FOOD COMPOSITION AND ADDITIVES

Classification of Wines from Five Spanish Origin Denominations by Aromatic Compound Analysis

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Wine is a complex matrix in which aroma compounds play an important role in the characterization of the flavor pattern of a given wine. Twelve volatile compounds were determined in 244 samples of Spanish red wines from different denominations of origin: Rioja, Navarra, Valdepeñas, La Mancha, and Cariñena. The samples were analyzed by GC using headspace solid-phase microextraction. The concentration (mg/mL) intervals obtained were 3-methyl-butyl acetate (3.9 to 116), 3-methyl-1-butanol (93 to 724), ethyl hexanoate (0.8 to 39), 1-hexanol (0.3 to 6.7), ethyl octanoate (1.4 to 41), diethyl succinate (0.2 to 13), 2-phenyl ethyl acetate (0 to 5.3), hexanoic acid (0 to 8.3), geraniol (0 to 3.0), 2-phenylethanol (1.5 to 56), octanoic acid (0 to 20), and decanoic acid (0 to 3.3). Wines were classified by multivariate statistical methods: principal component analysis, and lineal discriminant analysis. A correct differentiation among wines according to their origin was obtained by lineal discriminant analysis.

W ine is a widely consumed global beverage and has obvious commercial value and social importance. Wine composition is very complex and the knowledge of many parameters, such as volatile compounds, polyphenols, carbohydrates, and metals is important for wine characterization. Sometimes the identification of these compounds is difficult because they are found in trace quantities. In previous studies, Maarse and Visscher (1) detected more than 680 volatile compounds in different white wines, and more than 1000 aroma compounds were identified by Etiévant (2) in several types of wine.

Throughout the years, for isolation and preconcentration of wine flavor compounds, several analytical techniques have been developed, such as liquid–liquid extraction (3–6), SPE (6–8), ultrasound extraction (9–11), supercritical fluid extraction (12, 13), microextraction by demixing (14), or purge and cold trapping (15). In the last decade, the solid-phase

microextraction (SPME) technique has been largely used (16–18); its main advantages are ease of use, short preparation time, economy of solvent, and selectivity (19).

The determination of volatile compounds extracted from wine samples is normally carried out by capillary GC with a flame ionization detector (GC-FID; 5, 6, 14, 17) or capillary GC/MS (20).

Different denominations of origin (DO) have been created in Spain; their main purpose is to guarantee the origin and quality of the wines to prevent fraud. Wines labeled with the same DO should have a similar and typical composition that gives them particular characteristics to be differentiated from other DO. The Rioja qualified denomination of origin (DOCa Rioja) is a prestigious denomination both at the national and international level and, thus, the possibility of creating a model to accept or reject a given wine from the DOCa Rioja will be very important to prevent potential fraud. It has been reported that several cases of wines were sold under the name of the DOCa La Rioja, although the grapes used to produce them really came from another DO. This is a serious fraud for consumers, and one that can appear in other DO of recognized prestige.

In order to achieve effective fraud control, it is very useful to know the values of the physicochemical parameters that allow a link to be established between the wine samples and their geographical place of origin. In food chemistry, the application of chemometric methods for characterizing or classifying products according to their geographical origin, quality or variety is very attractive and, in many cases, necessary (21, 22).

The capability of multivariate analysis techniques for classifying and differentiating wine and alcoholic beverages has received great attention and been widely used (23, 24). Some of the techniques used are principal component analysis (PCA) to examine the relationships between variables (25–27), linear discriminant analysis (LDA) to select the most useful variables for differentiating wines or cluster analysis to confirm the separation between groups with selected variables (28, 29), soft independent modeling class analogy as a modeling technique (30), or artificial neural networks to generate patterns to achieve various classification tasks (24, 26).

Different types of variables (chemical or physical) have been used in combination with these statistical methods: the content of volatile compounds to classify wines from specific

method of volatile compounds in wine, before their determination by GC-FID.

Experimental

Apparatus

The analyses were performed in a Varian (Walnut Creek, CA) CP-3800 gas chromatograph equipped with a FID and capillary column HP-INNOWAX (Agilent Technologies, Palo Alto, CA; 30 m   0.25 mm id, 0.25 mm film thickness).

The GC conditions were as follows: 220 C as injector and detector temperature; the oven temperature program was: initial temperature, 60 C for 4 min, raised 4 C/min to 170 C, this final temperature maintained for 12 min, with total time of chromatogram of 43.5 min; helium was used as the carrier gas (0.9 mL/min); the flow rates for gases in FID were hydrogen (30 mL/min), air (300 mL/min), and the auxiliary gas, He (25 mL/min). The injector was operated in split mode (ratio 0.15 to 1).

An automatic Varian CP-8200 injector was used. The fibers and the SPME syringe accessory were purchased from Supelco (Bellefonte, PA). The fiber was CW/DVB (coated with 65 mm Carbowax /divinylbenzene, orange color) and conditioned in accordance with the manufacturer's instructions. The SPME conditions were as follows: a CW/DVB fiber was installed in the SPME syringe accessory and the vials containing 0.8 mL were placed in the automatic injector. The HS mode was used and the adsorption and desorption times were 10 and 2 min, respectively. Magnetic stirring with no heating was used during the extraction procedure.

The compounds to study were previously identified in the wine with an HP 5989B Mass Spectrometer (Agilent

Figure 1. Geographical situation of denominations of origin.

regions (29, 31–34), metal content to characterize Spanish wines (28, 30, 32), aromatic index to differentiate white wines (35), or psychophysical parameters, such as color, to characterize Rioja wines (36).

The aim of this study was to classify chemometrically red wines belonging to various Spanish DO (Navarra, Valdepeñas, Cariñena, Rioja, and La Mancha) using PCA and LDA. Headspace (HS)-SPME was applied as a sampling

Table 1. Parameters for the calibration graphs

Compound	Slope[a]		Intercept[a,b]		$r_s{}^c$, %	No. of points
3MBA	0.49	0.02	0.028	0.001	5.3	5
DA	0.84	0.04	0.17	0.04	4.7	5
PEA	0.85	0.04	0.12	0.04	4.8	6
HA	0.73	0.02	0.020	0.001	3.2	4
OA	0.68	0.01	0.021	0.001	3.0	5
3M1B	0.58	0.02	0.028	0.002	5.8	5
EH	1.92	0.20	0.42	0.02	4.9	6
2PA	0.97	0.10	0.28	0.03	3.3	4
G	0.28	0.02	0.088	0.023	2.2	6
1H	0.51	0.02	0.020	0.001	4.0	4
EO	1.2	0.1	0.32	0.02	3.9	5
DS	0.61	0.01	0.019	0.001	4.5	4

[a] Slope and intercept with 95% confidence interval.

[b] Mean   2s.

[c] Relative standard deviation.

Table 2. Concentration intervals (mg/L) for the compounds studied

Compound	Navarra	Valdepežas	Cariñena	Rioja	La Mancha
3MBA	3.9–9.7	40–116	13–19	20–32	36–50
DA	0–3.3	0–2.3	0–1.3	0–2.4	0–0.3
PEA	0–5.3	0–0.8	0–0.13	0–0.23	0–1.2
HA	1.1–7.8	0–8.3	0–4.7	0–5.9	0–5.9
OA	0.7–7.3	0–20	0.3–3.6	0.36–4.0	1.4–9.9
3M1B	111–602	124–724	93–334	119–377	117–253
EH	0.8–12	1.3–39	4.6–18	0.99–23	3.5–16
2PA	5.2–53	1.5–56	3.8–29	3.2–20	4.7–24
G	0–2.4	0–3.0	0–0.5	0–1.1	ND[a]
1H	1.2–4.9	0.3–6.7	1.3–3.7	0.4–4.6	0.6–2.60
EO	2.4–13	3.4–41	3.5–12	1.4–15	6.2–31
DS	1.1–9.1	0.4–13	1.1–3.2	0.3–11	0.2–10

[a] ND = Not detected.

Technologies) coupled to an HP gas chromatograph 5890 Series II Plus. A 2 L volume of wine was injected in split mode in a capillary column, and the chromatographic conditions were the same as described above. The spectrometric conditions were: electronic impact (ionization energy, 70 eV); source temperature 250 C; 45 to 700 as mass range, and resolution was 1 atomic mass unit.

Wine Sample

The samples for the present study were 244 Spanish red young wines (2002 vintage) kindly donated by five DO (54 from Navarra, 52 from Valdepeñas, 36 from Cariñena, 60 from Rioja, and 42 from La Mancha) to guarantee the geographical origin of the wines (Figure 1).

A stock wine was prepared by dissolving all the flavor compounds studied in aqueous ethanol (12%, v/v). The final concentration for each compound was approximately similar to that of natural wine (2). The pH was then adjusted to 3.5 with tartaric acid (approximately 0.09 g/L). This solution will be hereafter referred to as synthetic wine.

Chemicals

All reagents were of analytical grade or better. Volatile compounds with purities ranging from 99.0 to 99.5% [(3-methyl-butyl acetate (3MBA), 3-methyl-1-butanol (3M1B), ethyl hexanoate (EH), 1-hexanol (1H), ethyl octanoate (EO), diethyl succinate (DS), phenyl ethyl acetate (PEA), hexanoic acid (HA), geraniol (G), 2-phenylethanol (2PA), octanoic acid (OA), decanoic acid (DA), and 3-octanol (internal standard)] were obtained from Fluka (Buchs, Switzerland) or Aldrich (Milwaukee, WI). Solid anhydrous ammonium sulfate, sodium chloride, and tartaric acid were purchased from Merck (Darmstadt, Germany) and ethanol from Panreac (Barcelona, Spain). Ultrapure water was obtained from a Milli-Q system (Millipore, Bedford, MA).

HS-SPME Procedure

We previously optimized the extraction procedure (18), and it was as follows: 10.0 mL synthetic wine or natural wine was placed in a tube of approximately 20 mL. The internal standard (3-octanol, 15.8 mg/L) and 0.1 g each of sodium chloride and ammonium sulfate were added. Finally, the solution was shaken and three aliquots of 0.80 mL were taken and placed in three capped vials, completing one chromatographic injection per vial; thus, three replicates were obtained for each sample.

Statistical Methods

(**a**) *Univariate analysis.*—To determine which variables were statistically significant between the different DO, one-way analysis of variance (ANOVA) was applied.

(**b**) *Principal component analysis (PCA).*—An unsupervised pattern recognition technique was used to reduce the dimensionality of the original data matrix, retaining the maximum amount of variability in a multivariate problem, and providing a new set of variables obtained as a linear combination of the original descriptors. The new variables, the PCs, are the eigenvectors of the data matrix that are orthogonal to one another. The magnitude of each eigenvector is expressed by the own eigenvalue which gives a measure of the variance related to a principal component. The value taken by an object for a principal component is called the score, and the interpretation of the results is usually carried out by visualization of the scores.

(**c**) *Linear discriminant analysis (LDA).*—In this supervised technique the number of categories and samples belonging to each category are defined beforehand. LDA renders a number of orthogonal linear discriminant functions equal to the number of categories minus one, in order to provide a method for predicting the group into which a new case is most likely to fall, or to obtain a small number of useful predictor

Table 3.   Signification of the variables from ANOVA study

| Compound	F[a]	a[b]	Signification[c]
3MBA	435	0.00	S
DA	3.39	0.01	S
PEA	4.84	0.00	S
HA	7.89	0.00	S
OA	10.9	0.00	S
3M1B	14.9	0.00	S
EH	0.958	0.43	NS
2PA	7.48	0.00	S
G	11.9	0.00	S
1H	1.88	0.12	NS
EO	6.91	0.00	S
DS	4.54	0.00	S

[a] Fisher's *F*.
[b] a = Signification ($P > 0.05$).
[c] S = Significant and NS = not significant.

Table 4.   Loadings, eigenvalues, percentage of explained variance, and cumulative variance for the first four principal components

Compound	PC 1	PC 2	PC 3	PC 4
3MBA	−0.035	0.336	0.569	−0.135
DA	0.166	−0.093	0.101	0.077
PEA	0.057	−0.107	−0.063	0.426
HA	0.170	−0.179	−0.014	−0.142
OA	0.188	−0.020	0.354	−0.177
3M1B	0.163	0.146	−0.342	0.024
EH	−0.018	0.051	0.221	0.850
2PA	0.201	0.136	−0.064	0.090
G	0.216	−0.082	0.090	−0.049
1H	0.122	0.380	−0.357	0.069
EO	0.001	0.509	0.040	−0.032
DS	0.182	−0.065	0.216	0.060
Eigenvalue	3.874	1.608	1.223	1.001
% Variance	32.283	13.403	10.195	8.338
% Cumulative	32.283	45.686	55.881	64.220
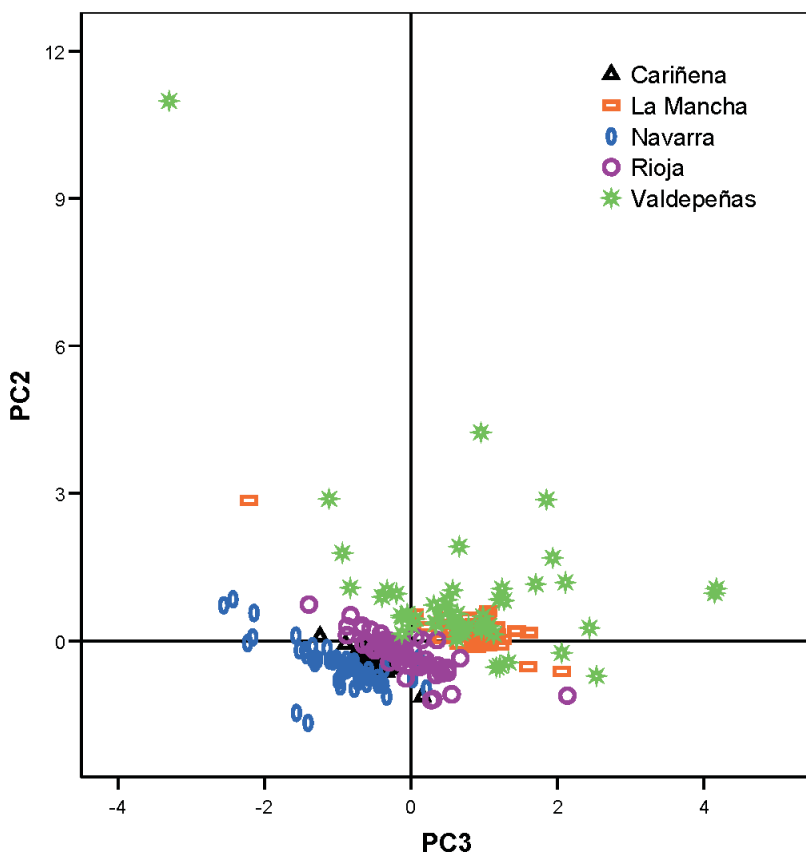
Figure 2.   Projection of 244 objects on the plot defined by the second and third principal components.

Table 5. Eigenvalues, explained variance, cumulative variance, and canonical correlation of the discriminant functions

Functions	Eigenvalue	Explained variance, %	Cumulative variance, %	Canonical correlation
1	12.799	84.4	84.4	0.963
2	1.934	12.7	97.1	0.812
3	0.419	2.8	99.9	0.543

Table 6. Coefficients of the standardized canonical discriminant functions

Compound	1	2	3
3MBA	0.980	0.299	−0.027
3M1B	−0.252	0.596	0.686
DS	−0.001	−0.599	0.483
HA	−0.206	0.447	0.079
G	−0.647	1.527	−0.612
OA	0.621	−1.448	0.534

variables. This method maximizes the variance between categories and minimizes the variance within each category. To decide which variables have a major discriminant capacity, a stepwise LDA using Wilk's lambda was applied.

(**d**) *Software.*—Chemometric evaluations were performed using the statistical software package STATGRAPHICS (Version Plus 4.0; Manugistics, Inc., Rockville, MD) and SPSS (Version 14.0; SPSS Inc., Chicago, IL).

Results and Discussion

The calibration graphs were constructed with three replicates of seven standard solutions of synthetic wine, using 3-octanol (15.8 mg/L) as the internal standard, and samples were analyzed by HS-SPME-GC. This sampling method of volatile compounds from wine samples is good, simple, fast, and reproducible.

A_c/A_{is} *vs* c_c/c_{is} was considered to represent these graphs, where A_c = peak area of volatile compound; A_{is} = peak area of internal standard; c_c = concentration of volatile compound and c_{is} = concentration of internal standard. The equations obtained are indicated in Table 1. The wine samples of different DO were analyzed and the concentration intervals obtained for each volatile compound are shown in Table 2. In general, the maximum concentrations obtained in the wines from Valdepeñas are higher than the other DO, probably due
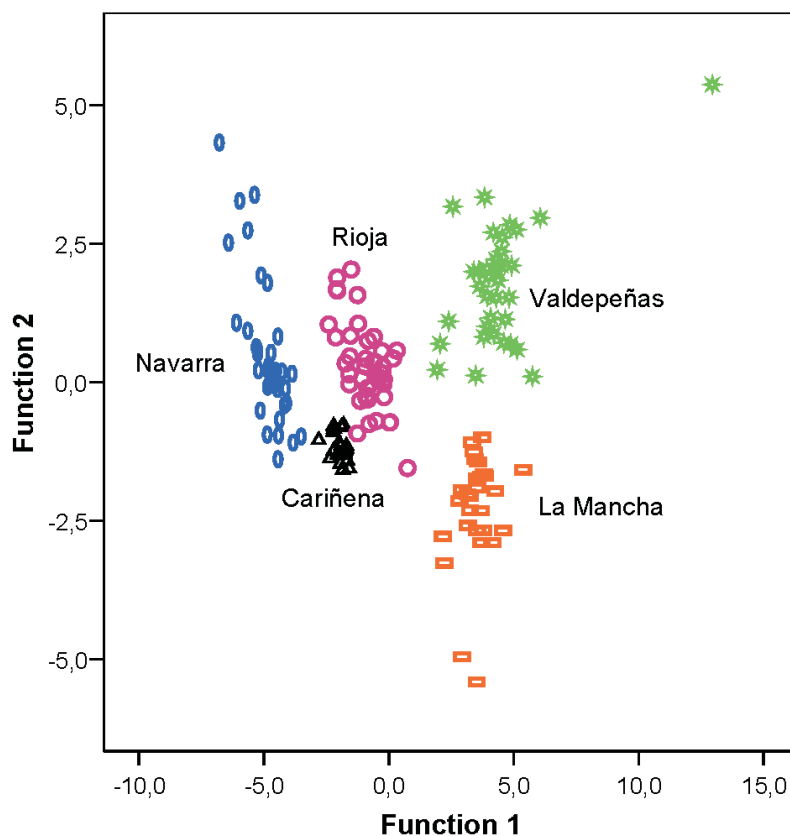

Figure 3. Plot of the samples on the plane defined by the two first canonical discriminant functions.

Table 7.   Recognition and prediction ability of wines according to DO using LDA

True category	Recognition ability, %					Prediction ability, %				
	Navarra	Valdepeñas	Cariñena	Rioja	La Mancha	Navarra	Valdepeñas	Cariñena	Rioja	La Mancha
Navarra	94.4	0	5.6	0	0	72.2	0	27.8	0	0
Valdepeñas	0	100	0	0	0	0	100	0	0	0
Cariñena	0	0	100	0	0	16.7	0	58.3	25.0	0
Rioja	0	0	2.5	97.5	0	0	0	40.0	60.0	0
La Mancha	0	0	0	0	100	0	14.3	0	0	85.7
Cases correctly classified: 98.2%.						Total prediction ability: 75.3%				

to the presence of *Airén* grapes in the elaboration of red clarete, a typical wine of this DO.

The aromatic composition of wine depends on many factors: climate (temperature and rainfall); soil (limestone, calcareous, or alluvial); grape variety (*Garnacha*, a high yielding grape that produces vigorous wines in Navarra, Cariñena, and La Mancha; *Tempranillo*, mentioned as the star of Spanish grapes, in Valdepeñas, La Rioja, and La Mancha; and *Airén* in Valdepeñas), wine-making technologies (similar in these DO); yields by hectane (from 2 tons in La Mancha to 6.5 tons in La Rioja, Navarra, or Valdepeñas); and other less important factors. However, a small variation in the soil or in the particular microclimate of the zone can cause wines produced in next vineyards to be different.

The mean concentration values obtained for each compound were compared by one-way ANOVA, assuming that there were significant differences between mean values when statistical comparison yielded $P < 0.05$. Table 3 shows that all variables, except ethyl hexanoate and 1-hexanol, are significant.

The application of PCA to our data set revealed that the four first principal components with an eigenvalue higher than 1 (Kaiser's rule), explain 64.2% of total variance (Table 4). The variables with higher loading (positive or negative; Table 4) have a great contribution to explain the meaning of each principal component. For example, the dominant features in the first principal component are geraniol and 2-phenylethanol; ethyl octanoate dominates in the second principal component, 3-methyl-butyl acetate in the third, and ethyl hexanoate in the fourth.

The scores were calculated from the principal component equations obtained. A plot of the scores of PC2 (13.4%) versus PC3 (10.2%) is reported in Figure 2, showing that the wines of Navarra, Cariñena, and Rioja are separated from that of Valdepeñas and La Mancha. On the other hand, a partial overlapping among samples from Valdepeñas and La Mancha is observed, possibly due to their geographical proximity (Figure 1). In the plot of PC1 versus PC2, the wines are more overlapped, although the cumulative variance of both PCs is 45.7%. This chemometric tool for exploratory analysis has

not presented great power of classification, but it has given good results in other studies (26, 29, 31, 32, 34, 37).

An LDA was applied to find an operative classification role for grouping together the wines of the five DO. A total of 164 samples (2/3) were use for modeling, leaving the other 80 samples (1/3) for predicting the validity of the model.

LDA, using Wilk's lambda as a selection criterion, was used to determine which variables should be included in the analysis. The final model selected six variables with the highest *F*-values: 3-methyl-butyl acetate, 3-methyl-1-butanol, diethyl succinate, hexanoic acid, geraniol, and octanoic acid that permit the separation of wines according to their origin.

Only the three first discriminant functions are statistically significant, explaining 99.9% of variability. The eigenvalues, variances (explained and cumulative), and canonical correlations for these functions are shown in Table 5.

The coefficients of the variables in the three first discriminant functions revealed which variables had a greater influence on the functions (Table 6). For the first function, the variable that displayed the biggest discriminatory power was 3-methyl-butyl acetate; in the second function, geraniol and octanoic acids, and in the third, 3-methyl-1-butanol and geraniol.

The graphical representation of the wines on the plane defined by the first two canonical functions is presented in Figure 3, where a good separation among DO wines can be visualized. La Mancha and Valdepeñas wines present a value of function 1 greater than 0 and the other wines, less than 0.

The obtained classification matrix indicates a global classification of 98.2% (Table 7). The total prediction ability of the model was 75.3%. All of the Valdepeñas wines were allotted to their group. The results obtained for Cariñena (58.3%) and La Rioja (60.0%) were not acceptable. However, the results for Navarra (72.2%) and La Mancha (85.7%) wines can be considered satisfactory. The percentages of noncorrect classification and prediction among DO wines may probably be due to the geographical proximity of the regions (Figure 1): on the one hand, Navarra, Cariñena, and Rioja; on the other, Valdepeñas and La Mancha. This conclusion is similar to other published data (28, 38).

Of both chemometric methods applied, PCA and LDA, only LDA has permitted an acceptable classification of wines.

This technique has high classification power and is widely used in the bibliography to characterize wines according to their origin (31, 34), wine-making technology (39), or grape variety (40).

Consequently, the aromatic composition of wines studied is a tool for differentiating their geographic origin, and thus, avoiding potential commercial fraud.

Acknowledgments

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