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Contribution of autochthonous lactic acid bacteria to the typical flavour of raw goat milk cheeses

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ABSTRACT

In this study, a collection of 298 wild strains of lactic acid bacteria (LAB) isolated from raw goat milk cheeses, belonging to 8 genera and 24 species, was investigated for their ability to generate volatile compounds. Sensory evaluation showed that goat milk cultures of *Leuconostoc* and *Lactococcus* strains reached the highest scores for dairy odour attributes. Fifty six LAB strains with the highest sensory evaluation scores (one per each ten, or fraction, of those belonging to the same species) were selected for gas chromatography–mass spectrometry (GC–MS) analysis. A total of 34 volatile compounds (five carboxylic acids, eleven alcohols, six aldehydes, six ketones, one ester, and five miscellaneous compounds) were detected in lactic curds made with each one of 56 selected LAB strains. The number of volatile compounds in individual curds varied from 15 to 33 while the total abundance of volatile compounds in individual curds ranged from 1.98- to 17.50-fold the total abundance in uninoculated curd. Major differences in volatile generation were related to amino acid catabolism.

1. Introduction

Goat milk, due to its compositional characteristics, is considered as a high-quality raw material for the production of baby food, food for the elderly and for certain sectors of the population with particular needs (Sanz Ceballos et al., 2009). Compared to cow milk, its protein fraction is more digestible and less allergenic, its lipid fraction is more digestible, and its mineral content and nutritional availability are of higher quality than those of cow milk (Kumar et al., 2016; Sanz Ceballos et al., 2009).

The microbiota of raw goat milk plays an important role in the sensory and textural characteristics of raw milk cheeses (Delgado et al., 2011; Litopoulou-Tzanetaki and Tzanetakis, 2011) and it may also provide beneficial effects for consumers' health (Foulquié Moreno et al., 2006; Montel et al., 2014). However, decarboxylase-positive bacteria present in raw milk are also responsible for biogenic amine production, and accumulation of biogenic amines in cheese is a matter of public health concern (Calzada et al., 2013). Raw goat milk microbiota includes different genera of lactic acid bacteria (LAB), mainly “wild” strains of lactococci, lactobacilli and enterococci (Badis et al., 2004; Delavenne et al., 2012; McInnis et al., 2015; Tormo et al., 2015).

The characterization of lactococci isolated from raw goat milk cheeses has been the subject of previous studies (Pavlidou et al., 2011; Picon et al., 2016; Psoni et al., 2007; Requena et al., 1991). There is also a growing interest in the characterization of lactobacilli isolated from raw goat milk cheeses and in the use of probiotic strains of lactobacilli as adjunct cultures for cheese production (Georgieva et al., 2009; Lavilla-Lerma et al., 2013; Sánchez et al., 2005). Enterococci are frequently isolated from raw goat milk cheeses, and their contribution to flavour characteristics has been acknowledged (Giraffa, 2003; Martín-Platero et al., 2009; Psoni et al., 2006; Suzzi et al., 2000). However, reports on some *Enterococcus*, *Carnobacterium* and *Lactobacillus* strains as tyramine producers have been published (Bover-Cid and Holzapfel, 1999; Martín-Platero et al., 2009; Picon et al., 2016; Suzzi et al., 2000).

Cheese flavour is one of the most relevant attributes influencing consumers' acceptance and preference (Arora et al., 1995). More than 600 volatile compounds have been identified in cheese, most of which have been associated with particular odour and aroma notes (Curioni and Bosset, 2002). During cheese manufacture and ripening, glycolysis, proteolysis and lipolysis, followed by the secondary catabolism of the resulting products give rise to compounds that, in many cases, have higher flavour impact than their respective precursors (Collins et al., 2003; McSweeney and Sousa, 2000; Yvon and Rijnen, 2001).

Consumer demands for new flavours and tasty dairy products has renewed the interest for LAB strains with novel properties. Autochthonous LAB isolated from raw milk or raw milk cheeses are frequently associated with more complex volatile profiles and higher scores for some sensory attributes than LAB strains found in commercial

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starters (Baruzzi et al., 2016; Montel et al., 2014; Morales et al., 2003b). Metabolic routes usually not present in commercial strains lead to the production of different flavour compounds in cheese by wild LAB strains, separately used or combined for cooperative metabolism (Ayad et al., 2001; Peláez and Requena, 2005).

The objective of the present study was to gain a better understanding of the contribution of autochthonous LAB strains isolated from raw goat milk cheeses to their flavour characteristics. For this purpose, the ability of 298 LAB strains to generate volatile compounds when grown as goat milk cultures was evaluated by sensory analysis. Thereafter, the volatile compounds produced by 56 selected strains individually used for the manufacture of lactic curds from pasteurized goat milk were extracted by headspace solid phase microextraction (HS-SPME) and analyzed by gas chromatography–mass spectrometry (GC–MS), to elucidate their contribution to the odour and aroma of raw goat milk cheeses.

2. Materials and methods

2.1. Lactic acid bacteria

A collection of 298 wild LAB strains belonging to 8 genera and 24 species was investigated. They were isolated from 1-day raw goat milk cheeses, manufactured from raw goat milk collected in spring and autumn at 11 dairy plants located in Cádiz and Málaga provinces (Andalusia, Spain), and identified at the molecular level (Picon et al., 2016). Strains and their origin are listed in Table 1. For comparison, three commercial cheese starters were included in the experiments. They were CHOOZIT™ MA11 and MA16 cultures (Danisco A/S, Copenhagen, Denmark) and H13 culture (Biostar S.A., Valmojado, Spain).

Table 1

Bacterial strains used in this study, their origin and the strains selected for the GC–MS characterization of their volatile profile in inoculated curds.

Genus	No.	Species	No.	Selected strains[a]
Lactococcus	76	*lactis* ssp. *cremoris*	12	C3, M870
		lactis ssp. *lactis*	59	C2, C57, C387, M5, M13, M18, M899
		garvieae	3	M12
		raffinolactis	2	C374, M881
Lactobacillus	88	*brevis*	5	C521
		casei	33	C176, C530, C541, M81
		delbrueckii	9	C187, M961
		fermentum	3	C184, M981
		gallinarum/helveticus	7	C156, M971
		ingluviei	7	M82
		plantarum	17	C194, M87
		rhamnosus	7	C542, M960
Leuconostoc	61	*citreum*	2	M938
		holzapfelii	1	M924
		lactis	2	M882
		mesenteroides	56	C112, C490, C496, M53, M117, M923
Enterococcus	60	*casseliflavus/ gallinarum*	7	C99, M147
		durans	8	C455, M156
		faecalis	40	C77, C442, M139, M167, M1002
		faecium/hirae	5	C75, M169, M1022
Carnobacterium	2	*divergens*	2	M30
Pediococcus	4	*acidilactici*	1	C168
		pentosaceous	3	C549
Streptococcus	5	*gallolyticus*	5	C28, M906, M994
Weisella	2	*cibaria*	2	C150

[a] C, strains from Cádiz cheeses; M, strains from Málaga cheeses.

2.2. Microbiological analysis of commercial starters and strain identification

The content of each sachet of lyophilized commercial starter was dissolved in 200 mL of a sterile 100 mM potassium phosphate solution pH 6.6 and decimal dilutions were prepared in sterile 1 g L^{-1} peptone solution. Mesophilic LAB and presumptive lactobacilli were respectively determined in MRS agar (Biolife, Milano, Italy) incubated at 30 and 37 °C for 48 h and Rogosa agar (Biolife, acidified at pH 5.4 with acetic acid) incubated anaerobically at 37 °C for 48 h. From each medium, 10–15 colonies were randomly chosen. Purification, DNA extraction and molecular identification of strains were performed as previously described (Campos et al., 2011).

2.3. Preparation of milk cultures

Wild LAB strains and commercial starters, maintained as frozen glycerol stocks, were inoculated into MRS broth (Biolife) and grown at 30 °C for 24 h. They were activated by two successive transfers in UHT skim milk prior to inoculation at 1% (v/v) in 100 mL of pasteurized goat milk in 250 mL flasks. Grown cultures were obtained by incubation at 30 °C for 24 h, in two independent experiments.

2.4. Sensory evaluation of milk cultures

Milk cultures were presented to a panel of 10 trained assessors (4 males and 6 females aged 25 to 62) who had a minimum 2-year experience in the sensory analysis of dairy products. After selection of the most representative sensory attributes in previous trials, panellists were asked to evaluate, using a 0 to 7 scale, the intensity of six “dairy” odour attributes (fresh cheese, yogurt, butter, cream, cooked milk and whey), four “vegetal” odour attributes (grass-hay, floral, fruity and nuts) and four “roasted” odour attributes (caramel-toffee, coffee, chocolate and burned). In each session, eight flasks containing milk cultures were randomly presented to panellists together with a flask containing 100 mL of uninoculated pasteurized goat milk (kept for 22 h at 4 °C followed by 2 h at 30 °C) as control. Panellists were asked to open the flask after gentle shaking and evaluate the 14 odour attributes. The data were reported as mean score values with standard deviations of two sensory evaluation sessions.

2.5. Preparation of lactic curds

Lactic curds were prepared in duplicate from 56 selected wild LAB strains (those with the highest sensory evaluation scores, picking one per ten or fraction of strains from each species) and three commercial starter. Pasteurized goat milk (100 mL in 250 mL flasks) was supplemented with 1 mL L^{-1} of a 100 g L^{-1} $CaCl_2$ solution prior to inoculation. Milk was individually inoculated (1% v/v) with 56 selected LAB strains (Table 1) and the three commercial starters, and incubated at 30 °C for 24 h. Coagulated milk cultures were transferred with the help of sterile spatulas to centrifuge tubes and centrifuged at 14,000*g* for 20 min in an Eppendorf 5810R centrifuge equipped with a F34-6-38 rotor (Eppendorf, Hamburg, Germany). Non-coagulated grown milk cultures were acidified with a 2 N HCl solution to a pH of 4.6, to achieve the isoelectric precipitation of caseins, before centrifugation. Uninoculated curd was prepared from pasteurized goat milk following the same acidification procedure as for non-coagulated grown milk cultures. Curd pellets were wrapped in aluminium foil, vacuum packed in HT3050 plastic bags (Cryovac Sealed Air Corporation, Milano, Italy) and kept at −35 °C until analysis, which took place within 1 month.

2.6. Analysis of volatile compounds

Volatile compounds were extracted in duplicate from each curd by automated HP-SPME using a CombiPAL autosampler (CTC Analytics,

Table 2
Scores[1] for selected individual odour attributes obtained in the sensory evaluation of milk cultures of 298 strains of autochthonous lactic acid bacteria and three commercial starters.

Genus	Species	No.	Fresh cheese		Yogurt		Butter		Cooked milk		Whey		Fruity		Nuts		Burned	
Lactococcus	*lactis* ssp. *cremoris*	12	0.48	± 0.46^{b}	1.58	± 0.98^{b}	0.28	± 0.16^{ab}	0.24	± 0.11^{b}	0.10	± 0.06^{ab}	nd		0.01	± 0.04^{b}	1.34	± 1.11^{a}
	lactis ssp. *lactis*	59	0.24	± 0.22^{b}	1.43	± 0.93^{b}	0.30	± 0.26^{ab}	0.29	± 0.18^{b}	0.14	± 0.22^{ab}	0.01	± 0.03^{b}	0.01	± 0.02^{b}	1.56	± 1.15^{a}
	garvieae	3	0.23	± 0.19^{b}	0.74	± 0.65^{b}	0.81	± 0.75^{ab}	0.81	± 0.39^{ab}	0.10	± 0.11^{ab}	0.02	± 0.03^{ab}	nd		0.02	± 0.03^{b}
	raffinolactis	2	1.50	± 1.77^{ab}	1.53	± 0.15^{b}	0.81	± 0.06^{ab}	0.86	± 0.61^{ab}	1.23	± 1.45^{ab}	nd		nd		0.73	± 1.03^{ab}
Lactobacillus	*brevis*	5	0.42	± 0.49^{b}	1.23	± 1.33^{b}	0.04	± 0.06^{b}	0.73	± 0.67^{ab}	0.19	± 0.13^{ab}	0.02	± 0.04^{ab}	nd		0.09	± 0.12^{b}
	casei	33	0.28	± 0.22^{b}	0.86	± 0.55^{b}	0.41	± 0.37^{ab}	1.05	± 0.38^{a}	0.15	± 0.09^{ab}	0.04	± 0.06^{ab}	nd		0.08	± 0.17^{b}
	delbrueckii	9	0.29	± 0.22^{b}	1.13	± 0.25^{b}	0.18	± 0.14^{ab}	1.10	± 0.44^{a}	0.10	± 0.05^{ab}	0.03	± 0.07^{ab}	nd		nd	
	fermentum	3	0.16	± 0.04^{b}	0.63	± 0.50^{b}	0.20	± 0.09^{ab}	1.29	± 0.30^{a}	0.10	± 0.05^{ab}	nd		nd		nd	
	gallinarum/helveticus	7	0.43	± 0.45^{b}	0.77	± 0.59^{b}	0.31	± 0.15^{ab}	1.30	± 0.41^{a}	0.11	± 0.03^{ab}	0.05	± 0.05^{ab}	nd		0.09	± 0.17^{b}
	ingluviei	7	0.15	± 0.10^{b}	0.58	± 0.54^{b}	0.27	± 0.02^{ab}	0.93	± 0.15^{a}	0.13	± 0.07^{ab}	0.05	± 0.05^{ab}	nd		0.09	± 0.17^{b}
	plantarum	17	0.26	± 0.24^{b}	0.66	± 0.53^{b}	0.19	± 0.15^{ab}	0.99	± 0.46^{a}	0.12	± 0.03^{ab}	0.05	± 0.08^{ab}	nd		0.01	± 0.04^{b}
	rhamnosus	7	0.33	± 0.31^{b}	0.48	± 0.45^{b}	0.27	± 0.05^{ab}	1.32	± 0.13^{a}	0.12	± 0.03^{ab}	0.05	± 0.06^{ab}	nd		0.05	± 0.03^{b}
Leuconostoc	*citreum*	2	0.23	± 0.08^{b}	0.09	± 0.04^{b}	0.98	± 0.84^{ab}	0.68	± 0.37^{ab}	0.59	± 0.65^{ab}	0.25	± 0.00^{a}	nd		0.12	± 0.16^{ab}
	holzapfelii	1	1.68	± 0.03^{ab}	3.13	± 0.04^{ab}	0.28	± 0.07^{ab}	nd		1.12	± 0.06^{a}	nd		nd		0.09	± 0.00^{b}
	lactis	2	1.37	± 0.07^{ab}	3.25	± 1.50^{ab}	0.61	± 0.15^{ab}	0.53	± 0.27^{ab}	1.13	± 0.02^{a}	0.11	± 0.15^{ab}	nd		nd	
	mesenteroides	56	0.51	± 0.69^{b}	1.17	± 1.34^{b}	0.45	± 0.40^{ab}	0.92	± 0.46^{a}	0.45	± 0.66^{ab}	0.05	± 0.09^{ab}	nd		0.04	± 0.14^{b}
Enterococcus	*casseliflavus/gallinarum*	7	0.29	± 0.11^{b}	1.83	± 0.55^{b}	0.35	± 0.43^{ab}	0.79	± 0.58^{ab}	0.43	± 0.45^{ab}	nd		nd		0.09	± 0.10^{b}
	durans	8	0.20	± 0.04^{b}	1.02	± 0.92^{b}	0.52	± 0.45^{ab}	1.16	± 0.51^{a}	0.30	± 0.42^{ab}	nd		nd		0.06	± 0.10^{b}
	faecalis	40	0.31	± 0.23^{b}	1.55	± 1.40^{b}	0.37	± 0.33^{ab}	0.77	± 0.58^{ab}	0.54	± 0.58^{ab}	0.05	± 0.03^{ab}	0.03	± 0.03^{b}	0.13	± 0.25^{ab}
	faecium/hirae	5	0.61	± 0.57^{b}	0.43	± 0.53^{b}	0.22	± 0.36^{ab}	1.02	± 0.76^{a}	0.41	± 0.55^{ab}	nd		nd		0.06	± 0.14^{b}
Carnobacterium	*divergens*	2	0.32	± 0.16^{b}	1.23	± 0.14^{b}	0.21	± 0.06^{ab}	0.03	± 0.00^{b}	0.04	± 0.06^{b}	nd		0.24	± 0.34^{a}	0.77	± 0.64^{ab}
Pediococcus	*acidilactici*	1	0.57	± 0.02^{b}	1.49	± 0.03^{b}	0.36	± 0.05^{ab}	0.05	± 0.00^{b}	0.12	± 0.03^{ab}	nd		0.13	± 0.02^{a}	nd	
	pentosaceous	3	0.16	± 0.03^{b}	1.04	± 0.05^{b}	0.15	± 0.03^{ab}	0.31	± 0.24^{ab}	0.09	± 0.03^{ab}	nd		nd		nd	
Streptococcus	*gallolyticus*	5	0.17	± 0.07^{b}	1.46	± 0.82^{b}	0.40	± 0.28^{ab}	0.61	± 0.36^{ab}	0.13	± 0.10^{ab}	nd		0.12	± 0.24^{ab}	0.63	± 1.08^{ab}
Weisella	*cibaria*	2	0.18	± 0.06^{b}	nd		0.23	± 0.03^{ab}	1.47	± 0.21^{a}	0.13	± 0.01^{ab}	0.10	± 0.14^{ab}	nd		nd	
Commercial starters	MA11		3.18	± 0.10^{a}	5.76	± 0.18^{a}	1.00	± 0.05^{ab}	0.44	± 0.05^{ab}	0.14	± 0.02^{ab}	nd		nd		nd	
	MA16		2.18	± 0.15^{a}	2.72	± 0.07^{ab}	1.56	± 0.05^{a}	0.66	± 0.09^{ab}	0.12	± 0.06^{ab}	nd		nd		nd	
	H13		2.24	± 0.08^{a}	4.24	± 0.11^{a}	0.84	± 0.05^{ab}	nd		0.36	± 0.10^{ab}	nd		nd		1.22	± 0.20^{ab}

[1] Mean values (± SD) of individual odour attributes obtained using a 0- to 7-point scale in two sensory evaluation sessions for all the strains belonging to the same species; nd, not detected. Means in the same column with different superscripts differ significantly ($P < .05$).

Zwingen, Switzerland) fitted with a vial heater and analyzed by gas chromatography–mass spectrometry (GC–MS) using a HP 6890 gas chromatograph coupled to a mass selective detector HP 5973 (Hewlett-Packard, Agilent, Palo Alto, CA, USA). Curd samples (7 g) were homogenized in a mechanical grinder with 14 g of anhydrous Na_2SO_4 (Merck, Darmstadt, Germany) and 25 μL of an aqueous solution of 495 mg L^{-1} cyclohexanone (Sigma-Aldrich, Alcobendas, Spain) added as internal standard. Five gram of the mixtures were weighed in 40 mL glass vials with magnetic steel caps and polytetrafluoroethylene (PTFE) faced silicone septa (Agilent Technologies, Wilmington, DE, U.S.). A 2 cm × 50/30 μm StableFlex divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) coated SPME fibre (Supelco, Bellefonte, PA, U.S.) was used for headspace extraction. Before use, the fibre was conditioned according to manufacturer instructions in the GC injection port with Merlin Microseal™ at 270 °C for 1 h and afterwards placed into the SPME adapter of the autosampler. Vials were placed on the autosampler rack and, after equilibration at 37 °C for 20 min, the SPME fibre was exposed to the vial headspace for 30 min and then desorbed in the GC injection port at 260 °C for 10 min under splitless conditions and 2.5 split vent. Chromatographic separation was carried out in a Zebron 100% polyethylene glycol capillary column (60 m long; 0.25 mm internal diameter; 0.50 μm film thickness; ZB-WAXplus, Phenomenex, Torrance, CA, USA) with 1 mL min^{-1} Helium flow, with the following temperature program: 16 min at 45 °C, a first ramp at 4 °C min^{-1} to 110 °C, 9 min at 110 °C, a second ramp at 15 °C min^{-1} to 230 °C and 3 min at 230 °C, a final ramp at 10 °C min^{-1} to 250 °C and 2 min at 250 °C. Detection was performed with electron impact ionization, with 70 eV ionization energy operating in the full-scan mode at 1.74 scans s^{-1} from 33 to 280 amu. Interface, source and quadrupole temperatures were 280, 230 and 150 °C, respectively. External standards were regularly injected to corroborate the absence of instrumental drift. The absence of carry-over effects was tested by intercalating empty vials between samples. Compound identification was carried out by injection of commercial standards and by spectra comparison using the Wiley7Nist05 Library (John Wiley & Sons Inc., Hoboken, NJ, USA). The sums of the abundances of selected characteristic ions were used for the semi-quantitative determination of volatile compounds. To ease result comprehension by the reader, areas were multiplied by 10^{-5} (AU × 10^5).

2.7. Statistical analysis

Statistical treatment of data was performed by means of SPSS Win 19.0 program (SPSS Inc., Chicago, IL, USA). After testing the normality assumption of the dataset by the Shapiro-Wilk test and rejecting the null hypothesis, the Kruskal-Wallis test (non-parametric alternative to one-way ANOVA) was applied and pairwise comparisons on each pair of groups were calculated by the Dunn-Bonferroni test. Principal component analysis (PCA), with Varimax rotation, was carried out on the scores of sensory attributes and the levels of volatile compounds using the same statistical package.

3. Results and discussion

3.1. Lactic acid bacteria in commercial starters

Mesophilic LAB counts respectively were 14.21, 14.07 and 13.40 log CFU g^{-1} in MA11, MA16 and H13 lyophilized cultures. No growth of colonies was observed for MA11 or MA16 cultures when plated on MRS agar at 37 °C or Rogosa agar, but counts of 13.34 and 11.69 log CFU g^{-1} were obtained for H13 culture on those media, respectively.

MA11 culture isolates were identified as *Lactococcus lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris*, in a 80:20 ratio, whereas those from MA16 culture were all identified as *L. lactis* ssp. *lactis*. According to the manufacturer, both starters are composed by *L. lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris*. H13 culture isolates from MRS agar were all identified as *L. lactis* ssp. *lactis*, while those from Rogosa agar were identified as *Lactobacillus delbrueckii* and *Lb. helveticus*, in a 50:50 ratio.

3.2. Sensory evaluation of milk cultures

Significant ($P < .05$) differences for five of the six “dairy odour” attributes (fresh cheese, yogurt, butter, cooked milk and whey), two of the four “vegetal odour” attributes (fruity and nuts) and one of the four “roasted odour” attributes (burned) were recorded between bacterial species (Table 2), with a high variability among strains belonging to the same species. Dairy odour attributes were similar to those used by other authors for the characterization of fermented cow milk (Baruzzi et al., 2016; Gutiérrez-Méndez et al., 2008). The highest scores of those five dairy odour attributes generally corresponded to strains belonging to the different species of the genera *Leuconostoc* and *Lactococcus*. Sensory analysis of *L. lactis* strains isolated from different ecosystems revealed that wild strains of dairy origin presented intense yogurt-like and fresh cheese-like aromas while industrial strains presented low intensity for both odour descriptors (Gutiérrez-Méndez et al., 2008). In the present study, commercial starters generally obtained higher values for dairy odour attributes than wild strains (Table 2).

Vegetal odour attributes were not detected or reached scores below 0.5 in milk cultures of most wild LAB strains, and were not detected in milk cultures of commercial starters. Three of the four roasted odour attributes also reached low scores in milk cultures of most wild LAB strains and were absent from milk cultures of commercial starters. “Burned” odour notes were particularly intense in the case of *L. lactis*, with 43 positive strains which received scores ranging from 1.2 to 4.3, while one *Carnobacterium divergens* received a 1.2 score, one *L. raffinolactis* a 1.4 score, one *Streptococcus gallolyticus* a 2.2 score, and commercial starter H13 a 1.2 score.

3.3. Volatile profile of curds

A total of 34 volatile compounds were detected in lactic curds made with each one of the 56 selected wild LAB strains and the three commercial starters, by means of HS-SPME followed by GC–MS. Compounds grouped by chemical families, together with their retention times, the ions used for quantification and the identification method are listed in Table 3. They included five carboxylic acids, eleven alcohols, six aldehydes, six ketones, one ester, three terpenes and two miscellaneous compounds. The number of volatile compounds in individual curds ranged from 15 compounds for *Leuconostoc lactis* strain M882 to 33 compounds for *L. lactis* ssp. *lactis* strain M5. Variations in number and levels of volatile compounds showed differences in metabolic pathways and/or in enzyme activities among wild LAB strains and commercial starters. A higher number of volatile compounds were found in experimental small-size cow milk cheeses made with 32 wild *L. lactis* strains by Morales et al. (2003a) or with a *L. lactis* MG1363-derivative by Picon et al. (2008). The higher number of peaks found in those studies could be attributed to the use of rennet, the effect of cheese ripening, and the use of dynamic headspace extraction procedure vs the HS-SPME method used in the present work. Although freshly made curds of several types of cheese have similar bland flavours, ripening results in characteristic flavour generation through different chemical and biochemical reactions (McSweeney and Sousa, 2000; Smit et al., 2005). Many of the volatile compounds identified in the present study have been previously found in the volatile fraction of goat milk cheeses (Bontinis et al., 2012; Delgado et al., 2012; Di Cagno et al., 2007; Hayaloglu et al., 2013; Kondyli et al., 2016).

3.3.1. Acids

Acetic, butanoic, hexanoic and octanoic acids were detected in the volatile fraction of curds from all wild LAB strains and commercial starters (Table 4). Fatty acids (FA) are important components of the

Table 3
Volatile compounds identified in the headspace of curds made with selected strains of autochthonous lactic acid bacteria.

Compound	RT (min)	QI[a]	ID[b]
Acids			
Acetic acid	44.64	60, 45, 43	ST, MS
Butanoic acid	48.26	60, 73, 42	ST, MS
Hexanoic acid	51.59	60, 73, 87, 41	ST, MS
Octanoic acid	54.91	60, 73, 101	ST, MS
3-Methyl-butanoic acid	49.30	60, 43	MS
Alcohols			
Ethanol	13.15	45, 46, 43	ST, MS
1-Propanol	21.58	59, 42, 60	ST, MS
1-Butanol	28.28	56, 46, 44	ST, MS
1-Hexanol	39.14	56, 55, 43	ST, MS
2-Propanol	12.64	45, 43, 41, 39	ST, MS
2-Butanol	20.61	45, 49	ST, MS
2,3-Butanediol	47.44	43, 45, 61	ST, MS
2-Methyl-1-propanol	25.22	43, 41, 42, 33	ST, MS
2-Methyl-1-butanol	31.40	41, 57, 56, 70	ST, MS
3-Methyl-1-butanol	31.46	55, 70, 42, 57	ST, MS
Benzene-ethanol	52.60	91, 92, 122, 45	MS
Esters			
Ethyl acetate	10.50	43, 61, 70, 88	ST, MS
Aldehydes			
Ethanal	5.31	44, 43	ST, MS
2-Methylpropanal	7.35	43, 41, 72	ST, MS
2-Methylbutanal	11.46	57, 58, 41, 39	ST, MS
3-Methylbutanal	11.70	44, 41, 58, 71	ST, MS
Benzaldehyde	46.71	106, 105, 77	ST, MS
Benzeneacetaldehyde	49.01	91, 120	MS
Ketones			
2-Propanone	7.43	43, 58	ST, MS
2-Butanone	10.78	43, 72, 57, 42	ST, MS
2-Heptanone	30.17	43, 58, 71	ST, MS
2,3-Butanedione	16.32	43, 86	ST, MS
3-Hydroxy-2-butanone	35.57	45, 43, 88, 73	ST, MS
3-Hydroxy-2-pentanone	36.20	59, 43, 41	MS
Miscellaneous			
α-Pinene	19.85	93, 91, 92, 77	MS
β-Pinene	23.63	93, 69, 41	MS
δ-3-Carene	28.28	93, 91, 77, 79	MS
Butyrolactone	48.71	42, 86	MS
Naphthalene	50.50	128	MS

[a] QI: Ions used for the identification of compounds.

[b] ID: Peak identification: ST, comparison of spectra and retention time (RT) with commercial standards; MS, tentatively identified by spectra comparison using the Wiley Library.

flavour of many cheese types. They are not only aroma compounds by themselves, but also serve as precursors of methyl ketones, alcohols, lactones and esters (Curioni and Bosset, 2002). Short-chain ($C_{4:0}$-$C_{8:0}$) free FA are generated through the action of lipolytic enzymes. They have relatively high flavour perception thresholds (at ppm levels) and impart rancid, cheesy, pungent, goaty, soapy or waxy flavour notes (Holland et al., 2005). Milk contains a potent indigenous lipase that preferentially releases short- and medium-chain FA (Deeth, 2006). However, its relative heat instability precludes its contribution to lipolysis in pasteurized milk cheeses (Deeth, 2006; McSweeney and Sousa, 2000). LAB strains contribute to the production of short-chain ($C_{4:0}$-$C_{8:0}$), and medium-chain ($C_{10:0}$-$C_{14:0}$) free FA, as shown by comparing 1-d-old Cheddar cheeses acidified with lactic acid and glucono-δ-lactone, or inoculated with LAB strains (Hickey et al., 2006). Esterases of several LAB have been purified and characterised to various degrees, including those from *Lactococcus*, *Lactobacillus* and *Streptococcus* strains (reviewed by Holland et al., 2005). Although LAB are weakly lipolytic, they appear to be the principal lipolytic agent in pasteurized semi-hard cheeses (Collins et al., 2003). Apart from lipolysis, acetic, pyruvic and lactic acid are also formed from lactose and citric acid (Urbach, 1995). Only acetic and decanoic acids were detected in cow milk cheese-based media after growth of two *Lactobacillus casei* and two *Lb. rhamnosus* strains (Sgarbi et al., 2013).

The compound 3-methyl-butanoic acid was only detected in curds made with 25 of the 56 wild strains (8 *Lactococcus*, 7 *Lactobacillus*, 6 *Enterococcus*, 1 *Carnobacterium*, 2 *Pediococcus* and 1 *Streptococcus*) and was not found in curds made with commercial starters (Table 4). Differences were also detected among strains belonging to the same species. This compound was detected in curds made with four out of seven *L. lactis* ssp. *lactis*, one out of two *L. raffinolactis*, one out of four *Lb. casei*, one out of two *Lb. plantarum*, two out of five *E. faecalis*, two out of three *E. faecium/hirae* and one out of three *S. gallotyticus*. Branched-chain acids as 1-methyl-propanoic and 3-methyl-butanoic acids are generated through amino acid catabolism (Urbach, 1995), and are characteristic impact compounds of goat and sheep cheeses (Curioni and Bosset, 2002). Furthermore, 3-methylbutanoic acid, with its rancid, cheesy, sweaty and putrid odour, probably contributes to the very-ripened-cheese aroma (Yvon and Rijnen, 2001).

The amount of total volatile acids in curds made with wild LAB strains (Table 9) ranged from 148.75 AU × 10^5 for *Lb. brevis* to 917.96 AU × 10^5 for *E. faecalis* vs. 82.96 AU × 10^5 in uninoculated curd. A remarkable case in total volatile acid production, with a value of 1753.38 AU × 10^5, was that of commercial starter H13. Within wild LAB species, acids represented between 15.0 and 65.5% of total volatile compounds in curds, percentages which corresponded to *L. raffinolactis* and *Lb. fermentum*, respectively. In most curds, contribution to total volatile acids was, in decreasing order: acetic acid (derived from lactose and lactate metabolism), FA derived from lipolysis, and 3-methylbutanoic acid (derived from amino acid catabolism).

3.3.2. Esters

Ethyl acetate (Table 4) was the only ester identified in the volatile fraction of curds, being present in curds made with 50 of the 56 wild LAB strains, excepting three out of seven *L. lactis* ssp. *lactis*, one out of two *L. raffinolactis*, the *Lb. brevis* and the *Leuc. lactis* strains. Esters are common cheese volatile compounds, formed by esterification between short- to medium-chain fatty acids and primary and secondary alcohols derived from lactose fermentation or from amino acid catabolism (Curioni and Bosset, 2002). Due to their low flavour perception thresholds (at ppb levels), esters are key odorants in cheese, imparting fruity flavour notes (Holland et al., 2005). Ethyl esters are the dominant esters in cheese since ethanol is the most abundant alcohol (McSweeney, 2004). Ethyl acetate, described as juicy fruit, gum and apple (Curioni and Bosset, 2002), is produced by esterification of ethanol and acetic acid. Although both precursors were detected in curds made with all the wild LAB and commercial starters, ethyl acetate was not detected in curds made with six of the wild LAB strains. This compound was also detected in the volatile fraction of experimental cheeses made with wild *L. lactis* strains from sheep or cow milk (Centeno et al., 2002; Morales et al., 2003a), but not in cheese-based media after growth of two *Lb. casei* and two *Lb. rhamnosus* strains (Sgarbi et al., 2013).

The amount of total esters in curds ranged from 1.11 AU × 10^5 for *L. lactis* ssp. *lactis* to 15.02 AU × 10^5 for *Pediococcus pentosaceous* vs. 0.26 AU × 10^5 in uninoculated curd. Within wild LAB species, esters represented between 0.04 and 1.93% of the total volatiles in curds, percentages which corresponded to *L. lactis* ssp. *lactis* and *P. pentosaceous* respectively.

3.3.3. Alcohols

Ethanol was identified in curds made with all wild LAB strains and all commercial starters (Table 5). Ethanol is derived from lactose metabolism or from amino acid catabolism (Urbach, 1995). Primary alcohols 1-propanol, 1-butanol and 1-hexanol were detected in the volatile fraction of curds made with 43, 53 and 11 of the 56 wild LAB strains, respectively (Table 5). Primary alcohols are formed by enzymatic reduction by an alcohol dehydrogenase of the corresponding aldehydes (McSweeney and Sousa, 2000). Secondary alcohols 2-propanol

Table 4
Levels of acids[a] and esters[a] in the volatile fraction of curds made with selected strains of autochthonous lactic acid bacteria. Data (AU × 10^5) are reported as mean ± SD of the strains producing that specific compound (duplicate curds of each strain).

Genus	Species	Acetic acid			Butanoic acid			Hexanoic acid			Octanoic acid			3-Methyl-butanoic acid			Ethyl acetate		
Lactococcus	*lactis* ssp. *cremoris*	286.21	± 100.40	(2/2)	86.06	± 60.11	(2/2)	72.69	± 27.54	(2/2)	28.90	± 12.75	(2/2)	11.75	± 8.49	(2/2)	2.80	± 2.09	(2/2)
	lactis ssp. *lactis*	358.89	± 136.41	(7/7)	67.27	± 33.00	(7/7)	50.38	± 19.26	(7/7)	20.15	± 8.25	(7/7)	3.74	± 4.37	(4/7)	1.11	± 1.70	(4/7)
	garvieae	82.27	± 0.76	(1/1)	26.98	± 8.51	(1/1)	26.02	± 12.95	(1/1)	17.45	± 10.89	(1/1)	18.58	± 16.20	(1/1)	2.06	± 0.43	(1/1)
	raffinolactis	206.92	± 68.93	(2/2)	58.68	± 28.59	(2/2)	49.47	± 15.18	(2/2)	20.35	± 7.74	(2/2)	2.78	± 3.36	(1/2)	1.31	± 1.56	(1/2)
Lactobacillus	*brevis*	51.68	± 14.62	(1/1)	29.84	± 8.44	(1/1)	30.38	± 8.59	(1/1)	12.98	± 3.67	(1/1)	23.87	± 6.75	(1/1)	nd		(0/1)
	casei	253.01	± 213.86	(4/4)	52.04	± 28.78	(4/4)	45.47	± 25.53	(4/4)	22.51	± 10.50	(4/4)	4.03	± 8.43	(1/4)	1.61	± 1.85	(4/4)
	delbrueckii	79.97	± 19.47	(2/2)	40.45	± 9.79	(2/2)	38.42	± 9.16	(2/2)	22.96	± 8.58	(2/2)	12.06	± 2.92	(2/2)	1.14	± 1.50	(2/2)
	fermentum	532.64	± 210.65	(2/2)	57.04	± 18.26	(2/2)	50.47	± 16.66	(2/2)	25.42	± 6.22	(2/2)	nd		(0/2)	2.89	± 2.09	(2/2)
	gallinarum/helveticus	89.66	± 41.10	(2/2)	49.14	± 15.82	(2/2)	48.26	± 10.10	(2/2)	31.24	± 6.82	(2/2)	42.58	± 18.78	(2/2)	5.36	± 6.93	(2/2)
	ingluviei	259.85	± 124.19	(1/1)	23.26	± 6.47	(1/1)	21.54	± 3.94	(1/1)	12.46	± 5.89	(1/1)	nd		(0/1)	2.85	± 4.02	(1/1)
	plantarum	89.89	± 58.45	(2/2)	49.46	± 32.38	(2/2)	42.06	± 23.25	(2/2)	18.47	± 10.03	(2/2)	3.76	± 4.45	(1/2)	2.66	± 1.96	(2/2)
	rhamnosus	286.39	± 124.16	(2/2)	64.96	± 16.73	(2/2)	46.45	± 12.63	(2/2)	21.66	± 3.31	(2/2)	nd		(0/2)	1.84	± 0.46	(2/2)
Leuconostoc	*citreum*	284.79	± 80.55	(1/1)	47.86	± 13.54	(1/1)	42.42	± 12.00	(1/1)	19.97	± 5.65	(1/1)	nd		(0/1)	9.17	± 2.59	(1/1)
	holzapfelii	214.42	± 165.26	(1/1)	35.55	± 12.05	(1/1)	27.83	± 13.36	(1/1)	11.85	± 5.86	(1/1)	nd		(0/1)	1.82	± 0.37	(1/1)
	lactis	273.33	± 102.52	(1/1)	212.87	± 122.20	(1/1)	25.83	± 5.43	(1/1)	9.71	± 4.08	(1/1)	nd		(0/1)	nd		(0/1)
	mesenteroides	778.57	± 474.13	(6/6)	50.02	± 21.10	(6/6)	36.35	± 15.53	(6/6)	16.35	± 7.50	(6/6)	nd		(0/6)	4.02	± 3.93	(6/6)
Enterococcus	*casseliflavus/gallinarum*	262.37	± 121.49	(2/2)	59.88	± 26.15	(2/2)	42.64	± 16.95	(2/2)	20.57	± 10.98	(2/2)	nd		(0/2)	2.55	± 1.83	(2/2)
	durans	159.78	± 63.52	(2/2)	46.50	± 15.78	(2/2)	39.63	± 10.68	(2/2)	19.49	± 9.15	(2/2)	38.09	± 45.61	(2/2)	2.40	± 2.84	(2/2)
	faecalis	739.19	± 373.89	(5/5)	92.77	± 55.88	(5/5)	56.95	± 32.14	(5/5)	25.98	± 16.50	(5/5)	3.06	± 4.94	(2/5)	3.42	± 2.23	(5/5)
	faecium/hirae	254.34	± 158.02	(3/3)	47.74	± 20.53	(3/3)	41.96	± 18.41	(3/3)	26.09	± 14.10	(3/3)	5.55	± 10.93	(2/3)	1.75	± 2.01	(3/3)
Carnobacterium	*divergens*	113.79	± 10.30	(1/1)	25.14	± 1.14	(1/1)	22.01	± 6.96	(1/1)	12.18	± 7.85	(1/1)	15.58	± 10.70	(1/1)	1.79	± 2.53	(1/1)
Pediococcus	*acidilactici*	42.31	± 8.85	(1/1)	52.20	± 22.17	(1/1)	38.27	± 4.35	(1/1)	22.08	± 8.35	(1/1)	24.14	± 2.47	(1/1)	8.79	± 12.44	(1/1)
	pentosaceous	132.03	± 83.05	(1/1)	41.79	± 20.60	(1/1)	48.50	± 25.27	(1/1)	32.50	± 17.32	(1/1)	15.31	± 5.28	(1/1)	15.02	± 18.58	(1/1)
Streptococcus	*gallolyticus*	103.78	± 62.79	(3/3)	49.87	± 16.84	(3/3)	35.67	± 13.86	(3/3)	17.64	± 5.35	(3/3)	8.75	± 15.16	(1/3)	1.74	± 1.64	(3/3)
Weisella	*cibaria*	232.24	± 210.53	(1/1)	231.55	± 254.28	(1/1)	46.57	± 10.16	(1/1)	19.42	± 0.41	(1/1)	nd		(0/1)	2.33	± 3.29	(1/1)
Commercial starters	MA 11	354.61	± 104.90		96.41	± 25.02		62.59	± 21.14		29.00	± 18.68		nd			1.68	± 2.37	
	MA16	336.77	± 44.32		56.41	± 11.46		41.76	± 11.52		17.71	± 0.85		nd			1.22	± 1.73	
	H13	1622.27	± 145.36		57.89	± 7.37		46.91	± 3.13		26.31	± 4.22		nd			2.18	± 3.09	
Uninoculated curd		6.56	± 3.82		40.35	± 16.84		25.18	± 9.58		10.88	± 2.82		nd			0.26	± 0.51	

[a] nd, not detected. Between brackets, proportion of strains producing the compound.

Table 5
Levels of linear alcohols[a] in the volatile fraction of curds made with selected strains of autochthonous lactic acid bacteria. Data (AU × 10^5) are reported as mean ± SD of the strains producing that specific compound (duplicate curds of each strain).

Genus	Species	Ethanol			1-Propanol			1-Butanol			1-Hexanol			2-Propanol			2-Butanol		
Lactococcus	*lactis* ssp. *cremoris*	239.00	± 34.18	(2/2)	2.19	± 1.66	(2/2)	1.86	± 1.28	(2/2)	nd		(0/2)	nd		(0/2)	nd		(0/2)
	lactis ssp. *lactis*	270.74	± 152.18	(7/7)	2.42	± 1.50	(7/7)	1.70	± 1.16	(7/7)	0.03	± 0.10	(1/7)	nd		(0/7)	nd		(0/7)
	garvieae	134.41	± 4.57	(1/1)	0.63	± 0.90	(1/1)	3.01	± 0.70	(1/1)	nd		(0/1)	2.01	± 0.37	(1/1)	nd		(0/1)
	raffinolactis	114.31	± 107.79	(2/2)	1.50	± 1.81	(1/2)	1.22	± 1.11	(2/2)	nd		(0/2)	0.77	± 0.89	(1/2)	1.38	± 1.60	(1/2)
Lactobacillus	*brevis*	243.93	± 68.99	(1/1)	20.67	± 5.85	(1/1)	23.58	± 6.67	(1/1)	5.53	± 1.56	(1/1)	11.72	± 3.32	(1/1)	26.57	± 7.51	(1/1)
	casei	99.46	± 58.61	(4/4)	1.63	± 1.13	(4/4)	3.76	± 1.27	(4/4)	0.18	± 0.33	(1/4)	2.78	± 2.81	(4/4)	nd		(0/4)
	delbrueckii	88.61	± 36.73	(2/2)	1.45	± 1.81	(2/2)	4.57	± 2.42	(2/2)	1.90	± 0.82	(2/2)	0.40	± 0.81	(1/2)	0.74	± 1.48	(1/2)
	fermentum	76.79	± 39.89	(2/2)	1.38	± 1.85	(1/2)	3.47	± 0.87	(2/2)	nd		(0/2)	28.44	± 16.00	(2/2)	18.67	± 16.76	(2/2)
	gallinarum/helveticus	122.61	± 110.68	(2/2)	2.69	± 3.77	(1/2)	14.48	± 8.73	(2/2)	2.18	± 2.50	(2/2)	1.02	± 1.39	(2/2)	0.61	± 1.22	(1/2)
	ingluviei	58.75	± 7.41	(1/1)	0.95	± 0.68	(1/1)	2.14	± 0.78	(1/1)	nd		(0/1)	18.43	± 0.14	(1/1)	5.55	± 0.51	(1/1)
	plantarum	83.26	± 54.00	(1/2)	1.08	± 1.49	(1/2)	3.18	± 1.11	(2/2)	0.46	± 0.53	(1/2)	6.66	± 6.68	(2/2)	2.56	± 0.80	(2/2)
	rhamnosus	85.89	± 51.19	(1/2)	1.01	± 2.02	(1/2)	2.97	± 0.35	(2/2)	nd		(0/2)	1.18	± 1.54	(1/2)	nd		(0/2)
Leuconostoc	*citreum*	1102.28	± 311.77	(1/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)	2.26	± 0.64	(1/1)	nd		(0/1)
	holzapfelii	479.81	± 553.07	(1/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)	1.90	± 0.05	(1/1)	nd		(0/1)
	lactis	1056.04	± 203.68	(1/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)
	mesenteroides	500.31	± 193.48	(6/6)	0.69	± 0.99	(3/6)	1.25	± 1.21	(4/6)	nd		(0/6)	4.82	± 9.41	(4/6)	2.08	± 2.72	(4/6)
Enterococcus	*casseliflavus/gallinarum*	149.38	± 43.90	(2/2)	2.84	± 1.60	(2/2)	2.31	± 0.78	(2/2)	nd		(0/2)	0.39	± 0.78	(1/2)	nd		(0/2)
	durans	129.45	± 75.82	(2/2)	2.46	± 1.72	(2/2)	8.15	± 5.11	(2/2)	1.49	± 2.03	(1/2)	1.08	± 1.35	(1/2)	1.15	± 1.34	(1/2)
	faecalis	209.98	± 80.52	(5/5)	1.46	± 1.44	(4/5)	2.27	± 0.58	(5/5)	nd		(0/5)	0.16	± 0.51	(1/5)	nd		(0/5)
	faecium/hirae	76.90	± 19.37	(3/3)	1.63	± 1.18	(3/3)	2.29	± 0.62	(3/3)	nd		(0/3)	1.11	± 1.08	(2/3)	0.77	± 1.06	(1/3)
Carnobacterium	*divergens*	83.32	± 27.04	(1/1)	4.41	± 1.62	(1/1)	1.74	± 0.54	(1/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)
Pediococcus	*acidilactici*	208.13	± 33.63	(1/1)	7.91	± 1.74	(1/1)	26.95	± 5.58	(1/1)	6.31	± 2.69	(1/1)	3.19	± 0.83	(1/1)	1.97	± 0.05	(1/1)
	pentosaceous	207.33	± 51.33	(1/1)	4.72	± 2.48	(1/1)	19.91	± 5.58	(1/1)	2.29	± 1.69	(1/1)	3.42	± 2.13	(1/1)	0.98	± 1.39	(1/1)
Streptococcus	*gallolyticus*	99.44	± 67.18	(3/3)	0.96	± 1.52	(2/3)	0.66	± 0.69	(3/3)	nd		(0/3)	0.99	± 1.09	(2/3)	nd		(0/3)
Weisella	*cibaria*	167.37	± 14.01	(1/1)	4.08	± 0.93	(1/1)	12.48	± 13.73	(1/1)	nd		(0/1)	1.14	± 1.62	(1/1)	3.36	± 1.39	(1/1)
Commercial starters	MA 11	265.35	± 46.69		2.98	± 1.50		1.98	± 0.69		nd			nd			nd		
	MA16	210.61	± 12.02		0.80	± 1.14		1.70	± 0.54		nd			nd			nd		
	H13	190.46	± 66.60		1.26	± 1.78		2.51	± 1.68		nd			1.82	± 2.57		5.98	± 8.45	
Uninoculated curd		40.57	± 19.60		0.92	± 1.17		2.26	± 0.73		nd			1.03	± 1.19		nd		

[a] nd, not detected. Between brackets, proportion of strains producing the compound.

Table 6
Levels of diol and branched-chain alcohols[a] in the volatile fraction of curds made with selected strains of autochthonous lactic acid bacteria. Data (AU × 10^5) are reported as mean ± SD of the strains producing that specific compound (duplicate curds of each strain).

Genus	Species	2,3-Butanediol			2-Methyl-1-propanol			2-Methyl-1-butanol			3-Methyl-1-butanol			Benzene-ethanol		
Lactococcus	*lactis* ssp. *cremoris*	24.59	± 11.68	(2/2)	44.81	± 51.85	(1/2)	198.85	± 271.40	(1/2)	442.25	± 510.67	(1/2)	12.24	± 14.27	(1/2)
	lactis ssp. *lactis*	32.11	± 22.62	(7/7)	49.22	± 51.13	(5/7)	115.13	± 143.37	(4/7)	577.76	± 548.46	(5/7)	12.76	± 12.98	(5/7)
	garvieae	1.31	± 0.65	(1/1)	nd		(0/1)	nd		(0/1)	1.94	± 2.74	(1/1)	nd		(0/1)
	raffinolactis	10.41	± 20.82	(1/2)	54.03	± 62.40	(1/2)	176.34	± 206.14	(1/2)	497.95	± 574.46	(2/2)	12.55	± 14.50	(1/2)
Lactobacillus	*brevis*	1.86	± 0.53	(1/1)	2.30	± 0.65	(1/1)	nd		(0/1)	3.40	± 0.96	(1/1)	nd		(0/1)
	casei	2.50	± 4.80	(3/4)	nd		(0/4)	nd		(0/4)	1.06	± 1.98	(1/4)	nd		(0/4)
	delbrueckii	nd		(0/2)	nd		(0/2)	nd		(0/2)	0.65	± 1.30	(1/2)	nd		(0/2)
	fermentum	2.27	± 4.54	(1/2)	nd		(0/2)	nd		(0/2)	0.63	± 0.79	(1/2)	nd		(0/2)
	gallinarum/helveticus	nd		(0/2)	1.12	± 1.30	(1/2)	nd		(0/2)	nd		(0/2)	0.38	± 0.76	(1/2)
	ingluviei	3.39	± 4.79	(1/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)
	plantarum	nd		(0/2)	nd		(0/2)	nd		(0/2)	0.22	± 0.44	(1/2)	nd		(0/2)
	rhamnosus	nd		(0/2)	nd		(0/2)	nd		(0/2)	1.06	± 0.97	(2/2)	nd		(0/2)
Leuconostoc	*citreum*	nd		(0/1)	8.65	± 2.45	(1/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)
	holzapfelii	8.10	± 11.46	(1/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)
	lactis	2.55	± 3.61	(1/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)
	mesenteroides	22.86	± 28.07	(4/6)	nd		(0/6)	nd		(0/6)	nd		(0/6)	nd		(0/6)
Enterococcus	*casseliflavus/gallinarum*	0.39	± 0.79	(1/2)	nd		(0/2)	nd		(0/2)	nd		(0/2)	nd		(0/2)
	durans	nd		(0/2)	nd		(0/2)	nd		(0/2)	nd		(0/2)	nd		(0/2)
	faecalis	0.46	± 1.39	(1/5)	nd		(0/5)	nd		(0/5)	nd		(0/5)	nd		(0/5)
	faecium/hirae	1.13	± 2.04	(2/3)	nd		(0/3)	nd		(0/3)	nd		(0/3)	nd		(0/3)
Carnobacterium	*divergens*	24.33	± 5.78	(1/1)	8.42	± 1.79	(1/1)	nd		(0/1)	126.12	± 23.28	(1/1)	nd		(0/1)
Pediococcus	*acidilactici*	2.93	± 0.98	(1/1)	2.32	± 0.19	(1/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)
	pentosaceous	nd		(0/1)	2.39	± 1.42	(1/1)	nd		(0/1)	3.71	± 3.03	(1/1)	nd		(0/1)
Streptococcus	*gallolyticus*	11.82	± 14.44	(2/3)	nd		(0/3)	nd		(0/3)	nd		(0/3)	nd		(0/3)
Weisella	*cibaria*	nd		(0/1)	nd		(0/1)	nd		(0/1)	0.78	± 1.10	(1/1)	nd		(0/1)
Commercial starters	MA 11	67.45	± 37.85		nd			nd			nd			nd		
	MA16	49.13	± 4.52		nd			nd			nd			nd		
	H13	170.67	± 77.48		nd			nd			nd			nd		
Uninoculated curd		nd			nd			nd			nd			nd		

[a] nd, not detected. Between brackets, proportion of strains producing the compound.

and 2-butanol were less frequently detected, appearing in the volatile fraction of curds made with 32 and 18 wild LAB strains, respectively (Table 5). Secondary alcohols are formed by enzymatic reduction by an alcohol dehydrogenase of the corresponding methyl ketones (Curioni and Bosset, 2002). The compound 2,3-butanediol (Table 6) was detected in the volatile fraction of curds made with 31 wild LAB strains (11 *Lactococcus*, 6 *Lactobacillus*, 7 *Leuconostoc*, 4 *Enterococcus*, 1 *Carnobacterium*, 1 *Pediococcus* and 2 *Streptococcus*). It is formed by reduction of 2,3-butanedione (diacetyl), which derives from lactose and citrate metabolism (Curioni and Bosset, 2002). Branched-chain alcohol 2-methyl-1-propanol (Table 6) was identified in curds made with 13 wild LAB strains (7 *Lactococcus*, 2 *Lactobacillus*, 1 *Leuconostoc*, 1 *Carnobacterium* and 2 *Pediococcus*) while 2-methyl-1-butanol was only identified in curds made with 6 *Lactococcus* strains and 3-methyl-1-butanol in curds made with 19 wild LAB strains (9 *Lactococcus*, 7 *Lactobacillus*, 1 *Carnobacterium*, 1 *Pediococcus* and 1 *Weisella*). No branched-chain alcohols were detected in curds made with the commercial starters. Branched-chain alcohols 2-methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol respectively derive from valine, isoleucine and leucine catabolism, via the corresponding branched-chain aldehydes. They are considered key-flavour components in many raw milk cheeses (Fernández-García et al., 2002). A pleasant aroma of fresh cheese has been attributed to 3-methyl-1-butanol (Curioni and Bosset, 2002).

Benzene-ethanol (Table 6) was only detected in the volatile fraction of curds made with 7 *Lactococcus* strains (one out of two *L. lactis* ssp. *cremoris*, five out of seven *L. lactis* ssp. *lactis* and one out of two *L. raffinolactis*) and one out of two *Lactobacillus gallinarum/helveticus* strains. Benzene-ethanol is formed via catabolism of phenylalanine (McSweeney, 2004). It is among the most odorous aromatic alcohols, with rose flower notes, and has been identified in goat cheese and Gruyère (Curioni and Bosset, 2002).

Ethanol, 1-propanol, 2-propanol, 2-methyl-1-propanol and 3-methyl-1-butanol were among the alcohols identified in the volatile fraction of experimental cheeses made with wild *L. lactis* strains (Centeno et al., 2002; Morales et al., 2003a). Ethanol, 1-hexanol, 2-heptanol, 1-octanol, 1-nonanol, 1-undecanol, 3-methyl-1-butanol, 3-methyl-1-pentanol and 2-ethyl-1-hexanol were detected in cheese-based media after growth of two *Lb. casei* and two *Lb. rhamnosus* strains (Sgarbi et al., 2013). Benzene-ethanol was also detected in cheese-based media after growth of two *Lb. casei* and two *Lb. rhamnosus* strains (Sgarbi et al., 2013). Alcohols together with free fatty acids act as precursors in ester formation through esterification reactions catalysed by esterases (Liu et al., 2004).

The amount of total alcohols in curds (Table 9) ranged from 76.73 AU × 10^5 for *Enterococcus faecium/hirae* to 1120.34 AU × 10^5 for *Leuconostoc citreum* vs. 50.72 AU × 10^5 in uninoculated curd. Within wild LAB species, alcohols represented between 6.5 and 66.1% of the total volatiles in curds, percentages which corresponded to *Lactobacillus rhamnosus* and *Leuconostoc citreum*, respectively.

3.3.4. Aldehydes

Ethanal (Table 7) was detected in all curds, with the exception of the curd made with the *P. pentosaceous* strain. Aldehydes originate from amino acids either by transamination or by Strecker degradation, and are rapidly reduced to primary alcohols or oxidized to the corresponding acids (Curioni and Bosset, 2002).

The three branched-chain aldehydes identified in this study (Table 7) were only detected in curds made with 7 *Lactococcus* strains, while low levels of 2-methyl-butanal and 3-methyl-butanal were detected in the curd made with the *C. divergens* strain and 3-methyl-butanal was detected in curds made with the *Lb. brevis* and *Lb. ingluviei* strains. None of these compounds were detected in curds made with the commercial starters. Branched-chain aldehydes probably originate from amino acid degradation via enzymatic processes as well as non-enzymatic reactions (Strecker degradation). Within this group, 2-methyl-propanal, 2-methylbutanal and 3-methylbutanal are formed from valine, isoleucine and leucine, respectively (Curioni and Bosset, 2002). They have been identified as potent odorants in different cheese varieties (Curioni and Bosset, 2002). Aldehydes 2-methyl-butanal and 3-methyl-butanal were present in the volatile fraction of experimental cheeses made with wild *L. lactis* strains (Centeno et al., 2002; Morales et al., 2003a).

Benzaldehyde was detected in all curds of the present study with the only exception of the curd made with one out of five *E. faecalis* strains. It is formed by phenylalanine degradation (McSweeney, 2004). This compound was only detected in a model cheese system inoculated with four (two *Lb. plantarum*, one *Lb. mesenteroides* and one *Lb. rhamnosus*) out of 12 LAB strains (Randazzo et al., 2007).

Benzeneacetaldehyde was detected in curds made with seven *Lactococcus* and two *Lactobacillus* strains (Table 7). It can be produced by Strecker degradation of phenylalanine (McSweeney and Sousa, 2000) and is considered a major odour-active compound in cheese (Curioni and Bosset, 2002). This compound was not detected in a model cheese system inoculated with each one of 12 LAB wild strains (Randazzo et al., 2007).

The amount of total aldehydes in curds (Table 9) ranged from 2.39 AU × 10^5 for *Lactobacillus ingluviei* to 1102.23 AU × 10^5 for *L. lactis* ssp. *cremoris* vs. 42.82 AU × 10^5 in uninoculated curd. Within wild LAB species, aldehydes represented between 0.2 and 34.9% of total volatiles in the curds made with *Enterococcus faecalis* and *L. lactis* ssp. *cremoris*, respectively.

3.3.5. Ketones

Ketones 2-propanone, 2-butanone, 2,3-butanedione and 3-hydroxy-2-butanone (Table 8) were detected in curds made with all the wild LAB strains as well as in curds made with the commercial starters, although levels greatly varied. Ketones are present in most dairy products. The formation of 2,3-butanodione and 3-hydroxy-butanone in cheese and other dairy products is usually linked to LAB citrate and lactose metabolism (McSweeney and Sousa, 2000), but they can also be generated through amino acid catabolism (Kieronczyk et al., 2004; Ott et al., 2000). In cheese, the reduction of 2,3-butanedione produces 3-hydroxy-2-butanone, and subsequently 2-butanone (Urbach, 1995) and 2,3-butanediol (Ott et al., 2000). Ketones 2-propanone, 2-butanone, 2,3-butanedione and 3-hydroxy-2-butanone have been identified as odour-active compounds in Cheddar cheese with odour notes described as wood pulp or hay (2-propanone), butterscotch (2-butanone), butter (2,3-butanedione) or sour milk (3-hydroxy-2-butanone) (Curioni and Bosset, 2002). These ketones were all detected in the volatile fraction of cheeses made with wild *L. lactis* strains (Morales et al., 2003a) while only 2,3-butanodione and 3-hydroxy-2-butanone were detected in cheese-based media after growth of two *Lb. casei* and two *Lb. rhamnosus* strains (Sgarbi et al., 2013). Conversely to the results generally obtained for the other ketones, 3-hydroxy-2-pentanone (Table 8) was detected in curds made with 31 wild LAB strains (9 *Lactococcus*, 11 *Lactobacillus*, 7 *Enterococcus*, 1 *Carnobacterium*, 2 *Pediococcus* and 1 *Streptococcus*) and in curds made with two of the three commercial starters. This ketone is produced by reduction of 2,3-pentanedione, a diketone presumably linked to amino acid catabolism (Picon et al., 2008).

The only methyl ketone found was 2-heptanone (Table 8), which was detected in most curds, with the only exception of those made with *Leuc. citreum*, *Leuc. lactis*, two out of six *Leuc. mesenteroides* and *W. cibaria* strains. Methyl ketones are formed in cheese by the β-oxidation pathway and the enzymatic oxidative decarboxylation of free FA (Alewijn et al., 2005; Engels et al., 1997). The presence of 2-heptanone has been detected in Cheddar, Edam, Gouda, Gruyère, Maasdam and Parmesan cheeses (Engels et al., 1997) and higher levels of this methyl ketone were recorded in full-fat than in reduced-fat Cheddar cheese (Dimos et al., 1996). It was also found in the volatile fraction of model cheeses made with wild *L. lactis* strains (Morales et al., 2003a) and in a model cheese system inoculated with one out of two *Lb. casei*, one *Lb.*

Table 7
Levels of aldehydes[a] in the volatile fraction of curds made with selected strains of autochthonous lactic acid bacteria. Data (AU × 10^5) are reported as mean ± SD of the strains producing that specific compound (duplicate curds of each strain).

Genus	Species	Ethanal			2-Methyl-propanal			2-Methyl-butanal			3-Methyl-butanal			Benzaldehyde			Benzene-acetaldehyde		
Lactococcus	*lactis* ssp. *cremoris*	5.14	± 2.99	(2/2)	29.80	± 34.52	(1/2)	186.85	± 216.34	(1/2)	858.23	± 998.50	(1/2)	7.16	± 4.37	(2/2)	12.92	± 14.93	(1/2)
	lactis ssp. *lactis*	3.13	± 1.99	(7/7)	13.56	± 16.33	(5/7)	34.75	± 32.11	(5/7)	508.26	± 445.41	(5/7)	5.00	± 3.19	(7/7)	5.41	± 5.61	(5/7)
	garvieae	2.52	± 0.08	(1/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)	2.54	± 1.22	(1/1)	nd		(0/1)
	raffinolactis	2.28	± 1.54	(2/2)	47.98	± 58.60	(1/2)	73.22	± 85.25	(1/2)	435.63	± 509.95	(1/2)	5.01	± 0.59	(2/2)	3.44	± 3.99	(1/2)
Lactobacillus	*brevis*	3.13	± 0.88	(1/1)	nd		(0/1)	nd		(0/1)	1.18	± 0.34	(1/1)	1.28	± 0.36	(1/1)	nd		(0/1)
	casei	1.73	± 0.99	(4/4)	nd		(0/4)	nd		(0/4)	nd		(0/4)	4.23	± 3.68	(4/4)	0.33	± 0.92	(1/4)
	delbrueckii	1.96	± 1.18	(2/2)	nd		(0/2)	nd		(0/2)	nd		(0/2)	1.98	± 1.44	(2/2)	nd		(0/2)
	fermentum	2.08	± 1.01	(2/2)	nd		(0/2)	nd		(0/2)	nd		(0/2)	3.95	± 1.21	(2/2)	nd		(0/2)
	gallinarum/helveticus	1.91	± 1.47	(2/2)	nd		(0/2)	nd		(0/2)	nd		(0/2)	6.31	± 1.62	(2/2)	3.89	± 7.78	(1/2)
	ingluviei	0.75	± 1.05	(1/1)	nd		(0/1)	nd		(0/1)	0.17	± 0.24	(1/1)	1.07	± 0.27	(1/1)	nd		(0/1)
	plantarum	1.76	± 0.96	(2/2)	nd		(0/2)	nd		(0/2)	nd		(0/2)	2.42	± 1.22	(2/2)	nd		(0/2)
	rhamnosus	1.82	± 0.93	(2/2)	nd		(0/2)	nd		(0/2)	nd		(0/2)	2.95	± 1.86	(2/2)	nd		(0/2)
Leuconostoc	*citreum*	6.35	± 1.79	(1/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)	1.36	± 0.39	(1/1)	nd		(0/1)
	holzapfelii	2.85	± 1.95	(1/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)	2.51	± 0.99	(1/1)	nd		(0/1)
	lactis	6.55	± 1.97	(1/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)	1.39	± 0.24	(1/1)	nd		(0/1)
	mesenteroides	3.04	± 1.29	(6/6)	nd		(0/6)	nd		(0/6)	nd		(0/6)	1.88	± 0.96	(6/6)	nd		(0/6)
Enterococcus	*casseliflavus/gallinarum*	1.71	± 0.56	(2/2)	nd		(0/2)	nd		(0/2)	nd		(0/2)	2.98	± 1.89	(2/2)	nd		(0/2)
	durans	2.23	± 0.97	(2/2)	nd		(0/2)	nd		(0/2)	nd		(0/2)	2.78	± 1.97	(2/2)	nd		(0/2)
	faecalis	2.49	± 0.78	(5/5)	nd		(0/5)	nd		(0/5)	nd		(0/5)	2.29	± 1.63	(4/5)	nd		(0/5)
	faecium/hirae	1.33	± 0.89	(3/3)	nd		(0/3)	nd		(0/3)	nd		(0/3)	3.08	± 2.47	(3/3)	nd		(0/3)
Carnobacterium	*divergens*	1.32	± 0.74	(1/1)	nd		(0/1)	2.04	± 2.89	(1/1)	61.77	± 28.23	(1/1)	1.19	± 0.16	(1/1)	nd		(0/1)
Pediococcus	*acidilactici*	3.27	± 0.25	(1/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)	1.58	± 0.18	(1/1)	nd		(0/1)
	pentosaceous	nd		(0/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)	2.11	± 1.61	(1/1)	nd		(0/1)
Streptococcus	*gallolyticus*	1.73	± 0.72	(3/3)	nd		(0/3)	nd		(0/3)	nd		(0/3)	2.70	± 1.33	(3/3)	nd		(0/3)
Weisella	*cibaria*	1.72	± 0.46	(1/1)	nd		(0/1)	nd		(0/1)	nd		(0/1)	3.01	± 2.00	(1/1)	nd		(0/1)
Commercial starters	MA 11	5.17	± 1.45		nd			nd			nd			1.70	± 0.65		nd		
	MA16	3.77	± 1.93		nd			nd			nd			2.52	± 0.21		nd		
	H13	3.89	± 0.32		nd			nd			nd			1.57	± 0.52		nd		
Uninoculated curd		0.58	± 0.67		nd			nd			nd			1.62	± 0.32		38.00	± 17.58	

[a] nd, not detected. Between brackets, proportion of strains producing the compound.

Table 8
Levels of ketones[a] in the volatile fraction of curds made with selected strains of autochthonous lactic acid bacteria. Data (AU × 10^5) are reported as mean ± SD of the strains producing that specific compound (duplicate curds of each strain).

Genus	Species	2-Propanone			2-Butanone			2-Heptanone			2,3-Butanedione			3-Hydroxy-2-butanone			3-Hydroxy-2-pentanone		
Lactococcus	*lactis* ssp. *cremoris*	54.24	± 14.31	(2/2)	26.35	± 8.38	(2/2)	1.77	± 0.78	(2/2)	80.54	± 23.40	(2/2)	399.21	± 145.51	(2/2)	4.20	± 3.29	(2/2)
	lactis ssp. *lactis*	78.56	± 40.81	(7/7)	32.39	± 11.38	(7/7)	2.24	± 1.04	(7/7)	86.52	± 37.30	(7/7)	296.87	± 205.67	(7/7)	2.03	± 2.49	(5/7)
	garvieae	100.32	± 2.67	(1/1)	26.18	± 5.59	(1/1)	0.99	± 0.11	(1/1)	23.13	± 3.24	(1/1)	132.83	± 6.63	(1/1)	11.82	± 4.12	(1/1)
	raffinolactis	98.42	± 22.82	(2/2)	64.04	± 43.81	(2/2)	1.12	± 0.24	(2/2)	56.20	± 8.63	(2/2)	220.31	± 14.80	(2/2)	6.83	± 7.88	(1/2)
Lactobacillus	*brevis*	78.23	± 22.13	(1/1)	21.59	± 6.11	(1/1)	0.61	± 0.17	(1/1)	18.04	± 5.10	(1/1)	64.55	± 18.26	(1/1)	14.31	± 4.05	(1/1)
	casei	83.08	± 41.15	(4/4)	30.67	± 10.32	(4/4)	1.36	± 0.64	(4/4)	243.40	± 70.18	(4/4)	404.95	± 120.43	(4/4)	6.85	± 10.64	(2/4)
	delbrueckii	81.31	± 19.17	(2/2)	48.00	± 34.78	(2/2)	0.98	± 0.38	(2/2)	29.48	± 10.48	(2/2)	108.90	± 68.07	(2/2)	6.77	± 2.90	(2/2)
	fermentum	63.22	± 13.64	(2/2)	49.72	± 18.66	(2/2)	1.30	± 0.49	(2/2)	27.52	± 15.16	(2/2)	41.45	± 30.25	(2/2)	nd		(0/2)
	gallinarum/helveticus	89.20	± 29.49	(2/2)	84.72	± 44.69	(2/2)	2.65	± 0.90	(2/2)	44.56	± 10.81	(2/2)	89.07	± 90.44	(2/2)	6.82	± 6.13	(2/2)
	ingluviei	91.79	± 3.75	(1/1)	24.65	± 6.23	(1/1)	0.72	± 0.14	(1/1)	15.43	± 2.77	(1/1)	19.40	± 0.61	(1/1)	0.89	± 1.26	(1/1)
	plantarum	130.40	± 39.01	(2/2)	60.23	± 32.14	(2/2)	1.30	± 0.57	(2/2)	15.66	± 4.79	(2/2)	60.95	± 48.83	(2/2)	5.89	± 5.47	(2/2)
	rhamnosus	82.94	± 15.44	(2/2)	48.86	± 28.60	(2/2)	1.66	± 0.83	(2/2)	333.12	± 174.93	(2/2)	532.88	± 226.96	(2/2)	0.81	± 1.62	(1/2)
Leuconostoc	*citreum*	77.06	± 21.80	(1/1)	23.51	± 6.65	(1/1)	nd		(0/1)	5.96	± 1.68	(1/1)	21.49	± 6.08	(1/1)	nd		(0/1)
	holzapfelii	110.78	± 36.60	(1/1)	30.18	± 14.77	(1/1)	0.38	± 0.54	(1/1)	19.58	± 10.41	(1/1)	159.59	± 94.49	(1/1)	nd		(0/1)
	lactis	66.92	± 12.76	(1/1)	30.74	± 6.83	(1/1)	nd		(0/1)	3.82	± 0.35	(1/1)	1.73	± 2.45	(1/1)	nd		(0/1)
	mesenteroides	61.31	± 28.19	(6/6)	28.14	± 5.60	(6/6)	0.54	± 0.64	(4/6)	47.81	± 52.35	(6/6)	301.61	± 419.62	(6/6)	nd		(0/6)
Enterococcus	*casseliflavus/gallinarum*	92.27	± 16.44	(2/2)	35.72	± 11.50	(2/2)	0.98	± 0.78	(2/2)	35.62	± 12.83	(2/2)	132.82	± 125.66	(2/2)	3.02	± 2.31	(2/2)
	durans	96.72	± 21.96	(2/2)	52.90	± 39.07	(2/2)	0.84	± 0.57	(2/2)	36.78	± 11.38	(2/2)	250.80	± 153.36	(2/2)	8.61	± 5.77	(2/2)
	faecalis	73.92	± 37.61	(5/5)	25.87	± 10.46	(5/5)	1.03	± 0.80	(5/5)	108.45	± 68.48	(5/5)	982.00	± 579.02	(5/5)	1.94	± 4.43	(1/5)
	faecium/hirae	113.20	± 43.75	(3/3)	35.21	± 8.54	(3/3)	1.15	± 0.48	(3/3)	34.53	± 13.03	(3/3)	343.57	± 185.50	(3/3)	12.15	± 15.68	(2/3)
Carnobacterium	*divergens*	96.20	± 4.59	(1/1)	32.43	± 1.18	(1/1)	1.05	± 0.41	(1/1)	24.03	± 5.46	(1/1)	168.59	± 59.81	(1/1)	9.02	± 2.56	(1/1)
Pediococcus	*acidilactici*	98.21	± 5.14	(1/1)	32.55	± 9.47	(1/1)	1.35	± 0.11	(1/1)	16.74	± 4.25	(1/1)	88.06	± 7.49	(1/1)	41.65	± 5.85	(1/1)
	pentosaceous	63.48	± 11.23	(1/1)	27.73	± 1.94	(1/1)	1.63	± 1.39	(1/1)	16.09	± 8.33	(1/1)	82.89	± 58.77	(1/1)	20.40	± 24.86	(1/1)
Streptococcus	*gallolyticus*	81.66	± 24.04	(3/3)	40.72	± 24.59	(3/3)	1.04	± 0.61	(3/3)	61.56	± 39.01	(3/3)	304.27	± 262.21	(3/3)	7.18	± 17.58	(1/3)
Weisella	*cibaria*	60.77	± 16.71	(1/1)	63.15	± 55.30	(1/1)	nd		(0/1)	2.82	± 3.99	(1/1)	1.91	± 0.15	(1/1)	nd		(0/1)
Commercial starters	MA 11	58.14	± 12.14		27.58	± 4.38		2.41	± 0.46		84.87	± 0.76		183.47	± 48.15		3.54	± 1.84	
	MA16	48.17	± 18.17		17.85	± 5.66		2.07	± 0.27		39.98	± 21.87		257.87	± 71.33		nd		
	H13	45.85	± 7.08		16.78	± 9.72		1.90	± 0.06		272.33	± 2.73		2132.30	± 314.54		3.91	± 0.83	
Uninoculated curd		58.31	± 39.34		19.84	± 12.59		nd			0.99	± 1.97		1.10	± 0.84		nd		

[a] nd, not detected. Between brackets, proportion of strains producing the compound.

Table 9

Levels of chemical groups[1] in the volatile fraction of curds made with selected strains of autochthonous lactic acid bacteria and volatile index (VI)[2] of curds. Data (AU × 10^5) are reported as mean ± SD of all the selected strains (duplicate curds of each strain).

Genus	Species	Total acids		Total alcohols		Total aldehydes		Total ketones		Total terpenes		Total volatiles		VI
Lactococcus	*lactis* ssp. *cremoris*	485.61	± 189.34^{ab}	976.60	± 795.85^{a}	1102.23	± 1269.48^{a}	568.09	± 142.70^{ab}	22.11	± 15.95^{a}	3153.66	± 2025.13^{a}	11.25
	lactis ssp. *lactis*	500.43	± 166.62^{ab}	1072.30	± 785.18^{a}	571.69	± 475.50^{ab}	502.61	± 238.14^{ab}	21.02	± 15.11^{a}	2662.45	± 1191.45^{a}	9.50
	garvieae	171.29	± 15.39^{ab}	153.04	± 10.43^{ab}	5.06	± 1.30^{ab}	296.60	± 3.44^{ab}	27.05	± 21.90^{a}	650.89	± 8.61^{ab}	2.32
	raffinolactis	338.19	± 29.63^{ab}	888.94	± 985.11^{ab}	572.93	± 654.93^{ab}	447.71	± 41.10^{ab}	18.38	± 14.31^{a}	2249.89	± 1646.99^{a}	8.03
Lactobacillus	*brevis*	148.75	± 42.07^{ab}	354.38	± 100.23^{ab}	5.59	± 1.58^{ab}	198.87	± 56.25^{ab}	41.19	± 11.65^{a}	734.23	± 207.67^{ab}	2.62
	casei	377.05	± 269.17^{ab}	120.71	± 62.70^{b}	7.40	± 4.73^{ab}	771.29	± 166.48^{a}	24.34	± 15.66^{a}	1298.47	± 381.84^{ab}	4.63
	delbrueckii	193.86	± 25.61^{ab}	104.14	± 38.14^{b}	5.27	± 2.20^{ab}	277.08	± 73.39^{ab}	32.37	± 30.61^{a}	609.49	± 79.31^{b}	2.17
	fermentum	665.57	± 243.19^{ab}	143.33	± 17.70^{ab}	9.16	± 2.44^{ab}	183.82	± 57.49^{ab}	19.81	± 15.52^{a}	1016.69	± 199.82^{ab}	3.63
	gallinarum/helveticus	260.87	± 63.91^{ab}	154.27	± 128.86^{ab}	14.78	± 8.59^{ab}	318.18	± 67.19^{ab}	21.98	± 16.29^{a}	767.58	± 155.93^{ab}	2.74
	ingluviei	317.11	± 107.89^{ab}	100.41	± 3.27^{b}	2.39	± 2.13^{b}	154.56	± 8.42^{ab}	24.40	± 20.14^{a}	594.41	± 103.21^{b}	2.12
	plantarum	203.63	± 118.28^{ab}	106.62	± 55.18^{b}	7.18	± 5.72^{ab}	275.62	± 75.77^{ab}	21.20	± 13.35^{a}	610.10	± 93.52^{b}	2.18
	rhamnosus	419.45	± 150.25^{ab}	101.21	± 49.54^{b}	5.93	± 3.38^{ab}	1001.30	± 383.75^{a}	27.57	± 18.45^{a}	1548.63	± 435.62^{ab}	5.52
Leuconostoc	*citreum*	395.04	± 111.73^{ab}	1120.34	± 316.88^{a}	7.71	± 2.18^{ab}	128.02	± 36.21^{ab}	41.08	± 11.62^{a}	1696.00	± 479.70^{ab}	6.05
	holzapfelii	289.65	± 133.99^{ab}	496.64	± 567.60^{ab}	5.36	± 0.96^{ab}	323.89	± 161.59^{ab}	21.97	± 17.11^{a}	1134.11	± 555.07^{ab}	4.05
	lactis	521.74	± 234.23^{ab}	1062.45	± 212.74^{a}	7.94	± 2.22^{ab}	103.20	± 8.72^{ab}	23.33	± 22.16^{a}	2202.27	± 785.08^{a}	7.86
	mesenteroides	881.28	± 462.75^{a}	541.93	± 201.04^{ab}	5.48	± 1.21^{ab}	439.83	± 479.52^{ab}	21.65	± 14.31^{a}	1888.69	± 620.34^{ab}	6.74
Enterococcus	*casseliflavus/gallinarum*	385.46	± 161.54^{ab}	164.86	± 40.37^{ab}	6.97	± 2.73^{ab}	301.91	± 122.58^{ab}	21.75	± 14.46^{a}	878.19	± 302.07^{ab}	3.13
	durans	303.49	± 84.91^{ab}	151.97	± 86.37^{ab}	6.34	± 2.92^{ab}	447.88	± 156.25^{ab}	26.79	± 13.68^{a}	933.21	± 289.86^{ab}	3.33
	faecalis	917.96	± 407.55^{a}	223.06	± 78.13^{ab}	5.47	± 2.61^{ab}	1193.55	± 614.65^{a}	25.52	± 14.65^{a}	2365.67	± 969.36^{a}	8.44
	faecium/hirae	313.06	± 219.58^{ab}	76.73	± 40.19^{b}	4.97	± 3.88^{b}	451.09	± 292.38^{ab}	18.65	± 17.12^{a}	862.57	± 536.41^{ab}	3.08
Carnobacterium	*divergens*	188.71	± 5.05^{ab}	261.05	± 16.40^{ab}	66.32	± 30.22^{ab}	332.18	± 71.12^{ab}	22.17	± 20.26^{a}	864.65	± 67.71^{ab}	3.08
Pediococcus	*acidilactici*	179.00	± 46.19^{ab}	269.03	± 39.21^{ab}	4.85	± 0.43^{b}	281.04	± 21.66^{ab}	25.03	± 20.00^{a}	763.32	± 53.51^{ab}	2.72
	pentosaceous	270.13	± 140.96^{ab}	255.31	± 60.59^{ab}	2.11	± 1.61^{b}	213.21	± 78.81^{ab}	24.89	± 22.17^{a}	776.79	± 122.38^{ab}	2.77
Streptococcus	*gallolyticus*	215.72	± 87.98^{ab}	123.85	± 65.48^{b}	6.71	± 1.94^{ab}	502.81	± 302.85^{ab}	25.31	± 15.69^{a}	865.36	± 273.54^{ab}	3.09
Weisella	*cibaria*	529.79	± 475.37^{ab}	197.61	± 9.61^{ab}	7.20	± 2.07^{ab}	130.20	± 67.20^{ab}	27.70	± 11.91^{a}	885.67	± 539.43^{ab}	3.16
Commercial starters	MA 11	542.62	± 169.74^{ab}	343.63	± 79.60^{ab}	8.54	± 0.14^{ab}	360.84	± 38.51^{ab}	22.23	± 15.99^{a}	1276.81	± 201.57^{ab}	4.55
	MA16	452.65	± 66.45^{ab}	269.12	± 9.34^{ab}	7.45	± 3.36^{ab}	367.36	± 116.68^{ab}	21.85	± 17.10^{a}	1115.68	± 175.08^{ab}	3.98
	H13	1753.38	± 130.65^{a}	380.80	± 163.59^{ab}	6.59	± 1.80^{ab}	2473.07	± 334.96^{ab}	27.45	± 24.94^{a}	4639.51	± 23.61^{a}	16.55
Uninoculated curd		82.96	± 28.88^{b}	50.72	± 16.87^{b}	42.82	± 17.83^{ab}	82.65	± 50.68^{b}	27.26	± 12.77^{a}	280.33	± 67.24^{b}	1.00

[1] Values in the same column with different superscripts differ significantly ($P < .05$).

[2] Volatile index (VI) was obtained by dividing the total volatiles in each of the curds by the total volatiles in the uninoculated curd.

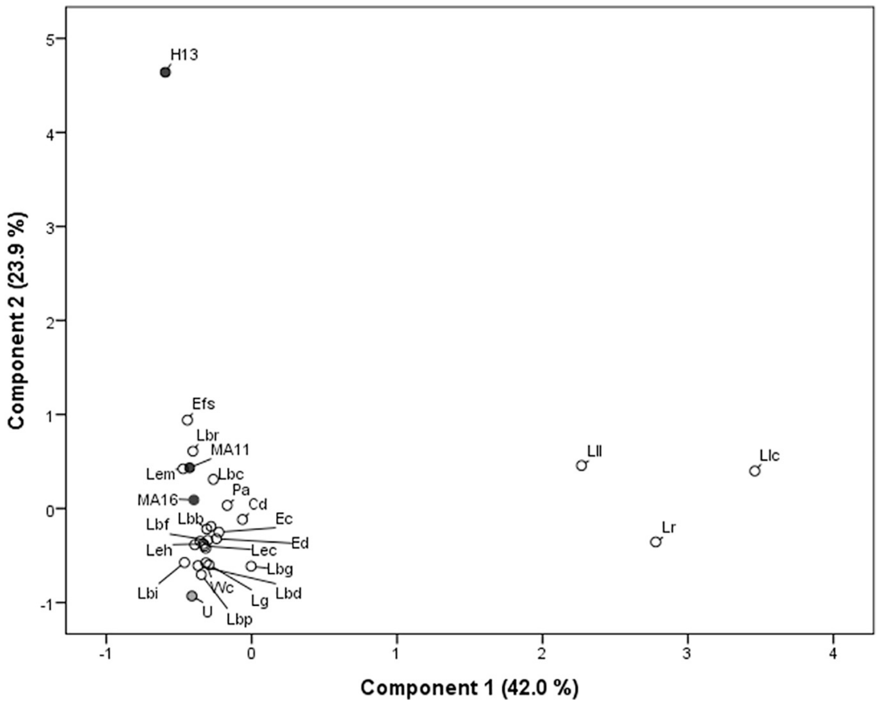

Fig. 1. Principal component analysis on the levels of 17 selected volatile compounds, the sum of roasted odour attributes and the volatile index of curds made with different strains of lactic acid bacteria and commercial starters.

Abbreviation: Llc, *Lactococcus lactis* ssp. *cremoris*; Lll, *Lactococcus lactis* ssp. lactis; Lg, *Lactococcus garvieae*; Lr, *Lactococcus raffinolactis*; Lbb, *Lactobacillus brevis*; Lbc, *Lactobacillus casei*; Lbd, *Lactobacillus delbrueckii*; Lbf, *Lactobacillus fermentum*; Lbg, *Lactobacillus gallinarum/helveticus*; Lbi, *Lactobacillus ingluviei*; Lbp, *Lactobacillus plantarum*; Lbr, *Lactobacillus rhamnosus*; Lec, *Leuconostoc citreum*; Leh, *Leuconostoc holzapfelii*; Lel, *Leuconostoc lactis*; Lem, *Leuconostoc mesenteroides*; Ec, *Enterococcus casseliflavus/gallinarum*; Ed, *Enterococcus durans*; Efs, *Enterococcus faecalis*; Efm, *Enterococcus faecium/hirae*; Cd, *Carnobacterium divergens*; Pa, *Pediococcus acidilactici*; Pp, *Pediococcus pentosaceous*; Sg, *Streptococcus gallolyticus*; Wc, *Weisella cibaria*; MA11, commercial starter; MA16, commercial starter; H13, commercial starter; U, uninoculated curd.

rhamnosus and one *E. hirae* strains after incubation for 60 days (Randazzo et al., 2007).

The amount of total ketones in curds (Table 9) ranged from 128.02 AU × 10^5 for *Leuconostoc citreum* to 1193.5 AU × 10^5 for *Enterococcus faecalis* vs. 82.65 AU × 10^5 in uninoculated curd. A remarkable case in total ketone production was commercial starter H13, with a value of 2473.07 AU × 10^5. Within wild LAB species, ketones represented between 4.7 and 64.6% of total volatiles in the curds made with *Leuconostoc lactis* and *Lb. rhamnosus*, respectively.

3.3.6. Other compounds

Terpenes α-pinene, β-pinene and δ-3-carene were detected, at low levels, in most curds, including those made with commercial starters and in uninoculated curd (data not shown). Terpenes, coming from plants, are transferred to the milk of grazing animals and end up in cheese. Their contribution to cheese flavour remains controversial (Curioni and Bosset, 2002). The compound α-pinene, one of the most frequently identified terpenes, with odour notes described as pine and green (Curioni and Bosset, 2002), was detected in the volatile fraction of experimental cheeses made with wild *L. lactis* strains (Centeno et al., 2002; Morales et al., 2003a). The relative abundance of α-pinene in raw milk cheese was not influenced by starter composition (Centeno et al., 2002). However, α-pinene relative abundance in pasteurized milk cheeses varied with the *L. lactis* strain used as starter, reaching higher levels in cheeses made with two out of four groups of *L. lactis* strains than in control cheese, and lower levels in cheeses made with the other two groups of strains (Morales et al., 2003a), a result which points to microbial bioconversion by *L. lactis* strains. The only terpene detected in a model cheese system inoculated with one out of five *Lb. plantarum* and one out of two *Lb. mesenteroides* strains after incubation for 60 days was limonene (Randazzo et al., 2007).

Total terpenes (Table 9) ranged from 18.38 AU × 10^5 for *L. raffinolactis* to 41.19 AU × 10^5 for *Lb. brevis* vs. 27.26 AU × 10^5 in uninoculated curd. Within wild LAB species, terpenes represented between 0.7 and 5.6% of total volatiles in the curds made with *L. lactis* ssp. *cremoris* and *Lb. brevis*, respectively.

Butyrolactone was the only lactone detected in most curds. Lactones are cyclic compounds that are generated by the esterification of hydroxy FA (McSweeney and Sousa, 2000). They are important contributors to cheese aroma due to their low perception thresholds, and are generally associated with peach and coconut odour descriptors (Curioni and Bosset, 2002). The presence of lactones in raw and pasteurized milk has also been reported (Castellani et al., 2017; Zhang et al., 2011). Butyrolactone levels varied from 0 for curds made with *L. brevis* and *L. rhamnosus*, *Leuc. citreum*, *holzapfelii*, *lactis* and two out of six *Leuc. mesenteroides* strains to 6.21 AU × 10^5 for curds made with *L. lactis* ssp. *lactis* vs. 2.34 AU × 10^5 in uninoculated curd.

3.3.7. PCA analyses

The first three components of a PCA including the 34 individual volatile compounds, the volatile index (Table 9) and the sums of dairy, vegetal and roasted odour attributes only explained 51.8% of the variance (data not shown). When only 17 selected volatile compounds, the volatile index and the sum of roasted odour attributes were included in the PCA, the first three components jointly explained 81.3% of the variance. Component 1, including most of the volatile compounds originated from amino acid catabolism (2-methyl-1-butanol, benzene-ethanol 3-methyl-1-butanal, 2-methyl-1-propanol, 3-methyl-butanol, 2-methyl-propanal, 2-methyl-butanal, benzaldehyde) and the sum of roasted odour attributes, explained 42.0% of the variance. Component 2, including volatile compounds originated from lactose and citrate metabolism (3-hydroxy-2-butanone, acetic acid, 2,3-butanediol, 2,3-butanodione) and the volatile index, explained 23.9% of the variance, and component 3 (including 1-hexanol, 1-butanol, 3-hydroxy-2-pentanone, 1-propanol and 3-methyl-butanoic acid) 15.4% of the variance. Most of the wild LAB strains together with commercial starters MA11 and MA16 were grouped in the second and third quadrants close to the intersection of the X and Y axes, whereas *L. lactis* subsp. *lactis*, *L. raffinolactis* and *L. lactis* subsp. *cremoris* were located in the first and fourth quadrants close to the X axis and far from the Y axis, and commercial starter H13 was located in the second quadrant far from the X axis and close to the Y axis (Fig. 1).

The levels of chemical groups of volatile compunds performed better than the levels of individual volatile compounds in the PCA. Thus, the first three components of a PCA including the levels of chemical groups of volatiles, the volatile index and the sums of dairy, vegetal and roasted odour attributes jointly explained 80.6% of the variance (Fig. 2). Component 1 included total acids, total ketones, the

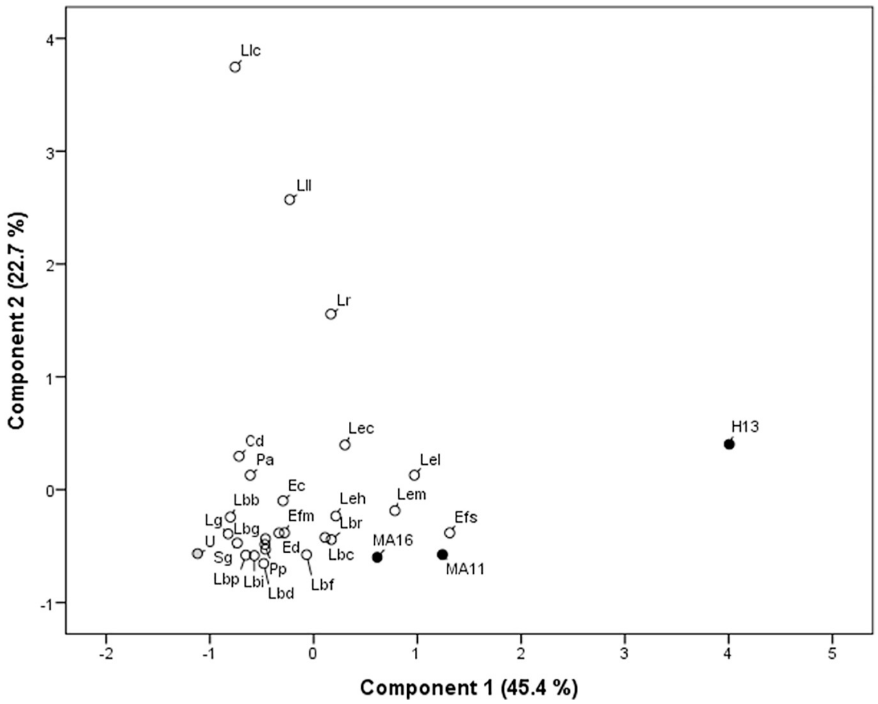

Fig. 2. Principal component analysis on the levels of chemical groups of volatile compounds, the sums of vegetable and roasted odour attributes and the volatile index of curds made with different strains of lactic acid bacteria and commercial starters.

Abbreviation: Llc, *Lactococcus lactis* ssp. *cremoris*; Lll, *Lactococcus lactis* ssp. lactis; Lg, *Lactococcus garvieae*; Lr, *Lactococcus raffinolactis*; Lbb, *Lactobacillus brevis*; Lbc, *Lactobacillus casei*; Lbd, *Lactobacillus delbrueckii*; Lbf, *Lactobacillus fermentum*; Lbg, *Lactobacillus gallinarum/helveticus*; Lbi, *Lactobacillus ingluviei*; Lbp, *Lactobacillus plantarum*; Lbr, *Lactobacillus rhamnosus*; Lec, *Leuconostoc citreum*; Leh, *Leuconostoc holzapfelii*; Lel, *Leuconostoc lactis*; Lem, *Leuconostoc mesenteroides*; Ec, *Enterococcus casseliflavus/gallinarum*; Ed, *Enterococcus durans*; Efs, *Enterococcus faecalis*; Efm, *Enterococcus faecium/hirae*; Cd, *Carnobacterium divergens*; Pa, *Pediococcus acidilactici*; Pp, *Pediococcus pentosaceous*; Sg, *Streptococcus gallolyticus*; Wc, *Weisella cibaria*; MA11, commercial starter; MA16, commercial starter; H13, commercial starter; U, uninoculated curd.

volatile index and the sum of lactic odour attributes, and accounted for 45.4% of the variance. Component 2 included total aldehydes, the sum of roasted odour attributes and total alcohols, and explained 22.7% of the variance. Component 3 included the sum of vegetable odour attributes and accounted for 12.4% of the variance. Most wild LAB strains and commercial starters MA11 and MA16 were located in the third and fourth quadrants, close to the intersection of the X and Y axes. *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis* and *L. raffinolactis* were located on the positive Y axis and commercial starter H13 on the positive X axis.

4. Conclusions

This study contributes to elucidate the role of wild LAB strains in the development of goat cheese flavour. Differences in the volatile profiles of wild LAB strains belonging to 24 species (some of them not previously investigated) and commercial starters are highlighted. Variations in the number and levels of volatile compounds illustrate differences in metabolic pathways and/or in enzyme activities between wild LAB strains and commercial starters. Major differences in volatile generation were related to amino acid catabolism. Volatile production differed significantly even among LAB strains of the same bacterial species, and points to the feasibility of strain selection and the use of wild LAB strains to modulate cheese flavour and aroma. Complex interactions among cheese microbiota may influence flavour production by individual strains and must be taken into account before the industrial use of strains, what would require further studies.

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