

Varietal Differentiation of Red Wines in the Valencian Region (Spain)

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Ninety-one young varietal wines from the Valencian community (Spain), made from Cabernet Sauvignon, Tempranillo, Monastrell, and Bobal grapes, were tested on the basis of 33 variables: 9 conventional parameters, 10 alcohols and polyols, and 14 esters. Discriminant analysis was used to identify and explain the differences among samples, as well as to determine whether it is possible or not to differentiate among varieties. This differentiation (100% of the samples) has been possible due to the new discriminant analysis based on only 11 main variables: total acidity, *cis*-3-hexenol, methanol, glycerol, 2,3-butanediol, isobutyric alcohol, 1-pentanol, acetaldehyde, ethyl propionate, ethyl decanoate, and γ -butyrolactone, which allow differentiating 100% of the 1994 vintage and 97% of the 1995 vintage.

KEYWORDS: Differentiation; varietal red wine; discriminant analysis; chemical composition

INTRODUCTION

The study of wine components made from different grape varieties, having different geographical origins and prepared by means of individual manufacturing methods, allows us to gather precise information regarding the influence of such variables on the character and final quality of the resulting wine.

The characteristics of a wine are mainly determined by the grape variety used in its production and, therefore, it is important to establish a pattern of common characteristics that would allow one to identify those wines produced with different grape varieties and at the same time create differential criteria to classify wines as belonging to one variety or another.

The grape variety used provides wines with specific varietal characteristics (1) because it conditions their chemical compositions and their organoleptic properties. Differentiating wines according to the variety of grape to which they belong can be performed by determining parameters directly related to variety, parameters such as protein content and polyphenol, amino acid, and aromatic composition. The advance in analytical techniques has facilitated the quantification of these parameters. By means of electrophoresis and FPLC, the protein and amino acid profiles are determined. The fraction of polyphenols and fatty acids is studied by means of HPLC, and the metallic composition is analyzed for atomic adsorption. However, aromatic composition is the method most widely used for differentiating varietal wines through the application of GC-MS techniques for volatile component quantification.

According to this, Cabezado et al. (2), using conventional parameters of polyphenol and aromatic compounds, and Pueyo et al. (3), using protein fractions, have differentiated several Spanish wines produced from different varieties. Similarly, Forcén et al. (4), using conventional parameters of polyphenol, glycerin, and sugar composition, differentiated varietal wines from Majorca. Forina et al. (5) based their study on the phenolic composition of wines to differentiate wines from the Piemonte region. Ortega-Meder et al. (6) and Almela et al. (7) based their studies on the anthocyanin composition to differentiate varietal wines. Also, Latorre et al. (8) used metallic composition for varietal and geographic differentiation of white wines from Galicia.

The protein profile is equally employed, being one of the most widely used factors for differentiating varietal wines (3, 9), because it is genetically established (10) and not influenced by edaphic or climatic characteristics. Likewise, the amino acid content of wines (11, 12) and their organic acid content (13) can be used as a differentiating parameter for varietal wines.

Symonds and Cantagrel (14), among others, used aromatic composition. Noble et al. (15) applied discriminant analysis to the volatile compounds of French varietal red wines to obtain their differentiation. Rapp et al. (16, 17) and Presa-Owens et al. (18, 19) obtained the varietal differentiation of white wines according to their aromatic composition.

In the present paper, we have differentiated wines obtained from Cabernet Sauvignon, Tempranillo, Monastrell, and Bobal varieties, according to their chemical composition.

Monastrell and Bobal varieties are Valencian autochthonous varieties, and they are traditionally used for red wine production. The Tempranillo variety has been used as improvement material,

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even though many varietal wines are currently being produced from it, and Cabernet Sauvignon has been recently introduced into the region and is acquiring increasing importance.

MATERIALS AND METHODS

To carry out this work, 44 red wines from the 1994 vintage and 47 red wines from the 1995 vintage have been studied. These are made from Cabernet Sauvignon, Tempranillo, Monastrell, and Bobal grape varieties, all of them belonging to Valencia, Alicante, or Utiel-Requena "Appellation d'Origine" from the region of Valencia. Cultivation and production techniques were not uniform for all wines studied; however, ripeness degree was optimum in all varieties, and they were gathered during the optimum moment of grape harvest for the manufacturing of young wines with equal maceration times.

The physicochemical analyses practiced on the wines allowed classification and quantification of those components influencing wine taste and aroma. Classifications have been performed twice, using mean results as a basis for the study.

The physicochemical analyses have been performed according to the official methods established by the Bulletin de l'Office Internationale de la Vigne et du Vin (20), which allow classifying density, ethanol, pH, reducing sugars, total and volatile acidity, and free and combined sulfurous content.

Volatile components have been quantified through a chromatography technique in the gaseous phase, with a Hewlett-Packard 5890 A chromatograph provided with an ionization smoke detector or an HP-3395 integrator with nitrogen as carrier gas.

Acetaldehyde, ethyl and methyl acetates, methanol, 1-propanol, and isobutyric and isoamyl alcohols have been determined by direct injection of 1 μ L of wine in a Carbowax 1500 capillary column over Chromosorb to 5%, with 80–100 meshes, 4 m long and $1/8$ in. internal diameter (21). Operative conditions were as follows: oven temperature, 90 °C; injector temperature, 200 °C; detector temperature, 200 °C; nitrogen flow, 30 mL min⁻¹.

A prior extraction is necessary to determine ethyl propionate, isobutyl acetate, ethyl butyrate, isoamyl acetate, 1-butanol, 1-pentanol, hexyl acetate, ethyl lactate, ethyl octanoate, ethyl decanoate, γ -butyrolactone, diethyl succinate, diethyl glutarate, ethyl laurate, *cis*-3-hexenol, and 2-phenylethanol. The liquid–liquid extraction of a 500 mL sample (499 mL of wine and 1 mL of 1-heptanol, internal pattern) was continuously led by means of a mixture of dichloromethane/pentanol in a 2:3 v/v proportion for 10 h. From this sample, 1 μ L of the extract (obtained after concentration by evaporation in a 60 m long and a 0.25 mm internal diameter Supelcowax 10 capillary column) was injected (22). The temperature was programmed to 60 °C for 5 min and to then 180 °C for 20 min, with a temperature slope of 2.5 °C/min. Nitrogen, hydrogen, and air flows were 1.25, 300, and 300 mL/min, respectively. The temperature of the injector and detector was 250 °C in both cases.

Glycerol and 2,3-butanediol were determined by direct injection of 1 μ L of wine into a 101 Chromosorb column, with 60–80 meshes and $1/8$ in. internal diameter (23). Operative conditions were as follows: oven temperature, 160 °C; injector temperature, 200 °C; detector temperature, 280 °C; nitrogen flow, 25 mL/min.

Compound quantification was based on the internal pattern method. The efficiency of this method has been verified by means of the analysis performed with pattern solutions of the components studied and with the help of an HP-5979 mass spectrophotometer associated with the chromatograph.

The statistical treatment consisted of a discriminant analysis with the 33 variables studied. Therefore, and to clarify the nature of the differentiation, mean difference has been studied for each discriminant function by means of Logiciel Statgraphic Plus (version 2.1 plus).

RESULTS AND DISCUSSION

The average of the obtained values for the 33 variables studied in wines from 1994 and 1995 vintages are illustrated in Tables 1 and 2. The discriminant analysis has been performed on the data obtained for each of the vintages studied so as to compare the variability among vintages.

Table 1. Average Values of the Variables Studied in Cabernet Sauvignon, Tempranillo, Monastrell, and Bobal Wines, 1994 Vintage

	variety			
	Cabernet Sauvignon	Tempranillo	Monastrell	Bobal
volatile acidity ^a	0.67	0.64	0.61	0.58
total acidity ^b	6.32	4.82	5.07	6.14
pH	3.46	3.82	3.76	3.51
density	0.993	0.993	0.994	0.995
ethanol ^c	12.71	12.57	12.77	11.30
sugar ^d	1.99	1.90	2.02	2.67
SO ₂ total ^e	58.20	60.24	65.63	36.13
SO ₂ free ^e	18.36	26.98	21.41	20.11
acetaldehyde ^e	36.04	26.50	31.55	19.57
methanol ^e	161.98	142.39	213.92	147.05
1-propanol ^e	21.09	27.25	22.50	24.09
isobutyric alcohol ^e	50.49	44.58	53.49	57.11
isoamyl alcohol ^e	337.40	225.62	305.84	246.16
glycerol ^e	12012.25	10145.69	10200.32	8932.38
2,3-butanediol ^e	591.15	684.46	569.75	525.74
1-butanol ^e	3.32	2.28	3.93	1.49
1-pentanol ^e	0.06	0.05	0.09	0.05
<i>cis</i> -3-hexenol ^e	0.08	0.24	0.04	0.09
2-phenylethanol ^e	65.88	44.93	76.55	34.78
methyl acetate ^e	11.99	11.27	13.00	11.71
ethyl acetate ^e	55.15	57.39	54.64	50.95
ethyl propionate ^e	0.15	0.18	0.180	0.16
ethyl butyrate ^e	0.83	0.98	1.34	0.81
isoamyl acetate ^e	0.67	1.19	0.79	0.48
isobutyl acetate ^e	0.05	0.06	0.05	0.04
hexyl acetate ^e	0.04	0.05	0.03	0.05
ethyl lactate ^e	78.94	69.72	70.37	71.13
ethyl octanoate ^e	0.56	0.59	0.50	0.58
ethyl decanoate ^e	0.43	0.65	0.30	0.34
γ -butyrolactone ^e	9.24	6.41	10.70	6.53
diethyl succinate ^e	5.20	6.53	5.99	6.17
diethyl glutarate ^e	0.16	0.07	0.11	0.07
ethyl laurate ^e	0.03	0.04	0.03	0.03

^a Grams per liter of acetic acid. ^b Grams per liter of tartaric acid. ^c Percent v/v. ^d Grams per liter. ^e Milligrams per liter.

For each of the vintages studied (1994 and 1995), applying the discriminant analysis has rendered three discriminant functions, with the first two representing 94% of the total variability. Using the three discriminant functions obtained, it is possible to accurately separate all of the wines studied according to the variety they belong to: Cabernet Sauvignon, Tempranillo, Monastrell, and Bobal (Figures 1 and 2).

The first discriminant function obtained, mainly represented by *cis*-3-hexenol, methanol, glycerol, 2,3-butanediol, isobutyric alcohol, pentanol, acetaldehyde, ethyl butyrate, ethyl propionate, ethyl decanoate, and γ -butyrolactone, allows separating the wines into the four groups studied. The second discriminant function, integrated by the variables acetaldehyde, glycerol, diethyl succinate, total acidity, 1-propanol, and isoamyl acetate, allows differentiating Tempranillo variety wines from Bobal variety wines, although it confuses Cabernet Sauvignon and Monastrell variety wines; and the third discriminant function differentiates Cabernet Sauvignon variety wines from the rest of the wines in this study by means of pH, methanol, *cis*-3-hexenol, 2-phenylethanol, and γ -butyrolactone.

The analysis performed with the mean value obtained for each discriminant function consists of a variance analysis, taking the corresponding discriminant function value as variable answer for each wine and the group to which it belongs as explicative variable. The variance analysis on the mean values of the three discriminant functions for each vintage confirms the results obtained by the discriminant analysis and allows an accurate differentiation of wines within their specific groups.

Table 2. Average Values of the Variables Studied in Cabernet Sauvignon, Tempranillo, Monastrell, and Bobal Wines, 1995 Vintage

	variety			
	Cabernet Sauvignon	Tempranillo	Monastrell	Bobal
volatile acidity ^a	0.55	0.59	0.60	0.50
total acidity ^b	5.38	5.10	5.33	6.08
pH	3.68	3.86	3.75	3.62
density	0.995	0.995	0.995	0.995
ethanol ^c	12.54	12.41	12.99	11.97
sugar ^d	1.77	2.11	2.063	1.89
SO ₂ total ^e	62.96	53.35	51.70	49.72
SO ₂ free ^e	16.36	11.90	12.37	14.78
acetaldehyde ^e	29.94	34.00	30.73	20.53
methanol ^e	139.14	137.91	193.91	137.61
1-propanol ^e	19.48	25.51	24.81	18.98
isobutyric alcohol ^e	61.47	54.72	57.18	61.09
isoamylic alcohol ^e	359.24	245.02	264.31	243.31
glycerol ^e	10550.37	10030.25	11731.12	9118.58
2,3-butanediol ^e	594.32	663.01	672.56	573.92
1-butanol ^e	2.99	1.76	2.42	1.14
1-pentanol ^e	0.07	0.04	0.10	0.06
cis-3-hexenol ^e	0.09	0.23	0.06	0.10
2-phenylethanol ^e	57.30	35.07	48.17	34.75
methyl acetate ^e	7.03	7.14	7.43	6.17
ethyl acetate ^e	44.57	61.72	47.75	45.4
ethyl propionate ^e	0.20	0.17	0.02	0.12
ethyl butyrate ^e	0.54	0.79	0.96	0.75
isoamyl acetate ^e	1.42	0.98	0.77	0.78
isobutyl acetate ^e	0.06	0.09	0.06	0.10
hexyl acetate ^e	0.05	0.06	0.07	0.03
ethyl lactate ^e	38.22	53.37	49.9	56.79
ethyl octanoate ^e	0.54	0.61	0.35	0.54
ethyl decanoate ^e	0.35	0.46	0.21	0.36
γ-butyrolactone ^e	5.81	5.15	8.48	6.05
diethyl succinate ^e	5.75	5.46	5.07	4.07
diethyl glutarate ^e	0.05	0.07	0.13	0.07
ethyl laurate ^e	0.04	0.04	0.03	0.04

^a Grams per liter of acetic acid. ^b Grams per liter of tartaric acid. ^c Percent v/v. ^d Grams per liter. ^e Milligrams per liter.

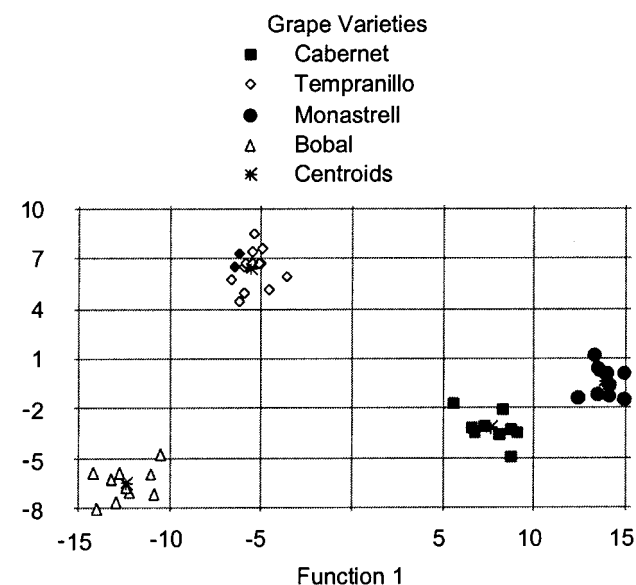


Figure 1. Projection according to the first and second discriminant functions of all variables.

By applying the discriminant analysis to the variables studied, it is possible to obtain an accurate differentiation and classification of wines into each of their respective groups, whereas the analysis of the variance corroborates this differentiation. How-

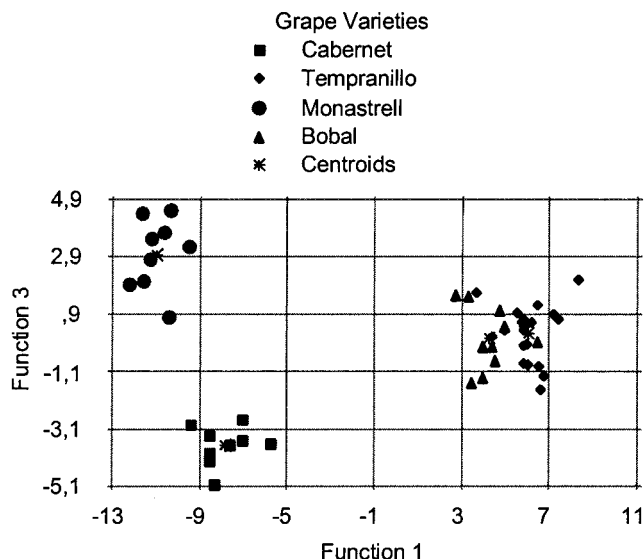


Figure 2. Projection according to the first and third discriminant functions of all variables.

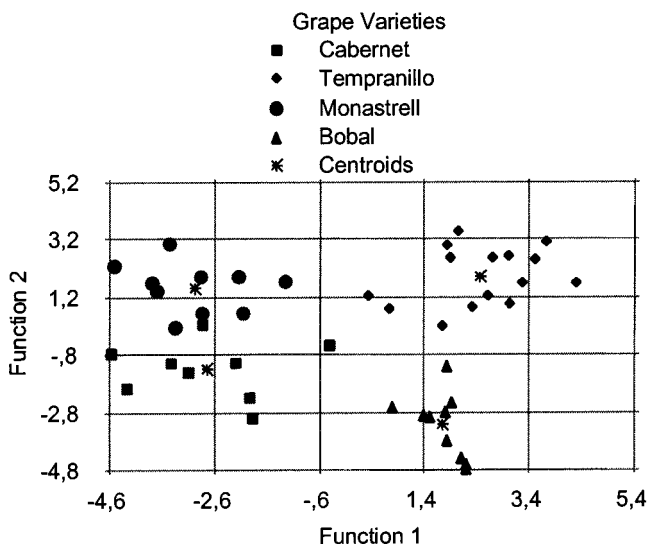


Figure 3. Projection according to the first and second discriminant functions for the 11 limit variables (vintage 1994).

ever, for the differentiation of the two vintages, a great number of variables are necessary.

In an attempt to limit the number of variables necessary for differentiating the wines, successive discriminant analyses have been performed with those variables considered as most important for the separate discrimination of the wines of each of the vintages. The results have shown that with only 11 of the 33 initial variables, it is possible to differentiate the wines obtained from the four different grape varieties.

The variables used for this new analysis were total acidity, cis-3-hexenol, methanol, glycerol, 2,3-butanediol, isobutyric alcohol, pentanol, acetaldehyde, ethyl propionate, ethyl decanoate, and γ-butyrolactone. The first discriminant function obtained allows separation of the wines into two groups: one group composed of Cabernet Sauvignon and Monastrell wines, and the other one composed of Tempranillo and Bobal wines. The second discriminant function forms two other groups, this time composed of Tempranillo and Monastrell wines in the first case and Cabernet Sauvignon and Bobal wines in the second case (Figures 3 and 4). The third discriminant function does not provide any new data to the differentiation. The contribution

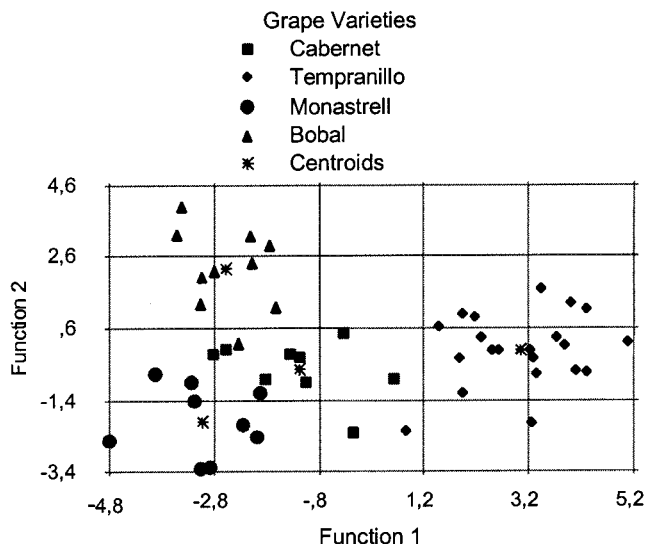


Figure 4. Projection according to the first and second discriminant functions for the 11 limit variables (vintage 1995).

of the two discriminant functions permits an accurate classification of 100% of the wines in the case of the 1994 vintage and of 97% of the wines in the case of the 1995 vintage (in the latter case, a Tempranillo wine was confused with the Cabernet Sauvignon).

The analysis of the variance performed on the averages corroborates the results obtained by the discriminant analysis, consequently allowing an accurate classification of the wines studied within each corresponding group.

The content of the 11 compounds that have permitted us to differentiate the wines made from the Cabernet Sauvignon, Tempranillo, Monastrell, and Bobal varieties shows a different and characteristic behavior for each variety.

In this way, wines made from Cabernet Sauvignon have the highest total acidity and glycerine values, with these two parameters being the ones most related to grape variety and especially to ripeness grade (4, 24).

Tempranillo wines have the lowest total acidity, which may possibly be attributed to a varietal factor due to the high potassium absorption observed in this variety (25). At the same time, these wines possess the highest contents of *cis*-3-hexenol and ethyl decanoate, and the lowest of isobutyric alcohol content, when compared with other wines examined in this work. According to Cabezudo et al. (26), *cis*-3-hexenol concentration is strongly related to grape variety because it is directly provided by the grape and metabolized by yeast. On the other hand, isobutyric alcohol in wine originates from valine amino acid, and so its amount greatly depends on the grape variety used for making the wine (4, 27). However, ethyl decanoate concentration is more related to the action of the yeasts fermenting the wines (28, 29).

Wines of the Monastrell variety possess higher methanol and γ -butyrolactone contents than do Cabernet Sauvignon, Tempranillo, and Bobal wines, whereas their contents in *cis*-3-hexenol and ethyl decanoate are lower. Methanol amount directly depends on pectin content in the grape peel, and so it is directly connected to the grape variety used, as illustrated by Ribereau-Gayon et al. (30).

Bobal wines present a higher content in isobutyric alcohol and the lowest contents in 2,3-butanediol and acetaldehyde.

Of the 11 compounds responsible for differentiating varietal wines, 5 of them (*cis*-3-hexenol, methanol, glycerol, isobutyric alcohol, and pentanol) belong to the group of superior alcohols

and polyols. Some researchers have found a connection between these compounds and grape variety (28, 31, 32) as well as a connection between acidity and grape variety (25).

Acetaldehyde, 2,3-butanediol, ethyl propionate, ethyl decanoate, and γ -butyrolactone are the compounds most connected to fermentation conditions and yeast types used in it, both of which constitute a determinant factor for grape variety (33, 34).

In addition to all of this, the present work illustrates how the compounds that differentiate wines according to a varietal criterion (except in the cases of Bobal variety and concrete geographical areas) belong indistinguishably to the three "Appellations d'Origine" existing within the Valencian community. According to these findings, the geographical factor is discredited as a differentiating parameter for these wines, although it is still considered as very useful for other differentiation studies.

Furthermore, it is also necessary to consider that cultural practices and manufacturing procedures are not uniform for all of the wines studied, so such factors should not influence the differentiation.

CONCLUSIONS

This study illustrates how a small number of variables related to the chemical composition of wines allow differentiating wines made from different varieties.

The fact that a small number of variables could allow clear differentiation of wines of different varieties establishes a link between chemical composition and varietal character because the wines belong indistinguishably to any of the three "Appellations d'Origin" from the Valencian community with the single exception of Bobal wines, which come from a specific geographic area. The geographical criterion, otherwise useful in the case of wine differentiation, cannot be considered here as a differentiating parameter.

On the other hand, the fact that such a differentiation is possible despite irregular cultural practices and manufacturing procedures indicates that, even though these two contributions are important, they do not have a definite influence on wine varietal differentiation.

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