TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2015) 39: 984-991

doi:10.3906/tar-1412-82

Seasonal variation in amino acid and phenolic compound profiles of three Turkish white wine grapes

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Received: 20.12.2014 • **Accepted/Published Online:** 20.06.2015 • **Printed:** 30.11.2015

Abstract: Changes in amino acids and phenolic compounds in Emir, Narince, and Sultaniye grapes were monitored by high-performance liquid chromatography for two consecutive seasons. Seasonal and varietal variations in amino acid content were observed among the cultivars. Arginine, histidine, and alanine were the most prominent amino acids in all 3 cultivars in both years, with arginine being the highest found in the Sultaniye cultivar, varying between 910 and 955 mg/L. The phenolic contents also showed seasonal and varietal variations. Of the phenolic compounds identified, catechin was the most abundant in all three cultivars, with the highest found in Narince ranging between 106 and 109 mg/kg. Procyanidin B1 and gallic acid were the second most prominent.

Key words: Amino acid, phenolics, seasonal change, grape, wine

1. Introduction

Free amino acids and ammonia account for the majority of the nitrogen-containing compounds that are, next to sugars, quantitatively the most important yeast nutrients in wine grapes for successful alcohol and/or malolactic fermentation. Yeast growth, fermentation rate, fermentation duration, fermentation bouquet, and the end products of yeast metabolism are all affected by the nitrogen content of musts. Yeasts can utilize amino acids and ammonia as a nitrogen source. The cells can utilize glutamate in the production of important amino acids for cell metabolism. The most important amino acid for *Saccharomyces* is arginine. Nitrogen concentration of the grape varies depending on growing conditions, environment, variety, and other factors. Winemaking practices also influence the nitrogen content of must. For example, nitrogen content increases with slow pressing and skin maceration (Ribereau-Gayon, 2006; Fugelsang and Edwards, 2007; Garde-Cerdan and Ancin-Azpilicueta, 2008; Lee and Schreiner, 2010; Moreira et al., 2011).

Although the nitrogen content of must usually suffices for fermentation, it can vary considerably and mainly consists of free amino acids. The minimum amount of assimilable nitrogen required for complete fermentation is 150 mg L^{-1}, which should be in the form of free amino acids. Arginine and proline are the predominant free amino acids in grape must; however, proline cannot be utilized by yeasts as a nitrogen source under anaerobic conditions (Jackson, 2008). Sluggish or stuck fermentation is often caused by a low nitrogen concentration. In such cases, diammonium orthophosphate can be added to the must to ensure complete fermentation (Valdes et al., 2011).

Phenolic compounds are divided into flavonoid and nonflavonoid compounds. Flavonoids can be further divided into flavonols, flavones, flavan-3-ols, flavanones, and anthocynidins. Nonflavonoids include phenolic acids, hydroxycinnamic acids and their conjugated derivatives, and polyphenolic stilbenes (Monagas et al., 2005; Perestrelo, 2012). Various attributes of wine such as color, taste, mouthfeel, fragrance, and antimicrobial and antioxidant properties are influenced by phenols and related compounds. The major phenolic compounds in white wines are caftaric acid and the related derivatives *p*-coumaric acid and ferulic acid (Jackson, 2008).

The phenolic content of grapes is affected by soil composition, cultivar, climate, cultivation practices, exposure to diseases, and degree of maturation. Grape phenolics are mainly distributed in the skin, stem, leaf, and seed of the grape. Phenolic compounds have beneficial effects on human health. Due to their biological

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and organoleptic properties, there have been numerous research studies done on the phenolics of grapes and wines (Perestrelo et al., 2012; Flamini et al., 2013; Garrido and Borges, 2013; Teixeira et al., 2013). Understanding the relationship between the quality of a particular wine and its phenolic composition poses a challenge in enological research (Garrido and Borges, 2013).

The Emir, Narince, and Sultaniye grapes used in this study are important white grape varieties for the Turkish wine industry. Emir, which is cultivated in the Nevşehir-Ürgüp (Cappadocia) region, is an important white grape variety for the wine industry (Ünal and Şener, 2006). It constitutes approximately 25% of the total vineyards of the region. Narince is another important white variety commonly grown in the Tokat region (Ünal and Şener, 2014). Sultaniye, which is mainly cultivated in the Aegean region, is marketed as a fresh fruit as well as being used in wine production (Ünal et al., 2007). In this study, the seasonal variations of the amino acid and phenolic compound profiles of the Emir, Narince, and Sultaniye cultivars were investigated. No such research has been carried out on these cultivars.

2. Materials and methods

2.1. Plant material

The investigation was conducted for the fruiting seasons of 2006 and 2007 using the Sultaniye, Emir, and Narince grape varieties. The Sultaniye grapes were obtained from Denizli, the Emir grapes were from Nevşehir, and the Narince grapes were from Tokat, all provinces of Turkey. The fruits were randomly collected at the time of optimum harvest maturity, as determined by the Turkish wine producers. Approximately 20 kg of fruit of each cultivar was collected. The grapes were transferred to the lab in a cool Styrofoam box, frozen at –25 °C, and stored in a freezer until further analysis.

2.2. Chemicals

The L-aspartic acid, L-glutamic acid, L-asparagine, DL-serine, L-glutamine, L-histidine, L-threonine, L-arginine, DL-alanine, L-tyrosine, γ-aminobutyric acid (GABA), ethanolamine, L-valine, DL-methionine, DL-tryptophan, L-phenylalanine, L-isoleucine, L-leucine, L-lysine, cysteine, cysteic acid, acetone, acetonitrile, (-)-epicatechin, (-)-catechin, chlorogenic acid, and caffeic acid used in this study were purchased from Sigma-Aldrich (USA). The gallic acid, ferulic acid, ellagic acid, (-)-epicatechin gallate, and procyanidin B1 were purchased from Fluka (USA).

2.3. Extraction and determination of amino acids by HPLC

2.3.1. Extraction of amino acids

First, berries of the Emir and Narince cultivars were manually deseeded. Deseeding was not required for Sultaniye as it is a seedless variety. The samples were then homogenized using a Waring blender. Each homogenate was filtered through cheesecloth and centrifuged at 8000 × *g* for 15 min. Then the supernatant was subjected to derivatization. To start, 30 mL of diethyl ethoxymethylenemalonate, 1.5 mL of methanol, 1 mL of juice sample, and 3.5 mL of borate buffer (1 mol/L, pH 9) were placed in a 10-mL tube with a screw cap. After closing, the tube was placed in an ultrasonic water bath at room temperature for 30 min. The derivatized sample was then kept at 70 °C for 2 h. For cysteine + cystine, 1 mL of supernatant was mixed with 1 mL of a performic acid-hydrogen peroxide mixture (9.5 mL of 99% formic acid : 0.5 mL of 30% hydrogen peroxide) and the mixture was heated to 50 °C for 15 min to oxidize the cysteine + cystine to cysteic acid. After heating, the content was immediately cooled and allowed to stand at –10 °C for 30 min. Then the pH was adjusted to 6.5 using 10 M sodium hydroxide. The content was filtered through a 0.45-µm membrane filter and analyzed by high-performance liquid chromatography (HPLC) (Hermosin, 2003; Gomez-Alonso et al., 2007).

2.3.2. Determination of amino acids by HPLC

An Agilent 1100 HPLC system (Agilent, USA) with a photodiode array detector was used. Separation was carried out with an ACE C18 column (Agilent, UK) (5 µm, 250 mm × 4.6 mm) thermostated at 16 °C. Detection was at 280 with a diode array detector. The elution solvents were acetonitrile (A) and an acetate buffer (25 mm) at pH 5.8 with 0.02% sodium azide (B). Elution was performed with a gradient program of 6% A, 16% A (13 min), 18% A (13.5 min), 18% A (17 min), 22% A (20 min), and 32% A (32 min). The flow rate was 0.9 mL/min and γ-aminobutyric acid was used as an internal standard. Identification was based on the retention times obtained from the pure compounds.

Quantification was achieved using calibration curves obtained from an amino acid of known concentrations. Cysteic acid was used in the preparation of a calibration curve for cysteine + cystine (Bozdoğan and Canbaş, 2011).

The cysteic acid concentrations were then converted to cysteine + cystine using a conversion rate of cysteine + cystine to cysteic acid that was experimentally determined from 5 different cysteine concentrations. The conversion rate was found to be 59.1% (Varga-Visi et al., 2000).

2.4. Extraction and determination of phenolic compounds by HPLC

2.4.1. Extraction of phenolic compounds

To start, 150 g of undamaged grape berries was snipped from clusters. Berries of the Narince and Emir cultivars were manually deseeded and then all of the cultivars were lyophilized using a freeze-dryer (Jouan LP3, France). Next, 4 g of each of the lyophilized berry samples was mixed

with 200 mL of an acetone/water mixture (70/30; v/v) and the extraction was performed by magnetically stirring under nitrogen for 12 h. Each slurry was then filtered with Whatman GF/F filter paper. The extraction was repeated 3 times as described for each cultivar. The extracts were combined and evaporated under vacuum using a vacuum evaporator (BUCHI, Switzerland).

2.4.2. Determination of phenolic compounds by HPLC

Separation of the phenolics was performed on a C18 cartridge (Bound, USA). The grape extracts were passed through the cartridge, which was preconditioned by passing 120 mL of methanol and 120 mL of distilled water through it. The phenol acids and neutral phenols were bound to the resin. Separation of the phenolic acids and sugars was achieved by passing 180 mL of distilled water through the cartridge. The bound neutral phenolic compounds were eluted with 180 mL of MeOH-HCl (99.9/0.1, v/v). Both fractions were dried under vacuum using a vacuum evaporator at 35 °C. The acidic fraction was dissolved in 2 mL of methanol/water/formic acid (40/55/5, v/v/v) and then filtered through a 0.45-μm filter. Analysis of the phenolic acids was carried out on this fraction. The fraction containing the neutral phenolic compounds was dissolved in a small amount of distilled water and then lyophilized. The lyophilized fraction was dissolved in 2 mL of methanol/water/formic acid (40/55/5, v/v/v) and was filtered through a 0.45-μm filter. Analysis of the neutral phenolics was carried out in this fraction (Bourzeix et al., 1986; Freitas et al., 2000; Montealegre et al., 2006).

The analyses were performed using an Agilent 1100 HPLC. Separation was performed on an ACE C18 column (250 mm × 4.6 mm, 5 μm). The elution solvents were water and acetic acid (95/5, v/v) (A) and methanol and acetic acid (95/5, v/v) (B). A gradient consisting of solvents A and B was applied at a flow rate of 0.9 mL/min as follows: 100% A (1 min), 15% A (1–30 min), 0% A (30–95 min), 0% A (95–115 min), and 100% A (115–120 min). For detection, a diode array detector monitored at 280 nm and 320 nm was used. Quantification was achieved by using calibration curves obtained by spiking known amounts of the phenolics compounds.

2.5. Statistical methods

A two-way analysis of variance (ANOVA) was used to test the effects of year and cultivar and a Tukey test was used for means comparison. Data processing was conducted using SPSS 18 for Windows (SPSS Inc., USA).

3. Results and discussion

3.1. Free amino acids

Amino acids are the building blocks of enzymes and other proteins. Yeast can use amino acids as nitrogen and energy sources, which may indirectly generate important flavor compounds such as organic acids, higher alcohols, aldehydes, phenols, and lactones. Despite the fact that some amino acids have bitter, sweet, or sour tastes, it is expected that they are unlikely to contribute to the sensory properties of wine because of their low concentrations (Jackson, 2008). The concentration of assimilable nitrogen in the must of grapes can affect the growth of the yeast and lactic acid bacteria during fermentation, thereby affecting wine quality (Valdes et al., 2011). Free amino acid content directly affects wine quality, because it interferes with the levels of some trace compounds that enhance quality, such as aroma compounds, or have physiological significance, such as ethyl carbamate, or can even be related to wine authenticity (Herbert et al., 2006).

Two-way ANOVA was used to study the differences between the amount of free amino acids due to year, cultivar, and year × cultivar interaction. The results are summarized as mean values and standard deviations in Table 1. Significant differences in amino acid content were found for all amino acids by the year factor, except for isoleucine. The differences between the cultivars occur for all amino acids. The year × cultivar interaction was also statistically different for all amino acids. The combined effect of year and cultivar significantly affected the amino acid content of the grapes.

There were seasonal variations among the cultivars with respect to concentration of total amino acids (Figure). The total free alpha amino acid concentration was determined by summing all of the free amino acids in each juice sample. The total amino acid concentration was highest in the Sultaniye cultivar in 2006 at 1924 mg/L, while the highest from 2007 was found in the Emir cultivar at 1942 mg/L. The total amino acid content and the concentrations of individual amino acids is an important parameter for wine grapes that ultimately influences the final quality of wine, while the amino acids found in table grapes make an important contribution to taste and quality (Jogaiah et al., 2010). It was reported that a range between 330 and 530 mg/L is optimal for normal fermentation of grape must. Must with insufficient nitrogen is associated with the production of hydrogen sulfide taint and arrested fermentation (Person, 2010).

The most abundant amino acid in all of the cultivars was arginine, followed by histidine and alanine. The highest arginine levels in 2006 and 2007 were found in the Sultaniye cultivar at 955 and 910 mg/L, respectively. The Emir cultivar had the highest histidine in 2006 and 2007 at 229 and 308 mg/L, respectively. The highest alanine concentration in 2006 was found in Emir at 99 mg/L, while in 2007 it was in the Narince cultivar at 110 mg/L. Hernandez-Orte et al. (2007) reported that arginine, proline, histidine, and glutamine were the most prominent amino acids in the Tempranillo variety. It was reported

Table 1. Mean values ± standard deviation of free amino acid content (mg/L) in grapes and results of two-way ANOVA.

Amino acid	Factor effect			Year		Cultivar		
	A	B	C	2006	2007	Emir	Narince	Sultaniye
Aspartic acid	**	**	**	41.21^{a} ± 12	29.54^{b} ± 8	33.88^{b} ± 15	27.77^{c} ± 3	44.46^{a} ± 6
Glutamic acid	**	**	**	81.94^{a} ± 27	55.22^{b} ± 17	43.19^{c} ± 12	69.60^{b} ± 5	92.94^{a} ± 26
Aspartic + serine	**	**	**	78.09^{a} ± 37	42.81^{b} ± 8	80.76^{a} ± 44	35.22^{c} ± 2	65.36^{b} ± 13
Glutamine	**	**	**	76.08^{b} ± 23	83.71^{a} ± 26	55.14^{c} ± 4	112.19^{a} ± 6	72.36^{b} ± 3
Histidine	**	**	**	192.44^{b} ± 36	210.21^{a} ± 74	268.43^{a} ± 48	156.73^{c} ± 9	178.81^{b} ± 25
Glycine	**	**	**	8.92^{b} ± 2	11.41^{a} ± 5	8.39^{b} ± 2	15.5^{a} ± 3	6.64^{c} ± 1
Threonine	**	**	**	15.49^{a} ± 6	11.17^{b} ± 1	17.21^{a} ± 6	11.79^{b} ± 1	10.99^{b} ± 1
Alanine	**	**	**	99.42^{a} ± 2	94.38^{b} ± 12	96.65^{a} ± 4	98.11^{a} ± 12	74.56^{b} ± 6
Arginine	**	**	**	685.87^{b} ± 225	863.62^{a} ± 78	784.17^{b} ± 141	607.27^{c} ± 175	932.79^{a} ± 68
GABA	**	**	**	102.77^{b} ± 48	118.42^{a} ± 34	54.95^{c} ± 18	140.69^{a} ± 6	136.13^{b} ± 4
Tyrosine	**	**	**	30.86^{b} ± 7	49.24^{a} ± 17	56.15^{a} ± 16	28.85^{c} ± 2	35.16^{b} ± 11
Ethanolamine	**	**	**	0.55^{a} ± 0	0.54^{b} ± 0	0.52^{b} ± 0.1	0.48^{c} ± 0.1	0.64^{a} ± 0.1
Valine	**	**	*	52.27^{b} ± 2	60.32^{a} ± 4	53.43^{c} ± 3	59.45^{a} ± 6	56.00^{b} ± 5
Methionine	**	**	**	6.85^{a} ± 3	4.14^{b} ± 1	7.22^{a} ± 3	2.65^{c} ± 0	6.61^{b} ± 2
Isoleucine	ns	**	**	16.91^{a} ± 6	17.17^{a} ± 4	20.77^{a} ± 4	18.64^{b} ± 3	11.71^{c} ± 1
Leucine	**	**	**	33.73^{b} ± 3	35.36^{a} ± 4	33.82^{b} ± 4	33.10^{b} ± 4	36.73^{a} ± 3
Tryptophan	**	**	**	50.39^{b} ± 36	60.07^{a} ± 43	108.76^{a} ± 10	22.41^{c} ± 4	34.52^{b} ± 2
Phenylalanine	**	**	**	46.40^{b} ± 10	50.68^{a} ± 15	61.27^{a} ± 10	40.02^{c} ± 8	44.33^{b} ± 11
Lysine	**	**	**	1.32^{b} ± 0.2	1.57^{a} ± 0.1	1.43^{b} ± 0.2	1.36^{c} ± 0.2	1.55^{a} ± 0.1
Cysteine + cystine	**	**	**	12.86^{b} ± 5	14.45^{a} ± 2	15.0^{b} ± 3	15.63^{a} ± 4	10.31^{c} ± 3

A = Year, B = cultivar, C = year × cultivar interaction, * = significant at the 0.05 significance level, ** = significant at the 0.01 significance level, ns = no significant difference, and GABA = γ-aminobutyric acid. Mean values in the same row with the same letter have significant differences between them ($P < 0.05$) for cultivar and year.

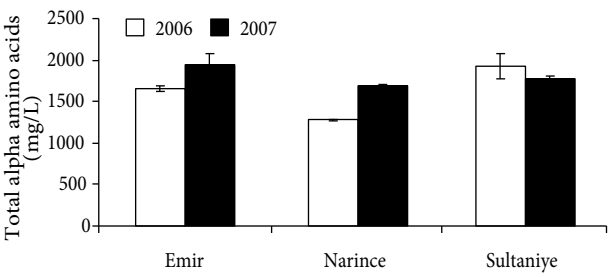

Figure. Seasonal changes in total free alpha amino acids. Error bars (vertical line with horizontal cap above the bar) represent the standard error of the mean.

that arginine is the main amino acid used as a nitrogen source (Jackson, 2008).

When yeasts were presented with a mixture of all amino acids in excess in a model medium, the most important source of nitrogen was arginine (Henschke and Jiranex, 1993). Stines et al. (2000) investigated the free amino acid profiles of the ripe berries of 6 grapevine cultivars (Cabernet Sauvignon, Grenache, Muscat Gordo, Pinot Noir, Riesling, and Sangiovese). They reported that there were compositional differences between the cultivars and that arginine and proline were always the major amino acids. According to their results, mature berries of Cabernet

Sauvignon contained a very high concentration of proline, but a much lower concentration of arginine, while those of the other cultivars contained moderate levels of both arginine and proline. Arginine level is used as an indicator for estimating the nitrogen needs of vineyards for many table grape cultivars (Kliewer, 1967). Many types of yeast cannot metabolize histidine (Henschke and Jiranex, 1993). However, histidine can be converted to histamine by the action of decarboxylating enzymes produced by lactic acid bacteria. It is a biogenic amine and can cause headaches, hypotension, and digestive problems (Moreno-Arribas and Polo, 2005).

The tryptophan level of the Emir cultivar was significantly higher than those of the Sultaniye and Narince cultivars, while the GABA concentration in the Emir cultivar was much lower than in the Sultaniye and Narince cultivars. The concentrations of lysine, ethanolamine, methionine, threonine, cysteine + cysteine, and glycine were considerably lower compared to the other amino acids in all cultivars in both harvest years. Arginine, glutamic acid, threonine, serine, and GABA are referred to as yeast-assimilable nitrogen (Valdes et al., 2011). Tryptophan and its metabolites are considered to be potential precursors of 2-aminoacetophenone, an aroma compound that causes an atypical aging off-flavor in *Vitis vinifera* wines (Hoenicke et al., 2001).

The highest cysteine + cystine level was in the Narince cultivar in 2006 at 21 mg/L, while that in 2007 was in Emir with 18 mg/L. In both 2006 and 2007, the Emir cultivar contained the highest methionine levels at 9.6 and 4.9 mg/L, respectively. Cysteine or cystine and methionine are the only amino acids containing sulfur. The metabolism of cysteine or cystine and methionine, the generation of H_2S, and the presence of cysteinylated conjugates in grapes appear to be the principal sources of thiol compounds in wine. Methionine seems to be less involved. Volatile organosulfur compounds include a wide diversity of straight-chain and cyclic molecules. They form principally during the yeast metabolism of sulfur-containing amino acids, peptides, and proteins (Jackson, 2008).

It has been shown that some grape varieties are typically more resistant to oxidative browning due to a higher content of reductive species that can react with quinones, such as glutathione and ascorbic acid (Moreno-Arribas and Polo, 2005). Similar effects are also postulated for cysteine, which has long been known to be a protoporphyrinogen oxidase inhibitor (Singleton et al., 1985; Ünal et al., 2010). Undesirable sulfur volatiles include hydrogen sulfide (H_2S) and other sulfides, thiols, and mercaptans. These compounds derive from the reduction of sulfates for biosynthesis (H_2S) or from the degradation of sulfur-containing amino acids as nitrogen sources. They can also arise late in fermentation due to the turnover of sulfur-containing components of the yeast, such as glutathione and S-adenosylmethionine, in addition to the sulfur-containing amino acids. Yeast esters, derived from amino acid degradation, confer generic fruity and floral characters to a wine as well as some yeast-specific notes such as toasty characters (Bisson, 2004).

The lysine level ranged between 1.1 and 1.6 mg/L in all cultivars in both years. Lysine is not considered a good nitrogen source for *Saccharomyces* yeasts (Henschke and Jiranex, 1993). It has been reported that there is a high incidence of bacterial strains that produce mousy off-flavors in wine, suggesting that the formation of these off-flavor compounds results from the catabolism of the sugars glucose and fructose and the amino acids ornithine and lysine in the presence of ethanol (Moreno-Arribas and Polo, 2005).

3.2. Phenolic compounds

The results of two-way ANOVA to study the differences between the amount of phenolic compounds due to year, cultivar, and year × cultivar interaction are summarized as mean values and standard deviations in Table 2. The year factor significantly affected all of the phenolic compounds except for gallic acid and chlorogenic acid. Significant differences in phenolic compounds were found between the cultivars. The effect of year × cultivar interaction significantly affected the phenolic content of the grapes ($P < 0.01$). Of the phenolic compounds studied, catechin was the most abundant in all of the cultivars. The highest catechin was found in the Narince cultivar in both years, varying between 106 and 109 mg/kg. Procyanidin B1 and gallic acid were the second most prominent phenolic compounds in all three cultivars. The highest levels of procyanidin B1 and gallic acid were in the Narince cultivar in both years, varying between 23 and 25 mg/kg and 10 and 13 mg/kg, respectively. No epicatechin gallate was detected in any cultivar.

Peinado et al. (2013) investigated the phenolic compounds of Spanish white Pedro Ximenez grapes. They reported a catechin value of 4.21 mg/L, which is much lower than those found in this study. According to their results, the second major phenolic compound was epicatechin with a mean value of 4.17 mg/L, which is higher than those found in this study. Hydroxycinnamic acids (HCAs) are one of the most representative classes of phenolic acids found in both grapes and wine. The main HCA found in grapes and wines are caftaric acid (caffeoyl tartaric acid), p-coutaric acid (coumaroyl tartaric acid), and fertaric acid (feruloyl tartaric acid) (Garrido and Borge, 2013). Meng et al. (2012) reported that (+)-catechin was the most abundant phenolic and the HCAs were the major phenolic acids in spine grapes (*Vitis davidii* Foex), with levels ranging between 13.60 and 29.31 μg/g of fresh sample.

Table 2. Mean values ± standard deviation of phenolic compounds (mg/kg) in grapes and results of two-way ANOVA.

Phenolic compound	Factor effect			Year		Cultivar		
	A	B	C	2006	2007	Emir	Narince	Sultaniye
Gallic	ns	**	**	$8.83^{a} \pm 1$	$8.69^{a} \pm 4$	$3.67^{c} \pm 15$	$6.98^{b} \pm 2$	$8.47^{a} \pm 2$
Chlorogenic	ns	**	**	$2.91^{a} \pm 0.3$	$2.61^{a} \pm 1$	$3.41^{a} \pm 0.7$	$2.28^{b} \pm 0.6$	$2.60^{b} \pm 0.7$
Caffeic	**	**	**	$0.25^{a} \pm 0.1$	$0.18^{b} \pm 0.1$	$0.26^{a} \pm 0.1$	$0.11^{b} \pm 0$	$0.27^{a} \pm 0.2$
Ferulic	**	**	**	$0.39^{a} \pm 0.1$	$0.24^{b} \pm 0.1$	$0.38^{a} \pm 0$	$0.34^{a} \pm 0.1$	$0.24^{b} \pm 0$
Ellagic	**	**	**	$0.13^{a} \pm 0$	$0.06^{b} \pm 0$	$0.16^{a} \pm 0$	$0.06^{c} \pm 0$	$0.08^{b} \pm 0$
Catechin	*	*	*	$79.25^{a} \pm 37$	$57.34^{b} \pm 40$	$72.68^{b} \pm 42$	$36.25^{c} \pm 10$	$95.93^{a} \pm 44$
Epicatechin	*	**	**	$1.60^{a} \pm 0.4$	$1.40^{b} \pm 1$	$2.05^{a} \pm 0.8$	$1.01^{c} \pm 0.4$	$1.43^{b} \pm 0.8$
Procyanidin B1	*	*	**	$19.11^{a} \pm 4$	$16.30^{b} \pm 7$	$20.15^{a} \pm 6$	$14.72^{b} \pm 4$	$18.25^{a} \pm 7$

A = Year, B = cultivar, C = year × cultivar interaction, * = significant at the 0.05 significance level, ** = significant at the 0.01 significance level, ns = no significant difference. Mean values in the same row with the same letter have significant differences between them ($P < 0.05$) for cultivar and year.

Environmental factors (topographical, agropedological, and climatic), usually described by the French term "terroir", have been acknowledged to influence grape and wine quality. A complex relationship exists between the factors that influence grape and wine composition. This is the result of complex relationships between temperature, sunlight, soil, water availability, and the physiological process of the vine variety. Soil and climate are two main factors taken into consideration with regard to influence on grape composition and subsequently wine quality (Kelebek et al., 2010). Climate changes are particularly important for grapevine cultivation, in which heat, drought, and light intensity are just some of the environmental stress factors that dramatically affect chemical composition (Teixeira et al., 2013). In this regard, climatic parameters such as rainfall, temperature, and solar radiation seem to be of special significance (Marais et al., 1999). The average rainfall, temperature, and hours of sunshine values are quite different (meteorological data not shown) in the regions of Cappadocia, Tokat, and Manisa, where the Emir, Narince, and Sultaniye cultivars were grown, respectively. In regard to vineyard characteristics, several researchers have found that soil impacts the overall quality of the grape and thus the resultant sensory quality of the finished wines. For instance, Sayed (1992) and Wiebe and Anderson (1977) found different wine compositions according to soil type. The soil types are also different in the Cappadocia (sand, sandstone, decomposed volcanic, and tufa), Tokat (river bed and glaciated alluvial fan), and Manisa (clay loam in the lower elevations and Akins series alternating with calcareous chalks) regions. Different behaviors of climate and soil type of these three different regions can be correlated with variations in the amino acid and phenolic content of the studied cultivars.

3.3. Conclusions

Both free amino acids and phenolic compounds in wine grapes affect fermentation and wine quality in a number of ways. Free amino acids and ammonia account for the majority of nitrogen-containing compounds that are, next to sugars, quantitatively the most important yeast nutrients in wine grapes for successful alcohol and/or malolactic fermentations. The nitrogen content of the must affects yeast growth, fermentation rate, and time to complete fermentation, and also influences the spectrum of end products of the yeast metabolism. Phenols and related compounds contribute to color, taste, mouthfeel, fragrance, and the antimicrobial and antioxidant properties of wine. Emir, Narince, and Sultaniye are important white wine grape cultivars grown in Turkey. Significant differences in the concentrations of individual and total amino acids were observed among the cultivars. Arginine, histidine, and alanine were the most prominent amino acids in all 3 cultivars in both years, with arginine being the highest found in Sultaniye, varying between 910 and 955 mg/L. The phenolic contents also showed seasonal and varietal variations. Of the phenolics compounds studied, catechin was the most abundant in all three cultivars, with the highest found in Narince, ranging between 106 and 109 mg/kg. Procyanidin B1 and gallic acid were the second most prominent. The differences in the amino acid and phenolic contents of the grape cultivars can be correlated with environmental factors, including mainly climate and soil type.

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