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Characterization of Wines through the Biogenic Amine Contents Using Chromatographic Techniques and Chemometric Data Analysis

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This paper describes a new method for wine characterization based on the analysis of the biogenic amine composition and the chromatographic profiles using chemometric methods such as principal component analysis and partial least-squares regression. Amine contents have been determined by liquid chromatography with a precolumn derivatization with 1,2-naphthoquinone-4-sulfonate. The corresponding chromatographic data have been advantageously exploited for extracting relevant information regarding some wine features such as elaboration procedure, vintage, or origin region. Results indicate that amines might be used as descriptors of the certain enological practices. Besides, younger wines can be reasonably distinguished from aged ones on the basis of the amine contents. The wine characterization through the analysis of raw chromatographic profiles is proven to be also effective, and patterns dealing with aging processes have also been encountered.

KEYWORDS: Wine characterization; biogenic amines; liquid chromatography; principal component analysis

INTRODUCTION

The characteristics and quality of wines depend on multiple parameters comprising climatic and geological factors of production regions, grape varieties and maturation, technological practices, etc. (*1*−*4*). The use of specific yeasts and bacteria strains (e.g., genetically modified microorganisms) may contribute to the quality and organoleptic properties of wines (*5*, *6*). As occurs with many other food products, analytical methods are being increasingly applied to wine characterization. For this purpose, methods for the determination of low molecular organic acids, polyphenols, amino acids, biogenic amines, and elemental and isotopic analysis have been proposed (*7*, *8*).

Biogenic amines occur in a wide variety of food products like fish, meat, fermented foodstuffs, and spoiled foods (*9*). These compounds participate in several metabolic processes of living organisms (*10*). However, the presence of high amounts of amines in food products is undesirable due to toxicological and organoleptic issues. First, some amines such as histamine and tyramine display severe adverse effects on the central nervous and vascular systems so that toxicity may be observed when they are ingested in high amounts. Second, certain amines such as putrescine and cadaverine may modify negatively the organoleptic properties of wines. Hence, analytical methods for the quantification of amines are needed to satisfy the demand of clinical and food analysis. In particular, high performance liquid chromatography (HPLC) (*11*−*13*), capillary electrophoresis (CE) (*14*−*16*), and gas chromatography (GC) (*17*−*19*) are the most common techniques used for the determination of biogenic amines.

Biogenic amines are present at low concentrations in grape juice although they are mainly formed in wines during alcoholic and malolactic fermentations as well as during aging process (*5*, *6*). Putrescine is the most abundant amine in wines and represents around a 50% of the overall amine levels (*20*). The malolactic fermentation seems to be the main mechanism contributing to the formation of some biogenic amines such as putrescine, tyramine, and phenylethylamine (*21*, *22*). At this stage, the increase in these amines occurs in parallel to a significant decrease of the corresponding amino acid precursors (*23*, *24*). Moreno et al. studied in detail the changes in amine concentration during aging of red wines in oak barrels (*25*). The behavior of each amine seems to be particular. For example, putrescine is mainly accumulated during this aging process as it does not undergo further degradation. In contrast, histamine, which is formed during the initial stages, could decline during the wine aging due to progressive decomposition. The amine composition also depends on other enological practices such as aging grape skin maceration which strongly increase their concentration (*26*). The importance of grape varieties, type of wine (*27*), and microorganism strains in the amine profiles have been pointed out by several authors (*28*, *29*). In consequence,

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Table 1. Features of the Red Wines Considered in the Study

wine class/type	no. of samples in each group
Origin Region	
Navarra	6
Penedès	6
La Mancha	6
Valdepeñas	6
Rioja	6
Ribera del Duero	6
Aging Period after Fermentation	
Reserva (≥12 months)	12
Crianza (≥6 months)	12
young (0 months)	12
Vintage	
1998–1999	4
2000–2001	13
2002–2003	8
2004–2005	11
total samples	36

novel natural winemaking practices to satisfy the demand of products with low biogenic amine contents are receiving increasing attention (*30*).

Despite the complexity of factors influencing the formation of amines, the correlation between amine amounts and aging processes seems to be quite evident (*31*). For this reason, several authors have proposed the use of biogenic amines as potential descriptors of wine characteristics and quality. However, extracting feasible information from wine analysis may result in a difficult task due to the multiple sources of variation commented above. As an example, Romero et al. have evaluated the possibilities of amine profiles for discriminating among wine types (red, white, and rose) using principal component analysis (PCA), cluster analysis (CA), and linear discriminant analysis (LDA) (*32*). Other authors applied PCA and LDA to classify red wines according to winemaking technology from the amino acid and amine contents (*33*).

Apart from amines, the elemental and isotopic composition (*8*), the terpenoid profiles (*34*), polyphenolic contents (*35*, *36*), global volatile fingerprint (*37*), UV−vis (*38*), infrared (*39*), fluorescence (*40*), and mass spectra (*41*, *42*) have also been utilized for the classification of wines as a function of vintage, origins, or other wine features. In this field, apart from more conventional methods, electronic tongues, noses, and other sensor array devices have been introduced as a novel approach for monitoring wines (*43*−*45*).

In this paper, a new method for the characterization of wines based on the analysis of amine contents and the use of the chromatographic profiles as wine fingerprint is proposed. Correlations between amines as well as possible relationships with some wine features, such as pH, aging period, and producing region, have been evaluated using PCA and related chemometric techniques (*46*). Results obtained may contribute to improve the knowledge on wine characteristics and to facilitate the extraction of relevant information.

MATHERIALS AND METHODS

Reagents and Solutions. Unless specified all reagents used were of analytical grade. Milli-Q water (Millipore, Milford, MA) was used to prepare all solutions. Cadaverine hydrochloride, putrescine hydrochloride, histamine dihydrochloride, tryptamine hydrochloride, tyramine hydrochloride, and phenylethylamine hydrochloride were purchased from Fluka (Buchs, Switzerland), and serotonin sulfate was from Sigma-Aldrich (Steinheim, Germany). Other chemicals were sodium 1,2-naphthoquinone-4-sulfonate (NQS) and sodium tetraborate from Carlo Erba (Milan, Italy), hydrochloric acid (37%, w/w) from Panreac (Barcelona, Spain), and sodium hydroxide, chloroform, and acetic acid (96%, w/w) from Merck (Darmstadt, Germany). HPLC grade methanol (Merck, Darmstadt, Germany) was used for the elution gradient.

Stock solutions of each amine were prepared at a concentration of 10^{-3} mol L^{-1} and stored at 4 °C. The reagent solution consisted of 0.07 mol L^{-1} NQS + 0.1 mol L^{-1} HCl, and the buffer solution for derivatization was 0.125 mol L^{-1} $Na_2B_4O_7$ + 0.1 mol L^{-1} NaOH.

Apparatus. The chromatographic systems consisted of an Agilent 1100 series HPLC instrument equipped with a G1311A quaternary pump, a G1379A degasser, a G1315B diode-array detector furnished with a 13 μL flow cell, and an Agilent Chemstation for data acquisition and analysis (Rev. A 10.02), all of them from Agilent Technologies

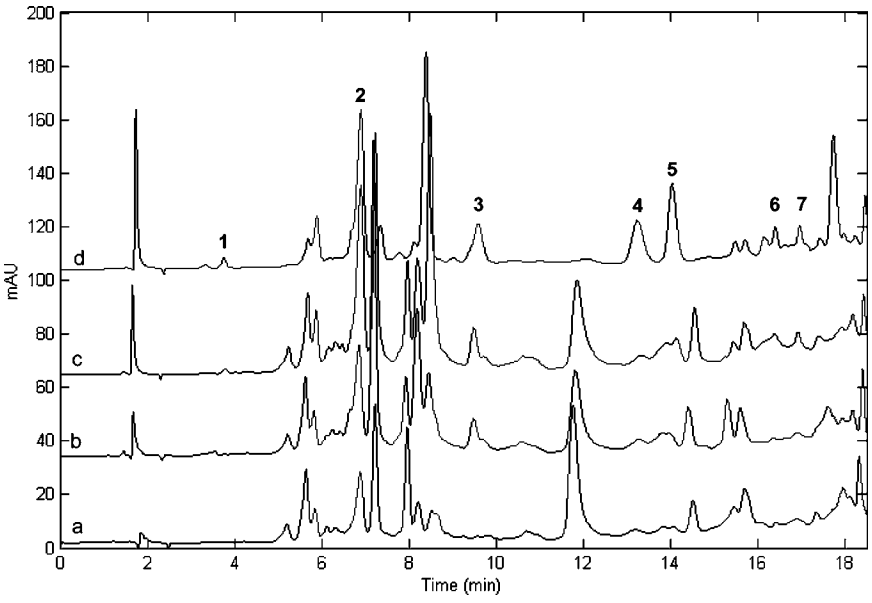

Figure 1. Chromatogram of commercial red wines and a biogenic amine standard mixture, obtained under the optimized experimental conditions described in Materials and Methods. (**a**) Young wine. (**b**) *Crianza* wine. (**c**) *Reserva* wine. (**d**) Biogenic amine standard mixture containing 350 μM putrescine, 250 μM cadaverine, 50 μM tryptamine, phenylethylamine, 75 μM histamine, serotonin, and tyramine. Peak assignment: **1** = histamine; **2** = putrescine; **3** = cadaverine; **4** = tryptamine; **5** = phenylethylamine; **6** = serotonin; **7** = tyramine.

Table 2. Biogenic Amine Contents Expressed in mg L^{-1} of the Red Wines Analyzed[a]

	overall samples ($n = 36$)		young wines ($n = 12$)		*Crianza* wines ($n = 12$)		*Reserva* wines ($n = 12$)	
amine	mean	range	mean	range	mean	range	mean	range
histamine	3.74	0.22–10.21	2.53 a	0.22–5.14	3.54 b	0.80–7.05	5.16 c	0.70–10.21
putrescine	18.82	5.28–44.35	16.83 a	5.28–42.65	22.34 a	8.92–44.35	17.29 a	7.23–36.60
cadaverine	11.59	0.82–30.39	4.59 a	0.82–13.33	16.88 b	7.94–30.39	13.31 c	4.24–25.48
tryptamine	0.51	0.008–1.78	0.36 a	0.008–0.80	0.57 b	0.008–1.13	0.60 b	0.008–1.78
phenylethylamine	1.04	0.006–7.11	0.70 a	0.006–3.89	1.28 a	0.006–5.65	1.14 a	0.07–7.11
serotonin	0.25	0.067–3.04	0.25 a	0.067–1.24	0.04 a	0.067–0.43	0.48 a	0.00–3.04
tyramine[b]	115.29	58.17–173.11	125.70 a	87.43–173.11	109.82 a	60.10–164.08	106.87 a	58.17–133.74

[a] a–c: Mean values of a given amine (in the same row) in each wine type with equal labels indicate that they are not significantly different ($\alpha = 0.05$). [b] Peak areas.

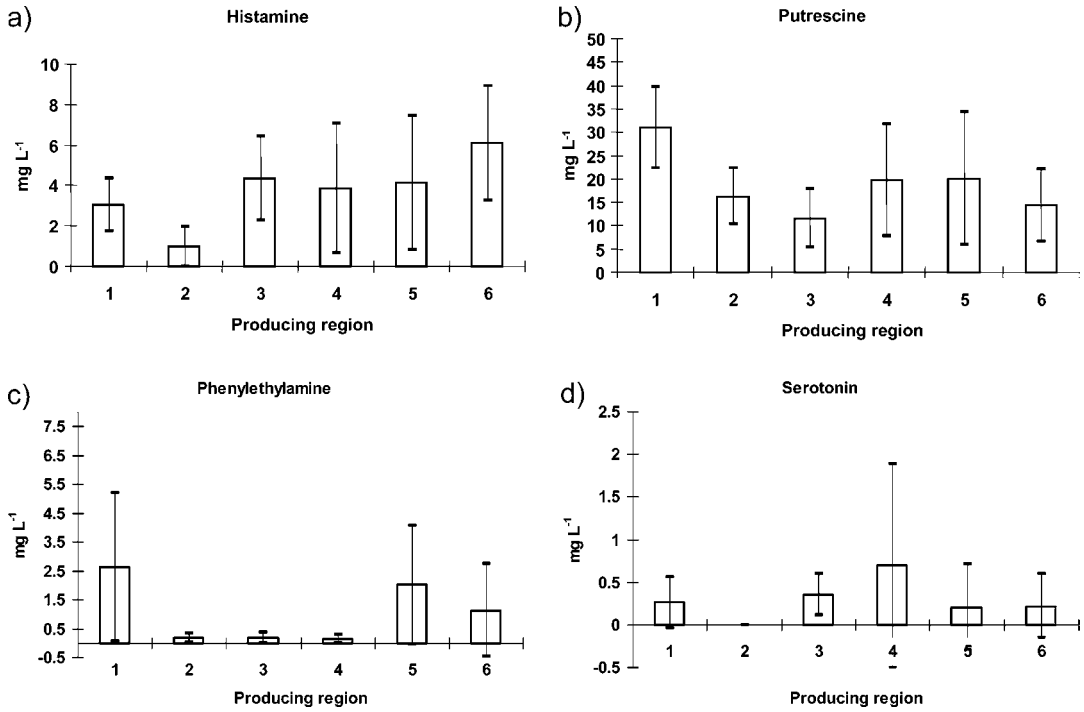

Figure 2. Concentration of selected amines in wines as a function of the producing region. (**a**) Histamine. (**b**) Putrescine. (**c**) Phenylethylamine. (**d**) Serotonin. Producing region assignment: **1** = Navarra; **2** = Penedès; **3** = La Mancha; **4** = Valdepeñas; **5** = Rioja; **6** = Ribera del Duero.

(Waldbronn, Germany). Samples were injected with a Rheodyne 7725-(i) (Rohnert Park, CA) injection valve equipped with a 20 μL sample loop.

A Stuart SBH200D block heater from Bibby Sterilin LTD (Stone, Staffordshire, U.K.) was used to perform the derivatization reaction. An evaporator Mini-Vap from Supelco (PA) was used to dry sample extracts. A Cyberscan 2500 pH meter from Eutech Instruments (Syngapore) with a Hamilton pH electrode (Bonaduz, Switzerland) was used for pH measurements of samples and buffers.

Samples. Thirty-six commercial red wines from the following Spanish wine producing regions were purchased in retail stores: Penedès, La Mancha, Rioja, Ribera del Duero, Navarra, and Valdepeñas (see **Table 1**). Six wines were chosen from each producing region. In parallel, these wines were also characterized according to their aging period after fermentation in barrels or bottles (12 months for *reserva* wines, 6 months for *crianza* wines, and no aging period for young wines) and their vintages, ranging between 1998 and 2005.

Derivatization Procedure. Wines were filtered through 0.45 μm nylon membranes (Cameo Nylon; Scharlab, Barcelona, Spain) and further treated as follows. Aliquots of wine samples, reagent, and buffer solutions of 250 μL each were mixed in the reaction vial. The reaction was developed at pH 9.2 and 65 °C for 5 min. The resulting mixtures were treated with 1.25 mL of chloroform, and derivatives were extracted by shaking for 1.5 min. The organic phases were recovered and the solvent evaporated to dryness through a nitrogen flow. Dry residues were reconstituted in 130 μL of a mixture 2% acetic acid aqueous solution and methanol (85:15, v/v) and injected into the chromatograph.

Chromatographic Analysis. A Synergi Hydro-RP C18 column (150 mm × 4.6 mm i.d., particle size 4 μm, 80 Å) and a guard column (4 mm × 3 mm i.d.), both from Phenomenex (Torrance, CA), were used. The injection volume was 20 μL. Amine derivatives were separated with an elution gradient using a 2% (v/v) acetic acid aqueous solution (mobile phase A) and methanol as organic modifier (channel B). Derivatives were detected spectrophotometrically at 305 and 270 nm. Spectra of chromatographic peaks were acquired in the range of 200 and 600 nm, and confirmation of peak identity was performed by using peak purity tests, which were available in the diode array detector used. More experimental details are given elsewhere (*14*).

Chemometric Software for Data Analysis. MATLAB (Version 6.5) was used for calculations. Principal component analysis (PCA) and partial least-squares regression (PLS) were from the PLS_Toolbox (*47*). A detailed description of these methods is given elsewhere (*46*).

The distribution of the samples on the principal components (PCs), the so-called scores' plots, was a successful strategy for classifying samples from the data measured. These graphs revealed patterns and other features that may be correlated to sample characteristics. In parallel, the study of the distribution of variables (loadings' plot) provided information dealing with their correlations and possible relationship with wine properties. Additionally, the simultaneous study of scores and loadings (bi-plot) was used to explore the relationships

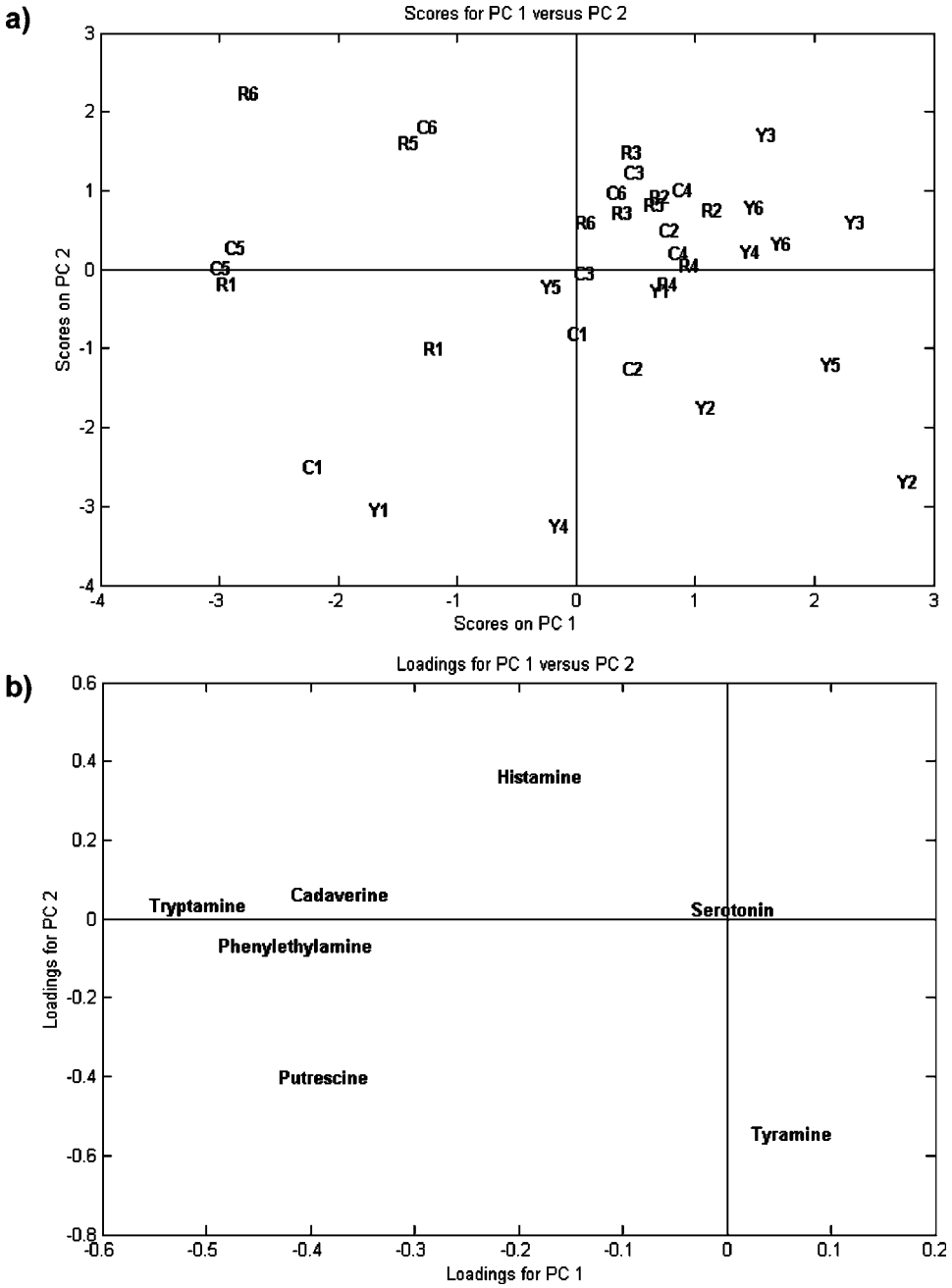

Figure 3. PCA characterization of wines using amine contents as analytical data. (**a**) Scores and (**b**) loadings of PC1 *versus* PC2. Sample codes: Y = young wine; C = *crianza* wine; R = *reserva* wine; 1 = Navarra; 2 = Penedès; 3 = La Mancha; 4 = Valdepeñas; 5 = Rioja; 6 = Ribera del Duero.

between samples and variables. In the case of PLS, the algorithm was focused on the prediction of some wine features such as vintage and producing regions (*Y*-block) using amine or chromatographic data as information (*X*-block). To validate both PCA and PLS models the leave-one-out cross-validation technique was used, in which the result for a particular sample is predicted by using the remaining samples as standards.

RESULTS AND DISCUSSION

As an example, **Figure 1** shows representative chromatograms of *reserva*, *crianza*, and young wines using the HPLC procedure described in Materials and Methods. As indicated in the figure, several peaks corresponding to biogenic amines such as phenylethylamine, putrescine, cadaverine, etc. were observed.

Preliminary Study of Amine Contents in Wines. Table 2 summarizes the overall contents of biogenic amines in wines according to the aging period. In general, putrescine was the most abundant compound with concentrations ranging from 5.28 to 44.35 mg L^{-1}. High concentration levels of cadaverine (0.82 to 30.39 mg L^{-1}) and histamine (0.22 to 10.21 mg L^{-1}) were also found. These findings are in concordance with other results reported in the literature (*24*, *26*, *27*, *48*). Regarding tryptamine, phenylethylamine, and serotonin, concentrations were much lower and the average values were 0.51, 1.04, and 0.25 mg L^{-1}, respectively.

An analysis of variance (ANOVA) was used to examine which biogenic amines varied significantly with the different features of the wines (e.g., age, vintage and origin region, pH) and to study the possible correlations between the different amines. A significance level $\alpha = 0.05$ was considered for the statistical evaluation. For certain compounds such as putrescine and serotonin, there was no apparent correlation between aging and level. For tryptamine and cadaverine, their concentrations

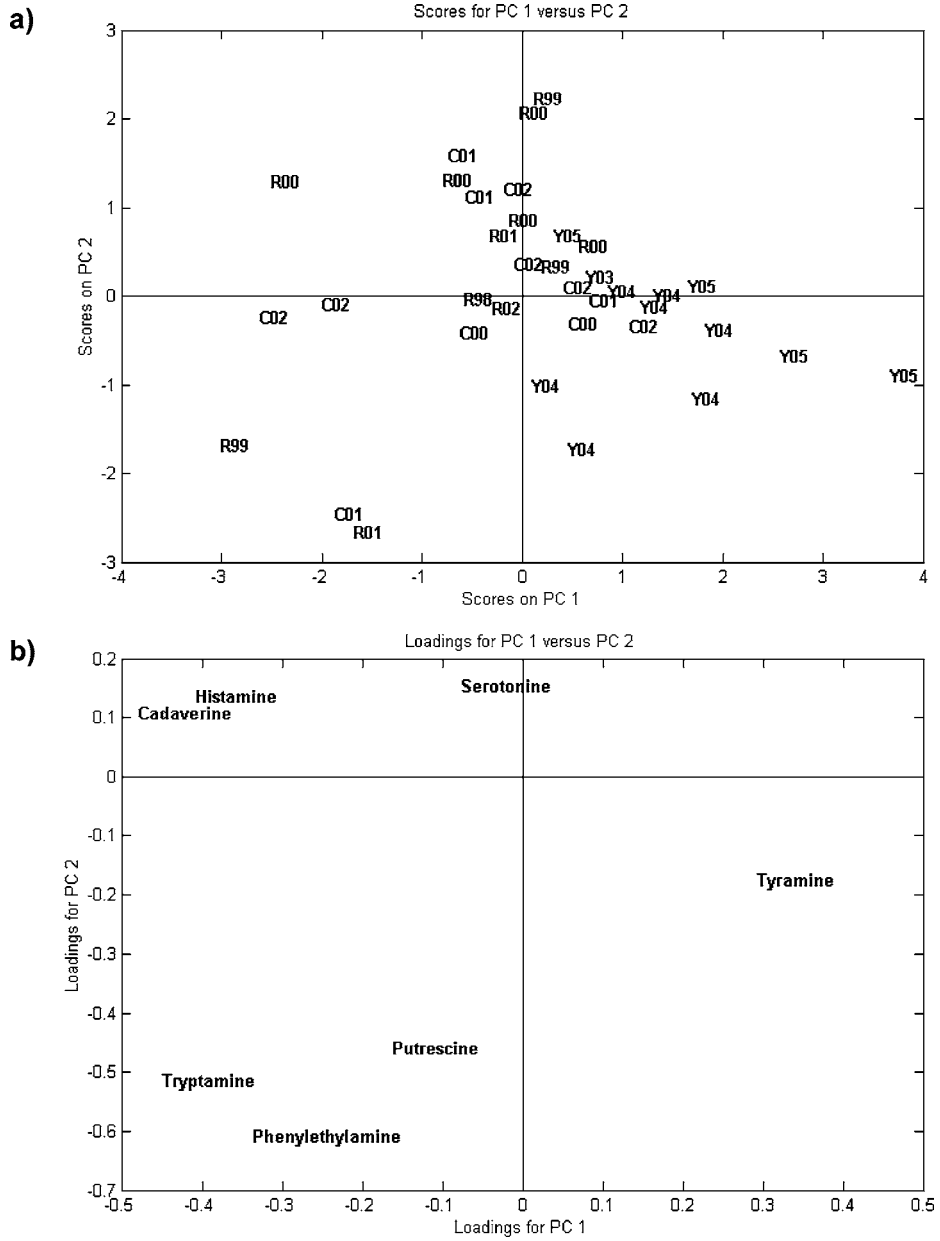

Figure 4. PLS characterization of wines using amine contents as prediction variables on the *X*-block and vintage data (*Y*-block) as a property to be predicted. (**a**) Scores and (**b**) loadings of PC1 *versus* PC2. Sample codes: Y = young wine; C = *crianza* wine; R = *reserva* wine.

in young wines were significantly lower than those found in older ones ($\alpha < 0.05$). In the case of histamine, a continuous increase in the concentrations with aging was observed (for young to *reserva*). From these results, the period of aging in barrels influenced the amine content as during wine storage histamine concentration increased because of an energy mechanism of cells involving histidine decarboxylation which is activated when fermentable substances (e.g., sugar or malic acid) get scarce.

A comparison of the dependence of contents of selected amines with respect to the origin region is shown in **Figure 2**. From these pictures, low amounts of histamine were found in Penedès wines in comparison with the others. Penedès wines were also characterized by quite low (or nondetectable) levels of serotonin. Besides, phenylethylamine permitted a certain distinction of wines from various regions into two groups: Penedès, La Mancha, and Valdepeñas with low and Navarra, Rioja, and Ribera de Duero with high concentrations. The rest of amines did not bring any noticeable information regarding producing zones.

Subsequent studies were focused on evaluating correlations between amines. These correlations were checked statistically. Significant (and positive) relationships ($\alpha < 0.05$) within the group phenylethylamine, tryptamine, and cadaverine were detected. As an example, the correlation coefficient between tryptamine and phenylethylamine was 0.57, which indicated that samples with high amounts of tryptamine also contained high amounts of phenylethylamine and *vice versa*.

Additional studies were carried out to check correlations between the concentration of a given amine and wine properties such as pH, age, vintage, producing region, etc. From these results, the histamine level was positively correlated with pH with a coefficient $r = 0.47$, which was statistically significant. As a result, it was found that the histamine amounts increased with pH. Similar conclusions regarding the correlation between histamine concentration and pH have been reported by other

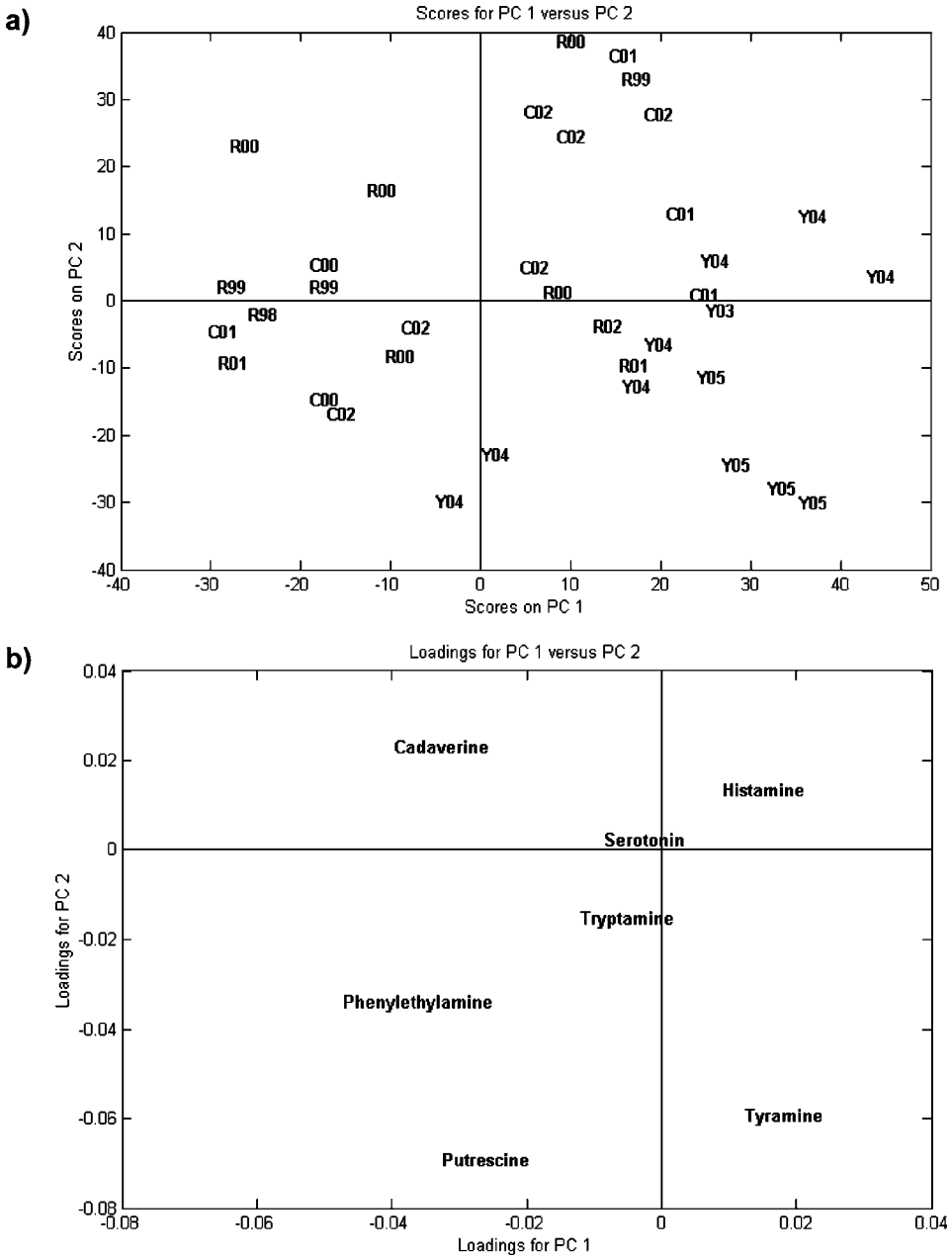

Figure 5. PLS characterization of wines using chromatographic profiles as prediction variables on the *X*-block and vintage data as a property to be predicted (*Y*-block). (**a**) Scores and (**b**) loadings of PC1 *versus* PC2. Sample codes: Y = young wine; C = *crianza* wine; R = *reserva* wine.

authors (*10*, *27*). This behavior is attributed to the higher activity of the microflora at higher pH and, thus, the microbial decarboxylation of histidine is more effective. Furthermore, as mentioned above, a dependence of the histamine amounts on the aging period was also noticed (see **Table 2**).

Principal Component Analysis. First PCA studies were carried out considering amine concentrations as analytical data. Dimensions of the corresponding matrix were 36 samples × 7 amines. Data were autoscaled for providing similar weights for all amines in the PCA model. The percentage of captured variance was 30% and 25% for PC1 and PC2, respectively. Three additional PCs were needed to retain more than 90% of information. The percentage of captured variance for PC3, PC4, and PC5 was 18%, 10%, and 9%, respectively.

The interpretation of the amine patterns and wine characteristics was mainly based on the representation of information contained in PC1 and PC2 (see **Figure 3**). Plots of scores and loading involving other combinations of PCs were also examined (graphs not shown here) although they did not bring additional or complementary conclusions of interest in wine description and characterization. Note that, however, this interpretation should be cautious as the percentage of variance retained with these two components was quite limited. The sample distribution on PC1 and PC2 reflected the influence of the aging period. As a result, aged wines (*reserva* and *crianza*) were mainly situated at the top left zone of the graph while young wine appeared to the right part. As a result, the wine aging process could be described as a tendency from the right bottom to the left top. Obviously, this behavior should not be understood rigidly as some samples appeared in intermediate zones and certain mixing of samples was detected. The study of the distribution of samples according to the producing regions did not show relevant patterns. However, it can be seen that samples belonging to certain regions (e.g., Valdepeñas, Penedès, and La Mancha) were found in rather compact areas. Con-

versely, Navarra and Rioja wines were widely spread with no predominant areas.

Regarding the loading plots, the distribution of variables on PC1 and PC2 showed that tryptamine, putrescine, and phenylethylamine were clustered to the left. This finding confirmed that, as mentioned above, these three amines were reasonably correlated and their behavior was similar. Histamine, serotonin, and tyramine were poorly correlated with each other.

Partial Least-Squares Regression. Although PLS has been currently devoted to prediction purposes, its potential usage for description tasks cannot be underestimated. In this paper, the application of PLS to characterization issues is based on the existing correlations between amine contents and wine features which have been found to be significant in ANOVA and PCA studies. The discrete nature of some wine features makes more difficult the establishment of efficient models to be used for prediction purposes.

PLS analysis was principally focused on generating models to fit the amine contents with the principal wine features. These models may lead to a better description of wine properties such as aging or origin. Here, PLS models were constructed considering amine concentration data as the prediction variables on the *X*-block and wine features (e.g., vintage) as a *Y*-block of predicted properties. The performance of these models for describing wines was even more satisfactory than results obtained with PCA. The correlation coefficient between *Y* actual values and *Y* predicted variables was 0.852. More than 47% of variance could be explained with 2 components. In principle, the vintage was modeled on PC1 and PC2 as young wines were mainly situated to the right side of the graph (see **Figure 4**). The distinction among *crianza* and *reserva* was not so clear. In contrast, the concentration of other amines, mainly histamine and cadaverine, increased during aging processes. At the same time, the amounts of tryptamine, putrescine, and phenylethylamine were reasonably higher for aged wines in comparison with young ones.

Study of Raw Chromatographic Data Sets. The analysis of chromatographic profiles may bring additional advantages with respect to the previous studies. First, no additional calculations of amine concentrations through the corresponding calibration procedure were needed. Hence, raw chromatogram vectors (i.e., absorbance values taken at each elution time) were directly used as multivariate data for PCA and PLS analysis. All chromatograms considered in this study were previously checked to see if all peaks of interest were co-incident in retention time and no significant oscillations in retention times were observed. The overall reproducibility of retention times was 0.6%, which represents a shift in retention times of approximately 3 s. Data were autoscaled in order to provide similar weight to certain relevant features. For instance, the small peaks of histamine, which otherwise resulted in a crucial source of information, got a significant role after scaling.

PLS models were constructed using information about aging period and vintage as *Y*-block data. The correlation coefficient between *Y* actual values and *Y* predicted variables was 0.882. More than 60% of variance could be explained with 2 components. In this case, the wine age was clearly described by PC1 as young wines were distributed at the bottom right side of the scores' plot, *crianza* wines appeared in general in intermediate regions, and oldest *reserva* samples were mainly placed at the top left part of the graph. Differences among young and aged wines were marked, although a less noticeable distinction was produced among *reserva* and *crianza*. The loadings' plot consisted of the representation of information dealing with chromatographic variables (i.e., retention time). As shown in **Figure 5**, the main features in the scatter plot were related with the principal chromatographic peaks. Wines to the left region were characterized by high putrescine and phenylethylamine contents while wines situated at the top of PC2 presented high histamine and cadaverine levels.

The method proposed here for the characterization of wines managed to extract relevant information around the samples analyzed as well as interesting relationships among amine concentrations, and certain wine features such as vintage and aging period were deduced. Simple chemometric techniques such as principal component analysis and partial least-squares regression were used for processing the data. The role of amine profiles in the characterization of wine origin was limited although certain patterns were encountered. Contents of certain amines were somewhat characteristic of given producing regions. The relationship between aging process or vintage and amines was certainly significant. In the case of tyramine, its concentrations decreased with aging. In contrast, the concentrations of phenylethylamine, putrescine, and tryptamine were higher in aged wines. The behavior of histamine was more systematic as they continuously increased with aging. The analysis from the raw chromatographic profiles results in a straightforward and powerful approach which did not require further amine quantification. Furthermore, additional features of chromatograms resulted in a richer source of variance. Loadings' plots were quite complex as they contained information of the whole chromatogram. Loadings interpretation demonstrated the relationship of mathematical factors with the amine contents.

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