Effect of Vegetation Time and Climatic Conditions on *trans*-Resveratrol Concentrations in Cabernet Sauvignon and Merlot Wines from Different Regions in Turkey

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In this study, 21 wine samples obtained from wine manufacturers in Turkey and produced from different grape varieties cultivated in different geographical regions, were analysed to determine their resveratrol concentrations. The HPLC method was used to analyse the trans-resveratrol concentrations of wines. Statistical analysis was done using IBM SPSS Statistics Version 20.0.0. Mean values were compared by using the Tukey test at a confidence level of p = 0.05. The resveratrol concentrations of Cabernet Sauvignon and Merlot grapes were also determined. Since these two varieties are grown widely in many regions of Turkey, they were studied in terms of the relationship of the resveratrol concentrations with parameters such as time of vegetation, total effective temperature, sunshine duration and rainfall. Positive correlations were observed between resveratrol levels and grape varieties, geographical regions and total effective temperature. The results of the statistical analysis showed that resveratrol concentrations also varied depending on vegetation time, sunshine duration and amount of rainfall in the vineyards. This study aimed to identify the relationships between (1) resveratrol concentrations of wines, (2) vegetation time of the grapes and (3) climatic conditions of the respective regions. Moreover, the effects of grape types on resveratrol concentrations were investigated. This study contributes to the body of knowledge by providing results on the utilisation of grapes that have high resveratrol levels for producing wines that have functional properties that are increasingly in demand by consumers.

INTRODUCTION

Wine contains high concentrations of polyphenolic compounds that are known to have protective properties against coronary heard diseases, cancer and inflammation. *In vitro* and *in vivo* investigations show that especially red wine has preventive effects against leukaemia, skin, lung and prostate cancers. Recent findings from *in vivo* investigations point out that *trans*-resveratrol is an effective agent during the initial, development and progressive phases of cancer (Bravo *et al.*, 2006; Anlı *et al.*, 2006; Stervbo *et al.*, 2007; Cottart *et al.*, 2010; Schaffer *et al.*, 2012; Singh *et al.*, 2015; Zielińska *et al.*, 2015).

Trans-resveratrol, a type of polyphenolic component of red wine, is produced in grape skins in reaction to biotic and abiotic stress in vineyards (Cui et al., 2002; İkizler et al., 2003; Olson et al., 2005; Cottart et al., 2010). Fungal infections are an example of biotic stress, whereas ultraviolet (UV) light exposure and climatic conditions are examples of abiotic stress. The effects of sunshine duration and UV light exposure on the resveratrol concentrations of red wines were investigated separately and were found to increase resveratrol concentrations to a considerable extent (Threlfall et al., 1999).

In many studies, several methods have been used to determine resveratrol concentrations. Several wines that were produced from different types of grapes and in different geographical locations have been analysed, and the results have been evaluated by researchers using comparable methods. The trans-resveratrol concentrations of 42 Hungarian wines from two different locations and producers were determined by Montsko et al. (2010). In another study, 12 red wines from commercial producers and three locations in Croatia were analysed to measure the polyphenolic concentrations, and then correlations between geographical conditions, such as the soil properties of the vineyard, sunlight exposure and temperature, and the resveratrol concentrations of the wines were investigated (Rastija et al., 2009). The trans-resveratrol concentrations of 186 Portuguese red wines from different grape types, origins and vintages were analysed (Paulo et al., 2011). Another study (Adıgüzel et al., 2010), on the relationship between geographical origin and climatic conditions of grapes, determined resveratrol concentrations. The highest resveratrol concentrations were found in red wine produced from the Öküzgözü black grape

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type from East Anatolia. The content of resveratrol and some related stilbenes in 21 Italian red grape varieties growing in the same edaphoclimatic conditions and at the same stage of ripeness were measured in another study. Some varieties, like Barbera, Schiava gentile, Corvina and Marzemino, showed a resveratrol content in the skin two to three times higher than the average concentration of all varieties. Considering the total concentrations of stilbenes, the highest content was found in Barbera, Franconia, Negroammaro, Corvina and Marzemino, whereas the other varieties, such as Montepulciano, Dolcetto, Croatina, Refosco and Primitivo, synthesised these phenolics in very low concentrations. Barbera grapes could be an interesting variety as a source of health-promoting molecules because relatively high concentrations of trans-piceatannol were analysed in this grape (Vincenzi et al., 2013).

The main aim of this research was to investigate the relationship between the resveratrol concentrations of red wines from different geographical and climatic conditions. It is known that resveratrol is a polyphenolic compound that has very important protective properties against coronary heart diseases, many cancer types, Alzheimer and inflammatory diseases. This polyphenolic compound is synthesised in the skin of grapes when the plant is under specific stress conditions, such as fungal infections, drought, insufficient sunlight exposure and insufficient temperature. In this study, the changes in resveratrol concentrations of wines with all these parameters were analysed using the HPLC method and the results were examined statistically. The reasons for the differences are presented and the correlations that influenced these differences are discussed.

MATERIALS AND METHODS

Materials

A total of 21 wines that had been produced from different grape varieties (Cabernet Sauvignon, Syrah, Merlot, Granache, Alicante, Cinsault, Öküzgözü, Boğazkere, Kalecik Karası and Çalkarası) cultivated in different geographical locations (Denizli, Çanakkale, Tekirdağ, Bozcaada, İzmir, Ankara, and Elazığ) were obtained from Turkish wineries. In this study, Cabernet Sauvignon (*Vitis vinifera L.*) and Merlot (*Vitis vinifera*) black grapes grown in Elazığ and Denizli were used.

Trans-resveratrol (Catalogue number: R5010) standardly used in calibration was purchased from Sigma (St. Louis, MO, USA). Methanol and acetonitrile solvents (HPLC grade) were purchased from Carlo Erba (Darmstadt, Germany).

Determination of resveratrol concentrations

For the quantitative analyses of the resveratrol concentrations of the wine samples, a Shimadzu (Tokyo, Japan) SCL-10 A VP HPLC instrument consisting of a UV-VIS detector (SPD-10A VP) was used. The system includes an LC-10 AT Liquid Chromatography (LC) pump (quaternary gradient) and degasser (DGU-14A) units. The column used was LiChrospher RP 5 μ m (250 X 4 mm I.D.). Wine samples (20 μ L) and calibration standards were injected directly into the column. For *trans*-resveratrol elution, distilled water (pump A) and acetonitrile solvent (pump B) were used as the

mobile phase in a ratio of 65:35 and at a flow rate of 1 mm/min. Wine samples were analysed with a UV-VIS detector at 289 nm wavelength (Denzer, 1991). Chromatographic peaks were determined according to retention times. The retention time of *trans*-resveratrol was 6.5 min for the analysis conditions. Triplicate measurements were performed for all wine samples. Stock solution was prepared by using 0.0005 g *trans*-resveratrol/10 mL methanol. Calibration solutions were prepared from this stock solution in the range of 0 to 10 mg/L. Resveratrol concentrations were obtained as milligrams per litre from a standard curve formula.

Resveratrol concentrations of the wine samples were compared with respect to their geographical origins, vegetation time (VT, in days), which refers to the ripeness of the grapes, total effective temperature (TET, in °C), which means the total temperature during the maturation process, sunshine duration (SD, in h), and rainfall (RF, in kg/m²).

Statistical analysis

Statistical analysis was done using the IBM SPSS Statistics Version 20.0.0 program. Mean values were compared by Tukey test at a confidence level of p = 0.05. The relationships between the parameters were determined by Pearson coefficient.

RESULTS AND DISCUSSION

In this study, a survey was conducted to determine the resveratrol values of red wines provided from different regions of Turkey. The resveratrol concentrations were obtained as a result of analysing different wines that were produced using the same vinification process in four different regions of Turkey: the Aegean, Central Anatolia, East Anatolia and Thrace. The different climatic conditions in the regions where the grapes were grown and the vegetation times of the vineyards were compared (Table 1).

Table 1 shows that the highest resveratrol concentration (2.59 mg/L) was obtained from the wines produced from Öküzgözü black grapes grown in Elazığ. This finding is in correlation with the study done by Adıgüzel *et al.* (2009), who determined the concentration of resveratrol in Turkish wines. They reported the highest resveratrol concentration as 6.47 mg/L from wine obtained from Öküzgözü black grapes that were grown in East Anatolia, whereas the range of resveratrol levels in the other wines varied between 0.15 and 4.04 mg/L. Similarly, in another study, the highest resveratrol concentration detected in wine was 4.403 mg/L, also obtained from Öküzgözü grapes, whereas the ranges for the other red wines were 0.176 to 4.403 mg/L (Gürbüz *et al.*, 2007).

The results show that high values of resveratrol concentration were found in wines produced from Öküzgözü and Kalecik Karası grapes. These cultivars are native varieties of Eastern Anatolia and Central Anatolia respectively. Moreover, when the climatic conditions of the regions where the grapes were grown were considered, the vegetation time of the Öküzgözü grape was 122 days, while it was 93 days for the Kalecik Karası. The total effective temperature was similar for both types of grapes, Cabernet Sauvignon and Merlot, while sunshine duration was slightly longer in the Elazığ region, but rainfall was much lower than in the Denizli

TABLE 1 Resveratrol concentrations according to regions and grape varieties.

					Total		
			Resveratrol	Vegetation	effective	Sunshine	Rainfall
Grape varieties	Region	Abbreviation	conc. (mg/L)	time (days)	temp. (°C)	duration (h)	(kg/m^2)
Cabernet Sauvignon	Denizli	CS-D	0.351	97	1 410	1 082	1 692
Öküzgözü	Denizli	O-D	1.49	113	1 579	1 232	1 904
Boğazkere	Denizli	B-D	0.441	126	1 681	1 355	2 075
Kalecik Karası	Denizli	KK-D	0.312	93	1 363	1 044	1 640
Syrah	Denizli	S-D	0.492	84	1 255	960	1 521
Merlot	Denizli	M-D	0.403	85	1 268	969	1 534
Çalkarası	Denizli	ÇK-D	2.28	144	1 765	1 524	2 313
Merlot	Çanakkale 1	M-Ç1	1.21	112	1 483	1 231	1 863
Merlot	Çanakkale 2	M-Ç2	1.52	103	1 376	1 151	1 670
Cabernet Sauvignon	Çanakkale 1	CS-Ç1	0.386	130	1 682	1 391	3 352
Merlot	Tekirdağ	M-T	0.477	103	1 370	942	2 850
Cabernet Sauvignon	Tekirdağ	CS-T	0.443	137	1 695	1 170	4 342
Grenache	Çanakkale 1	G-Ç1	0.569	141	1 784	1 489	3 967
Cabernet Sauvignon	Çanakkale 2	CS-Ç2	0.530	123	1 609	1 329	2 098
Cabernet Sauvignon	İzmir	CS-İ	0.319	103	1 624	1 201	866
Alcante	İzmir	A-İ	0.497	98	1 550	1 151	755
Cinsault	Tekirdağ	C-T	1.42	131	1 647	1 140	3 964
Syrah	İzmir	S-İ	2.08	129	2 199	1 459	1 471
Boğazkere	Elazığ	В-Е	0.601	122	1 875	1 366	1 068
Kalecik karası	Ankara	KK-A	0.371	144	1 533	1 387	3 192
Öküzgözü	Elazığ	O-E	2.59	122	1 875	1 366	1 068
Cabernet Sauvignon	Denizli	CS-D	0.351	97	1 410	1 082	1 692
Öküzgözü	Denizli	O-D	1.49	113	1 579	1 232	1 904
Boğazkere	Denizli	B-D	0.441	126	1 681	1 355	2 075
Kalecik Karası	Denizli	KK-D	0.312	93	1 363	1 044	1 640

region. The negative impact of rainfall on the resveratrol concentration of grapes was consistent with the higher secretion levels of resveratrol under abiotic stress conditions caused by rainfall. Moreover, excessive rainfall is known to increase fungal diseases that may occur in the vineyard. Jeandet et al. (1995) studied the relationship between fungal diseases, especially Botrytis cinerea infection, and the resveratrol concentrations of wine. According to their results, the main factor that determines the concentration of resveratrol in must and wine is the balance between the level of synthesized resveratrol of plants and degraded resveratrol concentration by pathogens. In the results of the abovementioned study, the resveratrol concentration did not increase significantly in the years in which severe Botrytis cinerea infection was seen (the resveratrol concentration was between 0.89 mg/L and 1.48 mg/L), while it was 2 mg/L to 2.49 mg/L when low or moderate fungal infections were observed. In another study, the detected resveratrol concentration of the red wines was between 0.96 and 3.93 mg/L, and the highest value, 3.93 mg/L, was found in the wine produced from black Shiraz grapes (Anlı et al., 2006). The resveratrol concentrations of Greek red wine were found to be between 0.38 and 2.14 mg/L (GerogiannakiChristopoulou et al., 2006)

In this study, significantly different resveratrol concentrations were found even in wines from similar geographical regions, so the interactions were explained by the differences in parameters such as grape variety, vegetation time, total effective temperature, sunshine duration and rainfall. Resveratrol concentrations varied between 0.312 and 2.59 mg/L and, when grape variety was considered, Öküzgözü had the highest values in both Elazığ and Denizli, followed by the Çalkarası, Shiraz and Cinsault varieties (Fig. 1). Among the varieties analysed in this survey, Kalecik Karası was common in Central Anatolia, Öküzgözü was common in East Anatolia, while Cabernet Sauvignon and Merlot were common in the Aegean region. The significantly higher resveratrol concentration, of 2.28 mg/L, in the wine produced from the Çalkarası black grape was thought to be due to its native variety in the Denizli region. Besides, the Kalecik Karası grape is native to Ankara while the Öküzgözü grape is native to Elazığ. Hence the resveratrol concentrations of the wines that are produce from these two varieties are also higher than the concentrations in the wines from the same varieties grown in both Denizli regions. A value of 0.298 mg/L was

obtained in the wine made from the Kalecik Karası variety grown in Denizli, which was lower than the concentration of resveratrol in the wine from the same variety grown in Ankara (0.372 mg/L). Similarly, the value of 1.49 mg/L in the wine from the Öküzgözü variety grown in Denizli was lower than the concentration of resveratrol in the wine from the same variety grown in Elazığ (2.59 mg/L) (p = 0.05). The resveratrol concentration of wine produced from a Boğazkere black grape, which is a native variety of East Anatolia, was 0.601 mg/L and 0.495 mg/L for the samples obtained from the Elazığ and Denizli regions respectively (Fig. 1). In this study, the resveratrol concentrations obtained from 21 wine samples were compared statistically using the Tukey method (Table 2).

The resveratrol concentrations of the Cabernet Sauvignon and Merlot grapes were concentrated in two statistical groups according to the Tukey analyses (group a and b), as shown in Table 2. Since these two varieties are grown widely in many regions of Turkey, Cabernet Sauvignon from five regions and Merlot from four regions were compared with respect to resveratrol concentrations.

In this study, meteorological data such as total effective temperature, sunshine duration and rainfall were obtained from the Meteorological Service, and the vegetation time was obtained from the producers; these are shown in Tables 5 and 6, respectively. The resveratrol concentrations of the wines detected by the HPLC method were evaluated separately for two varieties using Tukey's statistical method (Tables 3 and 4). Lastly, the effects of all these parameters on the resveratrol concentrations of wines are discussed.

According to the results, the resveratrol concentrations were found to be in the same group for Cabernet Sauvignon in the Denizli, İzmir and Çanakkale 1 regions; the Çanakkale 2 region was found to be in the other group, while the Tekirdağ region was between these two groups. Wines produced from the same kind of grapes varied in terms of sunshine duration, rainfall and total effective temperature when evaluated according to the different regions (Table 5 and Fig. 2).

Since the shortest vegetation time (97 days) was associated with the grapes grown in Denizli, the total effective temperature and sunshine duration were lower than expected. This is due to the fact that these two parameters are important for the ripeness of grapes, and thus resveratrol synthesis is dependent on the vegetation time of vineyards. Accordingly, limited time for growth decreases the effect of

these parameters on the synthesis of resveratrol in grapes compared to the ones that have had sufficient time to reach ripeness (Jeandet et al., 1995). It was noteworthy that the resveratrol concentration of wines produced from Cabernet Sauvignon grapes, which have the longest vegetation time in Tekirdağ (Table 5), was 0.44 mg/L. In this region, the length of the vegetation time also resulted in the highest total effective temperature. Moreover, the higher concentration of resveratrol obtained in the same area, despite the lower sunshine duration (1 170 hours) than in the Çanakkale 1 region, suggests that the total effective temperature is a more important parameter than sunshine duration. Referring to Table 5, the lowest resveratrol concentration observed in grapes grown in the area with the least amount of rainfall, namely İzmir (866 kg/m²), contradicted the knowledge about abiotic stress. Drought is an abiotic stressor for grapes, so this stress might be a reason for the low resveratrol concentration. Whereas low rainfall increases the resveratrol concentration due to drought stress on the plant, high rainfall activates the formation of pathogens on the plant. Jeandet et al. (1995) found that the factor that affects the resveratrol concentration in wines was the balance with the concentration of resveratrol synthesised in grapes and the degradation of resveratrol by pathogens such as *Botrytis cinerea*. Thus, high rainfall might increase fungal diseases, especially Botrytis cinerea infection, and resveratrol concentration will decrease due to degradation by these pathogens. However, it should be emphasised that data about the cultural processes applied to the vineyard should be obtained in order to represent the reasons for correlation with the resveratrol concentrations and parameters of different areas.

When another variety of grape, Merlot, was examined in terms of different vegetation time and meteorological conditions, it was found that there were no statistical differences between the resveratrol concentrations of the wines (0.403 and 0.477 mg/L) produced from Merlot grapes from the Denizli and Tekirdağ regions (Table 4 and Fig. 3). However, the resveratrol concentrations of the wines produced from Merlot grapes from the Çanakkale 1 and Çanakkale 2 regions were found to be statistically different, with values of 1.21 and 1.52 mg/L respectively (Table 4). The results in relation to the resveratrol concentrations of wines showed that the grapes grown in the Denizli region, which has the shortest vegetation time and sunshine duration, had the lowest resveratrol concentration (0.4 mg/L) (Table 6).

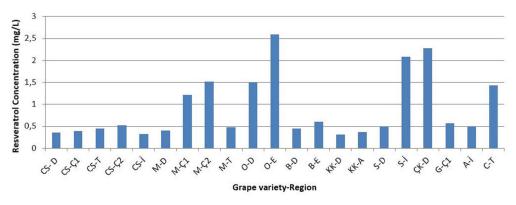


FIGURE 1
Resveratrol concentrations of wines produced from different grapes grown in different regions

TABLE 2 Statistical evaluation of the wines according to grape varieties and regions.

Grape	-	Resveratrol	Grape	-	Resveratrol
varieties	Region	conc. (mg/L)	varieties	Region	conc. (mg/L)
Cabernet Sauvignon	Denizli	0.3513 a	Cabernet Sauvignon	Tekirdağ	0.4437 a
Öküzgözü	Denizli	1.4933 b	Grenache	Çanakkale 1	0.5687 a
Boğazkere	Denizli	0.4950 a	Cabernet Sauvignon	Çanakkale 2	0.53 a
Kalecik Karası	Denizli	0.2980 a	Cabernet Sauvignon	İzmir	0.3197 a
Syrah	Denizli	0.45 a	Alicante	İzmir	0.4967 a
Merlot	Denizli	0.4033 a	Cinsault	Tekirdağ	1.4233 b
Çalkarası	Denizli	2.2783 cd	Syrah	İzmir	2.0830 c
Merlot	Çanakkale 1	1.2137 b	Boğazkere	Elazığ	0.6013 a
Merlot	Çanakkale 2	1.5157 b	Kalecik karası	Ankara	0.3717 a
Cabernet Sauvignon	Çanakkale 1	0.3857 a	Öküzgözü	Elazığ	2.5943 d
			Merlot	Tekirdağ	0.4773 a

TABLE 3 Statistical evaluation of the resveratrol concentration of Cabernet Sauvignon according to regions.

Region	Resveratrol concentration (mg/L)		
	0.378		
Denizli	0.420	0.3511 a	
	0.256		
	0.410		
Çanakkale 1	0.405	0.3859 a	
	0.342		
	0.386		
Tekirdağ	0.511	0.4434 ab	
	0.434		
	0.493		
Çanakkale 2	0.537	0.5300 b	
	0.560		
	0.305		
İzmir	0.312	0.3197 a	
	0.342		

Tukey HSD, subset for alpha = 0.05

TABLE 4 The statistical evaluation of the resveratrol concentration of Merlot according to regions.

Region	Resveratrol concentration (mg/L)		
	0.521		
Denizli	0.340	0.4031 a	
	0.349	0.4031 a	
	1.197		
Çanakkale 1	1.234	1.2140 b	
	1.210	1.2140 0	
	1.544		
Çanakkale 2	1.581	1.5159 c	
	1.422	1.3139 C	
	0.529		
Tekirdağ	0.469	0.4771 a	
	0.434	0.47/1 a	

Tukey HSD, subset for alpha (p = 0.05)

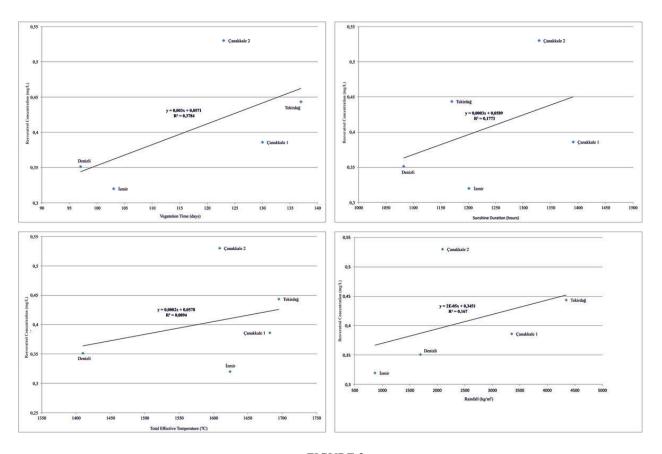


FIGURE 2
Resveratrol concentrations of wines produced from Cabernet Sauvignon according to vegetation time and climatic conditions.

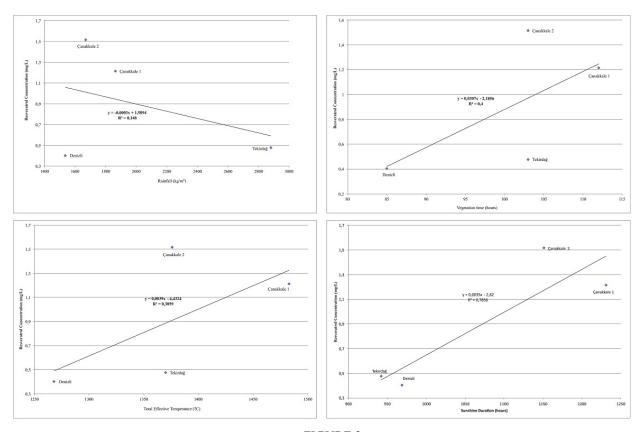


FIGURE 3 Resveratrol concentrations of wines made from Merlot in terms of vegetation time and climatic conditions.

TABLE 5
Resveratrol concentrations of the Cabernet Sauvignon according to different parameters.

	Resveratrol		Total effective		
	concentration	Vegetation time	temperature	Sunshine duration	Rainfall
Region	(mg/L)	(VT; days)	(TET; °C)	(SD; h)	$(R; kg/m^2)$
Denizli	0.351	97	1 410	1 082	1 692
Çanakkale 1	0.386	130	1 682	1 391	3 352
Tekirdağ	0.443	137	1 695	1 170	4 342
Çanakkale 2	0.530	123	1 609	1 329	2 098
İzmir	0.3197	103	1 624	1 201	866

TABLE 6
Resveratrol concentrations of Merlot according to different parameters

	Resveratrol		Total effective		
	concentration	Vegetation time	temperature	Sunshine duration	Rainfall
Region	(mg/L)	(VT; days)	(TET; °C)	(SD; h)	$(R; kg/m^2)$
Denizli	0.403	85	1 268	969	1 534
Çanakkale 1	1.21	112	1 483	1 231	1 863
Çanakkale 2	1.52	103	1 376	1 151	1 670
Tekirdağ	0.477	103	1 370	942	2 880

The vegetation time of Merlot grapes that were grown in Denizli was significantly short compared to all the others (85 days). Thus, the total effective temperature and sunshine duration were affected negatively (Table 6). In the Çanakkale 1 and 2 regions, the values of all three parameters (VT, TET and SD) were similar, thus high concentrations of resveratrol could be obtained from Merlot grapes that were grown in these regions, with values of 1.21 and 1.52 mg/L respectively. The reason for the higher resveratrol concentration in Çanakkale 2 (1.52 mg/L) can be explained by the rainfall parameter. In this region, the rainfall was 1 670 kg/m², whereas it was 1 863 kg/m² in Çanakkale 1 (Table 6). Water stress is an abiotic stress for grapes in ripeness, therefore resveratrol synthesis is expected to be higher than in drought conditions (Adıgüzel *et al.*, 2010).

The resveratrol concentrations also showed differences in relation to grape varieties. The results from the wines obtained from Merlot that were grown in Çanakkale 1 and 2 regions were significantly higher than the other concentrations from the same variety (Table 4) and the concentrations obtained from Cabernet Sauvignon wines (Table 3), with 1.21 and 1.52 mg/L respectively. It is known that an important ampelographic characteristic of Merlot grapes is the sensitivity to winter frost, and these regions are expected to experience more frost than all the other regions that were examined in this study (Çelik, 2006).

CONCLUSIONS

The results show that the differences in the resveratrol concentrations in wines obtained from different regions depend on grape variety, climatic conditions of the region, and vegetation time. In this study, resveratrol concentration in wines made from different grapes grown in the Aegean,

Marmara, Central Anatolia, Thrace and East Anatolia regions were determined, and the relationship of these values to the vegetation time applied to vineyards and climatic conditions was presented. The highest resveratrol concentration was found in wine produced from the Öküzgözü grape, unique to the East Anatolian region. The effects of different geographical conditions and vegetation time on resveratrol concentration were studied for the Cabernet Sauvignon and Merlot varieties, since they are grown more widely in Turkey, and statistically a greater correlation was found between these two varieties. The results show that the resveratrol concentration in Merlot wines was higher than that in Cabernet Sauvignon, without considering any other parameters. It was observed that there was a direct correlation between the resveratrol concentrations and the parameters of vegetation time, total effective temperature and sunshine duration, while the relationship between the resveratrol concentration and rainfall was found to be indirect. In this study, vegetation time and climatic conditions, which effect the resveratrol concentrations of grapes, were analysed and discussed. Future studies could focus on investigating the correlations between the effective parameters of resveratrol concentration and cultural processes such as pesticide programme, agricultural irrigation and fertilisation.

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