometric assays were repeated three times. The values were averaged and reported along with their standard deviation (S.D). The data regarding TPC, antiradical-antioxidant activity and color parameters were analyzed with One-Way ANOVA Post Hoc Tests, using Tukey's test for pairwise multiple comparisons with statistical significance ($P<0.05$). Regarding sensory scores, for all attributes assessed, F-value and P-values of each effect of the ANOVA and of the post-hoc Duncan discrimination test were calculated. The correlation among the results was performed by Spearman rank-order test. All statistical calculations including Principal Components and Classification Analysis procedure were performed with the STATISTICA package (STATISTICA software Statsoft Inc, 2004).

3. Results and discussion

3.1. Photometric analyses

The evolution of total phenolic content (TPC) of Malagousia wine samples, treated with different tannin addition levels, over storage time, is presented in Fig. 1a. TPC value of the studied wine (199.20 ± 6.36 mg/L) was similar with those reported by other researchers for white wines which ranging between 81 and 423 mg/L (Tourtoglou et al., 2014; Olejar et al., 2015; Jagatić Korenika et al., 2020; Tekos et al., 2021).

The addition of tannin resulted in a considerable and proportional increase of phenolic content, compared to the phenolic content of control and sulfited wines. This increase was remarkable until 100 d of storage period; however, at the end of experiment (125 d) a significant decrease in total phenolic content was observed at all tannin addition levels.

In addition to analysing the total phenolic content of wine samples, the ferric reducing/antioxidant power and $\text{ABTS}^{\bullet+}$ radical scavenging activity of the wines (expressed as mg Fe^{+2} /L and mg Trolox equivalents/L, respectively) were also measured at the same time intervals previously mentioned (Fig. 1c and e).

The addition of tannin resulted in a considerable increase of ferric reducing/antioxidant power and radical scavenging activity, compared to the respective ones of control and sulfited wines (Fig. 1c and e). However, after 25 d of storage time, the antioxidant power decreased gradually until 75 d of storage, whereas a considerable increase was observed at 100 d, and finally, at the end of experiment, the antioxidant power of all wine samples was reduced (Fig. 1c). The $\text{ABTS}^{\bullet+}$ radical scavenging activity of wine samples treated with tannin remained constant until 75 d of storage time, then it increased at 100 d of storage and at the final time period, the radical scavenging activity was reduced (Fig. 1e).

In accordance to the above findings, Jagatić Korenika et al. (2020), reported that the addition of ascorbic acid and enological tannins in Sauvignon blanc wine, resulted to significant increase of total phenolic content and antiradical activity. Moreover, enological tannins were suggested by other researchers to enhance the wine antioxidant activity (Ribereau-Gayon et al., 2006; Canuti et al., 2012; Rinaldi and Moio, 2018; Yildirim and Darici, 2020).

Model wine samples, prepared by adding 6 g/L (+)-tartaric acid in hydroalcoholic mixture 12% vol and treated with different concentrations of tannin, were also evaluated in comparison with the wine samples, in order to examine the antioxidant and antiradical activity of tannins without the interference of wine matrix. Control model wine (MW0) was prepared and analyzed for TPC, antioxidant and antiradical activity. However, the results showed negligible values (data not shown). It was observed that the addition of tannin in model wine solutions caused a similar effect in total phenolic content (Fig. 1b), ferric reducing/antioxidant power (Fig. 1d) and radical scavenging activity (Fig. 1f), compared with the respective of the wines. A remarkable finding was that the variations in total phenolic content, antiradical activity and antioxidant power followed the same trend in the model wine solutions as in the respective one of wine samples.

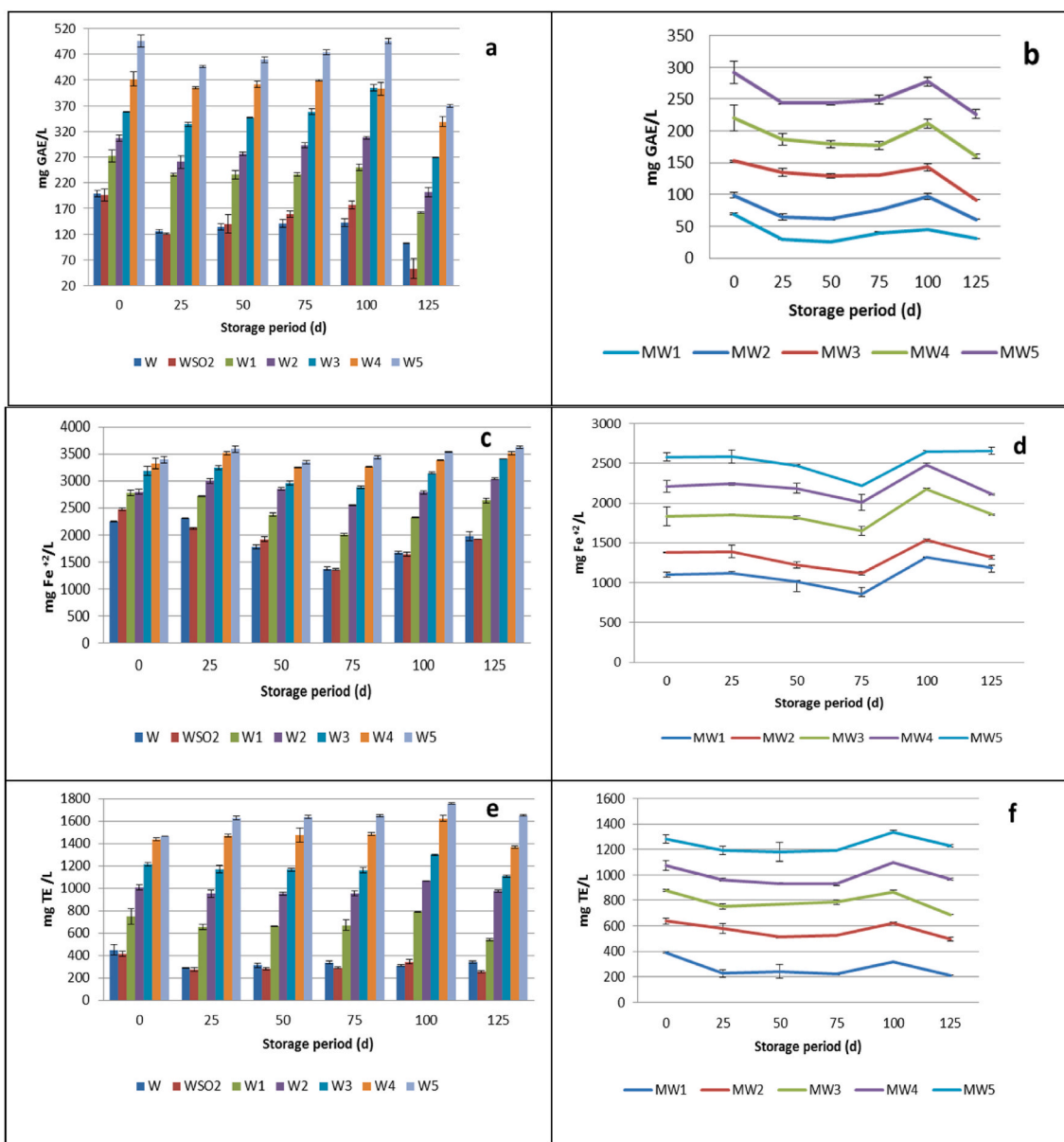


Fig. 1. Evolution of total phenolic content, Ferric Reducing/Antioxidant Power, and radical scavenging activity over storage time, of (a, c, e) Wine samples and (b, d, f) Model Wine samples, respectively.

“Vitanil B” tannin chosen for the experiment is a hydrolysable gallo-tannin consisting of polymers formed by esterification between D-glucose and gallic acid. Therefore, the increase of total phenolic content and antioxidant power observed over time could be explained by the hydrolysis of glycosylated forms of gallic acid, resulting to aglycone structures, whereas the subsequent decrease may be due to their susceptibility to oxidation (Ribéreau-Gayon et al., 2000).

The most important phenolic substances in white wines, both in terms of quantity and ability to participate in redox reactions, are hydroxycinnamic acids as well as hydroxybenzoic acids and flavanols (Pati et al., 2014). Among the phenolic acids, caffeic, caftaric and gallic acids predominate. Catechin is the most abundant flavonoid in white wines, and it commonly constitutes up to 20% of the total phenolic content (Abramovic et al., 2015). During the vinification process, phenolic compounds are susceptible to enzymatic and non-enzymatic oxidation. Enzymatic oxidation occurs entirely in grape must, whereas non-enzymatic oxidation occurs in wine in the presence of transition metal ions and involves the oxidation of polyphenols into quinones, which are unstable and participate in polymerization processes to

produce colored dimers (Oliveira et al., 2011). These compounds are possibly rearranged to form new dihydroxybenzene groups, more sensitive to oxidation due to their lower redox potential compared to their initial phenols (Singleton, 1987).

The main mechanisms of the action of sulfur dioxide as antioxidant agent are the direct oxygen scavenging, the reaction with hydrogen peroxide produced by the oxidation of polyphenols in wine and the reduction of the quinones formed during the oxidation process back to their phenol form (Yildirim and Darici, 2020). Gallotannins possess antioxidant capacities mainly originating by many phenolic hydroxyl groups and electron-donating groups at benzene ring; therefore, they protect the wine against chemical oxidation by direct consumption of dissolved oxygen and by scavenging peroxy radicals (Pascual et al., 2017). Moreover, the degree of polymerization, highly related to the molecular weight of tannins, is another characteristic that influences antioxidant properties. Tannins of high molecular weight and with a great amount of hydroxyl groups in their structure possess high antioxidant properties, while the formation of tannin–protein complexes reduces this antioxidant ability (Fraga-Corral et al., 2020).

Spearman Rank Order correlation coefficients showed a very strong positive relationship among total phenolic content (TPC) and antioxidant power (FRAP assay) ($r=0.869$) as well as between TPC and ABTS radical scavenging activity ($r=0.917$). Additionally, Spearman rank order correlation between ABTS antiradical activity and antioxidant power (FRAP assay) was also very strong ($r=0.904$). This finding indicates that the presence of phenolic compounds is strongly related to the antioxidant and antiradical activities of the examined samples of Malagousia wine. Similarly, high correlation coefficients were also observed between the total polyphenols and flavanols and the antioxidant activity values in selected Hellenic varietal white wines, including Malagousia, after 3 and 6 months storage (Kallithraka et al., 2009), as well as between TPC and Ferric reducing antioxidant power (FRAP) ($r=0.970$) and between TPC and DPPH (2,2-diphenyl-1-picrylhydrazyl) assays ($r=0.987$) of North Macedonian Wines (Mitrevska et al., 2020).

3.2. Chromatic parameters

In the CIELab color system, color is described by the five parameters L^* , a^* , b^* and h^* . L^* represents the color lightness, ranging from 0 (black) to 100 (white), a^* describes the green/red part of the color ($a^* < 0$ green, $a^* > 0$ red), b^* the yellow/blue part ($b^* > 0$ yellow, $b^* < 0$ blue) and h^* the tone (hue) of color. Table 2 displays the evolution of color parameters (L^* , a^* , b^* and h^*) of tannin enriched and sulfited wine samples, over the storage time. Some significant variations in lightness (L^*) were observed in control, tannin enriched and sulfited wine samples, over storage time, resulting in a slight decrease of lightness at the end of experiment in most cases (125 d). However, in tannin enriched

wine samples, lightness was not seriously affected until the fifth sampling date (100 d).

As regards the a^* values, the higher the value of a^* is, the more it tends to red. The addition of tannin did not seriously affect the a^* value of wine samples throughout the storage period, compared with the control. Nevertheless, after 25 d, a^* values decreased and were very slightly moved toward green area. Therefore, Tara tannin “Vitanil B” could confer protection from pinking phenomena, which is an undesirable result of white wines oxidative modifications (Cosme et al., 2019).

Unlike to changes of a^* , tannin addition led to increase of b^* (yellow/blue color component) values, more noticeable at the third level of tannin addition (300 mg/L-Wine sample W3) at 100 d of storage time. It was observed that tannin addition enhanced yellowness of wine samples in contrast to sulfur dioxide addition.

Finally, tannin addition caused a slight decrease in hue angle values (h^*) and movement to the yellow zone (90°), compared to control and sulfited wines. Interestingly and in accordance with the b^* values, this relocation to the yellow zone was more intense at the third level of tannin addition (300 mg/L-Wine sample W3) at 100 d of storage time.

Wine color deterioration, namely wine browning, is a major problem occurring in white wines during storage, affecting the shelf life of the product. This particular defect arises from the oxidation of phenolic compounds and the subsequent polymerization of the resulting products in order to generate colored compounds in the yellow-brown spectral region. Undesirable color changes in white wines may result after polymerization reactions between phenols and other compounds in the wine, such as acetaldehyde, or between phenols and oxidation products of tartaric acid (López-Toledano et al., 2006). More recently, Bührle

Table 2
Effect of tannin addition and storage time on the chromatic parameters (L^* , a^* , b^* and hue) of wine samples.

Wine samples	L^* (Lightness)					
	Storage period (d)					
	0	25	50	75	100	125
W	54,01±0,12 aA	53,71±0,05 aA	58,07±0,05bA	57,59±0,29 cA	57,24±0,11 cA	52,95±0,06 dA
WSO ₂	57,49±0,06 aB	58,87±0,14bB	55,34±0,21 cB	57,23±0,07adAC	55,35±0,40 cB	56,59±0,38dBC
W1	56,31±0,06 aC	56,99±0,03bCE	58,20±0,05 dA	57,38±0,08 cA	57,00±0,14bA	57,12±0,28bcC
W2	56,81±0,03aD	57,56±0,14bD	58,25±0,05 cA	54,76±0,03 dB	56,82±0,07 aA	56,49±0,34aBC
W3	57,24±0,03abE	57,36±0,36abD	57,60±0,10 aC	57,70±0,31 aA	57,16±0,10abA	56,97±0,02bC
W4	56,66±0,04acD	56,88±0,09abE	57,43±0,06bC	56,78±0,37 aC	56,08±0,30 cC	53,88±0,14dD
W5	57,05±0,2 aF	56,63±0,06 aE	57,67±0,15bC	56,15±0,06cD	56,69±0,22aAC	56,05±0,31 cB
a^* (redness/greenness)						
W	-0,13±0,02aAB	-0,60±0,00bA	-0,95±0,01 dA	-0,75±0,01cAB	-0,77±0,02 cA	-0,63±0,03bAC
WSO ₂	0,24±0,01 aC	-1,11±0,04bB	-0,65±0,02cdB	-0,71±0,01 dB	-0,61±0,02 cB	-0,70±0,02dABC
W1	0,03±0,00aD	-0,72±0,00cdC	-0,82±0,00eC	-0,81±0,02deAC	-0,57±0,04bB	-0,71±0,04cAB
W2	-0,08±0,02 aA	-0,91±0,03bDE	-0,86±0,02bD	-0,77±0,01cAB	-0,55±0,04 dB	-0,74±0,02 cB
W3	-0,23±0,01 aE	-0,95±0,02bE	-0,74±0,00 cE	-0,88±0,02 dC	-0,60±0,03eB	-0,70±0,0cABC
W4	-0,07±0,00 aA	-0,82±0,02bcF	-0,88±0,01bD	-0,73±0,01cAB	-0,46±0,04 cC	-0,62±0,03cC
W5	-0,20±0,02aBE	-0,85±0,01bDF	-0,85±0,01bCD	-0,56±0,02cD	-0,46±0,02 dC	-0,64±0,02eAC
b^* (yellowness/blueness)						
W	2,39±0,03 aA	3,56±0,00bA	5,97±0,03eA	5,03±0,04dAD	5,91±0,04eA	4,80±0,04 cA
WSO ₂	1,18±0,01 aB	5,14±0,03bB	3,73±0,07 cB	3,39±0,07 dB	3,76±0,07 cB	3,90±0,12 cB
W1	2,29±0,06 aC	4,20±0,02bC	5,32±0,03 cC	5,50±0,03 dC	5,37±0,03cdCD	5,30±0,07 cC
W2	2,27±0,03 aC	5,34±0,02bdD	5,69±0,02cD	5,24±0,02dD	5,54±0,02bcD	5,56±0,21bcCD
W3	3,07±0,04aD	5,94±0,02bcE	5,00±0,06 dE	6,01±0,10 cE	7,14±0,10eE	5,82±0,06bD
W4	2,45±0,02 aA	5,42±0,06bcD	5,66±0,03cdD	4,84±0,05eA	5,22±0,05cC	5,88±0,12dD
W5	3,05±0,03aD	5,20±0,04bB	5,93±0,05 cA	3,86±0,03 dF	4,64±0,03eF	5,26±0,13bC
h^* (hue angle)						
W	93,16±0,31aAD	99,54±0,09bAC	99,04±0,09bcA	98,45±0,11 cA	97,42±0,31 dA	97,29±0,33 dA
WSO ₂	78,45±0,50 aB	102,23±0,31 cB	100,24±0,45bB	101,85±0,31 cB	99,17±0,41bB	100,14±0,35bB
W1	89,19±1,38 aC	99,71±0,26 cC	98,70±0,15cdACD	98,34±0,15cdA	96,05±0,35bC	97,61±0,31bdA
W2	91,89±0,47 aA	99,65±0,30bAC	98,62±0,10cACD	98,32±0,14 cA	95,74±0,48dCE	97,54±0,18eA
W3	94,25±0,15aD	99,13±0,11bAD	98,41±0,13cCD	98,38±0,05 cA	94,79±0,23aD	96,93±0,38dAC
W4	91,65±0,14 aA	98,56±0,14bD	98,90±0,06bAD	98,60±0,42bA	95,03±0,28cDE	96,03±0,35 dC
W5	93,73±0,41aD	99,32±0,12bAC	98,16±0,02 cC	98,30±0,01 cA	95,70±0,23dCDE	96,98±0,46eAC

The results represent mean ± standard deviation (N=3). Different small letters after each value, in the same row, and different capital letters, in the same column, indicate statistically significant differences ($p<0.05$).

et al. (2017) studied the occurrence and importance of xanthylum derivatives, yellow to orange pigments formed by dimerization of flavanols, in white wine and they concluded that these compounds might play a role in color formation as intermediate products in polymerization and browning. Given the above concerns and in accordance with the results of the present study, Vignault et al. (2019) reported that enological tannins inhibit laccase activity and protect the color of white wines from browning.

Therefore, taking into consideration the results of photometric analyses as well as those of chromatic parameters, it can be assumed that the addition of tannin (300 mg/L) in Malagousia white wine enhances total phenolic content, ferric reducing/antioxidant power, radical scavenging activity and prevents color deterioration, for a storage period of 100 d.

3.3. Sensory analysis

The tannin enriched and sulfited Malagousia wine samples were evaluated for their sensory properties, at days 0, 25, 50, 75, 100, and 125, as explained in the Materials and Methods section. Taking into account the conclusions drawn by the aforementioned photometric and chromatic results, a characteristic radar plot listing 10 attributes and the panel scores obtained, after 100 d of storage, is presented in Fig. 2.

Color, taste intensity, aroma quality and body of wine samples were significantly affected after tannin addition, mostly at the upper levels of tannin addition. However, in the oxidation test results, the tannin enriched wine samples received lower scores than the control wine, nevertheless higher than the respective one of the sulfited wine, indicating the efficacy of tannin as antioxidant agent. Odor intensity was scored with 6 in sulfited wine, followed by the control wine and the tannin enriched wines. Therefore, the increased score of odor intensity in sulfited wine is possibly related to the presence of sulfur dioxide. Concerning the bitterness, a clear difference was observed in tannin enriched wines, compared to control and sulfited wine, whereas taste balance of tannin enriched wines was evaluated as comparable with the respective of control and sulfited wines.

Finally, the aroma quality, the body, the after taste and the overall acceptance of wine sample treated with 300 mg/L tannin, were highly appreciated and received the highest scores by the assessors (Fig. 2).

Moreover, a correlation analysis among photometric assays, color parameters and sensory attributes in Malagousia wine samples, after 100 d storage time, was listed in Table 3. Total phenolic content (TPC),

antiradical activity (ArA), and antioxidant power (AP) were very strongly positive correlated with chromatic parameter a^* and with bitterness; nevertheless, they were fairly strong negative correlated with hue. Lightness (L^*) was strongly positive correlated with chromatic parameter b^* and oxidation; while chromatic parameter a^* was strongly positive correlated with taste intensity and bitterness and b^* strongly related with aroma quality. Strong correlations were also observed between color intensity, either positive with bitterness, or negative with odor intensity. Aroma quality was strongly positive correlated with body and moderately with aftertaste and overall acceptance. Finally, overall acceptance was strongly positive correlated with aftertaste.

The oxidative degradation of white wines rapidly leads to a loss of their sensorial qualities, particularly the loss of characteristic floral and fruity aromas of young wines or the formation of new non typical aromas associated with the deterioration of wine and subsequently to undesirable chromatic changes (development of brown color) (Ferreira et al., 2002). Although it is well established that the addition of tannins contributes to the structure of wine and improves many sensory attributes, such as the mouth feel and the color stability of wine, they are not always perfectly integrated and wines may lose their equilibrium, resulting in a hardening of the wine and an increase in bitter sensations (Crespy, 2002).

Additionally, Li et al. (2020) have investigated the sensory qualities of tannin addition in red wines and concluded that the wine added hydrolysable tannin obtained high score in aroma and bouquet with balanced and well after taste; however, the wine had astringent flavor and bitterness which was consistent with the sensory properties of ellagitannins.

3.4. Principal Component Analysis (PCA)

Principal component analysis (PCA) was carried out in order to summarize the relative differences amongst wine samples, in relation to their photometric analyses, color and sensory analysis parameters. In PCA analysis, the first six PC explain 100% of the total variation in all parameters (Fig. 3). The first and third PCs (PC1 and PC3) accounted for 44.99 and 15.88% of the variation, respectively. The PC2 explained 24.33% of the variability, the PC4 10.16%, the PC5 2.71% and the PC6 1.93%. In the loading plot shown (Fig. 3a), odor intensity, oxidation, taste balance and hue were located on the right side, positively linked with PC1, whereas other color parameters (L^* , a^* , b^*), the rest sensory

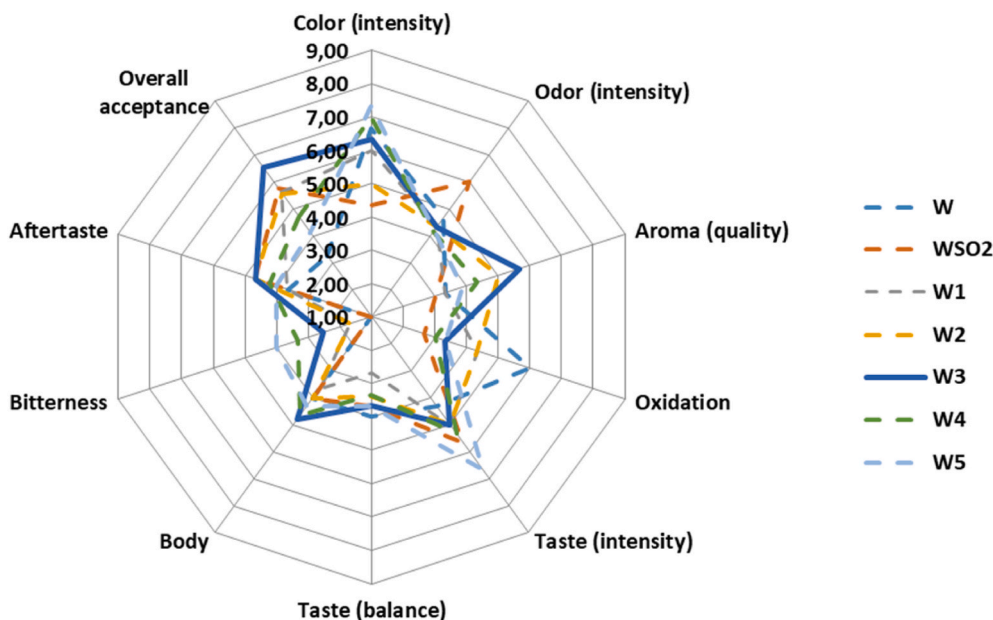


Fig. 2. Sensory scoring of tannin enriched and sulfited Malagousia wine samples, after 100 d of storage.

Table 3 Correlation analysis between photometric analyses, color parameters and sensory attributes in Malagousia wine samples, after 100 days storage time (Correlation is significant at the 0.05 level).

	Antiradical activity	Antioxidant power	L*	a*	b*	h*	Color (intensity)	Odor (intensity)	Aroma (quality)	Oxidation	Taste (intensity)	Taste (balance)	Body	Bitterness	Aftertaste	Overall acceptance
TPC	0,981	0,972	0,105	0,809	0,173	-0,773	0,570	-0,634	0,584	-0,517	0,631	-0,074	0,681	0,956	0,184	0,124
Antiradical activity	0,984	0,984	0,092	0,844	0,160	-0,815	0,607	-0,701	0,565	-0,471	0,566	-0,144	0,663	0,962	0,124	0,054
Antioxidant Power		0,202	0,202	0,792	0,305	-0,888	0,570	-0,754	0,685	-0,423	0,457	-0,179	0,677	0,911	0,175	0,157
L*				-0,318	0,795	-0,532	0,468	-0,693	0,379	0,702	-0,444	-0,031	-0,019	0,036	-0,430	-0,177
a*				-0,526	-0,286	-0,526	0,236	-0,388	0,270	-0,706	0,722	-0,430	0,357	0,801	0,218	0,228
b*				-0,638	0,344	-0,584	0,344	-0,584	0,716	0,382	-0,587	0,066	0,420	0,403	0,004	0,182
h*				0,922	-0,604	-0,760	-0,604	0,922	-0,760	0,078	-0,028	0,305	-0,568	-0,693	0,003	-0,158
Color (intensity)				-0,724	0,138	-0,724	0,138	-0,724	0,138	0,164	0,167	0,115	0,465	0,714	-0,576	-0,552
Odor (intensity)				-0,541	-0,237	-0,541	-0,237	-0,541	-0,237	0,045	0,045	0,298	-0,322	-0,616	0,328	0,163
Aroma (quality)				-0,178	-0,178	-0,178	-0,178	-0,178	-0,178	0,051	0,051	0,677	0,374	0,562	0,467	0,467
Oxidation				-0,679	-0,679	-0,679	-0,679	-0,679	-0,679	0,131	0,131	-0,476	-0,476	-0,602	-0,554	-0,554
Taste (intensity)				0,024	0,024	0,024	0,024	0,024	0,024	0,196	0,196	0,673	0,146	0,146	-0,004	-0,004
Taste (balance)				0,367	0,367	0,367	0,367	0,367	0,367	-0,041	-0,041	0,202	0,202	0,202	-0,413	-0,413
Body				0,648	0,648	0,648	0,648	0,648	0,648	0,412	0,412	0,412	0,412	0,412	0,153	0,153
Bitterness				-0,103	-0,103	-0,103	-0,103	-0,103	-0,103	-0,022	-0,022	-0,022	-0,022	-0,022	-0,103	-0,103
Aftertaste				0,683	0,683	0,683	0,683	0,683	0,683	0,683	0,683	0,683	0,683	0,683	0,683	0,683
Overall acceptance																

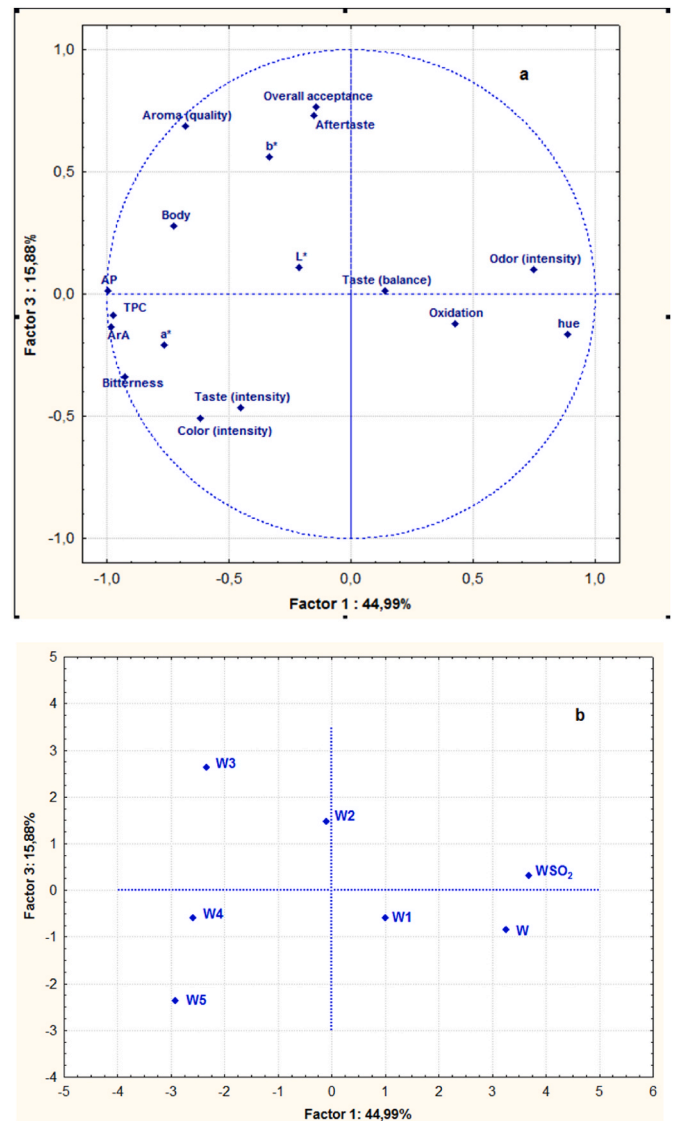


Fig. 3. (a) Projection of photometric analyses, color and sensory analysis parameters in the plane defined by the first and third principal components. (b) Projection of the variables of Malagousia wine samples studied in the plane defined by the first and third principal components (TPC: Total phenol content; AP: Antioxidant Power; ArA: Antiradical Activity). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

attributes and AP, TPC and ArA were projected at the negative values of PC1. Concerning the variables located far from the origin of PC1 and PC3 (Fig. 3a), odor intensity and hue were opposed to AP, TPC, ArA and a*, on the first factor axis and aroma quality was opposed to taste and color intensity on the third factor axis. The PCA of wine characteristics (Fig. 3a) showed a high correlation between overall acceptance and aftertaste, and among AP, TPC and ArA. In Fig. 3b, Malagousia wine samples were projected in the plane defined by the first and third principal components and were clearly differentiated from each other. Control wine, sulfited wine and the wine treated with 100 mg/L tannin were located on the right side of the figure and were characterized by odor intensity, oxidation and hue. Wines treated with the highest levels of tannin (W4 and W5) were located on the left side of the figure, where taste, color intensity, a* parameter and bitterness lay. Finally, the wine treated with 300 mg/L tannin (W3) was characterized with higher contributions of aroma quality, overall acceptance, aftertaste, body, b* parameter and L*.

4. Conclusions

The issue of reducing or supplementing the amount of SO₂ has always been considered as a challenge for wine industry. The intention of the present study was to apply an enological Tara tannin in Malagousia white wine, as a protective compound against oxidative damage, as well as to evaluate the wine quality, including antioxidant/antiradical activity and sensory characteristics. The results of the study demonstrated that the addition of tannin in Malagousia white wine influenced considerably total phenolic content, antioxidant power, radical scavenging activity and hindered color degradation, for a storage period of 100 d. The 300 mg/L tannin dosage was suggested as a better choice, because it combines high efficacy against oxidative degradation in the sulphite-free white wine coupled with better sensorial acceptance and color deterioration prevention, compared with the higher tannin dosages. Conclusively and based on the above findings, Principal Component Analysis pointed out the differences and enabled the discrimination among Malagousia wine samples, thus leading us to the hypothesis that the prospect of substituting sulfites with tannins is a promising alternative that might positively contribute to the production of wine with enhanced antioxidant and sensory profile.

CRedit authorship contribution statement

Irini F. Strati: Conceptualization, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing, Project administration. **Panagiotis Tataridis:** Resources, Data curation, Validation. **Adnan Shehadeh:** Investigation, Resources. **Arhontoula Chatzilazarou:** Investigation, Data curation, Validation. **Vasileios Bartzis:** Software, Formal analysis. **Anthimia Batrinou:** Investigation, Writing – review & editing. **Vassilia J. Sinanoglou:** Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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