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Chemometric Studies of Vinegars from Different Raw Materials and Processes of Production

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The phenolic composition, aroma compounds, and organic acid content of 83 vinegars have been determined. Multivariate analysis techniques have been used to classify these vinegar samples according to raw material (white wine, red wine, apple, honey, alcohol, balsamic, and malt) and production process (with and without aging in wood). Cluster analysis grouped the samples according to production process. Only apple and balsamic vinegars were separated from wine vinegars. Alcohol, honey, and malt vinegars were grouped with no aged wine vinegars. Linear discriminate analysis allowed a 88% differentiation according to raw material and 100% according to aging in wood. Besides, from the results obtained, a major role of the volatile compounds in the differentiation of the vinegar samples according to their aging period in wood can be seen.

KEYWORDS: Vinegar; multivariate analysis; polyphenols; volatile compounds; organic acids

INTRODUCTION

In the past few years, the importance of vinegar as a food product has been increasing. It is produced by a double fermentation process from a variety of raw materials (white and red wine, cider, malted barley, honey, pure alcohol, etc.) and by a variety of different methods. Its chemical and organoleptic properties are determined by the acetification system used, the raw material used as the substrate, and, in some cases, by the length of time it is aged in wood.

Methods of making vinegar can be divided in two groups: slow methods in which the culture of acetic acid bacteria, due to its requirement of oxygen, grows on the surface of the liquid contained in a wood barrel and quick processes in steel tanks with a submerged culture of bacteria where the oxygenation is favored by agitation. Traditional wine vinegars are produced by slow processes of acetification, which usually involve a certain period of aging in wood.

Sherry wine vinegar, from the Jerez-Xérès-Sherry, Manzanilla de Sanlúcar and Vinagre de Jerez Denomination of Origin (D. O.) region (SW Spain), produced from Sherry wines following traditional methods of acetification (*1*), is now a high quality product on a par with the wines and brandies typical of this region. This vinegar from Spain and balsamic vinegar from Italy are the highest recognized vinegars (*2*).

As important as obtaining a specific quality vinegar is the need to determine objectively the appropriate parameters that allow us to characterize and differentiate it from the other ones. Several studies have been carried out to characterize this product using different analytical parameters and several chemometric techniques (*3*−*7*). The polyalcohol content was an adequate tool to differentiate vinegars from different raw materials using multivariate variance analysis and cluster analysis (CA) (*3*).

García-Parrilla et al. (*4*) applied pattern recognition techniques to distinguish wine vinegars obtained from different wines and different acetification processes using, for this purpose, their polyphenolic content.

Benito et al. (*5*) demonstrated that it is possible to characterize the vinegars obtained from wine with certified denomination of origin Rioja and Jerez according to analytical parameters such as acidity, dry extract, ash, pH, chlorides, organic acids, proline, 3-hydroxy-2-butanone, glycerol, etc.

Four metals (Fe, Mg, Mn, and Na) were used to differentiate quick- and slow-processed vinegars (*6*). Excellent percentages of classification were obtained using LDA, KNN, and BPANN.

Some aroma compounds and organic acids were considered to carry out the differentiation of wine vinegars produced from different wine substrates and different acetification methods (*7*). As can be seen, the attempts to differentiate vinegars have been based either on the type of raw material employed or on the kind of process involved, but scarce literature about both criteria at the same time has been found. Besides, few studies have been carried out about vinegars from raw materials unlike wine or using volatile compounds as possible discriminant variables.

The main objective of this paper was to develop several pattern recognition approaches that permit classification of the vinegar samples according to raw material (wine, cider, alcohol, etc.) and production process using different analytical parameters, such as polyphenolic content, organic acids, and volatile compounds.

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Table 1. Vinegar Category and Identification Code

vinegar category		identification code	no. of samples
white wine	with aging in wood	WWW	28
	without aging in wood	WW	27
red wine	with aging in wood	RWW	2
	without aging in wood	RW	4
balsamic	with aging in wood	B	6
honey		H	2
alcohol		A	2
apple		AP	11
malt		M	1

MATERIAL AND METHODS

Vinegar Samples. Eighty-three vinegar samples obtained from different raw materials (white and red wine, cider, malted barley, honey, and pure alcohol) and produced by different methods (with and without aging in wood) were analyzed. All of them were commercial vinegars bought in Spain. In the case of the vinegar samples with aging in wood, the duration of this period was variable (at least a year); various ones were Sherry wine vinegars. The samples were divided in nine categories according to raw materials and aging method (**Table 1**).

The number of vinegars per category was not uniform because certain vinegar types (alcohol, malt, and honey) are commercialized by few or even just one brand.

Volatile Compounds. Volatile compounds were determined by SPME and gas chromatography (GC). All of the samples were analyzed in duplicate. The SPME method had been previously optimized for the determination of this type of compounds in vinegar (*8*, *9*). Gas chromatography was performed on a GC 8000 chromatograph with a FID detector (Fisons Instruments, Milan, Italy). The injection was made in the splitless mode for 2 min. For the desorption of the analytes inside the GC injection port, the temperature was 280 °C. The GC was equipped with a DB-Wax capillary column (J&W Scientific, Folsom, CA), 60 m × 0.25 mm i.d., with a 0.25 μm coating. The carrier gas was helium at a flow rate of 1.1 mL/min. The detector temperature was 250 °C. The GC oven was programmed as follows: held at 35 °C for 10 min, then ramped at 5 °C/min to 100 °C. Then it was raised to 210 °C at 3 °C/min and held for 40 min.

The compounds were identified by mass spectrometric analysis. In these analyses, the same GC coupled to a MD 800 mass detector (Fisons Instruments) was used. The mass detector was operated in the EI^+ mode at 70 eV in a range of 30−450 amu. GC analytical conditions were the same as described above.

The signal was recorded and processed with Masslab software supplied with the Wiley 6.0 MS library. Peak identification was carried out by analogy of mass spectra and confirmed by retention indices of standards. All of the aroma standards used in this study were supplied by Merck (Darmstadt, Germany) and Sigma (Steinheim, Germany).

Each compound was quantified by comparison with a calibration curve obtained with the corresponding standard. Quantitative data from the identified compounds were obtained by measuring the relative peak area in relation to that of 4-methyl-2-pentanol, the internal standard.

Organic Acids. The organic acid content of each vinegar was analyzed in duplicate by capillary electrophoresis using a method previously set up by the authors (*10*). A Waters Capillary Ion Analyzer (Milford, MA) was equipped with an UV−visible detector of wavelength set at 185 nm. The fused-silica capillary utilized had an effective length of 57 cm (60 cm of total length) with 75 μm i.d. Samples were introduced hydrostatically into the capillary (height 10 cm). The injection time was 30 s, and the temperature was 20 °C. The voltage applied was 7 kV, using a negative feed source. The conditions of the electrolyte were as follows: 10×10^{-3} M of sodium tetraborate at pH 9.3; 0.5×10^{-3} M of TTAOH as organic flow modifier; and 10 mg L^{-1} of Ca^{2+} and Mg^+ (added in the form of chlorides) as complexing agents.

Polyphenolic Compounds. Eighty microliters of vinegar after filtration (0.45 μm pore size) were analyzed by high-performance liquid chromatography (HPLC) in duplicate. The elution phases used were solvent A (95% water, 5% methanol) and solvent B (95% methanol, 5% water) at pH 2.5 (extra pure sulfuric acid). The elution gradient was from 100 to 85% solvent A in 5 min; from 85 to 50% solvent A in 40 min; and isocratic elution for 35 min. The analyses were carried out using a C_{18} column (Lichropher 100 RP-18, 250 mm × 3 mm, 5 mm particle size) at a flow rate of 0.5 mL/min and detection at 280 and 320 nm.

The various polyphenolic compounds present were identified by comparison with a library of DAD spectra and retention times of standards. Commercial standards were purchased from Fluka (Buchs, Switzerland) and Eastman Kodak (Rochester, NY). Caftaric and coutaric acids were isolated by the method described by Singleton et al. (*11*). Each compound was quantified by comparison with a calibration curve obtained with the corresponding standard, except GRP, which was quantified as caftaric.

Pattern Recognition Techniques. Univariate analysis and multivariate analysis of data, including CA and LDA, were performed using the Statgraphics Statistical Computer Package "Statgraphics Plus 5.0" for Windows 98.

RESULTS AND DISCUSSION

High standard deviations were found for some of the compounds considered (**Tables 2**−**4**). It could be explained on the basis of a close relationship between the content of this type of compounds and the raw material together with the specific conditions of the production process. With regard to volatile content found for the vinegar samples studied, **Table 2** shows the mean values and standard deviations calculated for each volatile compound concentration of all vinegar categories of **Table 1**.

The major volatile compounds quantified were 2- and 3-methyl-1-butanol, 3-hydroxy-2-butanone, 2-phenylethanol, 2,3-butanediol, and isopentanoic acid. 2- and 3-Methyl-butanol have been found in other wine vinegars in a range of 10−100 mg/L (*12*). The 3-hydroxy-2-butanone content found ranged from 18 mg/L for malt vinegar to 227 mg/L for the apple vinegars. A high content in 3-hydroxy-2-butanone for apple vinegars has been observed by other authors (*2*). It was justified as a consequence of a low aeration during the acetification process.

Wine and apple vinegars showed a high content in 2,3-butanediol. Wine vinegars aged in wood exhibited, in general, higher concentrations in 2,3-butanediol and 3-hydroxy-2-butanone than those without aging. Palacios et al. (*13*) found that the 3-hydroxy-2-butanone content increased in sherry vinegars during their aging in wood as a consequence of the transformation of butyleneglycol into this compound during the process of the acetic fermentation and of the general water loss produced during this period by evaporation.

4-Ethylguaiacol and 4-ethylphenol, which had already been identified in red wine vinegars (*14*), appear in a major amount for apple vinegars. Among the esters identified, which result from the fermentation of alcohols or by the reaction of acids with alcohols during aging, the major compounds were diethyl succinate, 2-phenylethyl acetate, isoamyl acetate, and *n*-butyl acetate.

The malt vinegar sample studied was very unaromatic. This could be explained due to a deficient raw material and/or poor process of aging in wood.

In relation to organic acid content, vinegars contain organic acids of the volatile type (acetic, etc.) and the nonvolatile type (tartaric, citric, malic, succinic, etc.). The means and the standard deviations found for each organic acid concentration of all vinegar categories studied are shown in **Table 3**. The acid that identifies the product as vinegar from the outset is acetic acid. Its content may vary depending on the carbohydrate substrate

Table 2. Means (mg/L) and Standard Deviations of Aromatic Compounds Found for the Different Vinegar Categories; N = Number of Samples for Each Category

compd	WWW ($n = 28$)	WW ($n = 27$)	B ($n = 6$)	RWW ($n = 2$)	RW ($n = 4$)	H ($n = 2$)	A ($n = 2$)	M ($n = 1$)	AP ($n = 11$)
n-butyl acetate	0.666 (0.444)$^{b-e}$	0.410 (0.607)^{d}	0.503 (0.282)^{a}	0.286 (0.025)	0.229 (0.102)^{c}	0.078 (0.022)^{e}	0.014 (0.017)^{a,b}	0.001	0.456 (0.570)
ethyl pentanoate	0.244 (0.216)$^{c,g-j}$	0.116 (0.273)^{f,i}	0.000 (0.015)^{b,c}	0.141 (0.088)	0.003 (0.026)^{e,h}	0.000 (0.007)^{d,g}	nd^{a,j}	0.001	0.278 (0.396)$^{a,b,d-f}$
2-methyl-1-propanol	5.55 (2.87)	5.89 (7.42)	6.53 (4.41)	3.05 (3.056)	4.34 (3.54)	1.78 (0.23)	2.74 (1.33)	0.649	6.32 (5.55)
isoamyl acetate	3.52 (1.78)^{c,i,k,m,n}	1.99 (2.73)^{g,n}	3.57 (1.43)$^{a,d-g}$	3.88 (2.40)^{b,h,j,l}	1.16 (0.44)^{f,l,m}	0.637 (0.067)^{e,j,k}	0.000 (0.002)$^{a-c}$	0.167	1.53 (1.30)^{d,h,i}
ethyl hexanoate	0.048 (0.069)	0.146 (0.262)	0.046 (0.041)	0.038 (0.036)	0.030 (0.023)	0.011 (0.005)	0.006 (0.006)	0.000	0.122 (0.106)
2-methyl-1-butanol	6.76 (9.45)^{f}	4.98 (7.42)^{f}	6.18 (3.46)^{b}	11.1 (34.2)$^{a-g}$	3.46 (2.30)^{e}	2.86 (0.46)^{d}	0.559 (0.567)^{a}	0.856	7.19 (7.76)^{c}
3-methyl-1-butanol	14.02 (11.82)^{f,h}	7.57 (10.26)^{b,g,h}	15.43 (11.05)^{a,b}	27.5 (81.2)$^{a,c-g,i}$	4.91 (2.30)^{e}	2.82 (0.58)^{d}	0.005 (0.846)^{j}	0.002	7.26 (11.64)^{c}
3-hydroxy-2-butanone	176.0 (155.9)^{b,h,i}	93.3 (65.9)^{g,i}	112.8 (41.5)^{c}	49.7 (23.1)^{e,h}	118.3 (56.2)^{f}	105.5 (69.5)^{d}	34.9 (13.5)^{a,b}	18.04	226.9 (115.6)$^{a,c-g}$
benzaldehyde	0.099 (0.200)	0.122 (0.182)	0.023 (0.025)^{a}	0.000 (0.002)^{b}	0.125 (0.083)	0.000 (0.013)^{c}	0.000 (0.002)^{d}	0.059	0.147 (0.191)$^{a-d}$
2,3-butanediol	197.4 (157.3)^{c,h,i}	47.1 (73.9)^{f,i}	128.5 (51.4)	239.3 (185.7)^{b,g}	99.6 (38.0)^{e}	19.7 (19.7)^{d,g,h}	nd^{a-c}	32.6	223.2 (132.0)$^{a,d-f}$
ethyl decanoate	0.002 (0.016)	0.000 (0.013)	0.001 (0.016)	0.000 (0.000)	0.000 (0.003)	0.000 (0.000)	nd	0.000	0.000 (0.000)
isopentanoic acid	32.0 (15.3)^{c,g}	30.6 (21.6)^{d,h}	8.18 (4.20)$^{e-h}$	15.1 (4.0)^{i}	34.6 (8.3)^{b,f}	17.3 (2.5)	0.328 (0.328)$^{a-d}$	7.13	35.0 (17.3)^{a,e,i}
diethyl succinate	1.63 (1.76)	3.75 (8.71)^{a}	1.44 (1.04)	2.25 (2.23)	1.07 (0.87)	0.348 (0.112)	0.000 (0.010)^{a}	0.001	1.61 (1.43)
benzyl acetate	0.106 (0.098)	0.179 (0.424)	0.023 (0.022)	0.000 (0.001)	0.036 (0.049)	0.000 (0.006)	nd	0.000	0.024 (0.063)
ethyl-2-phenyl acetate	0.061 (0.045)$^{a-g}$	0.005 (0.022)^{g}	0.002 (0.011)^{b}	0.000 (0.002)^{e}	0.002 (0.005)^{f}	0.000 (0.005)^{d}	nd^{a}	0.000	0.007 (0.025)^{c}
2-phenylethyl acetate	1.17 (0.79)$^{c,g-i}$	0.577 (0.696)^{f,i}	1.02 (0.38)^{a}	0.117 (0.111)^{e,h}	0.736 (0.460)	0.203 (0.058)^{d,g}	0.000 (0.006)$^{a-c}$	0.000	1.22 (1.10)$^{b,d-f}$
α-ionone	0.000 (0.004)	0.000 (0.002)	0.000 (0.003)	0.000 (0.002)	0.000 (0.003)	0.000 (0.002)	nd	nd	0.000 (0.002)
benzyl alcohol	0.511 (0.555)	1.01 (2.33)	0.826 (0.188)	0.000 (0.032)	0.236 (0.157)	0.000 (0.058)	nd	nd	0.331 (0.795)
2-phenylethanol	24.4 (12.2)^{c,h,j}	15.0 (14.1)^{i,j}	21.5 (8.8)^{a,d}	17.3 (9.5)^{f}	16.6 (6.0)^{g}	9.3 (0.7)^{e}	2.63 (0.03)$^{a-c}$	10.2	43.0 (34.4)$^{b,d-i}$
4-ethylguaiacol	0.002 (0.024)^{f}	0.000 (0.031)^{g}	0.020 (0.030)^{b}	0.000 (0.001)^{d}	0.000 (0.022)^{e}	0.087 (0.096)^{c}	0.000 (0.024)^{a}	0.000	0.239 (0.333)$^{a-g}$
octanoic acid	1.30 (0.77)^{f}	1.11 (1.71)^{g}	1.20 (0.52)^{b}	0.274 (0.142)^{d}	1.12 (0.60)^{e}	0.625 (0.351)^{c}	0.112 (0.048)^{a}	0.498	3.77 (2.72)$^{a-g}$
4-ethylphenol	0.130 (0.091)^{f}	0.079 (0.087)^{g}	0.103 (0.061)^{b}	0.064 (0.030)^{d}	0.084 (0.054)^{e}	0.067 (0.043)^{c}	0.000 (0.001)^{a}	0.000	0.484 (0.461)$^{a-g}$
decanoic acid	0.139 (0.103)^{f,h}	0.094 (0.110)^{f,h}	0.106 (0.165)^{b}	0.033 (0.029)^{d}	0.103 (0.057)^{e}	0.061 (0.037)^{c}	0.008 (0.005)^{a}	0.004	0.273 (0.189)$^{a-g}$

$^{a-n}$Mean values in the same row with the same superscript indicate that there are significant differences between them ($p < 0.05$).

used and on a possible dilution before going out to the market. As expected, levels of acetic acid obtained were higher in the wine vinegar samples, with the exception of balsamic vinegars, which showed a lower content in this acid.

With respect to nonvolatile organic acids, their class and content depend on the type of vinegar that is being analyzed (*15*). Vinegars derived from pure alcohol or from cereals present acetic as the only organic acid contained (*15*).

In apple vinegars, citric and lactic acids were the organic acids present in the largest proportion (**Table 3**). For the malt vinegar studied, lactic acid was the only nonvolatile organic acid found. As could be expected, wine vinegars were characterized by their content of tartaric acid and their relatively little content in malic acid. Red wine vinegars without aging in wood exhibited an abnormally low content in tartaric acid. The malic acid content in wine vinegars depends on the origin of the wine and on the enological techniques to which it has been subjected. This acid is converted into lactic acid during the malolactic fermentation; therefore, the ratio found for the vinegar's content of these two acids can be indicative of the degree to which this key fermentative process had developed in the particular raw material. It could explain the high content in lactic acid found for apple vinegars, taking into account that apples usually present high malic acid content. In turn, the content in lactic acid can be reduced during the acetic fermentation. Malic acid was not detected in red wine vinegars without wood, which presented a high content in lactic acid. A high content in malic acid was obtained for balsamic vinegars, as had already been pointed out by Plessi et al. (*16*).

The proportions in which citric acid, which comes from the grape, and succinic acid, which forms during the alcoholic fermentation, are present can sometimes be reduced by the presence of certain microorganisms, which have the effect of transforming both of these into acetic acid (*17*). In our case,

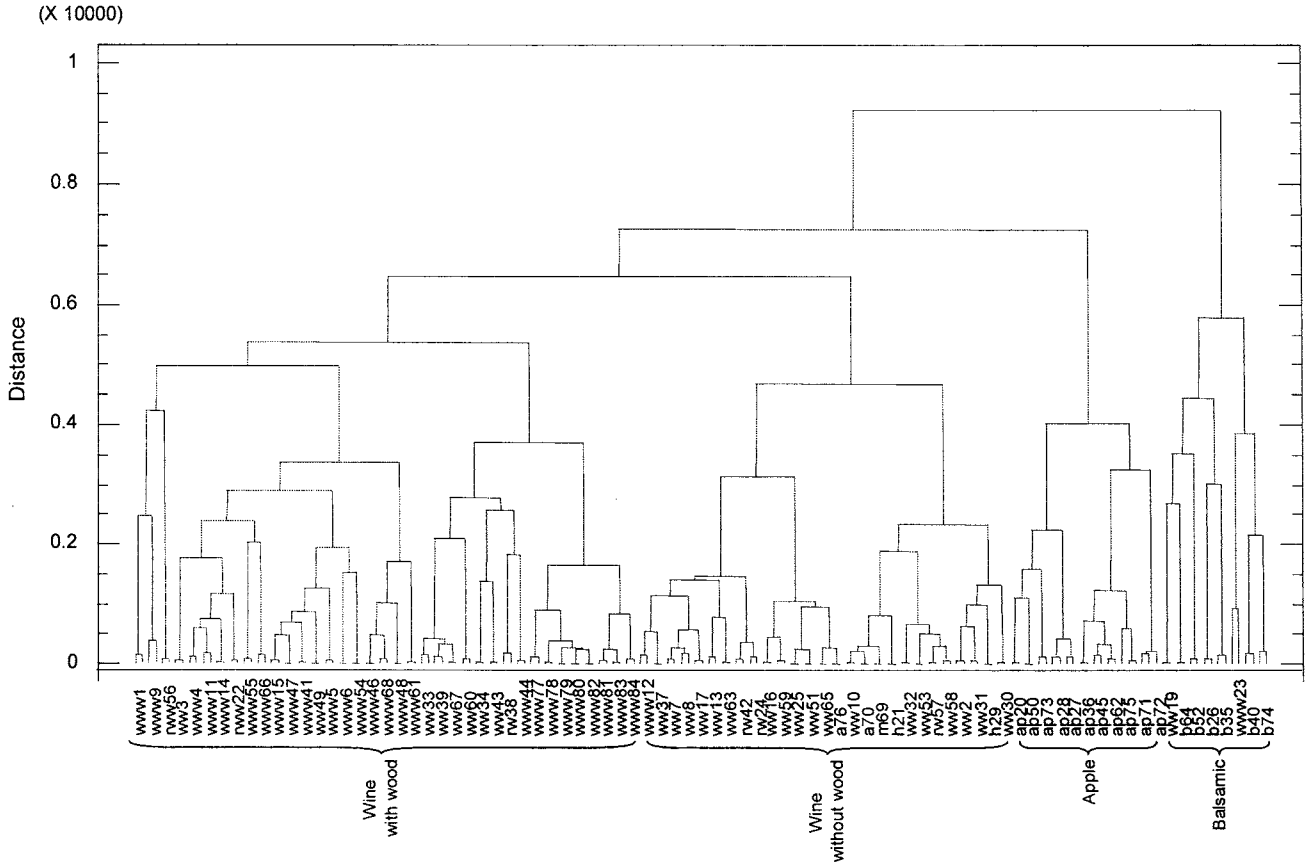

Figure 1. Dendrogram obtained after hierarchical agglomerative CA performed on polyphenols, volatile compounds, and organic acids of all samples studied. Sample codes in **Table 1**.

Table 3. Means (g/L) and Standard Deviations of Organic Acids Found for the Different Vinegar Categories; N = Number of Samples for Each Category

compd	WWW ($n = 28$)	WW ($n = 27$)	B ($n = 6$)	RWW ($n = 2$)	RW ($n = 4$)	H ($n = 2$)	A ($n = 2$)	M ($n = 1$)	AP ($n = 11$)
succinic acid	0.263 (0.134)^{e,k}	0.200 (0.113)^{f,h,l,m}	0.365 (0.212)^{a,g,h}	0.398 (0.120)^{c,j,l}	0.393 (0.282)^{d,i,m}	0.273 (0.048)^{b}	nd^{a-f}	nd	0.135 (0.157)$^{g,i-k}$
malic acid	0.107 (0.131)^{g,l,o}	0.066 (0.153)^{h,m,p}	0.722 (0.600)$^{a,d-h}$	0.337 (0.095)$^{c,e,j,n-p}$	nd^{f,k,n}	0.515 (0.381)$^{b,i,k-m}$	nd^{a-c}	nd	0.086 (0.195)^{d,i,j}
tartaric acid	1.02 (0.94)^{c,j,m,n,p,q}	0.537 (0.626)^{h,k,o,q}	1.37 (0.69)$^{a,d-h}$	2.20 (0.51)^{b,f,i,l,n,o}	0.098 (0.121)^{g,n,p}	0.212 (0.106)^{e,l,m}	nd^{a-c}	nd	nd$^{d,i-k}$
acetic acid	84.9 (17.2)^{a,c,f,h}	79.6 (25.1)^{d,g}	51.1 (21.5)$^{b-d}$	82.6 (17.4)	86.8 (29.2)^{b,e}	67.6 (20.4)^{h}	66.6 (13.9)^{a}	50.9	56.7 (12.8)$^{e-g}$
lactic acid	0.623 (0.436)^{j}	0.488 (0.420)^{j,k}	0.832 (0.727)^{a,e}	0.476 (0.021)^{g}	1.03 (0.87)^{d,h,k}	1.11 (1.11)^{c,f}	nd^{a-d}	0.305	2.02 (1.50)$^{b,e-j}$
citric acid	0.124 (0.192)^{b}	0.275 (0.889)^{c}	0.657 (0.666)$^{a-c}$	0.402 (0.402)	nd^{a}	nd	nd	nd	0.157 (0.378)

$^{a-q}$Mean values in the same row with the same superscript indicate that there are significant differences between them ($p < 0.05$).

the highest content in succinic acid was found for balsamic and red wine vinegars. For the honey vinegar samples, the only organic acid that was absent was citric acid.

In the case of the polyphenolic content found for the samples analyzed, the results obtained are shown in **Table 4**. The gallic acid content obtained for the wine vinegar samples with aging in wood (white wine with wood, red wine with wood, and balsamic) was the highest. According to the literature (*18*), the content in this polyphenolic compound increases with the aging in wood. This fact is explained by the hydrolysis of gallic tannins during aging.

The presence of 5-hydroxymethylfurfuraldehyde in vinegar has been traditionally attributed to must caramel addition. Some authors have found a clear increase of this compound during aging in wood (*18*−*20*), which implied that the presence of this could not be considered exclusively as an indicator of this addition. In our case, the highest content for this compound was found for balsamic vinegars. Two facts, the aging in wood and the process of production (with a slow heating process), should explain the high content in 5-hydroxymethylfurfuraldehyde and 2-furaldehyde found for this particular vinegar (*21*).

Especially remarkable are the mean levels of aldehydes (*p*-hydroxybenzaldehyde, protocatechualdehyde, and syringaldehyde) found for the categories white wine with wood, red wine with wood, and balsamic, all of them subjected to aging in wood. As can be seen, these data underline the analytical differences found for the different vinegars considered in this study in line with their raw material and production process.

Table 4. Means (mg/L) and Standard Deviations of Polyphenols Found for the Different Vinegar Categories; N = Number of Samples for Each Category

compd	WWW ($n = 28$)	WW ($n = 27$)	B ($n = 6$)	RWW ($n = 2$)	RW ($n = 4$)	H ($n = 2$)	A ($n = 2$)	M ($n = 1$)	AP ($n = 11$)
gallic acid	36.8 (23.8)^{c,g,k,m,p,q}	10.4 (10.8)^{h,j,q}	64.5 (10.2)$^{a,d-h}$	46.1 (9.5)^{b,f,i,l,n,o}	18.0 (10.0)$^{n-p}$	3.26 (0.50)^{e,l,m}	nd^{a-c}	5.31	4.02 (2.34)$^{d,i-k}$
hydroxymethyl-furfural	48.2 (55.6)$^{b,g,i-k}$	9.68 (32.06)^{h,k}	213.6 (58.5)$^{a,c-h}$	15.9 (15.9)^{e,f}	9.71 (13.87)^{j}	23.6 (18.5)^{d}	0.519 (0.521)^{a,b}	8.56	1.79 (1.82)^{c,i}
2-furaldehyde	6.53 (7.34)$^{b,g,i-l}$	1.14 (1.82)^{h,l}	18.5 (7.4)$^{a,c-h}$	2.74 (2.74)^{e}	0.837 (0.975)^{f,k}	0.995 (0.890)^{d,j}	nd^{a,b}	3.21	0.747 (0.887)^{c,i}
protocatechuic acid	9.02 (10.49)	7.45 (8.54)	13.40 (29.94)^{a}	15.3 (2.5)	10.1 (4.1)	4.60 (1.27)	nd^{a}	nd	8.72 (3.29)
tyrosol	54.4 (44.5)^{d,h,k,n,p}	30.0 (15.4)^{i,o,p}	109.4 (60.1)$^{a,e-i}$	105.5 (55.7)$^{b,j,l-o}$	41.6 (13.3)^{c,g,m}	31.6 (4.99)^{f,l}	nd^{a-d}	8.79	25.1 (9.4)^{e,j,k}
catechin	22.3 (40.2)^{g}	7.63 (9.10)^{c,h}	28.5 (41.0)^{b,c}	17.0 (17.0)^{e}	19.3 (19.0)^{f}	36.2 (32.5)^{d}	nd^{a}	nd	82.6 (46.0)$^{a-h}$
p-hydroxybenzoic acid	4.53 (6.40)$^{a,c-f}$	1.11 (2.24)^{f}	nd^{b,c}	nd^{d}	0.504 (0.943)^{e}	3.95 (1.37)	nd^{a}	nd	2.91 (2.88)^{b}
p-hydroxybenz-aldehyde	2.75 (2.18)^{c,g,j,m,n}	0.530 (1.048)^{h,l,n}	4.90 (4.32)$^{a,d-h}$	3.11 (0.15)^{b,i,k,l}	nd^{f,k,m}	0.929 (0.183)^{e}	nd^{a-c}	nd	0.195 (0.458)^{d,i,j}
vanillic acid	3.35 (5.25)^{d,g,k}	0.598 (1.669)$^{i-k}$	nd^{b-d}	5.34 (3.68)^{a,b,e,h,i}	3.52 (3.37)^{c,f,j}	nd^{h}	nd^{a}	nd	nd^{e-g}
epicatechin	15.7 (18.6)^{e,j}	10.0 (15.4)^{k}	nd^{c-e}	nd^{g,i}	12.0 (4.6)$^{b,d,h-k}$	nd^{f,h}	nd^{a,b}	nd	17.9 (13.9)^{a,c,f,g}
syringic acid	5.11 (5.23)^{b,d,f,h,j,k}	1.60 (3.60)^{i,k}	nd^{c,d}	3.45 (1.23)$^{a,c,e,g-i}$	nd^{g,j}	3.40 (3.04)	nd^{a,b}	nd	nd^{e,f}
caftaric acid	10.1 (5.9)^{c,i,l,o,q}	7.65 (8.26)^{d,j,m,r}	36.5 (7.0)$^{a,e-j}$	19.3 (6.0)$^{b,g,k,n,p-r}$	6.52 (3.88)^{h,p}	3.17 (1.44)^{f,n,o}	nd^{a-d}	nd	2.83 (5.12)$^{e,k-m}$
GRP	0.644 (0.784)^{d,g,l}	0.860 (1.075)^{b,e,h,j,m}	nd^{c-e}	2.39 (0.052)$^{a,c,f,i,k-m}$	0.640 (0.638)^{k}	nd^{i,j}	nd^{a,b}	nd	nd^{f-h}
protocatechu-aldehyde	1.60 (3.45)^{g,j,m}	0.400 (1.038)^{h,l,m}	15.7 (2.4)$^{a,c-h}$	3.26 (1.23)^{b,e,i,k,l}	nd^{f}	nd^{d,k}	nd^{a,b}	nd	nd^{c,i,j}
cis-*p*-coutaric acid	2.43 (1.89)^{a,c,e,g}	1.71 (1.92)^{b,d,f,g}	0.686 (1.534)^{c,d}	1.00 (1.00)	1.42 (0.38)	0.941 (0.229)	nd^{a,b}	nd	0.237 (0.316)^{e,f}
trans-*p*-coutaric acid	5.39 (2.46)^{c,i,l,o,q,s}	3.534 (3.37)^{d,j,m,r,s}	21.1 (6.4)$^{a,e-j}$	11.4 (3.7)$^{b,g,k,n,p-r}$	3.08 (1.44)^{h,p}	1.73 (0.77)^{f,n,o}	nd^{a-d}	nd	1.23 (0.68)$^{e,k-m}$
fertaric acid	1.03 (0.98)^{a,c,e,g,i,k}	0.770 (0.742)^{b,d,f,h,j}	nd^{c,d}	nd^{i,j}	0.502 (0.476)^{k}	nd^{g,h}	nd^{a,b}	nd	0.104 (0.234)^{e,f}
chlorogenic acid	nd^{c,f,h}	2.58 (10.78)^{d,g}	6.84 (9.68)$^{b-d}$	4.37 (0.74)	0.518 (0.476)^{b,e}	8.01 (3.38)^{h}	nd^{a}	nd	6.63 (6.83)^{a,e,fg}
caffeic acid	3.45 (1.74)^{f,h}	2.20 (1.92)^{g}	28.7 (12.4)$^{a-g}$	4.97 (1.54)^{d}	2.78 (1.80)^{e}	1.24 (1.11)^{b}	nd^{a}	nd	1.37 (1.18)^{c,h}
vanillin	3.49 (5.19)^{e,i,j}	nd^{c,h,j}	2.86 (6.44)$^{a-c}$	1.00 (0.46)$^{b,d,f-h}$	nd^{g,i}	nd^{f}	nd	nd	nd^{a,d,e}
syringaldehyde	2.19 (3.30)$^{a-g}$	nd^{g}	nd^{b}	nd^{f}	nd^{e}	nd^{d}	nd^{a}	nd	nd^{c}
cis-*p*-coumaric acid	3.03 (3.85)$^{a-c,e-h}$	1.61 (1.95)^{d,h}	nd^{b}	nd^{f}	nd^{g}	nd^{e}	nd^{a}	nd	0.033 (0.108)^{c,d}
trans-*p*-coumaric acid	2.33 (2.27)^{f}	0.629 (1.038)^{g}	22.4 (33.0)$^{a-g}$	4.93 (3.99)^{d}	1.40 (1.35)^{e}	0.774 (0.692)^{c}	nd^{a}	nd	0.947 (0.714)^{b}
i-ferulic acid	0.242 (0.635)^{g}	0.93 (2.32)$^{a-g}$	nd^{a}	nd^{c}	nd^{d}	nd^{e}	nd^{f}	nd	0.044 (0.144)^{b}
ferulic acid	0.492 (0.978)^{e}	0.171 (0.484)^{f}	9.3 (20.8)$^{a-f}$	6.14 (6.10)	nd^{d}	nd^{c}	nd^{a}	nd	0.066 (0.213)^{b}

$^{a-s}$Mean values in the same row with the same superscript indicate that there are significant differences between them ($p < 0.05$).

Univariate Analysis. An one way ANOVA was carried out according to aging in wood and raw material. Fisher's weight was calculated to establish the discriminant capacity of each variable. For the first possible criterion of differentiation, the aging in wood, the most discriminate variables (from the univariate point of view) were gallic acid, 5-hydroxymethylfurfuraldehyde, 2-furaldehyde, *p*-hydroxybenzaldehyde, 4-ethylphenol, and tartaric acid. For the classification according to raw material, these ones were protocatechualdehyde, caffeic acid, 5-hydroxymethylfurfuraldehyde, and malic acid.

ANOVA shows statistical differences among data according to one factor, but it is not an appropriate method to evaluate the discriminate power of one variable. Therefore, it is necessary to try the differentiation among the vinegar categories considered in this study using multivariate analysis methods.

CA. The data matrix was subjected to a hierarchical agglomerative CA of cases, taking the Euclidean distance as metric and the Ward method as the amalgamation rule. All of the analytical parameters were considered in this study. The dendrogram obtained is shown in **Figure 1**.

Four main clusters can be appreciated as follows: one cluster for wine vinegars with aging in wood (a few exceptions can be seen); another one for wine vinegars without aging in wood and vinegars from alcohol, honey, and malt; a third one for the vinegars from apple; and finally, a fourth one for balsamic vinegars. As can be seen, balsamic vinegars show a clear

Table 5. LDA According to Raw Material[a]

		forecast category						
actual category	no. of samples	white wine	red wine	balsamic	honey	alcohol	malt	apple
white wine	55	54						1
red wine	6	3	3					
balsamic	6			6				
honey	2				1			1
alcohol	2	1				1		
malt	1	1						
apple	11	1			1	1		8

[a] Reclassification of vinegar samples by the leave one out method.

differentiation from the rest of the vinegar samples, which could be due to their particular production process. Clusters were formed especially according to the production process. The groups obtained demonstrate that the variables possess sufficient explanatory power to the detection of the aged process and only some kind of raw material.

Thus, to obtain suitable classification rules for assigning categories to samples, supervised learning pattern recognition methods were applied. These methods assume an a priori knowledge of the number of classes and the sample class memberships.

LDA. A forward stepwise LDA was carried out. This was performed according to the Wilks' λ statistic (*22*) in order to choose the descriptors that best distinguish the different classes. The Wilks' λ statistic for the overall discrimination is computed as the ratio of the determinant of the within-group variance/covariance matrix over the determinant of the total variance/covariance matrix. A partial λ is computed for each variable as the ratio of Wilks' λ after adding the respective variable over the Wilks' λ before adding the variable. An F statistic is computed from the partial λ values leading to a p level. The maximum discriminatory power corresponds to minimum p level values. The so-called "leave one out" method has been employed (*23*). Two possible category classifications have been considered as follows: from the production process of vinegars (with and without wood) and from their raw material (white wine, red wine, balsamic, honey, malt, alcohol, and apple).

Grouping the samples according to the raw material used, the variables included in the discriminant functions obtained (cited according to their discriminant power) were tyrosol, catechin, syringic acid, protocatechualdehyde, *trans*-*p*-coutaric acid, fertaric acid, caffeic acid, *cis*- and *trans*-*p*-coumaric acid, ferulic acid, decanoic acid, succinic acid, malic acid, and tartaric acid. A high number of polyphenols and organic acids have been selected as discriminant variables. This fact should involve that the polyphenols and the organic acid content of vinegar are significantly affected by raw material. This relationship between raw material and phenolic composition of vinegar had already been observed in a previous study carried out in our laboratory (*24*). The scatterplot of the samples onto the plane defined by the first two discriminant functions is shown in **Figure 2**. At first glance, a group with balsamic vinegar samples and jungle constitutes by the rest of the samples can be observed (**Figure 2a**). After a zoom, a clear separation between vinegars from apple and vinegars from wine can be seen (**Figure 2b**). Eighty-eight percent of the samples were correctly classified in the check process by the leave one out method as can be seen in **Table 5**.

Regarding the aging period, another LDA, using only similar

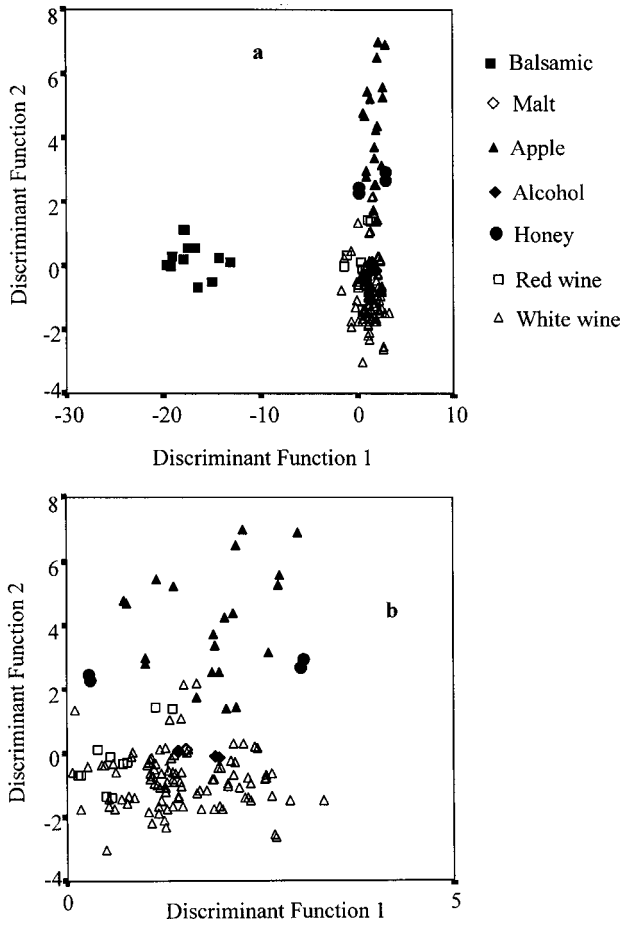

Figure 2. Forward stepwise LDA employing raw material as grouping criterion. (**a**) Projection of samples on the discriminant space selecting the first two discriminant functions as axes. (**b**) Zoom of the projection of samples on the discriminant space selecting the first two discriminant functions as axes.

samples (on the basis of the CA) to avoid interferences between factors, was carried out. The variables selected (according to their discriminant power) were protocatechualdehyde, 5-hydroxymethylfurfuraldehyde, gallic acid, syringic acid, decanoic acid, benzyl acetate, 2-phenylethanol, 2-phenylethyl acetate, 2,3-butanediol, and 2-methyl-1-propanol. A 100% correct classification was obtained in the check process.

From the results obtained, a major role of the volatile compounds considered in the differentiation of the vinegar samples according to aging period in wood can be seen. It should mean that important changes in the volatile content of vinegar take place during this period. Morales et al. (*25*) found that the volatile profile of vinegar underwent significant changes during the aging in wood and that it could be used to differentiate wine vinegar samples according to different aging times.

In conclusion, the analytical parameters selected are suitable descriptors to differentiate vinegar samples according to the aging period in wood, with a high role of volatile compounds. The results obtained in the case of the differentiation of vinegars from different raw materials were worse with honey, alcohol, and malt vinegars grouped with no aged wine vinegars.

ABBREVIATIONS USED

TTAOH, tetradecyltrimethylammonium hydroxide; DAD, diode array detector; GRP, 2-*S*-glutathionyl caftaric acid; ANOVA, analysis of variance; LDA, linear discriminant

analysis; KNN, nonparametric K-nearest neighbors; BPANN, artificial neural networks trained by back-propagation.

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