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# Impact of different techniques involving contact with lees on the volatile composition of cider

María José Antón-Díaz, Belén Suárez Valles, Juan José Mangas-Alonso, Ovidio Fernández-García, Anna Picinelli-Lobo *

*Area of Food Technology, SERIDA, Carretera de Oviedo s/n, 33300 Villaviciosa, Asturias, Spain*

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ABSTRACT

The effect of different treatments involving contact with natural lees on the aromatic profile of cider has been evaluated. Comparing with the untreated ciders, the contact with lees brought about a significant increase of the concentrations of most of the volatile compounds analysed, in particular fatty acids, alcohols, ethyl esters and 3-ethoxy-1-propanol. The opposite was observed among fusel acetate esters and 4-vinylguaiacol. The addition of β-glucanase enhanced the increase of ethyl octanoate, but produced a decrease in the contents of decanoic acid and all of the major volatiles excepting acetaldehyde, ethyl acetate and acetoine, whereas the application of oxygen influenced the rise of the level of 3-ethoxy-1-propanol only. The olfactometric profiles also revealed significant effects of the treatment with lees for ethyl propionate, diacetyl, *cis*-3-hexenol, acetic acid, benzyl alcohol, and *m*-cresol, while the addition of oxygen significantly influenced the perception of ethyl hexanoate, 1-octen-3-one, 3-methyl-2-butenol, *t*-3-hexenol and *c*-3-hexenol.



## 1. Introduction

Odour and flavour are the most important quality aspects of alcoholic beverages, paramount to determine the preferences of potential consumers. The development of these attributes throughout the making process is influenced by the succession of different and complex steps: selection of raw material, fermentation process and maturation or ageing.

Maturation and ageing are traditional oenological practices aiming at improving the sensorial characteristics of wines. Sparkling and some types of white wines are usually left on their lees but nowadays, the ageing over-lees is gaining relevance also in the production of red wines in all viticultural areas (Fornairon-Bonnedond, Camarasa, Moutounet, & Salmon, 2002; Pérez Serradilla & Luque de Castro, 2008).

The composition of lees is highly variable as they are mainly formed by microorganisms (yeasts and bacteria) and their autolysis products, together with organic and inorganic residues coming from the must. The autolysis of yeasts provides the must with valuable macromolecular components which play important roles in colloidal stability phenomena, stimulation of malolactic fermentation and sensory improving of wines (Fornairon-Bonnedond et al., 2002). Besides, lees are able to retain undesirable components such as sulphurs and volatile phenols (Chassagne, Guilloux-Benatier, Alexandre, & Voilley, 2005; Pradelles, Alexandre, Ortiz-Julien, & Chassagne, 2008; Vasserot, Steinmetz, & Jeandet, 2003), reinforcing existing evidences of the ability of lees to modify wine organoleptic characteristics.

Regarding aroma, the influence of a short contact with lees on the aromatic composition of wines is controversial. Bautista, Fernández, and Falqué (2007) observed a generalised significant increase of the volatile compounds of wines after contact with lees, while others (Loscos, Hernández-Orte, Cacho, & Ferreira, 2009) found the opposite effect. In the particular case of sparkling wine, there are typical profiles of volatile compounds related to ageing time (Francioli, Torrens, Riu-Aumatell, López-Tamames, & Buxaderas, 2003; Torrens, Riu-Aumatell, Vichi, López-Tamames, & Buxaderas, 2010). Ageing time has been identified as the main responsible for changes in the aromatic composition of sparkling ciders (Rodríguez Madrera, García Hevia, Palacios García, & Suárez Valles, 2008).

Cider is a worldwide popular drink which consumption figures have been increasing in the last years. Asturias, with making rates close to 80 M litres, is the fifth European producer (www.aicv.org). In this region, traditional cider is made by spontaneous fermentation (alcoholic and malolactic) of apple musts, followed by an optional step of maturation over-lees, which is claimed to bring

* Corresponding author.
*E-mail address:* apicinelli@serida.org (A. Picinelli-Lobo).

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about positive sensory characteristics, such as improved foaming properties and aroma complexity. Because of the cider-making process, lees are expected to be complex, since they include the presence of lactic bacteria.

The management of lees in winemaking has been the focus of many studies. Those reports include the application of microoxygenation to reduce sulphur off-odours or to improve wine colour and stability (Gómez-Plaza & Cano-López, 2011), and the use of enzymes to either accelerate the autolysis process or enhance the aromatic potential of wines (Masino, Montececchi, Arfelli, & Antonelli, 2008; Moreno-Arribas & Polo, 2005; Rocha, Coutinho, Delgadillo, Dias Cardoso, & Coimbra, 2005; Sánchez Palomo, Díaz-Maroto Hidalgo, González-Viñas, & Pérez-Coello, 2005; Torresi, Frangipane, & Anelli, 2011). However, to the best of our knowledge, these studies have not been made before in cider. Therefore, the purpose of this paper is to evaluate the effect of different treatments based on the contact with fermentation lees on the volatile composition and the olfactometric profiles of cider.

## 2. Materials and methods

### 2.1. Reagents and standards

The volatile standards were supplied by Sigma (St. Louis, MO), Aldrich (Gillingham, U.K.), and Fluka (Buchs, Switzerland). Pentane (Merck, Darmstadt, Germany), dichlorometane, absolute ethanol, ammonium sulphate and anhydrous sodium sulphate were from Panreac (Barcelona, Spain). All the reagents were of chromatographic quality. A β-glucanase commercial preparation (Enovin Glucan) was purchased from Agrovin (León, Spain).

### 2.2. Micro-oxygenation equipment

A multiple diffuser micro-oxygenation DosiOx equipment provided by Agrovin (León, Spain) was used. The oxygen employed was Ultrapure Plus from Carburos Metálicos (Barcelona, Spain).

### 2.3. Experimental design

A thousand litres of clean racked cider were obtained from a local cellar, and distributed between 100L-stainless steel tanks. This cider was made according to the usual procedure that is, pressing of a mixture of cider apples, spontaneous clarification and alcoholic and malolactic fermentation. The lees coming from the fermentation of this cider were also sampled, homogenised and used without further handling. Two tanks containing cider without treatment were taken as controls, and the other eight batches were added with lees (5% v/v) and different combinations of enzyme (β-glucanase, 5 g/hL) and oxygen (6 mL/L/month), as shown in Fig. 1. The experiment lasted 2 months at 12 °C, and after that, the ciders were sampled and analysed.

### 2.4. Analysis of major volatile compounds

Major volatiles were analysed by direct injection by GC-FID, as described elsewhere (Suárez Valles, Pando Bedriñana, Fernández Tascón, González García, & Rodríguez Madrera, 2005).

### 2.5. Analysis of minor volatile compounds

Minor volatiles were analysed by GC-FID, after a one-step liquid–liquid extraction, as described elsewhere (Antón, Suárez Valles, García Hevia, & Picinelli Lobo, 2014).

### 2.6. Olfactometric analysis

Olfactometric analyses were done by means of a Hewlett–Packard 5890 model fitted with a flame ionisation detector, coupled to an Olfactory Port 275 at 220 °C (Ingeniería Analítica, S.L., Barcelona, Spain), and a DB-WAX column (30 m × 0.32 mm; 0.50 μm from J&W Folson, CA, USA). The odorants were also injected on a DB-5 column (30 m × 0.32 mm; 0.25 μm from J&W Folson, CA, USA) for identification purposes. A panel of 6–8 people carried out the sniffings of the aforementioned cider extracts according to the methodology optimised by Antón et al. (2014).

### 2.7. Statistical analysis

A multivariate two-way analysis of variance was carried out to assess the influence of oxygen and enzyme on the chemical composition of ciders, and GC/O profiles by means of the SPSS v.12.0 for Windows.

Subsequently, an (ANOVA)-Simultaneous Component Analysis (ASCA) was performed to model the influence of the variation sources (application of microoxygenation and/or enzyme) on the chemical and olfactometric profiles of ciders by applying the following generalised model:

$$X_{ijk} = \mu + A_i + B_j + AB_{ij} + \varepsilon_{ijk} \quad (1)$$

where $X_{ijk}$ is the value of any variable (chemical or olfactometric) for the cider $k$ with $i$ and $j$ levels for A (oxygen) and B (enzyme) factors, respectively; $\mu$ is the general mean value; $A_i$ is the contribution of factor A at level $i$; $B_j$ is the contribution of factor B, at level $j$; $AB_{ij}$ represents the interaction between the factors A and B at levels $i$ and $j$, and $\varepsilon_{ijk}$ is the variation captured in the term error. In our case,

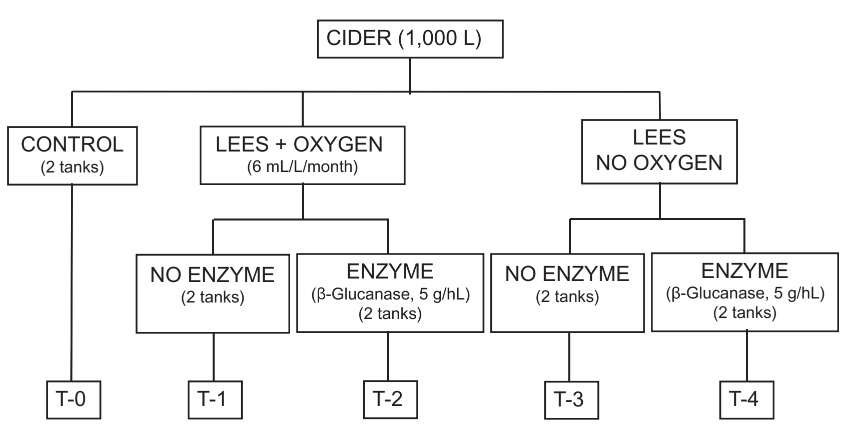


**Fig. 1.** Schematic representation of the experimental design.

the nomenclature used to name means of treatments was as follows: $X_{A1}$: oxygen; $X_{A2}$: no-oxygen; $X_{B1}$: enzyme; $X_{B2}$: no-enzyme. Four matrices were constructed: one for each factor, the interaction and error matrices, and PCA was subsequently applied on each one. The statistical program used was Unscrambler 9.2.

## 3. Results and discussion

### 3.1. Quantitative volatile component analyses

The ciders were analysed in duplicate for volatile composition, and data were statistically evaluated. Looking at Fig. 1, the factors evaluated were: the addition of lees only (T0 *vs* T3), or lees combined with oxygen (T1–T2 *vs* T3–T4) or enzyme (T1–T4 *vs* T1–T3).

Regarding major volatiles and compared with the control ciders, the contact with lees brought about a significant decrease of the contents of *iso*-butanol, amyl alcohols, and acetoine, and the increase of those of 1-propanol. Among the minor components, only seven components were not influenced by the contact with lees: decanoic acid, 4-ethylguaiacol (4-EG), *cis*-3-hexenol, ethyl 3-hydroxybutyrate and ethyl 4-hydroxybutyrate, γ-butyrolactone, and methionol. The general effect was the increase of the concentrations, those of 3-ethoxy-1-propanol, 4-ethylcatechol (4-EC), ethyl octanoate and octanoic acid being the most noticeable. Conversely, the contact with lees gave a significant decrease in the concentrations of the fusel acetate esters and 4-vinylguaiacol (4-VG). The levels of volatile phenols found in these ciders ranged between a hundred μg/L and 14 mg/L (Table 1), which are similar to those described for French ciders (Buron, Coton, et al., 2011). Some studies reported the influence of the yeast strains on the sorption of volatile phenols by lees (Chassagne et al., 2005; Pradelles et al., 2008), while others show significant increases of these components as a consequence of the contact with wine lees (Masino et al., 2008). In our case, the increase in the contents of 4-EP and 4-EC in the ciders with lees could be related to the presence of different strains of *Lactobacillus collinoides* lactic acid bacteria (data not shown), which are able to synthesize large amounts of those compounds from hydroxycinnamic acids, particularly 4-EC (Beech & Carr, 1977; Buron, Guichard, Coton, Ledauphin, & Barillier, 2011).

The effect of the application of microoxygenation or enzyme on the aromatic composition of treated ciders is summarised in Table 2. As seen, the presence of oxygen significantly influenced the content of 3-ethoxy-1-propanol only, while the addition of enzyme did affect the concentrations of decanoic acid, ethyl octanoate and all of the major volatiles excepting acetaldehyde and ethyl acetate. Contents of methanol and fusel alcohols decreased in the ciders submitted to enzymatic treatment. As reported in previous works, the effect of treatments of wines with lees alone or together with β-glucanase and/or microoxygenation is not straightforward (Bautista et al., 2007; Bueno, Peinado, Medina, & Moreno, 2006; Hernández-Orte et al., 2009; Masino et al., 2008; Rodríguez-Bencomo, Ortega-Heras, & Pérez-Magariño, 2010). This variability was explained on the basis of many factors: grape varieties (Bueno et al., 2006; Ortega-Heras, Rivero-Pérez, Pérez-Magariño, González-Huerta, & González-Sanjosé, 2008), nature and characteristics of lees or the time of contact with them (Bautista et al., 2007; Gallardo-Chacón, Vichi, López-Tamames, & Buxaderas, 2010).

Among the minor volatiles, the addition of β-glucanase had a negative effect on decanoic acid contents, while the application of microoxygenation produced the rise of 3-ethoxy-1-propanol concentration. Within the group of volatile phenols, the presence of oxygen increased the contents of 4-EC only, although a significant interaction between factors was observed. As said before,

**Table 1**
Effect of the contact with lees on the quantitative volatile composition (mean of treatments ± sd) of ciders.

| | Sig | Control | Lees |
|---|---|---|---|
| Major volatiles (mg/L) | | | |
| Acetaldehyde | ns | 10 ± 4 | 9 ± 3 |
| Ethyl acetate | ns | 41 ± 2 | 40 ± 3 |
| Methanol | ns | 50 ± 2 | 47 ± 5 |
| 1-Propanol | *** | 10 ± 1 | 14 ± 2 |
| *iso*-Butanol | * | 33 ± 1 | 31 ± 3 |
| 1-Butanol | ns | 3 ± 1 | 3 ± 1 |
| Amyl alcohols | * | 233 ± 8 | 218 ± 18 |
| Acetoine | *** | 35 ± 6 | 22 ± 6 |
| Ethyl lactate | ns | 56 ± 8 | 56 ± 6 |
| 2-Phenyl ethanol | ns | 150 ± 5 | 146 ± 11 |
| Minor volatiles (μg/L) | | | |
| *Fatty acids* | | | |
| Hexanoic | *** | 3198 ± 488 | 4232 ± 339 |
| Octanoic | *** | 5663 ± 669 | 13,543 ± 2724 |
| Decanoic | ns | 2268 ± 663 | 2400 ± 1124 |
| *Volatile phenols* | | | |
| 4-Ethylguaiacol | ns | 110 ± 75 | 159 ± 29 |
| 4-Ethylphenol | *** | 1735 ± 268 | 3036 ± 178 |
| 4-Vinylguaiacol | *** | 3173 ± 458 | 1832 ± 360 |
| 4-Ethylcatechol | *** | 3332 ± 1040 | 14,002 ± 3099 |
| *Alcohols* | | | |
| 3-Methyl-3-butenol | *** | 21 ± 3 | 28 ± 2 |
| 1-Pentanol | *** | 55 ± 13 | 70 ± 6 |
| 3-Methyl-2-butenol | *** | 17 ± 3 | 28 ± 4 |
| 3-Methyl-1-pentanol | * | 74 ± 12 | 82 ± 5 |
| Hexanol | *** | 3741 ± 607 | 4482 ± 296 |
| *trans*-3-hexenol | ** | 32 ± 4 | 36 ± 3 |
| *cis*-3-hexenol | ns | 450 ± 84 | 499 ± 34 |
| Benzylic | *** | 94 ± 21 | 179 ± 34 |
| *Ethyl esters* | | | |
| 2-Methylbutyrate | ** | 17 ± 6 | 26 ± 4 |
| Hexanoate | *** | 221 ± 41 | 317 ± 44 |
| Octanoate | *** | 510 ± 103 | 1841 ± 459 |
| 3-Hydroxybutyrate | ns | 47 ± 9 | 51 ± 6 |
| 4-Hydroxybutyrate | ns | 725 ± 150 | 710 ± 126 |
| *Acetate esters* | | | |
| Isoamyl | *** | 455 ± 96 | 333 ± 43 |
| 2-Phenylethyl | *** | 267 ± 75 | 102 ± 15 |
| *Others* | | | |
| 3-Ethoxy-1-propanol | *** | 70 ± 18 | 577 ± 169 |
| γ-Butyrolactone | ns | 659 ± 118 | 699 ± 171 |
| Methionol | ns | 514 ± 89 | 449 ± 73 |

Sig: significance; ns: not significant.
* Significant at 10% level.
** Significant at 5% level.
*** Significant at 1% level.

different strains of *L. collinoides* should be associated to the synthesis of 4-EC; these microorganisms are microaerophilic and therefore, their activity should be favoured by the presence of tiny amounts of oxygen (Table 2).

An ANOVA-Simultaneous Component analysis (ASCA) was used to reinforce the conclusions from multivariate variance analysis. This approach is useful when many variables are taken into account with only a few observations (Jansen et al., 2005). In this study, ASCA gave four vectors: $X_{A1}$, $X_{A2}$ (average of treatment with or without oxygen), $X_{B1,}$ $X_{B2}$ (average of treatments with or without enzyme), which were projected onto the only principal component computed. $X_{A1}$ and $X_{B2}$ vectors had positive loadings, while the opposite was detected for $X_{A2}$ and $X_{B1}$. Five chemical variables gave the most significant loadings onto this principal component. On the one hand, 4-EC was strongly associated with the treatments with oxygen (loading = 0.97), whereas the opposite was observed for 4-VG (loading = −0.06). On the other hand, the content of ethyl

**Table 2**
Effect of the application of oxygen and/or enzyme on the quantitative volatile composition (mean of treatments ± sd) of ciders.

| | Sig | | | Ox | no-Ox | Enz | no-Enz |
|---|---|---|---|---|---|---|---|
| | Int | Ox | Enz | | | | |
| Major volatiles (mg/L) | | | | | | | |
| Acetaldehyde | ns | ns | ns | 11 ± 3 | 11 ± 5 | 11 ± 5 | 11 ± 3 |
| Ethyl acetate | ns | ns | ns | 39 ± 6 | 38 ± 3 | 37 ± 5 | 39 ± 4 |
| Methanol | ns | ns | *** | 43 ± 7 | 45 ± 5 | 42 ± 5 | 47 ± 6 |
| 1-Propanol | ns | ns | *** | 14 ± 2 | 14 ± 2 | 13 ± 1 | 15 ± 2 |
| *iso*-Butanol | ns | ns | ** | 29 ± 4 | 30 ± 3 | 28 ± 4 | 31 ± 3 |
| 1-Butanol | ns | ns | ** | 2 ± 1 | 3 ± 0 | 2 ± 1 | 3 ± 0 |
| Amyl alcohols | ns | ns | *** | 205 ± 29 | 209 ± 19 | 196 ± 24 | 218 ± 20 |
| Acetoine | ** | ns | ** | 32 ± 11 | 37 ± 25 | 42 ± 23 | 27 ± 9 |
| Ethyl lactate | ** | ns | ** | 57 ± 22 | 56 ± 5 | 51 ± 7 | 62 ± 19 |
| 2-Phenyl ethanol | ns | ns | ** | 137 ± 18 | 139 ± 12 | 130 ± 14 | 146 ± 12 |
| Minor volatiles (μg/L) | | | | | | | |
| *Fatty acids* | | | | | | | |
| Hexanoic | ns | ns | ns | 4375 ± 654 | 3906 ± 435 | 4066 ± 731 | 4215 ± 433 |
| Octanoic | ns | ns | ns | 13,227 ± 2735 | 12,128 ± 2497 | 12,361 ± 3103 | 13,124 ± 2064 |
| Decanoic | ns | ns | ** | 2148 ± 452 | 1988 ± 922 | 1757 ± 400 | 2378 ± 839 |
| *Volatile phenols* | | | | | | | |
| 4-Ethylguaiacol | ns | ns | ns | 121 ± 30 | 123 ± 45 | 111 ± 33 | 133 ± 39 |
| 4-Ethylphenol | ns | ns | ns | 2985 ± 440 | 2772 ± 335 | 2812 ± 465 | 2945 ± 324 |
| 4-Vinylguaiacol | ns | ns | ns | 1586 ± 322 | 1716 ± 371 | 1557 ± 339 | 1745 ± 342 |
| 4-Ethylcatechol | * | *** | ns | 18,159 ± 3474 | 12,652 ± 2630 | 15,126 ± 4875 | 15,684 ± 3351 |
| *Alcohols* | | | | | | | |
| 3-Methyl-3-butenol | *** | ns | ns | 28 ± 4 | 24 ± 4 | 25 ± 6 | 37 ± 3 |
| 1-Pentanol | ** | ns | ns | 70 ± 10 | 62 ± 10 | 63 ± 12 | 69 ± 8 |
| 3-Methyl-2-butenol | ** | ns | ns | 29 ± 5 | 25 ± 5 | 26 ± 6 | 28 ± 3 |
| 3-Methyl-1-pentanol | ** | ns | ns | 88 ± 12 | 75 ± 11 | 79 ± 17 | 84 ± 7 |
| Hexanol | ** | ns | ns | 4412 ± 629 | 4041 ± 580 | 4107 ± 740 | 4347 ± 475 |
| *trans*-3-hexenol | ** | ns | ns | 36 ± 6 | 32 ± 5 | 33 ± 7 | 35 ± 4 |
| *cis*-3-hexenol | ns | ns | ns | 518 ± 78 | 452 ± 66 | 473 ± 98 | 497 ± 52 |
| Benzylic | ** | ns | ns | 168 ± 28 | 149 ± 39 | 147 ± 38 | 171 ± 27 |
| *Ethyl esters* | | | | | | | |
| 2-Methylbutyrate | ns | ns | ns | 24 ± 7 | 25 ± 3 | 25 ± 7 | 24 ± 4 |
| Hexanoate | ns | ns | ns | 315 ± 90 | 314 ± 40 | 334 ± 85 | 296 ± 42 |
| Octanoate | ns | ns | ** | 1922 ± 995 | 2069 ± 640 | 2343 ± 979 | 1647 ± 442 |
| 3-Hydroxybutyrate | ns | ns | ns | 56 ± 8 | 46 ± 7 | 50 ± 11 | 52 ± 6 |
| 4-Hydroxybutyrate | ** | ** | ns | 878 ± 147 | 628 ± 127 | 733 ± 226 | 773 ± 137 |
| *Acetate esters* | | | | | | | |
| Isoamyl | ns | ns | ns | 312 ± 62 | 315 ± 62 | 318 ± 73 | 310 ± 49 |
| 2-Phenylethyl | ns | ns | ns | 96 ± 29 | 94 ± 14 | 97 ± 27 | 93 ± 17 |
| *Others* | | | | | | | |
| 3-Ethoxy-1-propanol | ns | *** | ns | 1005 ± 134 | 515 ± 184 | 731 ± 337 | 788 ± 255 |
| γ-Butyrolactone | ** | ** | ns | 955 ± 151 | 607 ± 165 | 762 ± 283 | 801 ± 183 |
| Methionol | ** | ** | ns | 536 ± 89 | 399 ± 77 | 450 ± 128 | 484 ± 83 |

Sig: significance; Int: interaction; Ox: oxygen; Enz: enzyme; ns: not significant.
* Significant at 10% level.
** Significant at 5% level.
*** Significant at 1% level.

octanoate was correlated to the treatments with enzyme (loading = −0.50) while the production of octanoic (loading = 0.35) and decanoic acids (loading = 0.75) was higher without enzyme added. Lactic acid bacteria can profit the products released from the autolysis of yeasts to synthesize ethyl esters (Sumby, Grbin, & Jiranek, 2010), as well as many other volatile components, such as fatty acids, alcohols, methionol, and γ-butyrolactone, this activity being strain-dependent (Pozo-Bayón et al., 2005; Ugliano & Moio, 2005).

In general terms, the results obtained from ASCA were complementary with those from MANOVA so as to ascertain the effect of the application of oxygen or enzyme on the chemical profiles of ciders and the olfactometric perception of different components, where the MANOVA approach did not show significant differences for these factors. This was the case for octanoic acid, which contents were found to be higher in ciders without enzyme.

### 3.2. Olfactometric volatile analyses

Comparing with the control ciders, the contact with lees significantly increased the intensities of ethyl propionate, acetic acid, two alcohols (*c*-3-hexenol and benzyl alcohol), and *m*-cresol, and decreased that of diacetyl; this fact may be related to the synthesis of 2,3-butanediol by lactic bacteria (Swiegers, Bartowsky, Henschke, & Pretorius, 2005).

The effect of the subsequent applications of microoxygenation or enzyme on the olfactometric profile of ciders are summarised in Table 3. Two groups of odorants have been established on the basis of the average of their mean values for mean frequencies (MF).

In the first group were included those volatiles exhibiting a MF ⩾ 50% in at least one of the treatments. In this category there were components clearly perceived in all the samples, including

**Table 3**
Influence of the presence of oxygen and enzyme on the olfactometric profiles of ciders.

| $LRI_{WAX}$ | $LRI_5$ | Description | Identity | RefV | Sig | | | Ox | no-Ox | Enz | no-Enz |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Int | Ox | Enz | | | | |
| *Average MF ⩾ 50%* | | | | | | | | | | | |
| 1093 | 818 | Ripen fruit | Butyl acetate | V6 | *** | *** | *** | 63 | 57 | 65 | 55 |
| 1217 | 753 | Like fusel alcohols | Amyl alcohols | V8 | ns | ns | ns | 58 | 65 | 58 | 66 |
| 1240 | 1186 | Rippen fruit | Ethyl hexanoate | V10 | ns | * | ns | 60 | 44 | 59 | 46 |
| 1294 | 983 | Mushroom | 1-Octen-3-one | V12 | ns | * | ns | 34 | 51 | 44 | 42 |
| 1299 | 805 | Smoked, toasted | 3-Methyl-2-butenol | V13 | ns | *** | ** | 74 | 40 | 52 | 63 |
| 1383 | 887 | Flowery, geranium | *t*-3-Hexenol | V16 | ns | * | ns | 69 | 50 | 61 | 58 |
| 1448 | | Vinegar, like fusel alcohols | Acetic acid | V22 | ns | ns | ns | 63 | 52 | 58 | 58 |
| 1466 | | Dust, rancid, cooked vegetables | Methional | V23 | ns | ns | ns | 51 | 48 | 47 | 51 |
| 1635 | 954 | Rancid, varnish | γ-Butyrolactone | V34 | ns | ns | ns | 79 | 71 | 77 | 73 |
| 1681 | | Cheese, rancid | 2-Methylbutyric acid | V36 | ns | ns | ns | 75 | 76 | 74 | 78 |
| 1735 | 1013 | Cooked vegetables, sulphury | Methionol | V38 | ns | ns | ns | 47 | 48 | 45 | 50 |
| 1837 | 1267 | Stewed fruit, flowery | 2-Phenylethyl acetate | V43 | ns | ns | ns | 63 | 57 | 58 | 63 |
| 1860 | 1174 | Fatty, stable | Hexanoic acid | V44 | ns | ns | ns | 75 | 75 | 75 | 75 |
| 1903 | 1071 | Fruity, floral, sweet | Bencyl alcohol | V46 | ns | ns | ns | 50 | 44 | 44 | 50 |
| 1929 | 1175 | Roses | 2-Phenylethanol | V47 | ns | ns | ns | 81 | 76 | 79 | 77 |
| 2047 | 1291 | Spicy, sweet | 4-Ethylguaiacol | V52 | ns | * | ns | 52 | 61 | 71 | 41 |
| 2086 | | Sweet, spicy | ni | V55 | ns | ns | ns | 82 | 57 | 71 | 69 |
| 2194 | 1165 | Leather, stable | 4-Ethylphenol | V64 | ns | ns | ns | 64 | 55 | 63 | 56 |
| 2210 | 1095 | Smoked, spicy, curry | Sotolon | V66 | ns | ns | ns | 78 | 81 | 84 | 75 |
| 2246 | 1279 | Smoked, spicy | 4-Vinylguaiacol | V68 | ns | ns | ns | 57 | 33 | 41 | 50 |
| 2285 | 1451 | Fatty, stable | Decanoic acid | V71 | ns | ns | ns | 76 | 61 | 73 | 64 |
| *Average MF < 50%* | | | | | | | | | | | |
| 905 | 719 | Fruity | Ethyl propionate | V1 | ns | ns | ns | 9 | 22 | 18 | 13 |
| 931 | 721 | Fruity | Propyl acetate | V2 | ns | ns | ns | 44 | 48 | 47 | 46 |
| 972 | | Butter | Diacetyl | V3 | ns | ns | ns | 17 | 6 | 20 | 3 |
| 1074 | 805 | Fruity, sweet, apple | Ethyl 2-methylbutyrate | V5 | *** | *** | ** | 44 | 31 | 40 | 34 |
| 1234 | | Cooked vegetables | n.i. | V9 | ns | ns | ns | 18 | 16 | 11 | 22 |
| 1280 | | Butter, lactic | Acetoine | V11 | ns | ns | ns | 10 | 0 | 13 | 7 |
| 1364 | 889 | Flowery, fruity | Hexanol | V15 | ns | ns | ns | 16 | 15 | 13 | 17 |
| 1404 | 867 | Flowery, sweet | *c*-3-Hexenol | V17 | ns | *** | ns | 21 | 43 | 33 | 30 |
| 1440 | 1190 | Fruity, grass, resinous | Ethyl octanoate | V21 | ns | ns | ns | 17 | 24 | 19 | 22 |
| 1494 | 957 | Fruity, flowery | Ethyl 3-hydroxybutyrate | V27 | ns | ns | ns | 42 | 47 | 43 | 47 |
| 1529 | 909 | Fatty, heavy | *iso*-Butyric acid | V30 | ns | ns | ns | 9 | 19 | 14 | 14 |
| 1653 | 1090 | Flowery | Phenylacetaldehyde | V35 | ns | ns | ns | 34 | 26 | 32 | 28 |
| 1801 | | Flowery, sweet | Ethyl 4-hydroxybutyrate[a] | V42 | ns | ns | ns | 22 | 18 | 21 | 30 |
| 1880 | 1118 | Smoked, spicy, sweet | Guaiacol | V45 | ns | ns | ns | 22 | 29 | 21 | 30 |
| 2078 | 1358 | Fatty, stable, spicy | Octanoic acid | V54 | ns | ns | ns | 33 | 45 | 38 | 40 |
| 2098 | | Sweet | n.i. | V56 | ns | ns | ns | 30 | 34 | 40 | 24 |
| 2109 | 1099 | Leather, stable | *m*-Cresol | V58 | ns | ns | ns | 31 | 34 | 24 | 41 |
| 2171 | 1476 | Spicy, sweet, leather | γ -Decalactone | V62 | ns | ns | ns | 30 | 18 | 27 | 21 |
| 2183 | 1385 | Spicy, clover | Eugenol | V63 | ns | ns | ns | 35 | 25 | 34 | 27 |
| 2261 | 1913 | Resinous, dry grass | Ethyl hexadecanoate | V70 | ns | ns | ns | 19 | 16 | 22 | 16 |
| 2333 | | Phenolic, stable | n.i. | V76 | ns | ns | ** | 28 | 19 | 12 | 35 |
| 2360 | 1496 | Spicy, phenolic | Isoeugenol | V77 | ** | ns | ns | 13 | 20 | 22 | 12 |

$LRI_{WAX}$, $LRI_5$: lineal retention indices on the WAX and DB5 columns; RefV: references for variables; Sig: significance; Int: interaction; OX: oxygen; Enz: enzyme; ni: not identified; a: tentatively identified; ns: not significant.
[*] Significant at 10% level.
[**] Significant at 5% level.
[***] Significant at 1% level.

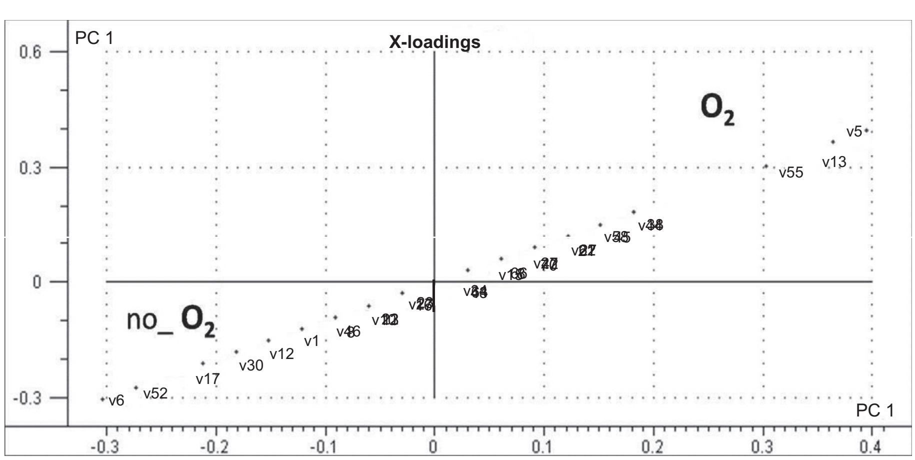


**Fig. 2.** Projection of olfactometric variables onto the principal component computed for oxygen factor. For identification of variables: see Table 3.

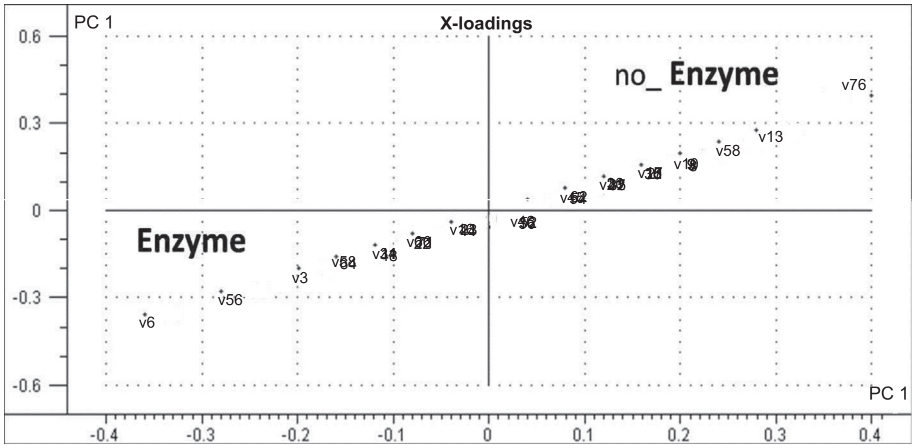


**Fig. 3.** Projection of olfactometric variables onto the principal component computed for enzyme factor. For identification of variables: see Table 3.

fusel alcohols (amylics and 2-phenylethanol), being major contributors to the aroma of cider from the quantitative point of view, or not quantifiable components, such as sotolon. In the second group, twenty-two odorants were included. From the qualitative point of view, almost all of these components have been described in Chinese and French ciders (Villière, Arvisenet, Lethuaut, Prost, & Serot, 2012; Xu, Fan, & Qian, 2007). Comparing with a previous report on Spanish ciders (Antón et al., 2014), different components have been found: diacetyl, acetoine, two sweet-like odorants (V55, V56), and one compound described as cooked vegetables (V9).

The influence of the application of microoxygenation and/or enzyme has been evaluated by a multivariate analysis of variance. As shown in Table 3, the presence of oxygen significantly increased the odour intensity of ethyl hexanoate and alcohols (3-methyl-2-butenol, *t*-3-hexenol), and decreased those of 4-EG, 1-octen-3-one, and *cis*-3-hexenol. On the contrary, the addition of the enzyme gave a significant decrease of the perception of 3-methyl-2-butenol and the odorant referred to as V76. No significant interactions were found between these technological factors excepting for butyl acetate, ethyl 2-methylbutyrate ($p < 0.001$), and isoeugenol ($p < 0.05$).

Figs. 2 and 3 show the projection of the olfactometric variables studied onto the principal component computed for oxygen and enzyme factors. As can be seen, ethyl 2-methylbutyrate (V5), 3-methyl-2-butenol (V13) and a sweet odorant (V55) were the most correlated with oxygen, while butyl acetate (V6), 4-EG (V52), *cis*-3-hexenol (V17), *iso*-butyric acid (V30), 1-octen-3-one (V12) and ethyl propionate (V1) were better correlated with the absence of oxygen (Fig. 2). On the other hand, one peak described as phenolic, stable (V76), 3-methyl-2-butenol (V13), and *m*-cresol (V58) were associated with the absence of enzyme, while butyl acetate (V6), diacetyl (V3), and another sweet odorant (V56) were linked to the enzyme treatments (Fig. 3).

The ASCA approach gave good correlations with several odorants for which no significant influence of the factors evaluated was observed by MANOVA analysis. This was the case of *iso*-butyric acid, ethyl propionate, diacetyl, *m*-cresol and the odorants referred to as V55 and V56. For other components, both statistical techniques were plenty coincident, for instance, 1-octen-3-one, 3-methyl-2-butenol, *cis*-3-hexenol and 4-ethylguaiacol. Moreover, the analysis of the olfactometric profiles by ASCA allowed to ascertain that both enzyme and microoxygenation significantly increased the perception of butyl acetate and ethyl 2-methylbutyrate respectively, even though a significant interaction was found (Table 3).

The quantitative and olfactometric data showed some differences for results of significant effects of treatments. In some cases, taking into account their corresponding detection thresholds (Escudero, Campo, Fariña, Cacho, & Ferreira, 2007; Ferreira, Petka, & Aznar, 2002), we should assume that the differences observed in the concentrations of one component could have not been enough to produce significant differences in its olfatometric perception. This explanation may be valid for amyl alcohols, or fatty acids. In other cases, the olfactometric signal can be saturated making it difficult to discriminate between olfactive stimuli; this situation may explain the lack of significance for methionol (Culleré, Escudero, Cacho, & Ferreira, 2004), 2-phenylethanol, 4-vinylguaiacol or ethyl esters of hexanoic and octanoic acids, as seen in Table 3.

## 4. Conclusions

The contact with lees brought about significant changes in the volatile composition of cider as evidenced the general increase in the contents of almost all of the minor volatile compounds. From the technological point of view, the addition of β-glucanase and/or microoxygenation may benefit the aromatic potential of cider, taking into account the effect of these factors on the perception of ethyl hexanoate, 4-EG, butyl acetate and ethyl 2-methylbutyrate. However, more research is needed to evaluate the convenience and consequences of the application of such treatments or their combinations in cider making.


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## References


Antón, M. J., Suárez Valles, B., García Hevia, A., & Picinelli Lobo, A. (2014). Aromatic profile of ciders by chemical quantitative, gas chromatography-olfactometry and sensory analysis. *Journal of Food Science, 79*, S92–S99.

Bautista, R., Fernández, E., & Falqué, E. (2007). Effect of the contact with fermentation-lees or commercial-lees on the volatile composition of white wines. *European Food Research & Technology, 224*, 405–413.

Beech, F. W., & Carr, J. G. (1977). Cider and Perry. In A. H. Rose (Ed.). *Economic microbiology* (Vol. 1, pp. 139–293). London: Academic Press.

Bueno, J. E., Peinado, R. A., Medina, M., & Moreno, J. (2006). Effect of a short contact time with lees on volatile composition of Airen and Macabeo wines. *Biotechnology Letters, 28*, 1007–1011.

Buron, N., Coton, M., Desmarais, C., Ledauphin, J., Guichard, H., & Barillier, D. (2011). Screening of representative cider yeast and bacteria for volatile phenol-production ability. *Food Microbiology, 28*, 1243–1251.

Buron, N., Guichard, H., Coton, E., Ledauphin, J., & Barillier, D. (2011). Evidence of 4-ethylcatechol as one of the main phenolic off-flavour markers in French ciders. *Food Chemistry, 125*, 542–548.

Chassagne, D., Guilloux-Benatier, M., Alexandre, H., & Voilley, A. (2005). Sorption of wine volatile phenols by yeast lees. *Food Chemistry, 91*, 39–44.

Culleré, L., Escudero, A., Cacho, J., & Ferreira, V. (2004). Gas chromatography-olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. *Journal of Agricultural and Food Chemistry, 52*, 1653–1660.

Escudero, A., Campo, E., Fariña, L., Cacho, J., & Ferreira, V. (2007). Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *Journal of Agricultural and Food Chemistry, 55*, 4501–4510.

Ferreira, V., Peťka, J., & Aznar, M. (2002). Aroma extract dilution analysis. Precision and optimal experimental design. *Journal of Agricultural and Food Chemistry, 50*, 1508–1514.

Fornairon-Bonnedond, C., Camarasa, C., Moutounet, M., & Salmon, J. M. (2002). New trends on yeast autolysis and wine ageing on lees: A bibliographic review. *Journal International des Sciences de la Vigne et du Vin, 36*, 49–69.

Francioli, S., Torrens, J., Riu-Aumatell, M., López-Tamames, E., & Buxaderas, S. (2003). Volatile compounds by SPME-GC as age markers of sparkling wines. *American Journal of Enology and Viticulture, 54*, 158–162.

Gallardo-Chacón, J. J., Vichi, S., López-Tamames, E., & Buxaderas, S. (2010). Changes in the sorption of diverse volatiles by *Saccharomyces cerevisiae* lees during sparkling wine ageing. *Journal of Agricultural and Food Chemistry, 58*, 12426–12430.

Gómez-Plaza, E., & Cano-López, M. (2011). A review on micro-oxygenation of red wines: Claims, benefits and the underlying chemistry. *Food Chemistry, 125*, 1131–1140.

Hernández-Orte, P., Lapeña, A. C., Escudero, A., Astrain, J., Baron, C., & Pardo, I. (2009). Effect of micro-oxygenation on the evolution of aromatic compounds in wines: Malolactic fermentation and ageing in wood. *LWT-Food Science and Technology, 42*, 391–401.

Jansen, J. J., Hoefsloot, H. C. J., van der Greef, J., Timmerman, M. E., Westerhuis, J. A., & Smilde, A. K. (2005). ASCA: Analysis of multivariate data obtained from an experimental design. *Journal of Chemometrics, 198*, 469–481.

Loscos, N., Hernández-Orte, P., Cacho, J., & Ferreira, V. (2009). Fate of grape flavour precursors during storage on yeast lees. *Journal of Agricultural and Food Chemistry, 57*, 5468–5479.

Masino, F., Montececchi, G., Arfelli, G., & Antonelli, A. (2008). Evaluation of the combined effects of enzymatic treatment and ageing on lees on the aroma of wine from *Bombino bianco* grapes. *Journal of Agricultural and Food Chemistry, 56*, 9495–9501.

Moreno-Arribas, M. V., & Polo, M. C. (2005). Winemaking biochemistry and microbiology: Current knowledge and future trends. *Critical Reviews in Food Science and Nutrition, 45*, 265–286.

Ortega-Heras, M., Rivero-Pérez, M. D., Pérez-Magariño, S., González-Huerta, C., & González-Sanjosé, M. L. (2008). Changes in the volatile composition of red wines during aging in oak barrels due to microoxygenation treatment applied before malolactic fermentation. *European Food Research & Technology, 226*, 1485–1493.

Pérez Serradilla, J. A., & Luque de Castro, M. D. (2008). Role of lees in wine production: A review. *Food Chemistry, 111*, 447–456.

Pozo-Bayón, M. A., G-Alegría, E., Polo, M. C., Tenorio, C., Martín-Álvarez, P. J., & Calvodela Banda, M. T. (2005). Wine volatile and amino acid composition alter malolactic fermentation: Effect of *Oenococcus oeni* and *Lactobacillus plantarum* starter cultures. *Journal of Agricultural and Food Chemistry, 53*, 8729–8735.

Pradelles, R., Alexandre, H., Ortiz-Julien, A., & Chassagne, D. (2008). Effects of yeast cell-wall characteristics on 4-ethylphenol sorption capacity on model wine. *Journal of Agricultural and Food Chemistry, 56*, 11854–11861.

Rocha, S. M., Coutinho, P., Delgadillo, I., Dias Cardoso, A., & Coimbra, M. A. (2005). Effect of enzymatic aroma release on the volatile compounds of white wines presenting different aroma potentials. *Journal of the Science of Food and Agriculture, 85*, 199–205.

Rodríguez Madrera, R., García Hevia, A., Palacios García, N., & Suárez Valles, B. (2008). Evolution of aroma compounds in sparkling ciders. *Lebensmittel-Wissenschaft und Technologie, 41*, 2064–2069.

Rodríguez-Bencomo, J. J., Ortega-Heras, M., & Pérez-Magariño, S. (2010). Effect of alternative techniques to ageing on lees and use of non-toasted oak chips in alcoholic fermentation on the aromatic composition of red wine. *European Food Research & Technology, 230*, 485–496.

Sánchez Palomo, E., Díaz-Maroto Hidalgo, M. C., González-Viñas, M. A., & Pérez-Coello, M. S. (2005). Aroma enhancement in wines from different grape varieties using exogenous glycosidases. *Food Chemistry, 92*, 627–635.

Suárez Valles, B., Pando Bedriñana, R., Fernández Tascón, N., González García, A., & Rodríguez Madrera, R. (2005). Analytical differentiation of cider inoculated with yeasts (*Saccharomyces cerevisiae*) isolated from Asturian (Spain) apple juices. *Lebensmittel-Wissenschaft und Technologie, 38*, 455–461.

Sumby, K. M., Grbin, P. R., & Jiranek, V. (2010). Microbial modulation of aromatic esters in wine: Current knowledge and future prospects. *Food Chemistry, 121*, 1–16.

Swiegers, J. H., Bartowsky, E. J., Henschke, P. A., & Pretorius, I. S. (2005). Yeast and bacterial modulation of wine aroma and flavor. *Australian Journal of Grape and Wine Research, 11*, 139–145.

Torrens, J., Riu-Aumatell, M., Vichi, S., López-Tamames, E., & Buxaderas, S. (2010). Assessment of volatile and sensory profiles between base and sparkling wines. *Journal of Agricultural and Food Chemistry, 58*, 2455–2461.

Torresi, S., Frangipane, M. T., & Anelli, G. (2011). Biotechnologies in sparkling wine production. Interesting approaches for quality improvement: A review. *Food Chemistry, 129*, 1232–1241.

Ugliano, M., & Moio, L. (2005). Changes in the concentration of yeast-derived volatile compounds of red wine during malolactic fermentation with four commercial starter cultures of *Oenococcus oeni*. *Journal of Agricultural and Food Chemistry, 53*, 10134–10139.

Vasserot, Y., Steinmetz, V., & Jeandet, P. (2003). Study of thiol consumption by yeast lees. *Antonie van Leeuwenthoek, 83*, 201–207.

Villière, A., Arvisenet, G., Lethuaut, L., Prost, C., & Serot, T. (2012). Selection of a representative extraction method for the analysis of odourant volatile composition of French cider by GC-MS-O and GC-TOF-MS. *Food Chemistry, 131*, 1561–1568.

Xu, Y., Fan, W., & Qian, M. C. (2007). Characterization of aroma compounds in apple cider using solvent-assisted flavour evaporation and headspace solid-phase microextraction. *Journal of Agricultural and Food Chemistry, 55*, 3051–3057.