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Polyphenolic compositions of Basque natural ciders: A chemometric study

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Abstract

Polyphenolic compositions of Basque natural ciders were determined by high-performance liquid chromatography, with diode array detection following thiolysis, in order to differentiate ciders according to the geographical origin of the main raw material used for their elaboration. Fifty percent of the apples used for cidermaking in the Basque Country are imported from France or Galicia (N.W. Spain); this gives beverages of different chemical compositions and sensory qualities. A data set, consisting of 64 cider samples and 33 measured variables, was evaluated using multivariate chemometric techniques. A preliminary study of data structure was performed by cluster analysis and principal component analysis. Different classification systems for the two categories were obtained on the basis of the chemical data by applying several supervised pattern recognition procedures, such as linear discriminant analysis (LDA), K-nearest neighbours (KNN), soft independent modelling of class analogy (SIMCA), and multilayer feed-forward artificial neural networks (MLF-ANN). KNN, SIMCA and the MLF neural network provided complementary results: KNN allowed the correct classification of almost all the ciders of the Galician category, SIMCA provided a model for the ciders of the French category that excluded all ciders made with Galician apples (50% of raw material), and the neural network achieved a level of hits for the classification of the ciders obtained from French apples (50% of raw material) above 95%. Polyphenolic profiles of the ciders provide enough information to develop classification rules for identifying ciders according to the geographical origin of the raw material used for cidermaking.

Keywords: Polyphenol; Cider; HPLC; Thiolysis; Chemometrics; Pattern recognition

1. Introduction

Polyphenols play an important role in cider quality, being related to several aspects. They are responsible for the colour and the balance of bitterness and astringency, which defines the "overall mouthfeel" of ciders (Lea, 1995). Moreover, they can be involved in the alcoholic and malolactic fermentations as metabolites, providing cider aroma, and as inhibitors of microorganism development (Salih, Le-Quéré, & Drilleau, 2000), controlling fermentation rates and cider spoilage (Cowan, 1999; Sponholtz, 1993). Phenolic compounds are also involved in the colloidal stability of cider, as well as in the inhibition of pre-fermentative clarification enzymes (Lea, 1990). In addition, polyphenols, as natural antioxidants constituents of human diet, are receiving increasing attention due to their health protective

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properties (Hertog, Feskins, Hollman, Katan, & Kromhout, 1993).

Polyphenolic composition of ciders mainly depends on the mixture of apple varieties and the cidermaking procedures used for their elaboration. In apples, a wide diversity of polyphenols is present: flavan-3-ols (catechins and procyanidins), hydroxycinnamic acids, dihydrochalcones, flavonols and anthocyanins (Amiot, Tacchini, Aubert, & Nicolas, 1992). Polyphenolic profiles of apples depend, not only on cultivars, but also on agronomic (Awad & De Jager, 2002) and climatologic factors (Lea, 1990), that can change between regions and/or seasons. Definitively, all these matters may influence polyphenolic composition of ciders. During manufacture of ciders, polyphenols are considerably modified, defining the sensory properties of ciders. Hence, knowledge of the precise composition of ciders may contribute to a better understanding of their implication in cider quality and diversity.

Traditional technology used in the Basque Country for cidermaking consists of the following steps: milling of cider apples; maceration of the pulp in the press for 24 h; pressing for 2 or 3 days; spontaneous must clarification and a natural fermentation to dryness. The stages of maceration and pressing can lead to certain problems, as uncontrolled proliferations of undesirable microorganisms (acetic and lactic bacteria, weakly fermentative and oxidative yeasts), as well as an important oxidation of the phenolic compounds of the milled apples take place during these steps of fruit processing. These phenomena can influence the development of fermentation because of the competition for the must nutrients between bacteria and non-fermentative yeast with fermentative yeast (Cabranes, Moreno, & Mangas, 1991). This may result in the generation of high levels of acetic acid, responsible for the typical high volatile acidity of Basque ciders (Irastorza, 1988). Traditional Basque practices for natural cidermaking are still maintained, but the ancient vertical screw presses of great mass and the wooden vats are slowly being replaced by more modern technologies. Thus, pneumatic presses and stainless steel vats are being introduced. With these kinds of presses, pressing times are shortened (2–3 h) and working conditions are more aseptic, allowing a better control of chemical and microbiological contaminations. In this sense, the new kinds of vats contribute to hygiene because they are easier to clean and disinfect. Moreover, in some cases, stainless steel vats are equipped with devices for controlling temperature and establishing inert atmospheres. These improvements allow a better control of the fermentative process, preventing the development of undesirable microflora. Furthermore, racking of ciders from yeast lees, once fermentation is complete, is becoming a common practice, thus guaranteeing a proper physicochemical and microbial stability of the ciders.

The production of cider apples in the Basque Country is not sufficient for supplying cidermakers the quantities of apples required. Therefore, significant amounts (about 50% of the total of the apples used for cidermaking) are brought from France (Brittany and Normandy) or Galicia (N.W. Spain). Basque ciders are made with 32% of Basque apples, and the rest of the apples used (18%) are from other European regions (e.g., Asturias (Spain), Czech Republic). Regarding the great influences that the raw material and polyphenols have on cider quality, it is interesting to distinguish ciders elaborated with apples cultivated in different regions, in order to select the most interesting apples for producing cider with the organoleptic properties and quality desired.

In this work, polyphenolic profiles of Basque ciders were analysed by chemometric techniques with the aim of differentiating ciders according to the geographical origin of the apples used for their elaboration: (France and Galicia). In this sense, classification rules were developed in order to predict the origin of the apples mainly used for making a Basque cider.

2. Materials and methods

2.1. Reagents and standards

Methanol (Romil Chemical Ltd., Heidelberg, Germany) was of HPLC grade. Water was purified on a Milli-Q system from Millipore (Bedford, MA, USA). Glacial acetic acid, toluene-α-thiol and fuming hydrochloric acid (37%), provided by Merck (Darmstadt, Germany), and ascorbic acid, provided by Panreac (Barcelona, Spain), were of analytical quality. All solvents used were previously filtered through 0.45 μm nylon membranes (Lida, Kenosha, WI, USA).

Polyphenol standards were supplied as follows: (+)-catechin, (−)-epicatechin, rutin, phloridzin, 5-caffeoylquinic acid, *p*-coumaric acid and caffeic acid by Sigma-Aldrich Chemie (Steinheim, Germany); hyperoside, isoquercitrin, avicularin and quercitrin by Extrasynthèse (Genay, France). (−)-Epicatechin 4R-benzylthioether and 4-p-coumaroylquinic acid were kindly provided by Dr. Guyot, and phloretin-2′-*O*-xyloglucoside and procyanidin B2 by Dr. F.A. Tomás-Barberán and Dr. C. Santos-Buelga, respectively. Stock standard solutions of (+)-catechin, (−)-epicatechin, (−)-epicatechin 4R-benzylthioether, rutin, phloridzin, 5-caffeoylquinic acid, *p*-coumaric acid and caffeic acid, at a concentration of 1 mg/ml and hyperoside, isoquercitrin, quercitrin and ideain at 0.6 mg/ml, were prepared in methanol and stored at 4 °C in darkness. The other standards were prepared in approximate concentrations and only used for chromatographic peak identification.