J. Agr. Sci. Tech. (2019) Vol. 21(6): 1507-1520

Amino Acid Composition of White Grape Juices as Affected by Soil Urea Fertilization

M. Karoglan[1*], L. Maslov Bandic[1], M. Osrecak[1], M. Mihaljevic Zulj[1], B. Kozina[1], and A. Jeromel[1]

ABSTRACT

The aim of this work was to test the effects of soil urea fertilization on yield, grape juice soluble solids, total acidity, and amino acid composition of Chardonnay, Welschriesling, and Riesling grape varieties. The experiment was conducted in a vineyard with repeated records of low yeast-assimilable-nitrogen content in must and the corresponding grape juices in years prior to the experiment. Urea was applied after completion of flowering. Treatments included the control without fertilization, 5.5, 16.8 and 28.1 g N vine^{-1}. Urea fertilization generally increased yield components and amino acid concentrations. Fertilization with 28.1 g N vine^{-1} prolonged grape ripening, regarding soluble solids and total acidity values. Fertilization with 28.1 g N vine^{-1} was not so effective in improving amino acid concentration compared to other fertilization treatments. This leads to conclusion that fertilization with 28.1 g N vine^{-1} seems excessive and unnecessary regarding delayed fruit ripening and inconsistent effect on amino acid composition.

Keywords: Chardonnay variety, Excessive fertilization, Riesling variety, Welschriesling variety, Vineyard.

INTRODUCTION

Grape juice amino acid concentrations depend upon variety, growing conditions, harvest date, soil nitrogen availability, and nitrogen fertilization (Kliewer, 1970; Bell *et al*., 1979). Amino acid profiles of grape juices are usually dominated by proline and arginine, followed by a range of other less abundant primary amino acids (Hernàndez-Orte *et al*., 1999; Hilbert *et al*., 2003; Hannam *et al*., 2016). Primary amino acids form an important part of Yeast Assimilable Nitrogen (YAN) whose low concentration may result in a stuck fermentation (Kunkee, 1991; Monteiro and Bisson, 1991; Spayd *et al*., 1995; Bell and Henschke, 2005). Grape juice amino acid profiles have an important implication in the formation of higher alcohols and esters in wine (Bell and Henschke, 2005). High rates of nitrogen fertilizers cause nitrate pollution of groundwater, affect excessive vine growth and, consequently, delay ripening and enhance diseases (Coombe and Dry, 1992; Spayd *et al*., 1994). Furthermore, high rates of N-fertilizers could cause nitrate pollution of groundwater (Bell and Henschke, 2005). This leads to low application rates of N-fertilizers resulting in insufficient grape juice YAN concentrations, especially if grapevines are grown on nutrient-poor soils (Hannam *et al*., 2014). However, soil applications of N-fertilizers can still be used to improve total nitrogen and amino acid concentrations in grape juice (Hilbert *et al*., 2003; Linsenmeier, *et al*., 2008). Many

[1]Department of Viticulture and Enology, Faculty of Agriculture, University of Zagreb, Svetošimunska cesta 25, Zagreb, Croatia.
*Corresponding author; e-mail: mkaroglan@agr.hr

papers have dealt with the problem of timing, doses, and forms of N-fertilization to improve grape juice amino acid composition (Lasa *et al*., 2012; Garde-Cerdàn *et al*., 2014; Hannam *et al*., 2016), thus showing different efficiency depending on treatment. This two-year study focused on the efficiency of urea soil application immediately after completion of flowering, in a vineyard selected for the experiment because of repeated records of low must YAN content in years prior to the study (personal unpublished data). The problem of low must nitrogen is common on anthropogenic pseudogley soil types which are poorly supplied with nitrogen. These soils are among the most widespread soil types in the northwestern region of Croatia. Common soil nitrogen fertilization practices rarely consider precise fertilization rates, and are often based on assessment of the individual grape producer.

The objective of this study was to determine the effect of different urea application rates on amino acid composition of grape juices and wines from Chardonnay, Riesling and Welschriesling (*Vitis vinifera* L.) grapevines.

MATERIALS AND METHODS

Experimental Site

A two-year study (2006 through 2007) on urea soil fertilization was conducted on Chardonnay, Riesling, and Welschriesling (*Vitis vinifera* L.) grape varieties at Jazbina Experimental Field (University of Zagreb, Faculty of Agriculture, long. 45˚ 51’ N, lat. 16˚ 0’ E). Soil type was anthropogenic pseudogley with clay texture and depth of 110 to 130 cm, characterized by low organic matter content, low pH, and limited nutrient content.

The growing season average temperature (April to October) in 2006 was 17.5°C, with 425.5 mm precipitation. The growing season in 2007 had similar average temperature of 17.6°C, but the measured precipitation was significantly higher, with 560.9 mm. Non-irrigated, 21 years old *Vitis vinifera* L. varieties grafted on SO4 (*Vitis riparia*×*Vitis berlandieri*) were trained to double Guyot, leaving 24 buds per vine. Plantation density was 4,167 vines per hectare (2 m between rows and 1.2 m between grapes). Vines were managed according to usual commercial practices for this viticultural area. Application of glyphosate was used along the vine row (1 m wide) to eliminate ground vegetation. Shoots exceeding the height of the trellis were hedged to 20 cm above the last wire, 4 weeks before veraison.

The vines received no fertilizer in the year preceding the experiment. Soil analysis was done prior to the first experimental year (March 2006) and showed that soil was very acid with a surface pH (in KCl) of 4.2 (0 to 30 cm depth). The soil was very poor in organic matter, ranging from 0.7% (0 to 30 cm depth) to 1.5% (30 to 60 cm depth). The deep horizon of soil was richer in organic matter due to the trenching of soil up to 60 cm depth, performed prior to the planting of vines. Available P and K were very low ranging from 6 mg of P_2O_5 100 mg^{-1} soil, and 14 mg of K_2O 100 mg^{-1} soil, respectively. The soil was moderately supplied with nitrogen, ranging from 0.07 (0 to 30 cm depth) to 0.12% (30 to 60 cm depth). Since all experimental varieties were grown in the same vineyard, three soil samples were pooled together to get the average data analysis.

Treatments and Experimental Design

The experiment used a random block design, with four treatments in three replications for each variety. Each plot consisted of 8 continuous vines, with 8 untreated vines as a separation between plots. There were also two non-treated rows between experimental ones. Thus, each treatment consisted of 24 vines. There were 12 grape samples per experimental variety. Urea was chosen for its high availability when dissolved in the soil. Fertilization was

performed immediately after completion of flowering, corresponding to stage 26 according to modified Eichorn and Lorenz system (Coombe, 1995). In both years of the study, Urea dissolved in water was applied manually to the soil surface, all across the plot and directly beneath the vines. Each experimental vine was watered to facilitate urea dissolution and movement to the roots. Treatments were four levels of fertilization as follows: (1) control without any fertilization ("C"), (2) 5.5 ("N1"), (3) 16.8 ("N2"), and (4) 28.1 g N $vine^{-1}$ ("N3"). The cumulative application rates were 23 kg N ha^{-1} $year^{-1}$ for N1 treatment, 70 kg N ha^{-1} yr^{-1} for N2, and 117 kg N ha^{-1} yr^{-1} for N3.

Yield Components and Juice Analysis

Grapes were harvested manually at their full maturity when the total soluble solids (SS) of 200 g of randomly collected berries remained constant for a few days. In 2006, Chardonnay was harvested on 27th September (270th Day Of the Year- DOY), while Riesling and Welschriesling were harvested on 30th September (273 DOY). In 2007, Chardonnay was harvested on 5th September (248 DOY), Riesling on 12th September (255 DOY) and Welschriesling on 13th September (256 DOY). Each treatment and replicate was harvested the same day and processed separately. Number of clusters and yield per vine were determined, so cluster weight and yield per vine were calculated based on collected data. After crushing and destemming, the grapes were pressed and put into 15-L vessels. Grape juice samples were collected immediately after pressing, for soluble solids, total acidity and pH analysis. Additionally, grape juice samples for amino acid analysis were frozen at -18°C. Total Soluble Solids (TSS) were determined using refractometer (expressed in °Brix) and Total Acidity (TA, g L^{-1}) was measured by titration with 0.1M NaOH according to OIV method (OIV, 2013).

Amino Acid Analysis

Chemicals and Reagents

The 15 standards of L-amino acids, *o*-phtaldehyde, iodoacetic acid, propionic acid, 2-sulphanylethanol, and disodium hydrogen phosphate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile, dimethylsulfoxide, and methanol were obtained from JT Baker (Derventer, Netherlands).

Preparation of Standard Solution, Reagents, and Sample Derivatization

Analysis of amino acids was performed according to the method described by Pripis-Nicolau *et al*. (2001) with suitable modifications for our analysis. Standard solutions of amino acids were prepared in water. Few drops of 1M HCl were added. Borate buffer was prepared by dissolving 6.2 g of H_3BO_3 in 800 mL of distilled water and adjusting the pH= 9.5 with 4M NaOH. The final volume of 1,000 mL was made up by water. The *o*-phtaldehyde (750 mg) was dissolved in 5 mL methanol, and 0.5 mL of 2-sulphanyletanol was added. The solution was made up to 50 mL with borate buffer. The iodoacetic solution was prepared by adding 3.5 mg of iodoacetic acid in 50 mL of borate buffer and pH was adjusted to 9.5 with 4M NaOH.

HPLC Analysis

HPLC analysis was performed using Agilent 1100 Liquid Chromatograph, equipped with a fluorescence detector (Agilent 1200). The excitation and emission wavelengths were 356 nm and 445 nm, respectively. Separation of amino acid derivates was obtained by Lichroshere RP 18 column (125 mm×4 mm×5 μm). Precolumn derivatization was done using *o*-phtaldialdehyde and iodoacetic acid. Mobile phase A was a mixture of 230 mL of 250 mM

disodium hydrogen phosphate, 200 mL of 250 mM propionic acid and 20 mL of dimethyl sulphoxide adjusted pH to 6.65 with 4M NaOH followed by the addition of 65 mL of acetonitrile and made up to 1,000 mL with water. Mobile phase B was a mixture of 400 mL of acetonitrile, 330 mL of methanol, 70 mL of dimethyl sulphoxide and 200 mL of water. Flow rate was 0.8 mL min^{-1}. The analysis time was 125 minutes. A sample of must was filtered through 0.45 μm PTFE (Phenomenex, USA) membrane filters prior analysis. Injection program of on-line derivatization and gradient elution program is described in the Table 1.

Statistical Analysis

All analyses were performed separately by cultivar using two-way Analysis Of Variance (ANOVA), with year, treatment, and year×treatment interaction as independent variables in the model. Multiple tests of differences between means of the significant factor levels were performed using Bonferroni correction ($P \leq 0.05$). When the interaction year*treatment was found significant in the model, multiple comparisons were made between means of different treatments within the same year with appropriate Bonferroni correction. A canonical discriminant analysis was performed to evaluate the utility of amino acids in grape juice samples for discrimination between treatments within each cultivar. Squared Mahalanobis distance was calculated between centroids of treatments based on amino acids composition, and significance of these differences was determined. Data were analyzed using SAS statistical software, version 9.4 (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Yield Components and Juice Quality

Yield and juice quality parameters are presented in Table 2. Higher yield was produced in the second year of the study, regardless of treatments. Yield parameters

Table 1. Injection program of on-line derivatization and gradient elution program.

a) Injection program					b) Gradient elution program		
Command	Amount (μL)	Vial	Reagent	Speed (μL min^{-1})	Time	% B	Flow
DRAW	10	VIAL 1	H_2O	300	0	5	0.800
EJECT	10	VIAL 100		300	2	10	0.800
DRAW	10	VIAL 1	H_2O	300	35	10	0.800
EJECT	10	VIAL 100			45	30	0.800
DRAW	2	SAMPLE VIAL		300	80	30	0.800
DRAW	0	VIAL 1	H_2O		85	40	0.800
DRAW	5	VIAL 2	IDA	300	110	80	0.800
DRAW	0	VIAL 1	H_2O		115	80	0.800
MIX	12	IN AIR		500, 15 TIMES	120	50	0.800
WAIT 2 MIN					125	5	0.800
DRAW	5	VIAL 3	OPA	300			
DRAW	0	VIAL 1	H_2O				
MIX	17	IN AIR		500, 15 TIMES			
WAIT 2.50 MIN							
MIX	17	IN AIR		500, 15 TIMES			
WAIT 2.50 MIN							
INJECT							

Table 2. Yield components and juice analysis as affected by soil urea fertilization in Chardonnay, Welschriesling and Riesling vineyard (2006 and 2007).[a]

		Yield per vine (kg)	Cluster weight (g)	Soluble solids (°Brix)	Total acidity (g L^{-1})	pH
Chardonnay						
Treatment						
2006	C	3.6 b	113.7 ab	24.2 a	11.7 c	2.98 a
	N1	3.6 b	100.0 b	24.0 ab	11.9 b	2.87 c
	N2	3.7 b	129.4 a	23.8 b	10.5 d	2.96 b
	N3	4.0 a	116.6 ab	22.2 c	12.8 a	2.96 b
	Sig	*	*	*	*	*
2007	C	4.2 a	138.6	22.0 b	7.4 c	3.14 ab
	N1	4.3 a	141.3	22.0 b	8.1 a	3.13 b
	N2	4.0 b	121.2	22.6 a	7.7 b	3.11 c
	N3	4.2 a	137.6	22.0 b	8.1 a	3.15 a
	Sig	*	ns	*	*	*
Year						
	2006	3.7 b	114.9 b	23.6 a	11.7 a	2.94 b
	2007	4.2 a	134.7 a	22.2 b	7.8 b	3.13 a
	Sig	*	*	*	*	*
Treatment×Year		*	*	*	*	*
Welschriesling						
Treatment						
2006	C	3.3 b	118.8	22.4 a	6.5 d	3.93 a
	N1	3.3 b	112.4	22.0 b	6.7 c	2.91 b
	N2	3.5 a	124.8	21.8 b	7.1 b	2.94 a
	N3	3.5 a	109.6	21.8 b	7.3 a	2.87 c
	Sig	*	ns	*	*	*
2007	C	3.8 c	128.0	20.2 a	6.2 b	3.32 c
	N1	3.8 c	125.1	20.2 a	6.4 a	3.37 ab
	N2	4.0 b	133.8	19.8 b	6.3 ab	3.36 b
	N3	4.3 a	121.0	19.6 b	6.4 a	3.38 a
	Sig	*	ns	*	*	*
Year						
	2006	3.4 b	116.4	22.0 a	6.9 a	2.91 b
	2007	4.0 a	127.0	20.0 b	6.3 b	3.36 a
	Sig	*	ns	*	*	*
Treatment×Year		*	ns	ns	*	*
Riesling						
Treatment						
2006	C	3.5 b	94.6 a	23.0 d	10.2 d	2.59 a
	N1	3.7 a	99.2 a	24.8 a	13.6 a	2.54 b
	N2	3.0 c	80.6 b	24.0 c	13.4 b	2.53 b
	N3	3.7 a	97.4 a	24.4 b	11.2 c	2.59 a
	Sig	*	*	*	*	*
2007	C	3.8 c	106.5 b	21.2 b	8.7 d	3.05 c
	N1	3.8 c	97.5 d	21.6 a	8.9 c	3.10 b
	N2	4.2 a	116.7 a	21.2 b	9.4 b	3.13 a
	N3	4.0 b	102.6 c	21.2 b	9.6 a	3.10 b
	Sig	*	*	*	*	*
	2006	3.5 b	93.0 b	24.1 a	12.1 a	2.6 b
	2007	4.0 a	105.8 a	21.3 b	9.2 b	3.1 a
	Sig	*	*	*	*	*
Treatment*Year		*	*	*	*	*

[a] N1, N2 and N3: Indicate treatments of soil urea fertilization of 5.5, 16.8, and 28.1 g N $vine^{-1}$, respectively. C indicates control treatment without fertilization. Means with the same letter are not significantly different within cultivars and years (mean separation by Bonferroni correction at $P \leq 0.05$). * and ns: Indicate significant at $P \leq 0.05$ and not significant, respectively.

were also slightly increased by nitrogen fertilization, similar to Hannam *et al*. (2016). The differences were caused by the differences in cluster number per vine (data not shown), and not by increased cluster weight. Cluster weight values were not so responsive to fertilization treatments, especially in 2007 year, but were generally higher in fertilization treatments. There was an effect of experimental year on yield parameters, except on Welschriesling cluster weight, the same as for the interaction between year and treatment.

Higher yield reflected on juice quality, since higher SS values were measured in 2006. This could be also related to meteorological conditions, since 2007 had significantly higher precipitation. For example, in 2007 September, 136 mm was recorded, while in 2006 September only 68 mm of precipitation was recorded. The highest SS values, indicating earlier ripening, were never found in N3 treatments. Riesling grape juice SS values were increased by N1 treatments in both years. N2 treatment affected the highest SS values in Chardonnay grape juices from 2007. This is not in accordance to many other reports that stated that nitrogen fertilization generally affected lower SS values in grape juice (Peacock *et al*., 1991; Spayd *et al*., 1994, Bavaresco *et al*., 2001).

Grape juice TA was affected by nitrogen fertilization, resulting in significantly higher TA levels in almost all experimental juices, when compared to the control. The only exception was Chardonnay N2 grape juice from 2006, which had the lowest TA level. This result is similar to previous works (Bell *et al*., 1979; Christensen *et al*., 1994). Regarding pH values, no consistent trends were observed. Generally, N3 grape juices had the lowest SS values and the highest TA level, so, it could be concluded that fertilization with 28.1 g N $vine^{-1}$ significantly prolonged grape ripening.

Year effect was observed for all grape juice chemical components and for each cultivar. The interaction between year and treatment was observed for all grape juice chemical components, except for Welschriesling SS values.

Grape Juice Amino Acid Composition

Grape juice amino acid compositions of experimental cultivars are presented in Tables 3-5. Cystein is the only amino acid that did not consistently responded to applied treatments. Year effect on grape juice amino acid composition was observed for all amino acids, except for glycine and phenylalanine in Chardonnay, and leucine in Riesling juices. The interaction between year and treatment was not observed only for cysteine in Welschriesling grape juice.

There were five major amino acids in grape juices, measured each year in grape juices from all cultivars: arginine, histidine, alanine, tyrosine, and glutamate. These amino acids accounted for approximately 65% of the total amino acid content, although this percentage varied from 52 to 75%, depending on the variety and year.

Arginine was the predominant amino acid, accounting for about 31% of the total free amino acid concentration. This is in accordance to many other authors (Bell and Henschke, 2005; Bouzas-Cid *et al*., 2015). The percentage of arginine in Welschriesling grape juice even exceeded 40% of the total amino acid concentration. Still, arginine concentrations in the present experimental juices were very low when compared to other works (Huang and Ough, 1991; Spayd and Andersen-Bagge, 1996). Significant quantities of alanine was found in Welschriesling and Chardonnay juices, the latter being in accordance to Huang and Ough (1991).

Cysteine followed by glycine and isoleucine were among the less abundant amino acids. They accounted for less than 5% of the total amino acid content.

Quantitatively, Chardonnay was the variety with the highest concentration of total primary amino acids (regardless of the fertilization treatments), while Riesling grape juices had the lowest concentrations.

Table 3. Grape juice amino acid composition (mg L^{-1}) as affected by soil urea fertilization in Chardonnay vineyard (2006 and 2007).[a]

		Asp	Glu	Cys	Ser	His	Gly	Thr	Arg	Ala	Tyr	Val	Phe	Ile	Leu	Lys	Total
Treatment																	
	C	7.0c	6.7d	0.1	6.1d	7.8d	5.5a	1.7d	28.7d	12.2d	18.6b	0.6d	1.3b	1.7a	1.6c	7.0b	101.4c
	N1	10.1b	15.6b	0.1	14.5b	22.6c	2.5b	8.2c	55.8c	45.3a	47.2a	1.5b	2.7a	1.4b	2.3a	8.6a	238.4a
2006	N2	10.0b	15.2c	0.1	13.7c	25.2a	1.7c	9.2b	60.0b	42.3b	11.1c	1.3c	2.6a	1.6a	2.0b	5.1d	201.1b
	N3	11.6a	21.2a	0.1	19.4a	24.4b	1.0d	10.7a	75.5a	41.8c	11.2c	1.7a	2.7a	1.1c	1.4d	5.8c	229.6a
	Sig	*	*	ns	*	*	*	*	*	*	*	*	*	*	*	*	*
	C	5.5c	17.1a	0.8c	1.2d	15.5a	1.2c	2.3c	19.7c	13.5a	3.8a	3.3c	1.6d	5.2c	9.7b	10.5c	110,9a
	N1	5.4c	13.3b	0.7c	1.4c	12.1b	1.0d	2.4c	19.6c	10.0c	3.2b	3.0d	2.0c	5.6b	8.1d	8.2d	96.0c
2007	N2	7.0b	10.0c	1.2a	2.5b	11.9c	3.6b	3.6b	23.2b	10.1c	1.7d	4.3b	2.2b	4.8d	8.4c	11.5b	106.0b
	N3	8.4a	8.3d	1.0b	2.7a	10.7d	5.2a	4.4a	24.4a	11.9b	2.2c	4.6a	3.3a	5.6a	10.4a	11.8a	114.9a
	Sig	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Year																	
	2006	9.7a	14.7a	0.1b	13.4a	20.0a	2.7	7.5a	55.0a	35.4a	22.0a	1.3b	2.3	1.5b	1.8b	6.6b	192,6a
	2007	6.6b	12.2b	0.9a	2.0b	12.6b	2.8	3.2b	21.7b	11.4b	2.7b	3.8a	2.3	5.5a	9.2a	10.5a	107.0b
	Sig	*	*	*	*	*	ns	*	*	*	*	*	ns	*	*	*	*
	Treatment×Year	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

[a] Means with the same letter are not significantly different within cultivars and years (mean separation by Bonferroni correction at $P \leq 0.05$). Abbreviations: Asp– Aspartate, Glu– Glutamate, Cys– Cysteine, Ser– Serine, His– Histidine, Gly– Glycine, Thr– Threonine, Arg– Arginine, Ala– Alanine, Tyr– Tyrozine, Val– Valine, Phe– Phenylalanine, Ile– Isoleucine, Leu– Leucine, Lys- Lysine.

Table 4. Grape juice amino acid composition (mg L^{-1}) as affected by soil urea fertilization in Welschriesling vineyard (2006 and 2007).[a]

		Asp	Glu	Cys	Ser	His	Gly	Thr	Arg	Ala	Tyr	Val	Phe	Ile	Leu	Lys	Total
Treatment																	
	C	9.3d	8.9d	0.2ab	2.1d	0.8d	0.8b	4.7a	28.2d	5.8d	2.2c	2.6c	2.6d	1.9c	2.8c	9.4a	82.3c
	N1	12.4b	13.9b	0.3a	5.8a	4.1c	0.8b	3.3c	65.3c	13.3c	2.8b	3.9b	3.7c	3.6a	4.5a	3.5c	141.2b
2006	N2	14.7a	14.7a	0.1b	4.0c	6.8a	1.2a	4.4b	77.8b	16.4a	3.1a	4.2a	5.0a	3.5a	4.4a	3.2d	163.5a
	N3	10.6c	11.7c	0.2ab	4.6b	5.4b	1.2a	3.1d	78.2a	15.7b	3.1a	3.8b	3.9b	2.9b	3.8b	6.0b	154.2a
	Sig	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	C	9.2d	9.5d	0.3	2.3d	9.7d	1.7c	4.4c	25.6d	10.6d	5.1c	3.5d	2.9c	1.7c	2.9c	3.7d	93.1d
	N1	10.6c	12.4c	0.4	3.6c	16.2c	2.3b	4.7b	68.5c	18.9b	4.0d	5.3a	5.9a	2.6b	3.2b	4.3c	162.9c
2007	N2	11.5b	15.1b	0.3	4.0b	16.6b	2.6a	6.1a	79.3b	17.9c	5.5b	4.4c	5.9a	3.7a	2.9c	4.5b	180.3b
	N3	15.2a	19.1a	0.3	4.3a	18.9a	2.5a	6.1a	90.1a	19.3a	8.6a	4.8b	4.3b	3.6a	3.6a	5.2a	205.9a
	Sig	*	*	ns	*	*	*	*	*	*	*	*	*	*	*	*	*
Year																	
	2006	11.8a	12.3b	0.2b	4.1a	3.4b	1.0b	3.9b	62.4b	12.8b	2.8b	3.6b	3.8b	3.0a	3.9a	5.5a	135,3b
	2007	11.6b	14.0a	0.3a	3.6b	15.4a	2.3a	5.3a	68.4a	16.7a	5.8a	4.5a	4.8a	2.9b	3.2b	4.4b	160.6a
	Sig	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Treatment×Year		*	*	ns	*	*	*	*	*	*	*	*	*	*	*	*	*

[a] Means with the same letter are not significantly different within cultivars and years (mean separation by Bonferroni correction at $P \leq 0.05$). Abbreviations: As in previous table. * and ns: indicate significant at $P \leq 0.05$ and not significant, respectively.

Table 5. Grape juice amino acid composition (mg L^{-1}) as affected by soil urea fertilization in Riesling vineyard (2006 and 2007).[a]

		Asp	Glu	Cys	Ser	His	Gly	Thr	Arg	Ala	Tyr	Val	Phe	Ile	Leu	Lys	Total
Treatment																	
	C	3.1d	3.0d	0.1b	2.7a	11.0a	1.3b	2.6d	10.5d	7.0a	8.0d	1.2d	2.1c	2.4b	2.6d	3.5d	61.1b
	N1	5.8a	6.0b	0.5a	2.2b	8.9b	1.5a	2.8c	26.9a	7.0a	11.1b	3.8b	3.3a	3.3a	3.4b	7.5a	94.0a
2006	N2	4.8b	6.3a	0.2b	1.7c	8.2c	1.4ab	3.9a	21.1c	5.9c	11.4a	4.6a	2.5b	2.5b	3.9a	6.7b	85.1a
	N3	4.5c	5.8c	0.1b	1.3d	5.4d	1.1c	3.3b	25.9b	6.4b	9.4c	3.1c	2.1c	2.1c	3.0c	5.8c	79.3a
	Sig	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
	C	5.3d	3.1c	0.2	5.5c	7.6d	1.6c	2.9d	9.1d	2.7c	7.3c	2.6d	2.0d	1.3b	2.4c	4.3c	57.9c
	N1	6.8c	3.9b	0.1	6.2b	7.9c	2.6a	4.0c	13.1c	2.2d	8.6a	3.6b	4.5c	1.8a	3.7a	4.6b	73.6b
2007	N2	7.3b	5.3a	0.2	5.2d	11.2a	2.3b	4.7b	15.5b	3.4b	8.7a	3.4c	4.7b	1.7a	3.4b	4.0d	81.0b
	N3	7.9a	5.2a	0.1	8.2a	10.1b	2.4b	5.5a	29.2a	4.4a	7.9b	4.4a	6.9a	1.8a	3.3b	5.1a	102.4 a
	Sig	*	*	ns	*	*	*	*	*	*	*	*	*	*	*	*	*
Year																	
	2006	4.6b	5.3a	0.3a	2.0b	8.4b	1.3b	3.2b	21.1a	6.6a	10.0a	3.2b	5.2b	2.6a	3.2	5.9a	79.9a
	2007	6.8a	4.4b	0.2b	6.3a	9.2a	2.2a	4.3a	16.7b	3.2b	8.1b	3.5a	4.5a	1.7b	3.2	4.5b	78.7b
	Sig	*	*	*	*	*	*	*	*	*	*	*	*	*	NS	*	*
	Treatment×Year	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

[a] Means with the same letter are not significantly different within cultivars and years (mean separation by Bonferroni correction at P≤ 0.05). Abbreviations: As in previous tables.
* and ns: Indicate significant at $P \leq 0.05$ and not significant, respectively.

This confirms previous work by Yokotsuka and Fukui (2002) who claimed that Riesling had the lowest amino acid concentration in grape juice among 6 experimental varieties.

Chardonnay and Riesling grape juices from 2006 were richer in amino acids than juices from 2007, but Welschriesling grape juices from 2007 had higher level of amino acids than those from 2006. In general, amino acid concentrations were greater in 2006, probably caused by favorable climatic conditions, since 2006 was a drier year. This trend is in accordance to Hannam *et al*. (2013) and Ortega-Heras *et al*. (2014) who observed greater amino acids concentrations in the drier year.

Nitrogen fertilization generally increased concentrations of amino acids over those of the control samples (Tables 3-5). This pattern is similar to previously reported results of Free Amino Nitrogen (FAN), ammonium ion (NH_4+) and Yeast Assimilable Nitrogen (YAN) values in the same grape juices (Karoglan *et al*., 2011). The concentration of total amino acids in grape juices was increased with urea fertilization by more than 100% in Chardonnay (in 2006) and Welschriesling (in 2007) grape juices. The N3 treatment was the most effective in increasing amino acid content, especially in 2007. This is in accordance with the study of Neilsen *et al*. (2010) who found out that application of 80 kg of urea-N ha^{-1} at bloom was sufficient to increase grape juice YAN status above the 140 mg L^{-1}.

Amino acids that were most responsive to nitrogen fertilization were aspartate, arginine, valine, phenylalanine, isoleucine, and leucine. The concentration of histidine was the highest in the control grape juices from Riesling 2006, and Chardonnay 2007. Other amino acids concentrations varied depending on variety, year, and fertilization rate. N3 treatment indeed increased amino acid concentration in grape juices over the control ones, but the treatment was not so effective when compared to other fertilization treatments, especially N2. It is notable that N2 treatment was even more effective in increasing amino acids concentration than N3 in 2006 year, especially regarding Riesling and Welschriesling juices. Furthermore, N1 juices of Chardonnay and Riesling from 2006 year had the highest total amino acid concentration. Therefore, having in mind the delayed ripening and not so consistent effect on increase of amino acid level in grape juices, N3 fertilization seems excessive and unnecessary. This is similar to work of Ough and Lee (1981), who found out that additional increase in fertilizer rates did not affect concentrations of total nitrogen and arginine in must. Furthermore, Nielsen *et al*. (2013) found out that soil N fertilization did not consistently improved grape juice YAN concentration, thus implying that soil N fertilization is not reliable for improving grape juice N status.

However, nitrogen fertilization did not always give consistent and clear results, regarding juice amino acid concentration. Accordingly, some specific amino acids as well as total amino acids concentration tend to decrease with the increase of nitrogen fertilization rates. Similar results are reported by Bell *et al*. (1979) and Monteiro and Bisson (1991) who claim that nitrogen compounds in grape juices do not always follow the increase in the nitrogen fertilization rates. Lasa *et al*. (2012) stated that increase of juice YAN and total amino acid concentration after foliar urea application depend on season and time of application. The same authors claim that later applications (veraison or 3 weeks after veraison) were more effective in increasing juice N compounds than application 3 weeks before veraison. The present study as well as many previous results (Lasa *et al*., 2012; Garde-Cerdan *et al*., 2014; Hannam *et al*., 2016) lead us to the conclusion that nitrogen fertilization does not always show clear and consistent effect on grape composition, since the results tend to vary in different experiments.

Therefore, modification of the time of soil urea application, in addition or in combination with foliar fertilization, could

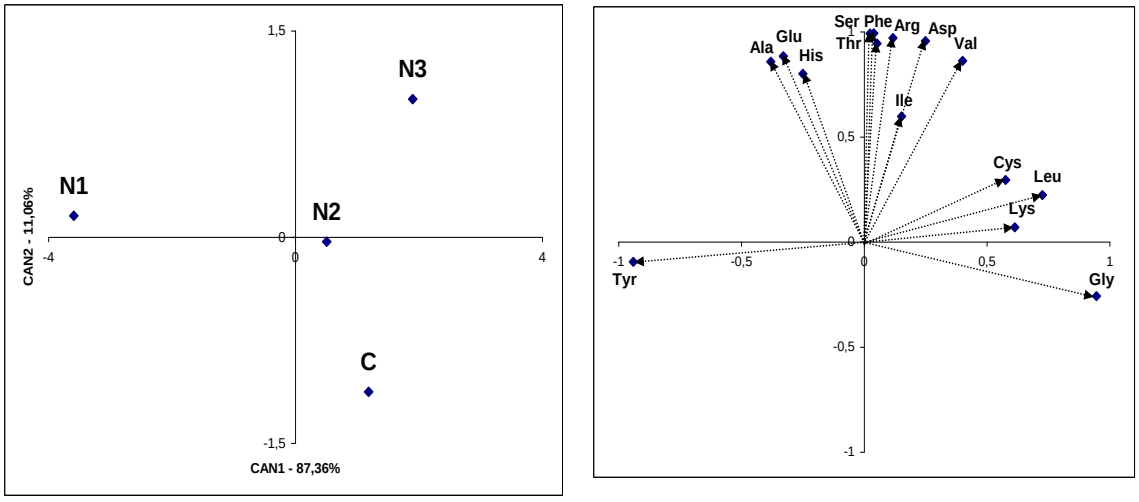
solve the problem of low N in grape juices from our experimental vineyard.

Many of these amino acids are precursors of higher alcohols and esters, important flavor compounds in wine (Bell and Henschke, 2005). Three nonpolar branched-chain amino acids, such as valine, leucine, and isoleucine, are direct precursors of higher alcohols and volatile fatty acids (Styger *et al*., 2011). In this study, these amino acids appeared in very low concentrations, accounting for an average of 7.3% in Welschriesling juices to 10.9% in Riesling juices. However, nitrogen fertilization increased the respective amino acids concentrations. Threonine greatly affects wine aroma composition, due to its relation to odorants from the fatty acid synthesis. Thus, Chardonnay exhibited the greatest aromatic potential regarding threonine concentration in the grape juice, irrespective of the treatments. Nitrogen fertilization, however, affected greater threonine concentrations. In the present study, Welschriesling juices had the highest concentrations of phenilalanine, amino acid known as a precursor of *β*-phenylethanol and isobutanol (Hernàndez-Orte *et al*., 2002). In this study, phenilalanine content was also strongly affected by vineyard nitrogen fertilization.

It has been reported that glutamine, asparagine, arginine, glutamate, serine, alanine and aspartate showed the best correlation with yeast cell growth (Monteiro and Bisson, 1991). Regarding this fact, nitrogen fertilization, particularly N3 treatment, caused significant increase in arginine and aspartate concentration in all juice samples and in both years. Concentrations of other mentioned amino acids varied within varieties and years.

Based on 15 amino acids, Canonical discriminant analysis between grape juices of all experimental cultivars from different treatments and the two years of research shows highly significant squared Mahalanobis distances between all treatments.

Based on position of Chardonnay grape juices from different treatments on scatter plot from the first and second canonical variables, and correlation of amino acids with the first two canonical variables (Figures 1-a and -b), it can be seen that distance between the control and N3 treatment is based on higher content of serine, threonine, phenylalanine, arginine,



(a) (b)

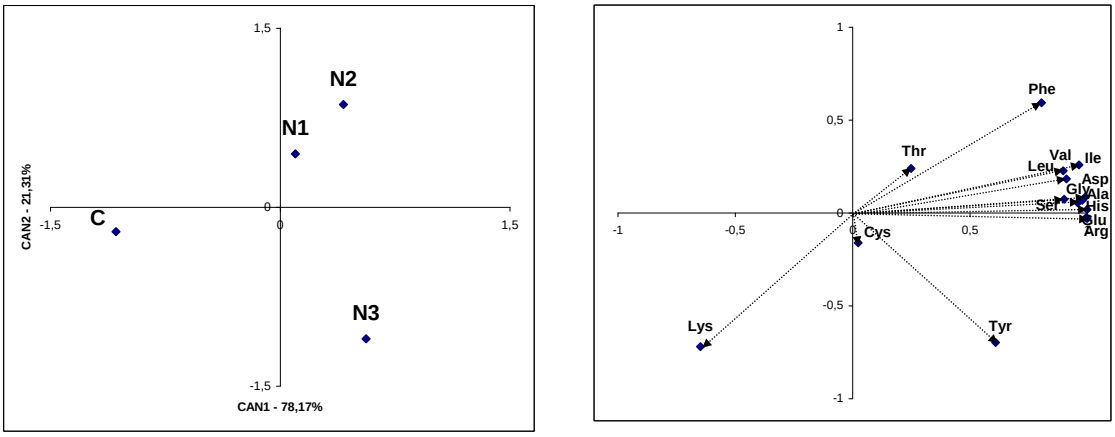
Figure 1-a). Position of *cv*. Chardonnay grape juice from different treatments in the space defined by the first two canonical variables from canonical discriminant analysis using their amino acid composition in the two years of study. **b)** Vector diagram of correlation of grape juice amino acid composition and the first two canonical variables based on different treatments shown in Figure 1-a.

aspartate and valine in N3 grape juices.

Regarding Welschriesling grape juices, distance between N3 and other treatments can be explained by the highest tyrozine concentration in N3 juices. Distance between the control and fertilization treatments is explained by lower concentration of all amino acids, except threonine and cysteine in the control grape juices (Figures 2-a and -b).

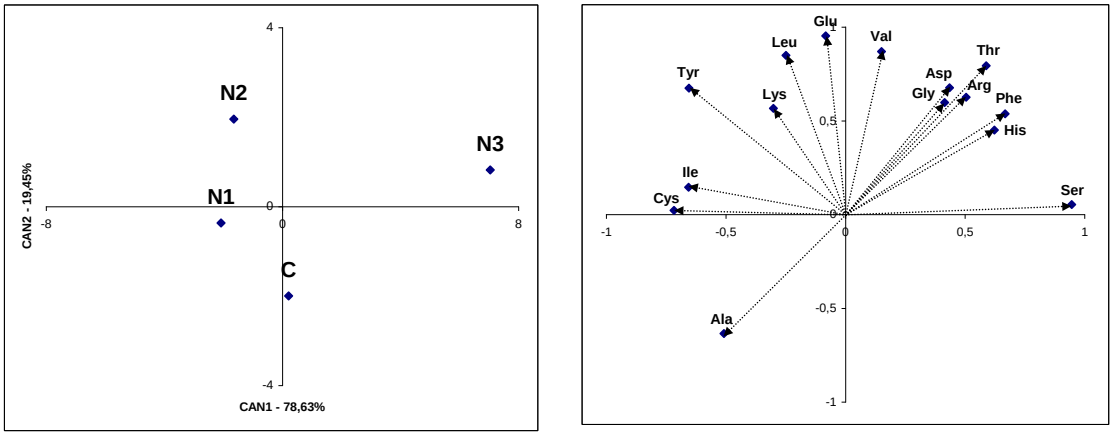
Regarding Riesling juices, distance between N3 treatment and all other treatments can be explained by the higher content of aspartate, arginine, threonine, glycine, histidine and phenylalanine in N3 grape juices. Distance between N2 and other treatments, especially the control ones, is explained by higher content of glutamate and leucine in N2 grape juices (Figures 3-a and -b).

Despite nitrogen fertilization, the presented concentrations of amino acids were very low and, probably, insufficient for alcoholic fermentation completion. This



(a) (b)

Figure 2-a) Position of *cv*. Welschriesling grape juice from different treatments in the space defined by the first two canonical variables from canonical discriminant analysis using their amino acid composition in the two years of study. **b)** Vector diagram of correlation of grape juice amino acid composition and the first two canonical variables based on different treatments shown in Figure 2-a.



(a) (b)

Figure 3-a) Position of cv. Riesling grape juice from different treatments in the space defined by first two canonical variables from canonical discriminant analysis using their amino acid composition from two years of study. b) Vector diagram of correlation of grape juice amino acid composition and first two canonical variables based on different treatments shown in figure 3a.

claim could be corroborated with previously reported results of Free Amino Nitrogen (FAN) and Yeast Assimilable Nitrogen (YAN) concentrations in the same grape juices (Karoglan *et al*., 2011). Furthermore, wines from such nitrogen-poor grape juices would probably have low concentrations of fermentative volatile compounds.

CONCLUSIONS

The effect of different rates of soil urea fertilization on yield components, grape juice soluble solids, total acidity, and amino acid composition of *Vitis vinifera* L. Chardonnay, Welschriesling, and Riesling vines was studied (Table 2). The results confirmed that yield parameters were slightly increased by urea fertilization. Fertilization with 28.1 g N $vine^{-1}$ significantly prolonged grape ripening, since those grape juices had the lowest soluble solids values and the highest total acidity level.

Chardonnay juices had the highest concentration of total primary amino acids, regardless of fertilization treatments. Amino acids aspartate, arginine, valine, phenylalanine, isoleucine and leucine were most responsive to soil urea fertilization. Urea fertilization strongly affected increase in amino acid composition, with 28.1 g N $vine^{-1}$ having the strongest effect, especially in more humid 2007 year. However, it should be mentioned that fertilization with 28.1 g N $vine^{-1}$ was not so effective when compared to fertilization with 5.5 and 16.8 g N $vine^{-1}$, respectively. This leads to the conclusion that fertilization with 28.1 g N $vine^{-1}$ seems excessive, expensive, and unnecessary regarding delayed fruit ripening and inconsistent effect on amino acid composition.

Finally, despite soil urea fertilization, the obtained grape juice amino acid concentrations remained very low and insufficient for successful alcoholic fermentation process. Further investigations should focus on finding more appropriate soil urea application time, possibly in combination with foliar application.

REFERENCES

1. Bavaresco, L., Pezzutto, S., Ragga, A., Ferrari, F. and Trevisan. M. 2001. Effect of Nitrogen Supply on *Trans*-Resveratrol Concentration in Berries of *Vitis vinifera* L. *cv*. Cabernet Sauvignon: Research Note. *Vitis*, ***40:*** 229-230.
2. Bell, A. A., Ough, C. S. and Kliewer, W. M. 1979. Effects on Must and Wine Composition, Rates of Fermentation and Wine Quality of Nitrogen Fertilization *Vitis vinifera* var. Thompson Seedless grapevines. *Am. J. Enol. Viticult.*, ***30:*** 124-129.
3. Bell, S. -J. and Henschke, P. A. 2005. Implications of Nitrogen Nutrition for Grapes, Fermentation and Wine. *Aust. J. Grape Wine Res.*, ***11:*** 242-295.
4. Bouzas-Cid, Y., Falqué, E., Orriols, I., Trigo-Córdoba, E., Díaz-Losada, E., Fornos-Rivas, D. and Mirás-Avalos, J. M. 2015. Amino Acid Profiles of Two Galician White Grapevine Cultivars (Godello and Treixadura). Ciênc. Téc. *Vitivinic*., ***30:*** 84-93.
5. Christensen, L. P., Bianchi, M. L., Peacock, W. L. and Hirschfelt., D. J. 1994. Effect of Nitrogen Fertilizer Timing and Rate on Inorganic Nitrogen Status, Fruit Composition, and Yield of Grapevines. *Am. J. Enol. Viticult.*, ***45:*** 377-387.
6. Coombe, B. G. and Dry, P. R. 1992. *Viticulture. Volume 2. Practices*. Winetitles. Adelaide, Australia.
7. Coombe, B. G. 1995. Adoption of a System for Identifying Grapevine Growth Stages. *Aust. J. Grape Wine Res.*, ***1:*** 104-110.
8. Garde-Cerdan, T., Lopez, R., Portu, J., Gonzalez-Arenzana, L., Lopez-Alfaro, I. and Santamaria. P. 2014. Study of the Effects of Proline, Phenylalanine, and Urea Foliar Application to Tempranillo Vineyards on Grape Amino Acid Content. Comparison with Commercial Nitrogen Fertilisers. *Food Chem.*, **163:**136–141.
9. Goldspink, B. and Gordon, C. 1991. Response of *Vitis vinifera cv*. Sauvignon Blanc Grapevines to Timed Applications of Nitrogen Fertilizers. In: *"Proceedings of International Symposium on Nitrogen in Grapes and Wine",* (Ed.): Rantz, J. M.

American Society for Enology and Viticulture, Davis, California, USA, PP. 255-258.
10. Hannam, K. D., Neilsen, G.H., Forge, T. and Neilsen, D. 2013. The Concentration of Yeast Assimilable Nitrogen in Merlot Grape Juice Is Increased by N Fertilization and Reduced Irrigation. *Can. J. Plant Sci.,* **93**: 37-45
11. Hannam, K. D., Neilsen, G. H., Neilsen, D., Rabie, W. S., Midwood, A. J. and Millard, P., 2014. Late-Season Foliar Urea Applications Can Increase Berry Yeast-Assimilable Nitrogen in Winegrapes (*Vitis vinifera* L.). *Am. J. Enol. Viticult., **65:*** 89-95.
12. Hannam, K. D., Neilsen, G. H., Neilsen, D., Midwood, A. J., Millard, P., Zhang, Z., Thorton, B. and Steinke, D. 2016. Amino Acid Composition of Grape (*Vitis vinifera* L.) Juice in Response to Applications of Urea to the Soil or Foliage. *Am. J. Enol. Viticult., **67:*** 47-55.
13. Hernández-Orte, P., Guitart, A. and Cacho, J. 1999. Changes in the Concentration of Amino Acids during Ripening of *Vitis vinifera* Tempranillo Variety from the Denomination d'Origine Somontano (Spain). *Am. J. Enol. Viticult., **50:*** 144-154.
14. Hernández-Orte, P., Cacho, J. and Ferreira, V. 2002. Relationship between Varietal Amino Acid Profile of Grapes and Wine Aromatic Composition. Experiments with Model Solutions and Chemometric Study. *J. Agr. Food Chem., **50:*** 2891-2899.
15. Hilbert, G., Soyer, J. P., Molot, C., Giraudon, J., Milin, S. and Gaudillère, J. P. 2003. Effects of Nitrogen Supply on Must Quality and Anthocyanin Accumulation in Berries of *cv*. Merlot. *Vitis, **42:*** 69-76.
16. Huang, Z. and Ough, C. S. 1991. Amino Acid Profiles of Commercial Grape Juices and Wines. *Am. J. Enol. Viticult., **42:*** 261-267.
17. Karoglan, M., Maslov, L., Matić, I., Brodski, A., Jeromel, A., Kozina, B. and Mijić, A. 2011. Total and Yeast Assimilable Nitrogen Composition in Grape Juices of Three Grapevine Cultivars as Affected by Vineyard Nitrogen Fertilization. *J. Cent. Eur. Agr., **12:*** 267-275.
18. Kliewer, W. M. 1970. Free Amino Acids and Other Nitrogenous Substances in Grape Wines. *J. Food Sci., **35:*** 17-21.
19. Kunkee, R. E. 1991. Relationship between Nitrogen Content of Must and Sluggish Fermentation. In: "*Proceedings of International Symposium on Nitrogen in Grapes and Wine"*, (Ed.): Rantz, J. M. American Society for Enology and Viticulture, Davis, California, USA, PP. 148-155.
20. Lasa, B., Menendez, S., Sagastizabal, K., Calleja Cervantes, M. E., Irigoyen, I., Muro, J., Aparicio-Tejo, P. M. and Ariz, I. 2012. Foliar Application of Urea to ''Sauvignon Blanc'' and ''Merlot'' Vines: Doses and Time of Application. *Plant Growth Regulat.*, **67:** 73–81.
21. Linsenmeier, A. W., Loos, U. and Löhnertz, O. 2008. Must Composition and Nitrogen Uptake in a Long-Term Trial as Affected by Timing of Nitrogen Fertilization in a Cool-Climate Riesling Vineyard. *Am. J. Enol. Viticult.,* **59:** 255-264.
22. Monteiro, F. F. and Bisson, L. 1991. Biological Assay of Nitrogen Content of Grape Juice and Prediction of Sluggish Fermentations. *Am. J. Enol. Viticult., **42:*** 47-57.
23. Neilsen, G. H., Neilsen, D., Bowen, P., Bogdanoff, C. and Usher, K. 2010. Effect of Timing, Rate and Form of N Fertilization on Nutrition, Vigor, Yield, and Berry Yeast-Assimilable N of Grape. *Amer. J. Enol. Viticult.,* **61:** 327–336.
24. Neilsen, G. H., Neilsen, D., Hannam, P., Millard, P. and Midwood A. 2013. Foliar Urea Applications Can Increase Berry Yeast-Assimilable N in Wine Grapes (*Vitis vinifera* L.). *Acta Hortic.*, **984:** 427–434.
25. OIV (Organisation Internationale de la Vigne et du Vin). 2013. *International Code of Oenological Practices*. Paris, France.
26. Ortega-Heras, M., Pérez-Magariño, S., Villar-Garrachón, V., González-Huerta, C., Moro González, L. C., Guadarrama Rodríguez, A., Villanueva Sánchez, S., Gallo González, R. and Martín de la Helguera, S. 2014. Study of the Effect of Vintage, Maturity Degree, and Irrigation on the Amino Acid and Biogenic Amine Content of a White Wine from Verdejo Variety. *J. Sci. Food Agric., **94:*** 2073-2082.
27. Ough, C. S. and Lee T. H. 1981. Effect of Vineyard Nitrogen Fertilization Level on the Formation of Some Fermentation Esters. *Am. J. Enol. Viticult., **32:***125- 127.

28. Peacock, W. L, Christensen, L. P. and Hirschfelt, D. J. 1991. Influence of Timing of Nitrogen Fertilizer Application on Grapevines in the San Joaquin Valley. *Am. J. Enol. Viticult.*, ***42:*** 322-326.
29. Pripis-Nicolau, L., Revel, G., Marchand, S., Anocibar Beloqui, A. and Bretrand, A. 2001. Automated HPLC Method for the Measurement of Free Amino Acids Including Cysteine in Musts and Wines; First Applications. *J. Sci. Food Agric.*, **81:** 731-738.
30. Spayd, S. E., Wample, R. L., Evans, R. G., Stevens, R. G., Seymour, B. J. and Nagel, C. W. 1994. Nitrogen Fertilization of White Riesling Grapes in Washington. Must and Wine Composition. *Am. J. Enol. Viticult.*, ***45:*** 34-42.
31. Spayd, S. E., Nagel, C. V. and Edwards, C. G. 1995. Yeast Growth in Riesling Juice as Affected by Vineyard Nitrogen Fertilization. *Am. J. Enol. Viticult.*, ***46:*** 49-55.
32. Spayd, S. E. and Andersen-Bagge, J. 1996. Free Amino Acid Composition of Grape Juice from 12 *Vitis vinifera* Cultivars in Washington. *Am. J. Enol. Viticult.*, ***47:*** 389-402.
33. Styger, G., Prior, B. and Bauer, F. F. 2011. Wine Flavor and Aroma. *J. Ind. Microbiol. Biotechnol.*, ***38:*** 1145-1159.
34. Yokotsuka, K. and Fukui, M. 2002. Changes in Nitrogen Compounds in Berries of Six Grape Cultivars during Ripening over Two Years. *Am. J. Enol. Viticult.*, ***53***: 69-77.

ترکیب آمینو اسید در آب انگورسفید تحت تاثیر کوددهی خاک با اوره

م. کاراوگلان، ل. ماسلو باندیک، م. اوسرشاک، م. میهالجویک زولج، ب. کوزینا، و ا. جرمل

چکیده

هدف این پژوهش بررسی اثر کوددهی خاک با اوره روی عملکرد، مواد جامد محلول در آب انگور، اسیدیته کل، و ترکیب آمینو اسید در انگور شامل ارقام Chardonnay، Welschriesling، و Rieslingبود. آزمایش در تاکستانی انجام شد که در سال های قبل از اجرای آزمایش، مکررا مقدار ازت قابل جذب مخمر (yeast) در انگور له شده مربوط به همان آب انگور از آن تاکستان کم گزارش شده بود. کود اوره بعد از کامل شدن مرحله گل به خاک افزوده شد. تیمار های آزمایش شامل: شاهد بدون کوددهی، ۵/۵ گرم N در هر تاک، ۱۶/۸ گرم N در هر تاک، و ۲۸/۱ گرم N در هر تاک بود. به طور کلی مصرف کود اوره باعث افزایش اجزای عملکرد و غلظت آمینو اسید شد. از نظر موادجامد محلول و اسیدیته کل، کوددهی با ۲۸/۱ گرم N در هر تاک منجر به طولانی شدن رسیدن انگور شد. این تیمار در مقایسه با دیگر تیمارهای کودی تاثیر چندانی در بهبود غلظت اسیدهای آمینه نداشت. نتیجه اینکه کود دهی با ۲۸/۱ گرم N در هر تاک زیاده از نیاز وغیر ضروری به نظر میرسد زیرا باعث تاخیر در رسیدن انگور شده و اثرات نا همخوان و بی ثباتی روی ترکیب آمینو اسیدها دارد.