

## White wines from Narince grapes: impact of two different grape provenances on phenolic and volatile composition

Mustafa Bayram<sup>1\*</sup> and Miyase Kayalar<sup>2</sup>

<sup>1</sup>Gaziosmanpaşa University, Faculty of Natural Sciences and Engineering, Department of Food Engineering, 60000, Tokat, Turkey

<sup>2</sup>Gaziosmanpaşa University, Graduate School of Natural and Applied Sciences, 60000, Tokat, Turkey

### Abstract

**Aim:** In this study, aroma and phenolics of wines produced from Narince grapes harvested from two different localities (Emirseyyit and Erbaa) of Tokat province (Turkey) were analyzed, and their effects on wine quality were assessed.

**Methods and results:** Samples were subjected to physicochemical, total phenolics, individual phenolics and aroma compounds analyses. Gallic acid content of the Erbaa and Emirseyyit wines at the end of fermentation was respectively 3.49 mg/L and 3.09 mg/L; (+)-catechin content 23.46 mg/L and 21.30 mg/L; and (-)-epicatechin content 9.46 mg/L and 8.74 mg/L. The differences in gallic acid and (-)-epicatechin contents of the wines produced from the grapes harvested from Erbaa and Emirseyyit were found to be significant at the end of fermentation. A total of 31 aroma compounds were also analyzed in the wines. The aroma substances were the same in both wines (with the exception of E-3-hexanol found exclusively in Erbaa wines), but the levels were different: the wines produced from the grapes harvested from Erbaa (205605.32 µg/L) had higher total aroma compounds than the wines produced from the grapes harvested from Emirseyyit (179547.85 µg/L).

**Conclusion:** There were no distinctive differences in total phenolics of Narince wines produced from two different localities, but there were differences in individual phenolics and aroma compounds.

**Significance and impact of the study:** The differences in some individual phenolics and aroma compounds of wines produced from grapes harvested from different localities are consistent with the concept of “terroir”.

**Keywords:** phenolic compounds, Narince, white wine, quality, aroma

Received : 9 November 2017; Accepted : 15 May 2018; Published : 25 June 2018  
DOI: 10.20870/oeno-one.2018.52.2.2114

## Introduction

Local differences may influence development of grapevines, ripening of grapes and composition and sensory characteristics of wines. Quality wines get their characteristic features from the places where the grapes are produced. Location of the vineyard or local conditions (soil, climate, topography) influence wine quality and style. The concept of “terroir”, used in origin check of the wines, is defined by the geographical location, topography, climate and solar radiation of the region in which the grapes are produced (Li *et al.*, 2011).

Phenolic compounds are secondary metabolites of plants, and they are the most common compounds in plants. They constitute a chemically heterogeneous group, and today there are almost 10000 compounds with already defined structure (Taiz and Zeiger, 2008). Phenolic compounds are one of the most significant quality criteria of wines, contributing specific flavors to the wine (Proestos *et al.*, 2005). Phenolic compounds of the wines mostly come from the grape (Ali *et al.*, 2010). They are influenced by many factors, mainly geographical origin. Phenolic compounds of the wines and grapes are thus greatly influenced by “terroir” (Li *et al.*, 2011).

It was reported in previous studies that phenolic compounds of white wines had higher absorption rates in human metabolism and may have positive contributions in prevention of ischemia-reperfusion injury of the heart. Also, phenolic compounds of white wines have higher antioxidant activity, are better at preventing blood serum lipid oxidation, and have higher cytotoxicity against normal peripheral mononuclear blood cells (Nardini *et al.*, 2009).

Aroma is another significant quality criterion in wines. Wines have a quite complex aromatic structure composed of several aroma compounds (San-Juan *et al.*, 2011). Grape cultivar, environmental factors (climate and soil), fermentation conditions (yeast flora, pH and temperature), technological processes used in wine production and wine aging conditions are the basic factors influencing formation of aroma compounds (Cabredo-Pinillos *et al.*, 2008).

There are several local grape cultivars grown for white and red wine production in Turkey. Narince is an indigenous white grape cultivar to Tokat province, and it is also grown in different parts of Turkey (Kiliç *et al.*, 2007). Narince grape cultivar is grown in several villages located in northern and southern parts of Kazova Valley 3 km away from the town of Turhal (Tokat province), in some villages of Turhal and Zile districts, and in several villages of Niksar and Erbaa

districts located in the Kelkit Valley region (Astan, 2006). Narince is a local grape cultivar processed into the best dry and semi-dry wines. Since it is a late ripening cultivar, harvest generally takes place in early October. Narince wines have a green-yellow color, fruity aromas, and a compact structure. Since their acid ratios are well, they are quite suitable for aging (Buhurcu, 2004).

There are several studies worldwide on phenolic compounds and aroma substances to classify the wines based on their geographical origins (terroir), but such studies are quite limited in Turkey. Therefore, there is a need for systematic studies dealing with local grape cultivars grown in different parts of Turkey. In the present study, aroma and phenolic compounds of wines produced from Narince grapes harvested from two different localities (Erbaa and Emirseyit) of Tokat province were determined, and their effects on wine quality were investigated.

## Materials and methods

### 1. Grapes and wines

Narince grapes harvested (2013) from two different localities of Tokat province (Erbaa and Emirseyit) were used in this study. Narince is a white table grape cultivar grown in Tokat province in the Middle Black Sea region of Turkey. Vines are cultivated using a bilateral cordon system. The altitudes of the vineyards from which the grapes were harvested in Erbaa and Emirseyit were respectively 360 m and 665 m. The vines are 12 years old. Two different wines were produced from the grapes harvested from Erbaa and Emirseyit. Wine production was performed at facilities of Diren Wines Co. Analyses of the wines were made at the laboratories of the Food Engineering Department of Gaziosmanpaşa University - Faculty of Engineering and Natural Sciences.

**Table 1. Gradient elution program for phenolic compounds**

Time	A (concentration) % (v/v)	B (concentration) % (v/v)
0	100	0
3	100	0
8	85	15
13	75	25
26	74	26
35	0	100
40	100	0

## 2. Winemaking

Wines were produced from the grapes harvested from Erbaa and Emirseyit. Following mechanical destemming and crushing, grapes were pressed, placed into 20000-L stainless steel fermentation tanks with temperature control and mixing apparatus, and left for fermentation. The must was supplemented with 30 ppm SO<sub>2</sub>. For ethyl alcohol fermentation, tanks were supplemented with 20 g/hL *Saccharomyces cerevisiae* (Oenobrand, Montpellier, France). Alcohol fermentation was performed at 21-24 °C. Temperature and density measurements were performed daily throughout the fermentation process. Samples to be analyzed were taken at the beginning and end of fermentation and at the end of clarification processes. Experiments were conducted in two replications.

## 3. Must and wine analyses

Total acidity, pH, reducing sugar, free and total SO<sub>2</sub>, density, alcohol content and volatile acid analyses were carried out in accordance with OIV (1990).

### 3.1 Total phenolic content

The Folin-Ciocalteu method as modified by Slinkard and Singleton was used to determine total phenolic

content. Spectrophotometric determination of the total phenolic content was done with the Folin-Ciocalteu micro method as adapted for wine analysis (Waterhouse, 2002) using gallic acid as the standard. The calibration curve of absorbance concentration of standard was used to quantify phenolic content. Calibration curve was prepared from gallic acid standard (at concentrations of 0, 50, 100, 150, 200 mg/mL in water). Results were expressed as mg gallic acid equivalents per liter of wine (mg GAE/L).

### 3.2 Individual phenolic compounds

Gallic acid, (+)-catechin, (-)-epicatechin, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid and quercetin contents were quantified by HPLC (High Performance Liquid Chromatography) (Bayram, 2011). All standards were supplied from Sigma-Aldrich. Samples were analyzed with a Shimadzu HPLC system. Detection and quantification was carried out with a CBM-20A Prominence system controller, a LC-20 AT Prominence pump, a CTO-10A SVp column oven and a SPD-M10AVP diode array detector with wavelengths set at 280 nm. Separation was performed on an Intersil C18 EPS-3 (250 x 4.6 mm, 3 μm ID) column. All chromatographic separations were carried out at 40 °C using gradient elution with mobile phases A

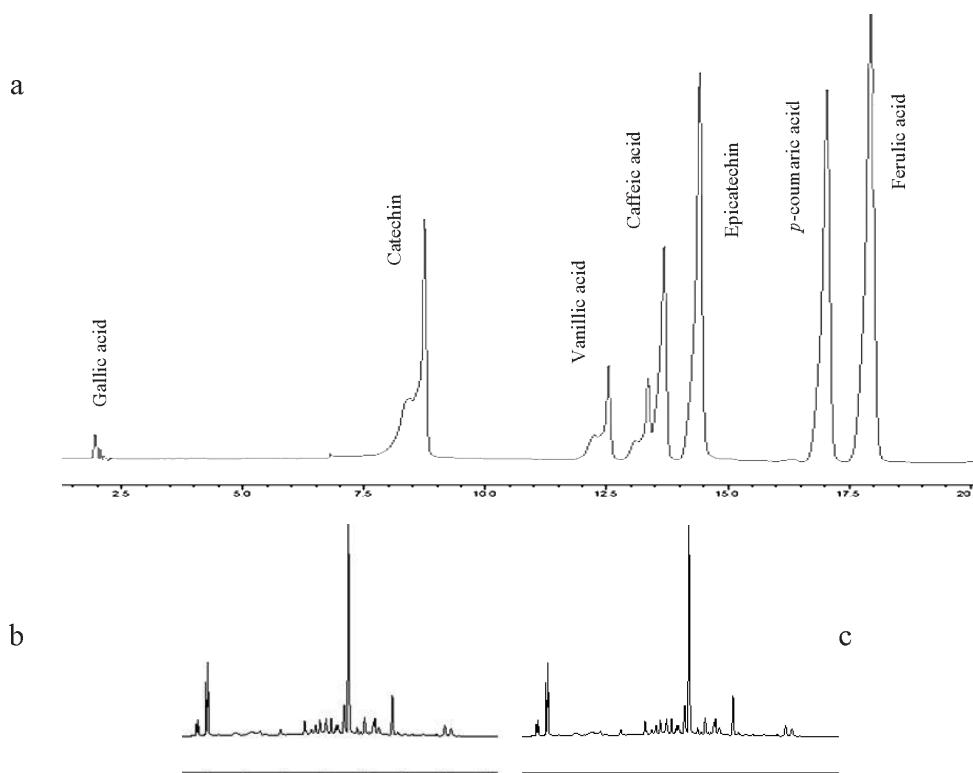


Figure 1. HPLC chromatogram at 280 nm of (a) phenolic standards, (b) Erbaa wines and (c) Emirseyit wines.

**Table 2. Physicochemical characteristics of grape musts**

	Erbaa	Emirseyyit
pH	3.63 ± 0.007	3.51 ± 0.006
Water soluble dry matter content (%)	19.80 ± 0.10	20.83 ± 0.29
Total acidity (g/L)*	3.66 ± 0.056	3.40 ± 0.056
Free SO <sub>2</sub> (mg/L)	23 ± 0.000	22 ± 0.000

Results are presented as mean ± standard error (n=3). \* expressed as tartaric acid equivalent

**Table 3. Physicochemical characteristics of wines**

	Erbaa	Emirseyyit
pH	3.58 ± 0.000	3.53 ± 0.005
Free SO <sub>2</sub> (mg/L)	41.83 ± 0.289	45.33 ± 1.527
Total SO <sub>2</sub> (mg/L)	103.67 ± 1.527	112.33 ± 0.577
Reducing sugar (g/L)	1.2 ± 0.000	1.2 ± 0.000
Alcohol (% v/v)	12.3 ± 0.100	12.4 ± 0.050
Volatile acidity (g/L)**	0.323 ± 0.000	0.392 ± 0.000
Total acidity (g/L)*	4.18 ± 0.028	4.21 ± 0.049
Density (g/mL)	0.990	0.990

Results are presented as mean ± standard error (n=3). \* expressed as tartaric acid equivalent, \*\* expressed as acetic acid equivalent

and B. Mobile phase A was formic acid:water (0.1%). Mobile phase B was acetonitrile. Flow rate was 1 mL/min.

Stock solutions (1 mg/mL) of all standards were prepared with methyl alcohol. The standards were kept at -18°C. Wine samples to be analyzed were filtered through a 0.45-µm (Millex-HV) membrane filter with a syringe. About 20-µL extract samples were directly analyzed. For quantitative analyses of phenolic acids, UV-Vis/DAD detector and internal standards were used at 280 nm. A calibration curve was drawn for these standard compounds and samples were quantitatively assessed through this calibration graph. Gradient elution programs for phenolic compounds in HPLC are given in Table 1.

### 3.3 Aroma compounds

Liquid-liquid extraction technique was used in aroma analyses. Extractions were performed in three replications for each sample with dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) solvent. In each extraction process, 100-mL wine sample was used. Wine sample was supplemented with 50 mL dichloromethane solvent and 40 µg internal standard (4-nonanol), and the resultant mixture was placed into 500-mL Erlenmeyer flask. The mixture was stirred under

nitrogen gas at 4-5.°C for 30 minutes with a magnetic stirrer. Then the sample mixture was centrifuged at 0.°C for 20 min (at 6000 rpm). Following centrifugation, the solvent phase containing the aroma substances was concentrated to 5 mL at 45.°C in a Vigreux concentrator. Then the 5-mL solvent phase was further concentrated to 0.5 mL in a micro-concentrator. The concentrated extract was injected (3 µL) into a GC-MS (Gas Chromatography-Mass Spectrophotometry) device, and aroma substances were determined. To identify aroma substances, Wiley 7.0 and NIST aroma substances library of GC-MS, standard substances and Kovats Index values were used. Following the identification of the peaks, quantity of aroma substances was calculated through internal standard method (Priser *et al.*, 1997).

### 4. Statistical analyses

Statistical analyses were carried out with SPSS (Version 20.0) software, and Duncan test was used to compare the means.

## Results and discussion

### 1. General chemical analyses for must and wines

Analysis results for the must obtained from Narince grapes harvested from Erbaa and Emirseyyit are

**Table 4. Total phenolic content of wines**

	Erbaa	Emirseyyit
Must	470.96 ± 56.615Aa	515.88 ± 1.821Ba
At the end of fermentation	443.39 ± 30.586Aa	403.39 ± 7.612Aa
At the end of clarification	383.39 ± 5.300Aa	412.56 ± 42.233Aa

Results are presented as mg/L gallic acid equivalent. Different capital letters in the same column indicate significant differences between wine production stages; different small letters in the same row indicate significant differences between localities ( $p < 0.05$ ;  $n=3$ ).

provided in Table 2, and analysis results for the wines after clarification are provided in Table 3.

Density of the wines produced from the grapes harvested from Erbaa and Emirseyyit was 0.990 g/mL, and free SO<sub>2</sub> content at the end of fermentation was respectively 23 mg/L and 22 mg/L. SO<sub>2</sub> plays a significant role in wine production, aging, and prevention of wine spoilage and defects (Cabaroğlu and Canbaş, 1994).

Reducing sugar content of the wines produced from the grapes harvested from Erbaa and Emirseyyit was 1.2 mg/L. Wines are classified based on residual sugar after clarification as: dry (0-4 g/L), semi-dry (4-12 g/L), semi-sweet (12-48 g/L) and sweet (>48 g/L). Wines with a sugar content below 4 g/L are included in dry wines (Turkish Food Codex, 2009). Based on this classification, all of the wines produced in this study can be classified as dry wine with full fermentation.

Alcohol content of the wines produced from the grapes harvested from Erbaa and Emirseyyit was respectively 12.3% and 12.4%. Alcohol is a significant component influencing characteristic taste and odor. Grape ripening level and variety may influence alcohol content of the wines (Jordão *et al.*, 2015). According to wine regulation of Turkish Food Codex (2009), actual alcohol content of wine in volume should be at least 9% and total alcohol content should be a maximum of 15%.

Volatile acid content (expressed as acetic acid equivalent) of the wines produced from the grapes harvested from Erbaa and Emirseyyit was respectively 0.323 g/L and 0.392 g/L. Volatile acids are formed during alcohol fermentation, and the majority of them forms acetic acid. The amount of volatile acid depends on must composition (acid, sugar, nitrous substances), yeast strain and fermentation conditions (Ough and Amerine, 1988). According to wine regulation of Turkish Food Codex (2009), volatile acid content (in acetic acid equivalent) should not be more than 18 meq/L for partially fermented grape

must, 18 meq/L for white and pink/rose wines, and 20 meq/L for red wines. Current findings were consistent with the literature and were lower than values specified in wine regulation.

Total acidity (expressed as tartaric acid equivalent) of the wines produced from the grapes harvested from Erbaa and Emirseyyit was respectively 4.18 g/L and 4.21 g/L; pH values of the wines were respectively 3.58 and 3.53. Acidity influences taste and resistance of wines and brings freshness to the wines. It is also effective on color tone, durability and taste of the wine (Navarre, 1988). According to wine regulation of Turkish Food Codex (2009), total acidity of wines (expressed as tartaric acid equivalent) should be at least 3.5 g/L or 46.6 meq/L.

## 2. Total phenolic content of wines

Total phenolics of the wines produced from Narince grapes harvested from the different localities are provided in Table 4. Total phenolic content of the must obtained from the grapes harvested from Erbaa and Emirseyyit was respectively 470.96 mg GAE/L and 515.88 mg GAE/L. Total phenolic content of the wines was respectively 443.39 mg GAE/L and 403.39 mg GAE/L at the end of the fermentation process, and 383.39 mg GAE/L and 412.56 mg GAE/L at the end of the clarification process (Table 4). The difference in total phenolics of the must and wines produced from the grapes harvested from two different localities were not found to be significant.

Shahidi and Naczki (1995) reported total phenolics of white wines as between 50-2000 mg/L. In another study carried out with Narince grapes of Tokat province, total phenolics of the wines was reported as 345 mg GAE/L (Şen, 2014).

Bisson and Ribéreau-Gayon (1978) investigated the effects of cultivar and environmental conditions on phenolic compounds of Cabernet Franc, Merlot, Pinot Noir and Gamay grape cultivars grown in two different regions and reported that total and individual phenolics of black grapes varied with the



**Table 5. Some individual phenolic compounds of wines**

		Erbaa	Emirsevit
Must		2.33 ± 0.130 Aa	2.45 ± 0.127 Aa
At the end of fermentation	Gallic acid	3.49 ± 0.028 Bb	3.09 ± 0.069 Ba
At the end of clarification		5.32 ± 0.470 Ca	4.57 ± 0.046 Ca
Must		1.79 ± 0.131 Ba	1.41 ± 0.023 Aa
At the end of fermentation	Ferulic acid	1.87 ± 0.010 Ba	1.44 ± 0.037 Aa
At the end of clarification		1.36 ± 0.031 Aa	1.51 ± 0.058 Ab
Must		0.43 ± 0.078 Aa	1.34 ± 0.060 Bb
At the end of fermentation	<i>p</i> -coumaric acid	0.54 ± 0.007 Aa	2.88 ± 0.064 Cb
At the end of clarification		2.33 ± 0.064 Cb	0.88 ± 0.117 Aa
Must		0.40 ± 0.038 Aa	0.47 ± 0.071 Aa
At the end of fermentation	Vanillic acid	0.62 ± 0.003 Ba	0.71 ± 0.049 Ba
At the end of clarification		0.39 ± 0.001 Aa	0.47 ± 0.004 Ab
Must		1.00 ± 0.200 Aa	0.69 ± 0.018 Aa
At the end of fermentation	Caffeic acid	3.10 ± 0.007 Bb	2.82 ± 0.062 Ba
At the end of clarification		9.74 ± 0.351 Ca	9.33 ± 0.251 Ca
Must total phenolic acids		5.95	6.36
Wine total phenolic acids (at the end of fermentation)		9.62	10.94
Wine total phenolic acids (at the end of clarification)		19.14	16.76
Must		25.44 ± 0.079 Cb	22.31 ± 1.307 Ba
At the end of fermentation	Catechin	23.46 ± 0.143 BCb	21.30 ± 0.038 ABa
At the end of clarification		18.25 ± 1.261 Aa	21.27 ± 0.610 ABa
Must		9.95 ± 0.935 Aa	8.85 ± 0.362 Aa
At the end of fermentation	Epicatechin	9.46 ± 0.330 Aa	8.74 ± 0.717 Aa
At the end of clarification		9.83 ± 0.198 Aa	11.96 ± 0.136 Bb
Must total flavonoids		35.39	31.16
Wine total flavonoids (at the end of fermentation)		32.92	30.04
Wine total flavonoids (at the end of clarification)		28.08	33.23

Results are presented in mg/L. Different capital letters in the same column indicate significant differences between wine production stages; different small letters in the same row indicate significant differences between localities ( $p < 0.05$ ;  $n = 3$ ).

cultivars; environmental conditions had significant effects on tannin and anthocyanin contents; same grape cultivars grown in different regions preserved their characteristics with regard to phenolic compounds.

Kelebek *et al.* (2010) characterized colored and colorless phenolic compounds of red wines produced from Öküzgözü grapes grown in different vineyard regions (Denizli and Elazığ) and investigated the effects of vineyard region on phenolic compounds. The researchers reported higher total phenolic contents for the wines produced from grapes of Denizli region than for the wines of Elazığ region and indicated the reason for the difference in phenolic compounds as the complex interactions between Öküzgözü grapevines and vineyard characteristics (location, climate, soil).

Phenolic compounds of the wines are mostly influenced by phenolic concentration of grapes, wine production technology, contact duration of berry skin and seeds, ethyl alcohol concentration, fermentation temperature, press pressure and transformations throughout the aging of the wines (Uylaşer and İnce, 2008). Besides these factors, the region where grapes are produced, soil characteristics and agricultural practices influence color components and phenolic compounds of the grapes (Ünsal, 2007).

### 3. Individual phenolic compounds of wines

Phenolic compounds of wines produced from Narince grapes harvested from the different localities are provided in Table 5. A total of seven phenolic compounds were quantitatively analyzed, namely two flavanols [(+)-catechin, (-)-epicatechin] and five phenolic acids (gallic acid, vanillic acid, caffeic acid,

*p*-coumaric acid, ferulic acid). HPLC chromatograms of phenolic standards and Erbaa and Emirseyit wines are presented in Figure 1.

Gallic acid content of the must obtained from the grapes harvested from Erbaa and Emirseyit was respectively 2.33 mg/L and 2.45 mg/L; (+)-catechin content 25.44 mg/L and 22.31 mg/L; (-)-epicatechin content 9.95 mg/L and 8.85 mg/L; ferulic acid content 1.79 mg/L and 1.41 mg/L; *p*-coumaric acid content 0.43 mg/L and 1.34 mg/L; vanillic acid content 0.40 mg/L and 0.47 mg/L; and caffeic acid content 1.0 mg/L and 0.69 mg/L.

Gallic acid content of the wines produced from the grapes harvested from Erbaa and Emirseyit at the end of fermentation was respectively 3.49 mg/L and 3.09 mg/L; (+)-catechin content 23.46 mg/L and 21.30 mg/L; (-)-epicatechin content 9.46 mg/L and 8.74 mg/L; ferulic acid content 1.87 mg/L and 1.44 mg/L; *p*-coumaric acid content 0.54 mg/L and 2.88 mg/L; vanillic acid content 0.62 mg/L and 0.71 mg/L; and caffeic acid content 3.10 mg/L and 2.82 mg/L (Table 5).

(+)-Catechin was the major phenolic compound in the must obtained from Narince grapes harvested from Erbaa and Emirseyit, followed by (-)-epicatechin and gallic acid. Only the difference in (+)-catechin and *p*-coumaric acid contents of the must was found to be significant.

At the end of clarification, the greatest (+)-catechin, (-)-epicatechin, caffeic acid and gallic acid contents were observed in Erbaa wines and the greatest *p*-coumaric acid and vanillic acid contents were observed in Emirseyit wines. The differences in gallic acid, *p*-coumaric acid, vanillic acid and (-)-epicatechin contents between Erbaa and Emirseyit wines were found to be significant at the end of fermentation, while the differences in ferulic acid, *p*-coumaric acid, vanillic acid and (-)-epicatechin contents were found to be significant at the end of clarification.

Total phenolic acids of Erbaa and Emirseyit must were respectively 5.95 mg/L and 6.36 mg/L. At the end of the clarification process, total phenolic acids of Erbaa and Emirseyit wines were respectively 19.14 mg/L and 16.76 mg/L. Phenolic acid content of wines was higher and flavonoid content of wines was lower than in must for both localities. As compared to the must, gallic acid, *p*-coumaric acid and caffeic acid contents of the wines of both localities were higher at the end of clarification.

Phenolic acids are classified as hydroxycinnamic and hydroxybenzoic acids. Although hydroxycinnamic acids exist in fruits as ester, various natural conditions or technological processes result in formation of hydroxycinnamic acids in free forms (Somers *et al.*, 1987). Lower *p*-coumaric acid and ferulic acid contents of the must can be explained by reduced polyphenoloxidase enzyme activity through SO<sub>2</sub> addition to the must before fermentation and prevention of enzymatic degradation of complex hydroxycinnamic acids. Similar results were also reported by Budic-Leto and Lovric (2002).

Increased *p*-coumaric acid and ferulic acid contents at the end of fermentation as compared to the must may be related to possible hydrolysis of hydroxycinnamic acid esters like caftaric, coutaric and fertaric acid. Similar findings on this issue were also reported by Budic-Leto and Lovric (2002).

Effects of terroir on phenolic compounds of various grape cultivars were reported in previous studies. Ünsal (2007) determined some phenolic compounds (gallic acid, (+)-catechin, (-)-epicatechin, vanillic acid and syringic acid) of the wines produced through classical maceration method from French and Turkish wine grapes (Kalecik Karası, Gamay and Cabernet sauvignon) harvested from Mürefte and Hoşkøy localities of Trachia with an HPLC and compared the wines for these phenolic compounds. Results revealed that gallic acid was the major phenolics in both localities and all three wines; the other phenolics varied in the different wines. It was also observed that gallic acid, (+)-catechin and (-)-epicatechin contents were higher than vanillic acid and syringic acid contents in both locations and all wines. It was concluded in that study that each three cultivars was well adapted to the region, especially Cabernet sauvignon which yielded quite strong wines rich in phenolic compounds.

In another study, Kelebek *et al.* (2010) investigated the effects of vineyard region (Denizli, Elazığ, Nevşehir, Ankara) on red grape (Öküzgözü, Kalecik Karası, Boğazkere) phenolic compounds. Öküzgözü cultivar was found to be rich in (+)-catechin and Kalecik Karası cultivar was found to be rich in (-)-epicatechin; Boğazkere cultivar had low (+)-catechin and (-)-epicatechin contents, but high procyanidin (B1, B2, B3 and B4) contents. With regard to colorless phenolic compounds, Boğazkere grapes of Elazığ region were richer than the grapes of Denizli region; the grapes of Nevşehir region were richer than the grapes of Ankara region. With regard to colored phenolic compounds, differences were observed in wines: Öküzgözü wines had high (+)-

catechin contents and Kalecik Karası wines had high (-)-epicatechin contents; Boğazkere wines had low catechin and epicatechin contents, but high *trans*-caftaric and *trans*-coutaric acid contents. With regard to colorless phenolic compounds, wines of Elazığ region were found to be richer than the wines of Denizli region.

Kumšta *et al.* (2012) analyzed 43 different Riesling wines from four vintages and 16 different localities in six sub-viticultural regions and reported that phenolic composition of the grapes and wines varied with the localities and the wines; wine regions were related to *trans*-resveratrol concentration. Lampíř and Pavloušek (2013) investigated the effects of regions on phenolic compounds of white wines produced from grapes grown in two different regions of Czech Republic and reported that protocatechuic acid, *p*-hydroxybenzoic acid, caftaric acid, *cis*-piceid, (+)-catechin and (-)-epicatechin were significantly influenced by terroir.

#### 4. Aroma compounds of wines

Description and olfactory perception thresholds of aroma substances and aroma compound amount of Erbaa and Emirseyit wines are provided in Table 6. While 31 aroma compounds were identified in wines produced from the grapes harvested from Erbaa, 30 aroma compounds were identified in wines produced from the grapes harvested from Emirseyit. The quantity of E-3-hexanol in Erbaa wines was 99.84 µg/L; the compound was not observed in Emirseyit wines. The other aroma substances were the same, but the levels were different: the wines produced from the grapes harvested from Erbaa had higher total aroma compounds (205605.32 µg/L) than the wines produced from the grapes harvested from Emirseyit (179547.85 µg/L). In both localities, alcohols were the greatest aroma compounds, followed respectively by acids and esters. The differences in levels between both wines were significant for 22 aroma compounds.

The levels of some of the volatile compounds are well correlated with the aromatic composition of wines made with grapes of the same varieties. Grape type and quality affect the chemical composition of the wines. Depending on the fermentation conditions and must treatments (temperature, micronutrients, vitamins and nitrogen composition of the must) *S. cerevisiae* produces different concentrations of aroma compounds (Carrau *et al.*, 2008). The microflora of the grapes and fermentation medium contribute to wine final aroma by mechanisms: firstly by utilizing grape juice constituents and biotransforming them

into aroma- or flavor-impacting components; secondly by bringing enzymes that transform neutral grape compounds into flavor-active compounds and lastly by the de novo synthesis of many flavor-active primary and secondary metabolites (Fengmei *et al.*, 2016). Also many of the aroma and flavor compounds found in the finished wine come not from the grape, but rather from compounds formed during primary (essential) or secondary metabolism of the wine yeast during alcoholic fermentation (Styger *et al.*, 2011).

The compounds with significant differences between localities were alcohols (1-propanol, 1-butanol, isoamyl alcohol, 3-hexanol, 1-hexanol, E-3-hexanol, methionol, phenylethyl alcohol, *p*-hydroxy phenyl ethyl alcohol), esters (isoamyl acetate, ethyl octanoate, ethyl-3-OH-butanoate, ethyl-4-OH-butanoate, phenylethyl acetate, diethyl DL malate, ethyl-H-succinate), acids (butanoic acid, hexanoic acid, octanoic acid, hexadecanoic acid), carbonyl compounds (acetoin), and lactones (□-butyrolactone).

Total quantity of 11 higher alcohol compounds was 173024.96 µg/L in Erbaa wines and 145831.14 µg/L in Emirseyit wines. Among these alcohol compounds, isoamyl alcohol, phenylethyl alcohol and isobutyl alcohol were the greatest in both localities. Phenylethyl alcohol content was 37225.28 µg/L in Erbaa wines and 21568.90 µg/L in Emirseyit wines; isoamyl acid content was 120251.57 µg/L in Erbaa wines and 109069.58 µg/L in Emirseyit wines. Higher alcohols exist in aliphatic (straight chain) and aromatic structure. Higher alcohols are the secondary products of yeast metabolism. While they give a sharp and bitter taste to wine at high concentrations, they contribute to fruity aroma of the wine at optimum concentrations (Lambrechts and Pretorius, 2000; Swiegers *et al.*, 2005). Ribéreau-Gayon *et al.* (2000) indicated that while higher alcohols give the desired aroma to wines at a total concentration below 300 mg/L, they negatively influence taste and odor at a total concentration above 400 mg/L. Higher alcohol content of the present study was lower than the value specified by Ribéreau-Gayon *et al.* (2000). Of the 11 aroma compounds, only two (isoamyl alcohol, phenylethyl alcohol) were determined above the odor perception threshold. Nykänen and Suomalainen (1989) indicated benzyl alcohol and phenylethyl alcohol as important alcohol compounds and stated that phenylethyl alcohol content was influenced by must composition, yeast strain and fermentation temperature. Phenylethyl alcohol and benzyl alcohol are aromatic alcohols which give a floral, pollen odor to wines. Higher alcohols with branched chain



**Table 6. Aroma compounds of wines**

Compounds	Threshold	Odor Description	Erbaa	Emirsevit
<b>Alcohols (µg/L)</b>				
1-propanol	306000	Floral, fruity, candy, sweet	2353.40 ± 102.814 A	3538.95 ± 40.275 B
isobutyl alcohol	40000	Fresh, banana	7923.70 ± 439.700 A	7482.80 ± 184.675 A
1-butanol	150000	Whiskey, medicinal	399.60 ± 5.187 A	510.68 ± 6.885 B
isoamyl alcohol	30000	Cheese, whiskey, malt	120251.57 ± 425.611 B	109069.58 ± 489.84A
2-hexanol	400	Green	242.15 ± 10.231 A	215.81 ± 12.500 A
3-hexanol	400	Green	270.92 ± 1.842 A	340.10 ± 6.792 B
1-hexanol	8000	Resin, green	893.41 ± 2.433 B	787.43 ± 3.773 A
E-3-hexanol	400	Green	99.84 ± 0.730	nd
methionol	1200	Potato	418.83 ± 9.165 B	330.80 ± 7.750 A
phenylethyl alcohol	14000	Floral, pollen	37225.28 ± 1631.156 B	21568.90 ± 37.268 A
<i>p</i> -hydroxy phenyl ethyl alcohol	14000	Floral	2946.26 ± 215.259 B	1986.09 ± 167.183 A
Total			173024.96	145831.14
<b>Esters (µg/L)</b>				
isoamyl acetate	30	Banana	3962.48 ± 28.307 A	5795.98 ± 226.460 B
ethyl hexanoate	5	Apple, banana, v-Violet	1223.91 ± 9.203 A	1120.65 ± 56.531 A
hexyl acetate	670	Fruity	241.87 ± 0.629 A	246.97 ± 11.859 A
ethyl acetate	7500	Lactic, raspberry	1688.82 ± 3.412 A	2400.32 ± 26.183 B
ethyl octanoate	2	Pineapple, pear, floral	1351.84 ± 25.669 B	1143.06 ± 65.838 A
ethyl-3-OH-butanoate	-	Dry fruit	192.13 ± 1.29 A	245.37 ± 0.499 B
ethyl decanoate	200	Fruity, grape, pleasant	369.01 ± 18.74 A	312.90 ± 24.451 A
diethyl succinate	120000	Fruity, apple	749.04 ± 43.116 A	752.73 ± 3.011 A
ethyl-4-OH-butanoate	-	Fruit, sweaty	1454.98 ± 10.493 B	714.89 ± 11.252 A
phenylethyl acetate	250	Rose, honey	832.83 ± 9.927 B	636.73 ± 6.232 A
diethyl DL malate	-	Caramel	289.195 ± 0.020 B	277.09 ± 3.663 A
ethyl-H-succinate	-		1053.94 ± 871.445 A	3186.17 ± 312.360 B
Total			13410.045	16832.86
<b>Acids (µg/L)</b>				
acetic acid	200000	Vinegar	998.84 ± 403.495 A	781.64 ± 68.825 A
isobutyric acid	200000	-	671.18 ± 78.719 A	730.76 ± 1.091 A
butanoic acid		Sweaty	676.67 ± 41.070 A	814.37 ± 5.369 B
hexanoic acid	3000	Sweaty	5363.81 ± 53.064 B	4869.89 ± 62.807 A
octanoic acid	500	Sweaty	8573.38 ± 17.427 B	7185.93 ± 317.900 A
hexadecanoic acid	10000	Waxy, fatty	745.285 ± 42.427 B	559.61 ± 11.385 A
Total			17029.165	14942.2
<b>Carbonyl Compounds (µg/L)</b>				
acetoin	150000	Buttery	244.67 ± 6.974 A	298.96 ± 3.831 B
<b>Lactones (µg/L)</b>				
$\gamma$ -butyrolactone	-	-	1896.48 ± 76.216 B	1642.69 ± 89.841 A

Results are presented in µg/L. Different letters in the same row indicate significant differences between localities ( $p < 0.05$ ;  $n = 3$ ). nd: not detected

structure like isoamyl alcohol are released from amino acids through the Ehrlich pathway by yeasts (Swiegers *et al.*, 2005). Selli *et al.* (2006) carried out a study to investigate the effects of maceration treatment (at 15°C for 12 hours) on aroma compounds of wines produced from Narince grapes

and reported isoamyl content of 73.737 µg/L in 1998 and 98.826 µg/L in 1999. It was argued that high 1-hexanol compound of wines mostly resulted from lipoxygenase enzyme activity of the grapes or air contact of the must (Rocha *et al.*, 2004). The 1-hexanol content of the wines was 893.41 µg/L in

Erbaa wines and 787.43 µg/L in Emirseyit wines.

Based on their origins, esters can be gathered under two groups. The first group is composed of the acetates of higher alcohols and includes isoamyl acetate, isobutyl acetate, methyl acetate and 2-phenyl acetate; the second group is composed of ethyl esters of fatty acids and includes ethyl hexanoate, ethyl octanoate and ethyl decanoate (Etiévant, 1991). Based on odor activity values, ethyl hexanoate adds ripe banana aroma, isomethyl acetate adds pineapple aroma, isoamyl acetate adds banana aroma and 2-phenylethyl acetate adds fruit jam aroma to the wines (Antonelli *et al.*, 1999). A total of 12 ester compounds were identified in wines of the present study. The total quantity was 13410.045 µg/L for Erbaa wines and 16832.86 µg/L for Emirseyit wines. Of the 12 aroma compounds, five (isoamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, phenylethyl acetate) were determined above their perception threshold value. Perception threshold value of isoamyl acetate in wine is 30 µg/L. Isoamyl acetate content of wines was 3962.48 µg/L for Erbaa and 5795.98 µg/L for Emirseyit. Isoamyl acetate gives banana aroma to wines. Perception threshold value of ethyl hexanoate in wine is 5 µg/L. Ethyl hexanoate content of wines was 1223.91 µg/L for Erbaa and 1120.65 µg/L for Emirseyit. Ethyl hexanoate gives apple and banana aroma to wines. Perception threshold value of ethyl octanoate in wine is 2 µg/L. Ethyl octanoate content of wines was 1351.84 µg/L for Erbaa and 1143.06 µg/L for Emirseyit. Ethyl octanoate gives pineapple and pear aroma to wines. Perception threshold value of ethyl acetate in wine is 7500 µg/L. Ethyl acetate content was 1688.82 µg/L for Erbaa and 2400.32 µg/L for Emirseyit. Selli *et al.* (2006) carried out a study to investigate the effects of maceration treatment (at 15°C for 12 hours) on aroma compounds of Narince grapes and reported isomethyl acetate content of 879 µg/L in 1998 and 640 µg/L in 1999, and ethyl lactate content of 1309 µg/L in 1998 and 3119 µg/L in 1999.

The amount of volatile acids in wines was 17029.165 µg/L for Erbaa and 14942.2 µg/L for Emirseyit. Octanoic acid content was 8573.38 µg/L for Erbaa and 7185.93 µg/L for Emirseyit; hexanoic acid content was 5363.81 µg/L for Erbaa and 7185.93 µg/L for Emirseyit. Perception threshold value of octanoic acid and hexanoic acid is 500 µg/L and 3000 µg/L respectively. Both of them give sweaty aroma to wines. Volatile acids are short-chain organic acids. Volatile acid concentrations of wines usually vary between 500-1000 mg/L. Acetic acid constitutes about 90% of volatile acids. The remaining volatile acids, including propionic acid and hexanoic acid, are

synthesized through lipid metabolism by bacteria and yeasts (Çelik, 2012). Acetic acid (vinegar aroma), propionic acid (goat aroma) and butanoic acid (rancid butter aroma) influence the aroma of the wines. Except for acetic acid, wine acids are usually present below the perception threshold levels (Rapp and Mandery, 1986; Costello, 2005). Selli *et al.* (2006), in a study carried out with Narince grapes, investigated the effects of maceration treatment (at 15°C for 12 hours) on aroma compounds of the wines and reported hexanoic acid content of 3019 µg/L in 1998 and 2932 µg/L in 1999 and octanoic acid content of 5245 µg/L in 1998 and 5260 µg/L in 1999.

Carbonyl compounds are synthesized through carbohydrate or citric acid metabolism, lipid oxidation or aminoacid reduction by microorganisms throughout the fermentation (Swiegers *et al.*, 2005). In the present study, acetoin content of the wines was 244.67 µg/L for Erbaa and 298.96 µg/L for Emirseyit. Acetoin content should not exceed the perception threshold value (150 mg/L). In the present study, wines of both localities had acetoin contents below the perception threshold value. Selli *et al.* (2006) reported acetoin content of Narince grapes of 296 µg/L in 1998 and 223 µg/L in 1999.

γ-Butyrolactone is the most significant lactone compound formed during the fermentation process. This lactone is formed by the lactonization of γ-hydroxybutyric acid formed through decarboxylation and deamination of glutamic acid through the Ehrlich pathway. This compound may also come directly from the grape (Ribéreau-Gayon *et al.*, 2000). In white wines, lactone quantities may significantly increase when the grapes are processed with their stems. It was also reported in a previous study that gamma lactones may greatly contribute to wine aroma; yeast strain and wine aging might significantly influence the quantities of these compounds (Rocha *et al.*, 2004). In the present study, γ-butyrolactone content was 1896.48 µg/L for Erbaa and 1642.69 µg/L for Emirseyit.

## Conclusion

In the present study, aroma and phenolic compounds of the wines produced from Narince grapes harvested from two different localities (Erbaa and Emirseyit) of Tokat province were analyzed. Results revealed that different localities and process stages influenced both chemical composition and phenolic compounds of wines. Considering the total phenolics of wines at the end of fermentation and clarification stages, it was observed that Emirseyit wines had higher total phenolic contents, although the differences between

the localities were not found to be significant. While the differences in (+)-catechin and caffeic acid content of the wines were found to be significant, the differences in (-)-epicatechin, ferulic acid and vanillic acid contents were not. The identified aroma substances were similar in both localities, but the wines produced from the grapes harvested from Erbaa had higher levels of aroma compounds than the wines produced from the grapes of Emirseyit. Therefore, it was concluded that the differences in some individual phenolics and aroma compounds of wines produced from the grapes harvested from different localities were consistent with the concept of “terroir”. In conclusion, there were no distinctive differences in total phenolics between wines produced from Narince grapes harvested from two different localities, but there were differences in individual phenolics and aroma compounds.

### Acknowledgements

The authors are grateful to the Scientific Research Commission of the Gaziosmanpaşa University for financial support.

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