Characterization of Elementary Wines of *Vitis vinifera* Varieties by Pattern Recognition of Free Amino Acid Profiles

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The free amino acid compositions of 42 elementary wines obtained from eight Portuguese *Vitis vinifera* varieties were studied over a 7-year period. The wine free amino acid profiles were obtained by capillary gas chromatography of the *N*-heptafluorobutyryl isopropyl esters. All the *V. vinifera* varieties were grown in the same vineyard, under identical conditions. The elementary wines were made under fixed standard procedures. Chemical data were treated by pattern recognition techniques, involving hierarchical clustering, principal-component analysis, and discriminant analysis. The free amino acid compositions of the elementary wines are correlated to the corresponding original grape varieties in the 42 cases studied.

Amino acid analysis plays a major role in food chemistry, not only in the assessment of food biological value but also, in many instances, as a characterization parameter. Amino acid composition has been used as a criterion for fruit juice evaluation (Wallrauch, 1985) as well as a quality control parameter for estimating juice content in commercial citrus juices and detection of adulterations (Vandercook and Price, 1972).

Wine characterization still relies heavily upon taste impressions. The subjective character of tasting is a strong stimulus to look for more objective procedures, based on chemical characterization. Pattern recognition techniques have been successfully used in enological research (Kwan and Kowalski, 1978; Kwan et al., 1979). Classification of wines by cluster analysis could be achieved through data from trace-element analysis (Siegmund and Bächmann, 1978), and gas chromatographic data on volatile compounds were used by Kwan and Kowalski (1978), who applied pattern recognition techniques to the classification of 42 wines of *Vitis vinifera* cv. Pinot Noir according to geographical origin.

Amino acids present in the mature fruit of *V. vinifera* accumulate in the berry during maturation (Poux, 1970). After vinification, the wine free amino acids profile is generally dominated by proline. Proline content in wine has been proposed as a genuinity parameter. However, it has been recognized that proline content depends on exogenous factors such as fertilization procedures. It seems to be rather indifferent to other factors such as the ones related to the fermentation techniques (Ooghe et al., 1981). Pattern recognition techniques have been attempted to correlate wine origin and amino acid profiles after acid hydrolysis of the wine peptides (Ooghe et al., 1981), but recent studies indicate that free amino acids may be more useful for the purposes of wine characterization (Vasconcelos and Chaves das Neves, 1985). The amino acid profile of the grape juice seems to be highly dependent on a variety of factors such as grape variety, type of soil, and climatic conditions (Flanzy and Poux, 1965). On the basis of those observations, we studied, during a 7-year period, the amino acid composition of 42 elementary wines, made under controlled vinification procedures, from 8 Portuguese grape varieties, grown under the same soil and climatic conditions in the same vineyard. In this work, we report the results of the studies on the free amino acid profiles of the elementary wines by pattern recognition techniques. It is shown that a correlation exists between the free amino acid profiles of the elementary wines produced during the period from 1977 to 1983 and the original grape varieties.

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EXPERIMENTAL SECTION

Wine Samples and Fermentation Techniques. The grape varieties were grown in the same vineyard at Reguengos de Monsaraz (Alentejo, South Portugal), a region known for its characteristic quality wines. The varieties studied are typical for that Portuguese wine-producing region. The elementary wines from four white and four red *V. vinifera* varieties were studied. The grapes were vinified at the winery of the University of Évora. The white destemmed grapes were vinified without skins, without temperature control or yeast inoculation. Prior to fermentation, 45 mg/L of tartaric acid and sodium metabisulfite, corresponding to 25 mg/L of SO_2, were added as the only additives used. The white varieties are designated as Roupeiro (1), Manteúdo (2) Tamarez (3), and Rabo de Ovelha (4). The red varieties, with the designations of Moreto (5), Trincadeira (6), Periquita (7), and Aragonez (8), were fermented with skins, stems, and stones. The wines were separated from the lies after alcoholic fermentation. All other conditions were identical with the ones used for the white grapes. The wines were bottled after 1 year in concrete. At bottling, malolactic fermentation was accomplished. The wines produced over a 7-year period from 1977 to 1983 were studied (Table I). Missing years for some varieties are due to grape spoilage caused by meteorological conditions at the harvest.

Reagents and Standards. Standard amino acids were chromatographic grade purchased from Supelco (Bellafonte, PA). All solvents were obtained from E. Merck (Darmstadt), purified by distillation after appropriate drying. Heptafluorobutyric anhydride (HFBA) was obtained from Pierce Chemical Co. (Rockford, IL) and distilled prior to use. The 3 M solution of HCl in 2-propanol was prepared every day by adding the corresponding amount of acetyl chloride to 25 mL of 2-propanol under ice-cooling. The OV-1 liquid phase used in capillary column preparation was obtained from Alltech Associates (Deerfield, IL).

Gas Chromatography. Capillary gas chromatography of the heptafluorobutyryl amino acid isopropyl esters was carried out with a Pye Unicam instrument, equipped with a split–splitless injector, a flame ionization detector, and a homemade 25 m × 0.25 mm (i.d.) borosilicate glass capillary, coated with OV-1, d_f = 0.25 μm. Analysis was performed under the following conditions: split ratio, 1:25; injector and detector temperatures, 300 °C; initial oven temperature, 110 °C, hold was isothermic for 5 min, followed by linear temperature programming at a heating rate of 3 °C/min until the final temperature of 270 °C was reached; carrier gas, hydrogen at an inlet pressure of 100 kPa. Quantitative calculations were performed by the internal standard method, using cycloleucine as internal standard, with a Shimadzu computing integrator, Model CR3-A, equipped with floppy disk and a CRT monitor. Whenever necessary, GC–MS experiments were performed with a Shimadzu instrument, Model QP-1000.

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Table I. Description of the Wine Samples

variety	sample identification		
	year	abbrev	code
Roupeiro	1977	R77	1
	1979	R79	1
	1980	R80	1
	1981	R81	1
	1982	R82	1
	1983	R83	1
Manteúdo	1977	M77	2
	1978	M78	2
	1979	M79	2
	1981	M81	2
	1982	M82	2
	1983	M83	2
Tamarez	1979	T79	3
	1980	T80	3
	1981	T81	3
	1982	T82	3
	1983	T83	3
Rabo de Ovelha	1977	Ro77	4
	1978	Ro78	4
	1981	Ro81	4
	1982	Ro82	4
Moreto	1978	Mo78	5
	1979	Mo79	5
	1981	Mo81	5
	1982	Mo82	5
	1983	Mo83	5
Trincadeira	1977	Tr77	6
	1978	Tr78	6
	1979	Tr79	6
	1980	Tr80	6
	1981	Tr81	6
	1982	Tr82	6
	1983	Tr83	6
Periquita	1977	P77	7
	1978	P78	7
	1979	P79	7
	1981	P81	7
	1982	P82	7
	1983	P83	7
Aragonez	1978	A78	8
	1981	A81	8
	1983	A83	8

Sample Preparation. Each wine sample was selected from randomly chosen bottles, to complete a total sample volume of 2 L. A 200-mL aliquot of the total sample volume was taken as the working sample. From this, four 20-mL samples were used for derivatization and quantitative amino acid determinations according to the following procedure: 20 mL of wine sample was deproteinized by addition of 80 mL of 95% ethanol and the resultant mixture left at −10 °C for 10 min. After that time, the sample was centrifuged at 3500 rpm for 10 min; the supernatant was transferred to a 50-mL round-bottom flask and concentrated to a final volume of 10 mL in a rotary evaporator. To the solution was added the amount of a standard stock solution corresponding to 1 mg of cycloleucine as an internal standard. The amino acids were isolated by ion exchange over Dowex 50W-X8, by elution with 4 M NH_4OH until no positive response to the ninhydrin test was observed. The eluate was concentrated to a final volume of approximately 2 mL in a rotary evaporator, from which a 250-μL aliquot was transferred to a Teflon-lined screw-cap derivatization vial and evaporated to dryness under a light stream of nitrogen. The residue was dried overnight over P_2O_5 in vacuo. To the dry residue was added 100 μL of a 3 M HCl/2-propanol solution. The solution was heated in the closed vial at 110 °C for 30 min. After the mixture was cooled to room temperature, the solvent was evaporated under a light stream of nitrogen and 50 μL of HFBA, 100 μL of CH_2Cl_2, and 10 μL of ethanethiol were added to the residue. This solution was heated at 150 °C for 15 min. After being cooled to room temperature, the solution was directly used for gas chromatography.

Pattern Recognition Analysis. The identity of the free amino acids in each sample, derivatized as the *N*-heptafluorobutyryl isopropyl esters, was assigned on the basis of their retention data relative to cycloleucine, as compared to the corresponding individual standards, and by gas chromatography–mass spectrometry. The amino acid concentration in each sample is the mean of four replicates (Table II). Each wine sample was then regarded as an assembly of features, each feature being the normalized percent amino acid composition as a mean of four replicates for each amino acid derivative. Principal-component analysis (PCA) and discriminant analysis were performed by means of the statistical software package Statgraphics (Statistical Graphics Corp., Rockville, MD). Hierarchical clustering was achieved with Clue (Apple IIe version; Elsevier Scientific Software, Amsterdam).

RESULTS AND DISCUSSION

Table I describes the elementary wines studied, according to the grape variety used for vinification. The grapes were grown in the same vineyard, under similar conditions. This eliminates the possible variations due to different soils, climatic conditions, and fertilization procedures. All grapes were vinified according to controlled vinification procedures. They correspond to the most typical varieties used in wine making in the Alentejo region, known in Portugal for its characteristic quality wines. The free amino acid composition of each wine sample is presented in Table II. The relative percent concentrations of the individual free amino acids in the elementary wines from the same grape variety in the different years show high relative standard deviations and fall outside the parameters of a normal distribution. Some amino acids are present only occasionally. These are the cases of sarcosine, present only in 1977 and 1982 wines of the Rabo de Ovelha variety, and α-aminobutyric acid, detected in most of the Rabo de Ovelha and in two Periquita wines. Those wines obtained the highest scores in sensory tests. Another interesting observation is the low proline content of the 1979, 1981, and 1982 wines of the Manteúdo variety. This fact confirms the inadequacy of the proline test for general wine authenticity. These results show that, on the basis of the amino acid composition alone, no typification of the wines can be achieved nor can any apparent relation to the original *V. vinifera* variety be established. For this to be achieved, it is necessary to ferret out more relevant information from the amount of data collected from the gas chromatographic amino acid analysis. Under the circumstances, this is a typical problem for multivariate analysis of data, for which pattern recognition studies are particularly suited.

Each wine sample (object) was considered as an assembly of variables (or features) represented by the relevant amino acids. Therefore, each amino acid was treated as a feature with a magnitude representing its relative percent concentration in each sample. Classification of the data vectors into categories according to the original grape varieties was first attempted by principal-component analysis. In the starting approach, 22 variables were considered as features forming a data vector representing a particular wine sample. A correlation matrix was constructed with the relative percent concentrations for each of the 22 features and the 42 wine samples as objects. Principal components and component weights were calculated by using a routine of Statgrafics. A scatter plot was obtained, which correlates the weighting factors of the variables in the first principal component versus the weighting factors in the second principal component. It can be seen from Figure 1 that Ile, Gaba, Pro, Met, and Tyr are the dominating features in the second principal component (12% of the total variability), while Ala, Gly, Val, Thr, Ser, Leu, Cys, Asx, Glx, Phe, and Lys strongly dominate the first principal component. Both components account for 49% of the total variability. Group classification by PCA af-

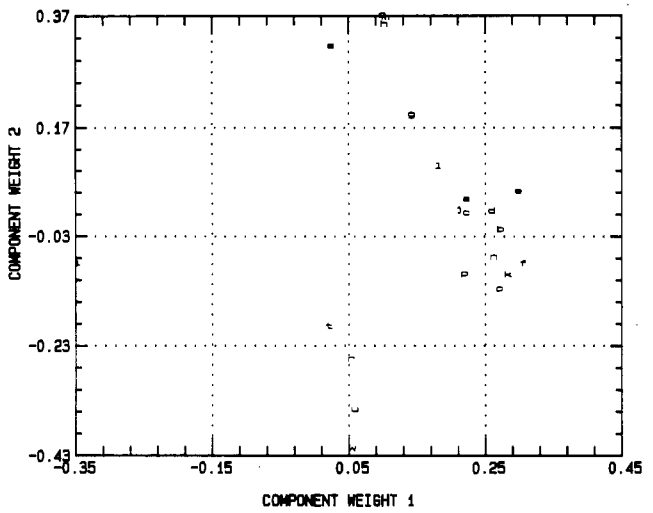

Figure 1. Plot of component weights in first principal component versus component weights in second principal component from PCA of free amino acid profiles in elementary wines from Portuguese *V. vinifera* varieties. Symbols: a, Ala; b, Gly; c, Val; d, Thr; e, Ser; f, Leu; g, Ile; h, Gaba; i, Pro; j, Cys; k, Asx; l, Hyp; m, Met; n, Glx; o, Phe; p, Lys; q, Tyr; r, His; s, Orn; t, Arg; u, Sar; w, Abu.

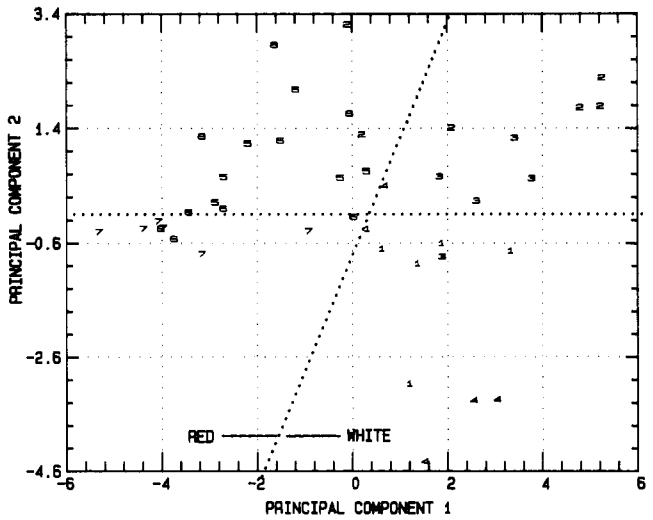

Figure 2. Plot of the first principal component versus second principal component in PCA of free amino acid profiles (22 variables) in elementary wines from Portuguese *V. vinifera* varieties. Sample labels as in Table I.

forded interesting results. When a two-dimensional plot of the first principal component versus the second principal component was drawn, a clear separation of the objects (wine samples) in two main groups was achieved, with two misclassified samples of variety 2 (Figure 2). One group (varieties 1–4) corresponds to the wines from the white varieties. The other group, constituted by the wines from the red varieties (5–8), is clearly separated from the former. A remarkable tendency for subgrouping according to original grape variety is observed. The white wines clearly form four independent clusters corresponding each one to the original grape variety. Only one sample corresponding to classification group 3 appears included within group 1. Interestingly, this corresponds to the wine obtained from the 1980 Tamarez variety. This was an exceptionally rainy year in the region, specially during maturation time, that led to the discarding of the varieties Manteúdo, Rabo de Ovelha, Moreto, Periquita, and Aragonez due to grape spoilage. The 1980 wines of the varieties Roupeiro and Trincadeira show a similar effect. It is interesting to note

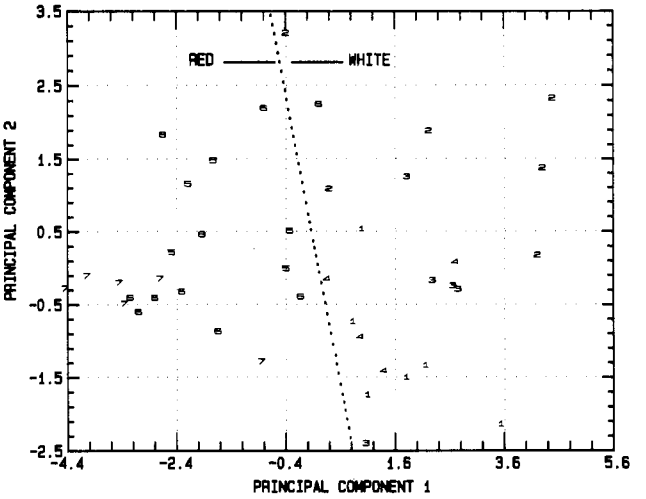

Figure 3. Plot of the first principal component versus the second principal component in PCA of free amino acid profiles (13 variables) in elementary wines from Portuguese *V. vinifera* varieties. The variables are Ala, Thr, Ser, Leu, Ile, Cys, Asx, Hyp, Met, Glx, Phe, Lys, and Tyr. Sample labels as in Table I.

that subgrouping of the red wines according to grape variety is not as clear as the subgrouping observed for the white wines. This agrees with the fact that the wines of the white varieties studied are more easily characterized and differentiated by sensory tests. Principal component analysis is a useful technique for reducing the number of variables in a data set by finding the linear combinations of those variables that explain most of the variability between objects. Selection of a smaller number of features increases the reliability of the results of mathematical classification. From the study of component weights, 13 variables can be extracted as highly significant. They are Ala, Thr, Ser, Leu, Ile, Cys, Asx, Hyp, Met, Glx, Phe, Lys, and Tyr. With the relative percent concentration of these amino acids as variables in PCA, a clear-cut separation of the red and white wines was obtained with one misclassified sample (Figure 3). The tendency for subgrouping according to variety is maintained, following a pattern identical with the one observed in Figure 1. However, varieties Roupeiro (1) and Rabo de Ovelha (4) group together, as do Moreto (5) and Aragonez (8), indicating that they are closely related. This agrees with the results of sensory evaluation. The outlier of group 2 corresponds to the 1978 Manteúdo wine. This sample deviates from the other members of the series by lower contents of Gly, Thr, Leu, Gaba, Asx, Hyp, and Lys and a much higher content of Tyr (Table II). Further attempts to improve classification by variable selection were not as successful. The fact that 100% success is not achieved, in spite of the strong tendency for clustering, may be explained by two reasons: The two principal components account for only 60% of the total variability; each set of wine samples within the same variety is best characterized by a certain number of features that are not qualitatively nor quantitatively the same in all varieties. The characteristic features for each classification group can be assessed by PCA performed for the wines originating from the same grape variety. Application of minimal spanning tree (MST) procedures leads to similar results. Table III compares the results obtained by both methods to the description of the wine samples by their most characteristic amino acids. The variables listed in Table III for each wine variety as profile defining in PCA were used for wine classification by hierarchical clustering by means of Clue. Examination of the dendogram in Figure 4 shows that the

Table II. Normalized Percent Free Amino Acids Composition of Elementary Wines from *V. vinifera* Varieties Roupeiro (R), Rabo de Ovelha (Ro), Tamarez (T), Manteúdo (M), Aragonez (A), Moreto (Mo), Periquita (P), and Trincadeira (Tr)

sample	amino acid,[a] %																				
	Ala	Gly	Abu	Sar	Val	Thr	Ser	Leu	Ile	Gaba	Pro	Cys	Asx	Hyp	Met	Glx	Phe	Orn	Lys	Tyr	His	Arg
R77	6.5	3.8			2.3	2.8	3.8	5.1	5.1	0.4	51.2	1.5	7.5	1.9	2.8	5.9	3.1		5.1		0.5	0.6
R79	6.5	3.8			2.3	2.8	4.0	7.8	0.2	0.2	32.5	0.8	7.6	2.0	1.1	4.8	4.0		4.8		1.3	1.6
R80	5.0	4.4			4.2	4.6	6.2	10.3		2.2	28.3	2.4	9.5	2.2	2.2	9.7	6.8		0.5	1.1		
R81	7.4	4.0			3.4	3.0	4.7	8.4		3.0	39.5	6.8	7.6	1.8	1.6	6.5	5.0		6.0	0.3		
R82	7.2	3.7			1.4	2.2	4.2	5.5		1.5	47.7	7.3	4.1	1.8	1.0	6.2	2.3		2.4	1.2		
R83	6.3	3.9			2.7	3.0	4.6	7.4			39.8	3.8	7.5	1.9	1.7	6.6	4.2		3.8			
mean	6.3	3.9			2.7	3.1	4.6	7.4		1.2	39.8	3.8	7.3	1.9	1.7	6.6	4.2		3.8			
SD[b]	14.5	6.4			35.8	3.1	18.8	25.9		72.6	21.8	72.7	24.0	7.7	39.2	24.8	37.0		53.6			
med[c]	6.4	3.9			2.5	2.9	4.4	7.6		1.0	39.7	3.1	7.6	1.9	1.7	6.4	4.1		4.3			
Ro77	7.0	4.6	5.2	0.6	4.5	3.7	4.2	6.1	1.2	0.9	43.5	5.0	5.6	0.8	0.9	5.0	3.6		4.0			
Ro78	8.2	2.7			4.7	2.6	3.0	5.7	2.7	2.2	36.8	2.6	5.5	0.4	1.6	3.3	2.1	11.1	5.0	0.4	1.2	
Ro81	12.5	5.6	2.5		9.7	1.3	2.5	9.4			31.3	4.1	5.6	0.8	1.5	5.9	4.4	0.7	10.9		2.3	
Ro82	7.4	6.0	3.8	0.6	2.0	4.0	6.0	7.0	4.5	1.5	29.0	4.2	5.7	1.1	2.1	6.4	5.6	0.7	8.7			
mean	8.8	4.7	3.8		5.2	2.9	3.9	7.1	2.8	1.5	35.2	3.9	5.6	0.8	1.5	5.2	3.9	4.2	7.2			
SD	28.9	31.2	28.7		61.7	42.2	39.6	23.5	48.1	34.6	18.3	25.2	1.4	37.1	32.3	26.5	37.4		44.9			
med	7.8	5.1	3.8		4.6	3.2	3.6	6.6	2.8	1.5	34.1	4.2	5.6	0.8	1.6	5.5	4.0	0.7	6.9			
T79	14.5	7.9			3.5	5.6	6.1	8.4	1.7		18.9	3.0	6.1	1.5	2.6	2.9	3.2		3.9	3.5		
T80	8.2	4.6			6.3	3.6	4.5	7.1	3.5		28.6	4.3	6.7	0.9	1.6	5.9	3.5	1.1	5.9	1.1		
T81	12.0	7.5			6.7	1.5	2.4	12.6	1.2		36.0	0.4	6.0	1.9	0.9	1.7	7.2					
T82	7.7	5.7			2.2	2.8	6.5	5.2	1.6		30.4	3.0	4.7	2.6	4.1	3.0	2.2		13.8	1.8		
T83	10.9	8.6			5.9	4.2	4.6	8.1	1.7		14.9	4.8	6.7	2.3	3.7	4.4	4.1		6.0			
mean	10.7	6.9			4.9	3.5	4.8	8.3	1.9		25.6	3.1	6.0	1.8	2.6	3.6	4.0		7.4	2.1		
SD	26.3	24.1			39.9	43.3	33.6	32.8	46.2		34.3	55.0	13.5	36.7	46.8	45.0	46.9		51.2	47.2		
med	10.9	7.5			5.9	3.6	4.6	8.1	1.7		28.6	3.0	6.1	1.8	2.6	3.0	3.5		6.0	1.8		
M77	5.5	2.6			3.0	2.6	3.6	4.2	2.0	3.8	42.1	4.3	4.1	1.6	2.9	4.6	2.2	2.4	3.1	4.2		
M78	6.9	2.5			4.7	2.0	0.7	5.9	2.8	2.3	31.2	3.0	0.8	0.3	3.1	3.5	2.7	8.2	0.6	15.8		
M79	10.3	6.1			4.6	4.8	7.5	8.6	3.1	6.5	5.0	5.3	8.5	2.4	3.6	5.5	4.1		7.5	8.6		
M81	7.9	7.9			7.8	5.4	7.2	9.5	3.7	3.4	2.4	5.2	5.6	15.2	3.1	5.7	5.0	6.9	2.3	1.5		
M82	8.5	9.2			4.1	4.9	10.7	7.4	3.5	3.6	1.9	3.6	6.9	3.4	5.9	5.1	4.6	4.0	5.7	6.9		
M83	6.9	4.8			3.1	3.5	6.3	7.8	3.9	3.3	31.5	3.1	6.5	1.0	4.1	6.1	3.9		5.9	9.0		
mean	7.7	5.5			4.6	3.9	6.0	7.2	3.2	3.8	19.0	4.1	5.4	4.0	3.8	5.1	3.8	5.4	4.2	7.7		
SD	21.4	49.7			38.4	35.7	57.6	26.5	22.0	37.0	94.1	24.8	49.7	140.5	29.7	18.3	29.0	42.7	62.1	63.7		
med	7.4	5.5			4.4	4.2	6.8	7.6	3.3	3.5	18.1	4.0	6.1	2.0	3.4	5.3	4.0	5.5	4.4	7.8		
A78	7.1	2.1			1.8	0.9	1.4	1.6	1.2	8.0	40.8	0.9	3.0	0.04	3.0	3.2	1.8	14.4	1.0	1.4		
A81	7.0	2.3			3.7	1.9	2.8	5.2	2.7	2.1	46.9	2.4	4.1	0.2	4.6	3.4	2.4	0.9	3.4	7.8		
A82	2.4	1.8			1.6	1.0	1.2	1.6	0.9	1.3	68.1	1.6	2.2	0.5	5.9	1.5	0.4	1.7	0.8	0.2		
mean	5.5	2.1			2.4	1.3	1.8	2.8	1.6	3.8	51.9	1.6	3.1	0.3	4.5	2.7	1.5	5.7	1.8	3.1		
SD	48.8	12.2			48.9	43.4	48.4	74.2	56.9	96.3	27.8	45.9	30.8	103.0	32.5	36.1	66.9	132.2	80.1	130.3		
med	7.0	2.2			1.8	1.0	1.4	1.6	1.2	2.1	46.9	1.6	3.0	0.2	4.5	3.2	1.8	1.7	1.0	1.4		
Mo78	4.7	1.6			2.8	0.6	0.7	2.4	1.8	0.8	70.5	3.0	2.9	0.3	4.0	1.8	0.8		1.3			
Mo79	7.1	2.3			6.1	2.4	2.4	5.6	3.8	2.6	51.7	2.6	4.1		2.4	2.5	2.1		2.2			
Mo81	3.4	2.2			2.7	1.2	1.3	2.3	1.1	2.1	73.1	5.1	2.8	0.1	1.1	0.7	0.7	0.5	0.8			
Mo82	4.5	1.6			2.5	1.4	1.8	2.3	1.2	2.1	64.5	3.5	2.2	0.6	5.2	2.6	0.9	1.7	1.3			
Mo83	9.0	6.8			4.8	2.3	2.6	4.4	2.8	4.9	45.8	2.5	4.2	0.2	2.2	3.8	1.5		2.1			
mean	5.7	2.9			3.8	1.6	1.7	3.4	2.1	2.5	61.1	3.3	3.2	0.3	3.0	2.3	1.2		1.5			
SD	35.3	67.9			37.7	43.2	39.7	40.0	47.9	53.6	17.4	28.3	24.5	62.3	35.5	44.6	44.1		38.7			
med	4.7	2.2			2.8	1.4	1.8	2.4	1.8	2.1	64.5	3.0	2.9	0.3	2.4	2.5	0.9		1.3			
P77	4.5	3.2	1.4		1.1	3.3	0.9	2.1	1.1	0.1	76.4		1.6		1.2	1.3	0.9		0.9			
P78	14.4	4.0	0.6		2.9	4.8	1.8	4.5	0.9	0.8	56.9	0.3	3.0		0.5	2.9	0.3	1.0	0.4			
P79	3.7	1.9			1.7	1.1	1.1	2.5	0.4	0.2	83.3	0.3	0.9	0.4	1.1	0.4	0.9					
P81	3.9	1.5			2.2	0.7	0.9	2.4	0.5	0.4	82.1	0.1	2.5	0.8	0.5	1.2	0.2					
P82	1.8	1.2			0.4	0.3	0.3	0.4	0.1	0.08	93.0	0.2	0.7	0.1	0.2	0.5	0.3	9.2				
P83	2.4	4.8			0.7	0.7	0.5	0.1	0.1	0.08	86.1	0.4	0.5	0.3	0.8	1.0	0.7	0.7				
mean	5.1	2.8			1.5	1.8	0.9	2.0	0.5	0.3	79.6	0.3	1.5	0.4	0.7	1.2	0.6					
SD	83.0	75.0			57.7	91.0	52.1	73.0	73.0	91.8	14.2	43.8	61.0	73.6	49.2	71.3	53.2					
med	3.8	2.6			1.4	0.9	0.9	2.2	0.5	0.2	82.7	0.3	1.3	0.4	0.7	1.1	0.5					
Tr77	4.4	1.4			1.5	1.0	1.2	1.4	0.9	2.3	77.3		3.5		0.9	2.5	0.8		0.8			
Tr78	8.9	2.1			2.7	1.9	1.7	1.9	1.2	11.1	58.3		4.2		0.7	4.3	1.1		0.8			
Tr79	5.3	2.2			2.4	1.4	1.1	2.1	2.0	2.2	71.6		4.8		0.9	1.8	1.1		1.0			
Tr80	6.0	1.1			2.9	1.7	1.7	3.0	1.5	2.0	50.5	1.6	3.3	0.9	6.3	3.4	2.1	5.1	2.2	4.5		
Tr81	4.6	1.4			1.2	1.2	0.9	0.8	0.6	0.1	83.3	0.2	0.4	1.2	0.5	1.1	2.1		0.6	0.1		
Tr82	6.3	4.5			5.8	5.9	0.4	4.3	4.6	0.4	55.6	0.8	3.8	0.2	0.2	1.9	7.6			1.5		
Tr83	3.2	1.9			1.6	1.5	0.5	1.2	0.7	0.2	80.1	0.3	2.8	0.2		0.7	2.0		1.0	0.7		
mean	5.5	2.1			2.6	2.1	1.1	2.1	1.6	2.6	68.1	0.7	3.3	0.6	1.6	2.3	2.4		1.0	1.7		
SD	30.4	50.5			55.7	76.6	44.8	53.0	78.0		17.8	76.4	40.1	70.0	134.0	87.7	90.0		49.2	99.4		
med	5.3	1.9			2.4	1.5	1.1	1.9	1.2	2.1	71.6	0.6	3.5	0.6	0.8	1.9	2.0		0.8	1.1		

[a] Amino acids RSD ≈1–3%. [b] Percent. [c] Median.

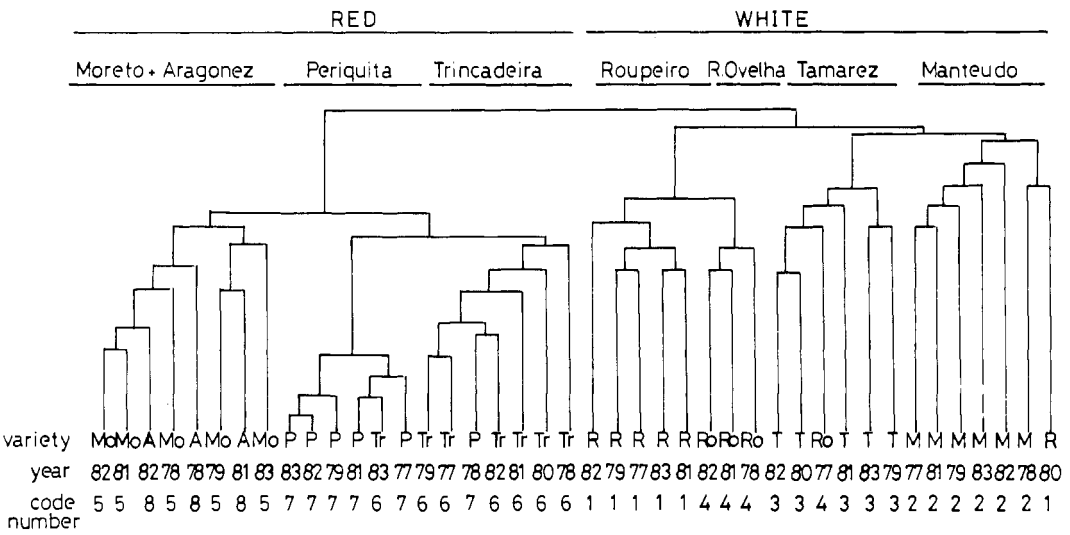

Figure 4. Dendogram of hierarchical clustering classification of elementary wines from Portuguese *V. vinifera* varieties by pattern analysis of free amino acid profiles. Profile-defining variables from Table III.

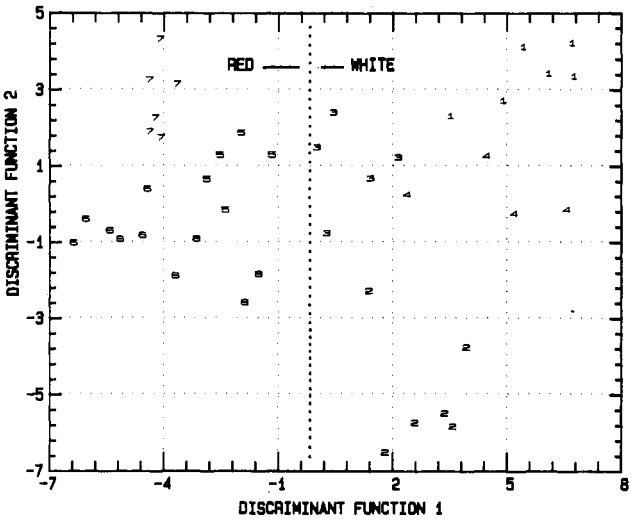

Figure 5. Classification of elementary wines from Portuguese *V. vinifera* varieties by discriminant analysis of free amino acid profiles. The data in Table II were used, except for sarcosine and α-aminobutyric acid.

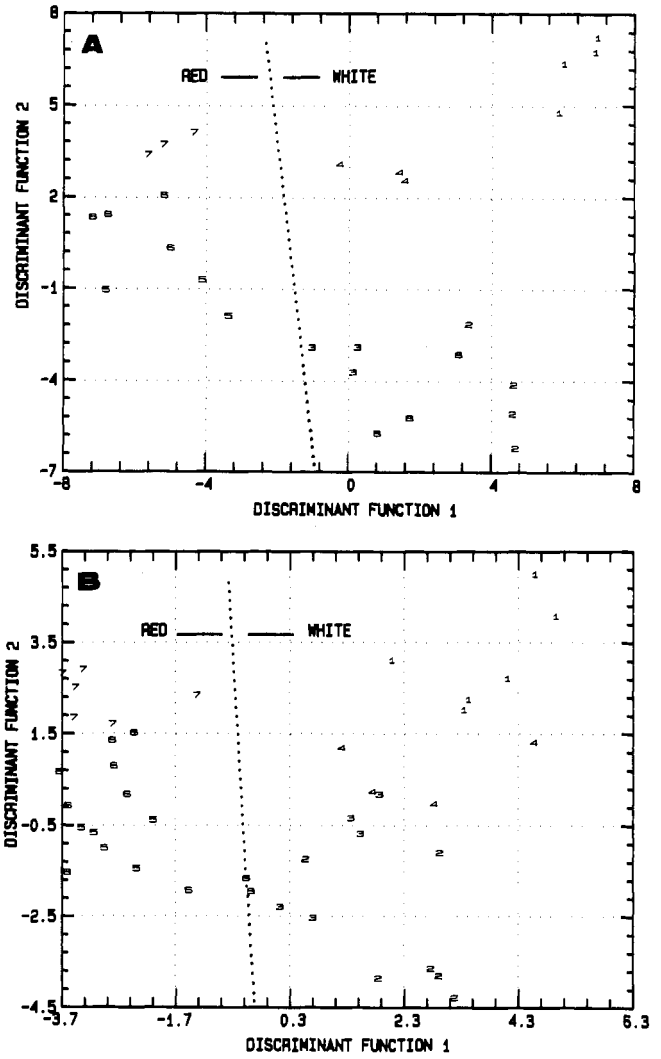

Figure 6. Classification of elementary wines from Portuguese *V. vinifera* varieties by discriminant analysis of free amino acid profiles. Two examples of plots of the first discriminant function versus the second discriminant function in a jackknifing test series: (A) 27 samples; (B) 42 samples. Variables used: Gly, Val, Thr, Leu, Gaba, Pro, Met, Glx, Lys, and Orn. Sample labels as in Table I.

amino acid profiles define two main groups corresponding to the red and the white varieties. The white varieties are classified in three well-defined clusters for Manteúdo, Tamarez, and the pair Roupeiro–Rabo de Ovelha. The red wines are grouped in two main clusters, Periquita and Trincadeira on one side and Moreto and Aragonez on the other. The last two show a high degree of similarity that does not allow the formation of characteristic subclusters. These results completely agree with the ones obtained by PCA and can be taken as a strong indication that the wine free amino acids may play an important role as enological parameters. It is interesting to note that wine amino acids have been referred to as contributing to the overall taste of wines (Amerine and Rössler, 1976). The application of the multivariate approach to treatment of analytical data on wine free amino acid composition yields results that are surprisingly coincident with the empirically accepted characteristics of the studied wines. The wines from the Aragonez variety are believed to be subject to larger variations from vintage to vintage and are more affected by climatic conditions than all the others. This is displayed in the PCA plot through the dispersion of the corresponding samples. The wines from the red varieties Moreto and Aragonez are commonly used for conferring finer aging qualities to wines. The most typical red wines of the Alentejo region are the ones from the Periquita and Trincadeira varieties. They are the preferred ones in the

Table III. Profile-Defining Amino Acids As Obtained by Principal Component Analysis (PCA) and Minimal Spanning Tree (MST)

	variety															
	white								red							
amino acid	Roupeiro		Rabo de Ovelha		Tamarez		Manteúdo		Moreto		Trincadeira		Periquita		Aragonez	
	PCA	MST	PCA	MST	PCA	MST	PCA	MST	PCA	MST	PCA	MST	PCA	MST	PCA	MST
Ala	yes	yes			yes	yes	yes		yes	yes	yes	yes	yes	yes	yes	
Gly		yes	yes	yes	yes	yes	yes	yes	yes						yes	
Abu			yes													
Sar			yes													
Val					yes	yes			yes	yes			yes	yes		yes
Thr			yes	yes	yes	yes		yes	yes	yes		yes	yes	yes	yes	yes
Ser		yes	yes	yes			yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Leu					yes	yes	yes	yes	yes	yes			yes	yes	yes	yes
Ile	yes						yes	yes	yes	yes				yes	yes	yes
Gaba		yes				yes			yes		yes	yes	yes	yes		
Pro	yes				yes											
Cys	yes	yes	yes	yes			yes				yes					
Asx	yes		yes		yes	yes	yes	yes	yes	yes	yes		yes	yes	yes	
Hyp	yes							yes			yes					
Met							yes				yes	yes				
Glx	yes	yes	yes	yes			yes	yes			yes	yes	yes	yes	yes	
Phe	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes					yes	
Orn											yes		yes			
Lys			yes						yes	yes	yes				yes	yes
Tyr											yes				yes	yes
His	yes															
Arg	yes															

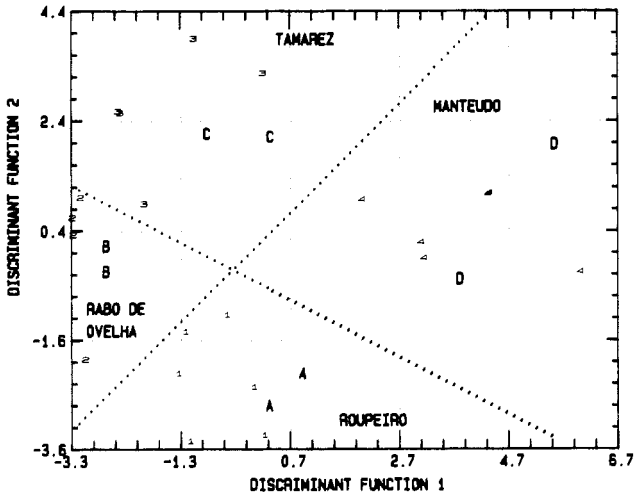

Figure 7. Classification of elementary wines of white Portuguese *V. vinifera* varieties by discriminant analysis on free amino acid profiles. The variables used are the same as in Figure 6. Samples labeled A–D correspond to correctly classified 1985 and 1986 wines of the same variety, used as tests.

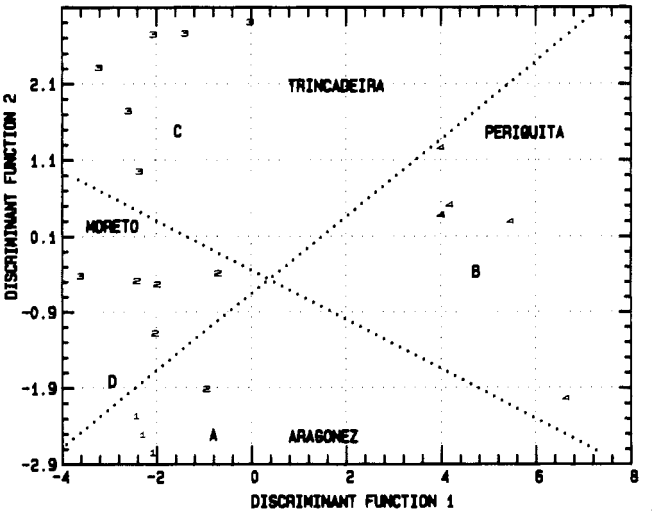

Figure 8. Classification of elementary wines of red Portuguese *V. vinifera* varieties by discriminant analysis of free amino acid profiles. The variables used are the same as in Figure 6. Sample labeled A–D correspond to correctly classified 1985 and 1986 wines of the same variety, used as tests.

production of one-variety quality wines. As for the white varieties, it is empirically accepted that they produce the most typical white wines in that Portuguese region, each one possessing well-defined individual sensory characteristics, Roupeiro being the variety "par excellence".

The results from discriminant analysis follow identical patterns. An initial matrix containing the 42 objects and the variables from Table II, except α-aminobutyric and sarcosine, was used as an exploratory training set. The recognition ability for the two main classes (red and white varieties) and for grouping within each class according to variety is shown in the plot of the first discriminant function versus the second discriminant function (Figure 5). However, both discriminant functions account for only 69% of the discriminatory information. The prediction ability of the discriminant function was under 50%. Feature reduction was accomplished by selecting the variables containing most of the discriminatory information. A new training set was derived from a new matrix with Gly, Val, Thr, Leu, Gaba, Pro, Met, Glx, Lys, and Orn as features. The training set was constituted by 25 objects from the initial set. A plot of the first discriminant function versus the second discriminant function, accounting for 81% of the discriminatory information, was obtained (Figure 6A). The objects are clearly separated into two main groups as before, within well-formed subgroups according to grape variety. The prediction ability of the new function was checked by a jackknifing procedure. Correct classification was achieved with a success rate of 78%. As an example, Figure 6B shows the results obtained in the classification of the wine samples.

For practical purposes, the deduction of a rule for classification of red wine samples, on one side, and white wine samples, on the other, according to the original grape

variety is more important. Thus, the wine samples were separated into two groups: red wines and white wines. For each group, the above set of 10 variables was used in deriving discriminant functions for group classification according to grape variety. The white wines (Figure 7) are classified according to two main groups, respectively Tamarez–Manteúdo and Roupeiro–Rabo de Ovelha. The function was tested by the leave-one-out method. The red wines behave identically (Figure 8). The function distinguishes in 80% of the cases between Aragonez–Moreto and Trincadeira–Periquita. This confirms the observations drawn from principal component analysis and hierarchical clustering.

The results of this study of pattern recognition of amino acid profiles in elementary wines show that there is a clear correlation between wine free amino acid content and the original grape variety. The use of elementary wines obtained under the same fermentation procedures from well-known Portuguese *V. vinifera* varieties, grown under the same soil and climatic conditions, eliminates the influence of these factors. Under this conditions, a direct correlation between grape variety and wine free amino acid content in the absence of extraneous factors was established for the 42 wines studied.

ACKNOWLEDGMENT

We thank Smith Kline & French International for a research grant that made this work possible.

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Received for review May 20, 1988. Revised manuscript received February 9, 1989. Accepted March 3, 1989.

A Direct Spectrofluorimetric Determination of the Herbicide Flurecol in Cultivated Soils

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A method for determining residues of the herbicide and plant growth regulator flurecol in soil is described. Soil is extracted with methanol. The organic extracts were evaporated to dryness and redissolved in *N,N*-dimethylformamide. The compound is determined with a spectrofluorimetric detector. A concentration range from 0.13 to 6.8 μg/mL with a detection limit of 40.90 ng/mL could be determined by normal, first, or second synchronous derivative spectrofluorimetric technique with a maximum relative standard deviation of 4.58%. Recoveries of spiked soil samples varied from 88.23 to 105.64%.

Since the advent of organic pesticides (insecticides, herbicides, fungicides) in the 1930s, numerous compounds have been developed for the control of different pests. During the past 15 years, there has been a trend toward the use of pesticides that would degrade more readily and thus be less detrimental to the environment.

This is the case of flurecol (9-hydroxyfluorene-9-carboxylic acid), introduced by Schneider (1964), which acts via leaves and roots as a growth-retarding and -suppressing agent with an effect limited to dicotyledoneous plants. The general symptoms are inhibition of natural growth together with dwarfing, inhibition of elongation of internodes, and breaking of apical dominances.

Because flurecol and its derivatives differ in their action from other plant growth regulators, the term morphactin has been proposed for them (Schneider et al., 1965). They are nontoxic to honey bees and are quickly and completely degraded in soil.

Trace analysis methods for the determination of pesticide residues in crops, animal tissues, soil, and water need to have both high sensitivity and selectivity (Roberts, 1985). As can be seen in the literature, most organic pesticides would be observed with a UV detector. Selective detection techniques are beginning to be used for improving the determination of these compounds. One of these detection techniques, fluorescence, is well regarded as an analytical tool because of its excellent sensitivity and added selectivity, as compared to classical colorimetric methods. Nevertheless, its application to organic residue analysis has been somewhat limited due to the fact that not too many pollutants are very fluorescent and that many naturally occurring compounds interfere. A number of pesticides have been reported to fluoresce naturally (Argauer, 1977; Addison et al., 1977). Recently, several

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