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Effects of Vinification Techniques Combined with UV-C Irradiation on Phenolic Contents of Red Wines

Hande Tahmaz and Gökhan Söylemezoğlu

Abstract: Red wines are typically high in phenolic and antioxidant capacity and both of which can be increased by vinification techniques. This study employed 3 vinification techniques to assess the increase in phenolic compounds and antioxidant capacity. Wines were obtained from Boğazkere grape cultivar by techniques of classical maceration, cold maceration combined with ultraviolet light (UV) irradiation, and thermovinification combined with UV irradiation and changes in phenolic contents were examined. Total phenolic and anthocyanin contents and trolox equivalent antioxidant capacity of wines were measured spectrophotometrically and phenolic contents (+)-catechin, (−)-epicatechin, rutin, quercetin, *trans*-resveratrol, and *cis*-resveratrol were measured by High Pressure Liquid Chromatography with Diode Array Detection (HPLC-DAD). As a result of the study, the highest phenolic content except for quercetin was measured in the wines obtained by thermovinification combined with UV irradiation. We demonstrated that the highest phenolic compounds with health effect, total phenolic compounds, total anthocyanin, and antioxidant activity were obtained from thermovinification with UV-C treatment than classical wine making.

Keywords: antioxidant capacity, red wine, *trans*-resveratrol, UV light, vinification

Introduction

Phenolic compounds found in grapes and wines are considered as the most important compounds in enology due to their organoleptic properties and antioxidative effects. Among these compounds, *trans*-resveratrol (3,5,4′-trihydroxy-trans-stilbene) is one of the most studied because it is believed to be the main responsible for red wine benefits on human health (Pendurthi and others 1999; Bath and others 2001). *trans*-resveratrol, a phytoalexin that belongs to the group of compounds known as stilbenes, is known to occur in grapes and consequently in grape products and wine. It is abundant in grape skin and present in higher concentration in red grape varieties compared with white varieties (Fremont and others 1999). Many significant *in vitro* and *in vivo* studies have been conducted in recent years on activation of SIRT 1-the enzyme which controls aging—by resveratrol (Kaeberlein and others 2005). The studies confirmed that antioxidant phenolic compounds demonstrate cardioprotective (Das and Maulik 2006), vasorelaxative (Pendurthi and others 1999), anti-inflammatory (Wang and others 2002), reactive oxygen scavenging (Şener and others 2006), and anticancer activities (Han and others 2010). The phenolic content in grapes and wines may significantly vary according to disease factors (Romero-Pérez and others 2001), exposure to UV (Langcake and Pryce 1977) and chemical treatments (Artés-Hernandez and others 2003), effects of cultivar and rootstock (Göktürk Baydar 2006), tissue differences (Poudel and others 2008), effects of climate (Melzoch and others 2001), effects of cultivation by pruning (Prajitna and others 2007), and vinification techniques (Pezet and Cuenat 1996).

Phenolic compounds in red wines are responsible for color, bitterness, astringency, and aging behavior of wine. Wine-making techniques have an influence on phenolic content of completely fermented red wines, which determines wine quality. Anthocyanins which are responsible for color of red wines are extracted from the grape skin during maceration. Other phenolic compounds in the grape skin and seeds pass into the wine during maceration which is the 1st stage of wine-making. The quantity of phenolic compounds which dissolve and pass into the wine during maceration varies according to ambient temperature, duration of maceration, and quantity of alcohol produced (Netzel and others 2003). The use of pectolytic enzymes and the intensity of maceration process (including the pumping over) are also factors that could contribute for final red wine phenolic content.

Thermovinification is a frequently used technique to increase phenolic and volatile compound contents in red wines. When temperatures are rapidly increased to 60 to 85 °C, cell walls in the grape skin are damaged and higher amounts of phenolic compounds pass into the must (Atanacković and others 2012).

Low-temperature maceration is another vinification technique that increases the amount of phenolic compounds in must. Cold maceration is the initial phase of fermentation conducted at 4 to 15 °C to reduce the risk posed by spoilage organisms and to limit the activities of enzymes that affect wine color and flavor (Kelebek and others 2010).

Synthesis of *trans*-resveratrol in grapes is facilitated by the enzyme stilbene synthase (STS) and the 2 substrates p-coumaroyl-CoA and malonyl-CoA (Fritzemeier and Kindl 1981). The same substrates also play a role in the enzyme chalcon synthase (CHS), which is involved in flavonoid synthesis (Melchior and Kindl 1990). UV irradiation enhances resveratrol synthesis in grapes. This is explained by the competition of both CHS and STS enzymes for the same substrates and the effect of UV irradiation in facilitating STS induction (Versari and others 2001).

There are not any studies in the literature that investigate the effect of different vinification techniques combined with UV-C irradiation on phenolic content of wines. The purpose of this study is to identify the vinification method for yield of wine with the highest phenolic content after harvest of the grapes. This is the

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1st study in the literature which is using a combination of these treatments.

Materials and Methods

Chemicals

Methanol, ethyl acetate, acetonitrile, potassium persulfate, sodium chloride, sodium phosphate monobasic and dibasic, (R)-(+)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Folin Ciocalteus phenol reagent, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, and phenolic standarts were obtained from Sigma-Aldrich (St. Louis, Miss., U.S.A.). Hydrochloric acid, formic acid, sodium carbonate, sodium chloride, sodium acetate, ethyl acetate, acetonitrile, and potassium chloride were obtained from Merck (Darmstadt, Germany).

Plant material

Boğazkere (*Vitis vinifera* L.) grape cultivar was used in the study which is one of the most widely used and best quality red wine grape cultivars in Turkey. One hundred thirty-five healthy vine stocks with good growth potential were randomly chosen from the vineyard of BAK Viticulture and Winery Co. located in Kalecik District of Ankara Province (40° 05′ 50.03″ N, 33° 27′ 00.78″ E, 688 m). The vine stocks were planted in 2005 and grafted onto 41 B rootstock. Guyot cultivation was used and row spacing and intrarow spacing was 2.50 × 1.25 m. Vine stocks had a stem height of 60 cm. The parcel was irrigated with drip irrigation and fertilized. The grapes were harvested on October 21, 2011 manually when they reached technological maturity, and the grapes showed following technological ripening indices: sugar content 24.3° Brix, pH 3.47, and total acidity 5.47 mg/g of tartaric acid equivalents.

Vinification

About 800 kg of grapes harvested from 135 vine stocks was divided into 3 groups and treatments were replicated 3 times.

Classical fermentation (group 1). The grapes were harvested at 1.094 density and processed into wine on the same day by maceration technique. During destemming and crushing, grapes were sulphurated with 60 g/ton granulated potassium metabilsulfite (Laffort, Bordeaux-France) and taken to a microvinification tank with a 250 L cooling jacket. Alcohol fermentation (20 to 24 °C) was started on the 1st d of maceration by addition of 200 g/ton yeast (Laffort FX10, Bordeaux-France) and 300 g/ton yeast nutrient (Laffort Dynastart, Bordeaux-France). After the completion of alcohol fermentation, grapes were pressed and malolactic fermentation was started by the addition of 250 g/250 hL bacteria (Laffort 450 PreAc, Bordeaux-France) and 1250 g/250 hL bacteria nutrient (Laffort Energizer PreAc, Bordeaux-France) at an ambient temperature of 20 to 24 °C. Fermented wines were aerated, taken to demijohns, sulphurated with 60 g/ton granulated potassium metabilsulfite, and bottled after a 5-mounth maturation period.

Cold maceration (group 2). The grapes were kept at cold storage room at 1 °C temperature and 95% relative humidity for 72 h and then processed into wine. Different from classical maceration, the grapes were exposed to cold maceration at 13 °C in the 1st 3 d of maceration. As done in the thermovinification, the grape pomaces in the tank were exposed to 254 nm UV-C irradiation at 20 cm height for 1 h. Four lamps each with 36 W lamp power were used during UV-C treatment (Philips TUV PL-L Somerset, NJ).

Thermovinification (group 3). As in cold maceration group, the grapes were kept at cold storage room at 1 °C temperature and 95% relative humidity for 72 h and then processed into wine. Different from classical maceration, on the 1st d of maceration, the grapes were 1st exposed to a rapidly increased temperature of 80 °C which was then decreased to 24 °C (Atanacković and others 2012) and were taken to a microvinification tank with a cooling jacket. On the 5th d of maceration, the grape pomaces in the tank were exposed to 254 nm UV-C irradiation at 20 cm height for 1 h. Four lamps each with 36 W lamp power were used during UV-C treatment (Philips TUV PL-L).

Phenolic compound analysis

The wines obtained with classical maceration, thermovinification, and cold maceration were filtered through a 0.45 μm pore size PVDF membrane and their total phenolic and anthocyanin contents and trolox equivalent antioxidant capacity were quantified with Analytik Jena Specord 200 (Analytik Jena, Germany) model spectrophotometer.

Total phenolic analysis. Total phenolic content analyses were done according to Singletton and Rossi (1965). The measurement results were given in terms of mg/L gallic acid. For this purpose, gallic acid curves were produced from concentrations of 1200, 1100, 1000, 900, 800, 700, and 600 mg/L ($R^2 = 0.9948$) gallic acid. The measurements were executed at 765 nm.

Total anthocyanin analysis. pH differential method of Giusti and Wrolstad (2001) was used in total anthocyanin analyses. Total anthocyanin contents were calculated in terms of malvidin-3-glucoside, the major anthocyanin grapes. The readings were executed at 520 and 700 nm in macro cuvettes, the results were calculated using the formula below and expressed in terms of mg/L.

$$\text{Total anthocyanin content (mg/L)} = [(A) \times (MW) \times (DF) \times 1000]/[(\varepsilon) \times (L)]$$

A: Difference of sample absorbance between pH 1.0 and 4.5
MW: Molecular weight
DF: Dilution factor
ε: Molar absorption coefficient
L: Pathlength (cm)

Antioxidant capacity analysis

Antioxidant capacities of wines were evaluated using the trolox equivalent antioxidant capacity assay based on the method of Re and others (1999). First, ABTS (2.2′-azinobis-(3-etilenbenzotiazolin-6-sulfonik asit) diammonium salt) ≥98-Sigma A1888 solution was prepared by using 2.45 mM potassium persulfate. The radical solution was allowed to stand in the dark for 12 to 16 h at room temperature and was used within 2 d of preparation. During analysis, the solution was kept in +4 °C. 0.1 M and phosphate buffered saline (PBS) buffer (pH 7.4) was prepared to dilute ABTS and extracts. Readings were executed in PBS solution and before each reading, the ABTS radical solution was diluted with PBS to an absorbance of 0.700 (±0.010) at 734 nm. The analyses were conducted with 10, 20, and 30 μL samples to obtain 3 different inhibition rates at the end of 6 min. The standard curve ($R^2 = 0.9996$) obtained with the trolox standard (R–(+)-6-Hydroxy-2,5,7,8-tetramethylchroman-2carboxylic acid %98-Aldrich 391913) in 5, 10, 15, and 20 μM concentrations was used for calculations and the results were expressed in the terms of μmol trolox/mL.

Table 1–Calibration parameters used for the HPLC-DAD determination of phenolic compounds.

Phenolic compounds	RT (Min)	Λ (nm)	Calibration curve	R^2	LOD (mg/kg)	LOQ (mg/kg)	Recovery (%)
(+)- catechin	28.6	280	$y = 15323x–160.89$	0.9997	0.96	2.91	89.76
(–)- epicatechin	33.7	280	$y = 33977x–7173$	0.9999	0.69	2.09	88.81
Quercetin	55.6	365	$y = 20153x–44559$	0.9999	0.46	1.53	94.60
Rutin	43.2	365	$y = 74629x–24943$	0.9999	0.45	1.37	88.92
trans-resveratrol	54.9	306	$y = 403404x–78716$	0.9998	0.28	0.86	89.72
cis-resveratrol	55.2	280	$y = 132264x+22.462$	0.9981	0.01	0.03	90.12

RT, retention time; λ, detection wavelength; R^2, correlation coefficients; LOD, limit of detection; LOQ, limit of quantification.

Table 2–General wine analysis.

Analysis	Classical maceration	Cold maceration	Thermovinification
Total acidity*	5.157	5.337	5.838
pH	3.70	3.62	3.53
Residual sugar (g/L)	1.2	1.1	1.3
Alcohol (% h/h)	12.77	12.80	13.38
Free SO_2 (mg/L)	27.33	28.67	27.67
Total SO_2 (mg/L)	42.43	43.04	42.97
Volatile acid (g/L)**	0.25	0.32	0.21

*In terms of tartaric acid.
**In terms of sulphuric acid.

Phenolic compound extraction. The wine samples were applied to C18 cartridges (Waters) in a vacuum manifold for purification before the HPLC analyses. The cartridges were 1st activated with 5 mL ethyl acetate and then with 5 mL methanol/HCl (99.99/0.01: v/v) and 2 mL water/HCl (99.99/0.01: v/v) prior to loading of the extracts. Then, 1 mL sample was loaded to the cartridge and the cartridge was washed with 5 mL ethyl acetate. Ethyl acetate was evaporated from the sample under nitrogen at 40 °C and the residue was dissolved in 2 mL distilled water (containing 0.01% HCl, v/v) in an ultrasonic bath. The extracts were then filtered through a 0.45 μm pore size PVDF membrane and transferred to amber vials.

HPLC-DAD analysis of phenolic compounds. The amount of (+)-catechin,(–)-epicatechin, quercetin, rutin, *trans*-resveratrol, and *cis*-resveratrol in wines was determined with an Shimadzu LC 10 AT VP system (Shimadzu Corp., Kyoto, Japan). The HPLC equipment was used with diode array detector (DAD). System consisted of a binary pump, degasser, and auto sampler. The column used was a Gemini Phenomenex C18 (Calif., U.S.A.): 4.6 mm × 260 mm. The mobile phase consisted of 2 solvents: solvent A, water/ formic acid (99/1: v/v) and solvent B, acetonitrile (100/100: h/h). Triplicate analyses were performed for each sample (Waterhouse 2005). The identification of phenolic compounds was obtained out by using authentic standards and by comparing the retention times and their visible spectra, while quantification was performed by external calibration with standards. No standard is available for *cis*-resveratrol. To identify the peaks for the *cis* form in the samples, standard of *trans*-resveratrol was exposed to UV-C light (254 nm). The limit of detection (LOD) was calculated with the equation $LOD = 3\,\sigma/S$ and the limit of quantification (LOQ) was calculated with the equation $LOQ = 10\,\sigma/S$. σ is the standard deviation of the y-intercepts of the calibration curves, and S is the average of the slopes of the concentration curves. Calibration parameters are given in Table 1.

Sensory analysis. Sensory analyses for determination of wine organoleptic profiles were performed according to Jackson (2002). Ten expert wine tasters evaluated the wines with respect to harmony, astringency, astringency aftertaste, bitterness, sourness, sweetness, body, flavor, and color. Panelists evaluated each descriptor on horizontal unstructured scale of 10 cm. The marks given for each descriptor by all the panelists were summed. The results were interpreted after variance analysis.

Statistical analysis. Statistical analyses of the data were done using SPSS (SPSS Inc.,Chicago, Illinois) statistical program version 11.5. General linear model procedures were used to determine treatment effects, and Duncan's multiple range tests were used to compare means. All analyses were performed in triplicate.

Results and Discussion

General wine analyses are shown in Table 2. The phenolic content of control (classical maceration) wines is compared with those of CM+UV (cold maceration combined with UV-C irradiation) and T+UV (thermovinification combined with UV-C irradiation) treated wines in Table 3.

Total phenolic content

Total phenolic content of control wines was measured as 2420 mg/L. As seen in Table 3, this value increased to 2856 mg/L with CM+UV treatment and to 8334 mg/L with T+UV treatment ($P < 0.05$). A 3.5-fold increase was recorded in total phenolic content of T+UV wines compared to that of control. Total phenolic content of Boğazkere wines was reported as 2213 μg/mL (Bayram and others 2014). Atanacković and others (2012) reported that total phenolic content in control was increased from 1040 to 1071 mg/L GAE in Merlot wines treated with 80 °C thermovinification and total phenolic content in control was increased from 912 to 1099 mg/L GAE in Cabernet Sauvignon wines treated with 80 °C thermovinification. Kelebek and others (2010) measured higher total phenolic content in cold maceration Öküzgözü wines compared to that of control wines. In our study, CM+UV and T+UV treatments induced higher level of increase in phenolic contents compared to that reported in the literature. This is attributed to the additional effect of UV irradiation combined with the treatments.

Total anthocyanin

Total anthocyanin content was measured as 109.1 mg/L in classical maceration wines (Table 3). CM+UV and T+UV treatments resulted in the same levels of increase statistically ($P < 0.05$). In their study on Öküzgözü wines, Kelebek and others (2010) measured total anthocyanin content as 243.62 mg/L in classical maceration wines and as 284.15 mg/L in cold maceration wines. Similar quantities were also measured in Syrah (Gomez-Miguez and others 2007), Tempranillo (Álvarezet and others 2009), and Cabernet Sauvignon (Gil-Munoz and others 2009) wines obtained by cold maceration. Increased total anthocyanin content was reported in these 3 types of wines in another study investigating the effects of 65 °C thermovinification (Netzel and others 2003).

Table 3–Phenolic content of the wines (mean ± SE) for treatment.

	C	CM+UV	T+UV
Total phenolic (mg/L GAE)	2420 ± 18c	2856 ± 78b	8334 ± 103a
Total anthocyanin (mg/L)	109.1 ± 0.18b	149.7 ± 0.62a	147.2 ± 0.85a
Antioxidant capacity (μmol trolox/mL)	9.65 ± 0.803c	10.33 ± 0.893b	18.13 ± 0.423a
Catechin (mg/L)	6.12 ± 0.066c	9.72 ± 0.175b	14.46 ± 1.060a
Epicatechin (mg/L)	0.98 ± 0.030c	1.39 ± 0.024b	2.84 ± 0.136a
Rutin (mg/L)	0.86 ± 0.003b	0.85 ± 0.008b	1.35 ± 0.003a
Quercetin (mg/L)	0.50 ± 0.002b	0.52 ± 0.003a	0.50 ± 0.003b
trans-resveratrol (mg/L)	0.48 ± 0.004b	0.49 ± 0.004b	0.58 ± 0.007a
cis- resveratrol (mg/L)	0.29 ± 0.002b	0.30 ± 0.0104b	0.34 ± 0.000a

Different superscripts in the same line indicate statistical differences at the $P < 0.05$ level.
C, classical maceration (control); CM+UV, cold maceration with UV-C treatment; T+UV, thermovinification with UV-C treatment.

Antioxidant capacity

As seen in Table 3, 9.65 μmol trolox/mL antioxidant capacity in control was increased to 10.33 μmol trolox/mL in CM+UV treatment group and to 18.13 μmol trolox/mL in T+UV treatment group ($P < 0.05$). Kondrashov and others (2009) stated that trolox equivalent antioxidant capacity in red wines ranged between 7.7 and 16.6 μmol trolox/mL. Atanacković and others (2012) reported that 80 °C thermovinification treatment both increased and decreased antioxidant capacity depending on grape cultivar. As increased total phenolic content induced by cold maceration may also result in increased antioxidant capacity, our findings are in agreement with the literature.

Phenolic compounds

As seen from Table 3, T+UV treated group had higher content of all phenolic compounds except for quercetin compared to that of control ($P < 0.05$). Only quercetin content was higher in CM+UV treatment group compared to those of control and T+UV group ($P < 0.05$). In their study on Boğazkere wines, Özkan and Baydar (2006) measured (+)-catechin content as 17.82 mg/L, (–)-epicatechin content as 5.62 mg/L, rutin content as 4.67 mg/L, and quercetin content as 2.37 mg/L by using direct injection HPLC method. Adıgüzel Çaylak and others (2009) measured *trans*-resveratrol contents of Boğazkere wines between 0.516 and 1.51 mg/L with direct injection HPLC method. In a different study on Boğazkere wines, (+)-catechin content was reported as 14.357 mg/L, (–)-epicatechin content as 0.873 mg/L, and resveratrol content as 1.495 mg/L (Gürbüz and others 2007). In a study on Spanish wines, *trans*-resveratrol content ranged between 8 and 0.60 mg/L and *cis*-resveratrol content ranged between 2.13 and 0.11 mg/L. In their study by using cold maceration and thermovification (Lamuela-Raventos and others 1995), Clare and others (2004) measured *trans*-resveratrol as 1.55 mg/L in control and as 2.40 mg/L in cold maceration wines. In total, 0.52 mg/L *cis*-resveratrol content in control was increased to 0.72 mg/L in cold maceration wines and no *cis*-resveratrol content was identified in thermovinification wines.

Some researchers examined phenolic contents by directly injecting the samples on HPLC without applying any preliminary procedures. However, separation of compounds is highly difficult in such complex chromatograms obtained this way and error rate is high in description of chromatograms (Proestos and others 2005). In our study, purification procedure was applied per the method for obtaining explicit chromatograms of phenolic contents. Although this might have resulted lower values compared to those reported by the literature, phenolic compounds were clearly identified.

This study explores which treatment has the most enhancing effect on phenolic content of wines. Figure 1 shows the rate of increase (%) in measured contents induced by CM+UV and T+UV treatments compared to those of control (classical maceration). The highest rate of increase (244%) in total phenolic content was recorded in T+UV treated wines compared to control wines. The 2nd highest rate of increase was also obtained by T+UV treatment in contents of (–)-epicatechin (190%) and (+)-catechin (136%). Flavonoids (+)-catechin and (–)-epicatechin are the main phenolic compounds in grapes and wines (Goldberg and others 1998). (+)-Catechin and (–)-epicatechins are important for human health due to their antioxidant and free radical scavenging properties (Salah and others 1995). These 2 phenolic compounds also have an important role in biosynthetic polymerization of free phenols that inhibit blood clotting (Kovac and others 1995). (+)-Catechin and (–)-epicatechin are produced through the same metabolic pathway and hence, go parallel in terms of quantity (Goldberg and others 1998). As seen from Figure 1, the results of our study indicate that the highest increase among phenolic contents occurred in (+)-catechin and (–)-epicatechin contents with parallel values.

trans-resveratrol (trans-3,5,4′-trihydroxystilbene) is the most studied phenolic compound due to its antioxidative, anti-inflammatory, and anticancer effects. In our study, CM+UV treatment resulted in 2% increase and T+UV treatment resulted in 21% increase in *trans*-resveratrol content compared to control. Additionally, content of *cis*-resveratrol, formed by hydroxylation of glycosidic-form or isomerization of *trans*-form under UV irradiation (Roggero and Garcia-Parrilla 1995), increased by 3% with CM+UV treatment and by 17% with T+UV treatment compared to control wines. Bertelli and others (1996) reported that besides the known health benefits of *trans*-resveratrol, *cis*-resveratrol also has a number of positive effects on human health.

The study results indicated that T+UV treatment increased the levels of the antioxidative compounds, rutin (57%) and quercetin (4%).

Both treatments increased total anthocyanin content and antioxidant capacity compared to control (Figure 1).

Sensory analysis

Evaluation of organoleptic profiles of wines is presented in Figure 2. Harmony, bitterness, and astringency of control wines were not significantly different from those of CM+UV treated wines. However, color, flavor, body, sweetness, sourness, and astringency aftertaste of control wines were significantly different from those of CM+UV and T+UV treated wines ($P < 0.05$). Expert wine tasters favored T+UV treated wines for body, sourness, bitterness, astringency, and harmony; CM+UV treated wines

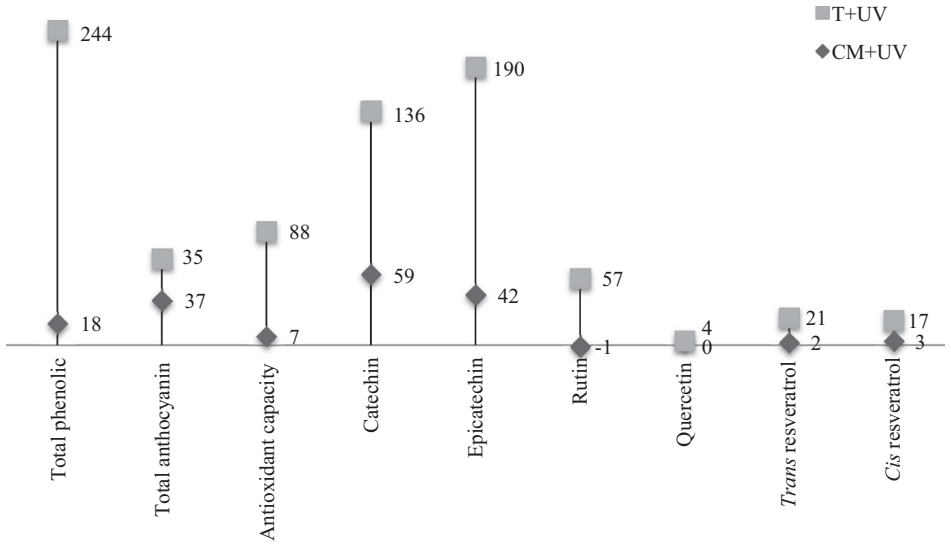

Figure 1–Rates of increase and decrease (%) in phenolic contents induced by CM+UV and T+UV treatments compared to control (classical fermentation) wines.

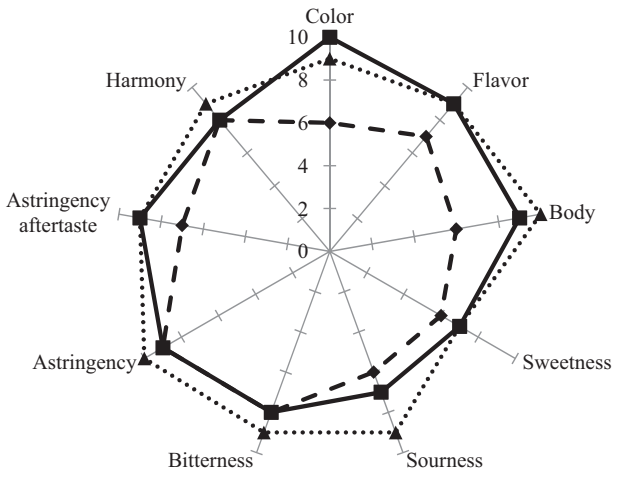

Figure 2–Sensory profiles of wines (significance at $P < 0.05$).

for color; and both CM+UV and T+UV treated wines for flavor and sweetness aftertaste.

Conclusions

This study has enabled the determination of the effect of ultraviolet light combined with vinification techniques on red wine phenolics. As a result, the yield of wine with the greater phenolic content and hence, with high antioxidant activity was obtained from both CM+UV and T+UV treatments. There are studies demonstrating the phenolic content increasing effects of thermovinification and cold maceration. However, in our study, this effect was further enhanced by the combination of these treatments with UV-C irradiation and wines with abundant phenolic content and favorable organoleptic profiles were obtained. Other grape varieties should be tested to confirm the results of our study.

Authors' Contribution

Author Gökhan Söylermezoğlu designed the study and interpreted the results. Author Hande Tahmaz carried out the studies, participated in collecting data, and drafted the manuscript. All authors read and approved the final manuscript.

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