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Evidence of 4-ethylcatechol as one of the main phenolic off-flavour markers in French ciders

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ABSTRACT

A new HPLC method using a diode array detector was developed and validated to quantify 4-ethylphenol, 4-vinylphenol, 4-ethylguaiacol, 4-vinylguaiacol and 4-ethylcatechol in cider. The procedure was linear up to 150 mg/l for each of the five volatile phenols, precise (RSD < 2.9%) and sensitive, with limits of detection between 0.03 and 0.10 mg/l; moreover, it did not require any sample preparation. This method was applied to 11 phenolic off-flavour defective ciders. In these ciders, the main volatile phenol corresponded to 4-ethylcatechol. Moreover, the observed concentrations (maximum of 164 mg/l) indicated, for the first time, that this compound is an important phenolic off -flavour marker in cider. Then, volatile phenols concentrations were determined for 47 French commercial ciders and showed mean quantities of 3.2 (4-EC), 0.8 (4-EP), 0.1 (4-EG), 0.2 (4-VP) and 0.3 mg/l (4-VG). The majority of the tested commercial ciders presented low volatile phenol levels.

1. Introduction

Volatile phenols are generally considered as major markers of organoleptic defects of many fermented alcoholic beverages. They are thought to be extracted from wooden materials during the ageing of wines (Rayne & Eggers, 2007). These compounds have been found to be mainly produced by microorganisms spoiling the matrix (Heresztyn, 1986). They generally have very low perception thresholds (Lopez, Aznar, Cacho, & Ferreira, 2002) ranging from approximately 30 to 440 μg/l for 4-ethylguaiacol (4-EG), 4-vinylguaiacol (4-VG), 4-vinylphenol (4-VP) and 4-ethylphenol (4-EP), respectively. Due to these low levels, they can have a major impact on the aroma of wine (Chatonnet, Dubourdieu, & Boidron, 1992; Etievant et al., 1989), beer (Halcrow, Glenister, Brumsted, & Lautenbach, 1966) or cider (Xu, Fan, & Qian, 2007). However, according to the matrices, the phenolic off-flavour defect thresholds can largely differ. For example, the defect threshold of 4-EP has been shown to be around 0.6 mg/l in Bordeaux red wines (Chatonnet et al., 1992) while it was 4.0 mg/l in Beaujolais red wines (Etievant et al., 1989). It was also previously shown that these compounds can be associated with "animal" or "spicy" aromatic notes in cider derived products, such as Calvados (Ledauphin, Guichard, Saint-Clair, Picoche, & Barillier, 2003). In this product type, 4-EP, 4-VP, 4-EG and 4-VP were generally the most concentrated, but traces of 4-methyl or 4-propylguaiacol and 4-methyl or 4-propylphenol were also detected. However, the small quantities of the latter compounds did not lead to an organoleptic effect.

The biosynthesis of volatile phenols has been mainly studied in wines. In this product, the main microorganism associated with volatile phenol-production corresponds to *Dekkera/Brettanomyces* yeasts (Chatonnet, Dubourdieu, & Boidron, 1995; Heresztyn, 1986; Martorell et al., 2006). The hydroxycinnamate decarboxylase and vinylphenol reductase activities (Fig. 1) of these yeasts enable them to produce 4-EP from *p*-coumaric acid (Chatonnet et al., 1992; Edlin, Narbad, Gasson, Dickinson, & Lloyd, 1998). However, the volatile phenol precursor contents have been shown to be very different in wines and ciders. In French ciders, the quantity of caffeic acid can reach 142 mg/l whereas the maximum level of *p*-coumaric acid was found to be 19 mg/l (Alonso-Salces et al., 2004). Caffeic acid can be metabolised to 4-EC via hydroxycinnamate decarboxylase and vinylphenol reductase activities. Moreover, caffeoylquinic acid, also known as chlorogenic acid, is the main hydroxycinnamic ester in cider and therefore a potential source for caffeic acid, thus 4-EC. However, the presence and quantities of 4-EC have never been studied in ciders while a recent study showed that it was present in wine (Larcher, Nicolini, Bertoldi, & Nardin, 2008).

A number of studies have described quantification methods that allow for volatile phenols to be measured in fermented alcoholic

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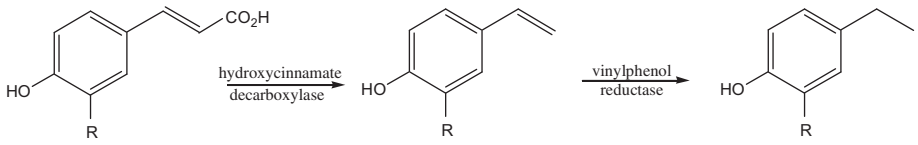

R	hydroxycinnamic acids	vinylphenols	ethylphenols
H	*p*-coumaric acid	4-vinylphenol (4-VP)	4-ethylphenol (4-EP)
OH	cafeic acid	4-vinylcatechol (4-VC)	4-ethylcatechol (4-EC)
OCH_3	ferulic acid	4-vinylgaïacol (4-VG)	4-ethylgaïacol (4-EG)

Fig. 1. Main hydroxycinnamic acids and phenolic by-products in cider.

beverages and especially in wines. The most common methods correspond to an extraction step, by either dispersive liquid–liquid microextraction (Farina, Boido, Carrau, & Dellacassa, 2007), solid-phase extraction (Lopez et al., 2002), solid-phase microextraction (Boutou & Chatonnet, 2007; Carrillo, Garrido-Lopez, & Tena, 2006; Castro Mejias, Natera Marin, de Valme Garcia Moreno, & Garcia Barroso, 2003; Martorell, Marti, Mestres, Busto, & Guasch, 2002; Monje, Privat, Gastine, & Nepveu, 2002; Pizarro, Perez-Del-Notario, & Gonzalez-Saiz, 2007) or stir-bar sorptive extraction (Diez, Dominguez, Guillen, Veas, & Barroso, 2004) and are generally associated with mass spectrometric detection, followed by gas chromatography for the separation procedure. However, without derivatising the alcohol functions with reactions, such as acetylation (Carrillo & Tena, 2007) or trimethylsilylation (Viñas, Campillo, Martínez-Castillo, & Hernández-Córdoba, 2009), the most hydrophilic and polar molecules, such as 4-ethylcatechol (4-EC) cannot be correctly recovered for adequate quantification. On the contrary, liquid chromatography does not require any high volatility for analytes. Nevertheless, only few HPLC methods focusing on the quantification of volatile phenols have been developed. They were optimised for the most part with specific detectors, such as coulometric (Larcher et al., 2007) or fluorimetric (Nicolini, Larcher, Bertoldi, Puecher, & Magno, 2007) ones.

In wines, volatile phenol analysis is generally focused on 4-EP and 4-EG levels (Caboni, Sarais, Cabras, & Angioni, 2007; Diez et al., 2004; Martorell et al., 2002; Monje et al., 2002; Pollnitz, Pardon, & Sefton, 2000), but more recently, 4-EC contents were also studied (Larcher et al., 2008). In ciders, only few studies have been devoted to the quantification of volatile phenols; these were limited to the characterisation of 4-EP and 4-EG. In this context, Pizarro, Perez-Del-Notario, and Gonzalez-Saiz (2009) studied five Spanish ciders using HS-SPME linked with the separation and detection by GC–MS/MS to quantify 4-EP, 4-VP, 4-EG and 4-VG without quantifying 4-EC.

In this work, a rapid method, using HPLC coupled with a diode array detector, for 4-EP, 4-VP, 4-EG, 4-VG and 4-EC quantification in ciders was validated. This method was applied to 11 ciders described as defective due to the presence of phenolic off-flavours. Moreover, 47 French commercial ciders from various origins, and produced by different fermentation procedures, were also analysed.

2. Materials and methods

2.1. Reagents and standards

HPLC gradient grade water and acetonitrile (ACN) were purchased from Fisher Scientific (Illkirch, France). Acetic acid and ethanol were of analytical quality (Grosseron, France). Standards for volatile phenols [4-EC, 4-EP, 4-EG, 4-VP (10% in propylene glycol), and 4-VG] and ascorbic acid were supplied by Sigma–Aldrich (Saint-Quentin Fallavier, France).

2.2. Analytical procedures

Prior to HPLC analysis, the cider samples were degassed in an ultrasonic bath for 15 min. Ascorbic acid (10 g/l final concentration) was then added to the sample in order to avoid oxidation of the targeted compounds as described by Mangas, Rodríguez, Suárez, Picinelli, and Dapena (1999). Ciders were then filtered through a 0.22 μm PTFE syringe filter (AIT, France) and transferred into 2 ml amber glass vials for automatic sampling. The data were obtained using an HPLC system (Varian, Les Ulis, France) equipped with an autosampler (model PS410), a column oven, a pump (PS230), a diode array detector (PS335) and the Galaxy software version 1.9 data system. Analytical separation of the volatile phenols was carried out on a reverse-phase Luna C18(2) column (250 mm × 4.0 mm I.D., 5 μm packing from Phenomenex, Le Pecq, France) equipped with a C18 guard column (4 × 3.0 mm). The temperature of the column oven was set to 35 °C. The injection volume was 20 μl, and a flow rate of 1.0 ml/min was used. Eluent A consisted of water/acetic acid (97.5:2.5 v/v) and eluent B was composed of ACN/solvent A (75:25 v/v). The elution conditions applied were: 0–5 min, mixture A/B (90:10) isocratic; 5–41 min, linear gradient A/B from (90:10) to (45:55); 41–44 min, linear gradient A/B from (45:55) to (0:100); 44–48 min, 100% B isocratic; finally, the stationary phase was then re-equilibrated for 5 min before performing another analysis.

The five volatile phenols were monitored and quantified at 280 nm, and the compound identification was achieved by comparing both the UV spectra (recorded between 230 and 430 nm with an acquisition speed of 1.0 s) and the retention times with those of pure diluted standards.

Quantification was performed using an external calibration method. Three standard solutions containing the five volatile phenols at different concentration levels (10, 50 and 100 mg/l) were analysed prior to analysis of each set of samples. These standard solutions were injected in duplicate to adjust the calibration slope between the concentrations and the corresponding peak areas of each volatile phenol, and also to verify their retention times. Then, each sample was injected in duplicate and the concentrations determined using the linear regression of each compound.

2.3. HPLC method validation

First, the linearity of the detector response was studied for each volatile phenol using standard solutions at eight different concentrations (0, 0.5, 1.0, 2.5, 10, 50, 100 and 150 mg/l; three replicates per concentration).

The matrix effect was investigated for four different ciders: two industrial ciders and two farm-style ciders (one cider at the beginning of fermentation and one bottled cider). Each product was analysed in duplicate with and without the addition of volatile phenols (standard solutions supplemented at two different concentrations: 5 and 50 mg/l). The precision of the method was studied using cider supplemented with a standard solution at ~10 mg/l for each volatile phenol; three series of six repetitions were analysed. The