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Evolution of the Aroma Profile of Sherry Wine Vinegars during an Experimental Aging in Wood

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Changes in the aroma profile of five Sherry wine vinegars submitted to an experimental static aging in wood were followed along 24 months. Eighteen volatile compounds were determined by GC-FID. The results were subjected to multivariate analyses: principal component analysis and linear discriminant analysis. The aroma profile of vinegar can be useful to discriminate vinegars produced from different substrates or with different aging times. During the experimental aging, volatile compounds such as methyl acetate, methanol, diacetyl, and γ-butyrolactone underwent significant concentration increases. Moreover, the initial ethanol content of vinegars is a factor in the final aromatic richness. The formation of ethyl acetate stood out in samples with an initial ethanol content of $\sim$2 alcoholic degrees.

KEYWORDS: Sherry wine vinegar; aging; volatile compounds; multivariate analysis; gas chromatography; aroma

INTRODUCTION

The flavor of wine vinegars is determined by the constituents formed during the two fermentation processes involved: alcoholic and acetic; maturation and aging play also important roles. To increase the aromatic quality of wine vinegar and to present new products to the consumers, manufacturers have to choose the best raw materials as well as the optimum acetification process (*1*). Although most of the volatile constituents are already present in the wine, the final content in wine vinegars is directly related to the genuine characteristics of the vinegar itself (*2*). They have a decisive effect on the vinegars' organoleptic quality and, ultimately, on their quality.

One of the most appreciated vinegars on the market is Sherry wine vinegar, thanks to the traditional method of production followed, the so-called "criaderas y solera" system. It is a slow acetification process that involves the growth of the acetic acid bacteria on the surface of the wine to be acetified involving the use of a system of wood casks. It is a dynamic method of production in which a fraction of a less aged vinegar is blended with a more aged vinegar. Thus, the final product has a very homogeneous quality. In lesser proportion, Sherry vinegars are also produced by static methods in which vinegar is matured in a single butt during quite a long period of time (*3*).

The volatile fraction of wine vinegars is mainly constituted by alcohols and esters. Major volatile compounds have been determined by gas chromatography with or without previous neutralization (*1*, *2*, *4*, *5*).

In general, few qualitative differences in aroma compounds seem to occur between Sherry vinegars and conventional wine vinegars; differences are mainly quantitative (*4*). In previous works, high concentrations in some aroma components (i.e., ethyl acetate) for Sherry wine vinegars have been pointed out (*2*, *4*). Volatile compounds were followed during the acetification of different wine substrates from La Mancha (Spain). Two different acetification methods employed different aeration rates (*6*). At the end of the acetification processes, no significant differences for volatile compounds were found between the vinegars. Very little is known about the formation of volatile compounds during the aging of vinegar but, indeed, there are important differences, from the sensorial point of view, between conventional vinegars and Sherry vinegars (*6*).

The purpose of this work is to follow the evolution of the major volatile compounds during the aging of Sherry wine vinegars submitted to an experimental static aging in small wood casks of 16 L capacity in order to ascertain the main changes in this process (loss or formation of volatile compounds).

MATERIALS AND METHODS

Samples and Aging Conditions. Five different wine vinegars were obtained by submerged culture in our laboratory fermentor from five different Sherry wines (A, B, C, D, and E) under the conditions previously established by the authors (*7*). The resulting vinegars (**Table 1**) were submitted to static aging in wood casks of 16 L capacity previously conditioned with Sherry wine. The casks were filled to three-fourths of total capacity. Successive samples were taken within the

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Table 1. Wine Vinegars Obtained in the Laboratory Fermentor and Submitted to Aging (Sample Coding and Initial Characteristics)

Sherry wine substrate		resulting vinegars		
code	alcoholic degree (% v/v)	code	acetic degree (% w/v)	alcoholic degree (% v/v)
A	9.4	SVA	8.3	0.9
B	12.2	SVB	7.5	0.1
C	9.5	SVC	7.4	0.1
D	10.4	SVD	8	2.1[a]
E	14.5	SVE	8.3	2.2

[a] Alcoholic degree resulting from ethanol addition.

interval of 90 days during the first year and every 6 months for the next year. Sampling was performed using a glass pipet. The extracted volumes (a total of 150 mL) were kept in amber bottles in a refrigerator. All of the vinegars had an initial acetic degree (grams of acetic acid/100 mL of vinegar) >7 (**Table 1**). Wine alcohol was added to sample SVD to check if this addition improved the formation of ethylic esters. Thus, samples SVD and SVE accounted for 2 alcoholic degrees (v/v) at the beginning of the study; the remaining samples (SVA, SVB, and SVC) accounted for an alcohol content between 0.09 and 0.9 degrees (**Table 1**).

Aging sampling points were numbered as follows: 0 for the samples at the beginning once casks were filled (i.e., SVA0), 1 for the first sampling point (90 days of aging) and so on until the final samplings numbered 6, accounting for 24 months of aging.

Gas Chromatographic Analysis. Volatile compounds were determined by GC. Gas chromatography was performed on a Hewlett-Packard 6890 with an FID detector. A capillary column, CP-Wax 57 CB, 50 m × 0.25 mm i.d. × 0.2 μm film thickness (Chrompack, Middelburg, The Netherlands), was used. Chromatographic conditions were as follows: initial temperature, 35 °C, during 5 min; program rate, 4 °C/min; final temperature, 150 °C; injector temperature, 220 °C; detector temperature, 250 °C; carrier gas, H_2 at 1 mL/min (*8*, *9*). Samples underwent direct injection into the split mode (1:60) of 1 μL; 102.14 mg/L of 4-methyl-2-pentanol was added as an internal standard. Reagents from Merck (Darmstadt, Germany) were employed to prepare the standard dissolutions of volatile compounds.

Others Parameter Analyses. Wine alcoholic degree was determined by densitometric method after distillation. Vinegar acetic degree was established by using the Official Method of volumetric titratition (*10*).

Statistical Analysis. Analysis of variance (ANOVA) was carried out for each quantified compound. Multivariate analysis methods were also performed: principal component analysis (PCA) and linear discriminant analysis (LDA). All statistical analyses were performed by means of Statistic software.

RESULTS AND DISCUSSION

Eighteen volatile compounds have been followed along the experimental aging of the five wine vinegar samples. These compounds have undergone different evolutions. **Tables 2**−**6** show the mean values obtained by triplicate analysis during the aging study.

To carry out ANOVAs, the samples were grouped in two sets, as follows, set A, samples accounting for <1 alcoholic degree (SVA, SVB, and SVC); set B, samples accounting for >1 alcoholic degree (SVD and SVE). Because a minimum period of 6 months of aging in wood is required for quality Sherry wine vinegars (*11*), ANOVAs between samples were performed (0−6 months). We could verify that during the first 6 months only methyl acetate and diacetyl significantly changed for set A; methyl acetate, ethyl acetate, and ethanol changed significantly for set B.

Sherry vinegars "Reserva" are those that have been aged for at least two years. For set A significant increases between the beginning and the end of the aging period (2 years) were found for methyl acetate, methanol, diacetyl, acetoin, hydroxyacetone, γ-butyrolactone, and diethyl succinate. For set B significant differences were found for methyl acetate, ethyl acetate, methanol, ethanol, diacetyl, 2-methyl-1-propanol, isoamylic alcohols (2-methyl-1-butanol and 3-methyl-1-butanol), γ-butyrolactone, and 2-phenylethanol. Therefore, aging plays an important role in the final volatile profile of vinegar.

For samples in set A, very low amounts of ethyl acetate were formed and even disappeared in the samples with the lower content of ethanol (**Tables 2** and **3**). On the contrary, in samples with ∼2 alcoholic degrees, the formation of ethyl acetate is favored. The concentration for this compound increased 5−7-fold. A maximum of 8 g/L was found for sample SVE6 (**Table 5**). This points out the importance of the original ethanol content

Table 2. Evolution of Volatile Compounds (Milligrams per Liter) in the Vinegar SVA

	samples						
volatile compound	SVA0 (0 months)	SVA1 (3 months)	SVA2 (6 months)	SVA3 (9 months)	SVA4 (12 months)	SVA5 (18 months)	SVA6 (24 months)
acetaldehyde	36.5 ± 1.0	42.1 ± 2.0	48.7 ± 1.9	51.6 ± 0.8	38.5 ± 1.7	39.2 ± 0.4	45.2 ± 0.0
ethyl formiate	–[a]	–	–	97.3 ± 0.4	–	108 ± 2	96.4 ± 0.4
methyl acetate	5.15 ± 0.16	9.86 ± 0.2	13.5 ± 0.2	13.7 ± 0.3	20.6 ± 0.4	34.0 ±1.1	42.9 ± 0.0
ethyl acetate	751 ± 39	1224 ± 20	1307 ± 61	1275 ± 3	1237 ± 25	1536 ± 44	1960 ± 22.0
methanol	28.3 ± 04	25.7 ± 0.6	32.9 ± 1.9	26.1 ± 0.9	27.4 ± 1.9	51.4 ± 0.3	48.9 ± 2.2
ethanol[b]	7.33 ± 0.18	9.13 ± 0.20	8.40 ± 0.25	7.13 ± 0.05	5.98 ± 0.20	5.86 ± 0.11	7.37 ± 0.0
diacetyl	–	14.2 ± 0.7	20.7 ± 0.1	13.4 ± 0.1	15.8 ± 0.3	39.1 ± 1.3	55.5 ± 0.8
1-propanol	0.82 ± 0.01	1.40 ± 0.0	1.55 ± 0.1	0.81 ± 0.01	–	–	0.66 ± 0.02
2-methyl-1-propanol	3.40 ± 0.0	4.03 ± 0.05	5.00 ± 0.1	4.47 ± 0.04	3.96 ± 0.3	3.75 ± 0.2	5.55 ± 0.22
isoamyl acetate	–	–	–	–	–	12.2 ± 0.79	14.1 ± 0.1
2-methyl-1-butanol	4.66 ± 0.01	5.13 ± 0.17	5.47 ± 0.19	5.20 ± 0.22	5.37 ± 0.05	5.97 ± 0.18	6.92 ± 0.12
3-methyl-1-butanol	23.0 ± 0.04	24.7 ± 0.34	25.3 ± 0.82	24.0 ± 0.15	21.9 ± 0.58	25.6 ± 0.40	28.3 ± 0.02
acetoin	607 ± 10	588 ± 7	601 ± 22	545 ± 2	634 ± 26	708 ± 5	976 ± 6
hydroxyacetone	23.2 ± 2.0	228 ± 13	75.4 ± 3.4	31.4 ± 1.6	160 ± 6	183 ± 8	278 ± 15
ethyl lactate	7.51 ± 0.0	–	–	–	–	–	–
γ-butyrolactone	29.9 ± 0.3	56.8 ± 0.7	47.5 ± 2.7	40.4 ± 0.1	57.3 ± 2.6	73.2 ± 0.9	98.9 ± 0.6
diethyl succinate	15.0 ± 0.1	12.9 ± 0.3	9.91 ± 0.0	3.10 ± 0.13	120 ± 4	107 ± 7	72.0 ± 2
2-phenylethanol	22.5 ± 0.3	22.9 ± 1.5	21.8 ± 0.6	20.6 ± 0.5	22.3 ± 0.7	22.3 ± 0.1	24.9 ± 0.0
total amounts							
esters	778	1247	1331	1389	1378	1796	2186
alcohols	90	93	100	88	87	115	123
compounds	1550	2042	2149	2127	2210	2771	3484

[a] –, not detected. [b] Grams per liter.

Table 3. Evolution of Volatile Compounds (Milligrams per Liter) in the Vinegar SVB

	samples						
volatile compound	SVB0 (0 months)	SVB1 (3 months)	SVB2 (6 months)	SVB3 (9 months)	SVB4 (12 months)	SVB5 (18 months)	SVB6 (24 months)
acetaldehyde	5.18 ± 0.28	66.9 ± 0.3	22.3 ± 0.5	9.78 ± 0.56	11. 8 ± 0.1	15.9 ± 0.7	20.7 ± 1.2
ethyl formiate	–[a]	–	–	–	118 ± 7	–	170 ± 0.1
methyl acetate	–	4.06 ± 0.15	9.71 ± 0.02	10.3 ± 0.2	22.0 ± 0.8	43.6 ± 2.0	62.2 ± 1.8
ethyl acetate	–	205 ± 1	114 ± 1	34.4 ± 0.5	–	–	–
methanol	1.56 ± 0.06	14.6 ± 0.1	26.8 ± 1.0	26.5 ± 1.0	39.5 ± 0.4	57.7 ± 3.1	69.3 ± 3.1
ethanol[b]	0.71 ± 0.02	2.95 ± 0.05	1.61 ± 0.05	1.18 ± 0.02	0.94 ± 0.02	0.95 ± 0.05	0.96 ± 0.01
diacetyl	–	8.24 ± 0.48	11.2 ± 0.36	8.32 ± 0.06	15.5 ± 0.5	43.0 ± 0.3	46.7 ± 2.8
1-propanol	–	–	–	–	–	–	–
2-methyl-1-propanol	–	–	–	–	–	–	–
isoamyl acetate	–	–	–	–	–	–	–
2-methyl-1-butanol	–	2.90 ± 0.03	2.96 ± 0.11	2.87 ± 0.02	2.64 ± 0.19	2.67 ± 0.02	3.32 ± 0.19
3-methyl-1-butanol	–	10.1 ± 0.2	8.97 ± 0.01	8.29 ± 0.06	8.10 ± 0.42	8.54 ± 0.01	9.83 ± 0.63
acetoin	306 ± 10	340 ± 4	347 ± 7	328 ± 3	426 ± 2	443 ± 4	559 ± 27
hydroxyacetone	50.5 ± 1.6	156 ± 0	67.1 ± 0.80	41.1 ± 1.9	106 ± 5	30.9 ± 0.0	115 ± 5
ethyl lactate	–	–	–	–	–	–	–
γ-butyrolactone	15.6 ± 0.4	26.8 ± 1.5	25.7 ± 0.4	25.3 ± 0.4	29.3 ± 2.2	26.9 ± 1.0	37.5 ± 1.1
diethyl succinate	–	5.58 ± 0.12	3.69 ± 0.03	–	78.3 ± 2.7	78.5 ± 3.0	101 ± 4
2-phenylethanol	8.06 ± 0.29	9.55 ± 0.31	8.70 ± 0.08	8.26 ± 0.01	8.17 ± 0.20	7.54 ± 0.09	7.99 ± 0.06
total amounts							
esters	0	215	127	447	218	122	333
alcohols	103	401	491	471	593	774	914
compounds	337	697	581	463	760	729	1089

[a] –, not detected. [b] Grams per liter.

Table 4. Evolution of Volatile Compounds (Milligrams per Liter) in the Vinegar SVC

	samples						
volatile compound	SVC0 (0 months)	SVC1 (3 months)	SVC2 (6 months)	SVC3 (9 months)	SVC4 (12 months)	SVC5 (18 months)	SVC6 (24 months)
acetaldehyde	126 ± 6	67.0 ± 0.3	41.7 ± 0.3	6.28 ± 0.0	–[a]	–	5.28 ± 0.52
ethyl formiate	–	–	–	–	–	–	153 ± 2
methyl acetate	1.80 ± 0.05	8.99 ± 0.11	12.2 ± 0.5	4.09 ± 0.09	14.5 ± 0.0.36	22.8 ± 0.3	36.5 ± 1.6
ethyl acetate	–	215 ± 6	191 ± 1	–	–	–	–
methanol	25.1 ± 1.4	37.9 ± 1.6	41.8 ± 0.3	39.4 ± 1.9	40.4 ± 2.2	52.3 ± 0.3	55.3 ± 1.2
ethanol[b]	0.86 ± 0.04	3.56 ± 0.03	2.53 ± 0.04	1.20 ± 0.03	0.72 ± 0.01	0.70 ± 0.04	0.71 ± 0.01
diacetyl	–	10.5 ± 0.0	14.0 ± 0.14	6.73 ± 0.22	13.9 ± 0.36	41.4 ± 0.2	52.2 ± 1.1
1-propanol	–	–	–	–	–	–	–
2-methyl-1-propanol	1.41 ± 0.03	3.35 ± 0.00	4.20 ± 0.06	1.65 ± 0.06	1.34 ± 0.08	0.49 ± 0.04	0.92 ± 0.01
isoamyl acetate	–	–	–	–	–	–	–
2-methyl-1-butanol	2.54 ± 0.03	5.26 ± 0.19	5.04 ± 0.27	4.99 ± 0.09	3.85 ± 0.03	4.08 ± 0.07	4.91 ± 0.38
3-methyl-1-butanol	15.1 ± 0.3	22.2 ± 1.0	19.4 ± 0.1	14.3 ± 0.0	10.9 ± 0.2	10.7 ± 0.5	12.8 ± 0.3
acetoin	621 ± 21	657 ± 11	629 ± 5	597 ± 3	641 ± 9	832 ± 41	1025 ± 33
hydroxyacetone	5.34 ± 0.36	66.4 ± 1.73	21.7 ± 0.1	5.21 ± 0.07	5.75 ± 0.13	8.21 ± 0.10	70.7 ± 4.0
ethyl lactate	–	–	–	–	–	–	–
γ-butyrolactone	23.8 ± 0.5	31.2 ± 0.1	29.2 ± 0.0	28.0 ± 0.9	29.6 ± 1.6	41.6 ± 0.4	55.1 ± 2.6
diethyl succinate	–	6.24 ± 0.30	5.87 ± 0.01	1.54 ± 0.04	40.8 ± 0.2	56.3 ± 1.2	68.6 ± 2.9
2-phenylethanol	16.8 ± 0.4	17.1 ± 0.1	15.9 ± 0.3	14.9 ± 0.3	13.0 ± 0.5	12.3 ± 0.2	14.2 ± 0.7
total amounts							
esters	18	230	208	56	553	792	258
alcohols	619	893	889	764	702	805	888
compounds	884	1086	1011	720	810	1074	1484

[a] –, not detected. [b] Grams per liter.

in the final concentration of ethyl acetate. When the ethanol content is ~1 alcoholic degree, the ethyl acetate concentration reaches >1000 mg/L, the same extent at which it has been found in other analyses of volatile compounds in commercial Sherry wine vinegars (*4*, *12*). Indeed, a high correlation has been observed between the initial ethanol values and the ethyl acetate final values in all of the samples ($r = 0.990$).

Methyl acetate undergoes a similar evolution in the five vinegars. At the end of the first year its content ranged between 15 and 20 mg/L; these figures were higher than those reported for commercial Sherry vinegars (*4*, *5*). This compound undergoes a substantial increase, especially in those vinegars with high acetic degrees (SVA, SVB, and SVC) because the formation of methyl esters is favored by the acidic environment (**Figure 1**).

Isoamylic alcohols (3-methyl-1-butanol and 2-methyl-1-butanol) are consumed during the acetification process (*6*, *13*); in our study the initial content is variable (**Tables 2**−**6**), increasing through aging, especially for set B.

Isoamyl acetate is present in those vinegar samples having high initial contents of isoamylic alcohols. Final values for isoamyl acetate in our samples are similar to those reported for Sherry vinegars (*4*, *12*). In our study, the formation of this ester takes place when isoamylic alcohols rise 31 mg/L, and a high correlation was found between the contents of this ester and its corresponding alcohols ($r = 0.98$ for SVE; $r = 0.94$ for SVD). Ethyl lactate, a characteristic compound for Sherry wine, is present in samples with the highest ethanol contents. During the first year, this compound suffered a decrease and then a slight rise (**Tables 2**, **5**, and **6**).

Table 5. Evolution of Volatile Compounds (Milligrams per Liter) in the Vinegar SVD

	samples						
volatile compound	SVD0 (0 months)	SVD1 (3 months)	SVD2 (6 months)	SVD3 (9 months)	SVD4 (12 months)	SVD5 (18 months)	SVD6 (24 months)
acetaldehyde	57.7 ± 0.4	41.5 ± 2.0	51.3 ± 0.8	53.9 ± 0.7	51.6 ± 0.3	50.4 ± 2.5	56.3 ± 0.8
ethyl formiate	–[a]	–	–	–	–	–	–
methyl acetate	4.34 ± 0.11	9.37 ± 0.11	14.6 ± 0.2	16.0 ± 0.91	15.5 ± 0.6	21.6 ± 0.9	33.8 ± 0.6
ethyl acetate	901 ± 8	2144 ± 30	2779 ± 44	4056 ± 51	4218 ± 147	5235 ± 136	6786 ± 153
methanol	28.0 ± 0.9	28.3 ± 0.3	30.5 ± 0.9	34.8 ± 1.2	36.2 ± 0.4	40.3 ± 1.9	59.0 ± 2.0
ethanol[b]	16.4 ± 0.2	22.7 ± 0.2	25.0 ± 0.3	25.5 ± 0.5	22.7 ± 0.2	24.4 ± 0.4	31.0 ± 0.8
diacetyl	–	–	–	–	13.9 ± 0.3	16.8 ± 0.4	31.0 ± 0.8
1-propanol	1.83 ± 0.07	3.51 ± 0.06	3.64 ± 0.22	4.18 ± 0.15	3.07 ± 0.07	4.06 ± 0.38	6.29 ± 0.00
2-methyl-1-propanol	5.21 ± 0.13	8.43 ± 0.10	7.78 ± 0.01	8.39 ± 0.05	9.18 ± 0.36	10.4 ± 0.27	14.9 ± 0.7
isoamyl acetate	9.49 ± 0.28	12.2 ± 0.2	15.2 ± 0.3	15.0 ± 0.1	17.0 ± 0.4	19.3 ± 0.3	24.6 ± 0.4
2-methyl-1-butanol	6.47 ± 0.09	8.94 ± 0.15	8.97 ± 0.18	10.0 ± 0.3	10.1 ± 0.0	11.5 ± 0.2	15.1 ± 0.9
3-methyl-1-butanol	39.2 ± 0.6	53.4 ± 0.8	55.2 ± 0.5	58.1 ± 1.0	57.1 ± 0.6	63.2 ± 1.5	80.0 ± 3.9
acetoin	214 ± 2	193 ± 5	253 ± 4	257 ± 7	232 ± 8	254 ± 7	399 ± 33
hydroxyacetone	4.14 ± 0.1	–	34.2 ± 0.1	32.4 ± 1.5	–	–	27.2 ± 0.8
ethyl lactate	8.64 ± 0.1	8.14 ± 0.04	–	–	5.18 ± 0.01	6.09 ± 0.13	9.97 ± 0.32
γ-butyrolactone	15.6 ± 0.2	19.4 ± 0.2	24.8 ± 1.0	25.8 ± 1.0	17.1 ± 0.1	21.1 ± 1.5	50.4 ± 0.4
diethyl succinate	4.14 ± 0.23	4.07 ± 0.06	–	–	6.39 ± 0.3	7.11 ± 0.1	7.03 ± 0.1
2-phenylethanol	16.8 ± 0.1	18.9 ± 0.6	21.4 ± 0.1	21.5 ± 0.04	18.4 ± 0.1	21.2 ± 0.6	27.6 ± 2.9
total amounts							
esters	928	2814	4086	4249	4930	5289	6861
alcohols	114	144	152	163	157	175	234
compounds	1337	3219	4565	478	5407	5812	7642

[a] –, not detected. [b] Grams per liter.

Table 6. Evolution of Volatile Compounds (Milligrams per Liter) in the Vinegar SVE

	samples					
volatile compound	SVE0 (0 months)	SVE1 (3 months)	SVE2 (6 months)	SVE3 (9 months)	SVE4 (12 months)	SVE5 (18 months)
acetaldehye	25.0 ± 0.1	32.6 ± 0.1	38.6 ± 0.6	49.7 ± 1.7	50.2 ± 0.5	61.0 ± 1.9
ethyl formiate	–[a]	–	–	–	–	–
methyl acetate	3.66 ± 0.23	5.16 ± 0.15	13.3 ± 0.3	21.2 ± 0.1	23.3 ± 0.8	35.7 ± 0.4
ethyl acetate	1547 ± 65	2428 ± 33	4423 ± 40	5604 ± 0	6092 ± 53	8259 ± 50
methanol	24.5 ± 0.5	24.2 ± 0.1	30.3 ± 0.9	39.5 ± 0.5	44.8 ± 1.1	63.6 ± 0.2
ethanol[b]	17.4 ± 0.4	24.6 ± 0.1	27.1 ± 0.1	28.0 ± 0.3	30.2 ± 0.6	35.5 ± 0.5
diacetyl	–	–	9.10 ± 0.23	22.0 ± 0.5	23.2 ± 1.1	51.4 ± 1.0
1-propanol	4.63 ± 0.16	5.80 ± 0.16	6.08 ± 0.26	7.37 ± 0.15	8.20 ± 0.13	11.0 ± 0.0
2-methyl-1-propanol	9.53 ± 0.34	10.6 ± 0.1	11.1 ± 0.7	13.7 ± 0.5	15.6 ± 1.0	21.1 ± 1.9
isoamyl acetate	10.3 ± 0.1	10.9 ± 0.0	18.5 ± 0.5	20.3 ± 0.0	21.4 ± 0.9	37.0 ± 0.1
2-methyl-1-butanol	7.92 ± 0.2	10.2 ± 0.2	10.7 ± 0.3	12.5 ± 0.1	13.8 ± 0.3	17.4 ± 1.0
3-methyl-1-butanol	46.3 ± 1.6	59.1 ± 0.2	63.8 ± 0.7	70.6 ± 0.2	78.1 ± 0.5	95.4 ± 4.3
acetoin	322 ± 9	289 ± 0	376 ± 1	407 ± 12	468 ± 17	660 ± 41
hydroxyacetone	2.54 ± 0.11	–	23.5 ± 0.6	32.8 ± 1.4	34.0 ± 1.7	22.5 ± 1.8
ethyl lactate	18.1 ± 0.3	16.7 ± 0.0	8.52 ± 0.01	9.27 ± 0.17	12.1 ± 0.4	17.6 ± 2.0
γ-butyrolactone	21.5 ± 0.1	20.9 ± 0.3	28.2 ± 0.4	35.6 ± 1.5	37.2 ± 4.4	53.9 ± 2.4
diethyl succinate	14.8 ± 0.4	12.8 ± 0.0	8.23 ± 0.27	5.00 ± 0.03	6.68 ± 0.09	9.27 ± 0.01
2-phenylethanol	26.2 ± 0.1	27.1 ± 0.7	30.5 ± 0.6	30.9 ± 1.1	31.5 ± 0.6	40.5 ± 5.2
total amounts						
esters	1593	2474	4472	5660	6156	8358
alcohols	136	161	179	202	222	285
compounds	2117	2995	5112	6385	6969	9487

[a] –, not detected. [b] Grams per liter.

Our results confirm that the synthesis of esters occurs in vinegars during maturation and aging as already pointed out by other authors (*13*).

Methanol underwent a significant increase in the whole set of samples, especially during the second year when the concentration phenomenon is more marked. The found final amount in these vinegars ranged between 50 and 70 mg/L, similar to or higher than that found in commercial Sherry wine vinegars (*4*, *5*).

Acetoin, a characteristic compound of acetification, increases mainly in the second year due to concentration. Its final content ranged between 400 and 1000 mg/L, which was twice the initial content.

Diacetyl is formed from acetoin in an oxidative environment. No diacetyl was formed in the initial stages of aging; for set A samples it was detected at 3 months of aging and in set B, later (**Figure 1**). This compound could be an indicator of the age of vinegars. γ-Butyrolactone increases during aging; the final contents ranged between 40 and 100 mg/L.

Principal Component Analysis. In this statistical analysis 14 volatile compounds were considered, leaving out those variables absent in most of the samples under study (ethyl formiate, 1-propanol, isoamyl acetate, and ethyl lactate).

Three PCs that accounted for 87.5% of the variance were chosen on the basis of Kraiser's criterion (eigenvalues >1). To ascertain the latent structure of the data, a Varimax rotation was carried out; the loadings of the first three varivectors obtained are shown in **Figure 2**. As can be seen, PC1 is closely related to higher alcohols and ethyl acetate, compounds that presented different evolutions between set A and B samples.

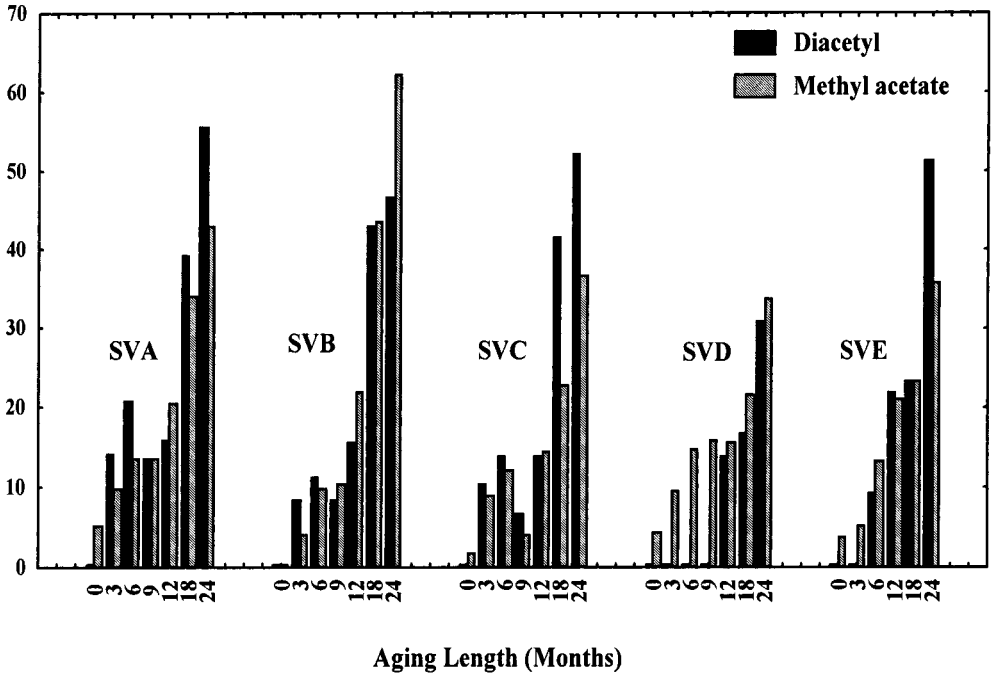

Figure 1. Diacetyl and methyl acetate evolution during experimental aging.

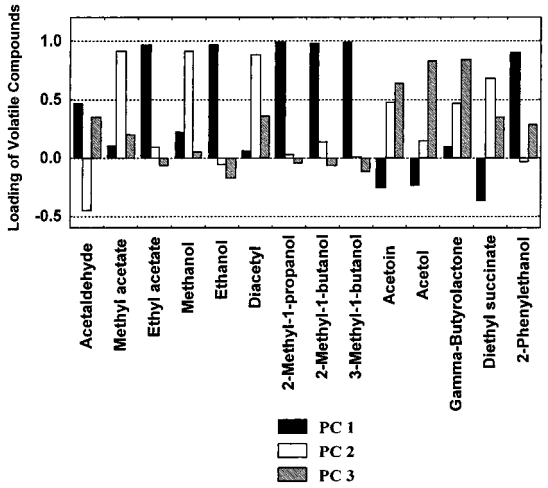

Figure 2. Loading of volatile compounds on principal components 1–3.

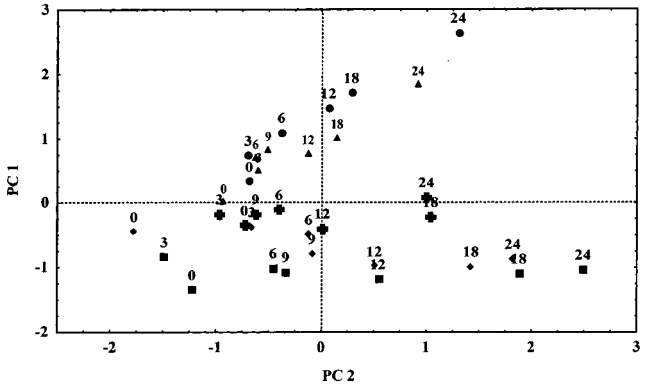

Figure 3. Score plot of the studied samples: SVA (+), SVB (■), and SVC (◆) belong to set A; SVD (▲) and SVE (●) belong to set B. Label numbers refer to sample aging (see text).

The second PC corresponds to methyl acetate, methanol, and diacetyl. These compounds significantly changed during aging in both sets of samples. These parameters are closely related to the aging process, being independent of the original composition of vinegar. The plot the score obtianed by selecting the first two PCs as axes (**Figure 3**) shows that the first component (PC1) allows us to differentiate between samples from sets A and B. The second component (PC2) is related to the length of the aging period.

Linear Discriminant Analysis. A priori knowledge of class membership is assumed when this chemometric method is applied. We have carried out LDA taking into account two criteria of sample grouping: wine substrate and aging time.

When LDA is applied to a set of samples, the samples are usually divided into a training set and a test set; the first one to find discriminant functions, and the second to check the utility of those discriminant functions to correctly classify new samples. In our case, we have used the so-called "leave one out" method (*15*) consisting of dividing the whole set of samples into two groups: a training set holding all of the samples except one, which is used then as test set. Thus, LDA was applied as many times as the number of samples.

Grouping the samples according to the wine substrate used as raw material (A−E) and by using the LDA standard method, we obtain four discriminant functions that include all of the variables under study. The scatterplot of the samples onto the plane defined by the first two discriminant functions is shown in **Figure 4**. As can be seen samples appear to be clearly separated into five groups according to the wine substrate, namely, A, B, C, D, and E. Moreover, we have obtained 100% correct classifications of samples in the check process by the leave one out method (*15*).

Regarding the second possible criterion for classifying our samples, the aging period, another LDA was performed. Samples were divided into five groups (0, 6, 12, 18, and 24 months), and the leave one out method was applied. These variables were able to discriminate all samples; a 100% correct classification of samples was obtained. **Figure 5** shows the samples plot onto the discriminant space formed by the first two discriminant functions as axes.

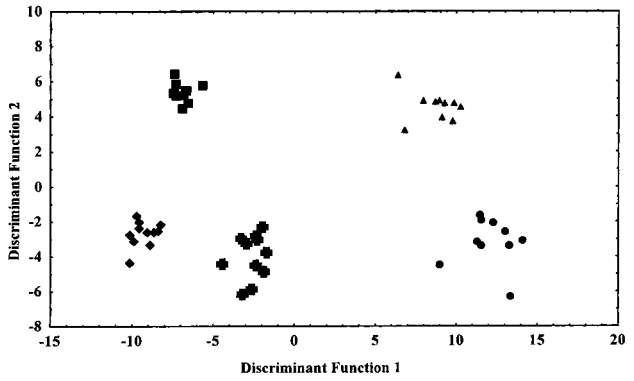

Figure 4. LDA employing wine substrate as grouping variable; projection of samples on the discriminant space selecting the first two discriminant functions as axes: SVA (+); SVB (■); SVC (◆); SVD (▲); SVE (●).

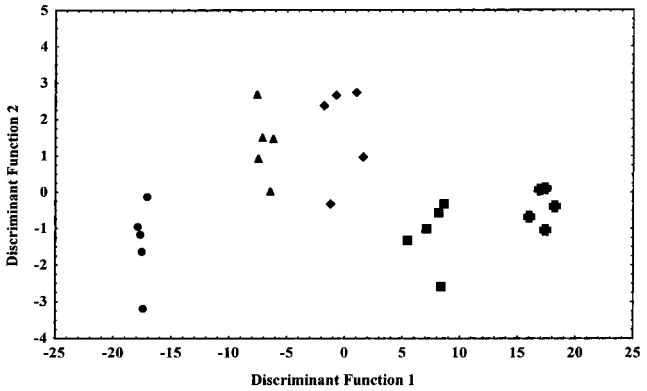

Figure 5. LDA employing aging period as grouping variable; projection of samples on the discriminant space selecting the first two discriminant functions as axes: 0 months (+); 6 months (■); 12 months (◆); 18 months (▲); 24 months (●).

Therefore, the volatile compounds can be used for classifying vinegars according to wine substrate or aging period when there are at least 6 months of difference.

Conclusions. The selected volatile compounds are suitable descriptors to differentiate vinegar samples according to the raw material and aging period. At 6 months of aging significant changes are found for a number of volatile compounds. Initial ethanol content determines the formation of certain compounds such as ethyl acetate. Thus, those samples with a high ethanol degree have the largest total content of volatiles. Methyl acetate and diacetyl present significant changes during aging independent of the initial composition of the vinegars. Multivariate analysis shows that for purposes of classification, the volatile composition is useful to distinguish wine vinegars according to the substrate wine employed or the length of aging.

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