APPLICATION OF MULTIVARIATE STATISTICAL ANALYSES TO THE STUDY OF FACTORS AFFECTING WHITE WINE VOLATILE COMPOSITION

VALERIA GALLO[1], RAFAEL BELTRÁN[1], FRANCISCO J. HEREDIA[2], M. LOURDES GONZÁLEZ-MIRET[2] and DOLORES HERNANZ[1,3]

[1]Department of Analytical Chemistry, Faculty of Experimental Sciences, University of Huelva, Avda. de las Fuerzas Armadas s/n 21007, Huelva, Spain
[2]Laboratory of Food Colour and Quality, Department of Nutrition and Food Science, Faculty of Pharmacy, University of Seville, Sevilla, Spain

[3]Corresponding author. TEL: 349-5921-9960; FAX: 349-5921-9942; EMAIL: vila@uhu.es

ABSTRACT

The influence of different storage conditions on the volatile composition of young white wine was evaluated during 1 year of storage. The wine was bottled and stored under different conditions of temperature, position and illumination. During storage, a significant increase in total content esters (mainly due to the increase in ethyl butyrate, ethyl acetate and diethyl succinate) and total content acids was detected. Also total content alcohols and carbonyl compounds decreased during storage. Storage time, temperature and illumination have a significant effect on esters content, whereas carbonyl compounds are affected by temperature, and alcohols content is affected by illumination. Stepwise linear discriminant analysis was applied to the experimental data. The results showed that the samples were well classified as a function of storage temperature. On the other hand, a certain trend was observed when the wine samples were classified according to storage time. The samples corresponding to two and four months of storage were well differentiated from the rest.

PRACTICAL APPLICATIONS

From a sensory point of view, the aroma influences greatly the quality of wines. The loss of aromatic characteristics in young white wine, as well as browning, take place over a variable period of time which ranges from one month to several years, depending on wine type and storage conditions. Assessment of possible factors affecting the useful lifespan of young white wine is of great interest for the wine making industry. Knowing the chemical composition of wine, specifically aromas, and its evolution relating to different storage and preservation conditions is an important tool in quality control.

INTRODUCTION

The loss of aromatic characteristics in young white wine, as well as browning, take place over a variable period of time, which ranges from one month to several years, depending on wine type and storage conditions. These chemical changes occurring during the wine storage are numerous and complex, and the process depends on grape factors, such as composition, pH or enzymatic activity (Ough and Ramey 1980; Singleton *et al.* 1985; Marais 1986; González-Viñas *et al.* 1996; Pérez-Prieto *et al.* 2003; Garde-Cerdán *et al.* 2008).

From a sensory point of view, the aroma greatly influences the quality of wines. The aromatic composition of wines is very complex because there is a large number of volatile components in the wine (most of them being at very low concentrations), as well as to the interactions among them. Alcohols and esters are the major volatile components, although there

are also variable amounts of aldehydes, acids, terpenes, volatile phenols and acetonic compounds. Aromas change over time; they evolve and become more complex, and wine becomes less fruity and gains bouquet due to acetalization and esterification reactions (Rapp *et al*. 1985). Acetals are products derived from the reaction of aldehydes with alcohols, with the most evident case being the formation of ethyl hemiacetal. Esterification reactions, which are very slow over time, produce highly characteristic compounds, such as ethyl acetate, which, when present at high concentrations (>140 mg/L), gives wine sour features (De Rosa 1998). The loss of fruitiness is often attributed to terpene oxidation reactions in wines with significant monoterpenol content (Ribereau-Gayon *et al*. 1975).

During wine bottle storage, a number of changes affecting wine stability and organoleptic characteristics occur (Recamales *et al*. 2006). These changes will depend not only on wine chemical composition, but also on storage duration and conditions, such as temperature, oxygen availability or illumination. Pérez-Coello *et al*. (2003) studied the influence of storage temperature on ester hydrolysis and its relationship with the loss of fruitiness in wine. Additionally, there is a relationship between dimethyl sulphide levels and sensory evaluation of stored wines, depending on storage temperature (Marais and Pool 1980). In white wines stabilized without sulfurous anhydride, temperature during storage is the most significant factor in the variation of volatile composition (Garde-Cerdán *et al*. 2008). Storage time also affects aroma (González-Viñas *et al*. 1996). According to González-Viñas *et al*. (1998), sensory characteristics of young white wines stored under usual commercial conditions remain up to 18 months. Pérez-Prieto *et al*. (2003) studied the evolution of major volatile compounds present in red wines submitted to different periods of storage in bottles (8 and 10 months) and in oak barrels (7 months and 4 years).

On the other hand, changes were observed during storage under controlled temperature (15.8C) and humidity (65.1%) for 5 years, depending on the type of bottle closure (cork or synthetic material) (Skouroumounis *et al*. 2005), the bottle position (horizontal and vertical position) during 36 months in white and red wines (Mas *et al*. 2002), and the type of glass used for the bottle (Selli *et al*. 2002).

Assessment of possible factors affecting the useful lifespan of young white wine is of great interest for the winemaking industry. Knowing the chemical composition of wine, specifically aromas, and its evolution relating to different storage and preservation conditions is an important tool in quality control. The aim of this study is to evaluate the effect of preservation environmental conditions (time, temperature, bottle position and illumination) on white wine quality characteristics. On the other hand, Stepwise Linear Discriminant Analysis (SLDA) was used to determine the most relevant changes in volatile composition in wine during storage.

MATERIALS AND METHODS

Samples

The wine was produced in wineries adhered to the DO Condado de Huelva according to traditional methods, fermented at controlled temperatures. Healthy ripe grapes of *Vitis vinifera* cv. Zalema (white grape variety) grown exclusively in southwest Spain were destemmed, crushed and the must subsequently transferred into a 30,000-L stainless steel tank. Enough sulfur dioxide solution (3.1%) was added to adjust the total sulfur dioxide content of the must to 80 mg/L. The must was kept in the tank at 12 ± 3C for 24 h, and then the residual solids were separated. Later on, the must was run into a fermentation tank, and the total acidity was adjusted to 6 g/L with tartaric acid. For the fermentation processes, no selected yeasts were used and the temperature was kept at 22 ± 2C. At the end of this stage, the wine was transferred to a 14,000-L stainless steel tank and it was stored for the stabilization process. The product was kept at 12 ± 3C during the stabilization process, and the free sulfur dioxide content was maintained at 25–30 mg/L. After cold stabilization, the wines were bottled in 750-mL green glass bottles and stored under two different conditions of temperature: "variable," where bottles were stored under variable conditions of temperature (daily and seasonal, within the approximate range from 8 to 30C), and "constant," where temperature ranged between 15 and 20C throughout the year. Within each of these lots ("variable" and "constant"), half of the bottles were placed in horizontal position and the other half in vertical position; both among the horizontal bottles and the vertical ones; half of the samples were stored in the dark and the other half under variable light conditions (daylight and night). The wine was stored for 12 months. The samples were taken at the moment of bottling, and then sampling frequency was done bimonthly. A total of 98 samples of bottled wine were analyzed.

Reagents and Standards

All reagents used were of chromatographic quality: dichloromethane, ammonium sulfate and tartaric acid (Merck, Darmstadt, Germany), ethanol (Panreac Química S.A.U., Barcelona, Spain) and potassium bitartrate (Sigma-Aldrich, St. Louis, MO). The standards used were acetaldehyde, 2-methylpropanoic acid and 3-methylbutanoic acid (Dr Ehresnstorfer GmbH, Augsburg, Germany); acetoin, butanoic acid, hexanoic acid, ethyl isovalerate, octanoic acid, isoamyl acetate, ethyl lactate, 2-phenylethanol and decanoic acid (Aldrich Chemical); furan-2-carbaldehyde, ethyl acetate, 2-methylpropyl ethanoate, 2-phenylethyl acetate, ethyl butyrate, ethyl octanoate, diethyl succinate, butan-1-ol, isobutanol, isoamyl alcohol and hexan-1-ol (Chem Service Inc); ethyl hexanoate and *cis*-3-hexen-1-ol (Fluka Chemika).

The internal standards used were 4-methyl-2-pentanol (Chem Service) and 2-octanol (Aldrich). All standards had a purity rate between 98 and 99%. The deionized water used was provided by the Milli-Q filtration system (Millipore model A-10).

The standard solutions were prepared using ethanol as solvent at a concentration of 1,000 mg/L. The concentrations of each internal standards compounds in the solution (just one solution for both standards) were 743 mg/L of 4-Methyl-2-pentanol and 620 mg/L of 2-octanol.

The wine model solution was prepared by weighing 1.16 g potassium bitartrate and 0.7 g tartaric acid dissolved in 1,000 mL of ultrapure water with 10% absolute ethanol (pH = 3.10) (Zhou *et al.* 1996). To achieve synthetic wine, the wine model solution (250 mL) was strengthened with a solution of aromas, so that concentration of the 25 compounds in the synthetic wine was 50 mg/L. The synthetic wine was used for optimization of the chromatographic method and for the precision study of the extraction method. The solutions were stored at 4C until use.

Analysis of Volatile Compounds

Preparation of the sample was carried out following the methods proposed by Ortega *et al.* (2001). 9 mL of sample were placed in a conical-bottom centrifuge tube, adding 13.5 g of ammonium sulfate, 21 mL of deionized water and 100 µL of the internal standard solution (final concentration 2.5 mg/L of 4-methyl-2-pentanol and 2.1 mg/L of 2-octanol). The mixture was shaken for 30 min, adding afterwards 600 µL dichlorometane, further shaken for 30 min, and centrifuged at 3,000 rpm for 10 min. The organic phase was collected and 1 µL was injected into the chromatograph for analysis. All analyses were performed in triplicate and the organic extract was injected in duplicate into the gas chromatograph. The result was expressed as mean value.

The volatile compounds were analyzed by gas chromatography using the chromatographic system Agilent Technologies 6,890 N Network GC System (Palo Alto, CA) equipped with a flame ionization detector and a Varian CP-Wax 52 CB column (60 m × 0.25 mm × 0.25 µm) (Walnut Creek, CA). The injection (1 µL of sample) was performed using the split mode (split ratio 1:20) and oven temperature was held constant at 40C for 5 min, then raised by 1.5C/min. to 200C, where it was held for 20 min. The injector and detector temperature was 250C. The carrier gas used was helium at a flow rate of 1 mL/min.

Analytical Parameters of the Extraction Method

Quantification of volatile compounds was performed using the internal standard method. From standard solutions, cali-

bration lines were constructed at six different concentration levels, taken into account the usual concentrations of these analytes in the wines (0.5 to 150 mg/L for esters and alcohols and 0.1 to 50 mg/L for acids and carbonyl compounds). The regression coefficients (r^2) ranged between 0.9830 (for butanoic acid) and 0.9998 (for decanoic acid).

The values of repeatability (precision) were assessed by calculating the relative standard deviation (R.S.D.) in six independent extractions from the synthetic wine, performed under the same analytical conditions as in the sample in the shortest time possible (UNE 82009–1:1998). Values ranged between 1.6% R.S.D for 2-phenylethyl acetate and 12.4% R.S.D. for hexanoic acid.

Multivariate Statistical Analysis

The various multivariate statistical treatments were performed using the STATISTICA® statistical package (version 6.0, StatSoft 2001).

Variance analysis was applied to the data obtained for the variables studied, which allows us to compare the mean values obtained for different groups or classes, thus establishing whether there are significant statistical differences between them among the wines stored under different conditions, or whether these differences are due to random error. On the other hand, SLDA was applied for the characterization and differentiation of the samples according to their aromatic composition.

RESULTS AND DISCUSSION

Twenty-five volatile compounds were identified in the wine samples, which could be classified into four different families: (1) esters (ethyl isovalerate, isoamyl acetate, ethyl lactate, ethyl acetate, 2-methylpropyl ethanoate, 2-phenylethyl acetate, ethyl butyrate, ethyl octanoate, diethyl succinate and ethyl hexanoate), (2) acids (2-methylpropanoic acid, 3-methylbutanoic acid, butanoic acid, hexanoic acid, octanoic acid and decanoic acid), (3) alcohols (2-phenylethanol, butan-1ol, isobutanol, isoamyl alcohol, hexan-1-ol and *cis*-3-hexen-1-ol); and (4) carbonyl compounds (acetaldehyde, acetoin and furan-2-carbaldehyde).

Tables 1 to 3 show the effect of the different storage conditions (time, temperature, illumination and bottle position) on the volatile contents (means and standard deviations) of the wines. Ethyl acetate, butanoic acid and acetoin concentrations in the wine are not affected significantly by any storage factor.

Considering the sum of all individual volatile compounds, which could mean the global aroma, the factors "storage time" and "temperature" have significant effect on the concentration of volatile compounds ($P < 0.01$ and $P < 0.001$,

TABLE 1. INFLUENCE OF TIME AND VARIOUS STORAGE CONDITIONS ON ESTERS (mg/L)†

Variable	Ethyl lactate	Ethyl hexanoate	Ethyl octanoate	Ethyl butyrate	Ethyl isovalerate	Diethyl succinate	2-Phenyl ethyl acetate	Isoamyl acetate	2-methylpropyl ethanoate	Ethyl acetate
Storage time (months)										
0	25.41 ± 4.58[a]	nd	0.04 ± 0.01[a]	0.02 ± 0.01[a]	0.01 ± 0.00[a]	6.70 ± 2.24[a]	0.11 ± 0.05[a]	0.40 ± 0.13[a]	0.46 ± 0.22[a]	15.51 ± 8.15[a]
2	21.31 ± 2.81[a]	0.03 ± 0.02[a]	0.04 ± 0.01[a]	0.03 ± 0.00[a]	0.01 ± 0.00[a]	4.78 ± 1.24[b]	0.09 ± 0.06[a]	0.84 ± 0.29[b]	0.27 ± 0.11[a]	17.35 ± 8.56[a]
4	25.28 ± 2.96[a]	0.03 ± 0.01[a]	0.05 ± 0.02[a]	0.04 ± 0.01[b]	0.01 ± 0.00[b]	5.69 ± 1.11[b]	0.08 ± 0.04[a]	0.70 ± 0.14[b]	0.38 ± 0.26[a]	18.67 ± 7.04[a]
6	26.13 ± 279[ab]	0.03 ± 0.01[a]	0.04 ± 0.01[a]	0.03 ± 0.01[a]	0.01 ± 0.00[b]	10.16 ± 3.44[c]	0.09 ± 0.05[a]	0.52 ± 0.15[c]	0.31 ± 0.27[a]	25.04 ± 9.59[a]
8	31.55 ± 2.95[b]	0.02 ± 0.02[a]	0.05 ± 0.01[a]	0.03 ± 0.01[a]	0.01 ± 0.00[b]	13.66 ± 4.69[d]	0.08 ± 0.06[a]	0.29 ± 0.32[d]	0.38 ± 0.26[a]	23.17 ± 10.07[a]
10	26.82 ± 3.41[ab]	0.03 ± 0.01[a]	0.05 ± 0.01[a]	0.04 ± 0.01[cb]	0.01 ± 0.00[c]	14.52 ± 3.35[d]	0.08 ± 0.04[a]	0.35 ± 0.23[d]	0.37 ± 0.21[a]	23.90 ± 8.28[a]
12	25.19 ± 3.23[a]	0.02 ± 0.02[a]	0.05 ± 0.02[a]	0.03 ± 0.01[a]	0.01 ± 0.00[c]	15.68 ± 3.77[d]	0.08 ± 0.04[a]	0.25 ± 0.18[d]	0.32 ± 0.23[a]	22.96 ± 10.59[a]
Significant effect	***	ns	ns	*	ns	***	ns	***	ns	ns
Temperature										
Variable	26.52 ± 4.08	0.02 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	13.02 ± 5.86	0.08 ± 0.06	0.31 ± 0.28	0.50 ± 0.18	23.21 ± 10.44
Constant	25.58 ± 5.09	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	8.47 ± 3.41	0.08 ± 0.03	0.68 ± 0.24	0.18 ± 0.12	20.49 ± 7.64
Significant effect	ns	***	***	***	ns	***	ns	***	***	ns
Illumination										
Light	27.50 ± 4.55	0.03 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	11.15 ± 4.32	0.08 ± 0.05	0.50 ± 0.26	0.35 ± 0.22	23.66 ± 9.69
Darkness	24.59 ± 4.22	0.03 ± 0.01	0.05 ± 0.02	0.03 ± 0.01	0.02 ± 0.00	10.34 ± 4.24	0.09 ± 0.05	0.48 ± 0.27	0.33 ± 0.21	20.03 ± 8.45
Significant effect	**	ns	ns	ns	*	ns	ns	ns	ns	ns
Position										
Horizontal	26.15 ± 5.05	0.03 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	10.85 ± 5.62	0.10 ± 0.05	0.48 ± 0.32	0.41 ± 0.26	19.92 ± 8.32
Vertical	25.95 ± 4.14	0.03 ± 0.01	0.05 ± 0.01	0.03 ± 0.00	0.01 ± 0.00	10.64 ± 4.93	0.07 ± 0.04	0.51 ± 0.31	0.27 ± 0.15	23.77 ± 9.71
Significant effect	ns	ns	*	ns	ns	ns	*	ns	***	ns

Significant differences at * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

† Data expressed as mean ± SD.

Note: Data followed by different letters for each column are significantly different according to Fisher's test, $P < 0.05$.

ns, not significant; nd, not detected.

TABLE 2. INFLUENCE OF TIME AND VARIOUS STORAGE CONDITIONS ON ACIDS (mg/L)†

Variable	2-methylpropanoic acid	3-Methylbutanoic acid	Butanoic acid	Hexanoic acid	Octanoic acid	Decanoic acid
Storage time (months)						
0	nd	0.44 ± 0.20[a]	16.96 ± 4.28[a]	5.58 ± 0.34[a]	2.17 ± 0.99[a]	1.54 ± 0.35[a]
2	nd	0.15 ± 0.23[a]	10.92 ± 6.07[a]	5.08 ± 0.44[a]	3.09 ± 1.10[a]	1.51 ± 0.20[a]
4	0.24 ± 0.16[a]	0.22 ± 0.21[a]	11.89 ± 4.81[a]	5.30 ± 0.19[a]	3.50 ± 0.61[a]	1.58 ± 0.09[a]
6	0.11 ± 0.10[a]	0.19 ± 0.15[a]	11.43 ± 3.27[a]	5.00 ± 0.40[a]	2.71 ± 0.95[a]	1.58 ± 0.39[a]
8	0.28 ± 0.27[a]	0.25 ± 0.19[a]	11.50 ± 4.74[a]	5.16 ± 0.44[a]	3.02 ± 1.10[a]	1.73 ± 0.45[a]
10	0.24 ± 0.25[a]	0.27 ± 0.23[a]	12.63 ± 3.74[a]	5.10 ± 0.24[a]	3.37 ± 1.26[a]	1.47 ± 0.10[a]
12	0.20 ± 0.17[a]	0.16 ± 0.18[a]	12.90 ± 3.42[a]	5.14 ± 0.30[a]	2.83 ± 0.87[a]	1.34 ± 0.58[a]
Significant effect	ns	ns	ns	ns	ns	ns
Temperature						
Variable	0.31 ± 0.29	0.29 ± 0.21	12.99 ± 2.49	5.11 ± 0.31	2.55 ± 0.70	1.68 ± 0.33
Constant	0.05 ± 0.10	0.13 ± 0.09	10.76 ± 5.36	5.15 ± 0.38	3.63 ± 0.94	1.39 ± 0.32
Significant effect	*	**	ns	ns	***	***
Illumination						
Light	0.22 ± 0.17	0.22 ± 0.19	12.50 ± 3.27	5.24 ± 0.28	3.26 ± 0.91	1.62 ± 0.32
Darkness	0.14 ± 0.26	0.20 ± 0.18	11.25 ± 5.10	5.02 ± 0.37	2.92 ± 1.06	1.44 ± 0.37
Significant effect	ns	ns	ns	*	ns	*
Position						
Horizontal	0.31 ± 0.29	0.26 ± 0.23	12.14 ± 3.87	5.18 ± 0.29	3.00 ± 0.93	1.67 ± 0.34
Vertical	0.05 ± 0.14	0.15 ± 0.14	11.61 ± 4.73	5.08 ± 0.39	3.17 ± 1.05	1.40 ± 0.32
Significant effect	*	*	ns	ns	ns	**

Significant differences at * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

† Data expressed as mean ± SD.

Note: Data followed by different letters for each column are significantly different according to Fisher's test, $P < 0.05$.

ns, not significant.

nd, not detected.

respectively), whereas bottle position and illumination have no significant effect on the variation of volatiles during storage (data not shown).

The variance analysis of the individual volatile compound data shows that temperature during storage (variable/constant) is a factor that significantly affects a greater number of volatiles, specifically 16 compounds (6 esters, 4 acids, 4 alcohols and 2 carbonyl compounds). Bottle position (horizontal/vertical) has a significant effect on 11 compounds (3 esters, 3 acids, 4 alcohols and furan-2-carbaldehyde); illumination (light/darkness) has a significant effect on 5 compounds (2 esters, 2 acids and isoamyl alcohol), and storage time on 5 compounds (isobutanol and 4 esters).

The total content of esters and acids (that is, considering the sum of all individual esters and acids compounds, respectively) increases during storage, whereas alcohol and carbonyl contents (considering the sum of all individual alcohol and carbonyl contents, respectively) decrease. The increase in ester content in the wines is mainly due to the increase in ethyl butyrate, ethyl acetate and diethyl succinate. Thus, after 12-month storage, wines have 1.5 times more ethyl butyrate and ethyl acetate, and 2.3 times more diethyl succinate. Ethyl lactate and isovalerate concentrations were the only component significantly modified by light intensity. Samples stored in the dark had lower concentration of ethyl lactate than those

exposed to light, whereas the wines stored in the dark had higher concentration of ethyl isovalerate than the wines exposed to light. Regarding 2-phenylethyl acetate, isoamyl acetate and 2-methylpropyl ethanoate concentrations, they decrease during storage. In the case of isoamyl acetate, the decrease is much higher in the wines stored at variable temperature, while in the case of 2-methylpropyl ethanoate, the decrease is higher in the wines stored at constant temperature (Table 1). Studies carried out in wines elaborated with the Airen, Viura and Macabeo white grape varieties show similar results in the evolution of esters during bottle storage (González-Viñas *et al*. 1996; Pérez-Coello *et al*. 1999, 2003).

Storage time does not affect acid content significantly, whereas temperature during storage significantly affects the content of 2-methylpropanoic, 3-methylbutanoic, octanoic and decanoic acids, with the wines stored at variable temperature showing higher concentration of 2-methylpropanoic, 3-methylbutanoic and decanoic acids (Table 2). Exposition to light affects decanoic and hexanoic acid concentration significantly, while bottle position affects the content of 2-methylpropanoic, 3-methylbutanoic and decanoic acids in the wines.

With respect to alcohols, temperature is the factor that most affects its concentration, with the wines stored at variable temperature and horizontal position showing higher

TABLE 3. INFLUENCE OF TIME AND VARIOUS STORAGE CONDITIONS ON ALCOHOLS AND CARBONYL COMPOUNDS (mg/L)†

Variable	hexan-1-ol	2-phenylethanol	Isobutanol	Butan-1-ol	Isoamyl alcohol	cis-3-Hexen-1-ol	Acetaldehyde	Acetoin	Furan-2-carbaldehyde
Storage time (months)									
0	1.83 ± 0.35[a]	123.07 ± 13.38[a]	1.56 ± 0.82[a]	0.71 ± 0.25[a]	123.81 ± 32.57[a]	0.38 ± 0.07[a]	95.08 ± 16.50[a]	4.72 ± 2.20[a]	2.87 ± 0.86[a]
2	1.10 ± 0.42[a]	75.67 ± 20.73[a]	0.52 ± 0.52[a]	0.70 ± 0.13[a]	125.59 ± 17.68[a]	0.31 ± 0.07[a]	91.05 ± 11.36[a]	4.84 ± 2.07[a]	1.47 ± 0.47[a]
4	1.01 ± 0.14[a]	67.02 ± 9.95[a]	1.51 ± 0.97[a]	0.93 ± 0.29[a]	100.29 ± 62.42[a]	0.25 ± 0.10[a]	90.24 ± 25.50[a]	4.32 ± 3.50[a]	1.79 ± 0.61[a]
6	1.07 ± 0.31[ab]	69.95 ± 25.55[a]	1.02 ± 0.92[a]	0.70 ± 0.27[a]	120.97 ± 10.48[a]	0.32 ± 0.09[b]	83.99 ± 16.36[a]	5.27 ± 1.70[ab]	1.86 ± 1.02[a]
8	1.22 ± 0.28[ab]	75.46 ± 27.80[a]	1.22 ± 0.98[a]	0.78 ± 0.22[a]	129.14 ± 11.88[a]	0.32 ± 0.05[b]	92.34 ± 14.78[a]	5.90 ± 1.54[ab]	2.29 ± 1.22[a]
10	1.02 ± 0.28[a]	62.52 ± 19.10[a]	1.38 ± 0.99[a]	0.80 ± 0.37[a]	121.94 ± 9.73[a]	0.29 ± 0.03[ab]	83.29 ± 15.71[a]	7.12 ± 1.76[b]	2.00 ± 0.74[a]
12	1.37 ± 0.48[b]	71.71 ± 19.45[a]	1.20 ± 0.92[a]	0.68 ± 0.18[a]	103.34 ± 43.37[a]	0.30 ± 0.03[ab]	78.81 ± 13.40[a]	5.86 ± 1.64[ab]	1.89 ± 0.91[a]
Significant effect	ns	ns	*	ns	ns	ns	ns	ns	ns
Temperature									
Variable	1.32 ± 0.36	83.94 ± 23.59	2.15 ± 0.96	0.79 ± 0.32	109.31 ± 34.21	0.33 ± 0.06	79.28 ± 12.62	5.53 ± 2.62	2.24 ± 1.01
Constant	0.94 ± 0.21	56.83 ± 11.04	0.14 ± 0.12	0.74 ± 0.17	124.44 ± 29.66	0.27 ± 0.06	93.96 ± 17.23	5.57 ± 1.71	1.52 ± 0.41
Significant effect	***	***	***	ns	ns	***	**	ns	**
Illumination									
Light	1.14 ± 0.32	72.52 ± 22.26	1.09 ± 1.00	0.76 ± 0.18	128.31 ± 13.51	0.29 ± 0.07	86.78 ± 19.39	5.78 ± 1.73	1.79 ± 0.78
Darkness	1.12 ± 0.39	68.26 ± 24.55	1.20 ± 1.06	0.77 ± 0.31	105.45 ± 41.82	0.31 ± 0.07	86.46 ± 13.25	5.32 ± 2.62	1.98 ± 0.94
Significant effect	ns	ns	ns	ns	*	ns	ns	ns	ns
Position									
Horizontal	1.20 ± 0.38	78.70 ± 28.22	1.42 ± 1.04	0.87 ± 0.28	118.09 ± 26.77	0.32 ± 0.07	85.71 ± 11.41	5.76 ± 2.36	2.12 ± 1.12
Vertical	1.06 ± 0.32	62.08 ± 11.37	0.86 ± 0.81	0.66 ± 0.17	115.67 ± 38.24	0.28 ± 0.07	87.53 ± 20.77	5.35 ± 2.05	1.65 ± 0.32
Significant effect	ns	***	**	**	ns	**	ns	ns	*

Significant differences at * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
† Data expressed as mean ± SD.
Note: Data followed by different letters for each column are significantly different according to Fisher's test, $P < 0.05$.
ns, not significant.

Factors	Esters		Alcohols		Acids		Carbonyl compounds	
	F ratio	P value	F ratio	P value	F ratio	P value	F ratio	P value
Time (T)	7.26	0.000*	1.24	0.307	0.31	0.905	0.74	0.600
Temperature (Ta)	7.79	0.008*	1.90	0.175	1.68	0.202	8.63	0.005*
Bottle position (P)	1.27	0.265	3.62	0.064	0.63	0.431	0.04	0.844
Illumination (I)	6.36	0.015*	6.60	0.014*	2.22	0.144	0.01	0.904
T × Ta	0.67	0.647	2.12	0.096	0.26	0.927	1.46	0.237
T × P	1.77	0.156	1.15	0.360	0.47	0.793	0.85	0.522
T × I	0.72	0.610	1.14	0.357	3.60	0.009*	0.28	0.916
Ta × P	0.08	0.772	2.29	0.143	4.14	0.053	28.72	0.000*
Ta × I	2.02	0.161	0.00	0.933	0.03	0.856	0.14	0.707
P × I	1.72	0.195	2.34	0.132	3.03	0.088	1.31	0.257
T × Ta × P	0.22	0.949	0.307	0.903	0.19	0.961	0.38	0.854
T × Ta × I	1.41	0.254	2.03	0.109	0.52	0.753	0.70	0.624
T × P × I	0.81	0.549	0.21	0.958	0.78	0.569	0.13	0.982
Ta × P × I	0.01	0.906	0.07	0.785	2.81	0.10	5.86	0.020*

TABLE 4. MULTIVARIATE ANALYSIS OF VARIANCE BY STORAGE CONDITIONS

* Significant effects at $P < 0.05$.

Note: *F* ratio and *P* values obtained by Fisher's test ($P < 0.05$).

contents in the studied alcohols. On the other hand, storage time and exposition to light have no significant effect on carbonyl compounds. These results agree with studies carried out by other authors in white wines at various storage temperatures (commercialization and 5C) (Marais 1986; Pérez-Coello *et al*. 2003).

Multivariate Statistical Analysis

The existence of statistical significant differences in the levels of volatiles compounds as a function of the conditions of storage surveyed, were evaluated by one-way analysis of variance (ANOVA) and two-way ANOVA The latter method is an extension to the former that evaluates the effect of two independent variables concurrently, that is, two-way ANOVA is a procedure that enables to assess whether the two independent variables interact with respect to their effect on the dependent variable.

The *F* values and significance levels of the factors as well as their interaction on the concentration of volatile compounds are shown in Table 4. The results show that esters have significant differences ($P < 0.05$) among the wines stored under the tested conditions (time, temperature and illumination during storage). On the other hand, acids concentration is not affected by the different storage conditions tested. Illumination exerts a significant influence on alcohol concentration, and during storage, temperature significantly conditions the composition of carbonyl compounds in the wine. When two-way ANOVA was applied, three significant interactions appeared: time/illumination for acids, and temperature/position and temperature/position/illumination for carbonyl compounds.

The aim of SLDA is to study to what extent it is possible to divide two or more sample groups. By applying SLDA it is possible to establish functions that discriminate between previously established groups, in order to apply them later to originally non-classified cases (Castino 1975; Schreier and Reiner 1979; Gorostiza *et al*. 1982; González-San José and Diez 1993). One of the most commonly used methods within SLDA is the iterative procedure, in which variables are added one by one to the discriminant functions, yielding a study of discrimination after each step. The process concludes when no discrimination significantly better than the last is obtained. The SLDA was performed to determine which variables are the most appropriate to discriminate among samples. As a result, four SLDA were performed taking into account the different storage conditions, time, temperature, illumination and bottle position.

Taking storage time into account, the mathematical model selects 11 variables (diethyl succinate, isobutanol, ethyl lactate, 2-phenylethanol, *cis*-3-hexen-1-ol, hexanoic acid, 2-methylpropyl ethanoate, ethyl octanoate, isoamyl alcohol, butan-1-ol and hexan-1-ol) with the greatest discriminatory power. The coefficients of the functions that classify the samples according to storage time are gathered in Table 5. By applying these functions, their validity is checked according to the success rate of the cases in their corresponding group. The classification matrix is shown in Table 6, with a mean classification rate of 89.6%. When the samples are graphically represented within the discriminant space (Fig. 1), it can be observed that the considered variables divide the wine samples by storage time. As observed in the chart, discriminant function 1 separates the samples corresponding to 2 and 4 months of storage from the rest. Discriminant function 1 is mainly related with diethyl succinate, *cis*-3 hexenol and hexan-1-ol with positive sign, and with isobutanol, 2-phenylethanol, ethyl octanoate and isoamyl alcohol with

TABLE 5. COEFFICIENTS OF THE
CLASSIFICATION FUNCTIONS (SLDA)
ACCORDING TO STORAGE TIME

	Storage time (months)					
	2	4	6	8	10	12
Diethyl succinate	−3.047	−3.879	−0.975	−0.301	0.246	1.070
Isobutanol	0.132	1.261	−2.895	−5.601	−5.039	−6.262
Ethyl lactate	−0.734	−0.266	0.243	0.981	0.460	0.371
2-phenyl ethanol	−0.005	−0.118	−0.306	−0.447	−0.449	−0.505
Cis-3-Hexen-1-ol	−12.797	−46.956	22.334	26.091	22.579	29.438
Hexanoic acid	58.679	65.842	53.504	52.831	53.949	54.911
2-methylpropyl ethanoate	−8.261	−26.626	−18.164	−27.759	−22.002	−21.686
Ethyl octanoate	820.605	838.479	563.093	602.710	582.633	408.273
Isoamyl alcohol	0.094	0.068	0.039	0.030	0.019	−0.037
Butan-1-ol	−5.609	1.332	1.338	9.387	7.503	3.991
Hexan-1-ol	2.935	2.652	9.219	13.289	11.101	17.791
Constant	−152.592	−168.946	−139.656	−160.938	−154.134	−156.672

TABLE 6. CLASSIFICATION MATRIX

		Storage time (months)					
	% correct	2	4	6	8	10	12
2	100.0	8	0	0	0	0	0
4	87.5	1	7	0	0	0	0
6	87.5	0	0	7	0	1	0
8	100.0	0	0	0	8	0	0
10	75.0	0	0	0	1	6	1
12	87.5	0	0	0	0	1	7
Total	89.6	9	7	7	9	8	8

negative sign. These volatiles compounds (isobutanol, 2-phenylethanol, ethyl octanoate and isoamyl alcohol) are impact odorants of Zalema wines (Gómez-Míguez *et al.* 2007) and are associated with fruit, floral, burned and fusel attributes, respectively (Campo *et al.* 2006). On the other hand, discriminant function 2 is related with 2-methylpropyl ethanoate with positive sign and ethyl lactate, hexanoic acid and butanol with negative sign.

Taking into account the temperature during the storage period, the mathematical model selects 9 variables having the greatest discriminatory power with a classification rate of 100% in all cases. The canonical function is related with isobutanol, 2-phenylethanol, ethyl octanoate and ethyl acetate with negative sign, and butan-1-ol, furan-2-carbaldehyde, hexanoic acid, acetaldehyde and diethyl succinate with positive sign. In the graphical representation of the samples within the discriminant space (Fig. 2), it can be observed that the variables used divide the samples into two clearly distinguishable groups, one corresponding to the wines stored at variable temperature and another to the wines
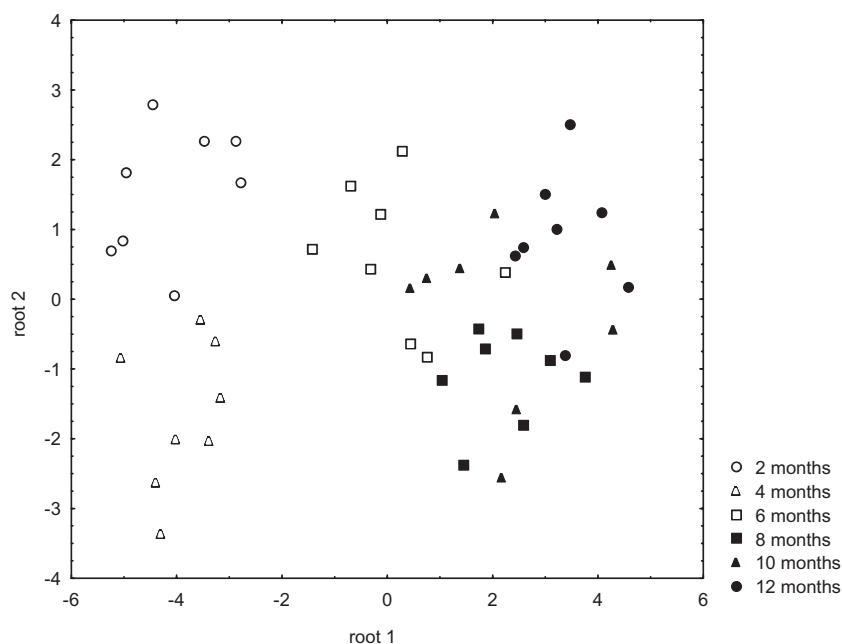
FIG. 1. SCATTERPLOT OF SAMPLES IN THE PLANE DEFINED BY THE CANONICAL FUNCTIONS WHEN THE TIME OF STORAGE IS CONSIDERED FOR DISCRIMINATION
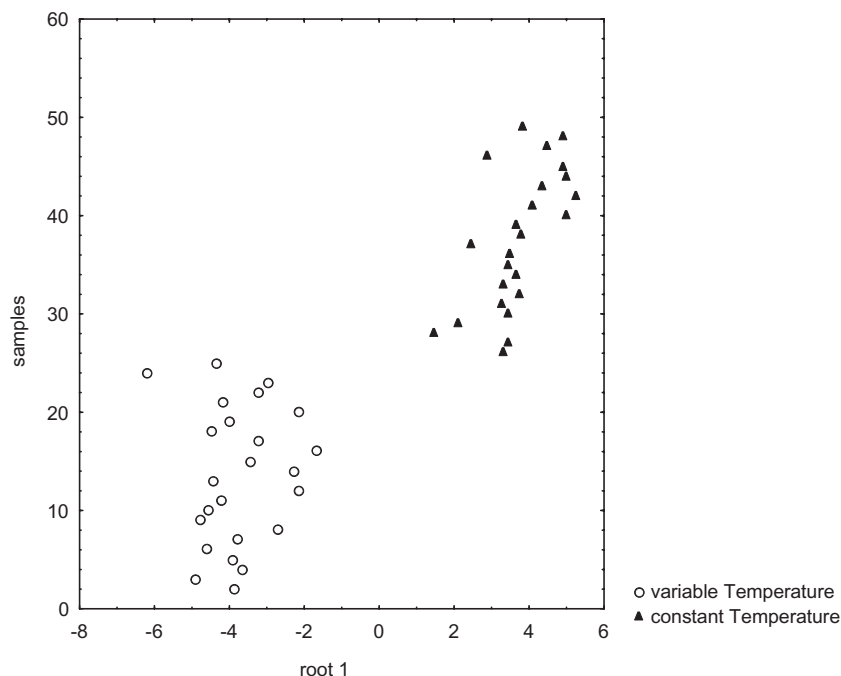
FIG. 2. DISPERSION CHART OF WINES CLASSIFIED BY STORAGE TEMPERATURE USING THE CALCULATED DISCRIMINANT FUNCTION

stored at constant temperature. The changes in the concentration of esters could be correlated with the loss of freshness aromas during the storage time and these sensory and chemical changes are less pronounced in wines stored at 5C (Pérez-Coello *et al.* 2003).

Taking into account the bottle position during the storage period, the variables with the greatest discriminatory power were decanoic acid, four alcohols (butan-1-ol, *cis*-3-hexen-1-ol, isobutanol and 2-phenylethanol) and five esters (isoamyl acetate, ethyl octanoate, 2-methylpropyl ethanoate, ethyl butyrate and ethyl isovalerate). The calculated discriminant function allows distinction between the wines stored in horizontal position and those stored in vertical position, as observed in Fig. 3. Finally, the discriminant analysis applied
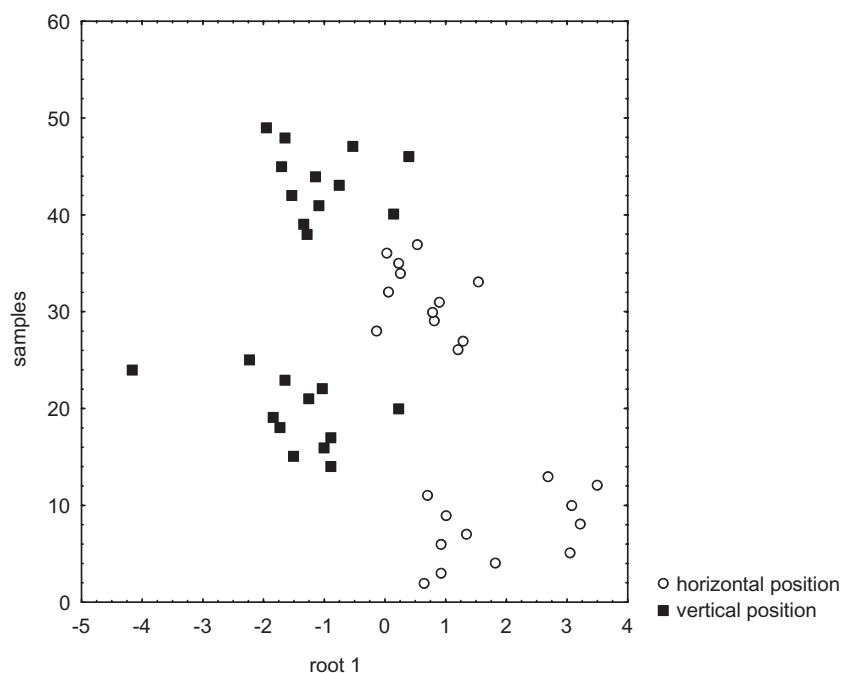

FIG. 3. SCATTERPLOT OF SAMPLES IN THE PLANE DEFINED BY THE CANONICAL FUNCTIONS WHEN THE BOTTLE POSITION IS CONSIDERED FOR DISCRIMINATION

using the illumination during the storage period as criterion for comparison, selected 9 variables (isoamyl alcohol, ethyl lactate, hexanoic acid, furan-2-carbaldehyde, ethyl butyrate, octanoic acid, ethyl acetate, ethyl isovalerate, isovalerate acid and isobutanol) and 75% of the samples were correctly classified. In this case, the successful classification rate was of 79% in the wines stored at a variable temperature and of 70% in those stored in the dark (chart not shown). Alcohols and esters are the variables with the greatest discriminatory power when bottle position and illumination are considered as criterion for comparison. These compounds produced during alcoholic fermentation, play an important role in the flavor of wines, depending on their concentrations (Valero *et al.* 2002). Isoamyl acetate, 2-phenylethanol and *cis*-3-hexen-1-ol are related to fruity and vegetable aromas in Chardonnay wines (Navarro *et al.* 2002), while isoamyl acetate, ethyl butyrate, ethyl isovalerate, ethyl octanoate and isoamyl alcohol can be considered the most powerful odorants of Zalema wines (Gómez-Míguez *et al.* 2007) and are associated with "fruity," "ripe fruity," "sweet" and "fresh" odor descriptors to this monovarietal wine.

CONCLUSIONS

Considering the sum of individual ester compounds (ester total content), an increase during storage is observed, mainly due to ethyl butyrate, ethyl acetate and diethyl succinate. Time, temperature and illumination factors exert significant influence. Total acid content (the sum of individual acids) increases during storage, showing no significant effect with the studied storage conditions. Alcohol and carbonyl compounds contents significantly decrease, affected by the illumination and the temperature, respectively.

Discriminant function analysis allows the wine samples to be classified by storage time into two groups: samples stored until the fourth month (0–4 months), and samples stored from the 6th to the 12th month. Also, high percentages of correct classification of cases were found for bottle position (92%) and illumination (75%) factors.

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