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Evolution of Sugars in Cider Brandy Aged in Oak Barrels: A Contribution to Its Characterization

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A chemometric study was carried out to typify cider brandies according to the type of wood employed in the maturation process and their aging time. Monosaccharides, previously derivatized with *p*-aminobenzoic ethyl ester, were analyzed using a reversed-phase high-performance liquid chromatographic method. Univariate data treatment was not sufficient to enable differentiation of the classes of cider brandies on the basis of wood type and maturation time. Two linear combinations of original variables, ascertained by principal components analysis, provided an adequate data structurization. A mathematical decision rule was established to classify cider brandies with prediction capacities of 92 and 97% using an LDA method and Bayesian analysis, respectively. The use of the PLS algorithm allowed the authors to differentiate cider brandies according to the age and type of oak used in the aging process.

KEYWORDS: Cider brandy; monosaccharides; American oak; French oak; aging; multivariate techniques

INTRODUCTION

Cider brandy is the drinkable spirit obtained by distillation of cider in a pot still, the freshly distilled spirits normally being 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 composition of cider brandies, namely, leaching of wood components, chemical processes such as oxidations and hydrolysis, evaporation of small molecules, and concentration (as a consequence of the former) of the larger molecules (*1*). For instance, acid ethanolysis of lignin and the subsequent action of oxygen promotes the accumulation of aromatic aldehydes and phenolic acids; gallic and ellagic acids and certain carbohydrates (glucose, galactose, xylose, arabinose, ribose, fucose, and rhamnose) are incorporated into the spirit as a consequence of the hydrolysis of tannins and hemicellulose, respectively. These chemical reactions occurring during the maturation process of cider brandy are very important for the quality of the final product; characteristics of taste, flavor, and color appreciated by consumers depend on the composition of the wood (origin of tree) (*2*−*4*), the different treatments used in the manufacture of oak barrels (charring level of wood), and the history of the oak barrel (how often it was used and for how long and for which liquid) (*5*, *6*).

Polysaccharides, that is, cellulose and hemicelluloses, are the primary constituents of wood (*7*). Cellulose is a homopolymer of D-glucose units, joined by β-(1−4) glycosidic linkages, whereas hemicelluloses are heteroglycans containing several different types of neutral (pentose and hexose) and acidic (uronic acid) monosaccharides as structural elements. Several authors have used the content in sugars as an indicator of the authenticity and age of wines and other alcoholic beverages stored in oak barrels (*8*−*11*), because the increase in monosaccharides is usually attributed to the acid hydrolysis of the wood hemicelluloses with the subsequent release of sugars.

Many different analytical techniques have been investigated for the separation and determination of sugars. The different enzymatic and chromatographic procedures available are those most widely used. Paper chromatography and thin-layer chromatography were the first chromatographic techniques used to separate individual sugars, but separations are limited to the number of recognized analytes, present poor resolution, and are not always quantitative (*12*).

Gas chromatography is the standard method used to determine sugars in food. However, although it is sensitive, it requires chemical modification of the sugars to produce volatile derivatives that can be detected (*13*).

Nowadays, high-performance liquid chromatography (HPLC) has been used as a powerful technique to separate and determine carbohydrates. Various stationary phases have been used for separation, including silica gel (*14*), amine-bonded silica (*15*), polystyrene-based anion- (*16*) and cation-exchange resins (*17*), and C18- and C8-bonded silica (*18*, *19*). Because saccharides do not have chromophores in their molecular structures, a pulsed amperometric detector (*20*) or refractive index detector (*21*, *22*)- can be employed for their analysis. Another alternative is

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spectrometric detection after chemical derivatization of the sugars (*23*−*25*). Each stationary phase and detection system presents certain advantages and drawbacks.

The aim of this paper was to gain an insight into the development of the monosaccharide components in distillates of ciders during aging and to ascertain which of them can be employed to classify spirits on the basis of the wood type used in the maturation process and their aging time.

MATERIALS AND METHODS

Chemicals. Aldose and uronic acid standards, *p*-aminobenzoic ethyl ester (ABEE), sodium cyanoborohydride, sodium citrate, and citric acid monohydrate were obtained from the Sigma Chemical Co. (St. Louis, MO). Acetic acid was obtained from Probus (Badalona, Spain). HPLC grade acetonitrile, methanol, chloroform, and tetrahydrofuran (THF) were purchased from Romil (Barcelona, Spain). Milli-Q water (Millipore, Milford, MA) was used throughout. All other chemicals and solvents were of analytical reagent or HPLC grade.

Samples. Cider (8500 L) was made from apple juice concentrate (AJC). AJC was diluted with water (density of reconstituted apple juice = 1056 g/L) and fermented by a starter of *Saccharomyces cerevisiae* belonging to the microorganisms collection of SERIDA. The cider was distilled in an industrial still rectification column, and the distillates were matured in wood casks of French and American oak for 69 months.

Coopering. White oak (*Quercus alba*) was chosen. Staves and heading were dried in the open air for 3 years. Wood, once brushed and cut, was subjected to a light toasting. The thickness of the staves and cask capacities were 28 mm and 100 L, respectively.

HPLC Analysis. Sugars were analyzed by HPLC using the methodologies previously described by the authors (*19*). A Shimadzu HPLC system equipped with two LC-10AD pumps, a UV−vis SPD-M10AD photodiode array detector, a Sil-10AD automatic injector, and a Gastor 150 LCD on-line degasser were used. A Kromasil C8 column (Teknokroma, 200 × 2.1 mm i.d., 3.5 μm) was used at 45 °C. The mobile phase consisted of solvent A (100 mM sodium citrate buffer, pH 5.5/THF = 88:12) and solvent B (acetonitrile), and the gradient program was as follows: 1−20 min, 1% B; 20−28 min, 20% B; 28−36 min, 1% B. The analysis was monitored at 307 nm, and the absorption spectra of compounds were recorded between 250 and 350 nm. The sample injection volume was 5 μL. Identification of compounds was carried out by comparing their retention time values and UV spectra with those of standards stored in a data bank. Quantitative determination was performed using the external standard method. All mobile phase solutions were filtered through a 0.45 μm membrane filter.

Derivatization Procedure of the Monosaccharides. The procedure employed for the derivatization of aldoses and uronic acids at their reducing end with ABEE was carried out according to the method of Wang et al. (*25*) as modified by the authors (*19*). Four milliliters of cider brandies was evaporated to just dryness, and the residues were reconstituted in 4 mL of water. Four hundred microliters of 1.4 M $NaBH_3CN$ in distilled water, 400 μL of glacial acetic acid, and 2 mL of 0.6 M ABEE in methanol were added to this solution, and the mixture was heated at 80 °C for 10 min. After cooling to ambient temperature, the aqueous phase was extracted with 4 mL of chloroform to remove excess ABEE and the aqueous layer was subjected to HPLC analysis.

Experimental Design. A factorial design with three replicates was used. Factors or independent variables were as follows: wood type (two levels, French oak and American oak) and aging time (6 samplings for 69 months). Response variables were as follows: glucose, galactose, xylose, arabinose, ribose, fucose, rhamnose, and D-glucuronic and D-galacturonic acids. In addition to 36 experimental cider brandies, we included 13 industrial cider brandies, to increase the information of database. Industrial samples were as follows: 5 fresh cider brandies aged in American oak, 1 old cider brandy aged in American oak, 4 fresh cider brandies aged in French oak, and 3 old cider brandies aged in French oak.

Data Processing. Data were processed using the PARVUS statistical package (*26*). A data matrix was constructed with rows (49) representing cider brandies, and columns (9) corresponding to chemical variables (glucose, galactose, xylose, arabinose, ribose, fucose, rhamnose, and d-glucuronic and D-galacturonic acids), monitored during the aging time of the spirits (3, 6, 12, 28, 63, and 69 months). Samples were categorized according to the wood type used in the maturation process [American (A) and French (F) oak] and aging time [fresh (f) ≤ 12 months and old (O) > 12 months]. This decision was made with consideration for the fact that the extractions and chemical processes such as oxidations, acetylations, and hydrolysis are more extended during the first 12 months of aging, especially if new barrels are used. The cider brandies were initially classified in four categories: 1 (14 observations, fresh brandies matured in American oak, fA); 2 (10 observations, old brandies matured in American oak, OA); 3 (13 observations, fresh brandies matured in French oak, fF); and 4 (12 observations, old brandies matured in French oak, OF). Several multivariate statistical techniques were employed to visualize the data structure and to typify the cider brandies, namely, principal component analysis (PCA), linear discriminant analysis (LDA), Bayesian analysis, and partial least squares (PLS).

Table 1. Descriptive Statistics of Variables (n = 49)[a]

variable	mean (mg/L)	SD	min (mg/L)	max (mg/L)	range (mg/L)
glucose	45.1	1310.7	6.1	133.8	127.8
galactose	10.5	84.0	0.9	38.0	37.1
xylose	15.0	71.1	3.5	34.8	31.4
arabinose	50.5	828.0	14.8	121.2	106.3
ribose	3.8	7.7	0.9	11.9	11.1
fucose	5.9	16.6	1.0	17.2	16.2
rhamnose	8.3	27.9	1.8	22.0	20.2
D-glucuronic acid	3.1	4.2	0.7	6.9	6.2
D-galacturonic acid	2.2	2.5	0.4	5.9	5.5

[a] SD, standard deviation; min, minimum values; max, maximum values.

RESULTS AND DISCUSSION

Univariate Analysis. Table 1 lists the mean, minimum, and maximum values together with range and standard deviation for each chemical variable monitored during the aging process of the cider brandies. Before multivariate data treatment was employed, a univariate analysis was carried out using Fisher's test. The most discriminant variables were rhamnose and ribose (Fisher's weights: 8.70 and 5.83, p < 5%, respectively). However, the use of the most discriminant variable, namely, rhamnose, did not allow us to differentiate between the cider brandies aged in different wood types and aging intervals, so multivariate treatment of the data was needed.

Principal Component Analysis. PCA was used to ascertain the structure of the data and to reduce the number of variables. The number of significant components was evaluated on the basis of double cross-validation of the components by means of the NIPALS method (*27*), using three cancellation groups. Two predictive components were chosen that accounted for 95.4% of the variance. As can be appreciated in **Figure 1**, a structurization of the data can be visualized. It can be seen that all of the variables have similar eigenvector loadings on the first predictive component, because under all of the aging conditions the sugars increase their concentration during the maturation process. At the same time, it can also be seen in **Figure 1** that D-galacturonic acid, xylose, and arabinose allow differentiation of old cider brandies (classes 2 and 4) on the basis of the wood type employed in their aging.

Linear Discriminant Analysis. The LDA method was used to classify the cider brandies according to their aging time and wood type. Each object is a datavector of two variables obtained by linear combination of the original variables using the PCA method. LDA computes a mathematical decision rule that allows

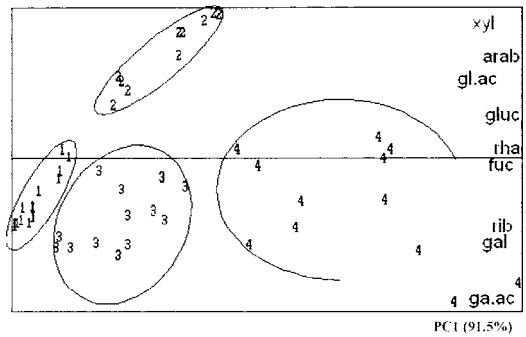

Figure 1. Projection of the variables and cider brandies onto the plane formed by the two first principal components: (1) fresh brandies aged in American oak; (2) old brandies aged in American oak; (3) fresh brandies aged in French oak; (4) old brandies aged in French oak; (gl.ac) D-glucuronic acid; (ga.ac) D-galacturonic acid; (gluc) glucose; (gal) galactose; (xyl) xylose; (arab) arabinose; (rib) ribose; (fuc) fucose; (rha) rhamnose.

Table 2. Classification Matrix for the Linear Discriminant Analysis Method[a]

	assigned category				
true category	fA	OA	fF	OF	hits (%)
fA	14	0	0	0	100
OA	0	10	0	0	100
fF	2	0	11	0	84.6
OF	0	0	0	12	100
overall					95.9

[a] Abbreviations: fA, fresh brandies aged in American oak; OA, old brandies aged in American oak; fF, fresh brandies aged in French oak; OF, old brandies aged in French oak.

Table 3. Prediction Matrix for Linear Discriminant Analysis Method Validation[a]

	assigned category				
true category	fA	OA	fF	OF	hits (%)
fA	14	0	0	0	100
OA	0	10	0	0	100
fF	2	0	11	0	84.6
OF	0	1	1	10	83.3
overall					92.0

[a] Abbreviations: see **Table 2**.

the category to which a sample belongs to be ascertained. As can be seen in **Table 2**, when all of the objects were included in the training set, 96% of classification hits were obtained. The method was validated by means of a cross-validation procedure using three groups for cancellation. **Table 3** shows the prediction matrix for each class; as we can see, the overall percentage of prediction hits was 92%. Hence, we may consider that the mathematical procedure computed is sufficiently robust for discriminating cider brandies on the basis of oak type and aging time.

Bayesian Analysis. We also used another classification method, namely, Bayesian analysis, to compare the results obtained with the LDA method. All of the cider brandies were correctly classified (100% hits). Category 1 includes 93% (sensitivity = 93%) of the observations assigned to this model; categories 2−4 include 100% of the samples assigned. Model 4 includes 15% (specificity = 85%) of cider brandies belonging to model 3, whereas classes 1−3 reject all cider brandies belonging to other categories (specificity = 100%). Internal validation of the Bayesian analysis using four groups for cancellation enabled us to correctly predict 97% of samples.

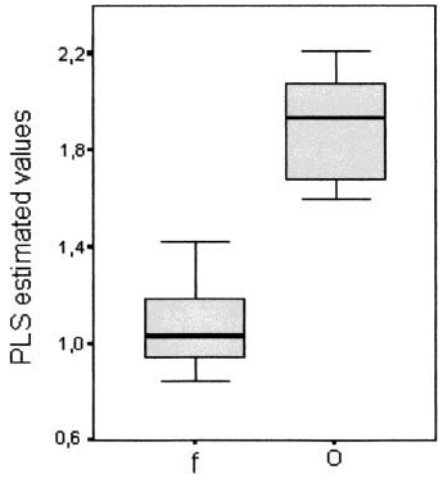

Figure 2. Multiple box-whisker plots for PLS estimated values of binary response (*Y*): f, fresh cider brandies; O, old cider brandies.

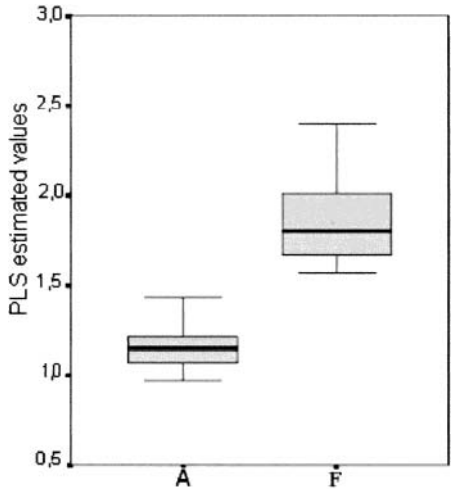

Figure 3. Multiple box-whisker plots for PLS estimated values of binary response (*Z*): A, American oak barrel; F, French oak barrel.

Partial Least Squares. PLS is a regression technique that is often used to model the relationship between two data matrices. If we define a binary response and establish a PLS model between this variable and the predictor variables set, PLS can be used as a discriminant technique. In our case, we define two binary response variables (*Y* and *Z*), namely, $Y = 1$ for fresh classs, $Y = 2$ for old class; $Z = 1$ for American class, and $Z = 2$ for French class. The PLS model established for aging time (*Y* as binary response variable and sugars as predictor variables) consisted of two latent variables estimated by cross-validation using three deletion groups. The percentages of the cross-validated variance, the explained variance, and the multiple linear correlation coefficient were 79.4, 80.1, and 81.0%, respectively. **Figure 2** shows multiple box-whisker plots for PLS values computed for the binary response (*Y*); as can be seen, the differentiation of both classes (fresh vs old) is accurate. The PLS model established for wood type (*Z* as binary response variable and sugars as predictor variables) consisted of five latent variables estimated by cross-validation using three groups for cancellation. The percentages of the cross-validated variance, the explained variance, and the multiple linear correlation coefficient were 68.3, 76.3, and 78.8%, respectively. **Figure 3** presents two box-whisker plots using PLS estimated values for binary response (*Z*). As we can see, the discrimination of both

classes (American vs French) is also accurate using this modeling technique.

Conclusions. Data obtained from HPLC analysis of chemical variables involved in the process of maturation of cider brandies, namely, sugars, together with chemometrics (PCA, LDA, Bayesian analysis, and PLS) enabled the authors to typify cider brandies. The PCA method provided an adequate data structurization using only two dimensions, and a feasible mathematical decision rule was established with the use of the LDA method (prediction capacity = 92%) and Bayesian analysis (prediction capacity = 97%) in order to classify cider brandies on the basis of wood type (American and French oak barrels) used and aging time (fresh and old brandies). Likewise, a linear model computed by means of the PLS algorithm allowed us to discriminate the spirits by their age and the type of oak used in the maturation process.

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