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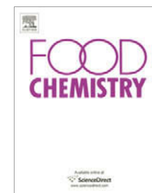

Analytical Methods

Characterization of cider by its hydrophobic protein profile and foam parameters

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ABSTRACT

This paper describes the characterization of ciders (both "natural" and sparkling cider) from the Principality of Asturias (northwest region of Spain) through the analysis of their protein content, based on their hydrophobic properties, and their foam characteristics. A reversed-phase high performance liquid chromatography (RP-HPLC) was applied to the protein analysis, and the foam parameters were measured with Bikerman's method. Multivariate techniques allowed the authors to differentiate ciders on the basis of the press and foam taking technologies, and foam sensory quality. Feasible and robust models were constructed for classifying purposes. Higher than 95% correct classifications were obtained for differentiating ciders on the basis of the factors studied (cider making technology and foam sensory quality). The multivariate regression model computed allowed the authors to predict (correlation coefficients higher than 0.8) the foam parameters related to foam stability and bubble average lifetime in "natural" cider.
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1. Introduction

Cider is a very important beverage in the Principality of Asturias (northwest region of Spain). Annual production is about 0.82 million hl, and 55% of this production corresponds to "natural" cider. The rest of the cider production corresponds to a sparkling beverage made either by carbonation of cider or by the "Champenoise" technology.

Foam is a quality parameter of great relevance from a sensory viewpoint, since it is the first attribute that consumers perceive. Moreover, high and significant correlations between foam, odor and taste qualities in cider have been detected (data not published). Good foaming behavior in "natural" cider includes the initial formation of a large amount of foam when it is poured in the glass, followed by its rapid disappearance forming little bubbles of carbonic gas; finally a rest of thin foam should stay in the glass. The typical visual attributes assessed in sparkling cider are initial foam, foam area persistence, number of nucleation sites, bubble size, and foam collar (Picinelli Lobo, Fernández Tascón, Rodríguez Madrera, & Suárez Valles, 2005).

Proteins contribute to form and stabilize foams. The important role of these molecules can be explained by their ability to decrease the interfacial tension, and increase the viscous and elastic properties of the film, as a result of the formation of hydrogen bonds, and electrostatic and hydrophobic interactions (Zayas, 1997).

Different authors have carried out studies on base wines used for making sparkling wine (Andrés-Lacueva, López-Tamanes, Lamuela-Raventós, Buxaderas, & De la Torre-Boronat, 1996; Brissonnet & Maujean, 1993; Robillard et al., 1993) and they have shown the existence of a relationship between protein concentration and foam quality.

On the other hand, proteins can become a valuable "finger print" for characterizing fermented beverages, since these macromolecules are not influenced by the nature of the soil or the climate, but are genetically defined. In fact, different authors have analyzed wine and must protein fractions in order to characterize them (González-Lara, Correa, Polo, Martín-Álvarez, & Ramos, 1989; Moreno-Arribas, Cabello, Polo, Martín-Álvarez, & Pueyo, 1999; Polo et al., 1989).

Our research group has characterized both "natural" and sparkling cider on the basis of the molecular weight profile of polypeptides having found a close association between sensory foam quality, cider making technology and polypeptides nature. Thus, in the case of "natural" cider, low and middle molecular weight polypeptides were associated with good foaming properties, while the presence of higher molecular weight polypeptides is linked to the fast press manufacturing technology (Blanco Gomis, Mangas Alonso, Junco Corujedo, & Gutiérrez Álvarez, 2007). On the other hand, in the case of sparkling cider manufacturing, the molecular weight of polypeptides was related to yeast type used in the foam generation step (Blanco Gomis, Mangas Alonso, Junco Corujedo, & Gutiérrez Álvarez, 2009).

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Since hydrophobic interactions are a very important factor for foam forming and stabilizing, it is necessary to know the protein composition based on its hydrophobic character. Therefore, knowledge of the influence of cider making technology (for example, press and foam taking technologies) on protein hydrophobic profile and foam quality is a very important question in order to improve and to suitably control cider quality. Other technological factors, such as addition of fining agents or thermally extracted yeast cell wall, can have an effect on the foam quality in sparkling wine (García, Aleixandre, Álvarez, & Lizama, 2009; Núñez, Carrascosa, González, Polo, & Martínez-Rodríguez, 2006).

Due to protein structural complexity and the great variability that exists, the use of modern analytical techniques such as reversed-phase high performance liquid chromatography (RP-HPLC) is required for protein analysis. This analytical technique is specially appropriate for quantifying polypeptides on the basis of hydrophobic properties (Bobe, Beitz, Freeman, & Lindberg, 1998; González & González-Lara, 1993; González-Lara & González, 1991; Knuutinen & Harjula, 1998; Santoro, 1995; Trujillo, Casals, & Guamis, 2000).

On the other hand, to carry out a study about cider foam is necessary to measure its foaming properties: Foam Height or foamability (FH), Foam Stability height (FS), a parameter related to bubble average lifetime and collar quality, and foam Stability Time (ST), a parameter related to the average lifetime of foam. These parameters have been positively correlated to descriptors evaluated in sensory analysis of sparkling wines as foam area, foam collar, and global impression (Gallart, Tomás, Suberbiola, López-Tamames, & Buxaderas, 2004).

The goal of this study was to characterize both ''natural'' and sparkling cider based on the hydrophobic profile (Blanco Gomis, Expósito Cimadevilla, Junco Corujedo, & Gutiérrez Álvarez, 2003) of polypeptides and foam properties (FH, FS, and ST). Chemometric techniques had to be applied to extract the information from a high number of observations obtained from the analytical measurements. Furthermore, with such number of analytical data, multivariate techniques are the most appropriate statistical methodologies for classifying purposes (Rodríguez-Nogales, García, & Marina, 2006). For example, while clustering techniques are used for searching natural grouping in the database, principal component analysis (PCA) is used to detect correlations between observations (samples) and original variables, in order to reduce their number. Modeling methods such as Bayesean analysis and Soft Independent Modeling of Class Analogy (SIMCA) are appropriate statistical tools for classifying purposes, and Partial Least Square (PLS) regression allows to establish mathematical relationship between a set of predictor variables and response ones.

2. Materials and methods

2.1. Ciders and foams

''Natural'' ciders ($n = 34$) were supplied by several cellars from the Principality of Asturias. Cider was elaborated by sequential juice extraction of Asturian cider apple varieties, using either a hammer mill, mechanical or hydraulic basket presses (slow press technology, $n = 16$) or pneumatic presses (fast press technology, $n = 18$). Afterwards, the extracted apple must was fermented by wild microflora in stainless steel, fiber, and/or chestnut wood casks.

Sparkling ciders ($n = 29$) were elaborated (''méthode champenoise'') in the ''Sidra el Gaitero'' cellar, in two vintages (2001–2002, $n = 14$, and 2002–2003, $n = 15$). The procedure of elaboration of sparkling cider was as follows: Asturian cider apples were first washed and milled, afterwards the pulp was macerated for 9–12 h, and finally it was pressed in an automated hydraulic press (Bucher-Guyer). Fermentation was conducted by wild microflora in stainless steel casks at 14–20 °C. When the first fermentation had ended, cider was matured until optimal sensory properties of cider (base cider) were achieved. Then, base cider was clarified through a ceramic microfilter of 0.22 μm of pore size. In order to carry out the foam taking in bottle, 18 g/L sucrose, 0.05% (w/v) nutritive solution [ammonium sulfate 96% (w/w), citric acid 3.3% (w/w), and thiamine 0.7% (w/w)], and 0.003% bentonite, used as fining agent, were added to the base cider. This cider was inoculated (2%) with two yeast types: cider yeast (C6, Saccharomyces bayanus) belonging to SERIDA yeasts collection, and a commercial wine yeast (Levuline CHP, Saccharomyces cerevisiae). Finally, cider was bottled (bottles of 0.75 and 1.5 L), and the second fermentation (1 month) and aging ''surlie'' was conducted at 12–15 °C during 16 months. Sampling was initiated in base cider (two samples in 2001–2002 vintage, and three samples in 2002–2003 vintage) and continued along the foam taking (two samples) and aging. During this last stage, 10 samples regularly distributed throughout 16 months of aging were taken.

2.2. Foams

Foam of ''natural'' cider was extracted following a procedure based on the method developed by Brissonnet and Maujean (1991): 250 mL of cider were added to a glass cylinder (diameter: 3.5 cm; length: 38.0 cm) with a glass-frit at the bottom (porosity 10 μm). Carbonic gas was sparged through the glass-frit at 6.0 L/h. The foam goes up along the glass cylinder, reaches the top, and collapses. The foam collapsed is denominated cider foam. The fraction contained inside the column after carbon dioxide has been sparged through it is the remainder cider. It is the cider without a significant part of the foam.

2.3. Sample preparation

Cider (40 mL) was sonicated for 5 min to free the proteins and centrifuged at 3000*g* for 40 min to eliminate solid substances. Afterwards cider was filtered at 4 °C through PVDF Durapore® (Millipore) 0.22 μm of pore size, and 15 mL of the filtrate were added to a PD-10 mini-column (6×2.5 mL void volume) of Sephadex® [Amersham Pharmacia Biotech (Uppsala, Sweden)] previously equilibrated with 25 mL of water. Subsequently elution of proteins was performed in each mini-column with water (6×3.5 mL), and the eluted volume was concentrated in the rotary vacuum evaporator until dryness. Sample was reconstituted in 300 μL of water.

2.4. HPLC equipment and conditions

HPLC analyses were performed on a Shimadzu HPLC system (Columbia, MD, USA) equipped with two LC-10AD pumps, a UV–Vis SPD-M10AD photodiode array detector, a Sil-10 AD automatic injector and a Gastor 150 LCD on-line degasificator.

A Supelcosil C_{18} column [Teknokroma (Barcelona, Spain), 250×4.6 mm id, 5 μm, with a pore size of 300 Å] was used. A guard column was inserted to protect the analytical column. The analysis of cider samples was monitored at 220 nm, and the absorption spectra were recorded between 190 and 370 nm. The mobile phase was, as eluent A, water (obtained through Millipore Milli-Q system, Milford, MA, USA) with 0.1% trifluoroacetic acid (Romil, Loughborough, UK), and as eluent B, water/acetonitrile (HPLC-grade, Romil) 5:95 (v/v) with 0.1% trifluoroacetic acid. HPLC elution was performed at a flow rate of 1 mL/min and at 38 °C in gradient mode. Percentage of eluent B ranged linearly from 0% to 50% for 45 min followed from 50% to 100% for 25 min. Afterwards, the column was equilibrated with 100% A for 15 min before the fol-

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