

Analysis of Polysaccharides in Cider: Their Effect on Sensory Foaming Properties

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A feasible spectrophotometric method for determining acidic and neutral polysaccharides in cider is described, and the advantages of this method are examined with respect to precision, accuracy, and detection and quantitation limits. The concentration of nonvolatile and volatile components in cider, together with chemometric techniques such as principal component analysis (PCA), soft independent modeling of class analogy (SIMCA), and partial least squares (PLS), allowed us to typify the ciders on the basis of their foaming properties. Acidic polysaccharide and 1-propanol were the most relevant variables for this purpose.

Keywords: *Polysaccharides; foaming properties; multivariate techniques; cider*

INTRODUCTION

The production of natural cider represents one of the major economic resources of Asturias. This beverage is made by the traditional method: slow pressing, spontaneous clarification and fermentation, and bottling without stabilization. The usual way of consuming cider is also very traditional; it is poured into a glass from a height of around 1.5 m, the cider beating against the inside wall of the glass. This handling generates some sensory foaming properties which are considered to be of major importance for cider quality. In fact, the majority of the cider-drinking public judges cider by eye as much as by taste and odor, as is the case for the beer-drinking public. In the case of cider, excessive and nonconsistent lacing and/or high foam stability cause a decrease in its sensory assessment.

There are many studies showing the influence of the chemical composition on the foaming properties of fermented beverages (Brissonet and Maujean, 1991; Pueyo et al., 1995; Andrés-Lacueva et al., 1996). Polysaccharides contribute to the flavor, color, and foaming properties of the product, as they are substances which stabilize foam. In this sense, it has been hypothesized that an increase in local viscosity, promoted by the presence of these macromolecules, hinders the rate of drainage of liquid between bubbles. At the same time, the redissolution of other surface-active components from the bubble wall is reduced by the presence of polysaccharides (Bamforth, 1985).

The polysaccharide level in fermented beverages is determined by several factors such as raw material (mainly the state of ripeness of the fruit), prefermentative technology, and the action of yeasts and bacteria during the biotransformation of fruit (Fleet, 1991; Charpentier and Feuillat, 1993; Sponholz, 1993).

The analysis of polysaccharides in fermented beverages and fruit juices is usually preceded by a purification step, using dialysis, ion-exchange columns, or ethanol precipitation as extraction procedures, as reviewed by Segarra et al. (1995). The chemical charac-

terization of polysaccharide fractions had been accomplished by chromatographic methods such as ion-exchange chromatography (Voragen et al., 1982), size-exclusion chromatography with refractive index detection (Dubourdieu et al., 1986; Hoagland et al., 1997), and gas-liquid chromatography (Ford, 1982; Ha and Thomas, 1988). However, spectrophotometric methods are the most frequently employed for determining polysaccharides, using phenol (Dubois et al., 1956) and orcinol (Tollier and Robin, 1979) as chromogen reagents for neutral polysaccharides, while an estimation of the acidic polysaccharide fraction may be ascertained by means of the *m*-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973; Robertson, 1979; Kintner and Van Buren, 1982). This reagent is highly selective, allowing to differentiate between acidic polysaccharides and other components present in fruit juices and fermented beverages, such as phenolic compounds (Robertson, 1981).

The objective of this present study was therefore to optimize an analytical procedure for determining acidic and neutral polysaccharides in cider and to study the influence of cider composition on its foaming properties using chemometric methods.

MATERIALS AND METHODS

Samples and Chemicals. Twenty samples of cider were collected from the corresponding cider-maker cellars and kept in the experimental cellar until sensory and global parameter analyses. For the rest of the instrumental data, samples were frozen ($-20\text{ }^{\circ}\text{C}$) at the same time as the sensory session and analyzed later on. Acidic polysaccharides (AP) were quantified as galacturonic acid, and neutral polysaccharides (NP) were quantified using arabinose as standard. All reagents, standards, and solutions were of analytical grade.

Analytical Procedures. *Global Parameters.* Total and volatile acidities and alcoholic content were determined according to the AOAC method (1984) and total polyphenols using the Folin-Ciocalteu procedure (Montreau, 1972).

Polysaccharides. The cider was vacuum-filtered through hydrophilic cotton and was then subjected to precipitation with ethanol (cider:ethanol, 1:5, v/v) in darkness during 24 h at room temperature. The precipitate was washed twice in ethanol (96%), using the Molish test to ascertain the absence

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of soluble sugars. The residue was dried in a stream of nitrogen and dissolved in Milli-Q quality water (cider:water, 1:2, for AP and cider:water, 1:5, for NP).

1. Acidic Polysaccharides. AP determination was carried out using *m*-hydroxydiphenyl as chromogen reagent, as described by Kintner and Van Buren (1982). The colored solution was degassed in an ultrasonic bath at 20 °C for 10 min to avoid the interference of bubbles formed when the reagent and sodium hydroxide were added. Colorimetric measurements at 523 nm were done after 15 min of color development.

2. Neutral Polysaccharides. NP analysis was carried out using the orcinol–sulfuric method. An aqueous extract (250 µL) was carefully mixed with 6 mL of orcinol–sulfuric reagent (0.2%, w/v, orcinol in 55%, w/v, sulfuric acid) for 2 min using a Vortex mixer. The tubes were then heated in an 85 °C water bath for precisely 15 min and immediately cooled in ice–water for 5 min. Subsequently, the tubes were vortexed thoroughly and degassed in an ultrasonic bath at 20 °C for 10 min, and the absorbance was measured at 423 nm. The orcinol–sulfuric method permits the estimation of total polysaccharides; NP are obtained by taking into account the interference of AP. This interference was evaluated from the calibration curve of galacturonic acid, obtained with the orcinol–sulfuric method, and the concentration of acidic polysaccharides estimated by the *m*-hydroxydiphenyl procedure.

Chromatography. Major organic acids (lactic and acetic) were analyzed by HPLC according to the method developed by Blanco et al. (1991), using a reversed-phase system and UV detection. Major volatiles (acetaldehyde, ethyl acetate, methanol, 1-propanol, 2-methyl-1-propanol, 1-butanol, and amyl alcohols) were determined by direct injection with a FID-GC system (Mangas et al., 1993).

Sensory Analysis. Seven cider makers were scheduled once a week for 2 months to carry out sensory analyses in 3-h sessions. The ciders were placed in water a few hours before the sensory evaluation so as to reach 14–15 °C and served to each judge by a professional cider pourer. Cider glasses (height: 12 cm; bottom diameter, 7 cm; top diameter, 9 cm) were cleaned up before each session with hot water, thoroughly rinsed with deionized water, and air-dried.

Participants were asked to score each sensory attribute, using a hedonic 10-point nonstructured scale, and to discuss their opinions afterward. Foaming properties (foam stability plus lacing) were assessed in duplicate. Nonsignificant differences were found among the judges at the 5% confidence level.

Data Processing. The data were processed using the PARVUS statistical package (Forina et al., 1988). The data matrix was structured with 20 rows representing ciders and 14 columns corresponding to chemical variables (total and volatile acidities, AP, NP, polyphenols, lactic and acetic acids, acetaldehyde, ethyl acetate, methanol, 1-propanol, 2-methyl-1-propanol, 1-butanol, and amyl alcohols). On the basis of the sensory evaluation, samples were categorized as LF (low foaming) and HF (high foaming). LF ciders were those that scored higher than 6, while HF were those that scored below 6. The data were autoscaled before statistical treatment.

RESULTS AND DISCUSSION

Polysaccharide Analysis. Three factors with four repetitive determinations were considered in the optimization of the extraction procedure of polysaccharides, namely, temperature (hot and cold extraction), the ratio of added ethanol (1:3 and 1:5 cider:ethanol), and the addition of hydrochloric acid. Hot extraction was conducted in boiling ethanol for 5 min, while cold extraction was carried out at 20 °C for 24 h in darkness. The polysaccharide concentration was significantly higher ($p < 5\%$) when the cold extraction procedure was applied instead of the hot extraction one and when the cider: ethanol ratio of 1:5 was used instead of the 1:3 cider: ethanol ratio ($p < 5\%$). Several researchers have carried out the precipitation of polysaccharides under acidic

Table 1. Analytical Characteristics of the Polysaccharide Analysis Procedure

repeatability ^a	<2%
reproducibility ^b	<3%
detection limit (mg/L)	0.22 (AP); 0.51 (NP)
quantitation limit (mg/L)	0.75 (AP); 1.71 (NP)
linearity (mg/L)	100 (AP); 60 (NP)

^a Determined as relative standard deviation from five measurements. ^b Estimated as mean of the relative standard deviation of each of the samples ($n = 20$) using three replicates; AP, acidic polysaccharide; NP, neutral polysaccharide.

conditions (Usseglio-Tomasset, 1976; Dubourdiu et al., 1981); our results showed a decrease in the polysaccharide concentration when 0.008 M hydrochloric acid was used, especially the acidic fraction. This could be due to the hydrolysis of these macromolecules, which is promoted by the action of the acid (Thibault, 1980). Other compounds such as proteins and polyphenols should not interfere in the determination of polysaccharides, in accordance with the findings obtained by Thibault (1979) and Segarra et al. (1995), since they are present at low concentrations in both cider (<200 mg/L for proteins) and the aqueous extracts (<40 mg/L for polyphenols). The accuracy of the precipitation procedure was tested by spiking a sample of cider with pectin from apples (Fluka 76282), reaching a percentage of recovery close to 97% (RSD < 3%). Other analytical characteristics of the method optimized are shown in Table 1.

Cider Composition and Foaming Properties.

Data Univariate Analysis. The descriptive statistics of chemical variables are shown in Table 2. Apparently, there exist some differences between LF ciders (positively assessed by the panel) and HF ciders (scored negatively by the panel) with respect to their chemical composition. LF ciders presented higher mean values for 1-propanol and lower mean contents for AP and methanol than HF ones. Fisher's test was applied so as to ascertain the most relevant variables for differentiating both categories (LF and HF), and these were found to be 1-propanol and acidic polysaccharide with Fisher weight values of 0.90 ($p < 5\%$) and 0.82 ($p < 5\%$), respectively. Figure 1 shows two Box–Whisker plots using the most discriminating variable, namely, 1-propanol; as can be seen in this figure, separation of the two categories may not be achieved by means of univariate treatment based on the 1-propanol content, and hence multivariate treatment was needed.

Factor Analysis of the Internal Structure. Principal component analysis (PCA) was used to reduce the dimensionality of the data matrix and to ascertain the data structure. Five eigenvectors that accounted for 85.3% of the variance were chosen on the basis of Kaiser's criterion. The analysis of the data structure was done by means of an orthogonal rotation. Figure 2 shows the projection of the original variables and ciders onto the plane formed by the first and third varivectors, respectively. Acidic polysaccharides, methanol, and 1-propanol were the most correlated to the third varivector (correlation coefficients, 0.58, 0.52, and -0.31 , respectively), while volatile acidity, acetic acid, and acetaldehyde were more correlated to the first varivector (correlation coefficients, 0.56, 0.51, and -0.38 , respectively). The distribution of data showed that ciders which belong to the low-foaming class were samples with high 1-propanol and low acidic polysaccharide and methanol contents. The influence of the polysaccharide

Table 2. Descriptive Statistics of Variables for Cider Categories (LF and HF)^a

	LF			HF		
	mean	min	max	mean	min	max
volatile acidity ^b	1.54	1.07	2.60	1.36	0.55	2.12
total acidity ^a	3.59	3.19	3.98	4.22	2.76	5.75
polyphenols ^d	1.03	0.64	1.94	0.94	0.53	1.13
acidic polysaccharide	28.25	14.04	44.42	184.76	25.24	539.00
neutral polysaccharide	165.67	106.75	244.17	172.92	118.53	234.28
alcoholic content (% v/v)	6.3	5.8	6.9	6.4	6.2	6.7
acetaldehyde	22.52	2.22	68.93	20.90	3.46	60.44
ethyl acetate	101.58	62.40	145.12	106.22	49.62	182.58
methanol	70.34	44.20	104.64	103.14	23.79	163.44
1-propanol	24.83	11.06	40.46	13.51	7.37	27.05
2-methyl-1-propanol	23.69	15.24	35.33	27.32	8.44	55.99
1-butanol	5.35	1.64	7.25	5.03	1.58	8.93
amyl alcohols	168.49	114.88	226.29	132.88	50.71	244.52
lactic acid ^e	4.13	3.25	5.05	3.62	3.29	4.10
acetic acid ^e	1.32	0.60	1.99	1.20	0.41	2.23

^a Concentration in mg/L ($n = 20$); LF, low foaming; HF, high foaming. ^b g of acetic acid/L. ^c g of sulfuric acid/L. ^d g of tannic acid/L. ^e Concentration in g/L.

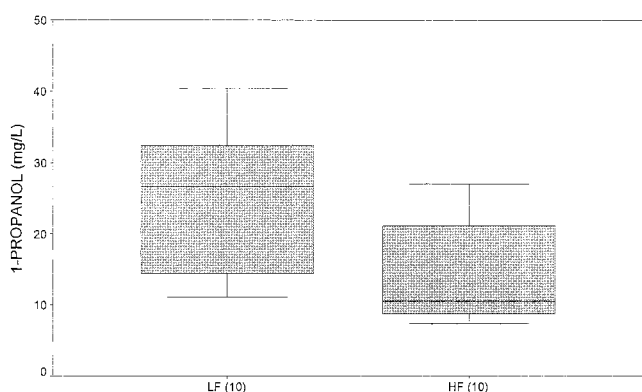


Figure 1. Box plots for each category (LF, low foaming; HF, high foaming) using the main discriminating variable (1-propanol). The number of samples for each category is included in parentheses.

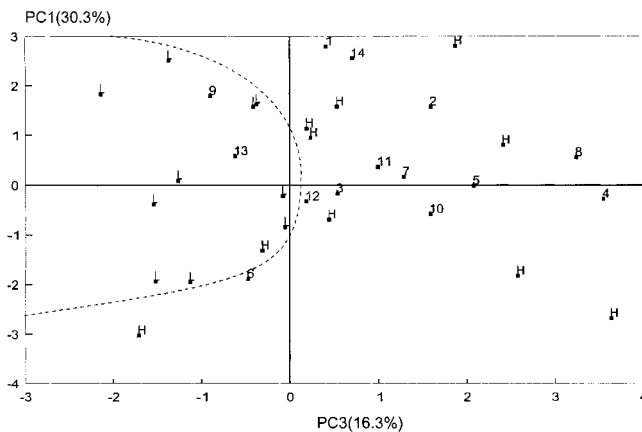


Figure 2. Eigenvector projection of chemical variables and ciders: 1, volatile acidity; 2, total acidity; 3, polyphenols; 4, acidic polysaccharide; 5, neutral polysaccharide; 6, acetaldehyde; 7, ethyl acetate; 8, methanol; 9, 1-propanol; 10, 2-methyl-1-propanol; 11, 1-butanol; 12, amyl alcohols; 13, lactic acid; 14, acetic acid; L, low-foaming category; H, high-foaming category.

level on foam stability in alcoholic beverages has been well-established (Bamforth, 1985). Obviously, the relationship detected between methanol content and foaming properties of cider is explained by the fact that this alcohol is chemically linked to apple pectins. The relationship detected between 1-propanol and the foaming properties of cider shows the necessity of controlling

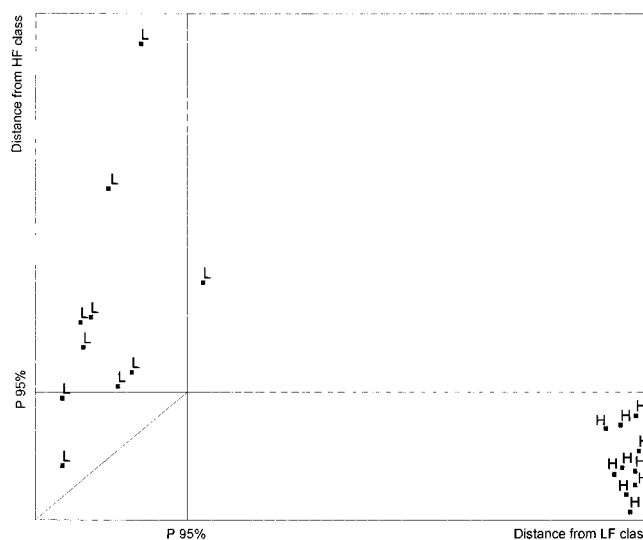


Figure 3. Coomans' diagram for SIMCA analysis (L, low-foaming category; H, high-foaming category).

the fermentation process by selecting an adequate starter, since it is well-known that the capacity of *Saccharomyces* to produce 1-propanol is basically controlled by genetic factors (Thornton, 1989).

Soft Independent Modeling of Class Analogy (SIMCA). Five and six principal components were computed for each classes (LF and HF, respectively) using a SIMCA augmented model, which accounted for 96.4% and 96.0% of the variance, respectively. Figure 3 shows a Coomans' diagram where the two SIMCA boxes are displayed. As can be seen, correct classifications were 100%, since all ciders belonging to the LF class (L in the figure) are placed above the diagonal, and all ciders belonging to the HF class (H in the figure) are placed below the diagonal, acidic polysaccharides being the most discriminating variable in accordance with the results obtained by PCA analysis. At the same time, the SIMCA models obtained were highly sensitive (90% and 100% for LF and HF, respectively) and specific (100% and 80% for LF and HF, respectively); thus, a high percentage of ciders classified initially within a category are correctly placed within their SIMCA box, while on the other hand, only a low percentage of ciders categorized initially within a class are accepted within the SIMCA box corresponding to another class.

Partial Least Squares (PLS). This modeling technique was also employed for typifying ciders on the basis of their foaming properties. The sensory attributes were then subjected to multivariate regression by PLS on the chemical variables. A cross-validation procedure was carried out using three groups for cancellation. The cross-validated explained variance (CDEV) showed a maximum value (75.0%) with one latent variable, the square of the multiple linear correlation coefficient (R^2) being 84.7%. The most relevant variables were as follows: acidic polysaccharide, 1-propanol, and total acidity. In agreement with PCA and SIMCA analyses, pectin content was also the most relevant variable in the model constructed from PLS-1 algorithm. This result is important from the technological point of view, showing the influence of the raw material on the foaming properties of ciders, as the pectin content depends on the degree of ripening of apples.

CONCLUSIONS

A reliable analytical procedure for analyzing acidic and neutral polysaccharides in cider was optimized. Cold precipitation with ethanol and the absence of hydrochloric acid promoted an increase in the polysaccharide content. The use of multivariate techniques of data analysis, namely, PCA, SIMCA, and PLS, allowed us to ascertain the important role played by the acidic polysaccharide fraction, methanol, and 1-propanol in the typification of ciders on the basis of their foaming properties. Thus, ciders positively scored in sensory analyses were those containing lower methanol and acidic polysaccharide contents and higher 1-propanol contents. Hence, the characteristics of the raw material (apple varieties and their degree of ripening), the prefermentative technology used, which directly influences the pectin level in apple juice, and the activity and type of fermentative yeasts which influence pectolytic activity developed in the fermentation media and the 1-propanol content are factors to be taken into account in order to control the foaming properties of cider.

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