Furanic and Phenolic Composition of Cider Brandy. A Chemometric Study

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A chemometric study based on furanic and polyphenolic substances is presented for typifying cider brandies of different ages obtained from ciders made from freshly pressed apple must and from reconstituted apple concentrate. Univariate treatment of the furfural content is sufficient for characterizing the brandies according to the raw material employed in their manufacture, but no single variable was found that by itself allowed correct discrimination of brandies on the basis of aging time in American oak barrels. Therefore, a number of pattern recognition techniques, namely linear discriminant analysis (LDA), *K* nearest-neighbor (KNN), soft independent modeling of class analogy (SIMCA), and partial least squares (PLS) were employed, which have allowed the typification of said spirits. Aromatic aldehydes, along with their oxidation products such as syringic acid, were the main modeling variables, which is in accordance with the model of lignin degradation by means of the mechanism of acid ethanolysis.

Keywords: *Furanic; polyphenol; chemometric; brandy*

INTRODUCTION

Cider brandy is the drinkable spirit obtained by distillation of cider in an alambic still; cider is generally manufactured from a mixture of cider apple varieties, the apple must produced being fermented by wild microflora (mainly *Saccharomyces* and *Kloeckera apiculata* yeasts and lactic and acetic bacteria) that spontaneously develop in the liquid medium (Cabranes et al., 1990). However, cider elaborated by fermentation of apple juice concentrate using a starter of the *Saccharomyces* strain could be used for making spirits to reduce the costs of the process, although the typical browning processes that develop during the concentration step of apple juice, such as the Maillard reaction and caramelization, accumulate furanic substances (Eskin, 1990); these components are generally a useful measure of the deterioration of foods.

Freshly distilled spirits are normally subjected to a maturation process in American or French oak casks for periods of time that depend on traditional practices. During the aging process, different mechanisms are involved in the changes in the composition of cider brandies, namely extractions, chemical processes such as oxidations and hydrolysis, evaporation of small molecules, and concentration of the larger molecules (Cantagrel et al., 1995). For instance, acid ethanolysis of lignin and the subsequent action of oxygen promotes the accumulation of aromatic aldehydes and phenolic acids such as syringic and vanillic acid (Puech, 1981; Puech and Jouret, 1982); gallic and ellagic acids and certain carbohydrates (xylose, arabinose, glucose, rhamnose, and fucose) are incorporated into the spirit (Belchior and Carneiro, 1972) as a consequence of hydrolysis of tannins and hemicelluloses, respectively.

At the same time, the relative proportion of alcohol−water determines the extension of chemical equilibria with regard to the accumulation of esters and acetals, the development of aged spirit aroma being closely associated with the alcoholic strength of the spirit (Singleton, 1995).

Charring and toasting, steps traditionally included in the coopering procedure, destroy carbohydrates; as a consequence, furanic components such as furfural, 5-methylfurfural, and 5-(hydroxymethyl)furfural are produced from the transformation of pentoses, rhamnose, and hexoses, respectively, and are later incorporated into the spirit. Villalón et al. (1991) and Quesada et al. (1996) established that the concentration of furanic aldehydes is related to the age of the cask, the charring process, the wood type, and the presence or absence of caramel but that these substances could not be employed as aging markers.

Multivariate data analysis (MDA) has traditionally been employed in food quality evaluation (Resurreccion, 1988) as well as for typifying and characterizing fruits (García-López, 1996), fruit juices (Page et al., 1988), fermented beverages (Etiévant et al., 1989), and distilled spirits (Cruz Ortiz et al., 1993). Different techniques of multivariate analysis can be employed to achieve these aims. Thus, exploratory data analysis (EDA) allows us to select variables and to search for "natural" groups (for example, by cluster analysis). Feature selection is a step of multivariate analysis that must be implemented to ascertain the most relevant variables, so that we can eliminate noise and thus visualize the structure of the data better and also carry out analyses at lower costs. Factor analysis (FA) is used to ascertain the basic structure of the data, the original data matrix being reduced to the most relevant components that account for a high percentage of variance of the system. The aim of classification techniques is to classify objects previously assigned to classes detected for cluster or factor analyses and also to predict the category of the samples whose class is unknown (level 1 pattern recognition). When boundaries are constructed for each class, level 2 pattern recognition is then defined; this is the case of Bayesean and soft

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independent modeling of class analogy (SIMCA) methods. Finally, correlation analysis allows us to estimate values of one variable from a set of controllable independent variables (level 3 pattern recognition) and to establish the relationship between two sets of predictor and response variables (level 4 pattern recognition). For instance, partial least-squares regression analysis (PLS) has been used to model relationships between sensory data (sensory descriptor scores linked to wood materials) and the nonvolatile composition (phenolic acids, aromatic aldehydes, and ellagitannins) of Scotch whiskey (Piggot et al., 1993).

The aim of this work was to utilize the furanic and polyphenolic profiles of cider brandies to identify patterns that allow us to distinguish cider brandies on the basis of raw material (cider) and aging time in casks.

EXPERIMENTAL PROCEDURES

Samples. Brandies were obtained from two types of cider: cider made from spontaneous fermentation of fresh apple must, which was manufactured from a mixture of cider apples with different sensory properties [Collaos (slightly sharp) 30%, Raxao (sharp) 40%, Durón Arroes (sweet) 10%, Coloradona (bittersweet) 15%, and Meana (bitter-sharp) 5%], and cider manufactured from controlled fermentation of apple juice concentrate by means of a starter of the *Saccharomyces cerevisiae* strain. Spirits were obtained from a batch distillation system equipped with a copper wash-still distillation vessel with a capacity of 500 L and a rectifying column that had 16 bubble-cap plates; steam was used as the heat source. Subsequently, the freshly distilled spirits were subjected to an aging process in oak casks for 15 months.

Coopering. The wood chosen was white oak (*Quercus alba*). The drying of staves and heading was conducted in the open air over 3 years. Once the wood had been brushed and cut, it was subjected to a light toasting. The thickness of the staves and the capacity of the barrels were 28 mm and 35 L, respectively.

Chromatography. The analytical procedure for determining furanic and polyphenolic compounds in cider brandy was optimized elsewhere (Mangas et al., 1996). Experimental data were obtained from a high-performance liquid chromatography (HPLC) system (Waters Associates) equipped with a 712 automatic injector, M510 pumps, a Millenium v. 2.0 software data module, and a 481 spectrophotometric detector. Separation of analytes was carried out on a Spherisorb ODS-2 column (250 mm × 4.6 mm; 3 μm) at 40 °C and detected at 280 nm, using 2% acetic acid and 0.02 M sodium acetate (solvent A) and methanol (solvent B) as mobile phases. Elution conditions were as follows: starting, 2.5% solvent A; isocratic for 4 min; linear increase of solvent B in solvent A to 30% B for 16 min; and isocratic for 25 min. Flow rate was 0.8 mL/min. Samples were previously subjected to an ethanol removal process under vacuum at 30−35 °C and then filtered through a 0.45 μm PVDF membrane; finally, 10 μL was injected into the HPLC system.

Experimental Design. A two-factor experiment (technology and time) with repeated measures on one of the factors (time) was carried out. Two levels (two cider types) were established for the technology factor and five levels for the time factor. The experiments for the aging of the spirits were performed in triplicate (cider made from apple juice concentrate) and duplicate (cider made from fresh apple must).

Data Processing. Data were processed by means of the PARVUS statistical package (Forina et al., 1988). A matrix was constructed with rows (25) representing cider brandies with different aging times manufactured from press cider and concentrate cider and columns (12) corresponding to furanic and polyphenolic components. On the basis of the raw material used for making the cider, spirits were categorized as category P (press cider) and category C (concentrate cider). Cider brandies were also classified on the basis of aging time in casks as category F (freshly distilled spirit, with <6 months of aging) and category A (aged distilled spirit, with aging time from 6 to 15 months). Likewise, to use the PLS technique to carry out modeling studies, we defined a binary response (Y), where $Y = 1.00$ for spirits included in category F and $Y = 2.00$ for spirits belonging to category A.

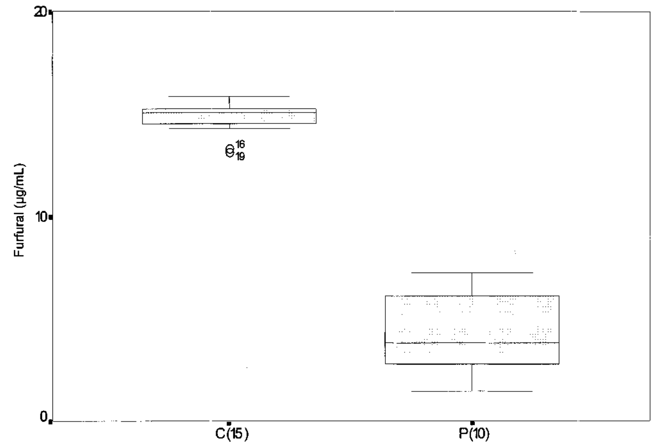

Figure 1. Box plots for each category: P, press cider; C, concentrate cider, using the main discriminant variable (furfural). The number of samples for each category is included in parentheses.

Table 1. Statistics of Freshly Distilled and Aged Categories (Concentrations in Micrograms per Milliliter)

	freshly distilled				aged			
	mean	SD	min	max	mean	SD	min	max
gallic	2.81	1.25	1.59	5.41	6.29	2.5	3.57	12.07
HMF	1.02	0.93	0.28	3.47	1.85	1.26	0.66	4.25
protocatechuic	0.38	0.13	0.22	0.66	0.84	0.25	0.46	1.40
p-hydroxybenzaldehyde	0.08	0.03	0.05	0.13	0.10	0.03	0.04	0.14
vanillic	1.35	0.69	0.64	2.97	2.60	0.82	1.26	4.04
caffeic	0.64	0.14	0.42	0.86	1.21	0.68	0.59	2.77
syringic	1.23	1.08	0.34	4.04	2.56	1.49	0.86	5.54
vanillin	1.77	1.16	0.73	4.78	3.52	1.87	1.47	7.16
syringaldehyde	4.15	3.62	1.19	13.58	8.38	5.49	2.70	18.66
ferulic	0.30	0.07	0.23	0.44	0.43	0.09	0.35	0.63
coniferyl aldehyde	2.76	2.08	0.76	8.08	4.94	2.52	1.81	9.72
furfural	10.01	6.08	1.48	15.26	11.08	5.16	2.79	15.88

RESULTS AND DISCUSSION

Univariate Analysis. Statistics of the spirits included in both categories are shown in Table 1. Before the multivariate techniques were applied to typify the cider brandies, a univariate analysis was carried out to determine whether any of the variables by themselves would allow us to distinguish the categories established. Univariate classification weights (Fisher weight) were computed for classifying the P and C classes; the highest discriminant values corresponded to furfural (24.786; $p < 1\%$), *p*-hydroxybenzaldehyde (2.435; $p < 1\%$), and (hydroxymethyl)furfural (1.479; $p < 1\%$), and the lowest corresponded to ferulic (0.008; $p > 10\%$) and cafeic (0.007; $p > 10\%$) acids. With regard to univariate typification and taking in account aging time (F and A classes), the highest values of Fisher weight corresponded to protocatechuic (2.959; $p < 1\%$), gallic (1.679; $p < 1\%$), ferulic (1.612; $p < 1\%$), and vanillic (1.490; $p < 1\%$) acids and the lowest to furfural (0.020; $p > 10\%$), so this last substance should not be employed as an aging marker.

Figure 1 shows two box-whisker plots for the categories P and C, using the most discriminant variable, namely furfural. As can be seen, separation of the P

Table 2. Prediction Matrix for LDA Technique Validation (Five Groups for Cancellation)

true category	assigned category F	A	hits (%)
F	10	0	100
A	4	11	73.3
overall			84

Table 3. Classification Matrices for KNN Method Using Different Values of *K*: 5, 8, 10, 12

	$K = 5$			$K = 8$			$K = 10$			$K = 12$		
true category	F	A	hits (%)	F	A	hits (%)	F	A	hits (%)	F	A	hits (%)
F	8	2	80	7	3	70	7	3	70	7	3	70
A	2	13	86.7	2	13	86.7	2	13	86.7	1	14	93.3
overall			84			80			80			84

and C categories (Figure 1) may be achieved by means of a univariate treatment based on the furfural content; however, the F and A categories are not sufficiently differentiated when the most discriminant variable, protocatechuic acid, is used. In consequence, a multivariate treatment was needed to typify spirits on the basis of aging time in terms of polyphenolic and furanic components.

Multivariate Analysis. Classification techniques used were as follows: linear discriminant analysis (LDA) and *K* nearest neighbor (KNN).

Linear Discriminant Analysis. The use of the LDA technique allowed us to correctly discriminate spirits categorized as freshly distilled and spirits included in the aged class (correct classifications: 100%).

To ascertain the most relevant variables for classification purposes, a stepwise LDA analysis using Wilks' lambda criterion was carried out. To do this, an *F*-to-enter of 3.84 and an *F*-to-remove of 2.71 were employed; these values correspond to a confidence level >90%. The selected variables were protocatechuic acid, *p*-hydroxybenzaldehyde, and ferulic acid, having detected a Wilks' lambda of 0.2365, so that 76.4% of total variance is explained by within-group differences. The LDA method was also validated using five groups for cancellation; the prediction matrix is displayed in Table 2, 84% of correct predictions having been obtained.

KNN Method. This classification technique is a nonparametric procedure that employs the Euclidean distance for selecting the *K* nearest objects to the sample to be classified. Once the *K* nearest objects are defined, the sample is classified in the category to which the majority of objects selected belong. We have used different values of *K* (5, 8, 10, and 12); the classification matrices are shown in Table 3. As can be seen, the overall percentage of hits ranged between 80 and 84%, which is lower than when the LDA method is applied.

Modeling Methods. Soft independent modeling of class analogy (SIMCA) and the partial least squares (PLS) methods were used.

SIMCA Method. Four components were computed for each class, which accounted for 98.6 and 96.0% of variance for the F and A classes, respectively. Sensitivities and specificities were computed for both classes from a reduced model. In the case of the freshly distilled spirit class (F), the sensitivity and specificity computed were 90 and 93.3%, respectively, so that only 10% of the objects belonging to category F were rejected from their class and only 6.7% of the objects belonging to category A were included in category F. With regard to the aged spirit category (A), the sensitivity and specificity estimated were 93.3 and 70%, respectively. In consequence, model A was less specific than model F, although its sensitivity was slightly higher. This lower specificity was foreseeable if we take into account the fact that no break in continuity exists in the assigning of objects to the F and A classes. In fact, the three F spirits that are included in class A correspond to the brandies with the longest aging time within class F. At the same time, correct classifications obtained from the SIMCA method were 88%, and vanillin and syringaldehyde were the main modeling variables for both models.

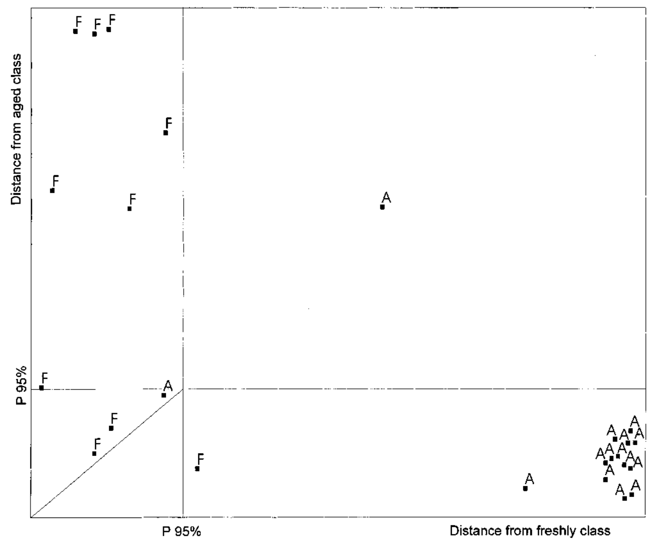

Figure 2. Coomans diagram obtained from SIMCA analysis: F, freshly distilled spirit; A, aged spirit.

The corresponding Coomans diagram is shown in Figure 2; as can be seen, 86.7% of A spirits and 90% of F spirits are correctly classified. Aged spirits are placed far from the model centroid for class F; in contrast, some freshly distilled spirits are placed next to the model centroid for class A, which is in accordance with the specificities detected for both models, that is, model A is less specific than model F.

PLS Method. This modeling technique was also employed to distinguish spirits on the basis of aging time in casks. The binary response variable, *Y*, was subjected to multivariate regression by the PLS method on the original variables (furanic and polyphenolic components). A cross-validation method using three deletion groups was carried out to validate the model constructed. The cross-validated explained variance (CVEV) showed a maximum value of 66.55% when five principal components were considered. The explained variance (EV) and the square of the multiple linear correlation coefficient (R^2) thus estimated were 73.36 and 78.91%, respectively. However, it should be pointed out that three latent variables (EV = 72.49%; R^2 = 75.92%; CVEV = 60.96%) are sufficient for discriminating both classes (Figure 3). Figure 3 shows multiple box-whisker plots for PLS values computed for both categories. As can be seen, we can correctly typify spirits on the basis of their aging time in small oak casks from the model constructed. The variables with the most modeling power were syringic acid, vanillin, and syringaldehyde. As is well-known, aromatic aldehydes such as vanillin and syringaldehyde arise from acid ethanolysis of the lignin of the cask, and the subsequent action of oxygen in the interior of the cask promotes the accumulation of phenolic acids such as syringic and

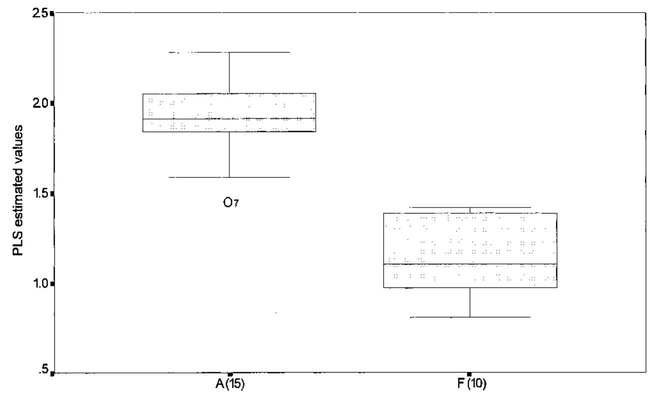

Figure 3. Multiple box-whisker plots for PLS computed values: F, freshly distilled spirit; A, aged spirit.

vanillic acid. In consequence, these variables could be considered as aging markers of cider brandy.

At the same time, when the aging time was established as a response variable, and it was subsequently related with the predictor variables (furanic and polyphenolic compounds) by means of the PLS-1 algorithm, the values obtained of EV, R^2, and CVEV from three latent variables were 94.77, 95.42, and 93.32%, respectively. As can be seen, the model estimated is highly predictive.

Conclusion. Furfural content alone allows us to distinguish classes of cider brandies according to the cider used for making the distilled spirits, namely press cider versus concentrate cider. However, furanic variables were not relevant for describing the maturation of spirits in oak casks.

None of the variables studied allowed us to discriminate cider brandies according to their length of maturation in casks, so a multivariate treatment was necessary. The typification of aged spirits in terms of polyphenolic substances was carried out using different pattern recognition techniques such as LDA, KNN, SIMCA, and PLS. It was possible to ascertain adequate markers of the aging of the cider brandy by means of the application of modeling techniques, such as SIMCA and PLS. It should be pointed out that the main modeling variables thus detected were aromatic aldehydes and syringic acid, which are linked to the process of lignin degradation. As is well-known, this process promotes the aging step of alcoholic beverages.

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