

Relationship between Varietal Amino Acid Profile of Grapes and Wine Aromatic Composition. Experiments with Model Solutions and Chemometric Study

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Synthetic solutions containing amino acids, sugar, water, and yeast nutrients have been fermented by *Saccharomyces cerevisiae*, and the volatile composition of the fermented media has been analyzed by GC. Eleven amino acid compositions imitating the characteristic amino acid profile of 11 different grape varieties were tested. Significant differences in the levels of some important volatile compounds (ethanol, ethyl acetate, acetic acid, higher alcohols and some of their acetates, methionol, isobutyric acid, ethyl butyrate, and hexanoic and octanoic acids) were found. The levels of some of the volatile compounds are well correlated with the aromatic composition of wines made with grapes of the same varieties. A multiple linear regression study produced good models for most of the odorants for which the level was related to the must amino acid composition. Partial least-squares regression models confirm that amino acid composition explains a high proportion of the variance in the volatile composition and show that the relationship between both sets of variables is highly multivariate. According to the different models, the levels of some byproducts of fatty acid synthesis are related to threonine and serine, the level of β -phenylethanol is closely related to the level of phenylalanine, and methionol is strongly correlated to the must methionine contents. The addition of selected amino acids to different musts confirms the previous observations.

KEYWORDS: Wine; grape varieties; amino acids; volatile compounds; aroma; PLS regression

INTRODUCTION

Different studies have shown that the levels in wine of volatile compounds biochemically related to the yeast amino acid metabolism are related to the variety of grape from which the wine was made (1–8). To name but a few examples, the levels of isobutyric and isovaleric acids, their ethyl esters, isobutanol, isoamyl alcohol, β -phenylethanol, methionol, and isoamyl and phenylethyl acetates were found to differ according to the variety of grape in Spanish red wines (6). The levels of isobutyric and isovaleric acids were also found to depend on the grape variety in the case of French red wines (2). Isoamyl acetate was identified as a key odorant of red wines made with the variety Pinotage (1). Some of these compounds have been necessary to build discriminant functions able to classify German wines (3, 5) or Spanish white and red wines (4, 6–8) with regard to their varietal origin. The existence of all these relationships made us think that the amino acid profile of a must had to be closely related to the wine content on the amino acid-related odorants and that this influence has, probably, a sensory impact.

Knowledge about the relationship between the must amino acid composition and the wine aroma composition was reviewed by Rapp and Versini in 1991 (9). Although that excellent review

states that “a strong correlation exists between the amino acid spectrum in must and the absolute and relative levels of the higher alcohols in wine”, such a correlation was not clear at that time. In fact, the only consistent result was that the addition of nitrogen, up to a certain (not known a priori) level, or of some individual amino acids, caused a decrease in the concentration of certain higher alcohols (10–13). These relationships were also found statistically by our research group (14). In the case of the higher alcohol acetates, the results were less clear. Increased use of nitrogen fertilizer on grapes produced an increase in the levels of isoamyl acetate (15), whereas a decrease in β -phenylethyl acetate was noted. Other authors noted a constant level in this last ester under similar conditions (9, 11). No clear relationship was found in our own studies (14, 16). The reasons results are often (apparently) contradictory have to be related to the complexity of the amino acid uptake system by yeast (17) and to the inadequate simplicity of univariate linear models (13). On the one hand, it has been described that Monod-like equations are more adequate to describe the relationship between the amino acid content and the production of volatile compounds in simple microbiological systems (18). On the other hand, at least 15 different amino acid transport systems have been described in *Saccharomyces cerevisiae*, and their activity is regulated by the presence of ammonium and the relative concentration of the different amino acids (17). In this context,

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Table 1. Composition (in Milligrams per Liter) of Amino Acids Related to the Variety of Grape of the 11 Synthetic Media Resembling 11 Types of Must

	Asp	Glu	Ser	Gly	His	Thr	Arg	Pro	Met	Phe	Lys	Gln
Cabernet Sauvignon	22.25	46.71	34.98	4.14	314.8	39.72	79.65	1718.0	43.64	5.61	0.00	71.52
Merlot	22.00	52.00	46.50	5.00	23.60	36.00	28.50	738.0	10.60	19.25	14.95	4.50
Grenache	34.82	61.83	21.21	1.11	109.20	18.80	199.50	241.5	29.85	11.15	3.33	104.80
Tempranillo	86.52	85.24	60.08	6.47	137.40	72.27	673.10	302.3	25.20	7.53	13.69	177.30
Chardonnay	39.00	114.00	86.00	3.00	39.43	65.50	159.30	418.7	10.70	28.33	9.73	84.00
Pinot Noir	52.33	139.30	60.00	5.67	38.67	96.33	332.70	118.7	12.80	24.43	15.40	71.33
Riesling	76.30	101.60	88.53	9.05	181.30	70.61	224.70	273.2	45.38	11.99	0.00	247.90
Moristel	26.63	66.21	22.38	0.00	65.00	20.02	189.90	224.4	24.42	5.39	13.37	66.95
Sauvignon Blanc	49.50	167.70	71.50	2.50	40.63	69.00	447.20	351.7	9.25	35.98	11.90	145.70
Carignan	28.96	96.16	34.93	0.00	68.00	27.55	154.30	539.0	14.33	5.54	0.85	54.64
Macabeo	71.90	72.28	57.58	8.42	125.50	51.53	326.00	286.4	40.35	30.16	0.00	323.30

it is not surprising that linear relationships should be held only for a certain amino acid composition and a narrow concentration range.

As to the amino acid profile of grape varieties, there exists a more or less specific amino acid profile for each variety (19–21). Of course, variations due to the area can be important (20), as are those related to the vintage or the maturity level (21). Bringing all of these ideas together, we could envision a must having an amino acid profile that is more or less similar to those of other musts from the same grape variety. That amino acid profile would influence the order in which the different amino acids are taken by the yeast, which in turn would influence the ratio of secondary metabolites produced. Therefore, the amino acid profile of a grape variety would be related to the aroma profile of the wine. This paper presents the results of a series of experiments carried out to check this hypothesis. The main goal of the present paper is to check if specific amino acid compositions imitating the amino acid profile of different grape varieties can induce a specific wine aroma profile.

MATERIALS AND METHODS

Reagents. Vitamins, growth factors, ergosterol, Tween 80, and individual amino acids were from Sigma (St. Louis, MO). Inorganic salts and glucose were of analytical reagent quality from Panreac (Barcelona, Spain). The pure reference compounds used in the quantitative analysis of volatile compounds were purchased from Aldrich (Gillingham, U.K.), Sigma (St. Louis, MO), Fluka (Buchs, Switzerland), Poly Sciences (Niles, IL), Lancaster (Strasbourg, France), or Chemservice (West Chester, PA). Water was obtained from a Milli-Q purification system (Millipore, Bedford, MA). The solvents used were dichloromethane of HPLC quality from Fischer Scientific (Loughborough, U.K.) and absolute ethanol ARG from Riedel-de-Häen (Seelze, Germany).

Solutions. Internal standard solution 1 was 2-butanol, 4-methyl-2-pentanol, 4-hydroxy-4-methyl-2-pentanone, and 2-octanol at 140 µg/mL in absolute ethanol.

Internal standard solution 2 was acetone 10% (v/v) in water.

Calibration Solutions. Exact weights of the chemical standards dissolved in absolute ethanol were diluted with water/ethanol to form a synthetic wine containing the aroma compounds at concentrations typically found in wine. These synthetic wines were 12% (v/v) in ethanol and 5 g/L tartaric acid at pH 3.2 (adjusted with 1 M NaOH).

Nonvarietal Amino Acid Solution. This solution contained amino acids found at fairly constant levels in all of the grape varieties: γ -aminobutyric acid (44.37 mg/L); alanine (58.51 mg/L); tyrosine (13.34 mg/L); valine (17.73 mg/L); isoleucine (13.43 mg/L); leucine (13.42 mg/L).

Varietal Amino Acid Solutions. This set of solutions consisted of 11 different amino acid compositions containing variable amounts of amino acids at the levels described in the literature for 11 different grape varieties. The different compositions are given in **Table 1**.

Table 2. Loss of Mass and Alcoholic Degree Reached by the Different Synthetic Media Used in the Experiment

synthetic must	wt lost (g)	alcoholic degree (%)
Moristel 1	5.52	11.7
Moristel 2	5.48	11.6
Carignane 1	5.62	11.8
Carignane 2	5.82	11.8
Merlot 1	5.52	11.7
Merlot 2	5.64	11.8
Sauvignon Blanc 1	5.38	11.9
Sauvignon Blanc 2	5.41	11.6
Macabeo 1	5.98	12.0
Macabeo 2	5.57	11.8
Grenache 1	5.13	10.8
Grenache 2	5.18	11.2
Tempranillo 1	4.89	11.2
Tempranillo 2	5.05	11.3
Chardonnay 1	4.87	11.1
Chardonnay 2	5.05	11.1
Pinot Noir 1	5.13	10.9
Pinot Noir 2	5.05	11.0
Riesling 1	4.95	11.3
Riesling 2	5.03	11.0
Cabernet Sauvignon 1	5.11	11.1
Cabernet Sauvignon 2	5.05	11.0

Fermentations. The synthetic medium for yeast development has been described by Albers et al. (22). The nitrogen source consisted of a mixture of amino acids prepared from the two different sets of solutions described above. Mineral components and glucose were autoclaved separately at 121 °C for 20 min. Amino acid, vitamins, and growth factors solutions were filtered through Acrodisk amicrobial disks from Pallgelman Sciences (Ann Arbor, MI). Ergosterol and Tween 80 were kept at 100 °C in a heater for 10 min just before their use. Fifty-four milliliters of the mixture containing all of the nutrients except the amino acids were put into 100 mL Erlenmeyer flasks. Then, the two amino acid solutions were added (the second one with the amino acid formulation characteristic of a grape variety), and the mixture was inoculated with the yeast, capped with a sterile cap, and incubated at 21 °C. This experiment was done in duplicate. The dry active yeast was *S. cerevisiae* from Uvaferm (Novo Nordisk) and was activated before its use by hydrating it in sterile water at 35 °C. The progress of the fermentations was monitored by weighting. At the end of the fermentation, the solutions were centrifuged at 5000 rpm for 10 min, and the supernatant was taken for analysis.

Three different musts from the varieties Grenache, Carignane, and Moristel were obtained by standard enological methods (destemmed grapes were crushed, pressed, and clarified). Each must was divided into six lots. The first lot was the control, and to each of the five other lots were added different amounts of amino acids: lot 1, 114 mg/L His; lot 2, 103 mg/L Thr plus 15 mg/L Lys; lot 3, 31 mg/L Phe; lot 4, 65 mg/L Ser; lot 5, 900 mg/L Pro plus 31 mg/L Met. The musts were inoculated with yeasts and fermented, and the obtained wines were analyzed as described below.

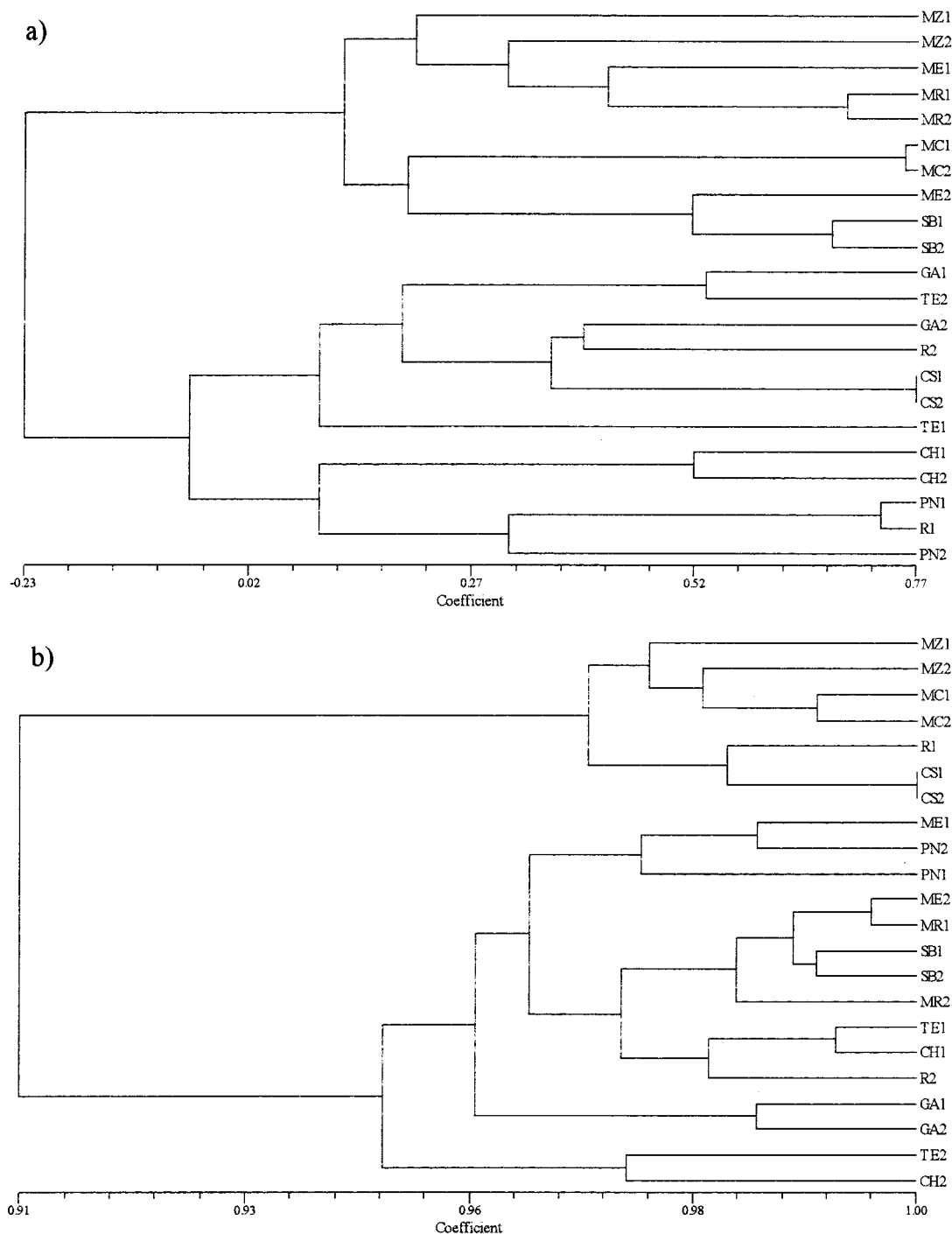


Figure 1. Dendrograms from the cluster analysis (unweighted pair-group method average solution) relating samples through their volatile composition. The similarity coefficient used was (a) the Pearson correlation coefficient or (b) the Spearman correlation coefficient. Sample codes: CH, Chardonnay; CS, Cabernet Sauvignon; GA, Grenache; MC, Macabeo; ME, Merlot; MR, Moristel; MZ, Carignane; PN, Pinot Noir; R, Riesling; SB, Sauvignon Blanc; TE, Tempranillo.

Analysis of Volatiles. The volatile compounds were analyzed following the procedure recently proposed by Ortega et al. (23). According to that method, 3 mL of the sample to be analyzed was transferred into a 15 mL screw-capped centrifuge tube and the following were added: 7 mL of water, 4.5 g of ammonium sulfate, 15 μL of internal standard solution 1, and 0.2 mL of dichloromethane. The tube was shaken mechanically for 1 h and later was centrifuged at 2500 rpm for 5 min. The dichloromethane phase was recovered with a 0.5 mL syringe, transferred to the autosampler vial, and analyzed.

The chromatographic analysis was performed in a Hewlett-Packard 5890 series II gas chromatograph (Avondale, PA) equipped with a DB-Wax column (50 m \times 0.32 mm \times 0.5 μm) from J&W (Folsom, CA) preceded by a 2 m \times 0.53 mm uncoated precolumn. The column was

initially at 40 $^{\circ}\text{C}$, and after 5 min was raised at 3 $^{\circ}\text{C}/\text{min}$ to 200 $^{\circ}\text{C}$. The carrier gas was hydrogen at 3 mL/min. The injection was in split mode (injection volume = 3 μL), the split flow being 30 mL/min. The detector was a FID.

The chromatographic peaks of the analytes were normalized by one of the internal standards (see ref 23), and the relative area was then interpolated in the calibration graphs built by the analysis of synthetic wines of known concentration of volatile compounds.

Ethanol Analysis. One milliliter of the sample and 1 mL of internal standard solution 2 were transferred into a 10 mL volumetric flask and brought to volume with water. One microliter of that solution was directly analyzed by GC in a Shimadzu GC-9A (Kyoto, Japan) equipped with a 2 m \times 0.55 mm column from Teknokroma (Barcelona, Spain)

Table 3. Values of *F* and *p* Obtained in the One-Way Analysis of Variance^a

	absolute concn		relative concn	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
ethanol	13.185	0.000	na ^b	na
ethyl acetate	4.755	0.012		
ethyl propanoate	3.141	0.046		
isobutyl acetate	6.543	0.003	10.370	0.001
ethyl butyrate			6.520	0.004
isobutanol	5.482	0.007		
isoamyl acetate	2.675	0.072	5.163	0.009
isoamyl alcohol			3.838	0.024
acetic acid	8.151	0.002	6.141	0.004
propanoic acid	3.071	0.047		
isobutyric acid	4.009	0.021	3.110	0.046
diethyl succinate	4.826	0.011		
methionol	57.203	0.000	28.590	0.000
hexanoic acid			9.805	0.001
benzyl alcohol	3.601	0.029	3.614	0.029
β-phenylethanol	8.301	0.001	6.350	0.004
octanoic acid	4.439	0.015	8.320	0.001

^a The must amino acid composition is the factor, and the replicates were used to measure the error. ^b na, not applicable.

filled with Carbowax (with 5% Carbowax 20M) at 100 °C. The peak area of the ethanol was normalized by that of the acetone and interpolated in calibration graphs built by the analysis of synthetic wines of known alcoholic degree.

Chemometric Study. Cluster analysis was performed with NTSYS from Exeter software (Setauket, NY). One-way ANOVA and stepwise multiple linear regression analysis were carried out with SPSS release 10.0 for windows (Chicago, IL). Partial least-squares regression models were carried out with Unscramble from CAMO ASA (Oslo, Norway).

Cluster Analysis. Dissimilarity matrices were obtained by using different similarity coefficients [Pearson, Spearman, and Kendall correlation coefficients and corrected (or taxonomic) Euclidean distance] on the standardized (centered and normalized by *S*) absolute concentration data matrix. In all cases a sequential agglomerative hierarchical nested cluster analysis (SAHN) was carried out. Complete linkage and unweighted pair-group (UPGMA) clustering methods were studied.

Multiple linear regression was carried out by a stepwise method on the nontreated data matrices containing absolute concentration data or relative data (amount of compound as percent of the total aroma quantified). The probability of the *F* quotient was taken as criterion to enter ($p < 0.05$) or leave ($p > 0.1$) the model.

Partial least-squares regression was carried out on both sets of standardized data: absolute and relative concentrations. The number of components for each *Y* variable was determined from the explained

validation variance plots. If the model failed in the prediction of a compound, this was excluded from the model and the process repeated. The method validation was carried out by full cross-validation.

RESULTS AND DISCUSSION

The fermentations of the different synthetic musts with specific amino acid compositions resembling those of 11 varieties of grape were carried out at a small scale and in duplicate. The progress of the fermentations was controlled by weighing the fermentation flasks daily. The fermentations lasted ~13–14 days, and the final alcoholic degree obtained can be seen in **Table 2**. The taxonomic relationships between the samples are shown in the plots of **Figure 1**. This figure shows the dendrograms obtained in the cluster analysis of the sample matrix using the aromatic composition as data (34 aroma compounds). In that representation, the samples having similar (highly correlated) aroma compositions are clustered together. As can be seen, only in some cases does the influence of the must amino acid composition outperform that due to random factors. For instance, replicates from the samples imitating musts from Cabernet Sauvignon, Macabeo and Sauvignon Blanc were very similar and are well clustered in both dendrograms. On the other hand, replicates from Riesling, Tempranillo, and Merlot and, to a lesser extent, from Chardonnay and Pinot Noir, are placed in different areas in both representations. The taxonomic study reveals, therefore, that the effect of the amino acid composition on the overall aroma composition can be noticed but that it is not big enough to classify all of the samples.

The effect of the amino acid composition on the content of the fermented media in the different aroma compounds was determined by one-way ANOVA. This experiment was carried out on either the absolute or relative (aroma content as percent of the total aroma produced, ethanol excluded) concentration data sets. Both sets of data were studied because in previous works (4, 8) it was found that the influence of grape variety on aroma was more noticeable for relative than for absolute concentrations. The results of the ANOVA are summarized in **Table 3**, which shows only significant results. The concentration of nearly half of all the volatile compounds analyzed significantly depended on the must amino acid composition. Not surprisingly, most of the amino acid-related odorants are in **Table 2**. The strongest relationship was found in the case of methionol. Samples imitating the amino acid composition of Macabeo grapes (richest in methionine) had the highest content

Table 4. Mean Aroma Composition (Milligrams per Liter) of Those Odorants for Which the Level Significantly Depends on the Must Amino Acid Composition

	Cabernet Sauvignon	Merlot	Grenache	Tempranillo	Chardonnay	Pinot Noir	Riesling	Moristel	Sauvignon Blanc	Carignane	Macabeo
ethyl acetate	45.0	52.53	41.78	42.37	59.83	47.83	44.24	48.29	54.09	48.07	49.56
ethyl propanoate	0.11	0.12	0.10	0.08	0.10	0.09	0.10	0.09	0.13	0.10	0.10
isobutyl acetate	0.03	0.10	0.05	0.05	0.03	0.15	0.02	0.07	0.09	0.08	0.09
ethyl butyrate	0.10	0.12	0.10	0.09	0.11	0.10	0.11	0.09	0.11	0.08	0.10
isobutanol	16.99	19.70	17.25	15.92	16.52	15.26	15.96	19.07	18.66	17.11	18.79
isoamyl acetate	0.21	0.22	0.23	0.31	0.31	0.18	0.27	0.15	0.18	0.17	0.16
isoamyl alcohol	74.14	73.03	84.90	73.28	69.51	67.51	76.29	61.35	69.80	60.13	65.12
acetic acid	143.13	190.01	122.60	117.95	137.52	89.85	94.69	191.26	148.91	184.81	174.59
propanoic acid	2.06	2.68	2.23	0.95	0.54	1.63	1.62	1.95	2.45	2.46	1.67
isobutyric acid	0.37	1.17	0.43	0.65	0.61	0.77	0.42	1.31	1.31	0.41	0.77
diethyl succinate	0.27	0.37	0.26	0.28	0.24	0.18	0.26	0.34	0.29	0.31	0.29
methionol	0.06	0.07	0.05	0.05	0.04	0.04	0.24	0.06	0.05	0.49	7.46
hexanoic acid	1.59	1.41	1.76	1.64	1.75	1.62	1.83	1.29	1.48	1.22	1.29
benzyl alcohol	0.08	0.06	0.24	0.14	0.07	0.10	0.10	0.01	0.03	0.02	0.02
β-phenylethanol	6.10	7.02	8.91	6.39	9.18	7.94	7.17	5.57	9.63	4.67	8.09
octanoic acid	3.27	2.90	3.60	3.34	3.81	3.43	3.43	2.34	2.84	2.41	2.25

Table 5. Stepwise Multiple Linear Regression Models Relating the Aroma Composition (Absolute Content) to the Must Amino Acid Composition

compound	model		r^2	F	p	observation
ethyl acetate	55.7 -0.173[His] +0.048 [Gln]	2.1 0.039 0.017	0.54	10.0	0.001	
ethyl propanoate	0.102 -9.176×10^{-4} [Phe] -4.09×10^{-4} [Asp]	0.009 3×10^{-4} 2×10^{-4}	0.48	7.9	0.004	Monod type
isobutanol	18.8 -0.056[Thr] +0.0795[Phe]	0.75 0.014 0.032	0.48	7.9	0.004	Monod type
acetic acid	157 -0.870[Thr] +0.0999[Pro]	21 0.27 0.033	0.62	14.0	0.000	
propanoic acid	2.96 -0.0369[Ser] +0.0418[Phe]	0.43 0.011 0.019	0.42	6.2	0.010	
diethyl succinate	0.306 -0.00128[Thr] + 0.000132[Pro]	0.029 5×10^{-4} 5×10^{-5}	0.63	14.7	0.000	
methionol	-0.744 +0.0304[Gln] -0.0067[Arg]	0.32 0.003 0.001	0.89	70.1	0.000	caused by Macabeo-like amino acid composition (see Table 4)
β -phenylethanol	4.80 +0.136[Phe]	0.43 0.02	0.72	47.3	0.000	

Table 6. Stepwise Multiple Linear Regression Models Relating the Aroma Composition (Relative Content) to the Must Amino Acid Composition

compound	model		r^2	F	p	observation
ethyl acetate	12.0 +0.876[Thr] -0.748[Asp]	1.2 0.20 0.30	0.53	9.5	0.002	
isobutyl acetate	0.032 -0.00175[His]	0.006 0.0005	0.21	4.9	0.040	Monod type
ethyl butyrate	0.0176 +0.00186[Thr]	0.001 0.0004	0.76	55.6	0.000	
isoamyl acetate	0.0296 +0.00972[Ser] -0.0115[Phe]	0.01 0.003 0.005	0.46	7.2	0.006	
isoamyl alcohol	11.325 +0.778[Thr] +0.75[Asp]	1.3 0.22 0.32	0.69	19.3	0.000	
acetic acid	52.0 -5.247[Asp] +5.011[Met]	3.4 0.81 1.31	0.71	21.0	0.000	
isobutyric acid	0.203 -0.406[His] +0.113[Met] +0.0033[Arg]	0.049 0.009 0.034 0.004	0.58	7.3	0.003	
methionol	-1.64 + 0.124[Gln] +0.0199[Pro]	0.19 0.012 0.004	0.82	38.9	0.000	caused by Macabeo-like amino acid composition (see Table 4)
hexanoic acid	0.228 +0.0362[Thr]	0.029 0.005	0.73	47.7	0.000	
benzyl alcohol	-0.0123 +0.011[Asp] -0.0017[Gln]	0.011 0.003 0.001	0.48	7.8	0.004	Monod type
β -phenylethanol	0.599 +0.063[Thr] +0.364[Phe] +0.017[Arg]	0.19 0.035 0.085 0.006	0.81	22.1	0.000	
octanoic acid	0.394 +0.084[Thr]	0.07 0.013	0.69	40.3	0.000	

Table 7. PLS 2 Regression Model: Basic Statistics of the Models and Regression Coefficients of the Amino Acids (*X* Variables) with the Best Model Found for Each Aroma Compound (*Y* Variables)^a

	ethyl acetate	ethyl propanoate	isobutyl acetate	isobutanol	acetic acid	isobutyric acid	diethyl succinate	methionol	benzyl alcohol	β -phenyl-ethanol	octanoic acid
Asp	-0.15	-0.16	-0.28	-0.22	-0.21	-1.05	-0.18	-0.12	1.39	0.54	0.99
Glu	0.09	0.05	0.31	-0.12	-0.09	-0.73	-0.10	-0.08	0.95	0.52	0.53
Ser	0.15	0.11	-1.26	-0.28	-0.27	0.03	-0.28	-0.27	-0.35	0.18	0.42
Gly	-0.06	-0.08	0.32	-0.15	-0.25	0.58	-0.22	0.01	-0.65	-0.40	-0.66
His	-0.23	-0.21	-0.89	-0.23	-0.19	-1.68	-0.14	-0.24	2.17	0.85	1.59
Thr	-0.05	-0.09	1.20	-0.44	-0.31	-0.91	-0.26	0.46	0.48	-0.06	0.54
Arg	-0.06	-0.07	0.19	0.16	0.21	1.78	0.18	-0.15	-1.93	-0.91	-1.61
Pro	0.30	0.32	0.53	0.44	0.55	-0.51	0.43	0.27	0.68	0.22	0.32
Met	-0.04	-0.02	0.54	0.32	0.25	-0.48	0.20	0.71	0.54	0.32	0.36
Phe	0.37	0.33	-0.01	0.53	0.22	-0.13	0.11	0.22	0.79	1.01	0.46
Lys	-0.08	-0.09	-0.15	0.36	0.06	0.11	0.09	-0.30	0.92	0.64	0.73
Gln	0.15	0.15	0.27	0.44	0.38	1.23	0.26	0.45	-1.44	-0.36	-1.19
components in the model	3	3	8	7	4	9	4	9	9	9	9
explained variance (%)	45.70	31.00	48.30	54.50	71.90	21.70	32.30	93.10	14.90	57.40	27.70
<i>r</i> (calibration)	0.78	0.73	0.92	0.89	0.92	0.88	0.80	0.99	0.87	0.94	0.89
<i>r</i> (validation)	0.63	0.49	0.71	0.72	0.83	0.57	0.56	0.96	0.53	0.76	0.60

^a Input data: standardized absolute concentrations.**Table 8.** PLS 2 Regression Model: Basic Statistics of the Models and Regression Coefficients of the Amino Acids (*X* Variables) with the Best Model Found for Each Aroma Compound (*Y* Variables)^a

	isobutyl acetate	ethyl butyrate	isoamyl acetate	isoamyl alcohol	acetic acid	methionol	hexanoic acid	β -phenyl-ethanol	octanoic acid
Asp	-0.39	0.09	1.88	0.14	-0.14	-0.33	0.29	-0.02	0.24
Glu	0.40	0.15	-1.73	0.12	-0.12	-0.16	-0.03	0.16	-0.01
Ser	-1.13	0.18	0.20	0.17	-0.17	-0.46	0.31	0.05	0.30
Gly	0.51	0.10	-2.20	0.14	-0.14	0.23	0.19	0.07	0.17
His	-0.83	-0.09	0.98	-0.01	0.01	-0.48	0.11	-0.27	0.06
Thr	1.19	0.26	1.20	0.21	-0.21	0.42	0.30	0.26	0.30
Arg	-0.07	0.08	-0.42	0.06	-0.06	-0.18	0.18	0.12	0.18
Pro	0.07	-0.08	0.22	-0.12	0.12	0.02	-0.13	-0.16	-0.11
Met	0.56	-0.14	-1.00	-0.05	0.05	0.57	-0.21	-0.08	-0.24
Phe	-0.21	0.10	0.75	0.13	-0.13	0.12	-0.17	0.33	-0.16
Lys	-0.22	0.14	0.27	0.07	-0.07	-0.41	0.11	-0.02	0.12
Gln	0.12	-0.13	-0.37	-0.01	0.01	0.68	-0.24	0.20	-0.26
components in the model	8	2	9	1	1	8	5	5	5
explained variance (%)	66.2	60.3	36.0	54.0	54.0	85.9	74.2	62.9	61.6
<i>r</i> (calibration)	0.95	0.92	0.91	0.79	0.79	0.98	0.92	0.90	0.89
<i>r</i> (validation)	0.81	0.74	0.65	0.70	0.70	0.91	0.85	0.78	0.77

^a Input data: standardized relative concentrations.

of this aroma, as is shown in **Table 4**. Surprisingly, the levels of other yeast byproducts, such as ethanol, ethyl acetate, acetic, and hexanoic and octanoic acids, were also related to the must amino acid composition. This finding is in agreement with data reported by other authors (2, 14, 16).

Table 4 gives the mean aroma composition of the odorants for which the concentration was found to be related to the must amino acid composition. Comparison of data in the first four columns in **Table 3** with those given in ref 6 is particularly interesting. Levels of isoamyl acetate, isobutanol, isobutyric acid, and methionol in the table are significantly different among varieties ($p < 0.001$, < 0.05 , < 0.05 , and < 0.05 , respectively), and in both experiments the maxima are coincident (Tempranillo in the case of isoamyl acetate and Merlot in the other cases). There are also some discrepancies because in that reference isoamyl alcohol and β -phenylethanol were found maxima in Merlot, but not in Grenache, as **Table 4** shows (differences in the table were nonsignificant). The levels of hexanoic and octanoic acids given in **Table 4** are also significantly different in the four first varieties in the table (at $p < 0.05$), and the maxima belong to solutions imitating Grenache, in agreement with data reported in ref 2.

Generally speaking, results in **Table 4** show a lesser content in higher alcohols in the fermented media than in the wines reported in ref 6. This may be due, at least in part, to the fact that red wines are richer in these compounds than wines fermented without skins (4). The levels of fatty acids are similar to levels reported in wines, whereas the levels of fatty acid ethyl esters are somehow lower, which could be due to the larger evaporation of these compounds from a small vessel (24). Despite these discrepancies, data in **Tables 3** and **4** confirm that the amino acid profile of the grape variety has a significant role in the levels of important odorants in the wine. Some of these odorants are formed as byproducts of yeast amino acid metabolism, but data in **Table 4** suggest that the amino acid composition influences the fatty acid synthesis as well. To our knowledge, there is no satisfactory explanation for this at present.

All of these observations, together with knowledge about vintage and geographical variations in must amino acid composition (19–21), suggest that most vintage and geographical variations observed in wine fermentative aroma are due to the differences in the must amino acid composition induced by these factors. However, additional research will be necessary to

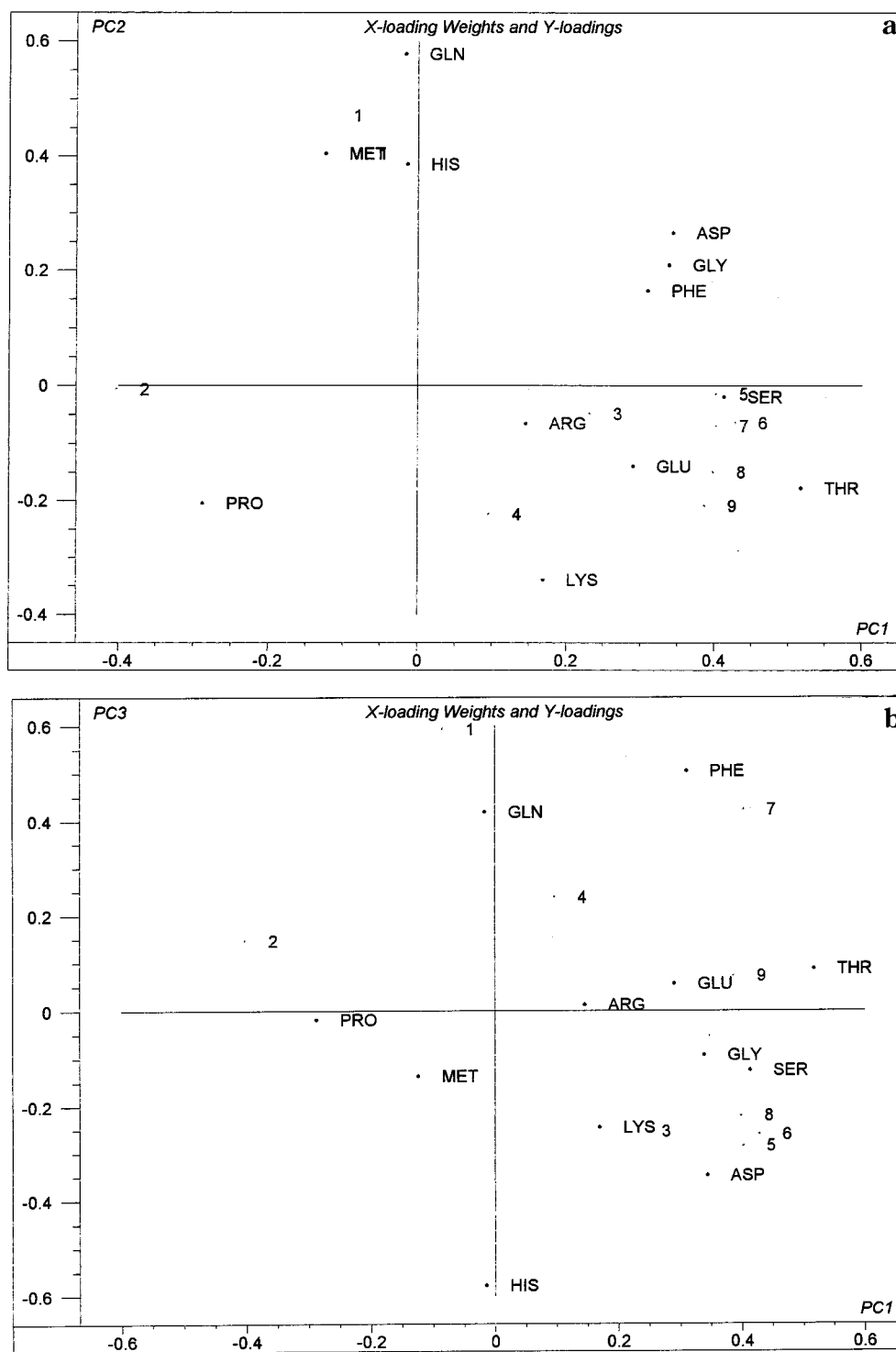


Figure 2. Partial Least-squares regression plots showing the X loading weights and the Y loadings for (a) the first (33% variance for X, 45% for Y) and second (26% variance for X, 9% for Y) components and (b) the first and third (9% variance for X and Y) components. Codes: 1, methionol; 2, acetic acid; 3, isoamyl acetate; 4, isobutyl acetate; 5, isoamyl alcohol; 6, hexanoic acid; 7, β -phenylethanol; 8, octanoic acid; 9, ethyl butyrate.

evaluate which proportion of must amino acid variability is translated into wine aroma composition variability and the sensory impact it has.

In a second part of the work, different statistical techniques have been used to see how those differences were related to the must amino acid composition. The first strategy was a stepwise multiple linear regression, and the results obtained with this technique can be seen in **Tables 5** and **6**. The modeling power of this technique is not very high, but it produces very

simple models that can be easily interpreted. This technique has produced satisfactory linear regression models as is shown in the tables, although in four cases a Monod-type model was observed (18). According to this model, the linear relationship held only at lower concentrations up to a certain constant level. In the rest of the cases, the linear relationship was observed along the whole interval of concentrations. Threonine seems to be the amino acid with maximum influence on the wine aroma composition. This amino acid, together with phenylalanine and

Table 9. Partial Validation of the Models Previously Developed^a

compound	addition of				
	His (%)	Thr + Lys (%)	Phe (%)	Ser (%)	Pro + Met (%)
methionol	NS	NS	NS	NS	400***
acetic acid	NS	NS	NS	NS	35*
isoamyl acetate	NS	27*	18	NS	NS
isobutyl acetate	-22	NS	NS	NS	NS
isoamyl alcohol	NS	NS	NS	NS	NS
hexanoic acid	NS	29*	NS	35**	NS
β -phenylethanol	-28**	NS	45***	NS	NS
octanoic acid	NS	37**	NS	33**	NS
ethyl butyrate	NS	16*	NS	21*	NS

^a Average effect of the addition of some selected amino acids on three different grape musts on the volatile composition of the wines produced with those musts. Differences are significant at (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$.

aspartic acid, is enough to model 8 of the 16 compounds in the tables. On the other hand, glycine, lysine, and glutamine did not participate in any of the models.

Threonine levels are strongly correlated with odorants related to the fatty acid synthesis. The models in **Tables 5** and **6** show that the higher the threonine levels, the higher the concentration of ethyl acetate, ethyl butyrate, and hexanoic and octanoic acids. At the same time, this amino acid seems to influence the proportion of higher alcohols of the wine. The higher the must in threonine is, the higher the proportions of isoamyl alcohol and β -phenylethanol and the lesser the proportion of isobutanol. Phenylalanine is related to the higher alcohol absolute and relative contents of wine. The higher the must content in phenylalanine, the higher the wine relative contents of β -phenylethanol and isobutanol and the lesser the wine relative contents of isoamyl alcohol (this last model is not shown in the table). Surprisingly, the levels of phenylalanine in the must are closely related to the β -phenylethanol concentration in wine, a result which contradicts that obtained in previous research (11). A possible explanation for this discrepancy is the difference in the experimental procedure. Whereas we have tried to emulate the amino acid composition of 11 different musts, the other researchers added phenylalanine to a given must.

A final data treatment aiming at a further understanding of the influence of must amino acid composition on wine aroma has been based on partial least-squares regression. The selected algorithm is called PLS 2, which is able to correlate a block of Y variables with a block of X variables. This is particularly useful in multivariate situations in which the Y variables are correlated. A summary of this data treatment performed on both the standardized absolute and relative concentrations of the amino acids (the X variables) and the aroma compounds (Y variables) is presented in **Tables 7** and **8**.

As to absolute concentrations, the complete model in **Table 7** explained 39% of the total Y variance of the 11 aroma compounds. The best-explained compounds were methionol, acetic acid, β -phenylethanol, isobutanol, isobutyl acetate, and ethyl acetate. On the contrary, the model failed in the interpretation of propanoic acid (not finally included in the model) and benzyl alcohol. **Table 8** summarizes the PLS 2 models obtained for the sets of relative data. The total variance explained is now 52.2%, and nearly all of the aroma compounds in the table are satisfactorily explained in terms of the amino acid composition. Isobutyric acid was not included in the model because its interpretation was not possible. This result confirms, therefore, that the amino acid profile influences the aroma profile (relative concentrations) more than the absolute content in aroma

compounds. **Figure 2** shows the loadings of both X and Y variables on the plane formed by the first two PLS components. Three patterns in the behavior of aroma compounds can be seen. Methionol is correlated with methionine, histidine, and glutamine. Acetic acid is correlated with proline, and the other aroma compounds (isoamyl alcohol, β -phenylethanol, hexanoic and octanoic acids, ethyl butyrate, isobutyl acetate, and isoamyl acetate) are mainly correlated with serine, threonine, and glutamic acid. The third component (not shown) splits the third group of aromas into two small groups: β -phenylethanol and isobutyl acetate were correlated with phenylalanine and glutamine, and the rest were correlated with aspartic acid and lysine.

The validity of this last model has been partially checked by the addition of selected amino acids to three different musts. The results of the experiment are shown in **Table 9** and corroborate some trends observed in the model in **Table 8** and **Figure 2**. The addition of histidine provokes the diminution of the levels of isobutyl acetate and β -phenylethanol. The addition of threonine and lysine increases the levels of hexanoic and octanoic acids, ethyl butyrate, and isoamyl acetate. Nearly the same effect is observed in the case of the addition of serine. The addition of phenylalanine brings about an important increment of β -phenylethanol. Finally, the combined addition of proline and methionine produces an amazing increment of wine content in methionol and a more moderate increment of acetic acid.

In summary, the present work has demonstrated that there is a close relationship between the must amino acid composition and the wine content on some important volatile compounds and that this relationship satisfactorily explains previous observations about the influence of the variety of grape on the volatile profile of wine.

ABBREVIATIONS USED

GC, gas chromatography; ANOVA, analysis of variance; GC-MS, gas chromatography-mass spectrometry; PLS, partial least squares.

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