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Influence of controlled inoculation of malolactic fermentation on the sensory properties of industrial cider

Ainoa Sánchez · Gilles de Revel · Guillaume Antalick · Mónica Herrero · Luis A. García · Mario Díaz

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Abstract Given the lack of research in the traditional cider making field when compared to the efforts devoted to winemaking, this work focused on the effects of controlled inoculation of the malolactic fermentation (MLF) on the sensory properties of cider. MLF develops spontaneously in cider making at industrial level. In this work, industrial cider samples were inoculated with selected indigenous *Oenococcus oeni* strains and the benefits on the aroma and flavour in cider production compared to non-inoculated ciders were evaluated. Randomly amplified polymorphic DNA PCR was used to monitor strain colonization ability, outnumbering the indigenous microbiota, after completion of the alcoholic fermentation at industrial scale (20,000 l). Aroma-active compounds of experimentally inoculated ciders were analysed by HPLC and GC–MS, and sensory profiles were determined by fractioning aroma extracts using reversed-phase HPLC. Principal component analysis allowed the identification of relationships and differences among ciders with or without inoculation, including several highly appreciated commercial ones obtained under spontaneous conditions. Under controlled inoculation conditions, not only could MLF be shortened by half but, interestingly, enhancement of aroma complexity and flavour resulted in ciders enriched with a higher fruity note. In addition, important aromatic groups analysed here had not been previously described, thus affording deeper knowledge on aroma characterization of apple cider.

Keywords Cider · Malolactic fermentation · Starter cultures · Aromatic compounds · Sensory properties

A. Sánchez · M. Herrero (✉) · L. A. García · M. Díaz
Department of Chemical Engineering and Environmental Technology, Faculty of Chemistry, University of Oviedo, C/Julián Clavería s/n, 33071 Oviedo, Spain
e-mail: herreromonica@uniovi.es

M. Díaz
e-mail: mariodiaz@uniovi.es

G. de Revel · G. Antalick
ISVV, EA 4577, Unité de recherche Oenologie, Univ. de Bordeaux, 33882 Villenave d'Ornon, France

G. de Revel · G. Antalick
INRA, ISVV, USC 1366 Oenologie, 33882 Villenave d'Ornon, France

Introduction

Although the use of starters to control industrial fermentations is well established in the brewing and wine industry it has not been adopted in cider making yet [17] mainly because of the profound lack of research in this traditional field. Therefore, scarce information is available and thus awareness about the advantages of applying this technology at industrial level is lacking. Malolactic fermentation (MLF) in cider making is usually carried out by indigenous lactic acid bacteria (LAB) [40] present in apples and cellars. Even though MLF can take place spontaneously, it starts uncontrolled, so any delay in the initial stage may lead to alterations in the product quality [16], and thus to economic losses. The MLF process is difficult to accomplish and may give rise to stuck or sluggish fermentations, mainly because of the strong combined inhibitory effect of ethanol and acidity, without achieving satisfactory results [47, 48]. These two factors, along with low temperatures and lack of nutrients, contribute to diminish the success of the biological deacidification [36]. In addition, it was previously reported that MLF had a positive effect promoting reduction of acetaldehyde levels in cider making [19], thereby diminishing at the same time health concerns and risk of product spoilage.

Cider making is a process similar to winemaking, employing nearly the same production techniques [51]. In winemaking a desirable control over the production of aromatic compounds and flavour is achieved by controlled MLF [4]. In the wine industry, different MLF starter cultures have been marketed, selected from spontaneous wine fermentations. Nevertheless, upon inoculation with wine starters in cider, MLF generally failed owing to a loss of cell viability prior to adaptation to the environment. Selection of proper starters according to the specific characteristics of the product and the common technological practices is essential because environmental factors will interact, ultimately selecting only those strains competitive enough to conduct the process. Nowadays the main challenge in food fermentations, besides improving safety, is to preserve the typical sensory qualities of traditional products [38, 46] after selection of suitable indigenous microorganisms [12, 34].

As has been previously established in wine, controlled MLF in cider may well enhance the complexity of the aroma and flavour of the product. Aroma compounds in apples and apple juice have been studied extensively over the past 30 years, although little information is available regarding the aroma of apple cider [50]. Major volatile compounds in apple cider have been identified as alcohols, esters, fatty acids, and carbonyls [50]. MLF contributes to the flavour complexity of cider by producing compounds such as ethyl lactate, diacetyl, acetoin, and butane-2,3-diol [11].

In wine, changes suffered during MLF are highly complex and may often involve the reduction of vegetable and herbaceous notes and the appearance of other fruity, floral, nutty or milky notes. The influence of the LAB strains used as starters and the scale of elaboration (industrial or laboratory scale) influence the chemical composition and subsequent aromatic complexity of the final product [20], such as esterase activity in *Oenococcus oeni* that changes the ester composition of wines after MLF [2, 45].

The aroma profile in fermented alcoholic beverages is determined through the combined effects of several hundred chemically different compounds at concentrations that, in some cases, can be lower than parts per billion (microgram per litre) [22]. The analysis of these compounds demands highly selective and efficient enrichment steps, such as preparative liquid or gas chromatography (GC), or multidimensional GC [14]. Reversed-phase HPLC has been described as a robust technique to fractionate the extracts and allow selective separation of aromatic compounds. This separation allows not only an easy recovery of several fractions containing aroma compounds of equivalent polarities, but also fractions that can be sensorially tested without problems of toxic and odorous solvents [14, 30].

To assess the influence of controlled inoculation of the MLF on the sensory properties of cider, four different indigenous strains showing interesting technological traits for cider production were independently inoculated in industrial samples obtained under the common cider making practices, testing their colonizing ability over the indigenous microbiota, and then analysing the aromatic compounds and sensory properties of the final products. These strains do not carry genes related to organoleptic spoilage such as exopolysaccharides and acrolein production or genes related to food safety such as biogenic amine production [41]. Besides, all of them were able to conduct MLF when inoculated in green cider as pure cultures at 15 and 22 °C [42], showing specific malic uptake rates around 0.3 and 0.6 g malic acid g/cell/h, respectively. As a result of the scarce information available regarding the aroma and sensory properties of apple cider, seven highly appreciated, non-inoculated commercial ciders were chemically and sensory analysed along with the experimentally inoculated ciders.

Materials and methods

Microorganisms

O. oeni strains used in this work were previously isolated from cellars located in the four main areas of traditional cider making in Asturias (Spain) and stored at −20 °C in glycerol [20 % (v/v)].

Fermentation processes

Alcoholic fermentation at industrial scale

Alcoholic fermentation (AF) was carried out in an industrial cellar. Apple juice was obtained from protected designation of origin (PDO) Sidra de Asturias apples using an automatic hydraulic press. Fermentations were carried out in 20,000-l refrigerated stainless-steel tanks, at 11–13 °C, without SO_2 addition. When the industrial AF was completed (density reached 1,005 g/l approx.), green cider samples were taken under aseptic conditions, and immediately transported to the laboratory in order to be inoculated with the selected LAB strains. A non-inoculated control was also included corresponding to the spontaneous cider fermentation from the industry, kept in the same conditions as the inoculated cider samples.

Malolactic fermentation

Frozen stock cultures of the pre-selected *O. oeni* strains were re-activated and incubated in liquid MRS at 30 °C.

After 48 h, pre-cultures were grown in reconstituted apple juice (concentrated apple juice diluted with distilled water to a final density of 1,030 g/l) supplemented with yeast extract 0.5 % (w/v), and sterilized by means of a tangential flow filtration device (Pellicon 2, Millipore), using polyethersulphone membranes (0.22 μm). *O. oeni* pre-cultures were incubated in static conditions for 96 h at 30 °C. Samples of industrial green cider were inoculated with 10^7 CFU/ml of each bacterial pre-culture, previously centrifuged, washed twice in saline solution and resuspended in the fermentation media. For each strain and for the non-inoculated control, fermentations were carried out in two pre-sterilized 2-l bottles filled to capacity, as independent experiments, at 15 °C in static conditions. Results presented for each sample point corresponded to the average of the two independent fermentations analysed. Throughout the process, pH and organic acids (malic and lactic acids) were monitored.

Microbiological methods

To follow the MLF evolution, samples were collected approximately each 24 h. For each sampling time, duplicates were taken and submitted to microbiological and chemical analyses. Enumeration of LAB was performed by the standard plate-counting method in MRS (Biokar Diagnostics) supplemented with 5 % (v/v) ethanol (Sigma) and 100 ppm of pimaricin (VGP Pharmachem) to inhibit yeast growth. Before plating, because LAB tend to form chains, samples were sonicated (5 min) and vortexed. Previously, the effectiveness of this disaggregation procedure was checked following microscopic observations and enumeration by plating, testing different sonication times. For each sampling time, total bacterial counts were performed in triplicate, in statistically significant dilutions (25–250 colonies per plate) and incubated under anaerobic conditions (CO_2 gen, Oxoid) to inhibit acetic acid bacteria, at 30 °C for 15 days.

To test the colonizing ability, samples were taken when malic acid was being degraded at a high rate (after 2, 3 and 4 days of incubation). For each sample, colonies were randomly picked from Petri dishes containing approximately 50 colonies, ensuring that all different colony morphologies were considered. A number of colonies corresponding to the square root of the total Petri dish population [38] were typed by randomly amplified polymorphic DNA (RAPD)-PCR. DNA was extracted and purified with a NucleoSpin Tissue kit (Macherey–Nagel) following the manufacturer's instructions. RAPD-PCR reactions were performed following the protocol as described [35]. Patterns were compared and the implantation value (the average of two independent experiments) was calculated as the number of colonies with the specific RAPD-PCR pattern divided by the total number of colonies picked, and expressed as a percentage [40].

Analytical methods

Ciders analysed (either by analytical methods and sensory analysis) and their origins are summarized in Table 1. Each sample was analysed three times, independently. A pH meter (Crison micropH 2001) was employed.

Organic acids

Samples were filtered through a membrane (0.45 μm) and 2-ml samples were frozen (−20 °C) until

Table 1 Ciders used in this work: nomenclature, origin and characteristics

Nomenclature	Type of cider	Characteristics
Commercial		
T 1	Traditional	Traditional cider making practices
T 2		
T 3		
PDO 1	Protected designation of origin	Traditional cider making practices but using only authorized local apples varieties
PDO 2		
PDO 3		
F	"New expression cider"	Traditional cider making practices but, at the end of the process, the cider is filtered
Experimental		
Cider 1.5	Protected designation of origin	Strain 1.5 used as starter culture
Cider 3.8a		Strain 3.8 used as starter culture
Cider 3.8b		Strain 3.8 used as starter culture with ageing period
Cider 5.4a		Strain 5.4 used as starter culture
Cider 5.4b		Strain 5.4 used as starter culture with ageing period
Cider 6.6		Strain 6.6 used as starter culture

chromatographical analysis was performed. Malic and lactic acids were analysed by HPLC (Alliance 2690; Waters) with a photodiode array detector (Waters 996). A Spherisorb ODS2 analytical column (4.6 mm × 250 mm, 3 μm, Teknokroma) was used following conditions described previously [29]. Solvent and reagents were HPLC grade. Analytical-grade organic acids (malic and lactic acids) were used as external standards (Fluka and Panreac). Quantification was based on peak area measurements, and data treatment was performed with Millennium software (Waters). Coefficients of variation obtained were less than 5 %.

Volatile phenols

Samples (10 ml) were placed in 25-ml vials containing NaCl (3.5 g). Deuterated 4-ethylphenol was added as internal standard at a concentration of 100 μg/l. Vials were then sealed with a septum-type cap and briefly shaken. For analysis, a Hewlett-Packard (HP) 6890N gas chromatograph was employed, equipped with an MPS2 autosampler for automated solid-phase microextraction (SPME) and coupled to an HP 5973 quadrupole mass spectrometer (Agilent, Santa Clara, CA, USA). SPME was carried out with 85-μm polyacrylate fibres from Supelco (Bellefonte, PA, USA) following the protocol described [37].

Volatile fatty acids with short and long chains, acetates, ethyl esters, diethyl succinate and phenylethanol

Cider samples (50 ml) were mixed with 200 μl of internal standard, octen-3-ol (20 mg/l). This mixture was extracted sequentially with 4, 2 ml, and again 2 ml of ether–isohexane (1:1, v/v), by stirring each extraction for 5 min. The organic phases were collected and injected into a gas chromatograph HP 5890 coupled to a flame ionization detector. The column was an FFAP type (BP 21, 50 m × 0.25 mm × 0.2 μm, SGE). Measurements were carried out following the protocol described [5].

Methanol and higher alcohols

These compounds were determined by direct injection of cider after addition of 4-methylpentan-2-ol (100 mg/l) into the HP 5890 GC (column CPWax 57CB, 50 m × 0.22 mm × 0.25 μm). The chromatographic conditions employed were those described elsewhere [6].

Carbonyl compounds

Sample preparation was carried out following the protocol described [13]. Briefly, 50 μl of internal standard (1.2 g/l hexane-2,3-dione in hydroalcoholic solution 50 % vol) was added to 50 ml of an aqueous solution of 1,2-diaminobenzene at 6.5 g/l, and the pH was adjusted to 8.0 (NaOH). After 3 h at 60 °C, the mixture was acidified with 2 M sulphuric acid to pH 2.0 and extracted twice with 5 ml of dichloromethane. The organic phase was dried on sodium sulphate and 2 μl of it was injected into the chromatograph.

GC–MS analyses were carried out in an HP 5890 gas chromatograph coupled with an HP 5970 mass spectrometer (electron impact 70 eV, 2.7 kV). The column was a BP21 (SGE 50 m × 0.32 mm × 0.25 μm). Quantitative determination was performed in selected ion monitoring (SIM) mode by selecting ions of *m*/*z* 76, 77, 117 and 158. Ions of *m*/*z* 76 and 77 were used for quantification and the others as diacetyl qualifiers.

Sulphur compounds

The volatile sulphur compounds (VSC) were determined according to the reported method [33] in a gas chromatograph (Hewlett-Packard 6890) coupled to a flame photometric detector (FPD). To measure the 2-mercaptoethanol, a gas chromatograph (Hewlett-Packard 5890) was coupled to a mass selective detector in the SIM mode (Hewlett-Packard 5972, electronic impact 70 eV, eMV 2.7 kV) and separations were carried out with a BP21 column (SGE 50 m × 0.32 mm × 0.25 μm). Chromatographic conditions used were as described elsewhere [33].

Sensory assays

Cider samples (100 ml) were extracted sequentially by using 8, 4 and 4 ml of dichloromethane with magnetic stirring for 5 min. Extracts were blended, dried over anhydrous sodium sulphate and concentrated. HPLC fractionation of cider samples was carried out with HPLC equipment consisting of one pump, an automated gradient controller and a manual injector. The column used was a Supelcosil LC18 (4.6 mm × 250 mm, 5 μm). The chromatographic conditions employed were as previously described [30]: flow rate, 0.5 ml/min; program gradient, min 0–2, with 100 % Milli-Q water, linear program until 100 % ethanol at 50 min. An automated fraction collector (Foxy JR) was connected to the end of the column collecting 1 ml of the eluted solvent each 2 min. The HPLC eluate was recovered in 26 separate fractions, which were evaluated for their smell. The alcohol content of the fractions eluted by HPLC was adjusted to 6 % (v/v). The fractions with remarkable odours were analysed. Samples were labelled with three-digit random codes.

The test panel was composed of eight experienced individuals (4 females and 4 males, non-smokers, between 26 and 50 years of age). In order to determine potentially odour-active compounds of the cider sample extracts, the

nasal impact frequency (NIF) method [32] was performed. Trained panellists were requested to evaluate each cider orthonasally and assigned each fraction to one or several aroma series, depending on their principal odour attributes. The panellists were also encouraged to describe the odour quality of each perception. Only descriptors cited by a minimum of three subjects (38 % of the panel) were considered for subsequent statistical analysis. In order to facilitate the judges' work, they were provided with a list of terms. These were arranged by general odour families: alcohol, butter/cream, fruity, floral, spicy, cheese, pharmaceutics, animal, sweet, sulphurous and "others". Judges were not informed about the nature of the ciders to evaluate. All tests were carried out in standardized booths.

Statistical analysis

Principal component analysis (PCA) was used to study attribute–sample relationships. By PCA, a sensory space was created, where samples were positioned in the attribute–sample space according to their characteristic sensory attributes. PCA were carried out using Xlstat software.

Results and discussion

Colonizing ability of *Oenococcus oeni* strains

As has been described for other fermentation products [25, 39] the best option to develop starter cultures should be based on the indigenous microbiota showing useful technological features. These strains should be well adapted to their environment and capable of competing successfully against potential food spoilage strains. In this work, in order to test their implantation capacity when competing with the indigenous microbiota in industrially produced cider, four *O. oeni* strains previously isolated and selected for their cider making traits were chosen.

Once alcoholic fermentation was completed at industrial scale, the MLF process was performed at laboratory level under the same controlled conditions, using each selected *O. oeni* strain as single-starter cultures. For each strain studied two independent experiments were carried out. Likewise, one cider sample taken and maintained under the same conditions but without starter inoculation was used as control, in order to check the evolution of the spontaneous MLF (carried out by the indigenous microbiota). As has been observed in cider [18] LAB growth is not necessary to perform the MLF, and low fermentation temperature is a widely preferred cider making practice because high fermentation temperatures would lead to the formation of undesirable volatile end-products due to yeast metabolism. Evolution of MLF carried out is shown in Fig. 1. Differences were found in the fermentation capacities among the *O. oeni* strains tested. When strains 1.5, 3.8 and 5.4 were inoculated, MLF were carried out successfully, lasting less than 8 days. Even strain 5.4 completed MLF in only 7 days. On the other hand, the MLF profile after inoculation with strain 6.6 showed no differences when compared to the non-inoculated control, needing, in both cases, 12 days to exhaust the malic acid content.

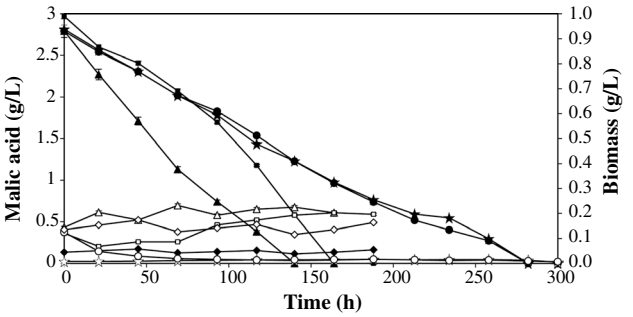

Fig. 1 Evolution of malic acid (*solid symbols*) and biomass concentration (*open symbols*) respectively, showing series error (ε), during MLF in "green cider" at 15 °C, carried out by *O. oeni* 1.5 (*filled square* $\varepsilon \pm 1.0$ %, *open square* $\varepsilon \pm 2.2$ %); *O. oeni* 3.8 (*filled diamond* $\varepsilon \pm 2.1$ %, *open diamond* $\varepsilon \pm 2.2$ %); *O. oeni* 5.4 (*filled triangle* $\varepsilon \pm 2.8$ %, *open triangle* $\varepsilon \pm 2.8$ %); *O. oeni* 6.6 (*filled circle* $\varepsilon \pm 0.9$ %, *open circle* $\varepsilon \pm 2.7$ %); and non-inoculated control (*filled star* $\varepsilon \pm 1.6$ %, *open star* $\varepsilon \pm 3.1$ %)

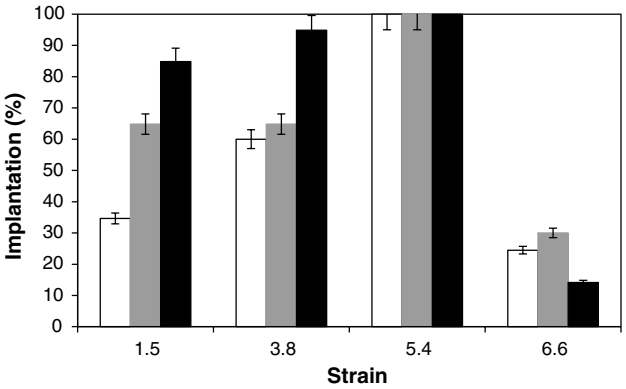

Fig. 2 Implantation average values (%) during MLF at 45 h (*white*), 69 h (*grey*) and 93 h (*black*) of the inoculated *O. oeni* strains

In order to assess the implantation capacity achieved by the different strains inoculated in the fermentation medium, RAPD analysis was performed. Clear distinctions among the different strains assayed were obtained on the basis of their electrophoretic band profiles [42]. The ability of the *O. oeni* strains 1.5, 3.8 and 5.4 to colonize the medium and to survive competitively throughout the fermentation process was assessed (Fig. 2), as long as they were able to outnumber the indigenous microbiota. For both the inoculated cider with strain 6.6 and the non-inoculated control, an

ecological succession of indigenous strains was observed, rather than a dominating colonization process. This fact could explain the different fermentation behaviour showed by these ciders. On the other hand, whereas strains 3.8 and 5.4 achieved an implantation value higher than 50 % after 45 h from inoculation, strain 1.5 took 24 h more to reach this colonization level. Moreover, the high capacity to colonize the medium shown by the *O. oeni* 5.4 strain, with an implantation value of 100 % only 45 h after inoculation and which was maintained throughout the fermentation process, could explain this particularly shortened MLF.

As, in previous work [42], the *O. oeni* 6.6 strain had shown the best fermentation capacity when used as pure culture in green cider at 15 °C, additional experiments conducted with this strain were repeated using a different industrial batch. The same behaviour was confirmed, because no differences could be observed when compared to the non-inoculated control (data not shown). From the results obtained it could be concluded that although strain 6.6, under the same operational conditions, was able to conduct MLF when used as pure culture (i.e. in the absence of indigenous microbiota), this strain did not show successful competitive colonizing ability in the complex and harsh environment of industrial cider fermentation conditions. This ability is essential in the development of suitable starter cultures, because selected strains ought to withstand the competition of the biodiversity present in cider microbiota.

In order to confirm this lack of competitive ability, another complementary assay was carried out. In this case, before inoculation with strain 6.6, a different batch of industrial cider after AF was kept cold (4 °C) for 24 h in order to slow down the metabolic activity of the indigenous microbiota present (the medium was left to temper before inoculation). Under these conditions, the MLF evolution did show differences between the inoculated cider and the control (Fig. 3). Whereas malic acid consumption began just after inoculation with strain 6.6, reaching a malic acid uptake rate of 0.11 g malic acid g/biomass/h and exhausting the malic acid, the non-inoculated control showed a delay in the onset of the process, with an approximately 1 day in lag-phase before starting malic acid degradation. Moreover, more than 50 % of the initial malic acid content still remained in the non-inoculated medium when in the inoculated one it was already fully depleted (in 4 days). Likewise, RAPD assays revealed that, under these conditions, the *O. oeni* 6.6 strain was able to colonize the medium reaching an implantation value of 90 % 3 days after inoculation (data not shown). Therefore, when the *O. oeni* 6.6 strain was inoculated under the same optimal conditions as in the previous fermentation experiments but the competitive microbiota in the spontaneous fermentation was subjected to increased stress conditions (represented by the induced precooled treatment), the starter strain was able to colonize the environment and consequently to conduct the MLF successfully.

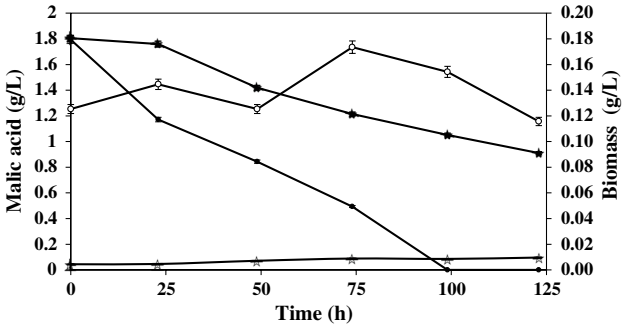

Fig. 3 Malic acid evolution (*solid symbols*) and biomass evolution (*open symbols*), respectively, during MLF after 24 h precooled "green cider" at 15 °C after *O. oeni* 6.6 inoculation (*filled circle* series error (ε) ± 1.5 %, *open circle* ε ± 2.8 %) and in non-inoculated control (*filled star* ε ± 1.3 %, *open star* ε ± 2.9 %)

Comparison of organoleptic characteristics between experimental and commercial ciders

Nowadays in Asturias, at least three different types of cider are locally produced: "traditional cider" (T), "PDO cider" (applying the same cider making practices but using only authorized local apple varieties) and "new expression cider" (after fermentation, cider is filtered in order to get microbiological stability) which will be referred to as filtered (F) from now on. Seven commercial cider samples produced without application of starter culture technologies (three T ciders, three PDO ciders and one F cider) and chosen owing to their good organoleptic properties were submitted to aromatic and sensorial characterization. The objectives were to obtain data from the marketed ciders comparing the properties of the resulting products after inoculation with non-inoculated ciders.

Volatile compounds

A total of 36 compounds were identified in the volatile fraction of cider samples: 11 esters, 10 alcohols, 7 acids, 4 phenols, 1 ketone and 3 sulphur compounds. Concentrations of the identified volatile compounds, grouped into chemical classes, are summarized in Tables 1 (commercial ciders) and 2 (inoculated ciders and non-inoculated control). Sample ciders inoculated with *O. oeni* strains 3.8 and 5.4 were taken just after malic acid was exhausted in the medium (ciders 3a and 5a), and, additional samples were taken 1 month later (ciders 3b and 5b) in order to evaluate the ageing effect. It was widely described that apple cultivars, fermentation technology and ageing processes will affect the

Table 2 Quantitative data of volatile compounds identified in the commercial ciders

Compound	T 1	T 2	T 3	PDO 1	PDO 2	PDO 3	F
Alcohols (mg/l)							
Methanol	44.41 ± 3.90	46.38 ± 3.39	54.57 ± 1.54	27.87 ± 2.41	19.83 ± 1.26	32.57 ± 2.17	64.89 ± 2.35
Propanol	29.71 ± 2.42	45.49 ± 2.61	65.93 ± 0.81	15.86 ± 1.64	20.01 ± 0.47	84.4 ± 4.68	13.38 ± 0.32
Allylic alcohol	10.74 ± 0.34	10.49 ± 0.17	8.36 ± 0.47	8.07 ± 0.35	11.79 ± 0.49	9.50 ± 0.27	<LOQ
1-Butanol	5.16 ± 0.34	5.36 ± 0.08	6.68 ± 0.14	4.84 ± 0.54	3.42 ± 0.05	5.27 ± 0.37	7.11 ± 0.63
2-Butanol	28.31 ± 1.35	12.84 ± 1.43	58.73 ± 0.25	2.06 ± 0.17	12.01 ± 0.62	5.52 ± 0.37	1.13 ± 0.10
Isobutanol	34.94 ± 1.87	35.67 ± 0.06	21.65 ± 0.58	34.47 ± 1.76	30.70 ± 0.13	85.81 ± 2.89	38.51 ± 0.22
2-Methyl-1-butanol	25.55 ± 1.09	30.15 ± 2.80	23.13 ± 0.30	28.37 ± 1.17	28.73 ± 0.44	43.86 ± 1.54	27.01 ± 0.38
3-Methyl-1-butanol	128.22 ± 5.61	166.21 ± 15.80	118.75 ± 0.98	145.76 ± 7.70	148.47 ± 4.11	226.03 ± 6.90	124.88 ± 1.74
1-Hexanol	7.69 ± 0.36	6.38 ± 0.51	7.26 ± 0.20	7.44 ± 0.18	7.21 ± 0.13	4.73 ± 0.06	8.25 ± 0.19
2-Phenylethanol	323.83 ± 20.47	435.75 ± 75.69	295.27 ± 9.17	344.49 ± 58.67	284.94 ± 13.20	409.50 ± 4.60	9.21 ± 0.67
Esters (mg/l)							
Isoamyl acetate	1.83 ± 0.22	0.87 ± 0.10	1.43 ± 0.10	0.63 ± 0.06	2.14 ± 0.03	1.47 ± 0.13	0.49 ± 0.02
Hexyl acetate	0.01 ± 0.003	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.003	0.01 ± 0.0004	0.01 ± 0.001
2-Phenylethyl acetate	0.15 ± 0.01	0.12 ± 0.002	0.10 ± 0.005	0.10 ± 0.01	0.12 ± 0.01	0.16 ± 0.01	0.02 ± 0.001
Ethyl acetate	76.77 ± 4.21	64.76 ± 9.79	105.71 ± 8.20	76.51 ± 2.93	59.14 ± 0.59	126.08 ± 6.5	86.90 ± 0.98
Ethyl butyrate	0.86 ± 0.11	0.92 ± 0.17	1.46 ± 0.25	0.38 ± 0.10	0.41 ± 0.08	1.95 ± 0.16	0.18 ± 0.02
Ethyl hexanoate	0.32 ± 0.01	0.37 ± 0.01	0.31 ± 0.01	0.35 ± 0.01	0.42 ± 0.01	0.51 ± 0.01	0.29 ± 0.0005
Ethyl octanoate	0.47 ± 0.01	0.49 ± 0.02	0.49 ± 0.03	0.58 ± 0.01	0.58 ± 0.02	0.60 ± 0.02	0.25 ± 0.006
Ethyl decanoate	0.31 ± 0.04	0.23 ± 0.02	0.22 ± 0.02	0.32 ± 0.02	0.32 ± 0.03	0.21 ± 0.01	0.04 ± 0.002
Ethyl dodecanoate	0.09 ± 0.01	0.05 ± 0.01	0.04 ± 0.003	0.07 ± 0.01	0.08 ± 0.01	0.04 ± 0.004	<LOQ
Ethyl lactate	233.10 ± 5.85	223.02 ± 4.37	205.03 ± 0.64	336.99 ± 5.48	164.70 ± 3.62	229.42 ± 8.38	271.16 ± 8.38
Diethyl succinate	4.95 ± 0.16	6.33 ± 0.46	5.20 ± 0.19	6.40 ± 0.31	4.53 ± 0.18	5.40 ± 0.11	6.92 ± 0.39
Ketones (mg/l)							
Diacetyl	0.13 ± 0.01	0.17 ± 0.003	0.12 ± 0.01	0.08 ± 0.0002	0.46 ± 0.03	0.19 ± 0.01	0.14 ± 0.01
Acids (mg/l)							
Butyric acid	3.30 ± 0.32	2.74 ± 0.61	3.17 ± 0.05	2.99 ± 0.45	2.16 ± 0.1	2.10 ± 0.06	3.10 ± 0.15
Isobutyric acid	4.14 ± 0.32	2.84 ± 0.44	2.63 ± 0.09	3.10 ± 0.45	2.64 ± 0.04	2.52 ± 0.05	2.55 ± 0.18
Isovaleric acid	4.10 ± 0.47	4.00 ± 0.33	4.69 ± 0.03	4.12 ± 0.51	3.83 ± 0.15	3.50 ± 0.05	4.56 ± 0.26
Hexanoic acid	4.22 ± 0.15	4.68 ± 0.34	3.94 ± 0.1	4.13 ± 0.28	4.68 ± 0.15	4.53 ± 0.06	3.20 ± 0.15
Octanoic acid	4.84 ± 0.25	4.68 ± 0.12	5.12 ± 0.32	4.60 ± 0.45	6.40 ± 0.29	4.93 ± 0.22	2.37 ± 0.13
Decanoic acid	3.43 ± 0.35	2.82 ± 0.18	3.36 ± 0.32	2.23 ± 0.25	3.80 ± 0.33	2.02 ± 0.21	1.10 ± 0.01
Dodecanoic acid	0.87 ± 0.10	0.55 ± 0.05	0.56 ± 0.06	0.23 ± 0.02	0.97 ± 0.13	0.27 ± 0.05	0.03 ± 0.0003
Volatile phenols (μg/l)							
4-Ethylphenol	3,805.04 ± 62.92	5,225.78 ± 93.36	3,368.41 ± 15.00	1,695.18 ± 9.81	3,223.29 ± 109.69	1,692.64 ± 23.02	2,707.57 ± 67.55

Table 2 continued

Compound	T 1	T 2	T 3	PDO 1	PDO 2	PDO 3	F
4-Ethylguaiacol	533.97 ± 21.34	965.89 ± 8.87	448.21 ± 3.89	474.02 ± 7.06	448.18 ± 22.86	740.35 ± 16.25	423.50 ± 6.23
4-Vinylphenol	59.73 ± 2.30	49.64 ± 1.45	34.24 ± 0.32	47.28 ± 0.32	65.30 ± 3.33	83.63 ± 1.77	88.45 ± 3.06
4-Vinylguaiacol	88.82 ± 0.47	129.33 ± 5.29	78.95 ± 0.94	90.28 ± 0.09	91.10 ± 0.39	88.94 ± 1.87	131.79 ± 2.54
Volatile sulphur compounds (μg/l)							
Hydrogen sulphide	2.93 ± 0.49	<LOQ	<LOQ	3.50 ± 0.57	<LOQ	<LOQ	1.95 ± 0.64
Dimethyl sulphide	9.15 ± 0.57	5.48 ± 0.28	4.20 ± 0.57	6.66 ± 0.57	4.94 ± 0.64	5.28 ± 0	4.69 ± 0.85
Carbon disulphide	<LOQ	<LOQ	1.22 ± 0.07	0.90 ± 0.07	5.88 ± 0.64	0.65 ± 0.07	0.65 ± 0.07

LOQ limit of quantification

formation of aroma compounds, thus affecting the quality and aromatic profile of cider [50]. As shown in Table 2, the technology used for F cider production implies an important reduction in the volatile components of cider. There are just a few studies reporting aroma compounds present in ciders, most of which are focused on sparkling cider, i.e. a different type from those analysed here. For traditional cider, only the concentration of some aromatic compounds (mainly alcohols and esters) could be found in the literature [28, 29]. Comparing those values with the ones obtained in this work, differences found were not significant.

As previously reported, the main important volatile compounds formed during fermentation, which are considered as key products affecting the organoleptic profile of cider, are higher alcohols, esters and carbonyl compounds [28]. Nevertheless, other aromatic compounds such as fatty acids, volatile phenols and sulphur-based compounds could also affect the cider profile.

Higher alcohols represent, from a quantitative point of view, the largest group of flavour compounds in alcoholic beverages, being the secondary products of the alcoholic fermentation [43]. On the basis of the results obtained, the most important alcohol was 2-phenylethanol, not only owing to its high concentration, but also its aroma intensity. 2-Phenylethanol gave rosy and honey aromas (its threshold in 10 % ethanol solution is 10 mg/l) [15]. The second most important alcohol, also based on concentration terms, could be 3-methylbutanol, which imparts fruity and nail polish-like odours. Moreover, its concentration in the analysed ciders was higher than its sensory threshold, which has been reported to be 30 mg/l [25].

The group of esters is essential for providing a fruity character [1]. Although many of the esters were originally present in apple and apple juice, most of them are formed from the esterification process taking place during fermentation [49]. In contrast with previously described data [28] ethyl lactate was the dominant ester instead of ethyl acetate. Ethyl hexanoate, ethyl octanoate and ethyl decanoate could become very important owing to their high aroma intensities [50]. It has been reported that even esters in significant lower concentrations can play an important contribution to the aroma, because most of them are present in concentrations around their threshold value, which implies that modest changes of their concentration might have a dramatic effect on wine flavour [30].

The most extensively studied carbonyl compound is diacetyl, which makes an important contribution to the flavour of cider, red wine, beer and some distilled products such as whisky and rum. It has been described that diacetyl can enrich cider flavour at levels lower than 600 ppb [21]. However, above these values, diacetyl contributes to a butter-like flavour of cider, deemed unpleasant by many consumers [28].

Short- and medium-chain fatty acids (C4:0–C12:0) analysed here presented similar concentrations among the commercial ciders tested, except F cider wherein the concentrations were considerably lower, especially for dodecanoic acid. These acids contribute to the overall cider aroma with rancid, cheese and sweat odours, and they have been taken into account owing to their high aroma intensities [50].

Another important aromatic group is the volatile phenolic compounds. Little is known about these compounds in cider; only qualitative but not quantitative data have been found in the literature. Several studies related to volatile phenolic compounds in wine have been published, because vinyl and ethyl-phenols are considered responsible for certain olfactory defects. Among the volatile phenolic compounds identified in apple cider, 4-ethylguaiacol and 4-vinylphenol had very high aroma intensities. 4-Ethylphenol and 4-vinylguaiacol had medium aroma intensities [50]. The main characteristic of these kinds of compounds is that their sensory thresholds are often lower than the habitual concentration ranges, so their contribution to wine aroma is usually significant [26]. The most unpleasant smelling are 4-vinylphenol (reminiscent of pharmaceuticals and paint) and 4-ethylphenol (stables, sweaty saddles, animal and leather). 4-Vinylguaiacol (clove, curry) and 4-ethylguaiacol (smoky, spicy aromas) are much less unpleasant, but they are unfortunately always associated with 4-vinylphenol and 4-ethylphenol, respectively. Their odour thresholds in hydroalcoholic solutions are 40 μg/l for 4-vinylguaiacol [15], 33 μg/l for 4-ethylguaiacol, 180 μg/l for 4-vinylphenol and 440 μg/l for 4-ethylphenol [8]. It is reported that as soon as they are detectable by tasters, vinyl- and ethylphenols have a negative impact on wine aroma. The concentrations of volatile phenolic compounds in the commercial ciders analysed (Table 2) were considerably higher than their odour threshold, but, as previously mentioned, the ciders selected for this work were highly appreciated by consumers. Even if that character is part of the bouquet of cider appreciated by consumers, this character could be sometimes very marked and it can also hide fruity notes which are generally appreciated.

In wine, the appearance of these compounds is associated with growth of certain *Pediococcus* and *Lactobacillus* strains, although the main microorganisms responsible for this defect are *Brettanomyces* and *Dekkera* yeasts [10]. One advantage of using malolactic starters in the winemaking process is avoiding the development of these microorganisms. It was reported [9] that during early stages of spontaneous MLF when LAB populations are low, *Brettanomyces bruxellensis* populations compete for available nutrients. Additionally, with greater tannic extractions, LAB exhibit a longer lag period. By inoculating a MLF starter culture, the ecological niche left by yeasts at the end of AF will be colonized by selected beneficial bacteria, thereby limiting the risk of development of spoiling LAB. The development of these species can not be prevented in spontaneous fermentations when present as indigenous microbiota, but it can be better controlled by using of an efficient MLF starter culture limiting their activity. This could be the reason why, as can be observed in Table 2, the commercial ciders undergoing spontaneous MLF turned out to be much richer in phenols than the experimental ciders inoculated with an MLF starter (Table 3). A lower level of *p*-coumaric acid in the experimental apple must could not be excluded, because the control showed a lower content of ethyl 4-phenol. However, interestingly, the results showed that it was possible to control and limit the risk of ethyl 4-phenol spoilage inoculating *O. oeni* starters (as shown in Tables 2 and 3).

Sulphur-containing compounds are also important because of their abundance and their aromatic impact. In fact, about 10 % of the volatile components detected in foods are sulphur compounds [7]. No references related to sulphur-containing compounds in cider have been found in the literature. In general, the aromatic contribution of these compounds is considered as detrimental to wine quality. The odour of these compounds can be described with terms such as cabbage, garlic, onion or rubber, which allude to their negative effects on wine aroma. Nevertheless, a low concentration of some sulphur compounds, such as dimethyl sulphide or carbon disulphide, has been reported to produce satisfactory wine aromas [27]. As shown in Table 1, the sulphur-containing compounds in the ciders analysed here varied widely. Some ciders contained all the sulphur compounds analysed, whereas in others, such as traditional 2, only dimethyl sulphide could be found.

The volatile compositions of the experimental ciders (Table 3) were quite similar to the corresponding commercial ciders (PDO ciders). So, when the indigenous strains tested in this work were used as starter cultures, it could be considered that they provided ciders with a quality comparable to the commercials. Variations in volatile compounds could be observed both in commercial and experimental ciders. As previously observed in wine [2], although LAB can modify the volatile composition, even the use of specific LAB strains could not imply complete control of the production of volatiles, taking into account that most of the volatiles analysed in this work derived mainly from yeast metabolism. Variations observed may be explained by a matrix effect (i.e. initial juice cultivar, yeast strains, even process parameters such as fermentation temperature known to alter fatty acids and their esters content). So changes in one or several parameters could modify the volatile composition among batches. In this work, more important variations observed in commercial ciders compared to experimental ciders (which originated from the same AF matrix) supported this effect.

Table 3 Quantitative data of volatile compounds identified in the experimental ciders

Compound	Cider 1.5	Cider 3.8a	Cider 3.8b	Cider 5.4a	Cider 5.4b	Cider 6.6
Alcohols (mg/l)						
Methanol	21.17 ± 3.36	17.56 ± 1.68	20.97 ± 3.31	17.10 ± 3.02	19.15 ± 3.92	25.41 ± 1.06
Propanol	13.69 ± 1.56	7.45 ± 0.32	12.37 ± 0.83	7.47 ± 0.34	12.78 ± 2.11	22.83 ± 0.05
Allylic alcohol	3.67 ± 0.62	<LOQ	<LOQ	<LOQ	<LOQ	4.61 ± 0.26
1-Butanol	3.23 ± 0.27	3.52 ± 0.08	3.74 ± 0.03	3.26 ± 0.14	3.51 ± 0.42	4.03 ± 0.24
2-Butanol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Isobutanol	42.48 ± 1.83	41.28 ± 1.70	44.16 ± 0.37	41.18 ± 2.07	41.32 ± 2.64	47.05 ± 1.47
2-Methyl-1-butanol	25.87 ± 0.82	23.99 ± 0.52	26.34 ± 0.79	23.88 ± 0.54	25.51 ± 1.84	27.87 ± 0.14
3-Methyl-1-butanol	139.47 ± 6.58	142.91 ± 6.96	149.79 ± 5.95	139.35 ± 3.25	141.04 ± 13.16	165.37 ± 1.08
1-Hexanol	8.15 ± 0.24	8.05 ± 0.43	8.52 ± 0.09	8.61 ± 0.15	7.80 ± 0.26	9.21 ± 0.02
2-Phenylethanol	51.85 ± 2.12	44.3 ± 1.85	49.52 ± 1.84	46.13 ± 0.40	46.93 ± 0.65	49.47 ± 2.36
Esters (mg/l)						
Isoamyl acetate	1.56 ± 0.01	2.19 ± 0.03	1.99 ± 0.02	2.35 ± 0.01	2.16 ± 0.05	1.74 ± 0.05
Hexyl acetate	0.02 ± 0.002	0.12 ± 0.01	0.05 ± 0.01	0.13 ± 0.004	0.07 ± 0.004	0.03 ± 0.01
2-Phenylethyl acetate	0.03 ± 0.001	0.023 ± 0.003	0.01 ± 0.001	0.02 ± 0.002	0.02 ± 0.0004	0.01 ± 0.0002
Ethyl acetate	169.68 ± 12.26	23.07 ± 1.68	22.32 ± 0.02	24.97 ± 3.41	24.30 ± 3.12	21.87 ± 0.62
Ethyl butyrate	0.29 ± 0.02	0.37 ± 0.03	0.35 ± 0.04	0.40 ± 0.01	0.38 ± 0.02	0.45 ± 0.05
Ethyl hexanoate	0.76 ± 0.05	0.92 ± 0.07	0.78 ± 0.02	0.96 ± 0.02	0.81 ± 0.03	0.81 ± 0.01
Ethyl octanoate	0.84 ± 0.03	0.86 ± 0.06	0.82 ± 0.04	0.86 ± 0.04	0.82 ± 0.01	0.88 ± 0.01
Ethyl decanoate	0.29 ± 0.04	0.31 ± 0.02	0.23 ± 0.02	0.28 ± 0.01	0.27 ± 0.03	0.23 ± 0.004
Ethyl dodecanoate	0.07 ± 0.001	0.089 ± 0.004	0.04 ± 0.001	0.07 ± 0.003	0.05 ± 0.01	0.04 ± 0.001
Ethyl lactate	135.6 ± 8.25	50.24 ± 2.69	131.72 ± 5.23	53.42 ± 1.01	128.54 ± 24.54	127.49 ± 2.08
Diethyl succinate	1.67 ± 0.04	0.5 ± 0.02	1.26 ± 0.05	0.64 ± 0.01	1.32 ± 0.05	1.24 ± 0.03
Ketones (mg/l)						
Diacetyl	0.10 ± 0.01	0.15 ± 0.01	0.16 ± 0.001	0.15 ± 0.01	0.07 ± 0.003	0.21 ± 0.01
Acids (mg/l)						
Butyric acid	6.17 ± 0.30	6.49 ± 0.42	6.93 ± 0.23	6.78 ± 0.09	6.34 ± 1.05	7.09 ± 0.42
Isobutyric acid	1.55 ± 0.08	0.94 ± 0.05	1.14 ± 0.02	1.04 ± 0.01	1.17 ± 0.21	1.18 ± 0.07
Isovaleric acid	3.57 ± 0.10	1.33 ± 0.07	1.95 ± 0.05	1.50 ± 0.004	2.26 ± 0.03	1.85 ± 0.07
Hexanoic acid	8.26 ± 0.11	5.82 ± 0.18	6.62 ± 0.17	6.04 ± 0.07	7.17 ± 0.13	6.64 ± 0.22
Octanoic acid	7.10 ± 0.22	7.07 ± 0.10	6.58 ± 0.39	6.64 ± 0.10	6.88 ± 0.08	6.68 ± 0.03
Decanoic acid	2.24 ± 0.06	2.53 ± 0.05	2.11 ± 0.27	2.18 ± 0.09	2.37 ± 0.14	1.92 ± 0.01
Dodecanoic acid	0.47 ± 0.03	0.72 ± 0.01	0.32 ± 0.09	0.56 ± 0.05	0.37 ± 0. 04	0.27 ± 0.01
Volatile phenols (μg/l)						
4-Ethylphenol	801.55 ± 2.91	798.15 ± 53.20	891.98 ± 20.25	770.35 ± 25.61	842.68 ± 8.93	838.40 ± 11.83
4-Ethylguaiacol	412.79 ± 2.30	450.48 ± 26.80	441.68 ± 13.31	423.77 ± 10.64	449.61 ± 4.05	506.53 ± 11.86
4-Vinylphenol	51.60 ± 7.02	89.69 ± 0.77	65.32 ± 0.80	104.13 ± 0.03	61.66 ± 1.12	50.76 ± 3.83
4-Vinylguaiacol	34.34 ± 2.05	26.30 ± 0.31	34.83 ± 1.06	25.44 ± 0.76	27.62 ± 1.30	36.20 ± 2.14
Volatile sulphur compounds (μg/l)						
Hydrogen sulphide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Dimethyl sulphide	3.22 ± 0.20	1.5 ± 0.12	3.80 ± 0.38	1.93 ± 0.57	3.10 ± 0.17	3.99 ± 0.49
Carbon disulphide	0.65 ± 0.07	<LOQ	0.43 ± 0.01	<LOQ	0.35 ± 0.06	0.38 ± 0.02

LOQ limit of quantification

Nevertheless, the high ethyl acetate concentration measured in cider 1.5, which is usually indicative of prolonged storage of the raw material and probable bacterial spoilage or even sample oxidation, is noteworthy [3]. From differences observed between values obtained in samples 3.8 and 5.4 (a, b), it could be concluded that, as in wine, many reactions occur during the aging phase which lead to significant changes in the composition of the product. As

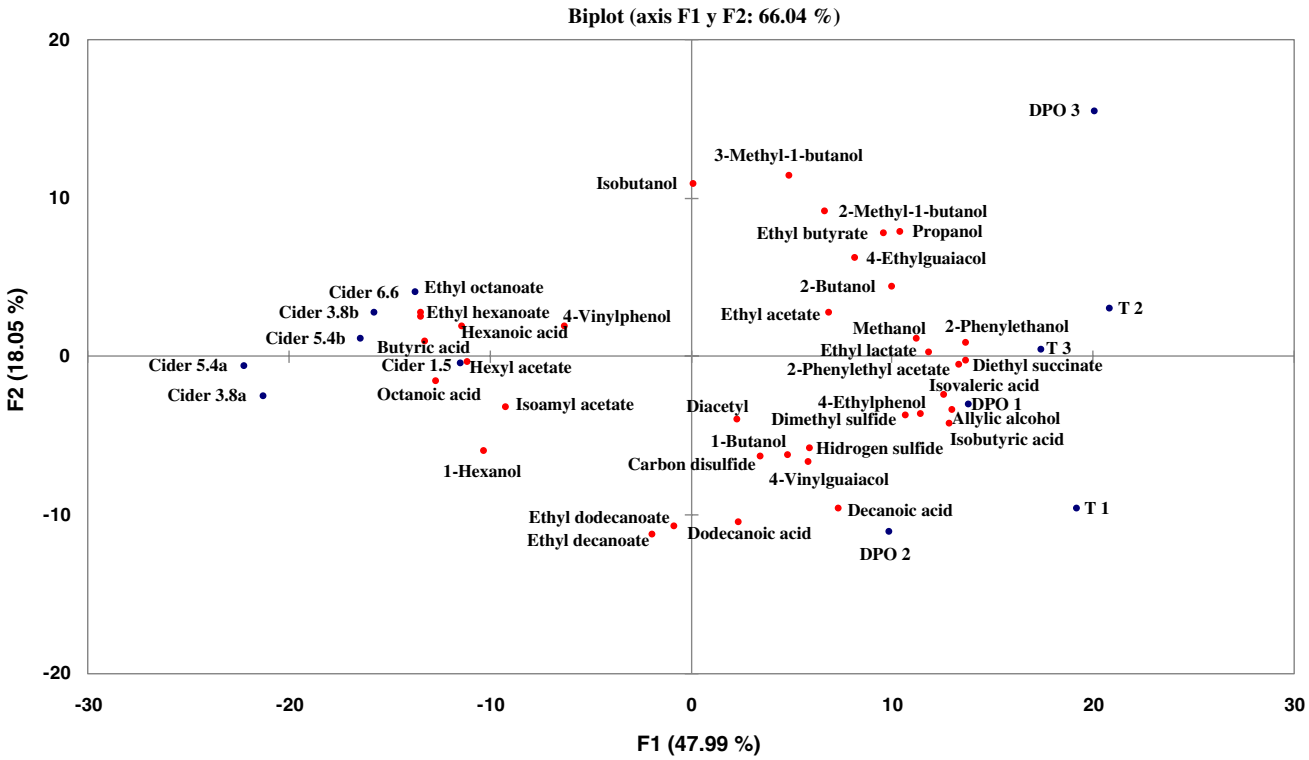

Fig. 4 Score plot of principal component analysis (PCA) applied to volatile compounds analysis of commercial and experimental ciders (*F*1 first principal component, *F*2 second principal component)

expected, during cider maturation, some esters were hydrolysed, and along with this degradation, a synthesis of new esters occurred (especially esters from organic acids such as ethyl lactate and diethyl succinate). A notably increase of carbon disulphide concentration was also observed.

Owing to the large number of variables studied, PCA analyses were performed in order to obtain a clearer comparison among ciders (Fig. 4). As shown by the analytical results, the technological procedure used in the cider called F (cider filtered) significantly affected its aromatic profile. The volatile composition of this type of cider was very different from both traditional and PDO ciders. F cider showed significantly lower concentrations of long-chain esters and fatty acids (C8, C10, C12), whereas differences in the content of short-chain esters and fatty acids among samples were minor. One possible explanation is that the filtering step in the process of this type of cider could have prevented the release of some metabolites during yeast autolysis, mainly apolar compounds bound to the cellular membrane, therefore affecting the final concentration of long-chain fatty acids and esters (C8, C10, C12). Therefore, F cider has not been included in the statistical study. Within the commercial group, both traditional and PDO cider samples presented a variable composition, these differences being notably for PDO ciders. The same behaviour was observed for experimental samples. Nevertheless, all the commercial samples presented clearly positive values on the axis corresponding to PC1, whereas the experimental samples had negative values. On the basis of statistical analysis, this separation seems to be mainly due to the concentration of esters present in the different ciders which is higher in the experimental ones. However, sulphur compounds, appearing on the positive part of the axis, could also contribute to this distinction between both commercial and experimental samples. In this work, experimental ciders were not sulphited, although it should be taken into account that this is a common practice in many cider industries to stop microbiological activity.

According to the analytical results, experimental ciders obtained not only presented an aromatic profile comparable to highly appreciated commercial ones, but also showed larger amounts of esters, the main compounds responsible of fruity aroma. These results assess the benefits of applying controlled inoculation of MLF, using proper starters, in industrial cider production.

Sensorial analysis

When the aroma of the alcoholic beverage analysed is characterized by a complex qualitative composition, its fractionation is sometimes advisable [23, 24]. This technique permits a more precise description of the aromatic profile

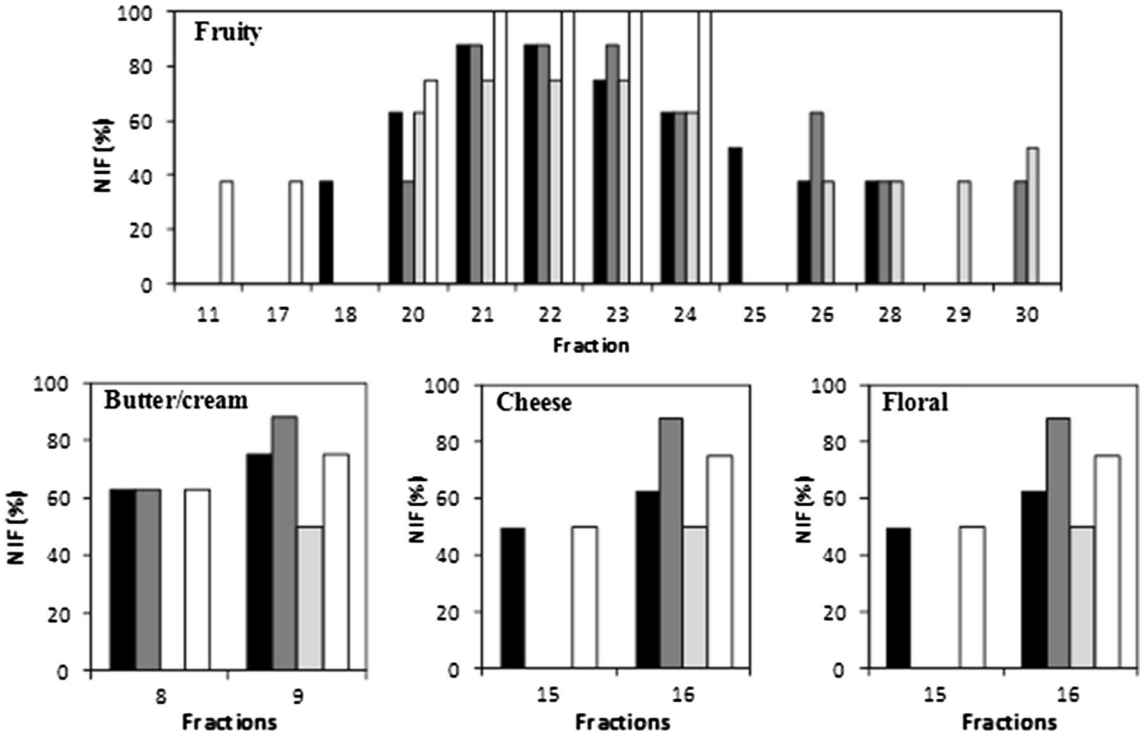

Fig. 5 Intensity scale obtained from HPLC fractions for the experimental ciders using detection frequency method for cider 1.5 (*black*), cider 3.8a (*grey*), cider 5.4a (*light grey*) and cider 6.6 (*white*)

and/or a better identification of the individual components of the global aroma.

Samples exhibited a high aromatic complexity, because at least four out of the nine descriptors evaluated were perceived in all of them. In ciders obtained by starter cultures inoculation, fruit aromatic descriptors were the most notable (between 20 and 40 % of fractions studied were described as fruity). In contrast, in commercial ciders only 16–20 % of the fractions were described using this term. This descriptor was responsible for aromas of positive nature, contributing to pleasant notes. The number of fractions described with negative notes, such as animal, pharmaceutical or sulphur, were approximately the same for both types of cider with the only exception of cider 5.4b. In this sample, 24 % of the fractions analysed were described as sulphur.

For NIF methods it is reported that compounds which are sensed more frequently than others are acknowledged as having the most important influence on the odour of the given sample [31]. The olfactograms obtained (Fig. 5) showed that the main descriptors of experimental cider samples were fruity (14 fractions), butter/cream (2 fractions), cheese (2 fractions) and floral (2 fractions). Although the fruity note was present in many fractions, there were 4 (21–24) in which this aroma was really intense. It is important to highlight that the analysis of only 10 fractions (out of the 30 fractions obtained from a cider extract) could lead to a sensory differentiation of cider samples because they have a great impact on cider aroma.

In addition, a statistical analysis of the olfactometry results allowed the comparison of both groups of samples (Fig. 6). This figure shows PCA results constructed from sensorial data (expressed as number of fractions) of the 14 samples analysed. On the positive side of the *F*1 axis, pharmaceutics, animal cheese and caramel descriptors are represented, whereas the negative side corresponds to butter/cream, fruity and floral. Spicy and sulphurous descriptors are located on the positive side of the *F*2 axis. As in the case of volatile compounds, the aromatic analysis showed an important differentiation between commercial and experimental ciders. This statistical analysis placed commercial samples on the positive side of the *F*1 axis (except PDO 2 and traditional 3 samples), whereas all experimental samples (excluding cider 3.8b) showed negative values on the axis corresponding to *F*1. The sensory properties detected could be explained by the concentration of volatile compounds. It could be observed that cider natural 2, which had the highest concentration of 4-ethylphenol, was the closest sample to the animal note. The high intensity of the butter and fruit note in the experimental samples is

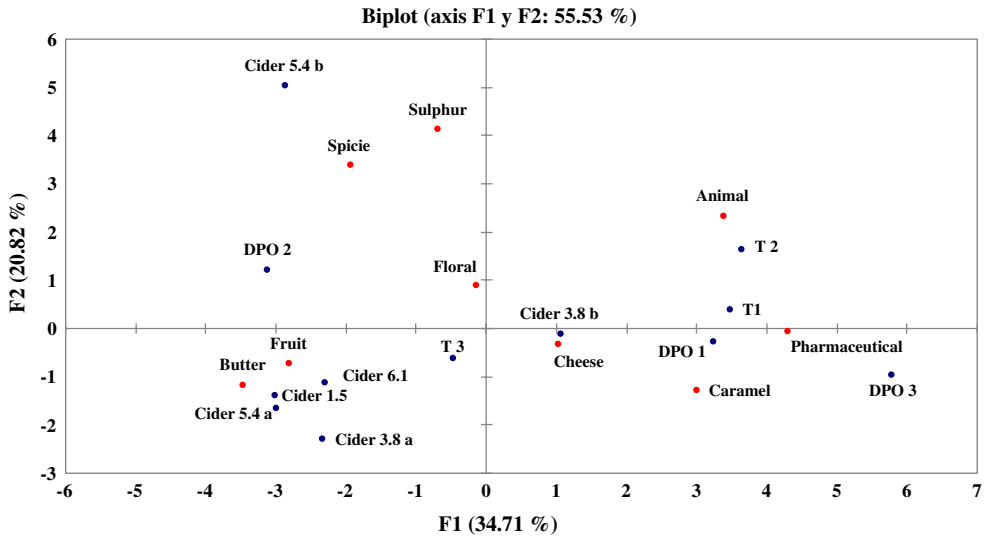

Fig. 6 Score plot of principal component analysis (PCA) applied to sensory analysis of commercial and experimental ciders. (*F*1 first principal component, *F*2 second principal component)

noteworthy. This fruity aroma could be explained, as mentioned, because this group showed a higher concentration of esters; nevertheless the diacetyl concentration is similar in all ciders analysed (Tables 2, 3). Sulphitation of commercial ciders certainly explains the lower butter aroma. SO_2 binds to diacetyl in a reversible reaction; the diacetyl–SO_2 complex is not volatile and so it is no longer perceptible. As shown in Fig. 5, the positions of both cider PDO 2 and cider 5.4b samples are very different compared with the rest of the samples. For PDO 2 sample, this position could be easily explained by the concentration of volatile compounds, which differs from the other PDO samples. However, in the case of cider 5.4b, results obtained in the analytical study could not explain this differentiation. During the olfactometry study the panel was capable of describing the cider 5.4b sulphur note as cabbage; therefore, the heavy sulphur compounds were also measured. When quantified (data not shown), it was noticed that the concentration of 2-mercaptoethanol (boxer, poultry, farmyard, alliaceous notes) in this sample was significantly higher than in the rest of samples, which could explain its position in the PCA diagram.

In wine, formation of VSC can occur during aging. The sulphur off-odours often arise from degradation of sulphur-containing compounds in the yeast lees, enzymatic formation, chemical reactions, or from re-release of chemically-entrapped sulphide during ageing [44]. For this reason, wine should be removed from heavy lees to minimize the formation of sulphur off-odours. Regarding the aging period, it could be observed that cider 3.8 taken immediately after MLF showed similar characteristics to the samples taken 1 month after. Nevertheless, in case of cider 5.4, concentrations of the less volatile VSC increased enormously during the aging period. So, the aging period could be reduced or even eliminated in industrial processes without losing the quality of the final product and shortening the global process by 1 month.

Conclusions

When applying starter culture technology using proper indigenous selected strains, MLF could be shortened almost by half compared with the spontaneous fermentation performed industrially, obtaining a desirable aromatic profile, showing a higher fruity note than the non-inoculated ones. Additionally, it was observed that cider aging period could be eliminated, thus shortening the global process without losing the quality of the final product. The results obtained should encourage further interest in applying starter culture technologies to control MLF in cider production at industrial level while ensuring a desirable control over the production of aromatic compounds and flavour. In addition, and on the basis of the sensorial analyses performed, it could be observed that consumer perceptions related to some compounds when present in cider differed from those reported when found in wine.

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