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Production and characterization of aroma compounds from apple pomace by solid-state fermentation with selected yeasts

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ABSTRACT

Volatile aroma profiles generated during apple pomace fermentation were characterized. The apple pomace was fermented with 4 yeast strains, using three strains of indigenous cider yeasts (*Saccharomyces cerevisiae*, *Hanseniaspora valbyensis* and *Hanseniaspora uvarum*) and a commercial *S. cerevisiae* combined with an enzymatic preparation as inocula. In all cases, the initial sugar content decreased until depletion within 21 days or less, yielding similar ethanol contents at the end of the alcoholic fermentation. Chromatographic analysis of the apple pomaces allowed the identification of 132 volatile compounds belonging to different chemical families. Quantitatively, the production of aromas was strongly strain-dependent. The apple pomaces inoculated with *Saccharomyces* strains accumulated higher levels of fatty acids and their corresponding ethyl esters, increasing throughout the period of study (28 days). On the other hand, the volatile profiles from experimental units fermented with *Hanseniaspora* genus yeasts were characterized by higher levels of acetic acid esters with a maximum at 7–14 days of fermentation. A significant decrease in the concentration of unsaturated carbonyls was also detected between 21 and 28 days in apple pomaces fermented with *Hanseniaspora* strains.

1. Introduction

Apple production is a major source of fruit worldwide with an estimated global production in 2013/2014 of 68.3 million tons (USDA Foreign Agricultural Service, 2014, http://apps.fas.usda.gov/psdonline/circulars/fruit.pdf). A substantial part of this production, around 15%, is intended for the manufacture of mainly juice and ciders. The residue from this process, known as apple pomace, comprises 20–30% of the initial apple weight and is made up of skin, pulp and seeds.

Apple pomace is a poor animal feed due to its low protein content and the large amount of anti-nutritional compounds it contains such as pectin and tannins (Hang, Lee, Woodams, & Cooley, 1981; Zhong-Tao, Lin-Mao, Cheng, & Jin-Hua, 2009). Moreover, one of the main drawbacks of this material is that it is difficult to conserve as a result of spontaneous fermentation, which can lead to environmental problems and a high oxygen demand (250–300 g/kg) (Shalini & Gupta, 2010).

Nevertheless, apple pomace is an interesting resource of healthy molecules such as phenolic compounds (Diñeiro García, Suárez Valles, & Picinelli Lobo, 2009), pectin and fibre (Chen, Ruben Thaler, Lung, & Baranowski, 1988; May, 1990). In this respect, our group has previously established positive correlations between the phenolic content of apple pomace and the antiviral effect on herpes simplex type 1 and 2 (Alvarez et al., 2012; Suárez et al., 2010). Apple pomace has also been put forward as a raw material for cultivating microorganisms producing enzymes (Berovic & Ostroversnik, 1997; Hang & Woodams, 1995), ethanol (Hang et al., 1981) and aroma compounds (Bramorski, Soccol, Christen, & Revah, 1998, Christen, Bramorski, Revah, & Soccol, 2000).

Aroma compounds constitute one of the most important segments in the food additives sector, comprising around 25% of the overall market (Longo & Sanromán, 2006). Chemical synthesis presents certain drawbacks because it is not environmentally friendly and it is sometimes not selective, thus reducing process efficiency. These reasons, in addition to consumer preferences for products labelled as “natural” (Ahmed et al., 2013; Longo & Sanromán, 2006), have led to increased interest in processes based on the use of microorganisms and enzyme preparations to generate natural aroma compounds, especially solid state fermentation (SSF) versus submerged fermentation (SmF), due to the former being a simpler technique with lower costs (Rodríguez

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Couto & Sanromán, 2006). In this sense, it is worth highlighting that apple pomace is an adequate substrate to obtain flavours labelled as "natural", either directly or after being processed (FDA, 2014, http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=501.22.

On the other hand, agricultural wastes are particularly attractive substrates for the production of flavours, as they constitute an ideal culture medium for the microorganisms involved in the required biocatalytic conversions. In addition, agricultural wastes present important levels of precursor molecules, allowing the accumulation of desired flavours (Schrader, 2007).

Accordingly, several studies have focused on the production of flavours from different agricultural wastes and microorganisms under SSF (Christen et al. 2000; Medeiros, Pandey, Freitas, Christen, & Soccol, 2000; Rossi et al., 2009). Almonsino and Belin (1991), Almonsino, Benssousan, and Belin (1996) used the apple pomace enzyme system to produce hexanal and deca-2,4-dienal from polyunsaturated fatty acids. Bramorski et al. (1998) and Christen et al. (2000) studied combinations of apple pomace, cassava bagasse and soybean fermented by *Rhizopus* strains and *Ceratocystis fimbriata*, respectively, to produce a pleasant fruity aroma, although no relationship was established with the volatile compounds detected. In this regard, the previous papers on the volatile components in apple pomace are limited to the determination of a few volatile compounds, without further analytical characterization of these compounds.

On the other hand, our group has conducted several studies aimed at studying the indigenous microflora in cider making, enabling the selection of autochthonous yeast strains characterized by their good aptitudes to carry out alcoholic fermentation and interesting enzymatic activities (Pando Bedriñana, Lastra Queipo, & Suárez Valles, 2011; Suárez Valles, Pando Bedriñana, Fernández Tascón, Querol Simón, & Rodríguez Madrera, 2007; Suárez, Rodríguez, Picinelli, Moreno, & Mangas, 2000; Suárez Valles, Pando Bedriñana, Lastra Queipo, & Mangas Alonso, 2008).

The objective of the present paper is to start with the characterization of aroma compounds from fermented apple pomace obtained by SSF as a new way of adding value to this agricultural waste. For this purpose, samples of apple pomaces were fermented by autochthonous and commercial yeasts and their aromatic profiles were determined by gas chromatography-mass spectrometry subsequent to stir bar sorptive extraction.

2. Materials and methods

2.1. Apple pomace

The apple pomace used in this study came from 15,000 kg capacity industrial hydraulic presses from the same cider mill. Nine batches of 50 kg were taken, each of which was dried in an oven with air circulation at 60 °C for 48 h. The different batches of dry pomace were homogenised and divided into 6.8 kg portions, subsequently keeping them in sealed bags to preserve the moisture until fermentation.

Table 1
Cultivation conditions in the bioreactor.

	Inoculum	Agitation	Temperature	pH	Air flow	Time
S.c. 3′	2%	300 rpm	30 °C	5.5	3.5 ml/min	24 h
H.u. 283	4%	300 rpm	25 °C	5.5	3.5 ml/min	72 h
H. v. 43	4%	300 rpm	25 °C	5.5	3.5 ml/min	72 h

rpm: revolutions per minute.

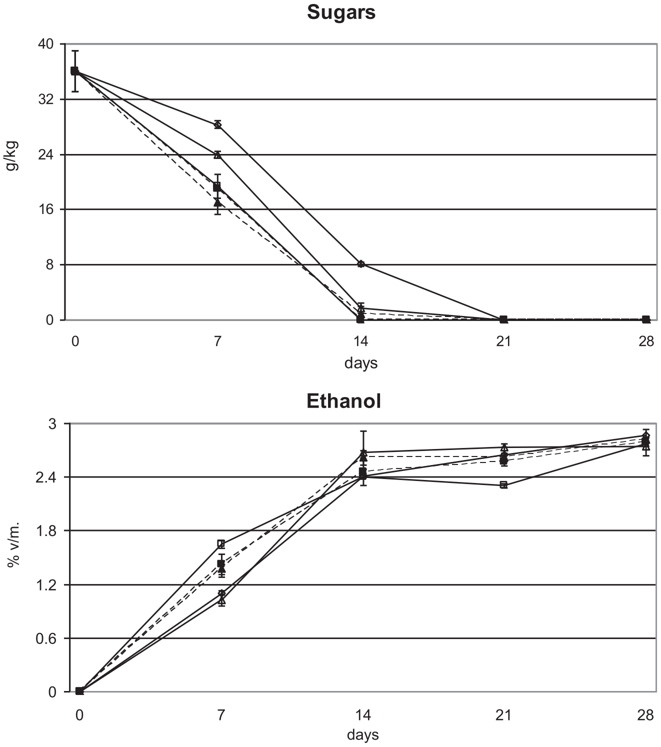

Fig. 1. Evolution of the content in sugars (sum of sucrose, glucose and fructose) and alcoholic strength during fermentation of apple pomaces. Open triangle: Levuline strain; open square: Levuline strain + enzyme; open diamond: *S.c.* 3′ strain; full triangle: *H.u.* 283 strain; full square: *H.v.* 43 strain. Solid line: *Saccharomyces* strain; dotted line: non-*Saccharomyces* strain. Mean of two experimental units and standard deviation.

2.2. Batch cultivation

Four yeast strains were used in this study. Three of them (*Saccharomyces cerevisiae*, *S.c.* 3′, *Hanseniaspora uvarum*, *H.u.* 283 and *Hanseniaspora valbyensis*, *H.v.* 43), characterized by their β-glucosidase activity form part of the SERIDA collection of pure cultures (Regional Service of Agrifood and Research and Development, Asturias, Spain) and a fourth was obtained from a commercial supplier (*S. cerevisiae*, Levuline CHP, OenoFrance, France).

Pure cultures of indigenous yeasts *S.c.* 3′, *H.u.* 283 and *H.v.* 43, stored at −80 °C, were used as inocula. The *S.c.* 3′ strain was inoculated for 24 h in liquid GPY medium (4% glucose, 0.5% peptone, 0.5% yeast extract) at 30 °C. An aliquot of this culture was added to 2% in 100 mL of liquid GPY medium and incubated for 16 h at 30 °C to obtain the inoculum used in the bioreactor. The *H.u.* 283 *and H.v.* 43 strains were inoculated on GPY agar and incubated at 30 °C for 48 h. Surface growth from one plate was transferred to 100 mL of liquid GPY medium and incubated for 72 h at 30 °C to obtain the

Table 2
Enzymatic activities detected in the apple pomace fermentations.

	β-Glu	PME	α-ARAF	β-Xyl
Levuline	−	−	−	+
Levuline + enzyme	+	+	+	+
S.c. 3′	+	−	−	−
H.v. 43	+	−	−	−
H.u. 283	+	−	−	−
Enzyme ENOVIN	+	+	+	−

β-Glu:β-glucosidase, PME: pectin methylesterase, α-ARAF: α-L-arabinofuranosidase; β-Xyl: β-xylosidase. +: activity. −: not activity.

Table 3
Volatile compounds detected in apple pomaces during fermentation.

	Linear retention index	Identification	Descriptor	Inoculation
Esters and lactones				
Isoamyl acetate	1077	a,b	Banana	1,2,3,4,5
Isobutyl 2-methylbutanoate	1130	a	Fruity	1,2,3,4,5
Ethyl hexanoate	1178	a,b	Apple peel fruit	0,1,2,3,4,5
Isoamyl butanoate	1193	a	Fruity, pear	1,2,3,4,5
Hexyl acetate	1212	a,b	Herbaceous	0,1,2,3,4,5
2-methylbutyl butanoate	1215	a	Fruity	1,2,3,4,5
Isoamyl 2-methylbutanoate	1222	a	Apple	1,2,3,4,5
2-methylbutyl 2-methylbutanoate	1226	a	Apple	1,2,3,4,5
Methyl octanoate	1327	a,b	Orange	0,1,2,3,4,5
Hexyl 2-methylbutanoate	1363	a	Strawberry	0,1,2,3,4,5
Ethyl octanoate	1377	a,b	Fruity fat	0,1,2,3,4,5
Isoamyl hexanoate	1403	a	Fruity	1,2,3,4,5
Methyl nonanoate	1433	a	Coconut	2
Ethyl nonanoate	1481	a	Mulberry	1,2,3,4,5
Isobutyl octanoate	1498	a	–	1,2,3
Decyl acetate	1507	a	Floral	1,2,3,4,5
Methyl decanoate	1535	a,b	Wine	1,2,3,4,5
Hexyl hexanoate	1551	a,b	Orange peel peach	0,1,2,3,4,5
γ -butyrolactone	1565	a,b	Caramel sweet	0,1,2,3,4,5
Ethyl decanoate	1574	a,b	Grape	0,1,2,3,4,5
Isoamyl octanoate	1588	a,b	Fruity	1,2,3,4,5
Diethyl succinate	1592	a,b	Wine fruity	1,2,3,4,5
Ethyl benzoate	1595	a,b	Floral camomile	0,1,2,3,4,5
Ethyl dec-9-enoate	1619	a	–	1,2,3,4,5
Phenylmethyl acetate	1657	a,b	Floral	0,1,2,3,4,5
Ethyl phenylacetate	1718	a	Fruity sweet	1,2,3,4,5
Methyl dodecanoate	1745	a	Coconut fat	1,2,3,4,5
Isobutyl benzoate	1747	a	Balsamic	1,2,3,4,5
2-phenylethyl acetate	1751	a,b	Roses honey	0,1,2,3,4,5
Ethyl dodecanoate	1781	a,b	Leaves	0,1,2,3,4,5
Isoamyl decanoate	1798	a	Brandy coconut	1,2,3,4,5
Butyl benzoate	1811	a	Balsamic	1,2,3,4,5
2-phenylethyl propanoate	1824	a	Fruity rose	1,2,3,4,5
Isoamyl ethyl succinate	1825	a	–	1,2,3,4,5
Isoamyl benzoate	1861	a	–	0,1,2,3,4,5
2-phenylethyl isobutanoate	1888	a	Fruity rose	1,2,3,4,5
2-phenylethyl butanoate	1897	a	Floral	0,1,2,3,4,5
Isoamyl phenylacetate	1921	a	Rose honey	1,2,3,4,5
Ethyl tetradecanoate	1957	a,b	Ether	1,2,3,4,5
γ-nonalactone	1967	a	Peach, coconut	0,1,2,3,4,5
Hexyl benzoate	2000	a	Woody balsamic	0,1,2,3,4,5
Ethyl cinnamate	2051	a,b	Cinnamon honey	0,1,2,3,4,5
γ-decalactone	2069	a,b	Peach	0,1,2,3,4,5
2-phenylethyl hexanoate	2091	a	Banana pineapple	1,2,3
Ethyl 4-ethoxybenzoate	2105	a	–	0,1,2,3,4,5
δ-decalactone	2116	a	Coconut	0,1,2,3,4,5
Ethyl hexadecanoate	2187	a,b	Fat	1,2,3,4,5
Methyl *trans*-dihydrojasmonate	2209	a	Floral	0,1,2,3,4,5
γ-dodecalactone	2292	a,b	Fruity sweet	0,1,2,3,4,5
2-phenylethyl octanoate	2303	a	–	1,2,3
δ-dodecalactone	2358	a	Fruit, sweet	0,1,2,3,4,5
Ethyl octadecanoate	2396	a	–	1,2,3,4,5
Ethyl oleate	2409	a	Mild waxy	1,2,3,4,5
Methyl linoleate	2416	a	–	2
Ethyl linoleate	2461	a,b	Fat	1,2,3,4,5
Methyl linolenate	2481	a	Fat	2
Ethyl linolenate	2518	a	Fat	1,2,3,4,5
Acids				
Acetic acid	1382	a,b	Vinegar	0,1,2,3,4,5
Propanoic acid	1459	a,b	Pungent rancid	0,1,2,3,4,5
Hexanoic acid	1762	a,b	Sweat	0,1,2,3,4,5
Octanoic acid	1981	a,b	Sweat cheese	0,1,2,3,4,5
Nonanoic acid	2063	a,b	Green fat	0,1,2,3,4,5
Decanoic acid	2165	a,b	Rancid fat	0,1,2,3,4,5
Benzoic acid	2331	a,b	Urine	0,1,2,3,4,5
Dodecanoic acid	2374	a,b	Fat	0,1,2,3,4,5
Aldehydes and Ketones				
Hexanal	1043	a,b	Grass	0,1,2,3,4,5
Heptanal	1139	a,b	Fat citrus	0,1,2,3,4,5
Hex-2-enal	1165	a,b	Green apple	0,1,2,3,4,5
Octan-3-one	1205	a	–	0,1,2,3,4,5
Octan-2-one	1222	a	–	0,1,2,3,4,5
Octanal	1231	a	Green fat	0,1,2,3,4,5

Table 3 (*continued*)

	Linear retention index	Identification	Descriptor	Inoculation
1-hydroxypropanone	1234	a,b	Cheese	0,1,2,3,4,5
Oct-1-en-3-one	1240	a,b	Mushroom	0,1,2,3,4,5
Nonan-5-one	1250	a	–	0,1,2,3,4,5
(E)-hept-2-enal	1264	a	Almond fruity	0,1,2,3,4,5
Nonan-2-one	1329	a,b	Green	0,1,2,3,4,5
Nonanal	1333	a,b	Green fat	0,1,2,3,4,5
Oct-2-enal	1368	a	Green walnut	0,1,2,3,4,5
Decan-2-one	1428	a	Orange	0,1,2,3,4,5
(E,E)-hepta-2,4-dienal	1433	a	Nuts fat	0,1,2,3,4,5
Decanal	1444	a	Orange peel tallow	0,1,2,3,4,5
Octa-3,5-dien-2-one	1465	a	Fruity mushroom	0,1,2,3,4,5
Benzaldehyde	1466	a,b	Almonds	0,1,2,3,4,5
Non-2-enal	1478	a	Cucumber fat	0,1,2,3,4,5
(E,E)-octa-2,4-dienal	1525	a	Cucumber	0,1,2,3,4,5
Undecan-2-one	1538	a,b	Orange green	0,1,2,3,4,5
(Z)-dec-2-enal	1578	a,b	Tallow	0,1,2,3,4,5
Acetophenone	1582	a,b	Floral almonds	0,1,2,3,4,5
(E,E)-nona-2,4-dienal	1633	a	Green fat	0,1,2,3,4,5
2-undecenal	1692	a	Sweet pungent	0,1,2,3,4,5
(E,Z)-deca-2,4-dienal	1703	a	Fried	0,1,2,3,4,5
(E,E)-deca-2,4-dienal	1747	a	Fried	0,1,2,3,4,5
dodec-2-enal	1805	a	–	0,1,2,3,4,5
Terpenoids				
β-ocimene	1191	a	Herbaceous	0,1,2,3,4,5
6-methylhept-5-en-2-one	1272	a,b	Green fat	0,1,2,3,4,5
2,6-dimethylhept-5-enal	1291	a	Melon green	0,1,2,3,4,5
3,7-dimethyloct-3-enal	1352	a	Fat	0,1,2,3,4,5
6-methylhept-5-en-2-ol	1400	a,b	–	4,5
Camphor	1477	a	Camphor	0,1,2,3,4,5
6-methylhepta-3,5-dien-2-one	1528	a	Cinnamon coconut	0,1,2,3,4,5
Isobornyl acetate	1529	a	Woody	0,1,2,3,4,5
β-cyclocitral	1560	a	Mint	0,1,2,3,4,5
2,6-dimethylhept-5-en-1-ol	1585	a	–	1,2,3,4,5
β-farnesene	1604	a	Citric sweet wood	0,1,2,3,4,5
β-citral isomer 1	1610	a	Lemon	0,1,2,3,4,5
β-citral isomer 2	1665	a	Lemon	0,1,2,3,4,5
α-farnesene	1696	a	Wood sweet	0,1,2,3,4,5
β-damascenone	1757	a,b	Apple roses honey	0,1,2,3,4,5
Geraniol	1781	a	Geranium	0,1,2,3,4,5
Nerylacetone	1790	a	Floral	0,1,2,3,4,5
Nerolidol isomer 1	1829	a	Wood floral	0,1,2,3,4,5
6,10-dimethylundeca-5,9-dien-2-ol	1869	a	Fruity sweet	0,1,2,3,4,5
β-ionone	1873	a	Violet raspberry	0,1,2,3,4,5
Nerolidol isomer 2	1949	a	Wood floral	0,1,2,3,4,5
Pseudoionone isomer 1	1967	a	–	0,1,2,3,4,5
Spathulenol	2042	a	Herbaceous, fruity	0,1,2,3,4,5
Pseudoionone isomer 2	2054	a	–	0,1,2,3,4,5
Farnesol isomer 1	2213	a,b	Floral	0,1,2,3,4,5
Farnesol isomer 2	2230	a,b	Floral	0,1,2,3,4,5
Farnesol isomer 3	2255	a,b	Floral	0,1,2,3,4,5
Alcohols				
Amyl alcohols	1145	a,b	Malt burnt	1,2,3,4,5
Hexan-1-ol	1276	a,b	Herbaceous	0,1,2,3,4,5
Oct-1-en-3-ol	1372	a,b	Mushroom	0,1,2,3,4,5
Octan-1-ol	1482	a,b	Oily fatty	0,1,2,3,4,5
Z-oct-2-en-1-ol	1516	a,b	Green	0,1,2,3,4,5
E-oct-3-en-1-ol	1546	a,b	–	0,1,2,3,4,5
Furfuryl alcohol	1586	a,b	Burnt	0,1,2,3,4,5
Decan-1-ol	1683	a,b	Fat	0,1,2,3,4,5
Dec-4-en-1-ol	1711	a	Floral	1,2,3
Phenylmethanol	1806	a,b	Floral	1,2,3,4,5
2-phenylethanol	1834	a,b	Rose floral	0,1,2,3,4,5
Eugenol	2099	a,b	Clove, spicy	1,2,3,4,5

a: identification by linear retention indices and/or mass spectrum (quality>85).

b: confirmed with pure standards. 0: unfermented pomace. 1: Levuline. 2: Levuline with enzyme. 3: *Saccharomyces cerevisiae* 3′. 4: *Hanseniaspora valvyensis* 43. 5: *Hanseniaspora uvarum* 283.

inoculum used in the bioreactor.

Biomass was obtained from a laboratory bioreactor (Biostat B Bonus, Sartorius) with a working volume of 2 L. Sterile apple juice (4 °Brix) and silicone antifoam (0.1%) were used for batch fermentation. The cultivation conditions in the bioreactor for autochthonous yeasts are shown in Table 1. Two batch tests were conducted for each strain. Yeast creams (3.5 g *S.c.* 3′ and 4.0 g *Hanseniaspora* strains) to 10^9 cfu/ml were stored, for less than

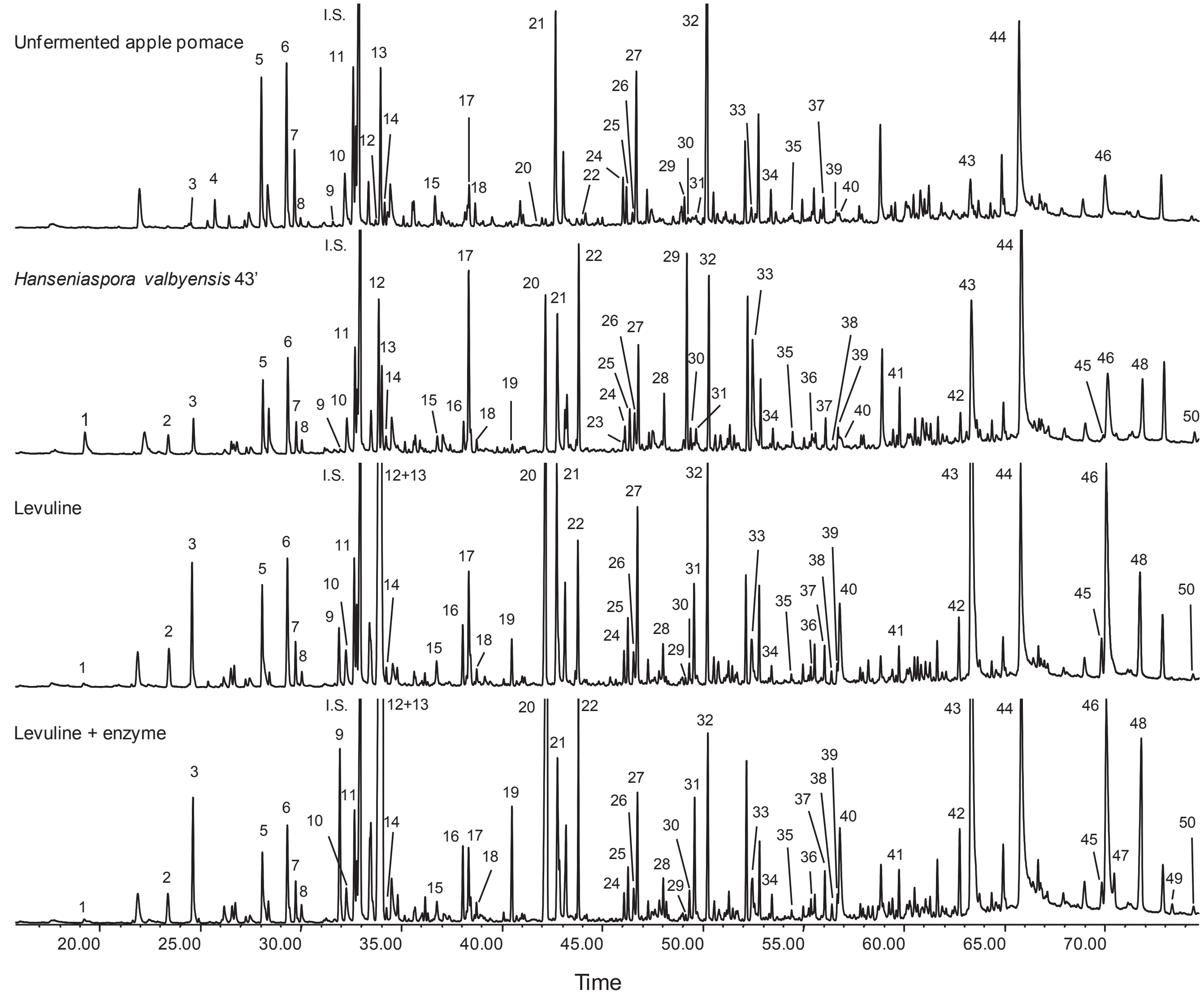

Fig. 2. Chromatograms of unfermented and fermented apple pomaces. 1: isoamyl acetate; 2: amyl alcohols; 3: ethyl hexanoate; 4: octan-3-one; 5: oct-1-en-3-one; 6: hept-2-enal; 7: 6-methylhepten-5-one; 8: hexan-1-ol; 9: methyl octanoate; 10: nonanal; 11: citronellal; 12: ethyl octanoate; 13: oct-2-enal; 14: oct-1-en-3-ol; 15: decanal; 16: ethyl nonanoate; 17: benzaldehyde; 18: octan-1-ol; 19: methyl decanoate; 20: ethyl decanoate; 21: decen-2-al; 22: ethyl benzoate; 23: phenylmethyl acetate; 24: β-citral isomer 2; 25: farnesene; 26: decan-1-ol; 27: 2-undecenal; 28: ethyl phenylacetate; 29: 2-phenylethyl acetate; 30: β-damascenone; 31: ethyl dodecanoate; 32: nerylacetone; 33: 2-phenylethanol; 34: β-ionone; 35: 2-phenylethyl butanoate; 36: isoamyl phenylacetate; 37: nerolidol isomer 2; 38: ethyl tetradecanoate; 39: γ-nonalactone; 40: octanoic acid; 41: ethyl cinnamate; 42: ethyl hexadecanoate; 43: decanoic acid; 44: farnesol isomer 3; 45: ethyl oleate; 46: dodecanoic acid; 47: methyl linoleate; 48: ethyl linoleate; 49: methyl linolenate; 50: ethyl linolenate.

1 month in sterile jars at 4 °C, and used in each fermentation tank.

The active dry yeast strain (Levuline CHP) was inoculated to 80 g/hL after rehydration in sterile water for 20 min, following the supplier's instructions.

2.3. Fermentation

Four fermentations were performed inoculating pure cultures (*S.c.* 3′, *H.u.* 283, *H.v.* 43 and Levuline CHP), while a fifth was performed with Levuline in combination with a β-glucosidase enzyme (10 g/hL, Enovin Varietal, Agrovin, Ciudad Real, Spain).

Each lot of 6.8 kg of dry pomace was rehydrated in 20 L of deionised water, adding the corresponding inoculum to this water. Fermentations took place in 30 L capacity high density polyethylene (HDPE) tanks equipped with an air-lock at 16 ± 2 °C for 4 weeks. Inoculations were carried out in duplicate, resulting in 10 experimental units.

2.4. Analysis of apple pomace

2.4.1. Microbiological counting

samples (10 g) were taken from each tank at the beginning and after 14 days of fermentation. These samples were then homogenized with 90 mL of a Ringer serum for 2 min in a Masticator 0410 (IUL Instrument). Quantitative determinations of yeasts, lactic and acetic bacteria were performed following the methodology described by Cabranes et al. (Cabranes, Blanco, & Mangas, 1997).

2.4.2. Implantation capacity

the implantation of the strains in the fermentations was evaluated after 14 days of inoculation by analysis of 10 isolated colonies. The isolates were analysed by mtDNA-RFLP (Querol, Barrio, & Ramón, 1992) for the tanks inoculated with *Saccharomyces* strains and by RAPD (Manzanares, Ramón, & Querol, 1999) for the experimental units inoculated with *Hanseniaspora* strains.

Table 4
Changes in esters and γ-nonalactone concentrations during apple pomace fermentation expressed as μg/kg 2-octanol (mean of two experimental units ± standard deviation).

Compound	Unfermented pomace	Fermentation time (days)	Levuline	Levuline + enzyme	S. c. 3′	H. u. 283	H. v. 43
ethyl hexanoate	2.5 ± 0.6	7	72.7 ± 2.7	44.5 ± 6.5	47.4 ± 2.4	16.5 ± 4.9	16.4 ± 1.4
		14	64.5 ± 10.6	59.1 ± 4.6	118.8 ± 19.8	25.4 ± 0.6	25.1 ± 1.9
		21	93.1 ± 0.6	74.4 ± 0.4	140.7 ± 40.4	36.5 ± 2.5	30.2 ± 2.8
		28	81.4 ± 14.3	84.4 ± 14.1	134.8 ± 10.8	39.2 ± 3.1	23.9 ± 4.8
ethy octanoate	7.3 ± 2.4	7	331.2 ± 17.8	935.0 ± 107.2	627.6 ± 30.9	47.1 ± 19.9	76.0 ± 22.8
		14	1010.4 ± 134.0	1348.7 ± 112.1	1374.1 ± 215.8	118 ± 1.1	127.8 ± 1.6
		21	1183.7 ± 13.4	1223.7 ± 134.6	1811.4 ± 698.1	183 ± 41.3	141.2 ± 11.6
		28	1116.5 ± 179.1	1644.1 ± 36.2	1716.8 ± 145.7	151.3 ± 17.9	106.3 ± 18.1
ethyl decanoate	2.2 ± 1.3	7	109.5 ± 4.1	315.1 ± 46.0	122.1 ± 2.1	8.5 ± 3.7	36.3 ± 2.8
		14	302.4 ± 23.9	507.4 ± 32.6	395.1 ± 36.2	23.4 ± 2.9	80.6 ± 6.5
		21	399.0 ± 17.1	466.4 ± 41.6	601.2 ± 226.9	49.4 ± 11.5	88.9 ± 11.8
		28	455.4 ± 48.3	660.8 ± 43.7	640.9 ± 97.2	44.0 ± 2.2	88.4 ± 20.3
ethyl dodecanoate	1.5 ± 0.3	7	9.4 ± 7.0	57.9 ± 6.6	11.5 ± 1.3	6.1 ± 2.3	3.8 ± 0.0
		14	32.7 ± 1.7	77.6 ± 5.4	36.3 ± 1.8	15.2 ± 2.6	8.3 ± 0.1
		21	42.7 ± 2.2	62.8 ± 8.7	42.7 ± 13.9	27.9 ± 6.0	11.1 ± 1.8
		28	51.5 ± 2.2	86.4 ± 8.2	59.5 ± 13.1	27.1 ± 2.0	10.4 ± 0.8
ethyl tetradecanoate	n.d	7	7.1 ± 10.0	6.1 ± 1.6	0.6 ± 0.1	0.8 ± 0.4	0.9 ± 0.0
		14	2.7 ± 0.1	8.2 ± 0.4	1.7 ± 0.2	1.7 ± 0.4	2.2 ± 0.2
		21	3.5 ± 0.1	6.6 ± 1.0	2.3 ± 0.0	2.3 ± 0.0	2.7 ± 0.1
		28	4.5 ± 0.5	9.7 ± 1.5	3.3 ± 1.0	2.2 ± 0.0	2.0 ± 0.2
ethyl cinnamate	2.3 ± 0.6	7	5.2 ± 0.2	14.9 ± 2.0	6.9 ± 0.2	10.5 ± 5.5	13.9 ± 1.9
		14	17.8 ± 2.2	22.2 ± 2.5	18.3 ± 4.0	22.8 ± 3.5	36.2 ± 1.7
		21	29.1 ± 0.0	27.6 ± 3.8	32.5 ± 8.2	36.9 ± 10.2	67 ± 4.7
		28	29.4 ± 5.8	41.5 ± 1.3	33.5 ± 1.0	43.1 ± 2.5	50.2 ± 4.5
ethyl phenylacetate	3.4 ± 0.2	7	30.6 ± 1.3	23.2 ± 3.4	24.2 ± 0.9	30.4 ± 6.7	28.6 ± 1.7
		14	37.7 ± 2.4	31.5 ± 1.6	43.4 ± 4.4	53.1 ± 4.6	62.1 ± 4.0
		21	59.1 ± 0.2	44.6 ± 1.6	69.4 ± 11.8	82.9 ± 5.5	87.2 ± 7.4
		28	60.4 ± 10.8	63.9 ± 4.5	80.6 ± 3.4	97.1 ± 12.9	80.3 ± 10.6
methyl octanoate	0.6 ± 0.3	7	6.6 ± 0.0	147 ± 15.8	25 ± 1.1	1.3 ± 0.4	2.1 ± 0.8
		14	49 ± 8.4	161.2 ± 18.1	41.6 ± 6.1	2.8 ± 0.3	2.8 ± 0.3
		21	70.9 ± 0.9	162.2 ± 12.8	56.9 ± 23.1	4.6 ± 1.3	3.2 ± 0.2
		28	71 ± 13.7	212.8 ± 9.7	54.1 ± 2.9	4.3 ± 0.3	2.8 ± 0.8
methyl decanoate	n.d	7	2.6 ± 0.0	52 ± 8.4	5.6 ± 0.2	0.0 ± 0.0	1.1 ± 0.2
		14	19.6 ± 2.3	71.8 ± 7.1	14.2 ± 1.1	0.8 ± 0.0	2.3 ± 0
		21	29.3 ± 0.7	69.6 ± 6.5	22.9 ± 8	1.7 ± 0.4	2.5 ± 0.4
		28	34.8 ± 4.9	109.2 ± 5.9	24 ± 3.2	1.6 ± 0.1	2.9 ± 0.9
methyl dodecanoate	n.d	7	0.9 ± 0.1	12.7 ± 1.5	0.9 ± 0	0.0 ± 0.0	0.0 ± 0.0
		14	3 ± 0.3	13.4 ± 0.8	2.1 ± 0	0.7 ± 0	0.4 ± 0.5
		21	4.2 ± 0.2	11.3 ± 1.2	2.1 ± 0.5	1.2 ± 0.3	0.5 ± 0.0
		28	5.4 ± 0.4	16.2 ± 1.6	2.7 ± 0.5	1.2 ± 0.1	0.6 ± 0.2
2-phenylethyl acetate	25.8 ± 1.3	7	10.6 ± 4.2	8.8 ± 0.2	11.7 ± 0.4	325.3 ± 2	4153.1 ± 289.6
		14	14.1 ± 4.6	14 ± 3.7	25.2 ± 8.8	434.1 ± 121.1	3870.9 ± 17.9
		21	11.6 ± 0.6	7.2 ± 0.2	65.2 ± 19.9	67.1 ± 1.3	1423.8 ± 54.1
		28	13.4 ± 8	7.8 ± 0.2	17.2 ± 2.6	29 ± 2.2	301.7 ± 56.5
2-phenylmethyl acetate	1.5 ± 1.1	7	0.8 ± 0.1	0.9 ± 0.1	1.2 ± 0.1	82.2 ± 13.5	1.1 ± 0.1
		14	0.7 ± 0.1	0.7 ± 0.1	1.3 ± 0.1	62.8 ± 3.1	1.8 ± 0.3
		21	0.7 ± 0.1	0.8 ± 0.2	1.9 ± 0.2	19.9 ± 0.1	1.5 ± 0.3
		28	0.8 ± 0.1	0.9 ± 0.1	2.2 ± 0.4	7.6 ± 0.1	1.5 ± 0.1
isoamyl acetate	n.d	7	5.2 ± 3.1	3.6 ± 0.2	4.5 ± 0.0	11.8 ± 5.3	75.1 ± 1.6
		14	6.5 ± 2.3	5.3 ± 0.2	5.4 ± 1.1	17.2 ± 1.1	79 ± 5.7
		21	5.6 ± 0.3	4.8 ± 0.6	5.7 ± 1.0	18.2 ± 3.1	65.8 ± 6.4
		28	4.4 ± 0.1	5.9 ± 0.4	6.5 ± 0.2	20.1 ± 0.2	34.6 ± 4.0
Isoamyl 2-methylbutanoate	n.d	7	4.9 ± 0.3	6.1 ± 1.2	3.3 ± 0.1	3.2 ± 1.4	2.9 ± 0.5
		14	9.9 ± 0.9	7.8 ± 0.8	6.5 ± 1.0	5.9 ± 0.5	7.5 ± 0.5
		21	13.8 ± 0.3	9.6 ± 1.9	11.6 ± 3.0	10.3 ± 1.7	11.2 ± 1.3
		28	15.2 ± 3.1	16.6 ± 0.1	15.9 ± 1.1	11.8 ± 2.3	10.5 ± 0.9
isoamyl hexanoate	n.d	7	5.5 ± 0.5	8.9 ± 0.7	6.5 ± 0.15	1.9 ± 0.7	1.71 ± 0.2
		14	7.5 ± 0.6	12.0 ± 0.8	18.7 ± 3.0	3.1 ± 0.1	2.4 ± 0.1
		21	9.7 ± 0.3	12.2 ± 1.6	25.4 ± 7.2	4.0 ± 0.6	2.8 ± 0.2
		28	10.6 ± 1.5	19.4 ± 0.6	32.7 ± 3.4	3.7 ± 0.4	1.9 ± 0.1
isoamyl octanoate	n.d	7	5.5 ± 0.1	23.6 ± 0.1	18.4 ± 2.0	1.7 ± 0.5	2.0 ± 0.0
		14	16.4 ± 0.5	32.8 ± 1.2	45.1 ± 4.1	3.0 ± 0.5	2.5 ± 0.2
		21	16.9 ± 1.3	27.7 ± 1.6	45.4 ± 17	3.0 ± 0.5	2.6 ± 0.4
		28	18.7 ± 1.3	38.4 ± 3.1	61.2 ± 13	2.6 ± 0.1	1.7 ± 0.0
isoamyl phenylacetate	n.d	7	6.5 ± 0.0	6.9 ± 1.2	14.9 ± 0.2	9.4 ± 2.9	6.0 ± 0.5
		14	11.3 ± 0.9	9.7 ± 0.9	25.7 ± 3.5	13.8 ± 0.8	11.8 ± 2.2
		21	13.6 ± 0.8	10.9 ± 1.6	33.4 ± 10.4	18.7 ± 1.8	17.1 ± 1.7
		28	13.7 ± 2.2	18 ± 0.0	38.5 ± 2.5	17.7 ± 2.7	11.3 ± 0.2
γ-nonalactone	10.6 ± 1.7	7	17.7 ± 0.2	25.8 ± 2.5	23.5 ± 0.8	22.5 ± 0.2	18.8 ± 1.2
		14	25.3 ± 1.6	27.9 ± 1.3	28.9 ± 2.9	32.5 ± 3.5	26.6 ± 0.7
		21	29.3 ± 0.4	29.8 ± 2.8	36.9 ± 3.3	37.1 ± 2.9	32.9 ± 0.5
		28	27.2 ± 4.2	36.0 ± 0.0	35.0 ± 0.4	28.9 ± 0.5	32.4 ± 2.6

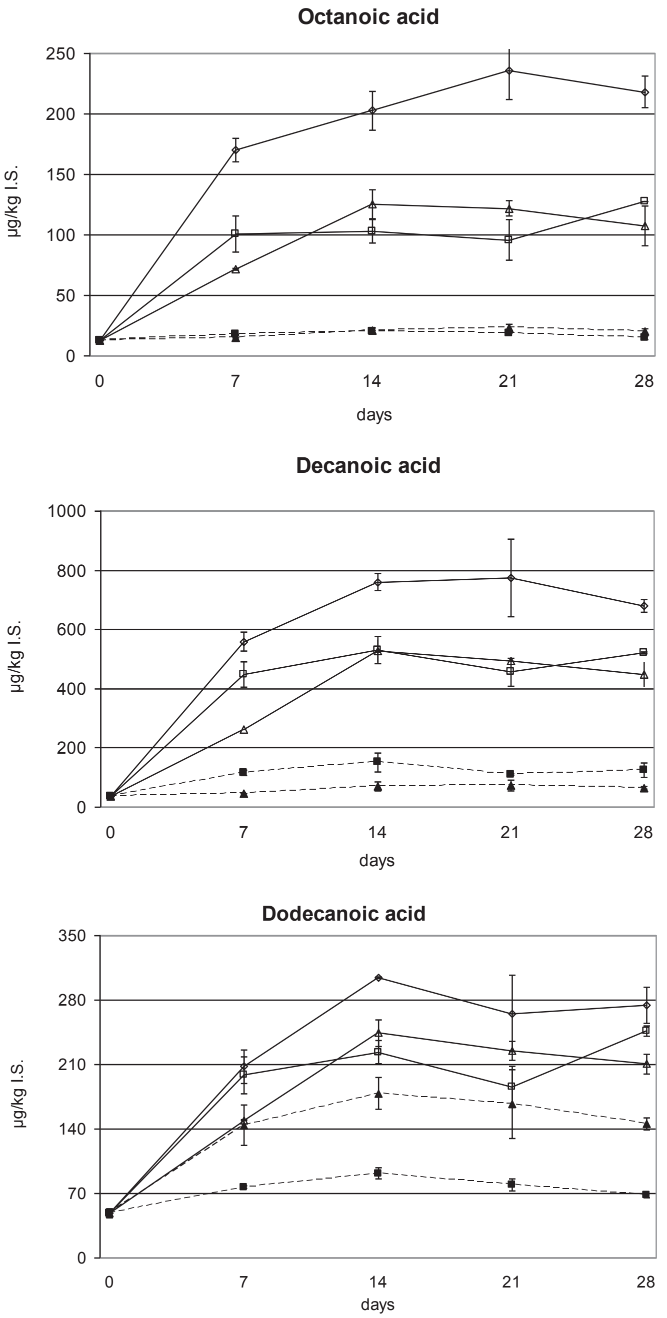

Fig. 3. Evolution of the content in fatty acids during fermentation of apple pomaces. Open triangle: Levuline strain; open square: Levuline strain + enzyme; open diamond: *S.c.* 3′ strain; full triangle: *H.u.* 283 strain; full square: *H.v.* 43 strain. Solid line: *Saccharomyces* strain; dotted line: non-*Saccharomyces* strain. Mean of two experimental units and standard deviation.

2.4.3. Enzymatic assays

Samples (50 g) were taken from each tank after 14 days of fermentation and homogenized in a Masticator 0410 (IUL Instrument) for 2 min. Aliquots of the extracts and the pure enzyme were used to evaluate the different enzymatic activities according to the following methods:

β-glucosidase activity was evaluated in agar slant tubes using arbutin as substrate. The extracts were inoculated in the basal medium following the methodology described by Pando Bedriñana et al. (21).

Pectin methylesterase activity was evaluated in liquid medium containing 6.7 g/L yeast nitrogen base (Difco), 1% glucose, 1% pectin from apples (Fluka) and 50 mM potassium phosphate buffer pH = 5.5. The medium was inoculated to 2% with extracts and incubated at 30 °C for 5 days. The methanol produced was measured by gas chromatography.

β-xylosidase activity was carried out on agar plates containing 1.7 g/L yeast nitrogen base without amino acids and ammonium sulphate (Difco), 5 g/L ammonium sulphate, 5 g/L D-xylose and 20 g/L agar (pH = 5.5) (Manzanares et al., 1999).

α-L-arabinofuranosidase activity was assayed according to the method described by Nurcholis et al. (2012), based on the hydrolysis of *p*-nitrophenyl-α-L-arabinofuranoside (pNP-A, Aldrich, USA).

Sugar content and alcoholic strength were determined at 0, 7, 14, 21 and 28 days of fermentation by HPLC analysis (Blanco, Gutiérrez, Mangas, & Noval, 1988) and NIR (Alcoholyzer Wine, Anton Paar, GmgH, Graz, Austria), respectively, subsequent to extraction following Pina and Hogg (1999).

Volatile compounds in apple pomace were determined at 0, 7, 14, 21 and 28 days of fermentation according to the method previously validated by our group (Rodríguez Madrera & Suárez Valles, 2011). Each sample (4.5 g) was transferred to a 50 mL vial containing 50 mg ascorbic acid and 2.8 ng octan-2-ol (internal standard) and 30 mL water. The mixture was homogenized in a Polytron PT 10–35 (Kinematica AG, Littau, Switzerland) for 2 min. A polydimethylsiloxane-coated stir bar (0.5 mm film thickness, 20 mm length, Twister, Gerstel GmbH & Co, Mülheim an der Ruhr, Germany) was then added to the mixture and extraction was performed at room temperature, stirring at 700 rpm for 3 h.

After the extraction, the stir bars were rinsed with distilled water, dried with a cellulose tissue and then placed in desorption tubes.

The desorption tubes were placed in a thermal desorption unit (TDU, Gerstel GmbH & Co, Mülheim an der Ruhr, Germany) and the stir bars were desorbed from 25 °C (1 min) to 295 °C (10 min) at a rate of 60 °C/min in solvent vent mode (helium flow: 50 mL/min). The desorbed aromas were cryofocussed in a CIS-4 Programmable Temperature Vaporizing (PTV) inlet (Gerstel GmbH & Co, Mülheim an der Ruhr, Germany) at −40 °C using liquid nitrogen, the inlet liner being packed with quartz wool. Analytes were transferred into the chromatographic column in splitless mode, raising the temperature from −40 °C to 320 °C at a rate of 10 °C/s. The gas chromatograph was an Agilent 7890A system equipped with an MSD 5975C (Palo Alto, California). The column employed was an FFAP (50 m × 0.2 mm i.d., 0.25 µm, Agilent Technologies). The oven temperature program was as follows: 40 °C (5 min) rising to 220 °C (25 min) at a rate of 3 °C/min. Helium was used as carrier gas at a flow rate of 1 mL/min.

Identification was carried out by comparing spectra with those registered in a Wiley 138 K mass spectral library and linear retention indices and confirmed by pure standards, whenever available. Results were expressed as µg internal standard (octan-2-ol) per kg pomace. All analyses were carried out in triplicate.

2.5. Statistical methods

Significant differences in the levels of the quantitated analytes were detected by means of a two-way ANOVA (yeast strain and fermentation time), with repeated measures for fermentation time. Duncan's test was carried out to detect significant differences for volatile compounds depending on the inoculated yeast strain. The program used was SPSS version 11.5.

Table 5
Changes in aldehyde and ketone concentrations during apple pomace fermentation expressed as µg/kg 2-octanol (mean of two experimental units ± standard deviation).

Compound	Unfermented pomace	Fermentation time (days)	Levuline	Levuline + enzyme	S. c. 3′	H. u. 283	H. v. 43
(E)-hept-2-enal	53.4 ± 3.6	7	50.4 ± 3.6	41.9 ± 3.1	41.8 ± 2.5	49.5 ± 0.7	56.3 ± 2.3
		14	36.9 ± 1.4	44.6 ± 3.0	40.9 ± 2.2	53.7 ± 2.2	54.5 ± 3.1
		21	49.1 ± 5.7	44.4 ± 0.0	46.2 ± 1.0	50.2 ± 6.7	58 ± 7.5
		28	50.3 ± 0.8	45.6 ± 1.4	52.5 ± 3.2	27.6 ± 4.5	44.2 ± 2.5
(E)-oct-2-enal	26.7 ± 2.8	7	29.0 ± 2.9	72.6 ± 6.6	50.5 ± 4.9	22.5 ± 0.2	31.9 ± 0.6
		14	74.2 ± 7.2	95.0 ± 8.3	97.0 ± 13.4	25.1 ± 2.4	32.5 ± 4.3
		21	90.4 ± 6.2	88.6 ± 8.6	109.6 ± 12.8	26.1 ± 8.6	34.7 ± 6.0
		28	69.6 ± 17.6	89.3 ± 39.4	113.8 ± 6.4	9.4 ± 1.6	23.4 ± 0.9
(E)-dec-2-enal	33.0 ± 3.1	7	33.0 ± 1.4	27.5 ± 4.0	28.4 ± 1.8	35.7 ± 0.1	45.3 ± 7.9
		14	27.7 ± 1.2	32.3 ± 3.0	33.3 ± 0.5	43.7 ± 0.2	48.7 ± 0.2
		21	37.8 ± 9.9	33.9 ± 3.2	45.5 ± 1.7	44.9 ± 8.1	49.7 ± 5.6
		28	41.1 ± 1.7	42.6 ± 0.2	49 ± 2.1	19.6 ± 4.0	36.2 ± 1.6
(E)-undec-2-enal	25.6 ± 2.0	7	27.9 ± 0.1	17.4 ± 0.7	18.4 ± 1.5	25.2 ± 1.2	29.4 ± 6.0
		14	17.0 ± 0.7	20.8 ± 1.7	18.7 ± 0.7	27.2 ± 1.2	24.5 ± 6.9
		21	22.9 ± 6.3	17.0 ± 0.9	23.9 ± 4.7	30.0 ± 7.1	28.7 ± 6.5
		28	29.5 ± 1.7	27.3 ± 1.2	34.5 ± 2.6	16.2 ± 0.1	22.2 ± 3.7
(Z)-undec-2-enal	14.0 ± 0.6	7	13.8 ± 1.0	10.5 ± 1.6	11.6 ± 0.1	11.7 ± 1.4	14.0 ± 1.3
		14	9.0 ± 0.1	8.2 ± 0.3	12.3 ± 0.7	11.3 ± 0.5	14.7 ± 3.5
		21	10.8 ± 3.2	7.3 ± 1.3	11.7 ± 0.8	11.8 ± 2.4	15.1 ± 1.9
		28	10.8 ± 0.5	11.6 ± 0.7	12.9 ± 0.8	8.6 ± 0.0	9.1 ± 0.7
(E,E)-hepta-2,4-dienal	16.0 ± 0.2	7	16.0 ± 0.5	9.9 ± 1.1	10.4 ± 1.5	11.3 ± 0.5	13.3 ± 0.6
		14	8.7 ± 0.1	10 ± 1.4	10.6 ± 0.4	11.8 ± 0.1	13.8 ± 1.1
		21	12.1 ± 1.9	9.5 ± 0.4	13.5 ± 0.0	11.8 ± 2.6	15.5 ± 3.1
		28	11.8 ± 1.2	11.4 ± 0.0	13.3 ± 0.2	8.6 ± 0.7	9.6 ± 0.1
(E,E)-nona-2,4-dienal	3.7 ± 0.2	7	2.8 ± 0.1	0.9 ± 0.0	1.2 ± 0.5	1.3 ± 0.0	2.4 ± 0.3
		14	1.2 ± 0.1	1.0 ± 0.0	1.1 ± 0.1	1.5 ± 0.0	2.1 ± 0.0
		21	1.5 ± 0.4	1.0 ± 0.0	1.7 ± 0.2	1.3 ± 0.4	1.8 ± 0.4
		28	1.3 ± 0.1	1.4 ± 0.0	1.7 ± 0.0	0.8 ± 0.1	1.3 ± 0.0
(E,E)-deca-2,4-dienal	21.0 ± 0.4	7	17.2 ± 0.6	10.7 ± 0.6	12.6 ± 3.1	15.6 ± 0.7	19.9 ± 4.3
		14	10.6 ± 0.2	11.7 ± 1.2	11.4 ± 0.3	18.7 ± 0.3	16.8 ± 4.2
		21	15.3 ± 4.6	10.4 ± 0.5	16.6 ± 2.8	17.5 ± 4.0	19.6 ± 6.4
		28	16.2 ± 0.4	14.9 ± 0.4	19.6 ± 0.9	10.0 ± 0.8	13.9 ± 0.8
(E,Z)-deca-2,4-dienal	10.1 ± 0.1	7	8.0 ± 0.6	4.6 ± 1.1	5.2 ± 0.8	8.8 ± 1.5	10.2 ± 1.3
		14	3.9 ± 0.2	3.8 ± 0.3	5.2 ± 0.4	7.4 ± 0.9	7.8 ± 0.8
		21	5.7 ± 1.7	4.3 ± 0.6	7.5 ± 0.2	7.4 ± 0.7	7.7 ± 0.1
		28	5.8 ± 0.7	5.0 ± 0.2	6.3 ± 0.1	6.3 ± 1.0	5.5 ± 0.0
nonanal	36.0 ± 1.7	7	36.4 ± 0.3	21.0 ± 1.6	22.4 ± 4.3	27.1 ± 3.2	24.5 ± 1.5
		14	18.7 ± 1.0	18.1 ± 0.7	19.3 ± 0.9	25.3 ± 2.2	23.9 ± 0.2
		21	20.5 ± 5.2	16.1 ± 0.5	24.5 ± 5.3	27.9 ± 8.6	21.3 ± 2.7
		28	21.4 ± 1.1	22.7 ± 0.0	25.6 ± 1.8	11.3 ± 1.4	20.9 ± 0.1
decanal	12.8 ± 1.2	7	13.1 ± 1.4	10.3 ± 1.3	7.3 ± 1.9	10.5 ± 1.0	11.6 ± 0.0
		14	8.6 ± 0.3	9.6 ± 1.6	8.3 ± 0.8	10.2 ± 0.4	8.7 ± 2.0
		21	8.6 ± 2.0	7.8 ± 1.2	7.9 ± 3.0	10.5 ± 1.9	10.5 ± 1.8
		28	10 ± 0.2	8.8 ± 1.1	9.6 ± 0.4	6 ± 0.3	6.6 ± 0.9
oct-1-en-3-one	98.0 ± 4.5	7	100.0 ± 11.1	74.1 ± 6.5	79.1 ± 11.8	89.6 ± 1.2	105.2 ± 3.3
		14	64.6 ± 1.9	72.5 ± 4.9	72.0 ± 0.1	95.4 ± 11.3	97.7 ± 12
		21	90.7 ± 23.3	71 ± 4.2	88.5 ± 2.7	94.1 ± 18.5	111.2 ± 19.2
		28	96.2 ± 1.5	76 ± 2.2	100.4 ± 2.8	22.6 ± 4.1	79.6 ± 4.5
benzaldehyde	33.9 ± 23.4	7	19.5 ± 1.8	49.5 ± 53.8	40.7 ± 26.3	63.2 ± 35.4	31.8 ± 9.9
		14	46 ± 23.0	22.1 ± 6.6	72.4 ± 29.7	99.3 ± 95.4	60.8 ± 7.8
		21	35.0 ± 6.4	39.9 ± 12.1	30.4 ± 9.9	42.0 ± 14.7	39.8 ± 10.6
		28	51.6 ± 8.4	53.5 ± 7.0	53.6 ± 20.9	116.1 ± 27.8	49.0 ± 11.3

3. Results and discussion

3.1. Fermentation kinetics and microbiological determinations

Firstly, it must be pointed out the degree of implantation of the inoculated yeast species was equal or higher than 90% in all cases, at yeast concentrations above 10^7 cfu/ml, which ensured that the inoculated strains carried out the fermentations.

Under these conditions, the initial sugar content (36 g/kg apple pomace) decreased until depletion in all inoculations before 21 days, yielding a similar ethanol content (2.80 ± 0.05% v/w) in all cases at the end of the alcoholic fermentation (Fig. 1). Although the genus *Hanseniaspora* is considered less efficient in producing ethanol than *Saccharomyces* (Diaz-Montaño, Délia, Estarrón-Espinosa, & Strehaiano, 2008), the fact of carrying out the fermentation in a favourable media with pure cultures and a low sugar content led to similar yields in all inoculations.

Table 2 summarizes the enzymatic activities detected in the apple pomaces during fermentation. As expected, β-glucosidase activity was detected in all inoculations except those conducted only with Levuline (Table 2), although the fermentation with the commercial enzyme showed both pectinmethylesterase and arabinofuranosidase activity. In this respect, it has been reported that the use of oenological enzymes of fungal origin may lead to the unexpected appearance of enzymatic activities due to inadequate purification (Wightman, Price, Watsom, & Wirolstad, 1997). β-xylosidase activity was only detected in the assays with the Levuline strain, however.

3.2. Volatile compounds in apple pomaces

Chromatographic analysis of the apple pomaces during

Table 6
Changes in terpenoid concentrations during apple pomace fermentation expressed as μg/kg 2-octanol (mean of two experimental units ± standard deviation).

Compound	Unfermented pomace	Fermentation time (days)	Levuline	Levuline + enzyme	S. c. 3′	H. u. 283	H. v. 43
β-damascenone	1.9 ± 0.1	7	2.6 ± 0.0	8.0 ± 0.7	6.2 ± 0.1	6.3 ± 1.1	9.0 ± 1.0
		14	9.7 ± 1.2	9.6 ± 1.4	8.2 ± 1.3	8.9 ± 0.0	14.2 ± 2.7
		21	10.0 ± 0.2	9.3 ± 1.2	11.5 ± 4.0	10.6 ± 0.5	17.4 ± 1.7
		28	9.1 ± 1.8	13.6 ± 0.5	10.6 ± 0.3	10.2 ± 1.1	9.6 ± 0.3
nerylacetone	105.6 ± 3.3	7	105.5 ± 5.9	77.5 ± 12.2	87.1 ± 0.3	81.9 ± 12.6	92.0 ± 9.1
		14	68.0 ± 0.6	61.3 ± 2.6	92.6 ± 4.5	78.5 ± 1.4	97.3 ± 24.2
		21	80.8 ± 25.4	53.5 ± 9.5	86.7 ± 7.5	81.7 ± 16.2	96.9 ± 15.0
		28	80.4 ± 3.8	84.2 ± 6.0	95.3 ± 6.0	55.1 ± 2.2	63.6 ± 6.0
β-citral isomer 2	25.4 ± 1.2	7	25.2 ± 1.7	17.4 ± 0.4	23.1 ± 6.2	20.1 ± 1.4	18.3 ± 2.3
		14	17.4 ± 0.8	14.4 ± 0.8	16.8 ± 1.7	21.6 ± 1.1	18.8 ± 1.3
		21	21.7 ± 6.1	12.4 ± 1.5	21.8 ± 6.2	23.5 ± 6.4	23.3 ± 6.0
		28	20.6 ± 0.4	20.1 ± 0.9	25.1 ± 0.7	12.6 ± 0.1	15.4 ± 0.7
β-ionone	20.1 ± 1.4	7	18.9 ± 0.4	16.8 ± 2.2	17.5 ± 3.2	15.7 ± 0.8	22.7 ± 1.4
		14	15.1 ± 0.4	15.3 ± 0.7	15.6 ± 1.6	17.1 ± 1.5	24.7 ± 5.4
		21	16.8 ± 1.0	13.1 ± 2.7	17.5 ± 4.1	17.5 ± 3.6	26.4 ± 3.8
		28	12.8 ± 2.2	20.9 ± 1.0	17.2 ± 1.2	14.2 ± 1.9	15.9 ± 1.0
6-methylhept-5-en-2-one	15.3 ± 0.7	7	13.7 ± 0.5	11 ± 1.6	12.9 ± 0.2	9.7 ± 1.1	14.0 ± 1
		14	8.9 ± 0.1	9.4 ± 0.2	11.1 ± 1.3	8.8 ± 0.4	14.0 ± 0.8
		21	10.9 ± 2.0	9.0 ± 0.3	11.1 ± 1.2	8.9 ± 1.4	14.1 ± 1.0
		28	10.0 ± 0.9	11.1 ± 0.0	9.7 ± 1.0	5.1 ± 0.3	8.3 ± 0.9
farnesol isomer 3	347.7 ± 73.1	7	453.3 ± 29.3	539.9 ± 67.9	387.9 ± 43.5	452.9 ± 21.3	323.0 ± 93.2
		14	442.0 ± 17.6	614.7 ± 0.7	380.2 ± 24	533.2 ± 50.6	377.5 ± 42.4
		21	424.9 ± 11.7	501 ± 85.3	376.0 ± 136.5	558.0 ± 45.5	418.5 ± 23.7
		28	367.4 ± 7.4	899.4 ± 78.9	474.3 ± 51.6	447.4 ± 26.7	407.4 ± 5.2

fermentation allowed the identification of 132 volatile compounds belonging to five chemical families, highlighting the potential of fermented apple pomace as a source of aromas (Table 3). Forty-four of these compounds were quantitated during apple pomace fermentation according to their quantitative and technological relevance. As can be seen in the chromatograms in Fig. 2, the aromatic richness of the apple pomace was markedly increased by fermentation, detecting significant differences in the levels of the analysed aromas depending on the inoculated yeast strain and fermentation time.

In all cases, the aroma of the fermented pomace was pleasant, with a strong smell of ripe fruit and raisins.

3.2.1. *Esters and lactones*

Esters and lactones comprise one of the most important groups of aromatic compounds in the agrifood/aromas industry, imparting pleasant odours (Table 3). Chromatographic analysis showed 51 esters in the fermented pomaces, 34 of which were not detected in the unfermented apple pomaces. In this respect, it should be noted that the absence of a characteristic apple marker in this substrate, such as isoamyl acetate, would be justified by the previous drying of the pomace.

The production of esters by yeasts, an intracellular process catalysed by acyltransferases from the respective alcohol and AcylCoA, resulted in a progressive increase in these compounds during the study period, especially in ethyl fatty acid ester contents (Fig. 2). The formation of esters of higher alcohols, absent in the unfermented pomace, was also detected. These included isoamyl and 2-phenylethyl esters, both coming from the major alcohols produced during alcoholic fermentation.

Table 4 shows the results for the quantitated esters. Significant differences ($p < 0.01$) were detected in ester production depending on the inoculated yeast (Table 4). Ethyl fatty acid esters were higher in fermentations with *Saccharomyces* strains versus *Hanseniaspora* strains, sometimes in ratios higher than 10:1. *S.c.* 3′ produced higher levels of ethyl hexanoate and ethyl octanoate ($p < 0.05$), while ethyl decanoate, ethyl dodecanoate and ethyl tetradecanoate were more abundant in fermentations with Levuline and the enzymatic preparation. Acetic acid esters, on the other hand, were higher in fermentation with *Hanseniaspora* strains. Isoamyl acetate and 2-phenylethyl acetate were more abundant in experimental units fermented with *H.v.* 43, while a higher concentration of phenylmethyl acetate was detected when apple pomace was inoculated with *H.u.* 283. Moreover, the subsequent decrease detected in 2-phenylethyl acetate levels for both inoculations is worth highlighting, as well as the decrease in isoamyl acetate for the *H.u.* 283 strain. These changes represented a decrease of more than 90% from the maximum values detected in the period of 7–14 days of fermentation as a result of hydrolysis and/or transesterification processes. Higher levels of acetic acid esters in fermentations with *Hanseniaspora* strains have been reported in previous papers by other authors during the fermentation of grapes, apples or/and synthetic media (Cabranes et al., 1997, 1998; Viana, Gil, Genovés, Vallés & Manzanares, 2008).

On the other hand, higher levels of methyl esters were detected in fermentations with Levuline and the enzyme (Table 4, Fig. 2), which could be justified by the release of methanol associated with the pectinmethylesterase activity detected in these experimental units (Table 2).

Other minor esters detected, such as isoamyl esters, ethyl cinnamate and ethyl phenylacetate, showed an increase during fermentation in all inoculations, in particular fatty acid isoamyl esters in fermentations with *S.c.* 3′ (Table 4).

Lactones are cyclic esters giving fruity aromas (Table 3). In this study, 6 lactones were detected in the unfermented apple pomace coming from oleic and linoleic acids by β-oxidation in intact tissues during apple ripening (Christensen, Edelenbos, & Kreutzmann, 2008). Moreover, the only one lactone quantitated in this study, γ-nonalactone, showed a significant increase ($p < 0.01$) during fermentation in all the experimental units (Table 4).

3.2.2. *Acids*

Under the analytical conditions employed in this study, 8 acids were detected in all the samples (Table 3), outstanding among which are the saturated fatty acids because of their interest. Saturated fatty acids are present in the unfermented pomace as a result of the β-oxidation of the unsaturated acids of apple. However, a significant increase ($p < 0.05$) was detected in all the experimental units for the quantified fatty acids (Fig. 3), resulting from the yeast metabolism during alcoholic fermentation via hydrolysis of their corresponding acyl-CoA. Moreover, significant differences ($p < 0.01$) were detected depending on the strain employed: the highest levels were detected in fermentations with *S.c.* 3′, followed by both inoculations with Levuline, while the lowest levels were found in fermentations by *Hanseniaspora* strains.

3.2.3. *Aldehydes and ketones*

Unbranched aldehydes and ketones constitute an important part of the flavour of apple pomace, with aromas usually defined as green (Table 3). These carbonylic compounds are generated in apple tissues in response to damage to the fruit during processing (milling) via the degradation of unsaturated C18 fatty acids by lipoxygenases (LOX) or autoxidation, depending on environmental conditions (Christensen et al., 2008). From the technological point of view, it should be noted that during the period of 21–28 days of fermentation, the levels of the quantified unsaturated carbonyl compounds decreased ($p < 0.01$) in fermentations with both *Hanseniaspora* strains, sometimes up to 60% (Table 5). This decrease may be associated with the presence of carbonyl reductases, reported in species of the genus *Hanseniaspora* (Goretti et al., 2011).

Significant differences ($p < 0.01$) were also detected during the period of fermentation in all the other strains. These changes were generally characterized by a decrease, which was sometimes followed by an increase back to initial levels (Table 5). These changes cannot be considered technologically relevant, however, except for the significant increase detected in concentrations of octen-2-al and decen-2-al in trials with *Saccharomyces* yeasts, reaching more than 300% of the initial value in the case of octen-2-al with *S.c.* 3′ (Table 5).

Furthermore, the presence of flavours such as oct-1-en-3-one has been associated with oxidative processes in the unsaturated fatty acids produced during cooking (Ashmore, Craske, & Srzednicki, 2014; Picardi & Issenberg, 1973). In our case, the presence of this ketone as a major compound of this group was due to the pomace drying stage.

Another major carbonylic compound detected in the apple pomaces, before and after fermentation, was benzaldehyde, a compound with a characteristic bitter almond-like *odour*. Unlike the above aldehydes and ketones, benzaldehyde originates from the hydrolysis of amygdalin in apple seeds, which could explain both its concentration was influenced neither by the inoculated yeast strain nor by fermentation time.

3.2.4. *Terpenoids*

Terpenoids from plants may have originated via anabolic processes, e.g. monoterpenes and sesquiterpenes, or via catabolic and/or oxidative processes, e.g. irregular terpenes (Christensen, Edelenbos & Kreutzmann, 2008). These compounds form part of the plant material and in some instances may be present either in their free form or glycosylated. Aglycones usually have very low thresholds, being perceived as a pleasant aromatic odour, and are accordingly used in different industries.

Several irregular terpenes: β-ionone, pseudoionone, β-damascenone, 6-methylhept-5-en-2-one, 6-methylhept-5-en-2-ol, 6-methylhepta-3,5-dien-2-one, 2,6-dimethylhept-5-enal, 2,6-dimethylhept-5-en-1-ol, nerylacetone and 6,10-dimethylundeca-5,9-dien-1-ol, were detected in this study, all of which are considered products of oxidative degradation of carotenoids

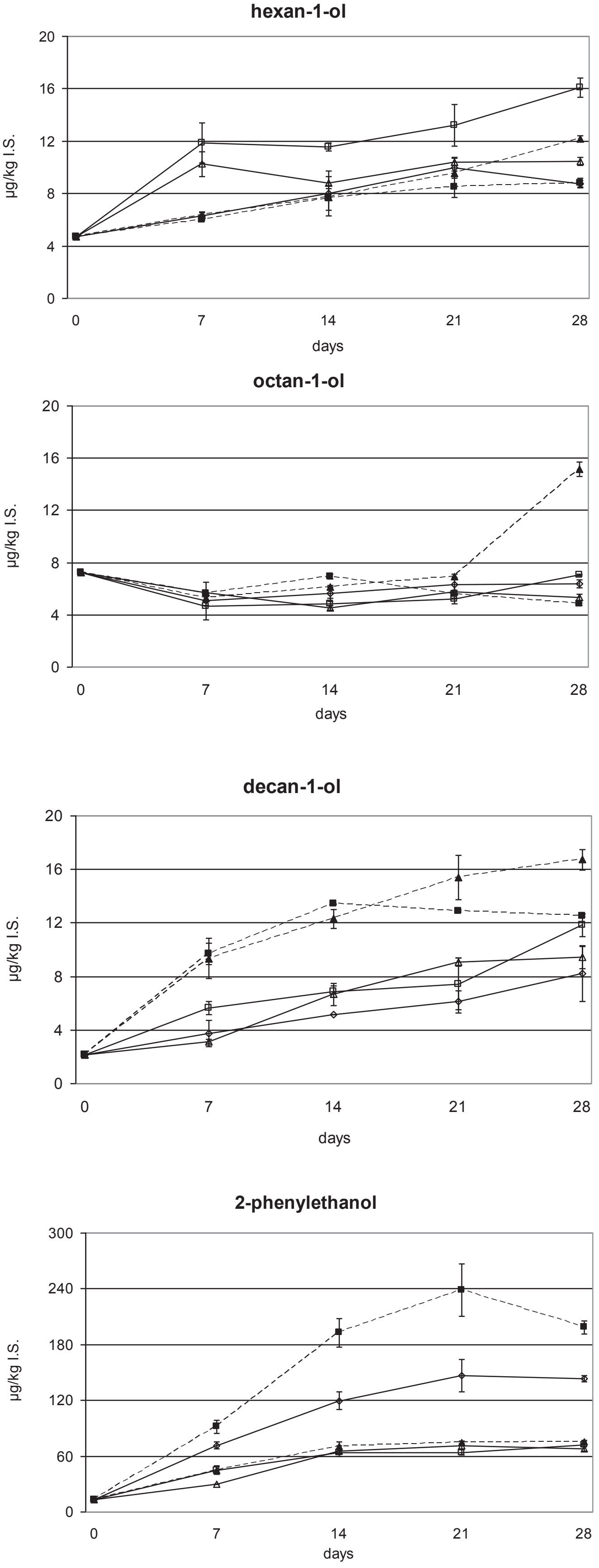

Fig. 4. Evolution of the content in alcohols during fermentation of apple pomaces. Open triangle: Levuline strain; open square: Levuline strain + enzyme; open diamond: *S.c.* 3′ strain; full triangle: *H.u.* 283 strain; full square: *H.v.* 43 strain. Solid line: *Saccharomyces* strain; dotted line: non-*Saccharomyces* strain. Mean of two experimental units and standard deviation.

constituting the plant structure. Cyclic carotenes from apple, such as β-carotene, produce the cyclic terpenoids ionone, pseudoionone or damascenone, while acyclic carotenes, such as lycopene, produce linear derivatives. As in the autoxidation of unsaturated fatty acids, oxidative activity occurs as a result of damage to the fruit during processing. Hence, these components are found in the apple pomace both before and after fermentation.

Furthermore, 17 mono- and sesquiterpenes were detected (Table 6), the major one being a farnesol isomer (Fig. 1, peak 44), an interesting compound with a floral aroma and antimicrobial activity against human and plant pathogens (Hornby et al., 2001; Jabra-Rizk, Meiller, James, & Shirtliff, 2006). Fermentation time and inoculation significantly affected ($p < 0.01$) the concentration of this terpene in the fermented apple pomace, the higher levels of this compound in the inoculations with the enzyme at 28 days of fermentation being especially noteworthy.

A significant increase ($p < 0.01$) in β-damascenone was likewise detected during fermentation in all inoculations (Table 6). This is an aroma used industrially for its characteristic smell of roses and is present in plant products both as a result of the enzymatic hydrolysis of its glycosides and due to the degradation of carotenoids (Winterhalter, 1992). In this case, the increase in β-damascenone may be associated with the hydrolysis of glycosylated precursors present in the apple, as reported by Roberts, Mordehai, and Acree (1994), bearing in mind that significant increases were not detected for other terpenoids from carotenoid degradation.

There was, in contrast, a significant decrease ($p < 0.05$) in 6-methylhept-5-en-2-one, citral and nerylacetone, more pronounced in the 21–28 day period in the fermentations with the two *Hanseniaspora* strains, which could be indicative of reductase activity, as already mentioned. In this regard, note that the alcohols 2,6-dimethylhept-5-en-1-ol and 6-methylhept-5-en-2-ol were detected only in fermented apple pomaces, the latter only being present in the fermentations with yeasts belonging to the genus *Hanseniaspora* (Table 3).

3.2.5. Alcohols

With regard to the content in non-terpene alcohols, the presence of a significant number of varietals alcohols was verified in the pomace both before and after fermentation (Table 3). These include the C6 alcohols responsible for a typical odour of grass, formed by degradation of linoleic and linolenic acids by LOX (38). Another interesting flavour employed in the food industry, due to its characteristic smell of mushrooms, is oct-1-en-3-ol; this alcohol was detected in the pomace before fermentation and has its origin in the breakdown of linoleic acid by the action of several enzymes (Tressl, Bahri, & Engel, 1980). On the other hand, dec-4-en-1-ol was only detected in fermentations with *Saccharomyces* strains, while eugenol, phenylmethanol and amyl alcohols were absent in unfermented pomaces.

Quantitatively, the higher concentration ($p < 0.01$) of 2-phenylethanol in the fermentation with the *H.v.* 43 strain (Fig. 4) is worth noting, justified by the hydrolysis and/or trans-esterification of 2-phenylethanol acetate discussed previously, followed by *S.c.* 3′, and finally inoculations with *H.u.* 283, Levuline and Levuline with the enzyme preparation. Hexan-1-ol and decan-1-ol (Fig. 4) increased their concentrations during fermentation in all inoculations ($p < 0.05$) while octen-1-ol showed a significant increase only in the experimental units inoculated with *H. u. 283*.

4. Conclusions

In summary, the fermentation of apple pomaces with autochthonous yeast strains is a suitable way of increasing the content in interesting aromatic compounds and of producing valuable levels of ethanol that may be recovered. Moreover, the production of aromas is strongly strain-dependent, the use of distinct yeast strains being able to favour the formation of desired compounds. Apple pomace or their extracts could be used as flavouring in the food industry as a natural additive, thereby enhancing consumer acceptance. Without previous treatment, fermented apple pomace could be suitable to flavour beverages by maceration or infusion or as a part of bakery products. The extracts of fermented apple pomace could be used as flavouring agent by collecting the whole flavour generated by different strains or after extraction and purification as single flavours.

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