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Evolution of Phenolic Compounds during an Experimental Aging in Wood of Sherry Vinegar

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Changes in the physicochemical composition of wine vinegars produced by submerged culture system and aged in wood were followed. Five Sherry wine vinegars and a model vinegar solution were aged in six new American oak butts of 16.6 L capacity. A total of 24 phenolic compounds were monitored during the maturation study (24 months), along with other physicochemical parameters (total extract, acidity, residual alcohol and total phenolic index). Multivariate statistical analysis was applied to the data. From the sixth month on, significant changes were produced in most of the phenolic compounds, mainly aromatic aldehydes and 5-(hydroxymethyl)-2-furaldehyde. When all the phenolic compounds were considered as variables, cluster analysis grouped samples according to the wine substrate employed in the elaboration of vinegars under study. Within each subcluster, samples are arranged according to their aging status when phenolic compounds accounting significative changes at 180 days of aging are considered. Discriminant functions were constructed from the phenolic compounds data set. The validity of these functions was tested using 13 samples of aged commercial Sherry wine vinegars and 25 unaged vinegars. A total of 97.4% of the test samples was correctly classified within its respective group.

KEYWORDS: Sherry vinegar; aging; oak barrels; phenolic compounds; discriminant analysis; pattern recognition; HPLC

INTRODUCTION

Traditionally, Sherry vinegars are elaborated in oak butts, being the bacterial culture placed on the surface of the wine substrate. Thus, oxygen availability to the bacteria is limited. This implies that a very long period of time is required to obtain a high acetic degree. As a consequence, aging occurs at the same time, and excellent organoleptic properties are acquired. A highly appreciated product is obtained, and it reaches high prices in the market. However, the volume of production is limited by the long period of time needed to acquire the desired properties (*1*).

On the other hand, the most common method for obtaining wine vinegar consists of a submerged culture where the bacteria are placed in an acetifying liquid (wine-vinegar mixture). Major capacity stainless steel vessels are used, and strong aeration is applied until the desired acetic degree (7% v/v) is reached within 24−36 h (*2*).

Hence, this work is part of the research project focused toward reducing the production time and cost of Sherry vinegar obtaining the required acetic degree by means of a submerged culture system followed by aging in wood. In this way a reduction in the total time needed is expected.

Sherry vinegar has its own checked denomination and origin (*3*). This regulation gives two categories for vinegars depending on their aging period: Vinagre de Jerez (products aged in wood for a minimum of six months) and Vinagre de Jerez Reserva (aged in wood for more than two years). This work intends to evaluate some aspects of the chemical composition in order to establish when significant changes take place.

The phenolic composition of aged products such as wine and its derivatives has been considered as a parameter to evaluate the quality, as these substances play an important role in the overall change in quality of, for example Armagnac (*4*), Cognac (*5*), and Vinegar (*6*). The influence of wood in the aging process has also been extensively studied by different authors for various products, i.e., wines (*7*, *8*) or rum (*9*). The extraction of components will presumably be very different from alcoholic beverages due to the different pH and ethanol concentration. Differences in phenolic composition between vinegars aged more than 2 years and with less aging time have already been pinpointed (*6*).

Despite the former results, it seems adequate to study the evolution of phenolic compounds during the aging process.

Many authors deal with the chemical nature of the wood extractables, both volatile (*10*, *11*) and nonvolatile components (*12*), and their contribution to the sensory characteristics of wine (*13*, *14*) and vinegar (*15*). The wood gives the product improved

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Table 1. Sherry Wine Substrates and Their Corresponding Vinegars Produced in the Laboratory by Means of Submerged Culture System

sherry wine substrate		sherry vinegars initial concentration	
substrate code	ethanol concentration (% v/v)	acetic degree (g/100 ml)	residual ethanol (% v/v)
A	9.4	8.3	0.91
B	12.2	7.5	0.09
C	9.5	7.4	0.11
D	10.4	8.0	2.02[a]
E	14.5	8.3	2.15

[a] Alcoholic degree after the addition of wine alcohol (ethanol)

qualities and an aroma of vanilla by releasing extractable substances (*16*). Vanillin, syringaldehyde, coniferaldehyde, and sinapaldehyde have been identified in oak-aged brandies (*17*). Brandy, cognac, armagnac, whisky, and rum are aged in oak barrels to improve their organoleptic properties (*18*). Vinegars with a marked woody odor were identified as Sherry wine vinegars in a sensorial characterization study of wine vinegars (*29*).

The rate and quantity of the extraction depends on factors such as oak variety, the geographic location of the forest (*20*, *21*), barrel age (whether the barrel is young or was used for various aging cycles), surface-to-volume ratio (*22*, *23*), the method used to obtain the staves (*24*), the stave drying technique (natural or artificial) (*25*), or treatments used during the cask-making process (*26*).

Moreover, during aging it must be taken into account that the enrichment of the product with substances released by the oak, the reaction with air which diffuses through pores in the wood, and the development of certain chemical reactions (esterification, acetal formation, etc) that take place slowly (*13*).

The aim of this study was to verify the evolution of vinegars produced by a submerged culture system and subsequently aged in barrels, using the same condition as that of Sherry wine vinegars, in terms of phenolic content as well as other parameters such as dry extract, total acidity, total phenolic content, alcoholic content and furanic aldehydes. From the results on phenolic composition, model classifying functions were constructed, and the validity of these functions were tested on commercially available Sherry and conventional wine vinegars.

MATERIALS AND METHODS

Vinegars Submitted to Aging. Five different wine vinegars were obtained using a submerged culture in our laboratory fermenter from five different Sherry wines (A−E, **Table 1**) in conditions previously established by the authors (*27*). The five substrate wines were supplied by different Sherry wineries with alcoholic degrees ranging 9.4−14.5. The resulting vinegars were submitted to static aging in American oak casks of 16.6 L capacity previously conditioned with Sherry wine. The casks were filled to $^3/_4$ of their total capacity.

Sampling was performed every 45 days during the first year and every 6 months for the next year (**Table 2**). Sampling was carried out using a glass pipet, the extracted volume (150 mL) being kept in topaz bottles in the refrigerator. All of the vinegars had an initial acetic degree (g acetic acid/100 mL vinegar) higher than 7 (**Table 1**). Wine alcohol was added to sample D up to 2.02° to reach the maximum level of alcohol concentration allowed by Sherry vinegar regulation. Hence, a wide range of ethanol concentration was used to assess the effect of alcohol on the extraction. Thus, samples D and E accounted 2 alcoholic degrees (v/v) at the beginning of the study, the remaining (A, B, and C) presented an alcohol content between 0.09% and 0.9% (v/v) (**Table 1**).

Table 2. Codification and Sampling of Sherry Vinegars and Model Vinegar Solution during the Maturation Study

sampling interval (days)	vinegar samples					model vinegar solution
0	A_0	B_0	C_0	D_0	E_0	T_0
45	A_1	B_1	C_1	D_1	E_1	T_1
90	A_2	B_2	C_2	D_2	E_2	T_2
135	A_3	B_3	C_3	D_3	E_3	T_3
180	A_4	B_4	C_4	D_4	E_4	T_4
225	A_5	B_5	C_5	D_5	E_5	T_5
270	A_6	B_6	C_6	D_6	*a*	T_6
315	A_7	B_7	C_7	*a*	E_7	T_7
360	A_8	B_8	C_8	D_8	E_8	T_8
540	A_9	B_9	C_9	D_9	E_9	T_9
720	A_{10}	B_{10}	C_{10}	D_{10}	E_{10}	T_{10}

[a] D_7 and E_6 sampling was not realized.

Sampling points were numbered as follows: 0 for the samples at the beginning once casks were filled, 1 for the first sampling point (45 days of aging), and so on; the last samples were numbered 10 for 24 months old. A total of 64 samples was analyzed throughout the study (**Table 2**).

Model Vinegar Solution. A model vinegar solution (T) which was used as a blank was prepared in those concentrations obtained from the literature (*10*) for the main chemical components of wine vinegar. The chemical composition was acetic acid 78.9 g/L, ethanol 20.02 mL/L, ethyl acetate 24.3 g/L, glycerol 3.74 g/L, methanol 1.58 g/L, 3-methyl-1-butanol 1 g/L, methyl acetate 0.923 g/L, acetaldehyde diethyl acetal 0.891 g/L, acetaldehyde 0.424 g/L, acetoin 0.4 g/L, proline 0.5 g/L, gallic acid 25.2 mg/L, tartaric acid 3 g/L, and distilled water 875 mL. The model vinegar solution was aged for the same period as that of vinegar's.

Vinegar Samples (Test Group). A total of 38 commercial and laboratory Sherry vinegars was used as a test group for the validation of classification functions (13 commercial Sherry vinegar samples elaborated by surface culture system and aged for more than two years, 25 vinegar samples elaborated by submerged culture system of these 13 commercial vinegars and 12 vinegars elaborated in our laboratory without aging).

HPLC Analysis of Phenolic Compounds. A high-performance liquid chromatographic system (Waters Associated Chromatography) was equipped with a pump (Waters 600E), system controller, and Model 7125 manual injector (Rheodyne, Cotati, CA); detection was carried out with a Photodiode Array Detector (Waters 996) connected to a Data Station (Millennium 2.0) for collection and mathematical treatment. The column was a Reversed Phase Merck Superspher 100 RP-18 (250 × 4 mm) protected by a Nova-Pak C_{18} guard cartridge module from Waters. The sample volume injected was 50FL. The flow rate was 0.5 mL.min^{-1}, and the temperature was set at 22.5 °C. Samples were not submitted to any treatment before being injected into the column, with the exception of filtration through a Millex-GV13 0.22μm filter (*28*). Duplicate analysis was carried out for each sample following the analysis procedure adapted by the authors (*28*) from the original method (*29*, *30*) for the wines. Identification was based on both retention time and UV−visible spectra matching with standards. In case of doubt, sample was spiked with the standard, and the peak purity was checked, while quantification was performed by external calibration. During the two-years period, external calibration was performed at each sample point in order to check the precision of the quantitative analysis.

A total of 24 phenolic compounds was identified in the vinegars studied. A chromatogram recorded for a vinegar sample is shown in **Figure 1**.

Other Parameters. Dry extract was determined gravimetrically according to Spanish Official Methods (*31*). Total acidity was determined by the volumetric titration Official Method (*31*). An enzymatic method was used for ethanol quantification (*32*, *33*) and Folin−Ciocalteu Method (*34*) for total phenolic content.

Statistical Analysis. Statistical analyses were performed by means of Statistica software (*35*). One-way analysis of variance (ANOVA) was used to ascertain at which stage of the aging period significative

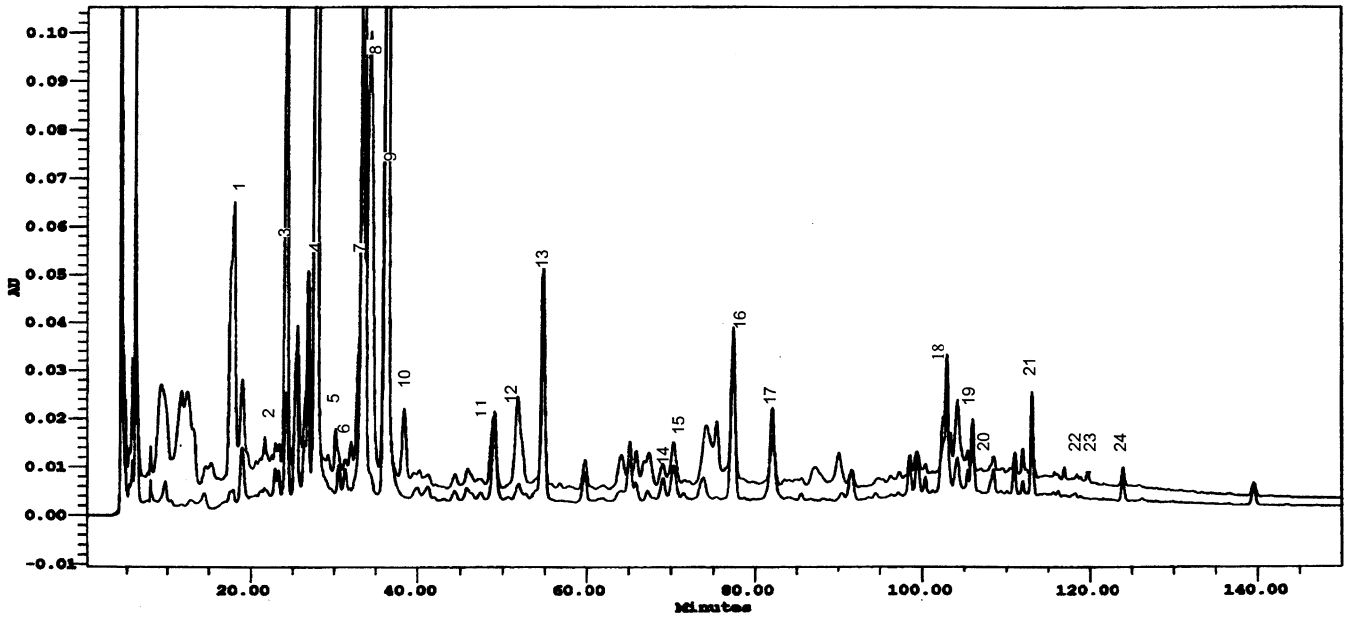

Figure 1. Chromatograms (at 280 and 313 nm) obtained for a Sherry wine vinegar for vinegars aged 1 year. 1, gallic acid; 2, 5-(hydroxymethyl)-2-furaldehyde); 3, protocatechuic acid; 4, caffeoyltartaric acid; 5, protocatechualdehyde; 6, 2-furaldehyde; 7, cumaroyltartaric acid glycoside; 8, p-hydroxybenzoic acid; 9, lumaroyltartaric acid; 10, tyrosol; 11, p-hydroxybenzaldehyde; 12, vanillic acid; 13, caffeic acid; 14, gallic ethyl ester; 15, vanillin; 16, p-coumaric acid; 17, syringaldehyde; 18, coniferaldehyde; 19, rutin; 20, isoquercitrin; 21, resveratrol; 22, t-cinnamic acid; 23, ferulic acid; 24, caffeic ethyl ester.

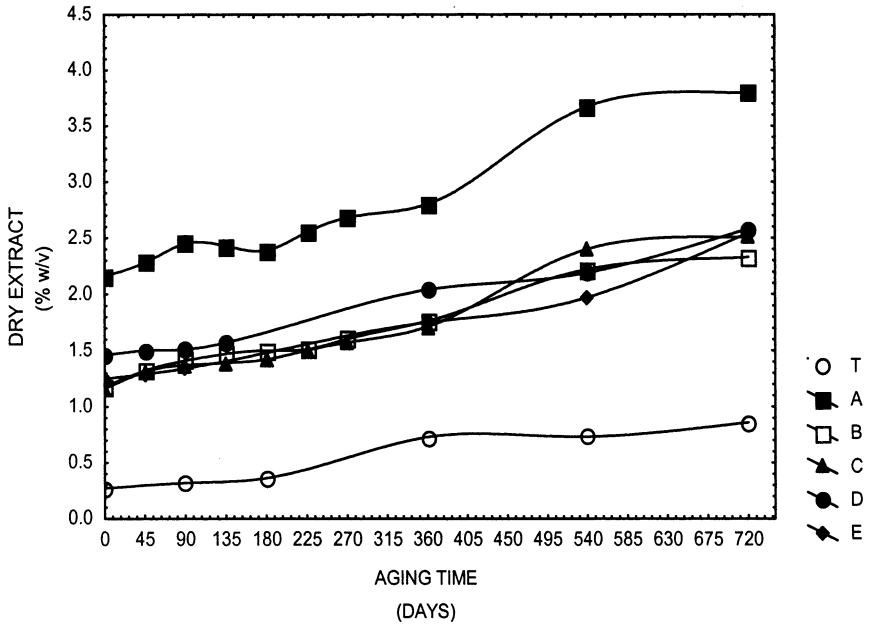

Figure 2. Evolution of dry extract during aging.

changes (significant at 5% level) would take place. Previously, normality of data was verified by the Kolmogorov−Smirnov test, and when the variables did not fit a normal distribution, the Mann−Whitney test was applied. Prior to the building of the classifying model functions, an exploratory analysis (cluster analysis) was carried out to see data trends.

RESULTS AND DISCUSSION

Dry Extract. This parameter increases along the whole aging period for all of the samples analyzed. This continuous increase was favored as the result of loss of water through the pores of the butt (diffusion and evaporation) as well as the extraction of wood components, principally polyphenols (**Figure 2**). In general, the increase for samples A−E was almost twice the initial value, while the model vinegar solution does three times more.

Total Acidity. As it was shown in **Figure 3**, values for total acidity increase slightly during the first year; however, during the second year, a strong increase was observed, reaching 14 acetic degrees by the end of the second year for the samples with a lower content of ethanol (A−C). Hence, our hypothesis lies on the fact that those samples with a lower content of residual alcohol would lose more water through the pores of the butt (diffusion and evaporation) due to the smaller molecular size of water in comparison with other components (*36*).

Alcoholic Content. The concentration of ethanol in vinegar samples with lower alcoholic content (A−C) was not varied during the aging period. However, for those samples with higher

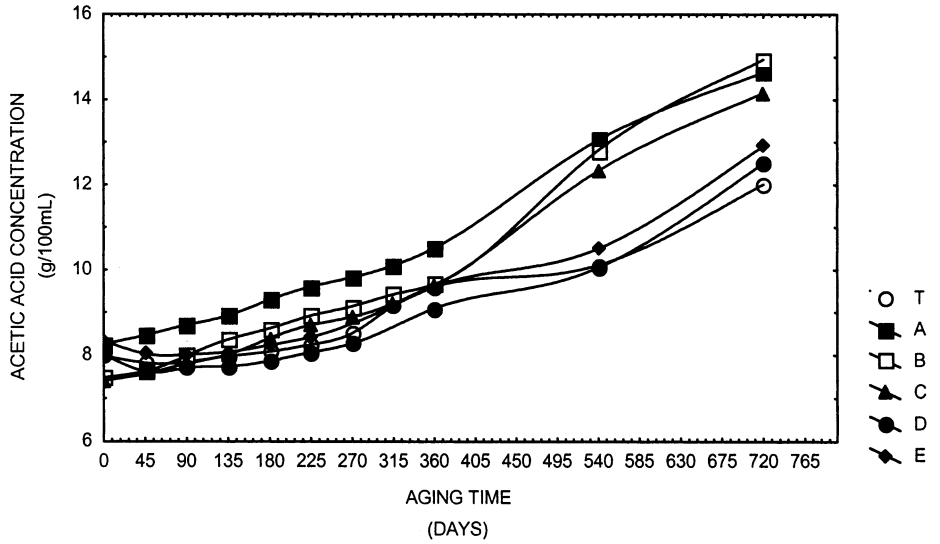

Figure 3. Concentration of acetic acid during aging.

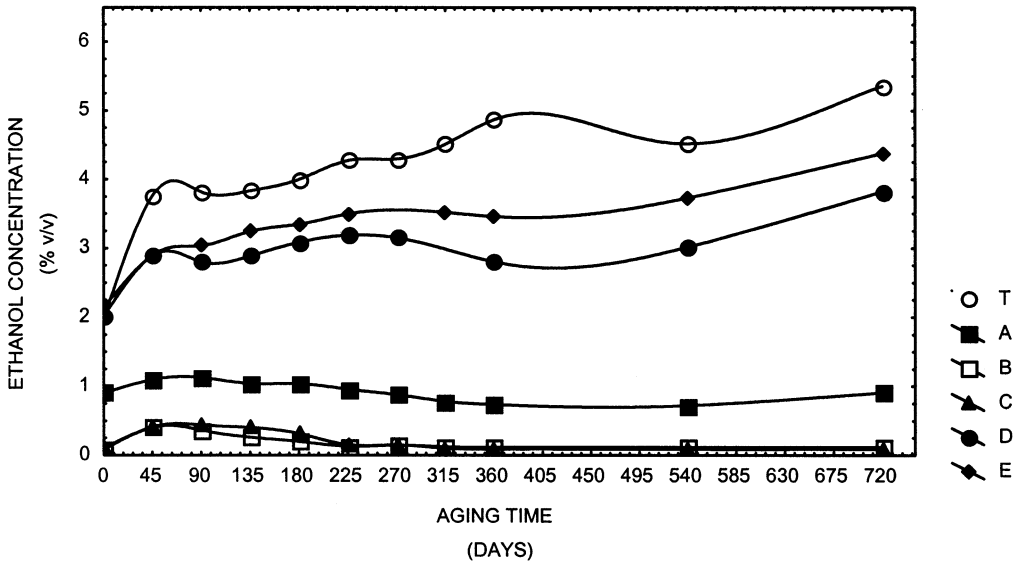

Figure 4. Evolution of ethanol concentration during aging.

alcoholic content (D and E), an increment at the beginning of the aging study was observed and remained more or less constant afterward (**Figure 4**).

Total Phenols. It is observed that the value for this parameter increases in all the vinegars during the aging study due to an important extraction of phenolic compounds from the butt. During the first three months, the concentration of phenolic compounds rises markedly for samples A, B, E, and T (**Figure 5**). At the end of two years, aging all the vinegars doubled their initial value, being 6 times greater for the model vinegar solution.

The degree of extraction of phenolic compounds should be related to the surface area/volume ratio. The polyphenolic index increases about 125 mg/L in a year, this ratio in our case (16.6 L capacity butts) being around 252 cm^2/L. Other authors (*23*) observed similar results for a model wine solution aged in a 20 L barrel, despite the higher alcoholic degree.

Phenolic Compounds. It was observed that some phenolic compounds follow similar trends in all the vinegars analyzed whereas another suffered variations from sample to sample.

Phenolic Aldehydes. Important increases were observed for syringaldehyde, coniferaldehyde, and vanillin during aging for all the samples analyzed. Other minor aldehydes (p-hydroxybenzaldehyde, protocatechualdehyde) are not present in all the samples analyzed. Interesting results are obtained for the model vinegar solution (T), since progressive increases occur for the main constituents of the oak i.e., vanillin, syringaldehyde, coniferaldehyde, and others like protocatechualdehyde, reaching considerable concentrations at the end of the two years aging (**Table 3**).

Hydroxybenzoic Acids (Gallic, Protocatechuic, p-hydroxybenzoic, Vanillic). Gallic acid clearly increases its concentration in three samples (A, D, and E) from 360 days onward of aging (**Tables 4**−**6**). In the remaining two samples (B and C), we did not observe significative changes (**Tables 7** and **8**). However, for the model vinegar solution, concentration remains constant throughout the aging period (**Table 3**). For protocatechuic, p-hydroxybenzoic, and vanillic acids, there was a great variability in the values observed. However, from the first year on, they were all present in appreciable concentrations.

Hydroxycinnamic Acids (Caffeic and p-Coumaric Acids). These compounds increase in concentration from the first year on for samples D and E (**Tables 5** and **6**). This can be explained by the hydrolyzes of their corresponding tartaric esters.

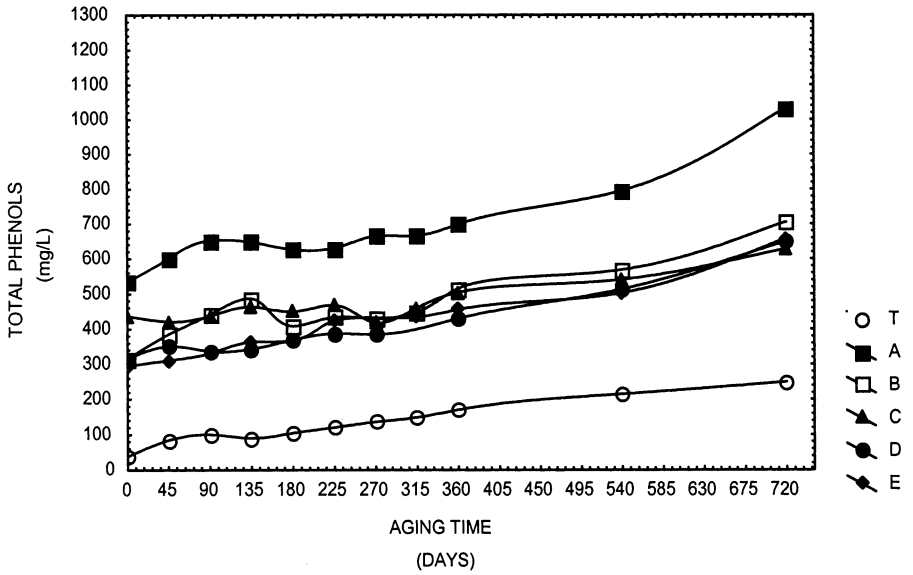

Figure 5. Evolution of total phenolics during aging.

Table 3. Evolution of Phenolic Compounds for Model Vinegar Solution T (mg/L) (–) Compounds Not Detected or Not Quantified

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
gallic acid	14.00	15.00	14.00	19.00	14.00	15.00	16.00	17.00	15.50	16.90
HMF	2.40	3.00	3.30	3.40	3.70	4.10	4.70	5.00	5.20	6.60
protocatechuic acid	–	–	–	–	0.50	0.57	0.60	0.70	–	–
caffeoyltartaric acid	0.30	0.37	0.37	0.37	0.43	0.47	0.54	0.62	–	1.20
protocatechualdehyde	0.99	1.03	1.03	1.02	1.09	1.07	1.18	1.17	1.54	1.80
p-hydroxybenzoic acid	0.04	0.05	–	–	0.08	–	0.01	0.08	–	–
2-furaldehyde	2.70	3.10	3.30	3.60	4.20	4.60	5.80	5.90	6.30	9.40
cumaroyltartaric acid	0.17	0.22	0.21	0.20	0.22	0.24	0.30	0.28	0.47	0.32
tyrosol	–	–	–	–	–	2.90	2.80	–	–	–
p-hydroxybenzaldehyde	0.35	0.39	0.41	0.44	0.45	0.48	0.53	0.56	–	0.77
vanillic acid	0.20	0.26	0.28	0.27	0.38	0.44	0.59	0.67	0.59	0.94
caffeic acid	–	–	–	–	–	–	–	0.17	–	–
gallic ethyl ester	0.59	0.74	0.89	1.01	1.28	1.56	2.61	2.72	4.92	7.36
vanillin	0.19	0.37	0.52	0.72	1.03	1.39	2.22	2.54	2.82	4.55
syringaldehyde	1.80	2.29	2.47	2.97	3.51	4.22	5.97	6.67	8.41	12.45
p-coumaric acid	0.37	0.40	0.38	0.42	0.44	0.48	0.51	0.50	0.23	0.40
coniferaldehyde	3.91	4.92	5.29	6.47	7.64	9.36	11.40	12.40	17.10	22.10
t-cinnamic acid	–	0.03	0.25	0.04	0.05	0.06	0.06	0.09	–	0.13

Table 4. Evolution of Phenolic Compounds for Vinegar A (mg/L) (–) Compounds Not Detected or Not Quantifiable

	A_0	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9	A_{10}
gallic acid	12.4	14.6	13.7	11.2	12.5	18.3	20.7	20.9	25.0	31.8	30.4
HMF	13.4	15.8	18.2	18.0	18.0	20.5	22.9	23.8	24.7	41.0	33.1
protocatechuic acid	1.01	–	–	–	–	–	1.57	–	–	–	–
caffeoyltartaric acid	24.1	19.8	18.6	14.0	14.5	18.5	19.4	19.1	24.7	29.1	10.7
protocatechualdehyde	1.4	1.7	1.6	–	–	–	1.7	–	–	–	–
cumaroyltartaric acid glycoside	8.8	–	8.2	–	–	9.0	9.2	–	10.1	20.4	1.6
2-furaldehyde	1.4	–	2.1	–	–	1.6	9.4	–	–	4.4	3.8
cumaroyltartaric acid	8.8	9.4	8.7	9.0	9.0	8.6	9.3	8.7	9.4	8.7	10.9
tyrosol	18.4	21.1	20.6	21.4	21.8	21.0	22.3	18.7	23.9	41.2	30.4
caffeic acid	1.8	1.9	2.0	1.9	2.1	2.0	2.0	1.5	0.4	–	–
gallic ethyl ester	1.7	1.7	1.8	2.3	2.9	1.7	3.3	2.9	2.4	–	–
vanillin	–	0.44	0.75	0.86	1.07	0.97	1.2	0.92	1.3	9.5	5.4
syringaldehyde	1.21	2.0	2.3	2.8	3.1	3.1	3.2	3.6	4.0	–	–
p-coumaric acid	2.2	2.2	2.2	2.2	2.0	2.0	2.1	1.9	3.0	–	–
resveratrol	0.12	0.13	0.08	0.04	0.39	0.1	0.11	0.09	–	0.18	0.08
caffeic ethyl ester	0.46	0.42	0.4	0.38	0.37	0.34	0.36	0.35	0.37	–	–
coniferaldehyde	–	5.41	9.71	10.0	9.55	9.69	10.0	12.2	13.2	34.9	16.1
t-cinnamic acid	0.03	0.05	0.07	0.09	0.10	0.11	0.11	0.12	0.13	0.14	–

Furanic Aldehydes. The concentration of 5-(hydroxymethyl)-2-furaldehyde rises in all cases. In the model vinegar solution, it rises from zero to a values of 6.6 ppm at the end of the second year of aging. It is obvious that this compound is produced from hemicellulose during the thermal treatment of staves in butt production. However, for commercial vinegars high concentrations of this compound are usual, due to the fact that the addition of must caramel is allowed in order to provide the desired color for Sherry vinegar (*3*).

2-Furaldehyde increases its concentration in those samples with higher alcoholic contents (T, D, and E) (**Tables 3**, **5**, and **6**), especially after 6 months of aging.

Statistical Analysis. *Analysis of Variance (ANOVA).* ANOVA was applied to all vinegar samples and the model vinegar

Table 5. Evolution of Phenolic Compounds for Vinegar D (mg/L) (–) Compounds Not Detected or Not Quantifiable

	D_0	D_1	D_2	D_3	D_4	D_5	D_6	D_8	D_9	D_{10}
gallic acid	5.5	7.3	7.6	6.9	6.1	6.3	7.4	10.9	13.3	17.5
HMF	–	3.3	3.9	4.4	4.9	5.3	6.0	7.4	8.1	13.9
caffeoyltartaric acid	70.7	72.8	68.8	71.5	71.8	69.9	69	64.9	50.2	55.9
cumaroyltartaric acid glycoside	18.4	19.1	17.1	18.1	18.8	18	17.8	16.6	–	–
p-hydroxybenzoic acid	1.4	–	–	–	–	–	–	–	–	–
2-furaldehyde	0.1	3.8	4.5	4.7	6.6	6.1	7.6	9.2	6.4	14.4
cumaroyltartaric acid	28.5	29.7	27.8	29.5	28.9	28.9	29	28.6	23.9	31.8
tyrosol	7.5	7.8	7.9	8.7	8.1	9.2	8.6	10.9	14.8	15.7
caffeic acid	2.7	2.9	2.7	2.7	2.7	2.9	3.1	3.6	1.8	1.5
gallic ethyl ester	1.45	–	–	–	–	–	–	2.9	–	–
vanillin	–	0.14	0.35	0.46	0.67	0.99	1.4	2.2	4.1	6.8
syringaldehyde	–	1.6	1.9	2.4	2.9	3.5	4.3	6.6	9.35	1.52
p-coumaric acid	2.7	2.6	2.4	2.5	2.4	2.6	2.4	3.4	6.1	14.3
ferulic acid	0.34	0.33	0.80	–	–	–	–	–	–	–
isoquercitrin	–	0.5	0.53	0.48	0.65	0.54	0.75	0.93	–	–
resveratrol	0.11	0.08	0.06	0.06	0.07	0.06	0.06	0.04	0.03	–
caffeic ethyl ester	0.21	0.17	0.14	0.13	0.20	0.22	0.20	0.30	–	–
coniferaldehyde	0.58	2.08	2.92	3.6	5.19	8.29	10.6	13.2	19.9	22.0
rutin	0.25	–	–	–	–	–	–	–	–	–
t-cinnamic acid	–	0.03	0.04	0.04	0.05	0.06	0.07	0.09	–	–

Table 6. Evolution of Phenolic Compounds for Vinegar E (mg/L) (–) Compounds Not Detected or Not Quantifiable

	E_0	E_1	E_2	E_3	E_4	E_5	E_7	E_8	E_9	E_{10}
gallic acid	5.1	5.7	5.1	5.9	6.2	6.9	8.03	8.4	12.8	5.5
HMF	1.3	3.6	3.7	4.8	5.0	5.7	8.0	8.2	9.4	10.7
protocatechuic acid	–	–	1.9	1.8	1.9	1.7	–	2.3	0.42	–
caffeoyltartaric acid	4.2	3.8	3.4	3.9	4.1	4.5	5.6	5.6	47.2	42.6
cumaroyltartaric acid glycoside	11.6	11.9	10.6	–	–	11.7	10.4	12.6	–	–
2-furaldehyde	0.16	3.2	4.7	5.4	6.7	7.7	10.9	11.8	9.0	10.2
cumaroyltartaric acid	13.7	14.1	12.9	14.4	14.1	13.8	13.9	14.2	8.6	10.1
tyrosol	17.6	20.6	18.9	21.2	21.8	21.8	22.3	25.0	22.7	19.1
vanillic acid	–	–	–	–	–	0.77	0.89	–	–	1.5
caffeic acid	2.3	2.0	1.8	2.1	2.2	2.4	2.9	3.0	3.0	3.0
gallic ethyl ester	0.59	1.1	1.1	0.79	1.9	2.1	2.2	3.2	–	–
vanillin	–	0.17	0.33	0.63	0.96	1.4	2.3	3.6	3.65	5.7
syringaldehyde	0.43	2.0	2.6	3.3	4.6	5.8	8.2	9.4	15.1	9.8
p-coumaric acid	2.4	2.5	2.1	2.4	2.4	2.06	2.8	6.3	12	12.4
ferulic acid	0.77	0.5	0.46	0.52	–	–	–	–	0.52	0.55
isoquercitrin	–	0.59	0.64	0.62	0.81	0.70	0.66	0.24	–	–
resveratrol	0.15	0.01	0.01	0.01	0.20	0.01	–	–	–	–
caffeic ethyl ester	1.2	0.99	0.84	0.89	0.59	0.62	0.64	0.55	–	–
conifealdehyde	–	2.2	2.86	4.57	6.66	9.17	12.7	13.9	17.7	30.3
rutin	0.16	–	–	–	–	–	–	–	–	–
t-cinnamic acid	–	0.02	0.03	0.03	0.07	0.05	0.08	–	–	–

Table 7. Evolution of Phenolic Compounds for Vinegar B (mg/L) (–) Compounds Not Detected or Not Quantifiable

	B_0	B_1	B_2	B_3	B_4	B_5	B_6	B_7	B_8	B_9	B_{10}
gallic acid	0.91	–	3.7	–	2.7	–	–	–	–	–	–
HMF	1.5	4.3	5.7	6.9	7.2	7.1	7.6	8.2	8.1	8.6	10.1
protocatechuic acid	0.42	–	–	1.4	4.5	–	–	–	–	–	–
caffeoyltartaric acid	–	–	–	0.56	–	–	–	–	–	–	–
protocatechualdehyde	–	–	1.3	1.1	1.7	1.1	1.0	1.3	1.2	1.9	2.5
cumaroyltartaric acid glycoside	1.8	2.4	2.0	–	–	1.6	1.8	–	2.2	0.83	0.5
p-hydroxybenzoic acid	0.09	3.2	–	–	0.13	–	0.15	0.21	–	–	–
2-furaldehyde	1.3	4.3	4.5	3.3	–	2.2	–	1.5	1.6	6.7	10.7
tyrosol	8.7	9.6	9.2	2.3	2.5	5.8	8.6	6.3	9.7	8.7	10.3
p-hydroxybenzaldehyde	0.97	1.02	1.1	0.67	0.34	0.79	1.2	0.37	1.1	1.7	2.3
vanillic acid	–	–	–	–	–	0.2	–	–	–	0.81	1.0
vanillin	–	0.31	0.67	1.04	–	1.3	1.4	0.01	1.7	2.1	5.8
syringaldehyde	–	–	0.74	1.4	0.49	0.84	1.1	0.86	1.5	–	–
p-coumaric acid	–	0.54	0.47	0.46	–	–	0.45	0.45	0.46	–	–
coniferaldehyde	–	–	–	0.35	0.41	0.47	0.66	1.65	9.8	7.8	2.6

solution to verify significative changes according to the aging time. For this purpose the phenolic compound data were used, and the normality was tested using the Kolmogorov−Smirnov test. Samples are divided into three groups according to three reference points for aging (90, 180, and 225 days). In each group samples were divided into two subgroups, that is, before and after the reference point. A reference point of 180 days for aging was selected on the basis of the Sherry vinegar checked denomination and origin regulation for vinegar, which states that to be considered as a Sherry vinegar it is necessary to have a minimum of of 180 days of aging in the oak butts. The other two points (90 and 225 days) were choosen to explore if significative changes could take place before and after the regulated period.

ANOVA results demonstrated that at 90 days of aging, 5-(hydroxymethyl)-2-furaldehyde, 2-furaldehyde, vanillin, syringaldehyde, coniferyl aldehyde, and cinnamic acid showed significative change, while at 180 days of aging, significative changes occur for 5-(hydroxymethyl)-2-furaldehyde, vanillic acid, 2-furaldehyde, vanillin, syringaldehyde, and coniferyl aldehyde; however, cinnamic acid is not suffer significant change. At 225 days, besides the former phenolic compounds

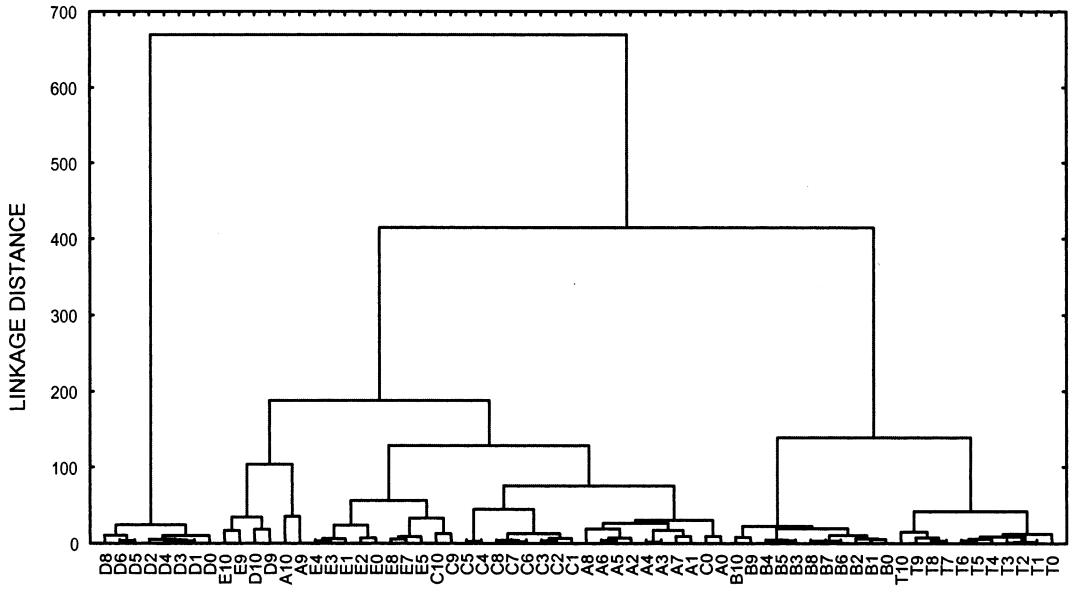

Figure 6. Dendogram obtained from cluster analysis (Ward's method) for 64 samples (including all the phenolic compounds).

Table 8. Evolution of Phenolic Compounds for Vinegar C (mg/L) (–) Compounds Not Detected or Not Quantifyable

	C_0	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
gallic acid	9.4	12.9	14.5	14.4	16.1	15.5	16.0	14.4	16.9	12.7	14.8
HMF	0.49	4.2	4.8	5.9	6.7	6.7	6.1	7.0	6.3	8.8	11.2
protocatechuic acid	–	–	–	–	2.2	2.1	–	–	–	–	–
caffeoyltartaric acid	25.5	26.4	24	23.5	23.4	23.1	24.6	23.6	21.5	16.1	12.4
protocatechualdehyde	–	12.5	–	–	1.9	1.7	–	–	–	–	–
cumaroyltartaric acid glycoside	12.2	12.3	11.3	11.2	–	–	11.4	11.9	11.1	11.8	12.3
2-furaldehyde	0.29	4.0	3.1	5.6	3.6	8.5	5.6	2.4	–	4.1	5.3
cumaroyltartaric acid	12.6	13.1	12	11.9	8.6	9.1	12.0	12.3	11.9	14	14.7
tyrosol	11.7	12.2	11.4	12.2	–	–	12.0	12.7	13.2	16.2	18.2
p-hydroxybenzaldehyde	–	1.0	–	–	–	–	–	–	–	1.9	2.6
vanillic acid	–	–	–	–	–	–	–	–	–	2.5	4.2
caffeic acid	1.3	1.3	1.5	1.5	1.5	1.6	1.3	1.6	1.5	1.2	0.82
gallic ethyl ester	–	1.0	1.3	1.8	1.9	1.9	2.0	2.1	2.1	3.0	–
vanillin	–	0.43	0.73	0.99	0.91	0.92	1.1	1.2	1.4	3.4	5.6
syringaldehyde	–	2.3	3.1	3.8	4.6	4.6	4.3	4.5	4.6	6.7	7.3
p-coumaric acid	2.8	2.6	2.5	2.4	2.6	2.5	2.2	2.2	2.3	2.8	9.2
ferulic acid	–	–	0.30	0.32	0.20	–	–	–	–	–	7.1
isoquercitrin	–	–	0.87	0.54	–	–	0.49	0.4	0.46	–	–
rutin	0.15	1.9	–	–	–	–	–	–	–	–	–
caffeic ethyl ester	0.57	0.5	0.36	0.35	0.35	0.35	0.38	0.38	0.33	–	–
coniferaldehyde	–	7.52	10.7	12.9	13.5	13.1	13.8	14.3	14.5	18.0	26.8
t-cinnamic acid	–	0.03	0.05	0.06	0.06	0.06	0.07	0.08	0.08	–	–

mentioned at 180 days of aging, gallic acid also attributes significative change. From these results we can conclude that at 180 days of aging, for all the samples as well as the model vinegar solution, significant changes occur statistically.

Cluster Analysis. Two cluster analyses were performed in the experimental vinegars following the Ward's method (*37*). In the first cluster analysis, all the phenolic compounds determined during two years were included as variables. Samples were divided into seven clusters, according to the wine substrate (**Figure 6**). There is a certain tendency for clusters to be divided into subclusters that amalgamate samples within the smallest distance following their aging time (i.e., sample D, **Figure 6**). From these results, we can conclude that the original substrate plays an important role on the characteristics of the finished products.

A second cluster analysis was performed considering only those phenolic compounds accounting significative changes at 180 days. In this case samples were grouped according to the aging time. The right extreme grouped samples at the "0" stage (**Figure 7**). In the opposite extreme, another cluster groups samples accounting for aging periods greater than or equal to 540 days.

Discriminant Analysis. The forward stepwise method was applied (*35*) to the whole data set of experimental vinegars to check the validity of phenolic compounds to classify samples according to the age of vinegars. As a training set, vinegar samples were divided into three groups according to aging time (0, 180 and 360 days). Once the method was applied, samples were all correctly classified (100%). Variables enclosed in the model by their discriminating power in accordance with Wilks' λ criterion are given in **Table 9**. The representation of samples in the discriminant space is shown in **Figure 8**. As can be seen, samples are clearly grouped according to aging time.

The utility of the discriminant functions obtained for the training set were then tested employing another 38 vinegar samples (Test set). After applying the classification functions to the test set, an overall success in classification of 97.4% was obtained. All the samples aged in wood are correctly classified (100%) and a 96% of nonaged samples. Hence, the constructed

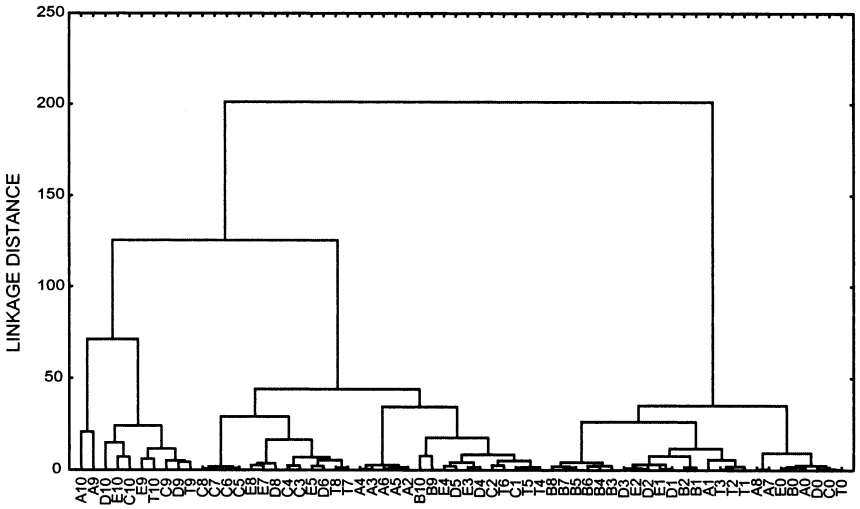

Figure 7. Dendogram obtained (cluster analysis) including those phenolic compounds accounting significative changes at 180 days of aging.

Table 9. Linear Discriminant Analysis[a]

variables	Wilks' λ	Parcial λ	F-remove	p-level	tolerance	1-tolerance (R^2)
vanillin	0.005435	0.01689	116.4116	0.000285	0.002741	0.997259
2-furaldehyde	0.014352	0.006397	310.6708	0.000041	0.001123	0.998877
HMF	0.009943	0.009233	214.612	0.000085	0.003845	0.996155
t-cinnamic acid	0.002789	0.032918	58.7568	0.001084	0.012333	0.987667
coniferyl aldehyde	0.004841	0.018962	103.4752	0.000360	0.004165	0.995835
protocatechuic acid	0.005399	0.017003	115.6283	0.000289	0.005745	0.994255
syringaldehyde	0.002264	0.040551	47.3209	0.001644	0.001512	0.998488
resveratrol	0.003523	0.02606	74.7446	0.000619	0.015759	0.984241
cumaroyltartaric acid	0.002264	0.041477	46.219	0.001720	0.01352	0.98648
p-hydroxybenzaldehyde	0.00063	0.146263	11.674	0.021393	0.03302	0.96698
p-hydroxybenzoic acid	0.00056	0.163203	10.2546	0.026635	0.09293	0.90707
vanillic acid	0.00024	0.379851	3.2652	0.144287	0.172907	0.827093

[a] Inclusion of Variables in the Model.

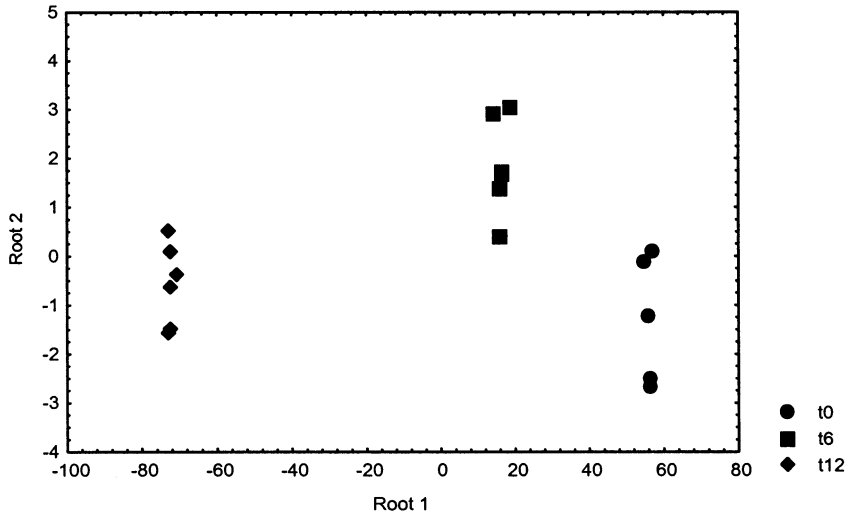

Figure 8. Linear discriminant analysis. Representation of training samples in the discriminant space.

model has a great utility for discriminating aged vinegars from nonaged ones.

CONCLUSION

During aging, acidity, dry extract, and total phenolic index increase for all the vinegars studied and the model solution. The last two parameters (dry extract and total phenolic index) clearly indicate the extraction of phenolic compounds from wood. The presence of residual alcohol in major quantities is determinant for minimizing losses of water.

Phenolic compounds are used to perform the statistical analysis. From ANOVA results, significative changes occur for 5-(hydroxymethyl)-2-furaldehyde, vanillic acid, 2-furaldehyde, vanillin, syringaldehyde, and coniferylaldehyde at 180 days of aging for all the samples as well as the model vinegar solution. Applying discriminant analysis, the models constructed from phenolic data set correctly classified the training samples (100%)

according to aging time. The validity of these classifying functions are checked with another 38 wine vinegar samples. A total of 97.4% of the samples is correctly classified, proving that the constructed models are useful to discriminate between aged vinegars and nonaged ones.

LITERATURE CITED

(1) Tesfaye, W.; Morales, M. L.; García-Parrilla, M. C.; Troncoso, A. M. Wine Vinegar: technology, authenticity and quality evaluation. *Trends Food Sci. Technol.* **2002**, *13*, 12−21.

(2) Adams, M. R. Vinegar. In *Microbiology of Fermented Foods*, 2nd ed.; Wood, B. J. B., Ed.; Thomson Science: London, 1998; Vol. 1, pp 1−44.

(3) Consejería de Agricultura y Pesca. Reglamento de la Denominación de Origen "Vinagre de Jerez" y de su Consejo Regulador. *Boletín Oficial Junta Andalucía* **2000**, *33*, 4015.

(4) Puech, J. L. Extraction and evolution of lignin products in Armagnac matured in oak. *Am. J. Enol. Vitic.* **1981**, *32*, 111−114.

(5) Puech, J. L.; Leauté, R.; Clot, G.; Nomdedeu, L.; Mondiés, H. Evolution de divers constituants volatils et phénoliques des eaux de vie de cognac au cours de leur vieillissement. *Sci. Aliments* **1968**, *4*, 65−80.

(6) García-Parrilla, M. C.; Heredia, F. J.; Troncoso, A. M. Sherry wine vinegars: phenolic composition changes during aging. *Food Res. Int.* **1999**, *32*, 433−440.

(7) Sauvageot, F.; Feuillat, F. The influence of oak wood (*Quercus robur L., Q. petraea Liebl.*) on the flavor of Burgundy Pinot noir. An examination of variation among individual trees. *Am. J. Enol. Vitic.* **2000**, *50*, 447−455.

(8) Spillman, P. J.; Pollnitz, A. P.; Liacopoulos, D.; Skouroumounis, G. K.; Sefton, M. A. Accumulation of vanillin during barrel-aging of white, red and model wines. *J. Agric. Food Chem.* **1997**, *45*, 2584−2589.

(9) Pino, J. A.; Marbot, R.; Perez, J.; Nuñez de Villavicencio, M. Application of multivariate statistical methods to differentiation of rum aged in two types of oak barrels. *J. Food Qual.* **1998**, *22*, 183−191.

(10) Morales, M. L. Cambios fisicoquímicos en las distintas etapas de producción de vinagres de Jerez. Thesis, Universidad de Sevilla, Sevilla, Spain, 1999.

(11) Lebrun, L. Used barrels and resulting aromas. The Australian Grapegrow. *Winemaker* **1991**, July: 21−22.

(12) Puech, J. L. Extraction of phenol compounds from oak wood in model solutions and evolution of aromatic aldehydes in wines aged in oak barrels. *Am. J. Enol. Vitic.* **1987**, *38*, 236−238.

(13) Pérez-Coello, M. S.; González-Viñas, M. A.; García-Romero, E.; Cabezudo, M. D.; Sanz, J. Chemical and sensory changes in white wines fermented in the presence of oak chips. *Int. J. Food Sci. Technol.* **2000**, *35*, 23−32.

(14) Pocock, K. F.; Sefton, M. A.; Williams, P. J. Taste thresholds of phenol extract of French and American oakwood: the influence of oak phenols on wine flavor. *Am. J. Enol. Vitic.* **1994**, *45*, 429−434.

(15) Tesfaye, W.; García-Parrilla, M. C.; Troncoso, A. M. Sensory evaluation of Sherry wine vinegars. *J. Sensory Stud.* **2002**, *17*, 133−144.

(16) Aiken, J. W.; Noble, A. C. Composition and sensory properties of Cabernet Sauvignon wine in French versus American oak barrels. *Vitis* **1984**, *23*, 27−36.

(17) Puech, J. L. Phenolic compounds in oak wood extracts used in the aging of brandies. *J. Sci. Food Agric.* **1988**, *42*, 165−172.

(18) Puech, J. L.; Mertz, C.; Michon, V.; Guernevé, C. L.; Doco, T.; Hervé du Penhoat, C. Evolution of castalagin and vescalagin inethanol solutions. Identification of new derivatives. *J. Agric. Food Chem.* **1999**, *47*, 2060−2066.

(19) Nieto, J.; González-Viñas, M. A.; Barba, P.; Martín-Álvarez, P. J.; Aldave, L.; García-Romero, E.; Cabezudo, M. D. Recent progress in wine vinegar R & D and some indicators for the future. In *Food Flavor, Ingredients and Composition*; Charalambous, G., Ed.; Elsevier: New York, 1993; pp 469−499.

(20) Marco, J.; Artajona, J.; Larrechi, J. M.; Rius, F. X. Relationship between geographical origin and chemical composition of wood for oak barrels. *Am. J. Enol. Vitic.* **1994**, *45*, 192−200.

(21) Towey, J. P.; Waterhouse, A. L. The extraction of volatile compounds from French and American oak barrels in Chardonnay during three successive vintages. *Am. J. Enol. Vitic.* **1996**, *47*, 63−172.

(22) Singleton, V. L. Some aspects of the wooden container as a factor in wine maturation. *In Adv. Chem. Ser.* **1974**, *137*, 254−277.

(23) Kadim, D.; Mannheim, C. H. Kinetics of phenolic extraction during aging of model wine solution and white wine in oak barrels. *Am. J. Enol. Vitic.* **1999**, *50*, 33−39.

(24) Artajona Serrano, J. Caracterización del roble según su origen y grado de tostado mediante GC y HPLC. *Vitic.-Enol. Profes.* **1991**, *14*, 62−71.

(25) Nivas, N.; Glories, Y. Sistema de secado de madera de roble para tonelería. *Viti—Vinic.* **1993**, *5−6*, 47−50.

(26) Chatonnet, P.; Boidron, J. N.; Pons, M. Incidence du traitement thermique du bois de chêne sur sa composition chimique. 2° Partie: Évolution de certains composés en fonction de Ìintensité de brûlage. *J. Int. Sci. Vigne Vin.* **1989**, *23*, 223−250.

(27) Tesfaye, W.; García-Parrilla, M. C.; Troncoso, A. M. Set up and optimization of a laboratory scale fermentor for the production of wine vinegars. *J. Inst. Brew.* **2000**, *106*, 215−219.

(28) García-Parrilla, M. C.; Heredia, F. J.; Troncoso, A. M. Phenols HPLC analysis by direct injection of Sherry wine vinegar. *J. Liq. Chromatogr. Relat. Technol.* **1996**, *19*, 274−258.

(29) Roggero, J. P.; Coen, C.; Archier, P. Wine phenolics: optimization of HPLC analysis. *J. Liq. Chromatogr.* **1990**, *13*, 2593−2603.

(30) Roggero, J. P.; Archier, P.; Coen, C. Wine phenolics analysis via direct injection: enhancement of the method. *J. Liq. Chromatogr.* **1991**, *14*, 533−538.

(31) Presidencia del Gobierno. Orden de 31 de enero de 1977, sobre Métodos Oficiales de Análisis de productos derivados de la uva. *Boletín Oficial Estado* **1977**, *178*, 15800−15808.

(32) Plessi, M.; Monzani, A.; Coppini, D. Determination of the monosaccharide and alcohol content of balsamic and other vinegars by enzymatic methods. *Agric. Biol. Chem.* **1988**, *52*, 25−30.

(33) Henniger, G. A Fast Enzymatic Method for the Determination of Ethanol in Alcohol Products, Poster-presentation in Association of Official Analytical Chemists Annual International Meeting, San Francisco, CA, 1987.

(34) Folin, C.; Ciocalteu, V. Tyrosine and tryptophan determination in proteins. *J. Biol. Chem.* **1927**, *73*, 627−650.

(35) Statsoft, Inc. *Statistica for Windows* [Computer Program Manual]. Tulsa, OK, 1995.

(36) Singleton, V. L. Maturation of wines and spirits: comparisons, facts, and hypotheses. *Am. J. Enol. Vitic.* **1995**, *46*, 98−115.

(37) Ward, J. H. Hierarchical grouping to optimize an objective function. *J. Am. Statis. Assoc.* **1963**, *58*, 236.

(38) Miller, J. C.; Miller, J. N. Error in instrumental analysis. In *Statistics for Analytical Chemistry*, 3rd ed.; Ellis Horwood Limited: Chichester, U.K., 1993; pp 115−117.

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