

EFFECT OF TIME AND STORAGE CONDITIONS ON MAJOR VOLATILE COMPOUNDS OF ZALEMA WHITE WINE

ANGELES F. RECAMALES^{1,3}, VALERIA GALLO¹, DOLORES HERNANZ¹,
MARIA LOURDES GONZÁLEZ-MIRET² and FRANCISCO J. HEREDIA²

¹Department of Analytical Chemistry, Faculty of Experimental Science, University of Huelva, Huelva 21007, Spain

²Lab. Food Colour and Quality, Department of Nutrition and Food Science, Faculty of Pharmacy, University of Seville, Seville, Spain

³Corresponding author. TEL: 34-959-219958;
FAX: 34-959-219942; EMAIL:
recamale@uhu.es

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ABSTRACT

Changes in quality attributes during bottled storage of white wine were studied. Different storage conditions, temperature, bottle position and exposure to light were studied over 1 year and their effects on major volatile compounds were evaluated. Ethyl lactate and diethyl succinate contents increased after 12 months of storage, whereas isoamyl acetate decreased over this period. The analysis of variance revealed that the temperature had a statistically significant effect on most volatile compounds studied.

Multivariate statistical techniques (specifically principal component analysis and linear discriminant analysis) confirmed that temperature is the most important factor, so the samples were distributed into two groups according to storage temperature. A rough differentiation among wines according to the length of storage was also obtained using linear discriminant analysis.

PRACTICAL APPLICATIONS

Aroma is one of the main factors contributing to the quality of wines and their acceptance by consumers. However during bottle storage, young white wines lose their fresh and fruity flavor and suffer a browning process. Among other factors, the time it takes a wine to spoil depends on the wine composition and on storage conditions.

Knowledge of the bottle storage stability of Zalema young white wines could allow us to predict their shelf life and establish the best commercial storage conditions to retain their organoleptic characteristics for longer periods.

INTRODUCTION

Wine is subject to continuous changes in composition during storage. These changes are numerous and complex and can affect aroma, phenolic and metal composition and the color (Gómez *et al.* 1995; González-Viñas *et al.* 1996; Recamales *et al.* 2006). The first change is generally toward higher quality; however, the quality of wines made from grape varieties with poor aroma decreases with storage time. In the young white wines, the loss of fresh and fruity characteristics and the browning take place in a period of variable time that oscillates from 1 month to several years, depending on the type of wine and the storage conditions.

Several studies have been performed with different purposes, such as the assessment of chemical changes during the storage of wine in bottles and their influence on the sensory perception (Rapp *et al.* 1985; Ferreira *et al.* 1997; González-Viñas *et al.* 1998; Pérez-Coello *et al.* 1999; Escudero *et al.* 2002; Pérez-Prieto *et al.* 2003), the research of environmental conditions (temperature, illumination, humidity, etc.) that are required to store the wine without loss of volatile compounds (Sivertsen *et al.* 2001; Mas *et al.* 2002; Pérez-Coello *et al.* 2003) or the conditions (closure type, bottle color and type, etc.) under which the wines must be bottled (Selli *et al.* 2002; Skouroumounis *et al.* 2005).

The most important flavor compounds in wine made from neutral varieties are those arising from the fermentation

process, which include mainly ethyl esters, acetate esters, higher alcohol, fatty acids and aldehydes. Ethyl esters of hexanoic, octanoic and decanoic acids, and isoamyl and isobutyl acetates are often considered to give wine much of its characteristic notes (Ferreira *et al.* 1995a). During storage, volatile compounds are modified due to different reactions taking place, especially esters hydrolysis/esterification reaction. The acetates are hydrolyzed more quickly than the ethyl esters of short chain fatty acids, and therefore, this decrease is the most important factor responsible for the loss of freshness and fruitiness of young white wine. This loss is reduced if the wine is stored at a low temperature (Sivertsen *et al.* 2001; Pérez-Coello *et al.* 2003). On the other hand, ethyl esters of diprotic acid concentration increase during wine storage as a result of chemical esterification. But these compounds do not appear to have any effects on wine aroma (Etiévant 1991).

The higher alcohol and fatty acid levels change very little during the storage. The content in 2-phenylethanol, one of the few higher alcohols that can have a smell impact (Carro *et al.* 1995), diminishes lightly. Marais and Pool (1980) found that this decrease was higher when the temperature increased.

The purpose of this study was to evaluate the influence of different storage conditions (temperature, illumination, position of bottles) on the volatile compounds of white wine during 12 months of storage. Statistical techniques, such as analysis of variance, principal component analysis and linear discriminant analysis were used to distinguish among wines with different conditions and lengths of storage.

MATERIALS AND METHODS

Samples

A total of 98 samples of bottled wines were analyzed. These wines had been elaborated with the white grape variety "Zalema," which is exclusively grown in "Condado de Huelva," a restricted wine-producing zone with certified brand of origin wines, situated in the southwest of Spain. The wines are produced according to traditional methods with fermentations to controlled temperatures. Due to the climatic conditions of the zone (sunny region with warm temperatures and low rainfall), an early grape harvest is carried out.

Grapes of *Vitis vinifera* cv. Zalema were manually harvested, and transported to a wine-production center. The grapes were destemmed and crushed, and the must then transferred into a 30,000-L stainless steel tank, where a 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 at 12 ± 3 C for 24 h. Then, residual solid parts were separated and the must was run straight into a fermentation tank, adjusting the total acidity to 6 g/L with tartaric acid. During fermentation processes, the temperature was kept at 18 ± 2 C. No selected yeasts were used. At the end of fermentation, the

TABLE 1. EXPERIMENTAL CONDITIONS FOR WINE STORAGE

Factor	Temperature*	Position	Illumination
Batch 1	Constant	Vertical	Dark
Batch 2	Constant	Vertical	Variable light
Batch 3	Constant	Horizontal	Dark
Batch 4	Constant	Horizontal	Variable light
Batch 5	Variable	Vertical	Dark
Batch 6	Variable	Vertical	Variable light
Batch 7	Variable	Horizontal	Dark
Batch 8	Variable	Horizontal	Variable light

* Constant temperature: 15–20C.

Variable temperature: 10.5–25.3C (minimum temperature of the coldest month was 6C and maximum of the warmest month 32C).

wine was transferred to a 14,000-L stainless steel tank and it was stored for the stabilization process. The wine was kept at 12 ± 3 C during the stabilization process and the free sulfur dioxide content was maintained at 25–30 mg/L.

After cold stabilization, the wine was filtered and bottled in green glass bottles of a capacity of 750 mL and stored in two different conditions: (1) one set of bottles was stored in a partly open warehouse in which the bottles were subjected to temperature variations (both daily and seasonal; the average temperature ranged between 10.5C and 25.3C throughout the year), this process is referred to as "variable;" (2) the other set of bottles was stored in a cellar where the temperature varied between 15C and 20C throughout the year, this process is referred to as "constant."

In each set, half of the bottles were placed in a horizontal position and the other half in a vertical position. Within each subset, half of the bottles were left in the dark and the other half in conditions of variable light. A total of eight lots (12 bottles each) were stored for 12 months. Table 1 shows the experimental conditions under which each set was stored.

Samples (two bottles of each set) were taken at the time of bottling, and then the same process was repeated every 2 months.

Volatile Compounds Analysis

The volatile compounds were extracted by liquid–liquid extraction following the method described by Ortega *et al.* (2001) slightly modified in our laboratory. According to this method, 13.5 g of ammonium sulphate was weighted in a centrifuge tube and 9 mL of wine sample, 21 mL of Milli-Q water (Millipore, Watford, UK) and 100 μ L of internal standard solution were added. The tube was shaken to mix for 30 min; 600 μ L of dichloromethane was added and then was shaken continuously for another 30 min. Finally, it was centrifuged at 3,000 rpm for 10 min. The organic phase was recovered and transferred to a vial, which was stored to -20 C until analysis.

Volatiles were analyzed using a GC HP-6890N (Agilent Technologies, Richmond, VA) equipped with a flame ionization detector and a fused silica capillary column of poly ethylenglycol (Tracer TRWax, Cole Parmer Instrument Company, Vernon Hills, IL) of 60 m, 0.25 mm internal diameter, 0.25 μm film thickness. Oven temperature was initially held at 40C for 5 min and then a temperature ramp of 1.5C/min was programmed up to 200C and maintained for 20 min. The carrier gas is helium at a flow of 2 mL/min.

Identification of the components was performed using chemical standards and mass spectra data. A Varian 3800 CG (Varian, Sunnyvale, CA) equipped with a Saturn 2200 mass detector and fused capillary column coated with wall-coated open tube from Varian (30 m \times 0.25 mm diameter, 0.5 μm film thickness) was used. The carrier was helium at 1 mL/min. The chromatographic oven was initially 45C for 3.5 min, and then was raised to 180C at 10C/min. A second temperature ramp of 4C/min was programmed up to 300C and maintained for 5 min. The detector was set to electronic impact mode (70 eV), with an acquisition range from 40 m/z to 220 m/z. The identification of volatile compounds was achieved by the comparison of the GC retention times and mass spectra with those of the pure standard compounds.

The quantification was carried out by the internal standard method. Thus, 2-octanol and 4-methyl-2-pentanol were chosen as internal standards. Quantitative data were obtained by interpolation of the peak relative areas in the calibration

graph built from analysis of pure reference compounds. The calibration linearity was good ($r = 0.999$, $n = 6$). The reproducibility of the results for the extracts is expressed as relative standard deviation (RSD). The data obtained were very good with RSD lower than 6%.

Statistical Analysis

Analysis of variance (ANOVA) was made, using the general linear model program to determine whether mean values for volatile compounds differed with conditions and storage time. The means were compared by the least significant difference (LSD) test at a significance level of 0.05. Pattern recognition (PR) techniques including principal component analysis (PCA) and linear discriminant analysis (LDA) were applied on experimental standardized data to distinguish among wines with different conditions and lengths of storage. Statistical analyses were performed using the statistical package STATISTICA (StatSoft, Inc. 2001).

RESULTS AND DISCUSSION

The samples of wines were analyzed to determine the concentration of the volatile compounds using the methodology and the chromatographic conditions proposed, which allow the separation of 33 compounds studied. Tables 2–6 show the

TABLE 2. INFLUENCE OF CONDITIONS AND STORAGE TIME ON ETHYL ESTERS (MG/L)^a

Source of variation	Ethyl lactate	Ethyl hexanoate	Ethyl octanoate	Ethyl butyrate	Ethyl isovalerate	Diethyl succinate
Storage period (months)						
0	21.41 ^c	ND	0.04	0.016 ^c	0.0105	6.70 ^{cd}
2	21.31 ^c	0.03	0.04	0.022 ^c	0.0092	4.78 ^c
4	25.28 ^d	0.03	0.05	0.030 ^d	0.0102	5.69 ^c
6	26.13 ^d	0.03	0.04	0.025 ^{cd}	0.0085	10.16 ^d
8	31.55 ^e	0.02	0.05	0.026 ^d	0.0097	13.66 ^e
10	26.82 ^d	0.03	0.05	0.031 ^d	0.0084	14.52 ^e
12	25.19 ^d	0.02	0.05	0.025 ^{cd}	0.0083	15.68 ^e
Significance ^b	***	Ns	Ns	*	Ns	***
Temperature						
Variable	26.52	0.02	0.05	0.03	0.01	13.02
Constant	25.58	0.04	0.04	0.02	0.01	8.47
Significance ^b	Ns	***	***	***	Ns	***
Exposition						
Light	27.50	0.03	0.04	0.03	0.01	11.15
Dark	24.59	0.03	0.05	0.03	0.01	10.34
Significance ^b	**	Ns	Ns	Ns	Ns	Ns
Position						
Horizontal	26.15	0.03	0.04	0.03	0.01	10.85
Vertical	25.95	0.03	0.05	0.03	0.01	10.64
Significance ^b	Ns	Ns	*	Ns	Ns	Ns

^a Data followed by different letters for each column and source of variation are significantly different by LSD test.

^b Ns, *, **, ***, not significant and significant at $P < 0.05$, 0.01 and 0.001, respectively.

ND, not detected.

TABLE 3. INFLUENCE OF CONDITIONS AND STORAGE TIME ON ACETATES (MG/L)^a

Source of variation	Phenylethyl acetate	Isoamyl acetate	Isobutyl acetate	Ethyl acetate
Storage period (months)				
0	0.11	0.40 ^{cd}	0.46	15.51
2	0.09	0.84 ^e	0.27	17.35
4	0.08	0.70 ^e	0.38	18.67
6	0.09	0.52 ^d	0.31	25.04
8	0.08	0.29 ^c	0.38	23.17
10	0.08	0.35 ^c	0.37	23.90
12	0.08	0.25 ^c	0.32	22.96
Significance ^b	Ns	***	Ns	Ns
Temperature				
Variable	0.08	0.31	0.50	23.21
Constant	0.08	0.68	0.18	20.49
Significance ^b	Ns	***	***	Ns
Exposition				
Light	0.08	0.50	0.35	23.66
Dark	0.09	0.48	0.33	20.03
Significance ^b	Ns	Ns	Ns	Ns
Position				
Horizontal	0.10	0.48	0.41	19.92
Vertical	0.07	0.51	0.27	23.77
Significance ^b	*	Ns	***	Ns

^a Data followed by different letters for each column and source of variation are significantly different by LSD test.

^b Ns, *, **, ***, not significant and significant at $P < 0.05$, 0.01 and 0.001, respectively.

TABLE 4. INFLUENCE OF CONDITIONS AND STORAGE TIME ON ACIDS (MG/L)^a

Source of variation	Isobutyric acid	Isovaleric acid	Butyric acid	Hexanoic acid	Octanoic acid	Decanoic acid
Storage period (months)						
0	ND	0.44	16.96	5.58	2.17	1.54
2	ND	0.15	10.92	5.08	3.09	1.51
4	0.24	0.22	11.89	5.30	3.50	1.58
6	0.11	0.19	11.43	5.00	2.71	1.58
8	0.28	0.25	11.50	5.16	3.02	1.73
10	0.24	0.27	12.63	5.10	3.37	1.47
12	0.20	0.16	12.90	5.14	2.83	1.34
Significance ^b	Ns	Ns	Ns	Ns	Ns	Ns
Temperature						
Variable	0.31	0.29	12.99	5.11	2.55	1.68
Constant	0.05	0.13	10.76	5.15	3.63	1.39
Significance ^b	*	**	Ns	Ns	***	***
Exposition						
Light	0.22	0.22	12.50	5.24	3.26	1.62
Dark	0.14	0.20	11.25	5.02	2.92	1.44
Significance ^b	Ns	Ns	Ns	*	Ns	*
Position						
Horizontal	0.31	0.26	12.14	5.18	3.00	1.67
Vertical	0.05	0.15	11.61	5.08	3.17	1.40
Significance ^b	*	*	Ns	Ns	Ns	**

^a Data followed by different letters for each column and source of variation are significantly different by LSD test.

^b Ns, *, **, ***, not significant and significant at $P < 0.05$, 0.01 and 0.001, respectively.

ND, not detected.

TABLE 5. INFLUENCE OF CONDITIONS AND STORAGE TIME ON ALCOHOLS (MG/L)^a

Source of variation	1-Hexanol	2-Phenylethyl alcohol	Isobutanol	1-Butanol	Isoamyl alcohol	<i>cis</i> -3-Hexen-1-ol
Storage period (months)						
0	1.83	123.07	1.56 ^d	0.71	123.81	0.38
2	1.10	75.67	0.52 ^c	0.70	125.59	0.31
4	1.01	67.02	1.51 ^d	0.93	100.29	0.25
6	1.07	69.95	1.02 ^{cd}	0.70	120.97	0.32
8	1.22	75.46	1.22 ^d	0.78	129.14	0.32
10	1.02	62.52	1.38 ^d	0.80	121.94	0.29
12	1.37	71.71	1.20 ^d	0.68	103.34	0.30
Significance ^b	Ns	Ns	*	Ns	Ns	Ns
Temperature						
Variable	1.32	83.94	2.15	0.79	109.31	0.33
Constant	0.94	56.83	0.14	0.74	124.44	0.27
Significance ^b	***	***	***	Ns	Ns	***
Exposition						
Light	1.14	72.52	1.09	0.76	128.31	0.29
Dark	1.12	68.26	1.20	0.77	105.45	0.31
Significance ^b	Ns	Ns	Ns	Ns	*	Ns
Position						
Horizontal	1.20	78.70	1.42	0.87	118.09	0.32
Vertical	1.06	62.08	0.86	0.66	115.67	0.28
Significance ^b	Ns	***	**	**	Ns	**

^a Data followed by different letters for each column and source of variation are significantly different by LSD test.

^b Ns, *, **, ***, not significant and significant at $P < 0.05$, 0.01 and 0.001, respectively.

mean concentration of compounds analyzed in the 98 wine samples grouped according to their time and conditions of storage. Benzyl alcohol, methionol, γ -butyrolactone, ethyl 3-hydroxybutyrate, ethyl isobutyrate and diacetyl were not included in these tables because, in all the samples analyzed, the levels were below the detection limit of the analytical method used.

The multifactor ANOVA was applied for testing the significance of the effects of storage time and conditions on different families of volatile compounds: higher alcohols, fatty acids, carbonyl compounds and ethyl esters and acetates. In multivariate analysis of variance, a new dependent variable that maximizes group differences is created from the set of variables dependent, and so it is used to ask whether a combination of the volatile compounds measures varies as a function of factors. The results showed that the time of storage, temperature and position of the bottle had a significant influence (P values of 0.0015, 0.0000 and 0.0046, respectively) on the parameters studied. The differences between dark and light storage were nonsignificant ($P = 0.397$). Moreover, the statistical significance of each factor on each volatile compound (univariate results) was included in Tables 2–6.

Even though individual volatile compounds were affected differently by the length and conditions of storage, it can be seen that the total contents of ethyl esters and acetates significantly increase during storage, whereas total concentrations

TABLE 6. INFLUENCE OF CONDITIONS AND STORAGE TIME ON CARBONYL COMPOUNDS (MG/L)^a

Source of variation	Acetaldehyde	Acetoin	Furfural
Storage period (months)			
0	95.08	4.72	2.87
2	91.05	4.84	1.47
4	90.24	4.32	1.79
6	83.99	5.27	1.86
8	92.34	5.90	2.29
10	83.29	7.12	2.00
12	78.81	5.86	1.89
Significance ^b	Ns	Ns	Ns
Temperature			
Variable	79.28	5.53	2.24
Constant	93.96	5.57	1.52
Significance ^b	**	Ns	**
Exposition			
Light	86.78	5.78	1.79
Dark	86.46	5.32	1.98
Significance ^b	Ns	Ns	Ns
Position			
Horizontal	85.71	5.76	2.12
Vertical	87.53	5.35	1.65
Significance ^b	Ns	Ns	*

*, **, *** Significant at $P < 0.05$, 0.01 and 0.001, respectively.

^a Data followed by different letters for each column and source of variation are significantly different by LSD test.

^b Ns, not significant.

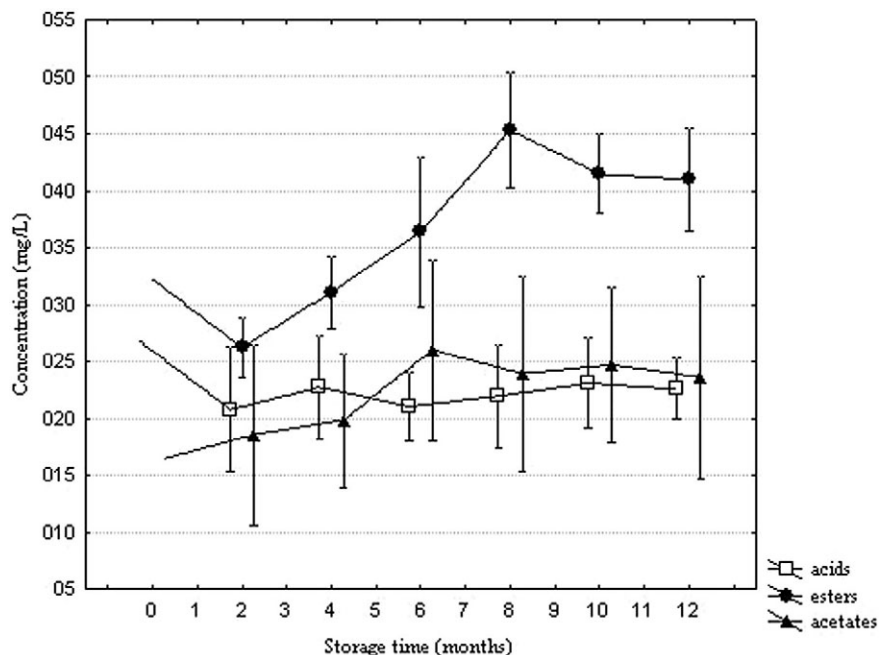


FIG. 1. EVOLUTION OF ACIDS, ESTERS AND ACETATES IN WINES DURING STORAGE

Vertical bars denote 0.95 confidence interval.

of alcohols and carbonyl compounds decreased during the same period (Figs. 1 and 2). Although the total ethyl ester content progressively increases in wines during storage time (Fig. 1), the study of individual behavior of these compounds indicates that only ethyl butyrate, ethyl lactate and diethyl succinate significantly increase their concentration (Table 2). The mean concentration of these compounds in wines stored for 12 months was 1.5, 1.2 and 2.3 times higher, respectively,

than in control wines. Figure 3 shows how the increment in diethyl succinate level was higher when the wine has been stored subjected to daily and seasonal temperature variations. These results are in accordance with the report by other authors (Rapp 1988). Analogous changes were found for ethyl octanoate and ethyl butyrate while the concentration of ethyl hexanoate was higher when the wines were stored at a constant temperature (0.04 mg/L) than at a variable temperature

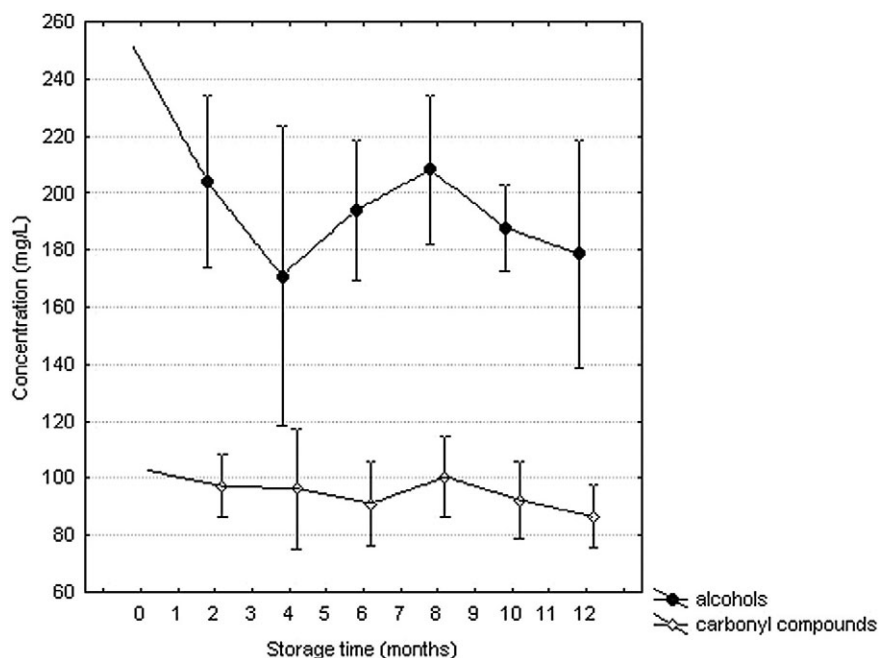


FIG. 2. EVOLUTION OF ALCOHOLS AND CARBONYL COMPOUNDS IN WINES DURING STORAGE

Vertical bars denote 0.95 confidence interval.

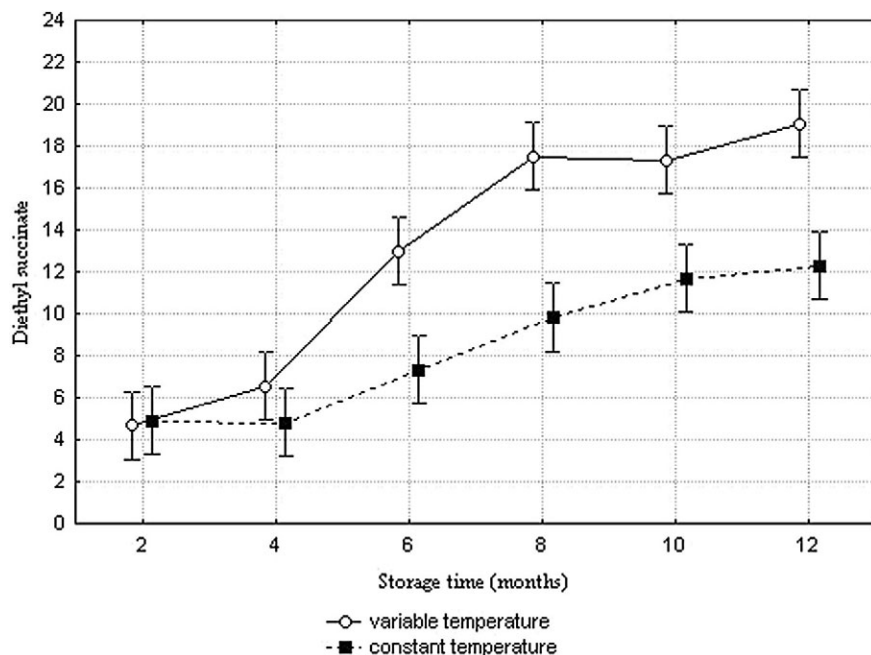


FIG. 3. EVOLUTION OF DIETHYL SUCCINATE IN WINES DURING STORAGE AT TWO CONDITIONS OF TEMPERATURE

(0.02 mg/L). The concentration of ethyl lactate was affected by the amount of light. So, the samples stored in the dark showed lower concentration (1.2 times lower) for this compound than the samples exposed to the light.

As can be seen in Table 3, after 12 months of storage, the samples show lower concentration of phenylethyl acetate, isoamyl acetate and isobutyl acetate relative to initial values, although these losses are not significant. Analogous changes

have been reported in white wines from variety Airén. After one-half to 3 years of storage in a bottle, a decrease in the content of isopentyl acetate and 2-phenylethyl acetate (González-Viñas *et al.* 1996) and isoamyl acetate (Pérez-Coello *et al.* 1999, 2003) was observed in Airén wines. Figure 4 shows the evolution of the concentration of isoamyl acetate in Zalema wines during the storage period. It can be observed how the levels of isoamyl acetate, which has been

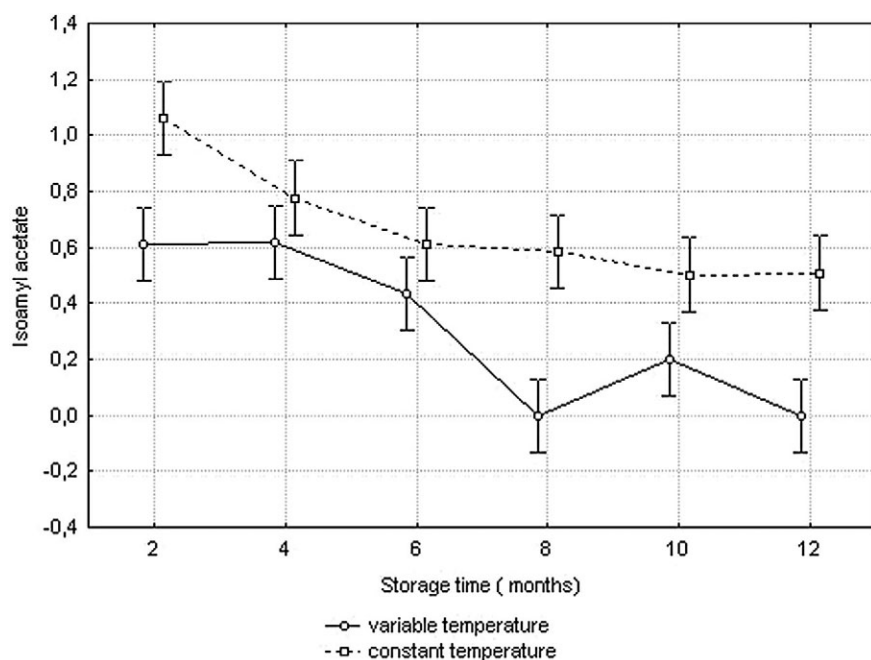


FIG. 4. EVOLUTION OF ISOAMYL ACETATE IN WINES DURING STORAGE AT TWO CONDITIONS OF TEMPERATURE

described as one of the most important compounds for wine aroma (van der Merwe and van Wyck 1981), significantly decrease during storage. However, it can be noted that their loss was significantly higher in the wine subjected to daily and seasonal temperature variations (100%) than in the wine stored at a constant temperature (52%) after 12 months.

With the exception of ethyl acetate, whose concentration increases lightly with time, the other acetate esters decreased during wine storage. Phenylethyl acetate and isobutyl acetate showed a lower pronounced concentration reduction, and in this case, their losses were significantly higher (1.5 times) when the bottles were placed in a vertical position. Isobutyl acetate concentration was significantly affected by the temperature.

On the other hand, the content of alcohols, acids and carbonyl compounds also changes during the period of storage, although these changes were not significant. These results are in agreement with those reports by other authors for wines from Spanish grape varieties (Pérez-Coello *et al.* 2003) and non-Spanish varieties (Marais 1986).

The changes in the ester content (decrease in acetates and increase in some ethyl esters) are due to hydrolysis/esterification reactions. Esters may be hydrolyzed, be formed through chemical esterification or remain at constant concentrations depending on their initial postfermentation level (Ramey and Ough 1980). Ethyl esters and acetates are formed enzymatically during fermentation, but while the ethyl esters are produced in concentrations below or closer to those of equilibrium, the acetates are produced in excess of their equilibrium concentrations. Therefore, the ethyl esters may be esterified and acetates hydrolyzed until they approach the equilibrium concentrations.

The ANOVA for data of acids (Table 4) indicated that temperature and position showed a significant influence on the concentration of isovaleric, decanoic and isobutyric acids. Their concentrations were higher when the bottles were stored to variable temperature and in a horizontal position. On the other hand, the temperature had an opposite effect on octanoic acid. The amount of light was a significant factor for decanoic and hexanoic acids. The content of butyric acid was not significantly affected by any main variable.

With regard to higher alcohols (Table 5), the content of 2-phenylethanol, *c*-3-hexen-1-ol and isobutanol was significantly affected by the temperature and position. 1-Hexanol, isoamyl alcohol and 1-butanol were affected by temperature, amount of light and position, respectively.

Another group of volatile compounds studied was carbonyl compounds whose concentrations decrease slightly with the time of storage (Table 6) and were affected mainly by temperature.

Besides the chemical changes, sensory changes are also observed according to other authors who report the loss of

wine fruity attributes (González-Viñas *et al.* 1998). The recently bottled wines were judged by assessors as wines with noticeable fresh, floral and unripe fruit odor. These attributes are modified during the storage, so after 12 months, the wines present a decrease in the floral and fresh aromas and an increase in intensity of the spicy and overripe fruit flavor. Esters contribute favorably to wine aroma as fruity notes. In particular, acetate esters are correlated with the freshness and fruitiness of young wines (Rapp 1990); therefore, the reduction in the concentrations of acetates observed during the storage period could be the main cause of the loss of fresh aroma (Ferreira *et al.* 1995b). The spicy and overripe odors could be correlated with an increase in the concentration of ethyl lactate that the studied wines present.

Multivariate Analysis

Another aim of this paper was to check the ability of the variables studied in order to differentiate among wines with different conditions and lengths of storage. For this reason, several PR techniques including PCA and LDA have been applied on experimental standardized data.

Principal component analysis was carried out to explore the relationships between samples and conditions and time of storage. The results showed that seven components have eigenvalues higher than 1 (Kaiser criterion) and accounted for 78.17% of the total variation. Among these, only the first two principal components, which retained 46.45% of the variance, were considered for graphical representation.

The scatter plot constructed with respect to time of storage display overlap of all the sample cases. However, as a function of the different temperature of storage, the sample classes were reasonably well separated. In fact, only a few samples were deviated from the overall distribution (Figure not shown).

These results were confirmed by LDA. Forward stepwise method, which includes variables step-by-step, beginning with the more significant until no significant changes are obtained, was chosen in this work. Variables were selected according to Wilk's lambda criterion. Thus, only a significant Wilk's lambda value was obtained when the wine samples were classified as a function of storage temperature. In this case, one discriminant function was estimated, which explains 100% of the variance. The standardized coefficients of this discriminant function show that 2-butanol, 2-phenylethyl alcohol and furfural have the higher weight in discriminating among two classes. The distribution of data expressed as discriminant scores along the function is presented in Fig. 5. In this representation, a good differentiation among classes can be visualized.

When the wine samples are classified as a function of storage time, five discriminant functions are obtained. The

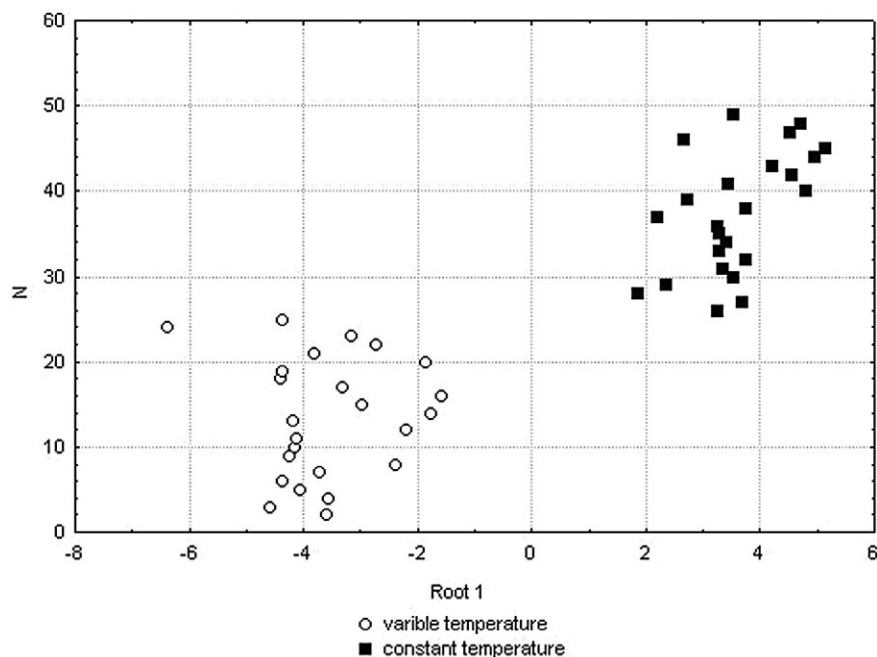


FIG. 5. SCATTERPLOT OF CANONICAL VARIATE WHEN THE SAMPLES WERE CLASSIFIED ACCORDING TO TEMPERATURE OF STORAGE

first two of them explain 99% of the variance and according to the standardized coefficients, the diethyl succinate in the first function and ethyl lactate in the second were the most important variables since they are the higher weight in discriminating among samples. The distribution of data expressed as

discriminant scores along the first two functions is presented in Fig. 6. This representation shows that the six classes are not clearly separated and the proportion of wine samples correctly assigned to their own group is 66.7%. However, a certain trend is observed in this figure. The samples stored in

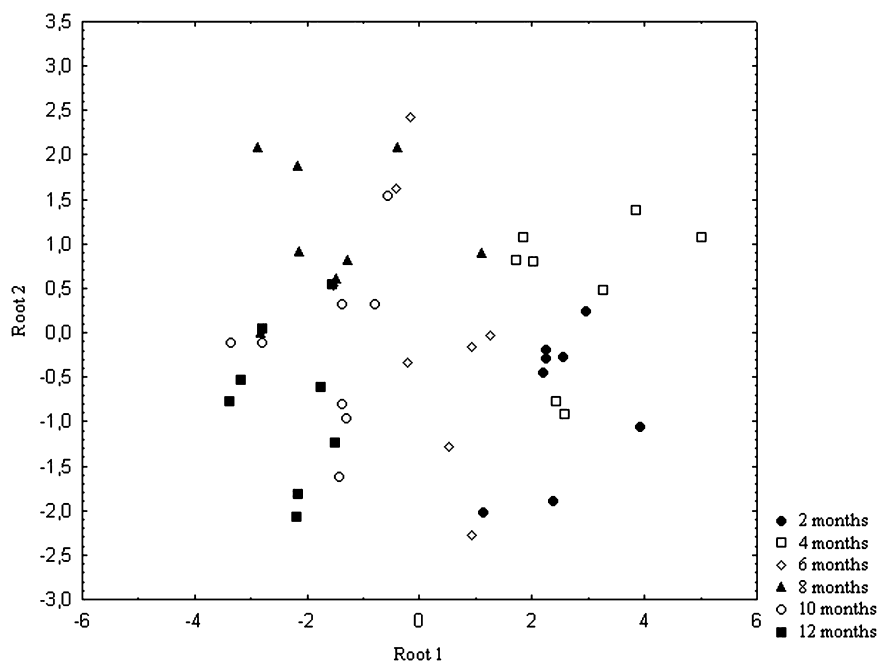


FIG. 6. SCATTERPLOT OF CANONICAL VARIATES WHEN THE SAMPLES WERE CLASSIFIED ACCORDING TO LENGTH OF STORAGE

bottled for more than 6 months were situated on the left of the graphic with negative values of the first discriminant functions, clearly separated from the rest of the samples, stored for 6 months or less, which were situated on the right side.

In conclusion, after 12 months of storage in a bottle, an increase in ethyl esters (ethyl butyrate, ethyl lactate and diethyl succinate) and a decrease in acetates, mainly isoamyl acetate, was observed. However, these changes depend not only on time but also on storage conditions, mainly temperature and position of the bottle. Thus, due to the interaction between these factors, the most suitable conditions to store this wine without loss of volatile compounds are a constant temperature and the bottle in a horizontal position. The PCA and LDA confirmed that the temperature is the most important factor; so the wine samples were separated in two groups according to their storage temperature. When the wine samples were classified as a function of storage time, two groups were also differentiated. Diethyl succinate was the variable with the most discriminant power among the wines stored for more than 6 months and those stored for 6 months or less.

REFERENCES

- CARRO, M., LÓPEZ, T. and GARCÍA, J. 1995. Contribución al estudio del potencial aromático de tres variedades de *Vitis vinifera* Muscat: Identificación de nuevos compuestos. *Food Sci. Technol. Int.* *1*, 105–116.
- ESCUADERO, A., ASENSIO, E., CACHO, J. and FERREIRA, V. 2002. Sensory and chemical changes of young white wine stored under oxygen. An assessment of the role played by aldehydes and some other important odorants. *Food Chem.* *77*, 325–331.
- ETIÉVANT, P. 1991. Volatile compounds in food and beverages. In *Wine* (H. Maars, ed.) pp. 483–546, Marcel Dekker, New York, NY.
- FERREIRA, V., FERNÁNDEZ, P., PEÑA, C., ESCUDERO, A. and CACHO, J. 1995a. Investigation on the role played by fermentation esters in the aroma of young Spanish wines by multivariate analysis. *J. Sci. Food Agric.* *67*, 381–392.
- FERREIRA, V., FERNÁNDEZ, P., GRACIA, J. and CACHO, J. 1995b. Identification of volatile constituents in wines from *Vitis vinifera* var Vidadillo and sensory contribution of the different wine flavour fractions. *J. Sci. Food Agric.* *69*, 299–310.
- FERREIRA, V., ESCUDERO, A., FERNÁNDEZ, P. and CACHO, J.F. 1997. Changes in the profile of volatile compounds in wines stored under oxygen and their relation with the browning process. *Z. Lebensm.-Unters. Forsch. A* *205*, 392–396.
- GÓMEZ, E., MARTINEZ, A. and LAENCINA, J. 1995. Prevention of oxidative browning during wine storage. *Food Res. Int.* *28*, 213–217.
- GONZÁLEZ-VIÑAS, M.A., PÉREZ-COELLO, M.S., SALVADOR, M.D., CABEZUDO, M.D. and MARTÍN-ALVAREZ, P.J. 1996. Changes in gas-chromatographic volatiles of young Airen wines during bottle storage. *Food Chem.* *56*, 399–403.
- GONZÁLEZ-VIÑAS, M.A., PÉREZ-COELLO, M.S., CABEZUDO, M.D. and MARTÍN-ALVAREZ, P.J. 1998. Sensory analysis of aroma attributes of young Airen white wines during storage in the bottle. *J. Food Qual.* *21*, 285–297.
- MARAIS, J. 1986. *The Shelf Life of Foods and Beverages*, Elsevier, Amsterdam, The Netherlands.
- MARAIS, J. and POOL, H.J. 1980. Effect of storage time and temperature on the volatile composition and quality of dry white table wines. *Vitis* *19*, 151–154.
- MAS, A., PUIG, J., LLADÓ, N. and ZAMORA, F. 2002. Sealing and storage position effects on wine evolution. *J. Food Sci.* *67*, 1374–1377.
- ORTEGA, C., LÓPEZ, R., CACHO, J. and FERREIRA, V. 2001. Fast analysis of important wine volatile compounds – development and validation of a new method based on gas chromatographic-flame ionisation detection analysis of dichloromethane microextracts. *J. Chromatogr. A* *923*(1–2), 205–214.
- PÉREZ-COELLO, M.S., MARTÍN-ALVAREZ, P.J. and CABEZUDO, M.D. 1999. Prediction of the storage time in bottles of Spanish white wines using multivariate statistical analysis. *Z. Lebensm.-Unters. Forsch. A* *208*, 408–412.
- PÉREZ-COELLO, M.S., GONZÁLEZ-VIÑAS, M.A., GARCÍA-ROMERO, E., DÍAZ-MAROTO, M.C. and CABEZUDO, M.D. 2003. Influence of storage temperature on the volatile compounds of young white wine. *Food Control* *14*, 301–306.
- PÉREZ-PRIETO, L.J., LÓPEZ-ROCA, J.M. and GÓMEZ-PLAZA, E. 2003. Differences in major volatile compounds of red wine according to storage length and storage conditions. *J. Food Compost. Anal.* *16*, 697–705.
- RAMEY, D.D. and OUGH, C.S. 1980. Volatile ester hydrolysis or formation during storage of model solutions and wines. *J. Agric. Food Chem.* *28*, 928–934.
- RAPP, A. 1988. *Studies of Terpenes Compounds in Wines*, (G. Charalambous ed.) pp. 799–813, Elsevier, Amsterdam, The Netherlands.
- RAPP, A. 1990. Natural flavours of wine: Correlation between instrumental analysis and sensory perception. *Fresenius J. Anal. Chem.* *337*, 777–785.
- RAPP, A., GÜNTERT, M. and ULLEMEYER, H. 1985. Changes in the content of single aroma substance during bottle maturation of wine. *Z. Lebensm.-Unters. Forsch. A* *180*, 109–116.
- RECAMALES, A.F., SAYAGO, A., GONZÁLEZ-MIRET, M.L. and HERNANZ, D. 2006. The effect of time and storage conditions on the phenolic composition and colour of white wine. *Food Res. Int.* *39*, 220–229.
- SELLI, S., CANBAŞ, A. and UNAL, U. 2002. Effect of bottle colour and storage conditions on browning of orange wine. *Nahrung* *46*(2), 64–67.

- SIVERTSEN, H.K., FIGENSCHOU, E., NICOLAYSEN, F. and RISVIK, E. 2001. Sensory and chemical changes in Chilean Cabernet Sauvignon wines during storage in bottles at different temperatures. *J. Sci. Food Agric.* *81*, 1561–1572.
- SKOUROUMOUNIS, G.K., KWIATKOWSKI, M.J., FRANCIS, I.L., OAKLEY, H., CAPONE, D.L., DUNCAN, B., SEFTON, M.A. and WATERS, E.J. 2005. The impact of closure type and storage conditions on the composition, colour and flavour properties of a Riesling and a wooded Chardonnay wine during five years' storage. *Aust. J. Grape Wine Res.* *11*, 369–377.
- STATSOFT, INC. 2001. Statistica for Windows v. 6.0. StatSoft, Inc. 2325 Street, Tulsa, OK.
- VAN DER MERWE, C.A. and VAN WYCK, C.K. 1981. The contribution of some fermentation products to the odor of dry white wines. *Am. J. Enol. Vitic.* *32*, 41–46.