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Primary amino acid profiles of Greek white wines and their use in classification according to variety, origin and vintage

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Abstract

Forty-two Greek white wines from six grape varieties and several geographic regions have been analysed for primary amino acids by reversed-phase high performance liquid chromatography, using precolumn derivatization with *o*-phthalaldehyde and fluorescence detection. A methodology was developed in order to enable the analysis of a large number of samples, in a quick and reliable way. The amino acid content of Greek white wines was within the range of values reported for other European wines. Wines from Chardonnay, Muscat white and Muscat d'Alexandrie grape varieties, had high amino acid contents, while wines from Asyrtiko, Moschofilero and Debina were characterized by substantially lower amounts of free amino acids. Arginine, γ-amino butyric acid, lysine, alanine, glycine, aspartic acid, and leucine were the most abundant amino acids. In about 29% of the white wine samples examined, the malolactic fermentation has occurred, resulting in lower arginine, γ-amino butyric acid and methionine values for these samples. The amino acid profiles have been useful in the classification of white wines according to grape variety, vintage, geographic origin and type of vinification by means of statistical methods.

Keywords: White wines; Amino acids; HPLC; Fluorescence; Discriminant analysis; Wine classification

1. Introduction

Amino acids represent 30–40% of the total wine nitrogen (Rizzon, 1985). Those, present in grape must, are used as nutrients for yeast growth since they are consumed as a nitrogen source during alcoholic fermentation (Juhász & Törley, 1985). In this context, several amino acids undergo a series of biotransformations, yielding higher alcohols, aldehydes, esters and ketonic acids. Being precursors of such compounds, they have an impact on the organoleptic properties of wine (Huang & Ough, 1989; Juhász & Törley, 1985; Tusseau, Benoit, & Valade, 1989). Moreover, it is generally accepted that amino acids may act as nutrients for bacterial growth during secondary fermentations.

Amino acids in wine have a variety of origins. Beyond those that are present in the grape and that can be partially or totally metabolized by yeast during the growth phase, some are excreted by living yeasts at the end of fermentation (Bidan, Feuillat, & Moulin, 1986), some are released by proteolysis during the autolysis of dead yeasts, and other are produced by enzymatic degradation of the grape proteins. Furthermore, it is well known that the amino acid content of grapes is dependent on the fertilization, the climatic conditions and the duration of skin maceration in the must (Etiévant, Schlich, Bouvier, Symonds, & Bertrand, 1988). Plant material, field treatments and viticultural practices also affect the amino acid content (Ough & Tabacman, 1979). Moreover, different winemaking conditions (e.g. fermentation temperature and speed) can influence the amino acid content of wine (Margheri, Versini, Pelligrini, & Tanon, 1986).

Despite this wide range of factors affecting the amino acids present in wine, some researchers have successfully employed the amino acid composition for differentiation of the product (Soufleros, Barrios, & Bertrand, 1998; Tapias, Callao, Larrechi, Guasch, & Rius, 1987).

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Soufleros et al. (1998) have managed to classify French wines of various regions (Bordeaux, Bourgogne, Alsace, Champagne) according to their origin, type and ageing by analysis of 21 amino acids, biogenic amines and volatile substances. Free amino acids were used for the characterization of Macabeo, Xarello and Parellada white wines from the Penedès region (de la Pressa-Owens, Lamuela-Raventos, Buxaderas, & de la Torre-Boronat, 1995a,b). Tyrosine, isoleucine, glycine, alanine and ethanolamine were significantly different among wines according to variety (de la Pressa-Owens et al., 1995b), while asparagine, proline and lysine were the most common compounds for distinguishing the wines according to the geographical origin (de la Pressa-Owens et al., 1995a). Tyrosine, γ-amino butyric acid, alanine, asparagine, histidine and methionine were the most significant variables in distinguishing wines according to vintage year (de la Pressa-Owens et al., 1995b), while glycine and tyrosine were responsible for the differentiation among musts according to vintage year (de la Pressa-Owens and Noble, 1995).

Amino acids have been successfully used to separate authentic champagnes from sparkling wines, as the second fermentation to produce the overpressure of CO_2 of the authentic champagnes is performed in the bottle, leading to an increase in amino acids. On the other hand, sparkling wines, some of which are produced by the method of "cuvees closes", exhibited lower amino acid concentrations (Tusseau, Valade, & Moncomble, 1994). In another study by Millery, Duteurtre, Boudaille, and Maujean (1986), 160 must samples from different champagne grape varieties (Pinot noir, Pinot meunier, Chardonnay) were separated according to variety by their amino acid composition. Serine, ornithine, citrulline, arginine and proline were amino acids employed for the differentiation. Moreover, the arginine content was suggested to reflect the genetic characteristics of the varieties (Millery et al., 1986). In other studies, amino acid composition has been employed to differentiate wines according to grape variety or to production area (Seeber, Sferlazzo, & Leardi, 1991; Symonds & Cantagrel, 1982; Vasconcelos & Chaves des Neves, 1990).

In recent years, product characterization by means of multivariate data analysis has been widely used in enology for differentiation and identifying purposes (Etievant et al., 1988; Neves & Vasconcelos, 1989). Principal component analysis (PCA) and discriminant analysis (DA) have been applied to anthocyanins, flavonoids and colour parameters determined in Spanish red wines aged in wood and showed considerable differences among samples produced in different regions (Gómez-Cordovés, González-San José, Junquera, & Estrella, 1995). Canonical discriminant analysis (CDA) has also been applied to identify similarities among wines from the same region and the main differences among wines from different regions. The application of this method, in conjunction with PCA, resulted in a satisfactory classification of 22 French red wines according to their geographical origin (Sivertsen, Holen, Nicolaysen, & Risvk, 1999). Alvarez, Casp, Zunica, Aleixandre, and Garcia (1997) used discriminant analysis to differentiate Spanish white wines according to the geographical origin, based on chemical composition of the wine samples).

The studies discussed earlier have demonstrated the importance of amino acids as discriminating factors for product differentiation. They have also shown the influence of variety, type of vinification, skin contact, wine ageing, fertilization, climatic conditions and area of production on the amino acid profile.

Quantitative determination of free amino acids can be done, either with ion-exchange liquid chromatography and post column derivatization (ninhydrin or OPA), or with precolumn derivatization and high performance liquid chromatography. The use of reverse-phase columns and precolumn derivatization is more efficient and faster than conventional ion-exchange techniques. Several reagents are applied to the amino acid analysis: the OPA/FMOC (Herbert, Barros, Ratola, & Alves, 2000; Linget, Netter, Heems, & Vérette, 1998), dansyl chloride (Krause, Bockhardt, Neckermann, Henle & Klostermeyer, 1995), PITC (phenylisothiocyanate) as PTC (phenylthiocarbamate) (Ancin Ayestaran, & Garrido, 1996; Marcè, Calull, Guasch, & Borrull, 1989) or PTH (phenylthiohydantoin) derivatives.

In our study, primary amino acids in wine were determined by derivative formation with OPA and fluorescence detection. OPA is a fluorophor, which reacts with the primary amino acids to form an isoindole. The isoindole derivatives are very amenable to reversed-phase chromatography and sensitive to small changes in mobile phase conditions. The major disadvantage of the OPA procedure is the lack of reaction with secondary amino acids and the low stability of the OPA derivatives (Fiorino, Frigo, & Cucchetti, 1989; Heems, Luck, Fraudeau, & Vérette, 1998).

The objectives of the present research were: (1) to identify amino acid profiles of Greek white wines made of indigenous, mainly, and French grape varieties cultivated in Greece, since amino acids have an important role in the progress of wine fermentations and in the biochemical formation of higher alcohols. Information on the amino acid content of Greek grape varieties is lacking. No systematic studies in this respect have been carried out until now. Only one study related to the influence of a viticultural practice to the amino acid composition of a specific Greek grape variety (Bena-Tzourou, Lanaridis, & Metafa, 1999) has been found. (2) To achieve a possible characterization of Greek white wine samples by means of their amino acid profiles.

Table 1
Distribution of the white wine samples studied according to grape variety, area of production and the type of vinification

Grape Variety	Geographic origin						Type of wine		
	Macedonia	Peloponnisos	Central Greece	Cyclades Islands	Samos Island	Lemnos Island	Dry wines	Sweet wines	Total
Roditis	5	5	5				15		15
Debina			3				3		3
Moschofilero		4					4		4
Asyrtiko				4			4		4
Muscat white					4		1	3	4
Muscat d'Alexandrie						8	5	3	8
Chardonnay		4					4		4
Total	5	13	8	4	4	8	36	6	42

2. Materials and methods

2.1. Wine samples

The selected 42 white wine samples represent a cross-section of Greek wine production (Table 1). Thirty-six of them were dry wines and six were sweet wines; among the latter, three samples are naturally sweet wines. All samples belong to six Greek white grape varieties, which are mainly cultivated in this country, and one French variety. Most of the wines were VQPRD and originated from the continental Greece as well as from the islands (Table 1).

2.2. Equipment

A liquid chromatograph consisting of two Marathon IV (State College, PA, USA) pumps and a SSI 502 programmable fluorescence detector were used for amino acid analysis. The excitation and emission wavelengths were 340 and 450 nm, respectively. Separation of amino acids was carried out using an Adsorbosphere XL C_{18} 90A 5u column, 25 cm×4.6 mm I.D. (Alltech Associates Inc., Deerfield, IL, USA) and a Kromasil C_{18} 5u, 150 mm×4.6 mm precolumn (Alltech Associates Inc., Deerfield, IL, USA). The ChromQuest Chromatography software (ThermoQuest Inc., San Jose, CA, USA) was used for data storage and integration.

2.3. Reagents and standards

The L-aspartic acid, L-glutamic acid, glycine, L-arginine, ethanolamine, L-leucine standards of the highest purity available, boric acid and potassiun hydroxide were obtained from Panreac Quimica SA (Barcelona, Spain). L-asparagine monohydrate, L-histidine, DL-alanine, DL-methionine, DL-tryptophan, L-phenylalanine and *o*-phthalaldehyde were obtained from Lancaster (Morecambe, England). DL-serine, DL-norvaline, were obtained from Fluka Chemie AG (Buch, Switzerland). L-tyrosine hydrochloride, γ-amino butyric acid, L-isoleucine, DL-ornithine hydrochloride and L-lysine were obtained from Sigma (St. Louis, MO, USA). Methanol (HPLC grade) and 2-mercaptoethanol were obtained from RiedeL-de Haën (Sigma Aldrich Laboratories, Seeize, Germany). Highly purified water (Milli-Q Millipore) was used throughout for preparation of all buffers and reagents.

2.4. Preparation of standard solution, reagents and sample derivatization

A standard solution of 21 amino acids was prepared by dissolving each amino acid in a 0.1 M HCl solution to provide a concentration similar to that found in wines. This solution was stored at 0 °C. The amino acid composition of the standard solution along with the amino acid code names, is given in Table 2.

Table 2
Amino acid composition of the standard solution

Amino acids	Codes	mg/l
L-Aspartic acid	ASP	77.2
L-Glutamic acid	GLU	74.0
L-Asparagine	ASN	80.4
DL-Serine	SER	68.4
L-Glutamine	GLN	49.6
L-Histidine	HIS	61.2
Glycine	GLY	53.6
L-Threonine	THR	61.6
L-Arginine	ARG	89.6
DL-Alanine	ALA	140
L-Tyrosine	TYR	59.6
γ-amino butyric acid	γ-AB	49.2
Ethanolamine	ETH	40.8
L-Valine	VAL	26.0
DL-Methionine	MET	24.4
DL-Tryptophan	TRP	56.4
L-Phenylalanine	PHE	59.2
L-Isoleucine	ILE	69.6
L-Leucine	LEU	82.0
DL-Ornithine	ORN	74.0
L-Lysine	LYS	92.0

Fifty milligrams of *o*-phthalaldehyde (OPA) were dissolved in methanol and 2.5 ml of 2-mercaptoethanol were added. The solution was adjusted to 50 ml with methanol and was left to settle for 24 h. The reagent was stored in dark glass vials at 4 °C and was prepared freshly every week; under these conditions the reagent was stable for 7 days. The borate buffer, pH 10.4, was prepared by dissolving 6.194 g H_3BO_3 and 6.524 g KOH in ultrapure milli-Q water and adjusting the pH to 10.4 with *o*-phosphoric acid. The final volume was adjusted to 200 ml with milli-Q water. At 4 °C this solution is stable for several months.

Fifty microlitres of internal standard solution (DL-norvaline, 1.488 g/l) were added to 5 ml of the wine sample and then filtered through a cellulose acetate membrane filter (0.2 μm average pore size) (Alltech Associates Inc., Deerfield, IL, USA); the final concentration of DL-norvaline in the wine sample was 14.73 mg/l. The derivatization was carried out in a 3-ml dark vial, according to the method of Soufleros and Bertrand (1998), with some modifications. The appropriate reagents (400 μl borate buffer and 800 μl OPA) were thoroughly mixed with 400 μl wine on a vortex-mixer for 5 s. A second mixing then took place after 2.5 min, and this was followed by a 2.5 min delay before injection (20 μl) of the derivatized sample into the HPLC column.

2.5. Chromatographic conditions

The mobile phase consisted of two different solvents, prepared daily: solvent A: 6.804 g sodium acetate 3 H_2O and 50 ml tetrahydrofuran (THF) were added to a 1l volumetric flask; the solution was made to volume with milli-Q water and well mixed. The pH was adjusted to 5.7 with acetic acid 2% (v/v): solvent B: absolute methanol. Both solvents were filtered through a 0.2 μm pore cellulose acetate membrane filter (Alltech Associates Inc., Deerfield, IL, USA) prior to use. Derivatized amino acids were eluted at a flow rate of 2 ml/min, using a linear multistep solvent gradient programme listed in Table 3, and were detected by spectrofluorometry. The analysis time was only 25 min. All 21 amino acids determined were well resolved and there was no interference from the derivatization reagents. The column clean up protocol, following the gradient elution program, is also outlined in Table 3. Fig. 1 presents a typical chromatogram of free amino acids present in a white wine sample.

2.6. Method validation

The **repeatability** of the method was examined by five consecutive injections of the same sample during a day. The coefficients of variance for the concentrations (mg/l) of amino acids ranged from 0.4 to 5.9% for the standard solution and from 2 to 8.3% for a wine sample in all cases.

Table 3
Gradient elution programme and column clean up protocol (flow rate 2 ml/min)

Time (min)	Eluent A (%)	Eluent B (%)
Gradient elution programme		
0.00	90	10
6.00	72	28
10.00	70	30
11.00	65	35
15.50	63	37
17.50	53	47
19.00	45	55
25.00	35	65
Gradient clean up programme		
25.00	35	65
27.00	35	65
28.00	90	10
30.00	90	10

The **reproducibility** on different days was also examined by injecting the same standard solution five times over a period of 20 days. The calculated concentrations of individual amino acids showed coefficients of variance (CV) less than 4% in all cases for repeated analyses of the same standard solution or a wine sample.

The **linearity** of the fluorescence response was tested using six different concentrations of the standard amino acid solution at concentration levels covering those of the wine samples. Calibration curves were thus established for all amino acids tested; linear regression analysis showed correlation coefficient values (R) within 0.970 and 0.998.

Quantification was performed by the internal standard method, using norvaline as internal standard and it was based on peak areas of the eluted amino acid derivatives.

2.7. Statistical analysis

Discriminant analysis (DA), and analysis of variance (ANOVA) of data were carried out using the SPSS version 10.0 software (SPSS Inc., 2000). The DA was used to provide information on the possibility of grouping wine samples according to variety, viticultural region and vintage. Such groups were defined in a multidimensional space by maximizing the distance between the gravity centres of each group. In order to measure the classification power of the analytical data, the number of individuals correctly predicted to belong to the assigned group was calculated (Day, Zhang, & Martin, 1995).

3. Results and discussion

3.1. General

The total primary amino acid content for the Greek white wines ranged from 68.4 to 2170 mg/l and had an

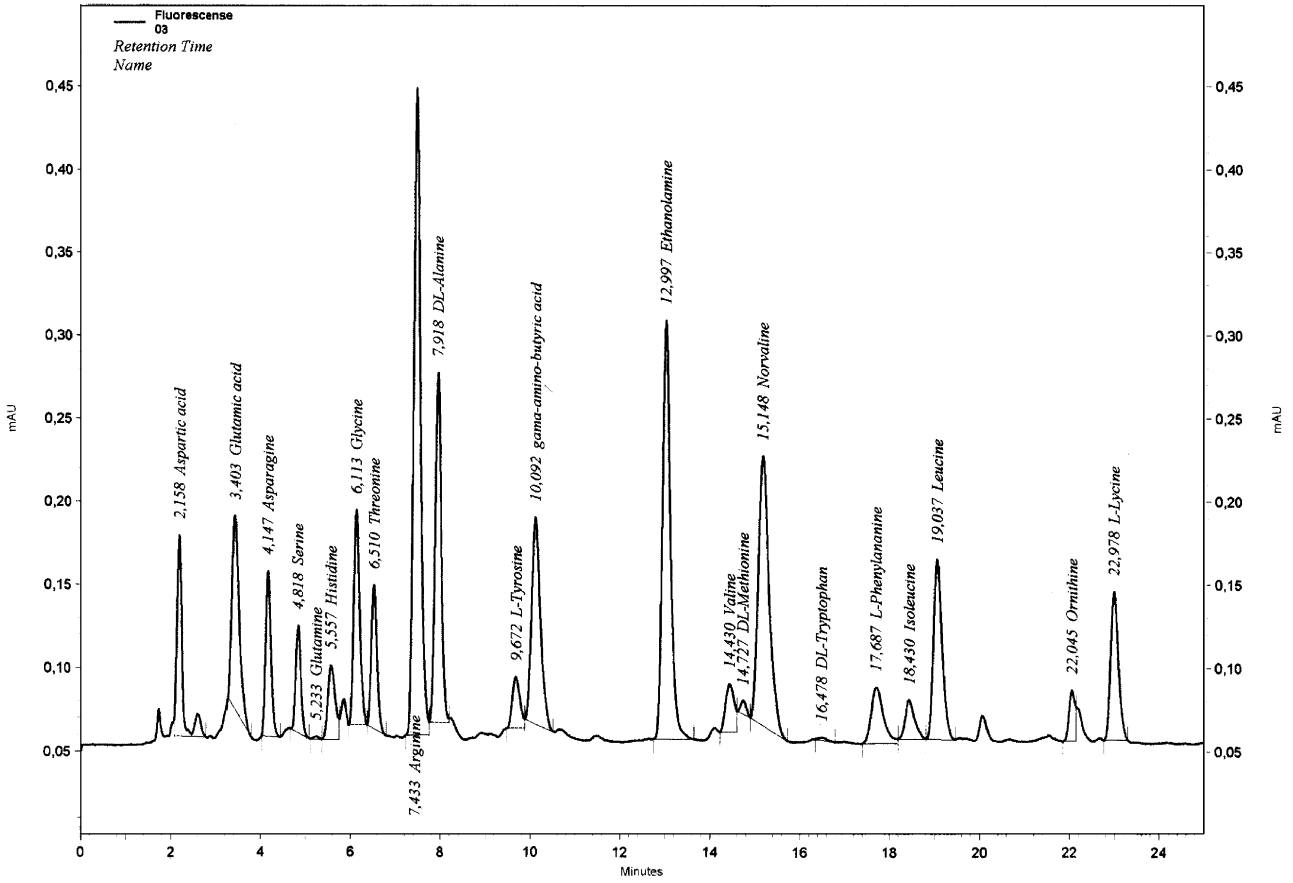

Fig. 1. Typical elution profile of amino acids present in a wine sample.

average value of 416 mg/l (Table 8). ARG, γ-AB, LYS, ALA, GLU, were the most abundant amino acids; among them, arginine (mean 91 mg/l) and γ-amino butyric acid (mean 39.7 mg/l) represented 31% of total primary amino acid content. Bena-Tzourou et al. (1999) also found that GLU, ARG, ALA and LYS were the major amino acids for wines made of the Greek variety Vilana, while Gallander, Cahoon, and Beelman (1969) found that ALA, ARG, GLU, γ-AB were among the major primary amino acids of eight American grape varieties.

In general, these results are in satisfactory agreement with the amino acid values determined for other varieties and viticultural regions. Soufleros et al. (1998) reported that the total values of primary amino acids in French Bordeaux wines, before malolactic fermentation were between 112 and 614 mg/l and, after malolactic fermentation, between 170 and 313 mg/l. Etiévant et al. (1988) have found similar primary amino acid contents (mean values) for 34 French red wines from the Narborne (126 mg/l), Bordeaux (137 mg/l) and Angers (172 mg/l) regions, respectively.

According to the results of Table 4, rather large standard deviation values are shown for some amino acids, which arise from large compositional differences among the wines analyzed. Such differences may originate from the type of fermentation, grape variety, geographical origin, climatic conditions and different viticultural and enological practices adopted during wine making. For example, sweet wines, in which winemaking technology includes stopping of the fermentation naturally or by addition of alcohol, present much higher amino acid values (810 mg/l) than dry wines (351 mg/l) (Table 4). This can be due to the fact that the higher amounts of amino acids in musts that are extracted from overripe grapes are not largely consumed by yeast. Herbert et al. (2000) also reported very high values of primary amino acids in Port wines (mean 1345 mg/l) and Port imitations (mean 2016 mg/l); these wines are produced with the addition of wine distillate before the completion of alcoholic fermentation. According to Margheri et al. (1986), the amino acid content of wines is largely dependent on yeast metabolism, varying widely with the conditions of fermentation (yeast strain, temperature, time of storage over yeast, NH_4^+ added etc.).

Other factors known to influence the concentration of amino acids in musts and wines are the variety of grapes, the region of cultivation, the malolactic fermentation and the vintage. These factors were examined in more detail, based on the data derived from amino acid composition of the wines analysed in the present study.

3.2. Grape variety

Wines from Chardonnay, Muscat white and Muscat d'Alexandrie grapes, more than the other varieties, were characterized by a definite preponderance of most amino acids, while wines from Asyrtiko and Moschofilero grapes were found to contain relatively small amounts of free amino acids. The mean values of concentrations, for all amino acids within each variety, are reported in Table 5.

Wines produced from Roditis, which is the most important Greek white grape variety according to the cultivation area, showed a high concentration of primary amino acids (total of means 430 mg/l) with ARG and LYS being the predominant amino acids. Wines produced from Debina showed a rather low concentration of primary amino acids (total of means 212 mg/l); GLU, LYS, ALA and LEU were the predominant amino acids of this variety, while the γ-AB and ARG values were quite low. The wine samples made from Moschofilero grapes also had very low concentrations of primary amino acids (total of means 163 mg/l); LYS, ALA, GLU and LEU were the most abundant amino acids, while the concentrations of γ-AB and ARG were quite low, just as in wines from the Debina variety. Wine samples from Asyrtiko showed quite low concentrations of primary amino acids (total of means 151 mg/l), with ALA, GLU, LYS and THR representing 40% of the total amino acids of this grape variety.

In the category of aromatic grape varieties, samples from the variety Muscat white showed very high concentrations of primary amino acids (total of means 597 mg/l); ARG and γ-AB were highest, followed by ALA, THR and ETH. Wines made from Muscat d'Alexandrie showed medium concentrations of primary amino acids. Except for one wine sample, that contained 2170 mg/l of total amino acids, the mean value for the remaining wine samples of this variety is 301 mg/l. In these wines, ARG was the most abundant amino acid, followed by γ-AB, LYS and GLU.

Wines made from Chardonnay grapes gave the highest mean concentration of primary amino acids (total of means 618 mg/l); in this variety, ARG was highest, followed by ALA, GLU and γ-AB. Hernández Orte, Guitart and Cacho (1997) reported 265 mg/l as a mean value of primary amino acid content of wines made from Chardonnay grapes of the Samontano Denomination of Origin in Spain. In the latter study, ARG, ALA, γ-AB, GLU were reported as the major amino acids.

Overall, in wines from varieties with low concentrations of primary amino acids GLU, LYS, ALA seem to be the predominant amino acids, whereas in wines from varieties with high total concentrations of amino acids, ARG and γ-AB appeared as the most abundant amino acids. Also, in all wine samples from different varieties, ALA was found among the five most abundant amino acids. Among all amino acids measured, ARG had the highest concentration and was the most variable in

Table 4
Free amino acid content (mg/l) of 42 Greek white wines and their respective composition according to their type

Amino acids	Minimum	Maximum	Average	S.D.	Dry wines ($n^*=36$)		Sweet wines ($n^*=6$)	
					Mean	S.D.	Mean	S.D.
L-Aspartic acid	3.90	74.8	21.9	13.2	22.3	13.8	19.4	9.22
L-Glutamic acid	6.74	140.2	31.0	21.7	32.0	22.7	25.4	14.9
L-Asparagine	0.66	45.1	8.93	7.52	9.91	7.66	3.07	2.23
DL-Serine	1.14	47.4	10.5	8.97	9.37	7.60	17.2	13.8
L-Glutamine	0.00	2.89	0.76	1.03	0.87	1.08	0.10	0.15
L-Histidine	1.02	79.4	13.9	13.0	11.7	7.84	26.7	26.9
Glycine	2.43	38.4	10.4	6.29	9.13	4.29	18.3	10.4
L-Threonine	4.88	62.6	18.9	11.1	17.0	8.41	30.6	17.9
L-Arginine	4.05	1075	91.5	192	53.7	95.8	318	409
DL-Alanine	3.79	238	32.1	35.0	31.3	36.8	36.8	23.3
L-Tyrosine	1.86	36.2	13.5	8.45	13.2	8.59	15.3	8.02
γ-Amino butyric acid	1.46	444	39.7	83.2	21.0	34.0	152	177
Ethanolamine	2.86	97.0	19.5	15.6	15.6	7.13	43.1	29.5
L-Valine	0.00	37.5	8.83	7.05	8.94	7.43	8.16	4.51
DL-Methionine	0.38	14.8	3.84	2.51	4.00	2.57	2.85	2.03
DL-Tryptophan	0.00	9.84	2.25	2.18	2.06	1.65	3.37	4.25
L-Phenylalanine	2.75	52.5	17.0	9.67	16.0	8.14	23.4	15.7
L-Isoleucine	0.00	18.0	6.54	3.38	6.49	3.55	6.81	2.35
L-Leucine	3.92	44.5	21.2	10.1	21.5	10.5	19.4	8.33
DL-Ornithine	0.00	54.7	10.8	13.0	11.1	13.8	8.51	6.36
L-Lysine	5.42	78.8	33.5	17.4	33.8	17.9	31.5	15.9
Total	68.4	2170	417		351		810	

* n: number of wine samples.

wines from varieties Roditis, Chardonnay, Muscat white and Muscat d'Alexandrie.

The large variation in ARG content among the wines most probably originates from the ARG levels present in the grape berry and the resulting must which are influenced by the inorganic nitrogen fertilization. Spayd, Wample, Evans, Stevens, Seymour, and Nagel (1994) have reported an increase of most free amino acids when 56 versus 0 kg N/ha were applied to White Riesling vines; the arginine concentrations increased linearly with increasing nitrogen fertilization. Bertrand, Ingargiola, and Delas (1991) also reported that application of 100 kg N/ha to own-rooted Merlot in Bordeaux, when compared with a no N fertilization regime, increased the wine arginine concentration from 57 to 110 mg/l. On the other hand, arginine is readily utilizable as a nitrogen source during anaerobic fermentation, but the extent of its degradation is variable, depending on concentration of other compounds, which can be preferentially utilized by yeast (Monteiro, Trousdale, & Bisson, 1989).

When discriminant analysis, by the use of 21 amino acids as variables, was applied, a marked tendency toward subgrouping in relation to varietal characteristics was achieved and six discriminating functions were obtained. The first two of them explained 72.4% 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 Muscat White grapes were positioned to the lower right quadrant, while wines of Muscat d'Alexandrie variety were positioned to the lower left quadrant (Fig. 2). Less apparent was the discrimination of wines from the other varieties. Thus, Chardonnay wines were positioned at the upper right quadrant, Debina at the upper left quadrant, quite close to wines from Moschofilero and Asyrtiko, whereas Roditis wines were located mainly at the upper section of the plot. The standardized coefficients of these first two discriminating functions showed that γ-AB, ALA and ETH had the higher weight in discriminating among white varieties. The γ-AB has been previously shown by several authors to discriminate varieties of red wines (Ooghe, Kastelijn, & de Waele, 1981; Polo, Martin-Cordero, & Cabezudo, 1984; Rizzon, 1985).

The percentage of samples correctly classified, on the basis of the derived discriminating functions, was 95.2%. Only one wine sample of Debina and one sample of Moschofilero were incorrectly classified. This can be due to the fact that the group centroids for wines produced of Roditis, Debina, Moschofilero and Asyrtiko are too close to each other (Fig. 2) and their concentrations of amino acids are much alike. In relation to

Table 5
Concentration of amino acids (mg/L) according to grape variety

Amino acids	Grape varieties													
	Roditis ($n^*=15$)		*Debina* ($n^*=3$)		*Moschofilero* ($n^*=4$)		*Asyrtiko* ($n^*=4$)		*Muscat d'Alexandrie* ($n^*=8$)		*Muscat white* ($n^*=4$)		*Chardonnay* ($n^*=4$)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
L-Aspartic acid	26.7	9.73	14.9	9.02	10.1	4.46	9.05	6.14	23.8	8.26	14.6	3.52	36.9	26.3
L-Glutamic acid	35.5	13.8	25.5	9.67	14.0	6.38	16.0	7.86	33.2	10.3	18.9	7.33	58.2	55.3
L-Asparagine	10.7	5.37	7.98	6.15	7.11	3.01	5.54	4.83	5.77	3.50	5.48	4.41	18.1	18.5
DL-Serine	10.4	4.00	6.23	4.41	5.29	1.09	4.10	1.65	13.2	11.9	12.0	8.89	18.6	19.4
L-Glutamine	0.84	1.11	0.84	1.46	0.81	1.14	0.47	0.86	0.69	1.08	0.84	1.30	0.71	0.88
L-Histidine	14.4	5.71	7.65	5.76	5.41	1.93	5.47	3.53	20.4	24.3	16.0	9.51	18.5	16.2
Glycine	10.6	3.62	5.50	2.76	6.03	0.70	5.82	1.80	12.9	10.8	14.3	4.87	13.8	6.52
L-Threonine	18.5	4.34	11.9	5.07	10.9	4.86	12.3	3.67	21.4	16.8	26.3	9.76	28.3	19.6
L-Arginine	75.1	101	12.5	9.74	10.93	5.09	11.0	6.35	158.1	371	199	204	132	202
DL-Alanine	30.9	9.49	15.7	11.0	16.3	4.92	16.3	7.31	30.2	15.8	32.6	22.5	83.6	103
L-Tyrosine	15.6	7.67	12.1	10.4	5.41	3.89	5.37	3.61	17.8	6.23	10.7	6.85	17.4	13.6
γ-amino butyric acid	28.6	34.0	5.20	0.85	6.46	2.27	5.53	2.88	59.6	156	125	110	49.6	71.4
Ethanolamine	16.4	5.22	7.14	3.88	11.2	4.12	12.2	1.53	24.7	29.3	39.1	8.40	26.0	10.4
L-Valine	12.6	8.23	6.49	5.56	3.68	2.06	2.00	1.99	7.80	2.83	6.46	4.60	12.9	9.41
DL-Methionine	5.11	3.27	3.80	2.50	2.36	0.63	1.93	1.02	4.12	1.47	2.08	1.30	3.67	2.01
DL-Tryptophan	2.24	1.70	2.47	2.33	2.10	1.48	0.74	0.88	3.20	3.02	1.88	3.77	2.25	1.92
L-Phenylalanine	20.0	7.19	11.6	7.73	8.28	4.19	7.24	4.35	23.7	13.2	15.0	6.81	17.2	9.02
L-Isoleucine	8.21	3.49	5.28	4.59	3.50	2.44	2.40	2.30	7.71	1.93	6.36	2.28	6.19	2.40
L-Leucine	27.7	8.78	15.5	10.2	11.7	5.88	9.12	5.61	23.6	6.75	14.9	4.89	24.0	11.7
DL-Ornithine	17.2	17.6	10.8	8.00	4.77	2.01	2.63	1.48	3.92	3.81	11.6	4.98	13.5	16.5
L-Lysine	42.8	17.8	23.0	12.8	17.5	8.22	15.7	8.24	39.2	15.0	24.6	11.3	37.1	16.4
Total	430		212		164		151		535		597		619	

* n: number of wine samples.

the variety, the concentrations of variables GLU, ETH, VAL, PHE, ILE, LYS showed statistically significant differences, at the 95% level, among wines, while the variables ASP and LEU showed statistically significant differences at the 99% level.

Overall, the results of amino acid analyses of the wines must be characteristic of the grape variety where they originate. This accords with the view of Larcheveque, Casanova, Dupuch, and Renard (1998) who concluded that differences in amino acid composition of musts are also transferred to the corresponding wines.

3.3. Region

According to the sampling protocol adopted in the present work, the wine samples were deliberately selected from several cultivation areas in Greece. In order to distinguish a possible group separation on the basis of the primary amino acid content, the wine samples were assigned to six groups, corresponding to different regions: Macedonia, Peloponnisos, Central Greece (Thessalia, Sterea Ellada, Epirus), Cyclades Island, Lemnos Island, Samos Island (Table 1).

Table 6 shows that the concentration mean values of individual amino acids and their sums, within each region, varied widely. Peloponnisos and Central Greece had close values of mean concentrations of primary amino acids (380 and 348 mg/l, respectively). It was also noted that wines from Samos and Lemnos Islands, and Macedonia were the richest in amino acids (means 597, 535 and 496 mg/l, respectively), while dry white wines from Cyclades Islands had the lowest concentration (mean 151 mg/l). The definite preponderance in free amino acids of wines from Samos and Lemnos Islands can be explained by the fact that most of the samples from these regions are sweet wines.

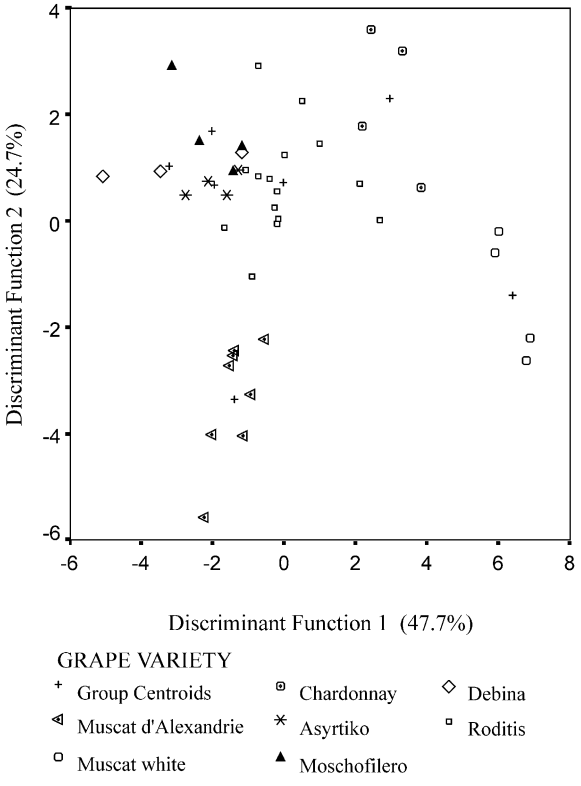

Fig. 2. Discriminant analysis of 42 individual Greek white wines by grape variety; the first two canonical discriminant functions are plotted.

Table 6 shows that wines from Macedonia are rich in ARG (101 mg/l on average), followed by LYS, GAB, GLU, and ALA. Wines from Peloponnisos had higher values of ARG, ALA, GLU, LYS, and γ-AB, while for wines of Central Greece, ARG, LYS, GLU, ALA and ASP were the most abundant amino acids. Wines from Central Greece and Peloponnisos exhibited the same average value of ARG and almost the same concentrations of total primary amino acids.

In the category of aromatic grape varieties, wine samples from Lemnos Island showed very high values of ARG (mean 158 mg/l) and, of the remaining amino acids, γ-AB, LYS, GLU, and ALA, ETH, PHE, LEU, THR were the most predominant. Wines from Samos Island also had high concentrations of ARG and γ-AB (means 198 and 125 mg/l, respectively), followed by ETH, ALA, THR. Samples originating from other Aegean Islands, for example, wines from Cyclades, showed very low total concentrations of primary amino acids (mean 150 mg/l) with ALA, GLU, LYS, THR, and ETH being the most abundant amino acids.

When discriminant analysis by region was applied to the data, five discriminant functions were obtained; the first two accounted for 41.7 and 35.5% of the total variance, respectively. Most of the regional separation occurred along discriminant function 1 (Fig. 3), which was most negatively correlated with ARG and most positively correlated with γ-AB. The amino acid ARG was also most negatively correlated with discriminant function 2 while γ-AB and PHE were positively correlated. The standardized coefficients of these two discriminating functions showed that γ-AB had the higher weight in discriminating among wines produced in different areas. Moreover, according to the geographical characterization of wine samples, only the variables MET, ILE and LEU could provide statistically significant differences among samples at 99, 95 and 95% levels, respectively.

Fig. 3 presents a plot of the scores in the coordinate plane defined by the first two canonical components of the functions with the greatest discriminating power for the wines considered. From the plot, it appears that differentiation and classification were good for regions like Lemnos and Samos Islands and less effective for Central Greece and Cyclades Islands. Furthermore, there was a definite overlap between samples from Macedonia and Peloponnisos regions in the discriminant space. Among the four samples misclassified (9.5%) two wines originated from Macedonia, one from

Table 6
Concentrations (mg/l) of amino acids of wine samples grouped according to origin

Region	Macedonia ($n^*=5$)		Peloponnisos ($n^*=13$)		Central Greece ($n^*=8$)		Cyclades Islands ($n^*=4$)		Samos Island ($n^*=4$)		Lemnos Island ($n^*=8$)	
Amino acids	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
L-Aspartic acid	25.0	9.60	24.2	18.7	24.2	11.1	9.05	6.14	14.6	3.52	23.8	8.26
L-Glutamic acid	40.6	15.7	35.2	35.0	29.6	6.58	16.0	7.86	18.9	7.33	33.2	10.3
L-Asparagine	12.4	7.86	11.7	10.9	8.87	3.75	5.54	4.83	5.48	4.41	5.77	3.50
DL-Serine	11.6	3.43	11.1	11.7	8.51	3.59	4.10	1.65	12.0	8.89	13.2	11.9
L-Glutamine	0.51	1.02	0.72	1.02	1.17	1.17	0.47	0.86	0.84	1.30	0.69	1.08
L-Histidine	15.6	4.98	12.5	10.6	11.7	6.48	5.47	3.53	16.0	9.51	20.4	24.3
Glycine	11.5	3.72	9.97	5.4	8.56	3.40	5.82	1.80	14.3	4.87	12.9	10.8
L-Threonine	17.8	3.29	18.5	12.7	17.6	6.48	12.3	3.67	26.3	9.76	21.4	16.8
L-Arginine	101	132	58.5	114	59.0	106	11.0	6.35	199	204	158	371
DL-Alanine	31.6	10.4	41.3	60.0	27.0	13.10	16.3	7.31	32.6	22.5	30.2	15.8
L-Tyrosine	17.7	7.06	12.2	10.1	14.2	8.29	5.37	3.61	10.7	6.85	17.8	6.23
γ-Amino butyric acid	42.7	53.5	25.5	41.4	15.5	18.4	5.53	2.88	125	110	59.6	156
Ethanolamine	17.8	5.13	17.4	8.74	12.6	7.19	12.2	1.53	39.1	8.40	24.7	29.3
L-Valine	13.3	4.44	10.2	10.3	9.38	5.56	2.00	1.99	6.46	4.60	7.80	2.83
DL-Methionine	7.13	4.95	3.28	1.38	4.24	1.98	1.93	1.02	2.08	1.30	4.12	1.47
DL-Tryptophan	2.28	1.92	1.89	1.68	2.81	1.53	0.74	0.88	1.88	3.77	3.20	3.02
L-Phenylalanine	20.4	6.03	15.8	9.63	16.3	6.85	7.24	4.35	15.0	6.81	23.7	13.2
L-Isoleucine	9.48	4.98	5.58	2.81	7.23	3.36	2.40	2.30	6.36	2.28	7.71	1.93
L-Leucine	30.7	8.93	21.2	11.3	22.0	9.31	9.12	5.61	14.9	4.89	23.6	6.75
DL-Ornithine	19.6	20.3	11.3	13.9	15.0	15.1	2.63	1.48	11.6	4.98	3.92	3.81
L-Lysine	47.7	15.1	33.0	20.7	32.9	14.1	15.7	8.24	24.6	11.3	39.2	15.1
Total	496		381		348		151		597		535	

* n: number of wine samples.

Peloponnisos and one from Central Greece. Such results may be related to the fact that wine samples from the islands of Samos and Lemnos belonged to two specific grape varieties (Muscat white and Muscat d'Alexandrie), which are not met in wines from all other regions (Table 1). On the other hand, some samples from Macedonia, Central Greece and Peloponnisos belong to a common grape variety (Roditis), which is cultivated almost all over the country. These samples show variations in total and individual amino acid concentrations among the regions; for example, the mean total amino acid concentration for wines from the Roditis grape variety was 496 mg/l for samples produced in Macedonia, 428 mg/l for samples produced in Central Greece and 394 mg/l for those produced in Peloponnisos. These values show a gradual increase in amino acid content from the South to Northern regions of cultivation for the same grape variety. Etiévant et al. (1988) also reported differences among French wines according to the latitude of the production region and that such differences correspond to increasing total nitrogen content in grapes from South to North.

3.4. Malolactic fermentation

Malolactic fermentation (MLF) is part of the traditional wine making techniques for red and white wines.

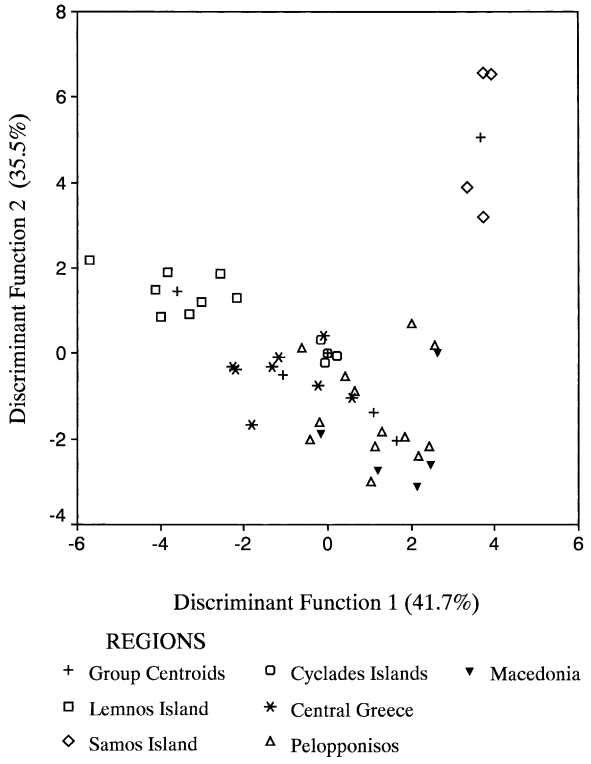

Fig. 3. Discriminant analysis of 42 individual Greek white wines by origin; the first two canonical discriminant functions are plotted.

It contributes strongly to aroma and aftertaste of some wines (i.e. Chardonnay), while its contribution in a Riesling wine may only be to give the wine a softer and rounder taste (Henick-Kling, Acree, Krieger, Laurent, & Edinger, 1994). However, malolactic fermentation is not usually pursued in all white wines.

In the present study, MLF had occurred in 29% of the wine samples examined. These samples were dry wines and were produced from grapes of Debina, Roditis and Muscat d'Alexandrie. According to Table 7, the concentrations of most amino acids in the samples are higher in wines that have not undergone malolactic fermentation. These findings accord with previous observations by Soufleros et al. (1998) who reported that following MLF, the amino acid concentration is generally reduced, except for phenylalanine and ornithine.

The most pronounced changes in amino acid composition in the malolactic-fermented wines, when compared with the no MLF-wines, were those of ARG and γ-AB, which showed nearly five-fold reductions. According to Ough, Crowell, and Mooney (1988), arginine, with the onset of lactic acid bacteria action, metabolizes into ornithine, which then converts quickly into proline and urea.

3.5. Vintage

From the 42 white wines analysed, 21 were produced in 1999, 11 in 1998, two in 1997 and the remaining eight were of unknown year of production.

Very small variations in the mean values of total amino acid content were found within the three years of production (Table 8). The wines of 1998 had higher percentages of ARG (27.3%) and γ-AB (11.4%) in relation to the total primary amino acid concentration, while wines of 1999 vintage were higher in ARG (13.5%), γ-AB (5.6%) and ALA (5.1%). Wines of 1997, based on the results only of two samples, had higher percentages of LYS (14.7%), ARG (11.2%) and GLU (9.2%). It seems that arginine percentages vary among years, which concurs with the findings of Spayd and Andersen-Bagge (1996) who showed that arginine concentrations varied widely between years, for all white grape varieties from Washington, studied. Moreover, the results (Table 8) indicate that each vintage is probably characterized by a specific amino acid profile, although in the present study wines from many varieties are implicated. Larcheveque et al. (1998), in their study of the Merlot grape variety, showed that each vintage had a specific amino acid profile. Huang and Ough

Table 7
Concentrations (mg/l) of amino acids in wine samples grouped according to the occurrence of malolactic fermentation

Amino acids	MLF[a] ($n^*=6$)	Without MLF ($n^*=15$)
L-Aspartic acid	22.9	23.7
L-Glutamic acid	36.5	36.3
L-Asparagine[b]	5.92	10.9
DL-Serine	9.19	11.5
L-Glutamine	0.47	0.79
L-Histidine	10.6	15.8
Glycine	8.58	11.2
L-Threonine	16.4	20.0
L-Arginine[b]	22.5	96.7
DL-Alanine	24.7	35.0
L-Tyrosine	14.9	16.1
γ-Amino butyric acid	9.33	48.8
Ethanolamine	11.7	16.4
L-Valine	12.3	12.3
DL-Methionine	3.87	5.74
DL-Tryptophan	2.36	3.62
L-Phenylalanine	17.4	20.8
L-Isoleucine	6.57	8.17
L-Leucine	23.2	27.7
DL-Ornithine	11.5	12.4
L-Lysine	37.9	41.4
Total amino acids	315	490

* n: number of wine sample.
[a] MLF, malolactic fermentation.
[b] Statistical difference among the two groups at 95% level.

Table 8
Concentrations (mg/l) of amino acids in 33 wine samples grouped according to their vintage

Amino acids	1997 ($n^*=2$)		1998 ($n^*=11$)		1999 ($n^*=20$)	
	Mean	% of total	Mean	% of total	Mean	% of total
L-Aspartic acid	33.7	7.8	21.0	4.7	21.5	3.0
L-Glutamic acid	39.7	9.2	29.1	6.6	32.5	4.5
L-Asparagine	8.58	2.0	7.75	1.7	10.7	1.5
DL-Serine	13.7	3.2	10.9	2.5	10.7	1.5
L-Glutamine	0.00	0.0	0.54	0.1	0.84	0.1
L-Histidine	18.9	4.4	15.5	3.5	13.4	1.8
Glycine	14.1	3.3	11.1	2.5	9.35	1.3
L-Threonine	18.3	4.3	19.2	4.3	19.2	2.6
L-Arginine	48.2	11.2	121	27.3	98.2	13.5
DL-Alanine	32.9	7.6	27.8	6.3	37.2	5.1
L-Tyrosine	24.3	5.6	12.2	2.8	13.2	1.8
γ-amino butyric acid	7.14	1.7	50.6	11.4	40.8	5.6
Ethanolamine	16.4	3.8	22.3	5.0	18.1	2.5
L-Valine	9.15	2.1	8.28	1.9	8.16	1.1
DL-Methionine	6.36	1.5	3.89	0.9	3.81	0.5
DL-Tryptophan	2.31	0.5	3.24	0.7	1.71	0.2
L-Phenylalanine	28.9	6.7	17.4	3.9	15.8	2.2
L-Isoleucine	9.45	2.2	6.06	1.4	6.59	0.9
L-Leucine	33.0	7.7	20.3	4.6	20.6	2.8
DL-Ornithine	2.39	0.6	7.04	1.6	12.6	1.7
L-Lysine	63	14.7	28.5	6.4	33.2	4.6
Total	431		444		428	

* n: number of wine samples.

(1991) reported that amino acid profiles appear to have similar patterns for a grape variety from the same location in different years, but there may be considerable variations in the levels of some amino acids from year to year. According to Flanzy and Poux (1965) and Schrader, Lemperle, Becker, and Bergner (1976), higher levels of amino acids can be found in musts of cool years than those of warm and sunny years. Apparently, in cooler years a smaller amount of proteins is synthesized from amino acids in the not-sufficiently ripened berries.

When discriminant analysis was applied to the data, a good discrimination among vintage years was obtained. The percentage success in classifying samples was 97.1%. The first two discriminant functions identified accounted for 63.1 and 36.9% of the variability in the data, respectively. Of the samples presented in Fig. 4 it appears that wines produced in 1997 are placed in the upper left quadrant, those of 1998 are located in the upper right quadrant, while most of wines of the 1999 vintage are clustered in the lower left quadrant. Wines of the 1997 vintage had higher concentrations of 12 amino acids, while wines of the 1998 and 1999 vintages exhibited higher concentrations of four amino acids each. The last two vintages are positioned symmetrically at opposite places on the imaginable diagonal axis that crosses the *y*-intercept of vertical and horizontal axes, which can be considered as an axis of the wine age.

The standardized coefficients of the two most important discriminating functions showed that the discriminant function 1 was most negatively correlated with ALA, γ-AB and HIS, and most positively correlated with ARG, SER. The discriminant function 2 was most negatively correlated with THR, and most positively correlated with ASP. Only LYS was found to differ among wines statistically at the 95% level. Consequently, differences in climate or grape maturity among vintages could have affected the concentration of LYS in the wines of different crop years.

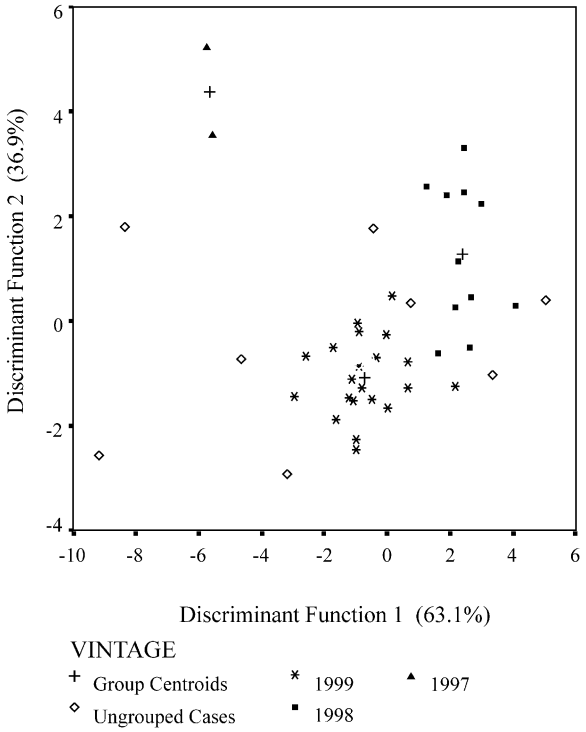

Fig. 4. Discriminant analysis of 42 individual Greek white wines by vintage; the first two canonical discriminant functions are plotted.

4. Conclusions

The amino acid concentrations of Greek white wines were within the range reported for other European white wines. The results indicate the influence of grape variety, geographic location and vintage on the amino acid composition of wine. The type of fermentation also has an impact on the concentration of certain amino acids; for example, sweet wines had generally higher amounts of most amino acids than dry wines, whereas wines which had undergone malolactic fermentation appeared to contain lower amounts of ARG, γ-AB and MET.

Discrimination of wines was attempted on the basis of amino acid composition and the use of statistical methods. Wines from Chardonnay, Muscat white and Muscat d'Alexandrie grapes, were characterized by high primary amino acid contents, while wines from Asyrtiko, Moschofilero and Debina grapes were found to contain relatively small amounts of total free amino acids. Moreover, wines from the Greek islands of Samos and Lemnos had the highest concentrations of amino acids, while those of Cyclades Islands had the lowest. An increase in amino acid content was observed for wines of the same variety originating from the Southern regions and towards the Northern areas of grapevine cultivation. Wines of the 1998 vintage exhibited higher percentages of ARG and γ-AB in their primary amino acid profiles, than wines of 1999 and 1997 vintages. Within all wine samples tested as a group, ARG and γ-AB were the most abundant amino acids, followed by LYS, ALA, GLU, ASP, LEU, ETH.

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