JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Study of the Evolution of Nitrogen Compounds during Grape Ripening. Application to Differentiate Grape Varieties and Cultivated Systems

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The aim of this work was to study the evolution of amino acids and ammonium during grape ripening and to evaluate its application to differentiate grape varieties and cultivated systems (organic and nonorganic). For this purpose, Monastrell, Syrah, Merlot, and Petit Verdot grapes produced using conventional agriculture and Monastrell grape cultivated using organic agriculture, collected during two consecutive harvests at different stages of ripening, were studied. These years of harvest were very different climatic years; even so, the grape varieties presented similar qualitative compositions. Therefore, the percentage of amino acids at harvest moment allowed differentiation of grapes according to variety and cultivated system, regardless of the year. The nitrogen composition could allow estimation of the fermentative aroma potential of grapes. Thus, Syrah was the grape with the greatest aroma potential at harvest. Monastrell nonorganic grape had a concentration of nitrogen compounds superior to that of Monastrell organic grape. In Monastrell, Syrah, and Merlot, traditional varieties in the area, the highest concentration of nitrogen compounds coincided with the highest °Baumé/total acidity ratio and color index during 2007. Consequently, technological and phenolic maturity of these grape varieties coincided with the maximum composition of nitrogen compounds. However, in 2008, this did not happen because grape ripening was irregular as a consequence of different climatological conditions.

KEYWORDS: Amino acids; ammonium; ripening; *Vitis vinifera*; grapevine cultivars

INTRODUCTION

The nitrogen compounds of must are essential for the growth and development of yeasts during alcoholic fermentation because nitrogen is, after carbon, the second element utilized during their growth. The ammonium and amino acids are the main sources of nitrogen for *Saccharomyces cerevisiae*. Also, the content of nitrogen compounds affects the kinetics of fermentation. Thus, nitrogen-deficient musts can cause slow and stuck alcoholic fermentation (*1*, *2*). In addition, these compounds are related to the formation of fermentative compounds, especially higher alcohols and esters (*3*). These compounds constitute the main group of compounds that form the "fermentation bouquet" (*4*). Therefore, the concentration of nitrogen compounds in the must can affect wine quality (*5*).

In the cellars it is common practice to add diammonium phosphate (DAP) to the must to prevent problems related to nitrogen deficiency. This addition should, however, follow some criterion, because the addition of large amounts of ammonium to the must can result in problems later on. Wines with higher amounts of residual nitrogen have more risk of microbiological instability, with formation of biogenic amines (*6*) and ethyl carbamate, which is a carcinogenic compound (*7*, *8*).

Therefore, to provide additional information on the optimal time of harvest it is very important to know the evolution of amino acids and ammonium during grape ripening, as the concentration of these compounds in the must can affect the development of fermentation and the formation of volatile compounds important for the aroma quality of wines (*9*). In addition, the analysis of ammonium and amino acids of the must gives information about the need or not need to enrich the must with nitrogen and thus avoid problems later on.

For all of these reasons, the aim of this work was to study the evolution of amino acids and ammonium content during grape ripening and to evaluate its application to differentiate grape varieties and cultivated systems (organic and nonorganic).

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10.1021/jf8037049 CCC: $40.75
Published on Web 02/19/2009

Table 1. Dates on Which the Samples of the Different Grape Varieties Were Taken

Monastrell nonorganic	Monastrell organic	Syrah	Merlot	Petit Verdot
		2007		
Aug 8	Aug 8	Aug 8g	Aug 8	
Aug 23	Aug 23	Aug 23	Aug 23	Aug 23
Sept 5	Sept 5	Sept 5	Sept 5	Sept 5
Sept 12	Sept 12	Sept 12	Sept 12 (harvest)	Sept 12
Sept 19	Sept 19	Sept 19 (harvest)		Sept 19
Sept 27	Sept 27 (harvest)			Sept 27
Oct 8 (harvest)				Oct 8 (harvest)
		2008		
Aug 28	Aug 28	Aug 28	Aug 20	Aug 28
Sept 27	Sept 11	Sept 20	Sept 28	Sept 27
Oct 3 (harvest)	Sept 20 (harvest)	Sept 27 (harvest)	Sept 4 (harvest)	Oct 3 (harvest)

The samples were collected during two consecutive harvests along the ripening of several grapes cultivated using conventional agriculture. In addition, the major variety in the region studied was cultivated using organic agriculture.

MATERIALS AND METHODS

Samples. For this study were used Monastrell, Syrah, Merlot, and Petit Verdot red grape varieties cultivated in the O.D. Jumilla (SE of Spain) according to the conventional agriculture system. The Monastrell variety was also cultivated according to organic agriculture. The parcels had similar soil and climatic characteristics as they are located in the same area. Jumilla is a warm region with maximum temperature of 40 °C and minimum temperature of 0 °C, average sunshine of 3000 h/year, and average annual rainfall of 300 mm (www.winesfromspain.com). In the conventional agriculture system, the vineyards were cultivated in trellis and were fitted with a drip irrigation system. They were fertilized with liquid fertilizer NPK 8−4−10 (%, w/w) (Agribeco, Spain), applied in total 250 g per vine. In the case of the organic system, the vineyards were cultivated in glass and were treated with fertilizer "cultivit ecológico" (Agribeco), consisting of dried granulated sheep manure, the composition of which was NPK 1.55−1.21−2.35 (%, w/w), applying in total 200 g per vine. The organic system was not irrigated.

The sampling was done during grape ripening, which took place between August and October 2007 and 2008. To ensure that the sampling was representative, this was carried out by choosing the first vine of the plot at random, of a row also chosen at random, and then in the same row was collected one grape cluster of every five vines, until 10 samples had been collected. The choice of grape cluster within the vine was so that there would be grape clusters facing south and north and different heights and depths within the same vine. The dates on which the samples of the different grape varieties were taken are shown in **Table 1**. The grapes reach maturity when they do not show any increase in weight for a few days. Controlling the weight of the berries at the end of ripening can be defined as vineyard maturation (*10*). In addition to this parameter, the evolution of °Baumé/total acidity and color index are evaluated to better determine the optimal time to harvest. To obtain the must, several grains of grapes were caught at random from the clusters collected for each sample. The musts were obtained manually. The musts were filtered, using cellulose acetate syringe filters nonsterile (Albet, Barcelona, Spain), for further analysis of their contents in amino acids and ammonium.

Enological Parameters. Total acidity, pH, and °Baumé of different samples were measured following the methods established by the ECC (*11*). The phenolic ripeness of the grapes was measured indirectly from the color intensity of the extract obtained by crushing 200 grains without breaking seeds and then it was centrifugated. The color intensity was determined by the sum of the absorbances at 420, 520, and 620 nm (*12*). This parameter is called the color index and has a high correlation with wine color (*13*).

Table 2. Enological Parameters of the Different Varieties during Grape Ripening

sample	wt of 100 berries (g)	°Baumé/total acidity[a]	pH	color index
		Monastrell nonorganic		
2007				
Aug 8	90	0.24	2.72	0.83
Aug 23	131	1.17	2.96	1.66
Sept 5	175	1.29	3.13	2.16
Sept 12	146	1.51	3.28	2.17
Sept 19	168	1.87	3.37	3.18
Sept 27	188	2.14	3.28	2.78
Oct 8	163	2.32	3.50	4.17
2008				
Aug 28	139	0.88	3.10	1.52
Sept 27	174	2.91	3.58	4.34
Oct 3	196	2.08	3.57	3.61
		Monastrell organic		
2007				
Aug 8	102	0.56	2.96	2.01
Aug 23	145	2.16	3.02	4.07
Sept 5	182	2.32	3.21	5.86
Sept 12	151	2.71	3.27	5.20
Sept 19	138	3.09	3.40	8.72
Sept 27	150	2.36	3.17	5.34
2008				
Aug 28	191	1.98	3.10	4.30
Sept 11	163	2.88	3.67	6.84
Sept 20	176	3.05	3.62	6.27
		Syrah		
2007				
Aug 8	118	0.62	2.82	1.07
Aug 23	111	2.02	3.21	8.02
Sept 5	142	1.79	3.40	7.31
Sept 12	102	1.88	3.24	9.44
Sept 19	132	3.15	3.59	9.64
2008				
Aug 28	147	1.13	3.28	6.73
Sept 20	166	2.47	3.78	9.44
Sept 27	175	3.94	3.93	7.71
		Merlot		
2007				
Aug 8	118	0.97	3.05	4.02
Aug 23	121	1.98	3.23	6.31
Sept 5	129	2.63	3.54	5.47
Sept 12	134	2.82	3.57	9.18
2008				
Aug 20	129	1.69	3.38	9.01
Aug 28	143	2.32	3.56	8.40
Sept 4	126	2.29	3.52	10.16
		Petit Verdot		
2007				
Aug 23	99	1.31	2.82	3.55
Sept 5	119	1.22	2.99	5.81
Sept 12	115	1.41	2.94	7.24
Sept 19	127	2.14	3.36	8.37
Sept 27	135	1.97	3.18	7.00
Oct 8	134	2.43	3.34	11.13
2008				
Aug 28	99	0.50	3.08	6.14
Sept 27	130	2.28	3.44	11.67
Oct 3	118	1.98	3.50	9.49

[a] Total acidity expressed as g/L tartaric acid.

Analysis of Amino Acids and Ammonium by HPLC. The analysis of amino acids and ammonium of the must obtained from the grapes was made using the method described by Gómez-Alonso et al. (*14*). The derivatization of amino acids and ammonium was carried out by reaction of 1.75 mL of 1 M borate buffer (pH 9), 750 μL of methanol (Merck, Darmstadt, Germany), 1 mL of sample (previously filtered), 20 μL of internal standard (2-aminoadipic acid, 1 g/L) (Aldrich, Gillingham, U.K.), and 30 μL of derivatization reagent diethyl

Table 3. Nitrogenous Fractions and Total Amino Acids of the Different Varieties during Grape Ripening[a]

sample	ammonium nitrogen (mg of N/L)	amino nitrogen (mg of N/L)	assimilable nitrogen (mg of N/L)	total amino acids (mg/L)
Monastrell nonorganic				
2007				
Aug 8	68.0 ± 1.0	85.0 ± 2.0	151.0 ± 4.0	501.0 ± 15.0
Aug 23	48.0 ± 1.0	156.0 ± 6.0	199.0 ± 6.0	746.0 ± 27.0
Sept 5	32.3 ± 0.4	141.0 ± 2.0	172.0 ± 2.0	645.0 ± 5.0
Sept 12	34.0 ± 1.0	141.0 ± 4.0	174.0 ± 5.0	628.0 ± 19.0
Sept 19	20.2 ± 0.3	62.1 ± 0.8	82.0 ± 1.0	285.0 ± 4.0
Sept 27	25.0 ± 1.0	123.0 ± 4.0	148.0 ± 5.0	557.0 ± 17.0
Oct 8	23.5 ± 0.0	201.4 ± 0.1	219.8 ± 0.2	920.0 ± 2.0
2008				
Aug 28	116.3 ± 7.9	163.9 ± 2.0	277.8 ± 9.6	809.0 ± 17.2
Sept 27	111.2 ± 3.6	168.7 ± 3.6	277.8 ± 7.1	777.4 ± 15.5
Oct 3	100.2 ± 7.3	154.9 ± 10.1	253.0 ± 17.4	682.6 ± 43.3
Monastrell organic				
2007				
Aug 8	18.8 ± 0.4	61.0 ± 1.0	78.0 ± 2.0	333.0 ± 5.0
Aug 23	14.9 ± 0.2	56.1 ± 0.5	69.9 ± 0.6	291.0 ± 3.0
Sept 5	22.2 ± 0.6	85.0 ± 2.0	106.0 ± 3.0	408.0 ± 11.0
Sept 12	15.6 ± 0.0	49.6 ± 0.0	65.1 ± 0.1	260.4 ± 0.2
Sept 19	28.5 ± 0.4	155.0 ± 2.0	183.0 ± 2.0	720.0 ± 7.0
Sept 27	15.7 ± 0.0	65.1 ± 0.1	78.8 ± 0.1	346.1 ± 0.2
2008				
Aug 28	101.6 ± 7.9	115.3 ± 8.4	213.6 ± 15.8	560.1 ± 40.8
Sept 11	92.9 ± 1.8	133.1 ± 4.1	223.6 ± 5.7	652.3 ± 19.4
Sept 20	100.4 ± 1.0	129.0 ± 1.2	226.5 ± 1.8	622.5 ± 5.8
Syrah				
2007				
Aug 8	44.1 ± 0.5	89.0 ± 1.0	133.0 ± 1.0	415.0 ± 4.0
Aug 23	33.8 ± 0.2	131.0 ± 1.0	164.0 ± 2.0	596.0 ± 6.0
Sept 5	20.6 ± 0.5	179.0 ± 3.0	199.0 ± 4.0	810.0 ± 16.0
Sept 12	21.3 ± 0.4	172.0 ± 3.0	192.0 ± 3.0	763.0 ± 12.0
Sept 19	17.6 ± 0.2	201.0 ± 2.0	218.0 ± 2.0	920.0 ± 10.0
2008				
Aug 28	91.4 ± 3.4	178.1 ± 5.6	268.0 ± 9.0	842.8 ± 25.8
Sept 20	93.5 ± 5.6	246.7 ± 14.0	337.9 ± 19.8	1172.5 ± 63.8
Sept 27	93.7 ± 5.8	252.6 ± 11.9	343.3 ± 6.7	1173.8 ± 10.4
Merlot				
2007				
Aug 8	39.9 ± 0.8	64.0 ± 1.0	103.0 ± 2.0	325.0 ± 6.0
Aug 23	25.3 ± 0.5	113.0 ± 1.0	136.0 ± 2.0	503.0 ± 5.0
Sept 5	17.6 ± 0.1	67.8 ± 0.1	84.9 ± 0.3	358.0 ± 0.1
Sept 12	17.2 ± 0.1	113.8 ± 0.2	128.4 ± 0.1	566.8 ± 0.8
2008				
Aug 20	98.6 ± 3.9	121.6 ± 5.6	216.9 ± 9.4	601.9 ± 25.9
Aug 28	96.2 ± 7.1	131.7 ± 11.5	223.4 ± 18.0	664.8 ± 57.4
Sept 4	90.0 ± 6.6	139.1 ± 11.1	225.8 ± 16.7	689.5 ± 58.7
Petit Verdot				
2007				
Aug 23	47.0 ± 1.0	95.0 ± 1.0	142.0 ± 2.0	469.0 ± 5.0
Sept 5	17.4 ± 0.2	108.6 ± 0.4	124.9 ± 0.7	519.0 ± 2.0
Sept 12	17.0 ± 0.3	92.0 ± 1.0	108.0 ± 1.0	452.0 ± 4.0
Sept 19	14.2 ± 0.2	95.4 ± 0.2	108.3 ± 0.3	470.0 ± 2.0
Sept 27	20.3 ± 0.5	157.0 ± 3.0	176.0 ± 4.0	730.0 ± 13.0
Oct 8	10.3 ± 0.2	74.0 ± 0.3	81.6 ± 0.6	443.5 ± 0.9
2008				
Aug 28	98.4 ± 2.3	196.2 ± 4.9	291.8 ± 7.1	983.2 ± 18.5
Sept 27	92.3 ± 3.4	195.7 ± 6.5	285.3 ± 9.8	900.8 ± 28.6
Oct 3	91.5 ± 5.6	163.6 ± 9.1	252.7 ± 14.1	764.6 ± 42.2

[a] All parameters are given with their standard deviation ($n = 2$ for 2007 year and $n = 4$ for 2008 year).

ethoxymethylenemalonate (DEEMM) (Aldrich). The reaction of derivatization was done in a screw-cap test tube over 30 min in an ultrasound bath. The sample was then heated at 70−80 °C for 2 h to allow complete degradation of excess DEEMM and reagent byproduct.

The analyses were performed on an Agilent 1100 HPLC (Palo Alto, CA), with a photodiode array detector. Chromatographic separation was performed in an ACE HPLC column (C18-HL) (Aberdeen, U.K.), particle size = 5 μm (250 mm × 4.6 mm). Amino acids were eluted under the following conditions: 0.9 mL/min flow rate, 10% B during 20 min, then elution with linear gradients from 10 to 17% B in 10 min, from 17 to 19% B in 0.01 min, maintained during 0.99 min, from 19 to 19.5% B in 0.01 min, from 19.5 to 23% B in 8.5 min, from 23 to 29.4% B in 20.6 min, from 29.4 to 72% B in 8 min, from 72 to 82% B in 5 min, from 82 to 100% B in 4 min, maintained during 3 min, followed by washing and reconditioning the column. Phase A was 25 mM acetate buffer (pH 5.8) with 0.4 g of sodium azide; phase B was 80:20 (v/v) mixture of acetonitrile and methanol (Merck). A photodiode array detector monitored at 280, 269, and 300 nm was used for detection. The volume of sample injected was 50 μL. The analysis of amino acids and ammonium was done in duplicate in the different samples from the 2007 harvest and in quadruplicate in the samples from 2008. The target compounds were identified according to the retention times and UV−vis spectral characteristics of corresponding standards (Aldrich) derivatizated. Quantification was done using the calibration graphs of the respective standards ($R^2 > 0.98$) in 0.1 N HCl, which underwent the same process of derivatization as the samples.

The amino nitrogen was calculated by determining free amino acids by HPLC, and the assimilable nitrogen was calculated as the amount of ammonium and amino nitrogen without taking into account the proline.

Statistical Analysis. The statistical elaboration of the data was performed using the SPSS version 15.0 statistical package for Windows (SPSS, Chicago, IL). Discriminant analyses were performed on data expressing amino acids, ammonium, and total amino acids concentration as well as the proline/arginine ratio in the grape samples during the ripening in order to classify the different grape varieties and the different cultivated systems. Also, discriminant analyses were performed on data expressing as percentage of each amino acid over the total amino acids in samples at 2007 and 2008 harvest.

RESULTS AND DISCUSSION

The years studied (2007 and 2008) showed very different climatologies, so the evolution of grape ripening was also different. In 2008, it rained in the spring and autumn, whereas the rainfall in 2007 was limited to spring, so it rained about 125 mm more in 2008 than in 2007, the latter being a typical climatological year in the Jumilla area.

Enological Parameters. The enological parameters studied (weight of berries, °Baumé/total acidity ratio, pH, and color index) increased during ripening of the different grape varieties, regardless of the year (**Table 2**). The highest weight of the berries corresponded to Monastrell nonorganic variety and the lowest to Petit Verdot. The grapes were harvested with a value of °Baumé/total acidity between 1.98 in Petit Verdot and 3.94 in Syrah. The evolution of this parameter depended on the variety and on the year, and its trend was the same during both years in only the Syrah variety (**Table 2**). The highest pH at harvest corresponded to Syrah variety in both 2007 and 2008. This parameter presented few changes during grape ripening, with a slight increase, similar for all varieties and years. The varieties that showed the highest color index at harvest were Petit Verdot in 2007 and Merlot in 2008, whereas Monastrell, both organic and nonorganic, was the variety with the lowest color index (**Table 2**). Ortega-Regules et al. (*15*) also observed that Monastrell variety had lower total phenolic compound content than other varieties (Cabernet Sauvignon, Merlot, and Syrah), probably due to differences in the cell wall composition between varieties, which influence the extraction of these compounds. In addition, Monastrell organic grape had higher color index than Monastrell nonorganic grape during ripening (**Table 2**), which could be due to the weight of the berries, which, being higher for the Monastrell nonorganic, resulted in a lower concentration of phenolic compounds. Color index increased substantially at the end of grape ripening in Merlot, but decreased in Monastrell organic and Petit Verdot grapes

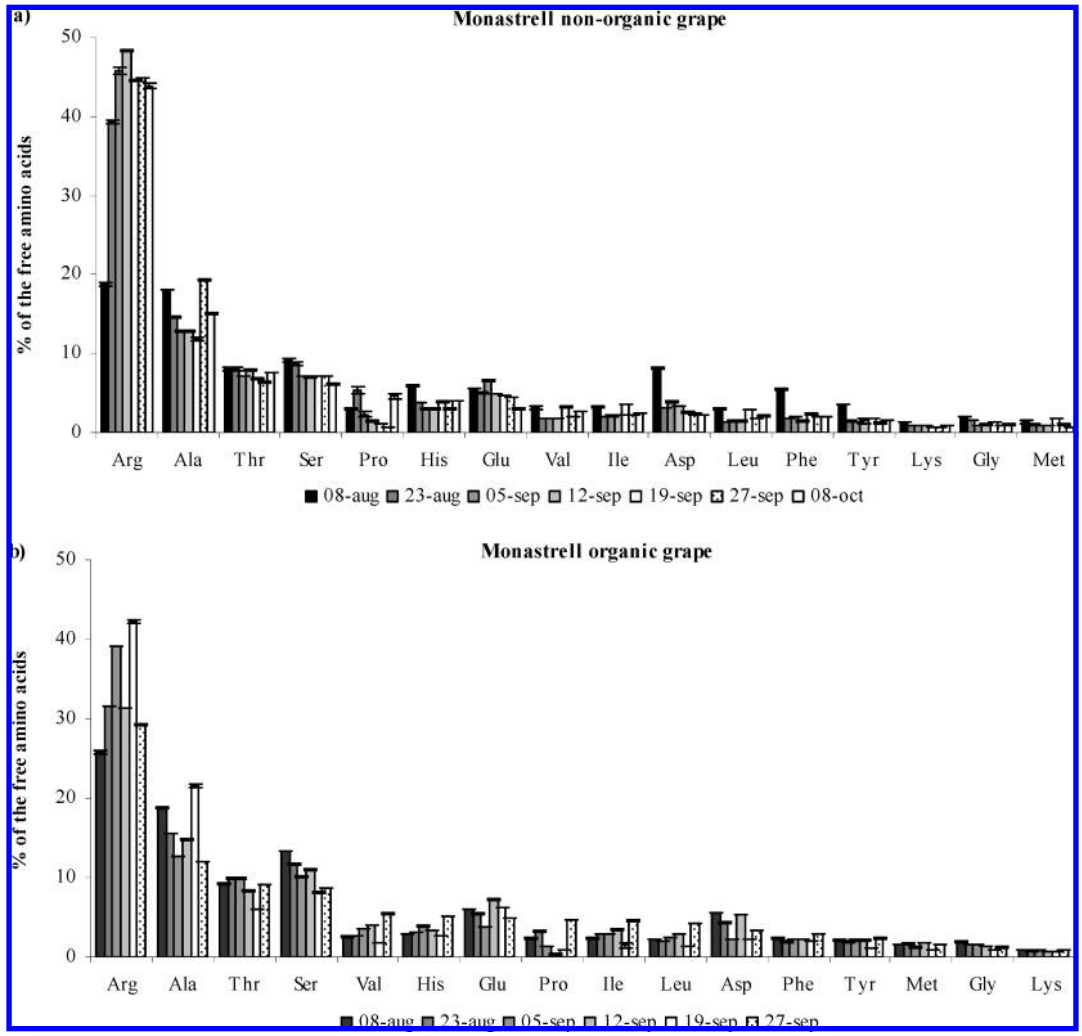

Figure 1. Percentage of each amino acid over the total amino acids in Monastrell nonorganic and organic grapes during ripening in 2007. All parameters are given with their standard deviation ($n = 2$), which are between 0.0001 and 0.487.

(**Table 2**). In general, the highest value of °Baumé/total acidity ratio during grape ripening coincided with the highest value of color index (**Table 2**). Navarro et al. (*16*) found that during grape ripening of different red varieties, the higher the °Brix, the higher the color intensity.

Nitrogenous Fractions. The general trend was that concentration of ammonium nitrogen decreased during grape ripening in all varieties studied during 2007 and 2008 (**Table 3**). This decrease was higher in the samples during 2007 than in 2008 for all varieties. Throughout grape ripening, the ammonium is transformed in amino acids (*17*), so its concentration decreased (**Table 3**). The content of ammonium in the grapes at 2007 harvest was lower than in 2008, being in all cases within the limits found for this compound (5−325 mg of N/L) (*18*). This difference could be due to the plant being better nourished in 2008 than in 2007, as the autumn rain mobilized soil nitrogen reserves and could facilitate the assimilation of nutrients.

The must should have a concentration of assimilable nitrogen of >140 mg of N/L in order for alcoholic fermentation to develop correctly (*19*), although it also depends on the sugar concentration of the must and on the capacity of the *S. cerevisiae* strain to ferment. At 2007 harvest, the concentration of assimilable nitrogen was above this value in Monastrell nonorganic and Syrah grapes, whereas it was lower than this value in Monastrell organic, Merlot, and Petit Verdot grapes. At 2008 harvest, all varieties showed a concentration of >140 mg/L (**Table 3**). This difference could be due to the better nutrition of the plant in 2008 than in 2007, as we have explained above.

If we compare the total concentration of amino acids at the beginning and at the end of ripening in both harvests, it increased in Monastrell organic, Syrah, and Merlot grapes, whereas in Petit Verdot it diminished (**Table 3**). At 2007 and 2008 harvests, Syrah was the variety that had the higher concentration of total amino acids, so this is potentially the more aromatic grape, as the amino acids are precursors of fermentative aroma compounds (*3*, *20*). The difference in the concentration of amino acids between Monastrell organic and nonorganic grapes at 2007 harvest (**Table 3**) could be due to the different irrigation and fertilization of the vineyards. In 2008, this difference was smaller, probably because it rained more than in 2007 and, therefore, it was not necessary to irrigate the Monastrell nonorganic, so that both cultivated systems received similar amounts of water. Lorenzo et al. (*21*) found that wines obtained from the Monastrell variety had lower concentrations of fermentative compounds than cowinemaking Monastrell wines with other varieties, which could be due to the fact that Monastrell had lower concentrations of amino acids than other varieties (**Table 3**).

The variety that had the highest pH and °Baumé/total acidity ratio at 2007 and 2008 harvests (Syrah) (**Table 2**) was the variety with highest content of nitrogen compounds (**Table 3**). In Monastrell, Syrah, and Merlot, varieties that grow tradition-

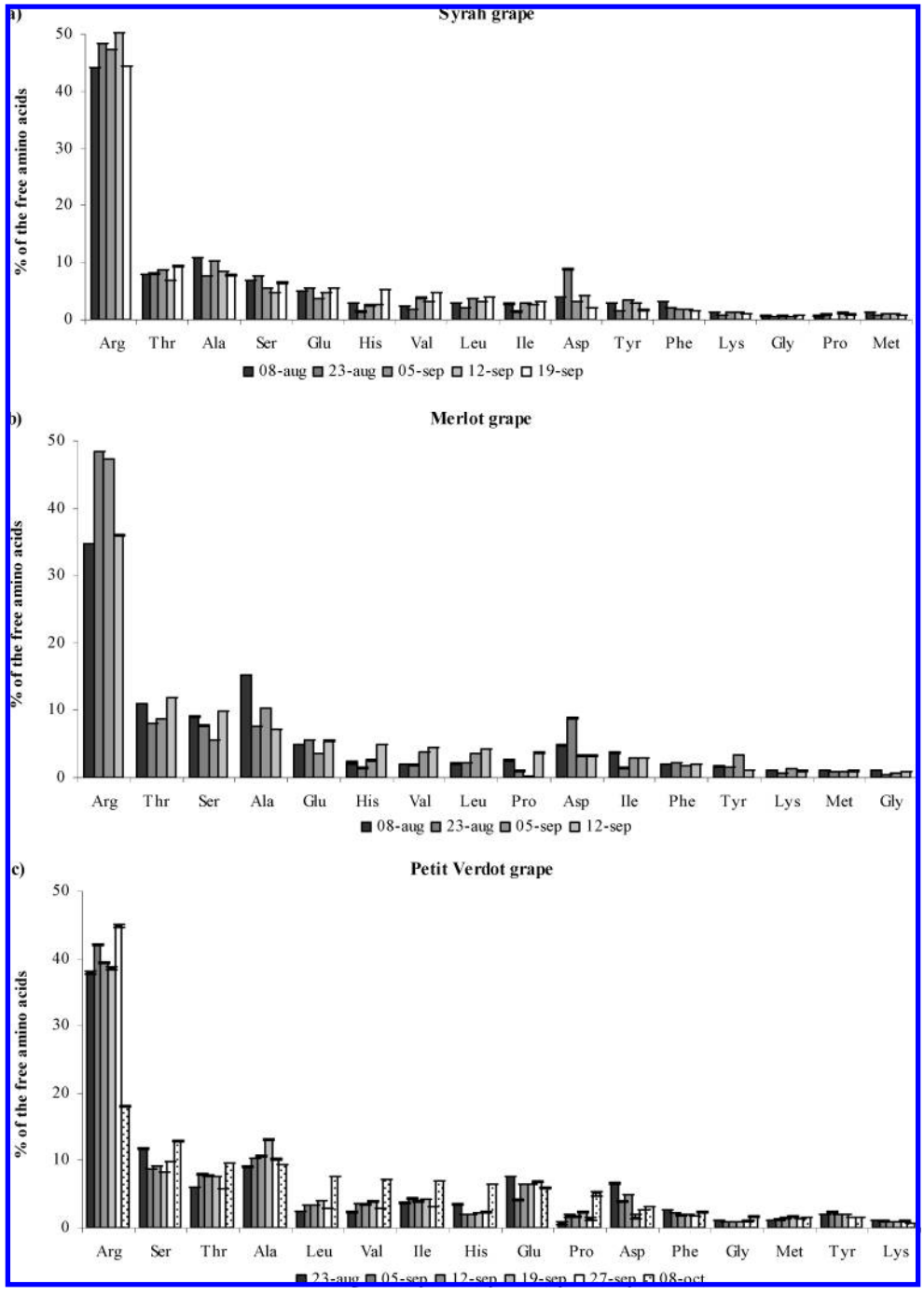

Figure 2. Percentage of each amino acid over the total amino acids in Syrah, Merlot, and Petit Verdot grapes during ripening in 2007. All parameters are given with their standard deviation ($n = 2$), which are between 0.0001 and 0.311.

ally in the area, it was noted that the highest concentration of nitrogen compounds during 2007 ripening (**Table 3**) coincided with the highest °Baumé/total acidity ratio and color index (**Table 2**), so the technological and phenolic maturity coincided with the maximum nitrogen composition of the grapes. Petit Verdot variety has begun to be cultivated in the zone only a few years ago, so it seems that this variety is less adapted to the area than the other varieties studied. As we have already mentioned, 2008 was a climatologically unusual year in the area, so grape ripening was quite irregular, and this is reflected in the different parameters of maturation evolved in a different way (**Tables 2** and **3**).

Amino Acid Content in the Different Grape Varieties during Ripening. Figures 1−4 show the percentages of each amino acid over the total amino acids in grapes during ripening in both harvests. For each variety and year, the amino acids have been ordered from highest to lowest percent in grapes at harvest. In all varieties, the majority amino acid was arginine, representing between 20 and 50%, approximately, of the amino acids present in grapes at harvest (**Figures 1−4**). This result is similar to those reported by other authors in must before the start of the alcoholic fermentation (*22*, *23*). Arginine and ammonium are the principal sources of nitrogen for the yeasts during alcoholic fermentation (*24*, *25*). The next three amino acids found in greater proportion in grapes (alanine, threonine, and serine) were also the same in the varieties studied, except in Merlot from 2008, although the order of importance was different depending on the variety (**Figures 1−4**). Valine, leucine, isoleucine, and phenylalanine, which are direct precursors of higher alcohols during the alcoholic fermentation,

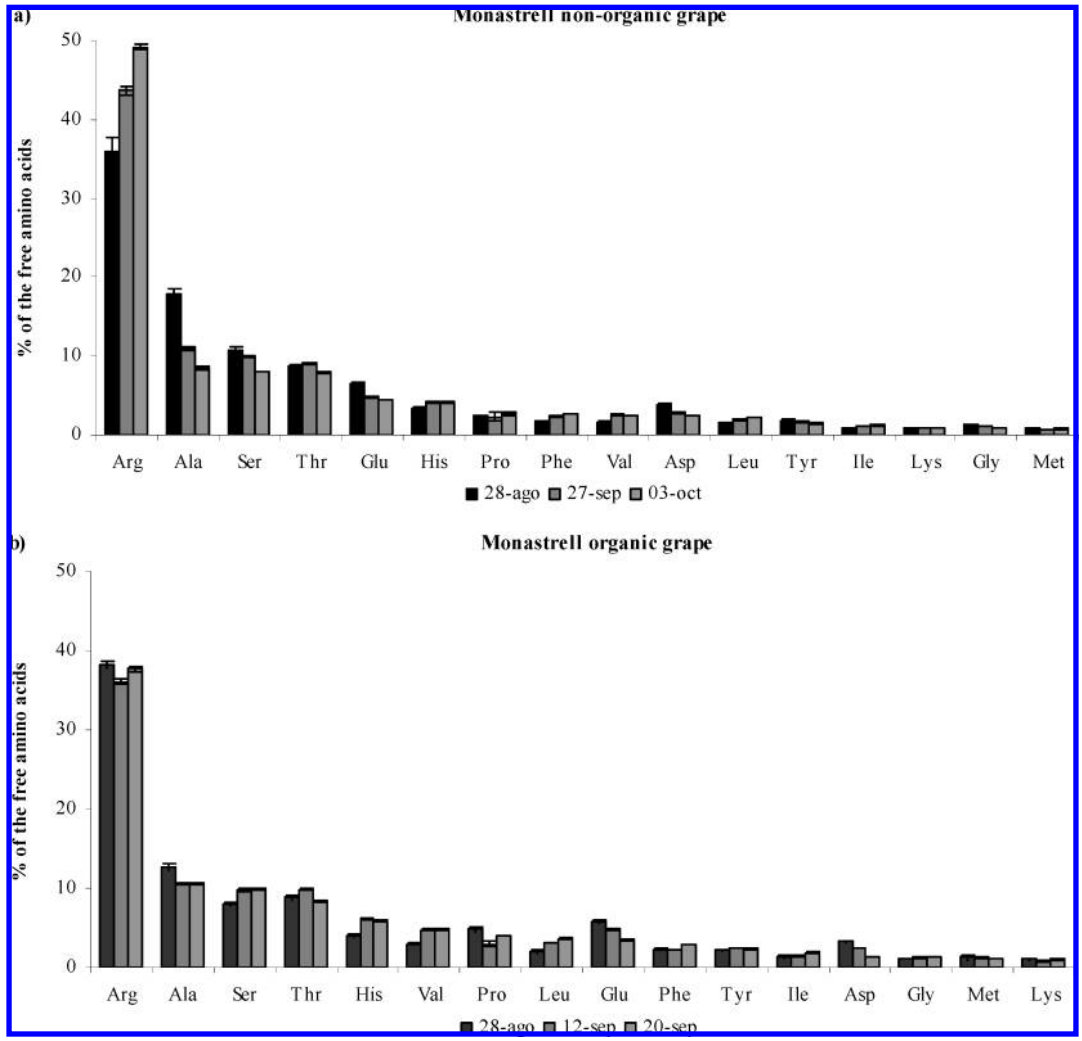

Figure 3. Percentage of each amino acid over the total amino acids in Monastrell nonorganic and organic grapes during ripening in 2008. All parameters are given with their standard deviation ($n = 4$), which are between 0.019 and 1.685.

presented proportions in grapes at harvest under 8% (**Figures 1**−**4**). All of these amino acids are good nitrogen sources for yeasts (*20, 26*). Also, the three amino acids present in smaller proportion in grapes (lysine, glycine, and methionine) were also the same in the different varieties, with the exception of Syrah variety in 2007, in which proline was also a minor amino acid (**Figures 1**−**4**). Lysine and glycine are not considered to be good nitrogen sources for *S. cerevisiae*, although they could be metabolized by other microorganisms, such as non-*Saccharomyces* yeasts (*24*). Therefore, the qualitative compositions of different grapes were similar, because the majority and minority amino acids were the same, which could indicate an adaptation of plants to the region.

The proline/arginine ratio in grapes at harvest was <1, being in 2007 between 0.02 in Syrah and 0.29 in Petit Verdot and in 2008 between 0.05 in Syrah and 0.11 in Merlot, indicating that these varieties are arginine accumulators. This ratio is used to classify grape varieties according to their ability to accumulate either one or the other of these two amino acids, so the varieties that have a proline/arginine ratio of <1 are arginine accumulators and vice versa. Thus, Chardonnay and Cabernet Sauvignon are proline accumulator varieties, whereas Garnacha and Pinot Noir are arginine accumulators (*27*).

During both years, arginine increased its proportion compared with the initial one, significantly in the Monastrell nonorganic grape (**Figures 1a** and **3a**) and slightly in Monastrell organic, Syrah, and Merlot grapes (**Figures 1b**, **2a**,**b**, **3b**, and **4a**,**b**). However, in Petit Verdot the percentage of this amino acid in the grapes in 2007 harvest was below the initial proportion (**Figure 2c**), whereas in 2008 the percentage of arginine was higher at the end than at the beginning of grape ripening (**Figure 4c**). In all grapes, with the exception of Monastrell nonorganic, there was a decrease in 2007 or slight changes during 2008 in the proportion of this amino acid at the end of the ripening (**Figures 1**−**4**). The reduction in arginine near the end of ripening in 2007 could indicate the remobilization of nitrogen toward the storage organs (i.e., roots), in times of low rainfall, in order to prepare for the following season. Alternatively, arginine could be converted to proline, so proline increased its proportion compared with the initial proportion in the varieties studied, with the exception of Syrah (**Figures 1** and **2**). This could be due to Syrah variety having the fewest proline accumulators, as it had the lowest proline/arginine ratio of the four varieties studied, and in the varieties that were greater proline accumulators, this amino acid increased at the end of the grape ripening as Stines et al. (*28*) found. In Monastrell variety, both organic and nonorganic, alanine and serine decreased their proportion, whereas the proportion of threonine varied slightly between initial and final time of grape ripening (**Figures 1** and **3**). In Syrah variety, these three amino acids showed little change during grape ripening (**Figures 2a** and **4a**). Of these three amino acids, alanine was the amino acid that

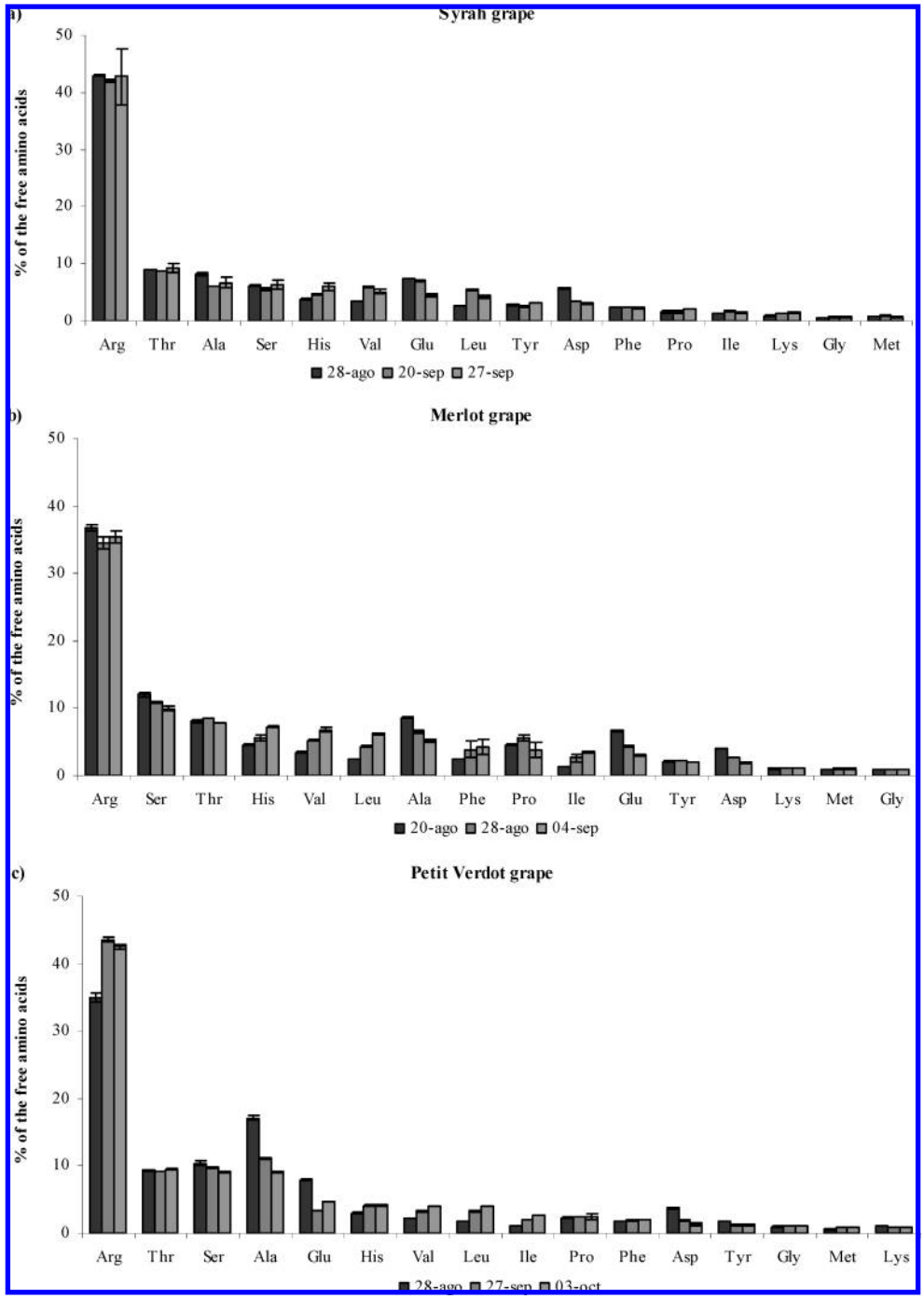

Figure 4. Percentage of each amino acid over the total amino acids in Syrah, Merlot, and Petit Verdot grapes during ripening in 2008. All parameters are given with their standard deviation ($n = 4$), which are between 0.004 and 4.847.

had the greatest differences in its proportion in the Merlot grape between the time of harvest and the beginning of the ripening (**Figures 2b** and **4b**). In the case of Petit Verdot variety, threonine increased its proportion, whereas alanine and serine presented similar proportions at the beginning and end of the period of ripening in 2007 (**Figure 2c**); alanine decreased its proportion, whereas threonine and serine presented similar proportions at the beginning and end of the period of ripening in 2008 (**Figure 4c**). The minor amino acids presented few changes during grape ripening (**Figures 1**−**4**).

To classify different grape varieties (Monastrell, Syrah, Merlot, and Petit Verdot) and different cultivation systems (nonorganic and organic), discriminant analysis was performed on data expressing as percentage each amino acid over the total amino acids in samples at 2007 and 2008 harvests (independent variables). The results are shown in **Figure 5**. Moreover, to classify different grape varieties (Monastrell, Syrah, Merlot, and Petit Verdot) and different cultivation systems (nonorganic and organic), discriminant analysis was performed on data expressing amino acids, ammonium, and total amino acid concentrations and proline/arginine ratio in the grape samples during ripening, in 2007 and 2008 (independent variables). The results are shown in **Figure 6**. In the first case (**Figure 5**), function 1 explained 68.2% of the variance and function 2 explained 17.0% of the variance, so the total of variance explained by these two functions was 85.2%. The variables that contributed most to the discriminant model were histidine, isoleucine, aspartic acid, and leucine (function 1) and leucine, valine, histidine, and

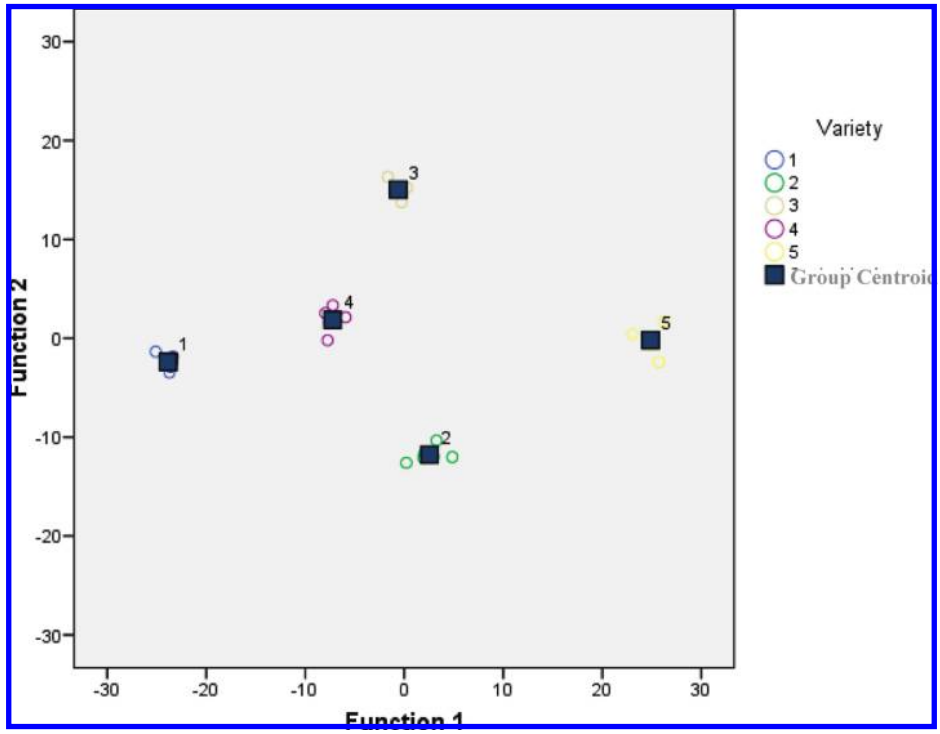

Figure 5. Application of discriminant analysis to the data expressing as percentage of each amino acid over the total amino acids of the different varieties (1, Monastrell nonorganic; 2, Monastrell organic; 3, Syrah; 4, Merlot; 5, Petit Verdot) at 2007 and 2008 harvests.

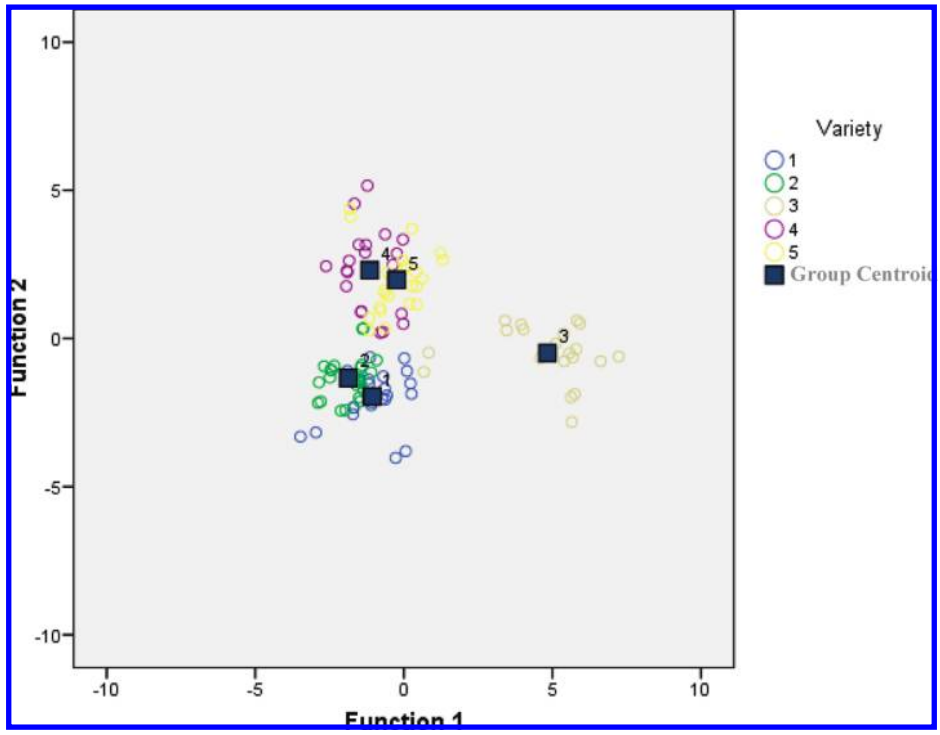

Figure 6. Application of discriminant analysis to data expressing amino acids (mg/L), ammonium (mg/L), total amino acids concentration (mg/L), and proline/arginine ratio in the samples of the different varieties (1, Monastrell nonorganic; 2, Monastrell organic; 3, Syrah; 4, Merlot; 5, Petit Verdot) at different times of grape ripening during 2007 and 2008.

glutamic acid (function 2). The two discriminant functions showed a good separation among different grapes (1−2, Monastrell; 3, Syrah; 4, Merlot; and 5, Petit Verdot) and different cultivation systems (1, Monastrell nonorganic grape; and 2, Monastrell organic grape). These discriminant functions allowed us to correctly classify 100% of the studies samples. Although these two years showed very different climatologies, the profile of amino acids depended mainly on the grape variety, a result that confirms what was observed in **Figures 1−4**. In the second case (**Figure 6**), function 1 explained 54.2% of the variance and function 2 explained 29.8% of the variance, the total explained by these two functions being 83.9%. In this case, the variables that contributed most to the discriminant model were threonine, aspartic acid, glycine, and serine (function 1) and serine, glycine, isoleucine, and arginine (function 2). The two discriminant functions show

a good separation between Syrah and the rest of the varieties. This could be because its concentration of amino acids was highest during both years (**Table 3**). Also, Merlot and Petit Verdot appear closer and separate from Monastrell organic and nonorganic, which were together in its turn (**Figure 6**). These discriminant functions also allowed us to correctly classify 92.2% of the studied grapes.

In conclusion, grape composition is the result of a series of interactions between genetic characteristics, environmental conditions, and cultural practices. The grape varieties studied (Monastrell, Syrah, Merlot, and Petit Verdot) presented similar qualitative compositions, because the major and minor amino acids were common, which could indicate an adaptation of plants to the region. The percentage of amino acids allowed differentiation of grapes according to the variety and cultivation system, regardless of year. At harvest, Syrah was the variety with greater aroma potential. The concentration of nitrogen compounds allowed differentiation of Syrah variety from the rest of the varieties. At harvest, Monastrell nonorganic grape had a concentration of nitrogen compounds much higher than that of the Monastrell organic grape. In Monastrell, Syrah, and Merlot, traditional varieties in the area, the highest concentration of nitrogen compounds coincided with the highest °Baumé/total acidity ratio and color index during 2007. Consequently, technological and phenolic maturity of these grape varieties coincided with the maximum composition of nitrogen compounds. However, in 2008, this did not happen because grape ripening was irregular as a consequence of different climatological conditions.

ACKNOWLEDGMENT

We express our gratitude to the Instituto de la Vid y el Vino de Castilla-La Mancha for facilitating the stay in this center of T.G.-C., Dr. Sergio Gómez-Alonso for help with the method of analysis of amino acids and ammonium, and Kathy Walsh for proofreading the English manuscript.

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Received for review June 16, 2008. Accepted January 21, 2009. T.G.-C. tos thank the Ministerio de Educación y Ciencia for the Juan de la Cierva contract.

JF8037049