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Primary amino acid composition and its use in discrimination of Greek red wines with regard to variety and cultivation region

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Summary

The primary amino acid content of 54 Greek red wines from several regions and grape varieties was determined by reversed-phase high performance liquid chromatography (HPLC) using precolumn derivatization with OPA (*o*-phthalaldehyde) and fluorescence detection. For each wine sample, 21 amino acids have been determined. Wine samples from the 4 most common Greek red grape cultivars, which are part of the Greek VQPRD (Vins de Qualité Produits dans des Régions Délimitées) wines, and from 4 foreign red grape varieties, were used. Wines from cv. Kotsifali had the highest amino acid content among the samples from indigenous varieties, followed by those originating from cvs Agiorgitiko, Mandilaria and Xinomavro. In contrast to wines from cv. Grenache rouge, which contained high amounts of amino acids, those from Cabernet Sauvignon, Syrah and Merlot had lower amounts. A classification of samples on the basis of variety and region was achieved by application of the discriminant analysis of the amino acid composition data. 22 % of the wine samples, originating from grapes cultivated in 'organic vineyards', had a low arginine content.

K e y w o r d s : Red wines; amino acids; HPLC; fluorescence; discriminant analysis; wine classification.

A b b r e v i a t i o n s : ALA, ARG, etc. (amino acids, see Tab. 1); γ-AB: γ-amino butyric acid.

Introduction

The cultivation of red grape varieties in Greece has an increasing tendency in the last few years and represents about 36 % of the Greek vineyards. In 90 % of the Greek vineyards, indigenous grape varieties are planted. However, over the last 30 years, several foreign varieties have been planted to improve Greek wines. Out of 94 red grape varieties (79 Greek and 15 foreign), 4 indigenous and 4 foreign varieties have been considered in the present study. While the indigenous varieties are covering the largest part of the production and also contribute to the production of several VQPRD and regional wines, the foreign varieties participate in many regional and table wines. Cv. Xinomavro is part of 4 VQPRD wines (Naoussa, Goumenissa, Amyntaio, Rapsani), cv. Mandilaria participates in the Paros, Rhodos, Archanes and Peza Denomination of Origin; cv. Kotsifali is the main variety of the Denomination of Origin Peza and Archanes. Nemea, one of the most important denominations of origin, is produced from cv. Agiorgitiko, which is widely cultivated in the homonymous region in Peloponnesos. Overall, these 4 indigenous varieties are covering 9 out of 12 Greek Denominations of Origin of red wines.

The variation of amino acid profiles in musts depends on grape variety, viticultural and enological management and environmental conditions (Feuillat 1974; Ough and Tabacman 1979; Etiévant *et al.* 1988; Bisson 1991; Jackson *et al.* 1993). Moreover, the amino acid content in wines varies with yeast strain, temperature, time of storage over yeast, NH_4^+ added etc. (Margheri *et al.* 1986). The concentration of most amino acids in wines is also a function of the technology used in wine making (e.g. Etiévant *et al.* 1988).

Although the amino acid profiles of some Greek white varieties have been examined (Bena-Tzourou *et al.* 1999; Soufleros *et al.* 2002), those from Greek red varieties have not been studied yet. The free amino acid content of wines has been studied to determine authenticity, geographical origin, substances of enological or toxicological interest, or fermentation kinetics (Monteiro and Bisson 1992; Herraiz and Ough 1993; Bauza *et al.* 1995; Soufleros *et al.* 1998; Herbert *et al.* 2000). Soufleros *et al.* (1998) used amino acids and other organic acids, volatile substances, biogenic amines and different sugars to discriminate among Bordeaux, Bourgogne, Alsace and Champagne wines and to classify them according to their type and age. Etiévant *et al.* (1988) have used only amino acids for the differentiation of 34 French red wines from three regions; among the 17 amino acids measured only proline, hydroxyproline and ethanolamine led to effective differentiation. Fourty two wines of 8 Portuguese grape varieties have been classified by Vasconelos *et al.* (1990) using amino acids as variables; ornithine, glutamine, ethanolamine and lysine contents were used to differentiate Spanish wines (de la Pressa *et al.* 1995). In several other studies, amino acid profiles have been used to discriminate wines with regard to variety or the production area (Symonds and Cantagrel 1982; Rizzon 1985; Seeber *et al.* 1991; Medina 1996; Carnevillier *et al.* 1999).

The aim of this study was to establish primary amino acid profiles of Greek red wines in order to discriminate them with regard to variety and origin.

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Material and Methods

Wine samples: The selected 54 red dry wines representing Greek wine production (Tab. 1): 4 most important Greek red grape varieties (Xinomavro, Agiorgitiko, Mandilaria, Kotsifali) and 4 French varieties cultivated in Greece (Cabernet Sauvignon, Merlot, Syrah, Grenache rouge). The wines were VQPRD and table wines, originating from continental Greece as well as from the islands. The samples purchased directly from the wineries and were typical samples from the retail market.

Wine analysis: The amino acids were determined by HPLC after precolumn derivatization of primary amino groups with *o*-phthaldehyde. The resulting isoindole derivatives were detected by spectrofluorimetry. The excitation and emission wavelengths were 340 and 450 nm, respectively. A binary gradient program was used for the mobile phase: solvent A was an aqueous solution of 0.68 % (w/v) $CH_3COONa \cdot H_2O$ and 5 % (v/v) tetrahydrofuran, adjusted to pH 5.7 with acetic acid; solvent B was absolute methanol. Amino acids were quantified using norvaline as internal standard. Details of derivatization, HPLC chromatographic separation and quantification of amino acids have been described elsewhere (SOUFLEROS *et al.* 2002).

Statistical analysis: Discriminant Analysis, with variables entering together in the analysis, was applied to the data using the SPSS, v.10 software (SPSS Inc.). Significant differences among wines were assessed with a One-way Analysis of Variance (ANOVA) using least significant differences (LSD-test) to distinguish among samples.

Results and Discussion

The total primary amino acid content for the Greek red wines ranged from 62.5 $mg \cdot l^{-1}$ to 756 $mg \cdot l^{-1}$. In Greek and French varieties, the average concentrations were 279 and 219 mgl^{-1}, respectively. The mean values of primary amino acids in red wines were generally lower than those of Greek white wines (SOUFLEROS *et al.* 2002). This concurs with data from literature, indicating that white wines generally have higher amounts of free amino acids than red wines (MILLERY *et al.* 1986). In Greek red wines GLU, ALA, ARG, ETH, -AB and LYS were most abundant (Tab. 2). SOUFLEROS *et al.* (1998) have reported that the mean primary amino acid content was 259 mgl^{-1} for red Bordeaux wines, where ARG, ALA, LYS, GLU, GLY, γ-AB and LEU dominated. MANCA DE NADRA *et al.* (1999) have reported higher primary amino acid values (481 mgl^{-1}) for Spanish red wine (cv. Cabernet Sauvignon), while ETIÉVANT *et al.* (1988) have reported lower amino acid values for 34 French red wines from various regions. In the latter study, ETH was a major amino acid.

As shown in Tab. 2, the standard deviation was relatively high for ARG, γ-AB, GLU and ALA. This reflects large differences between the wines with regard to grape variety, geographical origin, climatic conditions and enological and fertilization practices. Some of these factors were examined in more detail on the basis of the amino acid composition.

Grape varieties: Wines from cvs Kotsifali, Agiorgitiko and Grenache rouge had high amino acid contents, while those of cvs Syrah and Merlot had relatively lower total amino acid contents (Tab. 3). Wines made from Xinomavro, a Greek red grape variety mainly cultivated in the region of Macedonia, showed intermediate values of total amino acids. This variety had the highest average ETH values, which differed significantly ($p<0.05$) from other grape varieties (Tab. 4). Wines produced from cv. Agiorgitiko, the main Greek red variety mainly cultivated in Peloponnesos, showed a rather high concentration of primary amino acids, with GLU and ARG being predominant. Wines made from cv. Mandilaria showed intermediate primary amino acid content; GLU was the most abundant. Wines produced from cv. Kotsifali showed the highest values of primary amino acids, with ARG being predominant. Wines produced from Cabernet Sauvignon and Syrah showed rather low primary amino acid contents and quite low levels of γ-AB and ARG. ETIÉVANT *et al.* (1988) have also reported very low ARG values in French wines of Cabernet Sauvignon, containing on average 120 mgl^{-1} of primary amino acids. Merlot wines had substantially lower amounts of most amino acids. ETIÉVANT

Table 1

Number and origin of red wine samples of various varieties; numbers in brackets indicate organic wine samples

Grape Variety	Geographic origin					
	Macedonia	Peloponnesos	Central Greece	Aegean Islands	Crete	Total
Xinomavro	19 (6)					19
Agiorgitiko		9				9
Mandilaria				3		3
Kotsifali					2	2
Cabernet Sauvignon	2	4	5			11
Syrah	4 (3)					4
Merlot	3 (3)	1				4
Grenache rouge			2			2
Total	28 (12)	14	7	3	2	54

Table 2

Free amino acid content (mg·l^{-1}) of 54 Greek red wines

Amino acids	Codes	Minimum	Maximum	Average	Std. Dev.
L-Aspartic acid	ASP	3.1	32.1	14.2	7.3
L-Glutamic acid	GLU	3.8	79.3	30.5	17.1
L-Asparagine	ASN	1.5	25.8	9.3	4.6
DL-Serine	SER	1.1	20.3	6.9	3.8
L-Glutamine	GLN	0.0	2.8	0.8	1.1
L-Histidine	HIS	1.0	26.0	9.9	6.1
Glycine	GLY	2.4	30.3	11.4	5.9
L-Threonine	THR	0.0	24.4	12.3	5.2
L-Arginine	ARG	0.2	266.4	25.0	41.1
DL-Alanine	ALA	3.7	92.5	31.2	18.1
L-Tyrosine	TYR	0.0	23.0	7.9	5.2
γ-amino butyric acid	γ-AB	0.0	130.7	18.5	22.1
Ethanolamine	ETH	9.7	59.2	21.1	11.2
L-Valine	VAL	0.7	16.8	5.7	3.5
DL-Methionine	MET	0.0	4.1	1.6	1.1
DL-Tryptophan	TRP	0.0	5.7	1.4	1.6
L-Phenylalanine	PHE	1.0	20.4	8.3	4.3
L-Isoleucine	ILE	0.5	10.6	4.0	2.1
L-Leucine	LEU	1.7	36.2	10.1	6.1
DL-Ornithine	ORN	0.1	70.3	8.3	10.8
L-Lysine	LYS	0.0	54.2	17.2	10.3
Total				255.5	

et al. (1988) have reported a mean value of 124 mgl^{-1} for French Merlot wines. In contrast, samples from cv. Grenache rouge showed the second highest primary amino acid content, with ORN and GLU being most abundant.

Tab. 4 shows univariate statistics referred to grape variety. There were 12 amino acids that differed significantly ($p<0.05$) among the varieties. Thus, the high levels of ETH characterize wines made from Xinomavro grapes, while high ORN levels characterize wines made from Grenache rouge. Etiévant *et al.* (1988) have reported that ETH is generally lower in wines prepared from Grenache than in wines made from Cabernet Sauvignon or Merlot. This is confirmed by our results (Tab. 3), although the differences are less pronounced.

From the data of Tabs 2 and 3 it becomes apparent that the ARG values differ significantly among samples. This is probably due to the fact that the arginine content of grape juice is largely influenced by the inorganic nitrogen fertilization. Moreover, arginine is easily metabolized by yeasts and it may have been reduced during anaerobic fermentation (Monteiro *et al.* 1989).

When Discriminant Analysis was applied to the amino acid data a relatively good varietal association among the analyzed samples was achieved. Six discriminating functions were obtained. The first two explained 73.2 % of the total variance. In the plot of the scores in the coordinate plane, defined by the canonical components of the first two functions, the wine samples of cv. Xinomavro were positioned in the upper left quadrant (Fig. 1). The discriminant function 1 accounts for 38.2 % of the total variance and differentiates wines of cvs Grenache rouge, Kotsifali and Mandilaria, which are positioned in the right quadrants. The discriminant function 2 accounts for 35.0 % of the total variance and differentiates wines made of Xinomavro or Grenache rouge from the other varieties. Differentiation and classification were satisfactory for certain varieties (Xinomavro, Grenache rouge, Kotsifali, Mandilaria), whereas wines made of Merlot, Syrah, and Cabernet Sauvignon were not fully distinguishable at the plot. The standardized coefficients of these f irst two discriminating functions showed that ARG, ILE, PHE, ORN and γ-AB had the higher weight in discriminating among red varieties. The γ-AB has been previously cited by several authors as a discriminating parameter of red wines made from different varieties (Ooghe *et al.* 1981; Polo *et al.* 1984; Rizzon 1985).

While 92.6 % of the samples were correctly classified, only 72.7 % of the Cabernet Sauvignon wine samples could be classified. In fact, three wine samples of cv. Cabernet Sauvignon were incorrectly associated with the Syrah and Merlot groups. This can be due to the fact that the group centroids for wines produced from Cabernet Sauvignon, Syrah and Merlot grapes are too close to each other (Fig. 1) and their amino acid content is much alike.

R e g i o n : In the present work, the red wine samples originated from Macedonia, Peloponnesos, Central Greece (Thessalia, Sterea Ellada, Epirus), Aegean Islands and Crete

Table 3

Concentrations of amino acids ($mg \cdot l^{-1}$) according to grape variety

Amino acids	Xinomavro (n=19)		Agiorgitiko (n=9)		Mandilaria (n=3)		Kotsifali (n=2)		Cabernet Sauvignon (n=11)		Syrah (n=4)		Merlot (n=4)		Grenache rouge (n=2)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
L-Aspartic acid	12.4	5.8	19.3	8.2	15.0	8.0	18.3	7.8	14.5	6.7	12.0	11.2	8.2	5.4	20.9	0.7
L-Glutamic acid	26.3	14.7	44.4	21.1	30.2	18.3	42.1	13.7	27.5	15.7	33.2	19.9	22.2	13.7	37.9	3.1
L-Asparagine	11.0	6.3	8.7	3.3	7.7	3.4	10.8	2.4	8.0	2.2	6.1	1.6	10.7	4.7	9.1	0.2
DL-Serine	6.1	3.5	10.1	4.7	7.3	3.7	10.4	3.7	6.4	2.5	5.2	4.4	5.5	4.8	8.8	0.3
L-Glutamine	0.5	0.9	1.0	1.1	1.4	1.3	2.3	0.4	1.0	1.2	0.0	0.0	0.5	0.9	2.1	0.1
L-Histidine	7.9	5.4	12.6	5.8	13.2	7.0	18.7	7.7	9.7	2.5	6.9	9.1	12.2	11.4	11.9	0.5
Glycine	10.8	5.3	16.6	8.2	11.4	4.8	16.0	3.1	10.2	3.9	9.7	7.0	8.4	4.9	11.2	0.9
L-Threonine	12.6	4.8	16.5	5.4	14.4	2.8	16.6	1.5	10.3	3.4	8.2	5.7	6.7	5.7	16.7	2.8
L-Arginine	26.7	59.3	43.9	39.6	30.1	19.9	44.0	28.3	11.2	5.8	9.0	12.8	17.2	28.0	34.6	44.2
DL-Alanine	28.7	20.6	41.4	19.6	26.8	14.0	40.2	10.3	30.1	17.5	28.8	17.1	29.6	14.2	34.3	2.8
L-Tyrosine	7.9	4.8	7.6	4.2	10.9	9.0	17.0	8.3	8.3	5.4	6.0	6.0	5.5	4.0	8.7	0.4
γ-amino butyric acid	20.9	31.5	30.8	18.1	15.3	7.4	21.9	5.8	9.5	5.5	10.2	10.9	16.2	25.4	17.5	0.9
Ethanolamine	30.3	13.3	20.4	6.6	13.5	3.0	15.1	1.6	14.6	3.6	15.4	6.3	15.6	5.9	12.9	0.6
L-Valine	4.6	2.9	6.9	3.5	7.8	5.5	11.7	7.2	5.9	3.4	4.5	3.9	5.6	4.5	6.0	0.4
DL-Methionine	1.3	1.1	2.2	0.8	2.5	1.4	3.8	0.3	1.7	0.9	0.6	1.1	1.2	1.4	2.0	1.6
DL-Tryptophan	1.2	1.5	1.7	1.5	1.7	2.2	2.3	3.3	1.6	2.2	0.1	0.1	1.4	1.3	2.4	0.2
L-Phenylalanine	8.4	4.4	9.7	4.5	10.5	5.7	14.1	6.1	7.4	2.7	5.9	5.3	5.2	4.0	12.4	2.2
L-Isoleucine	3.4	1.8	5.3	2.3	4.4	2.2	6.2	1.9	4.4	1.9	1.8	1.6	3.1	2.2	6.0	0.4
L-Leucine	9.7	5.1	12.8	9.2	11.0	5.3	14.8	5.4	9.2	3.2	6.1	7.1	8.5	8.7	15.3	5.0
DL-Ornithine	4.4	3.6	12.9	10.3	10.6	8.1	18.5	3.1	7.8	4.5	2.6	3.4	5.1	4.6	39.5	43.6
L-Lysine	13.7	8.1	26.0	11.9	19.8	9.9	25.8	8.6	16.0	5.7	13.2	15.1	13.6	14.0	25.9	7.0
Total	248.6		350.7		265.3		370.6		215.3		185.3		202.1		335.8	

n: number of wine samples.
S.D.: Standard deviation.

Table 4

Grape variety and amino acid content; one-way ANOVA, showing significant differences among samples ($p<0.05$)

Variable	
ASP	4 *vs.* 1, 7; 7 *vs.* 8
GLU	4 *vs.*2, 5, 7
SER	4 *vs.* 1, 2, 5, 6, 7
GLN	3 *vs.* 1, 6, 7, 8; 8 *vs.* 6
GLY	4 *vs.* 1, 2, 5, 6, 7
THR	1 *vs.* 7; 3 *vs.* 6, 7; 4 *vs.* 1, 5, 6, 7; 8 *vs.* 6, 7
TYR	3 *vs.* 1,2, 5, 6, 7
γ-AB	4 *vs.* 5
ETH	1 *vs.*all
VAL	3 *vs.*1, 2, 5, 6, 7
MET	3 *vs.*1, 2, 5, 6, 7; 4 *vs.*1, 6
PHE	3 *vs.*5, 6
ILE	4 *vs.*1; 6 *vs.*3, 4, 5, 8
ORN	8 *vs.* all; 1 *vs.*3, 4, 8; 6*vs.* 3, 4
LYS	4 *vs.*1, 5, 11, 7

1 = Xinomavro; 2=Mandilaria; 3=Kotsifali; 4=Agiorgitiko; 5 = Cabernet Sauvignon; 6=Syrah; 7=Merlot; 8=Grenache rouge.

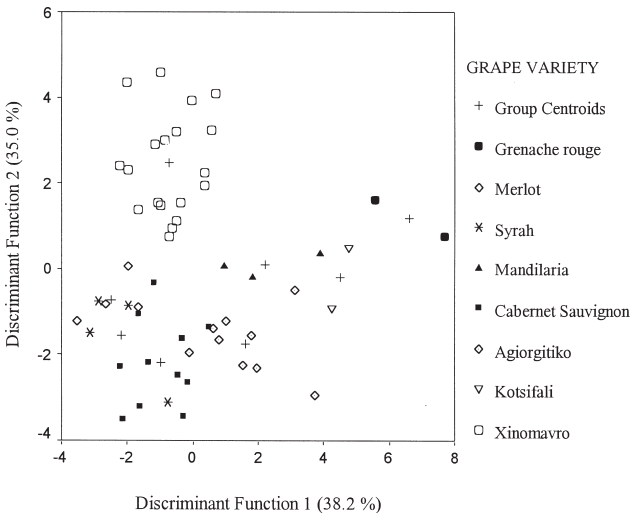

Fig. 1: Discriminant analysis of 54 Greek red wines by variety; the first two canonical discriminant functions are plotted.

(Tab. 1). Tab. 5 shows that the individual amino acid content within each region varied over a wide range. Red wines from Crete had the highest primary amino acid content, followed by wines of Peloponnesos region. These observations can be partially explained by the fact that the nitrogen fertilization levels in Crete are rather high. Wines originating from the Aegean Islands contained very low amounts of primary amino acids. Similar observations were made previously for white wines (Soufleros *et al*. 2002). This is probably due to the fact that in these areas soils are mostly rocky and unfertile and climatic conditions are semi-arid. Red wines from Central Greece and Macedonia had an intermediate primary amino acid content. Wines from Macedonia are characterized by high values of ALA, GLU and ETH and a deficiency in ORN, while wines from Peloponnesos show higher values of GLU, ALA, ARG and γ-AB. In wines from Central Greece GLU, ALA and LYS are most abundant, while the ORN values are also high. Wine samples from the Aegean Islands presented higher values of GLU, ARG, ALA and LYS. In wine samples from Crete, ARG, GLU and ALA were the predominant. Values of ORN were also high. Moreover, on the basis of a geographical characterization of the wines, only the variables MET, ILE, ORN and LYS could provide a statistically significant difference at the 95 % level.

When Discriminant Analysis was applied, a tendency towards subgrouping according to the region of grape production was noted, and 4 discriminant functions were obtained. The first two explain 84.7 % of the total variance. Fig. 2 presents a plot of the scores in the coordinate plane defined by the first two canonical components of the functions with the higher discriminating power for the wines considered. Discriminant function 1 was most negatively correlated with ALA and PHE and most positively correlated with ILE and GLU. The wines from Macedonia are positioned in the left part of the horizontal axis (Discriminant function 1) and discriminate from the wine samples of other regions (Fig. 2). PHE was also most negatively correlated with Discriminant function 2 while ASP was positively correlated. This function discriminates and differentiates wines produced on the Islands from the wines produced in Peloponnesos and Central Greece. Wines originating from Central Greece and most wines from Peloponnesos are positioned in the lower right quadrant. Finally, wines from the Aegean Islands and Crete are positioned in the upper right quadrant. Among the samples misclassified (5.6 %) two wines originated from Macedonia and one from Peloponnesos.

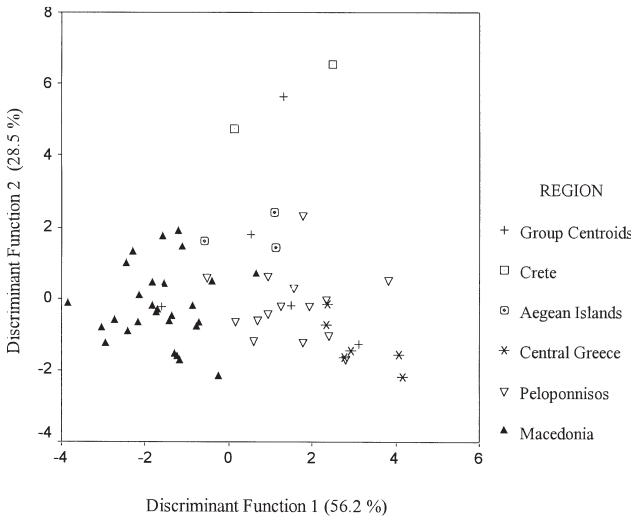

Fig. 2: Discriminant analysis of 54 Greek red wines by origin; the first two canonical discriminant functions are plotted.

The amino acid content among samples of the same cultivars varied; *i.e.* wines made of Cabernet Sauvignon cultivated in Macedonia, Central Greece and Peloponnesos contained 282.8, 138.5 and 152.6 mgl^{-1} of amino acids, re-

Table 5

Amino acid content (mgl^{-1}) of wine samples grouped according to their origin

Amino acids	Macedonia n=28		Peloponnesos n=15		Central Greece n=6		Aegean Islands n=3		Crete n=2	
	mean	st.dev	mean	st.dev	mean	st.dev	mean	st.dev	mean	st.dev
L-Aspartic acid	12.2	7.0	16.7	8.0	16.6	4.8	12.7	8.9	18.3	7.8
L-Glutamic acid	25.6	14.7	38.7	21.0	33.5	9.4	22.3	18.4	42.1	13.7
L-Asparagine	10.0	5.5	8.5	3.8	8.8	1.0	5.6	1.9	10.8	2.4
DL-Serine	5.9	3.6	8.6	4.5	7.2	2.2	5.2	2.1	10.4	3.7
L-Glutamine	0.6	1.0	0.8	1.0	1.2	1.2	0.9	1.5	2.3	0.4
L-Histidine	8.4	6.7	11.6	5.1	10.3	2.6	9.5	4.3	18.7	7.7
Glycine	10.2	5.2	14.0	7.6	10.9	3.1	8.3	2.6	16.0	3.1
L-Threonine	11.1	5.3	13.5	6.0	13.5	3.6	12.9	2.4	16.6	1.5
L-Arginine	20.7	49.5	34.4	34.4	17.3	24.3	20.8	8.5	44.0	28.3
DL-Alanine	30.1	19.6	35.9	19.4	28.1	8.2	17.9	6.5	40.2	10.3
L-Tyrosine	7.9	5.6	7.2	3.6	7.6	1.2	6.8	8.0	17.0	8.3
γ-amino butyric acid	16.5	26.9	25.4	18.9	12.7	5.4	10.9	4.4	21.9	5.8
Ethanolamine	25.0	13.7	18.4	6.4	15.8	3.6	12.4	3.4	15.1	1.6
L-Valine	4.9	3.3	6.6	3.7	5.1	1.1	5.2	3.1	11.7	7.2
DL-Methionine	1.3	1.1	1.9	1.0	1.9	0.7	1.7	0.9	3.8	0.3
DL-Tryptophan	1.3	1.7	1.4	1.3	1.5	1.8	1.3	2.0	2.3	3.3
L-Phenylalanine	7.6	4.5	8.5	4.2	9.2	3.0	8.1	5.0	14.1	6.1
L-Isoleucine	3.3	2.0	4.5	2.3	5.6	0.7	3.2	1.5	6.2	1.9
L-Leucine	8.7	5.4	11.6	8.0	11.7	4.1	8.5	4.2	14.8	5.4
DL-Ornithine	4.7	4.4	10.3	8.6	18.1	25.8	5.3	4.5	18.5	3.1
L-Lysine	13.2	8.8	22.7	11.5	19.5	7.6	15.7	9.9	25.8	8.6
Total	229.1		301.2		256.1		195.1		370.6	

n: number of wine samples.

spectively. This variation confirms that different geographical areas affect the amino acid content of must and corresponding wines; *i.e.* different climatic conditions, the composition of must, the grape variety and the yeast used for fermentation have an impact on the amino acid content of wines.

'Organic' viticulture: Twelve wine samples of 'organically' grown grapes of the cvs Xinomavro, Merlot and Syrah, originating from Macedonia and Peloponnesos, were among the samples studied. The amino acid profiles for 'organically' produced wines *vs.* those from conventional viticulture are shown in Tab. 6. The amino acid levels were almost double in wines from conventional viticulture; more specifically, the ARG and γ-AB concentrations were 6 and 3 times higher, respectively. Such differences most likely reflect the fertilization protocols in conventional viticulture; *i.e.* inorganic nitrogen fertilization is known to increase the amino acid levels of grape berries and the resulting musts, with arginine levels being mostly affected (Bertrand *et al.* 1991). The most abundant amino acids in 'organic' wines were ALA, GLU and ETH, whereas for wines from conventional viticulture GLU, ALA, ARG and γ-AB exhibited higher values.

Application of one-way ANOVA showed statistical differences between the two groups for the ASP, SER, THR, VAL, MET, PHE, ILE, LEU and LYS concentrations at $p<0.01$, whereas the concentrations of HIS differed statistically at the $p<0.05$ level.

Conclusion

For Greek red wines the most abundant amino acids were GLU, ALA, ARG, ETH, γ-AB and LYS, while ARG and γ-AB had the highest variation, due to the nitrogen fertilization practices adopted in different cultivation regions.

The amino acid content of red wines is strongly affected by grape variety and geographic area; *i.e.* the data can effectively be used to discriminate wines according to their varietal and geographical origin. Overall, and in accordance with previous studies (Soufleros *et al.* 2002), the amino acid profile of wines has a high discriminating ability, and can therefore be used as chemical tracer of product origin.

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Table 6

Amino acid content (mgl^{-1}) of wine samples grouped according to the type of cultivation

Amino Acids	Organic viticulture (n=12) Mean	Std. Dev.	% of total amino acids	Conventional viticulture (n=42) Mean	Std. Dev.	% of total amino acids
L-aspartic acid[a]	8.6	3.9	5.2	15.8	7.3	5.6
L-glutamic acid	22.1	7.1	13.3	33.0	18.4	11.7
L-asparagine	9.5	3.8	5.7	9.2	4.8	3.3
DL-serine[a]	4.1	1.8	2.5	7.7	3.9	2.7
L-glutamine	0.7	1.0	0.4	0.9	1.1	0.3
L-histidine[b]	6.8	6.9	4.1	10.8	5.6	3.9
glycine	9.4	4.7	5.6	12.0	6.1	4.3
L-threonine[a]	8.0	4.5	4.8	13.6	4.8	4.8
L-arginine	5.3	5.5	3.2	30.6	45.1	10.9
DL-alanine	26.6	11.4	16.0	32.5	19.5	11.6
L-tyrosine	5.7	3.5	3.4	8.6	5.4	3.0
γ-amino butyric acid	8.8	14.5	5.3	21.2	23.3	7.6
ethanolamine	19.6	11.4	11.8	21.5	11.3	7.6
L-valine[a]	3.4	1.1	2.0	6.3	3.7	2.3
DL-methionine[a]	0.7	0.8	0.4	1.9	1.1	0.7
DL-tryptophan	0.8	1.3	0.5	1.6	1.7	0.6
L-phenylalanine[a]	5.2	2.9	3.1	9.2	4.3	3.3
L-isoleucine[a]	2.3	1.4	1.4	4.4	2.1	1.6
L-leucine[a]	5.9	4.2	3.6	11.3	6.1	4.0
DL-ornithine	3.7	4.3	2.2	9.6	11.7	3.4
L-lysine[a]	9.5	6.1	5.7	19.3	10.2	6.9
Total	166.5			280.9		

a, b: significant difference at 99 % and 95 % levels, respectively; n: number of samples.

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