The individual contribution of starter and non starter lactic acid bacteria to the volatile organic compound composition of Caciocavallo Palermitano cheese

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ABSTRACT

The contribution of two starter (*Lactobacillus delbrueckii* and *Streptococcus thermophilus*) and nine non starter (*Enterococcus casselliflavous*, *Enterococcus faecalis*, *Enterococcus durans*, *Enterococcus gallinarum*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *Pediococcus acidilactici* and *Pediococcus pentosaceus*) species of lactic acid bacteria (LAB) to the volatile organic compounds (VOCs) of Caciocavallo Palermitano cheese was investigated. The strains used in this study were isolated during the production/ripening of the stretched cheese and tested in a cheese-based medium (CBM). The fermented substrates were analyzed for the growth of the single strains and subjected to the head space solid phase micro-extraction (HS-SPME) and gas chromatography – mass spectrometry (GC-MS). The 11 strains tested were all able to increase their numbers in CBM, even though the development of the starter LAB was quite limited. GC-MS analysis registered 43 compounds including seven chemical classes. A lower diversity of VOCs was registered for the unfermented curd based medium (CuBM) analysed for comparison. The class of ketones represented a consistent percentage of the VOCs for almost all LAB, followed by alcohols and esters. The volatile profile of *Pediococcus acidilactici* and *Lactobacillus delbrueckii* was mainly characterized by 2-butanol, butanoic acid and hexanoic acid and their esters, while that of *Lactobacillus casei* and *Lactobacillus rhamnosus* was characterized by 2,3-butanedione and 2-butanone, 3-hydroxy. In order to correlate the VOCs produced by Caciocavallo Palermitano cheeses with those generated by individual LAB, the 4-month ripened cheeses resulting from the dairy process monitored during the isolation of LAB were also analyzed for the volatile chemical fraction and the compounds in common were subjected to a multivariate statistical analysis. The canonical analysis indicated that the VOCs of the ripened cheeses are mainly influenced by *E. gallinarum*, *L. paracasei*, *L. delbrueckii*, *L. rhamnosus* and *L. casei* and that 1-hexanol, o-xylene and m-xylene were the cheese VOCs highly correlated with LAB.

Keywords: Lactic acid bacteria; Caciocavallo Palermitano; Ripened cheese; Volatile organic compounds; Cheese based medium.

1. Introduction

During cheese production, lactic acid bacteria can operate as starters or secondary cultures. Starter LAB (SLAB) are active in the fermentation process and basically convert lactose into lactic acid with the rapid acidification of the curd, while non starter LAB (NSLAB) are involved in cheese ripening (Gatti et al., 2014; Gobbetti et al., 2015). The characteristic aroma of ripened cheeses can be extremely variable because it is strongly influenced by the enzymatic activities of indigenous and/or added microorganisms (Oliszewski et al., 2013). Thus, although NSLAB strains are primarily responsible for the generation of aroma compounds, SLAB also contribute to this process (Carpino et al., 2017; Morea et al., 2007; Oliszewski et al., 2013; Randazzo et al., 2008).

The presence of different LAB species, necessary to transform milk into cheese (Guarcello et al., 2016), is generally detected through the entire traditional cheese making chains, including raw materials (milk and rennet) and dairy equipment (Cruciata et al., 2014; Di Grigoli et al., 2015; Franciosi et al., 2009; Settanni et al., 2012). In long-ripened cheeses, SLAB and NSLAB develop dynamically (Broadbent and Steele, 2005). Thus, the patterns of cheese volatile organic compounds (VOCs) are not only affected by raw materials and fermenting microorganisms, but also by the ripening time (McSweeney and Sousa, 2000). Cheese VOCs are strongly influenced by the microflora responsible for the ripening (Randazzo et al., 2008), but the role of each single member in the definition of the final volatile profile is difficult to retrieve in a complex microbial ecosystems.

Semi-hard stretched (pasta-filata) cheeses are characterized by a short ripening time. For “Caciocavallo” cheese typology, typical of Central and South Italy, this process lasts at least three or four months. Caciocavallo Palermitano cheese is one of the most widespread and marketed traditional cheeses in Western Sicily (Bonanno et al., 2013). Up to date, this cheese does not enjoy a ‘recognition of quality’ status and cheese makers have not restriction for production. Caciocavallo Palermitano cheese is made from raw milk of indigenous breed cows, Cinisara and Modicana, transformed in wooden vats with animal rennet without the addition of LAB cultures (Di Grigoli et al., 2015). In this conditions, the inoculation of the dairy LAB is ensured by the biofilms hosted onto the surface of the wooden vats (Licitra al., 2007; Scatassa et al., 2015).

Several studies have been published on the microflora of Caciocavallo and similar stretched cheeses (Mcsweeney and Sousa, 2000; Oliszewski et al., 2013). Most of these studies focused on the metabolic capabilities and the generation of VOCs with clear flavor descriptors of LAB, mainly SLAB such as *Lactococcus lactis*, *Lactobacillus delbrueckii* and *Streptococcus thermophilus* (Marilley and Casey, 2004; Morales et al., 2003; Randazzo et al., 2008). Recently, the contribution of NSLAB to the aroma of ripened cheeses has been object of a few studies (Bezerra et al., 2016; Lazzi et al., 2016; Sgarbi et al., 2013). In particular, Sgarbi et al. (2013) determined the VOCs generated by single strains of NSLAB in a synthetic medium that simulated a cheese environment, namely CBM. In that study, two strains of *L. casei* and two strains of *L. rhamnosus*, which dominate the NSLAB population during ripening of Parmesan cheese, where investigated. However, the strains of species not belonging to the group of LAB and present at subdominant levels during this process, have been found to contribute to the overall composition of cheese VOCs (Chaves-Lopez et al., 2006).

The aim of this work was to evaluate the generation of VOCs by LAB (SLAB and NSLAB) of traditional Caciocavallo Palermitano origin in a synthetic cheese medium (that mimics Caciocavallo Palermitano environment) and to correlate these compounds with those generated by cheese.

2. Materials and methods

2.1. Strains and growth conditions

Nine NSLAB (*E. casselliflavous* FMAC163, *E. faecalis* FMA721, *E. durans* FMAC134B, *E. gallinarum* FMA288, *L. casei* FMAC16, *L. paracasei* FMAC21, *L. rhamnosus* FMAC240, *P. acidilactici* FMAC31 and *P. pentosaceus* FMAC67) and two SLAB (*L. delbrueckii* FMAC8 and *S. thermophilus* FMA854) strains isolated from Caciocavallo Palermitano cheeses (Di Grigoli et al., 2015; Settanni et al., 2012) were used to evaluate the patterns of VOCs and retrieve their contribution to the aromatic profile of Caciocavallo Palermitano cheese.

Non starter *Lactobacillus* strains and *L. delbrueckii* were cultivated in MRS, while *S. thermophilus*, enterococci and pediococci in M17. The overnight incubation occurred at 44°C for SLAB and at 30°C for NSLAB. Growth media were purchased from Oxoid (Milan, Italy).

2.2. Growth of LAB in cheese based medium

After overnight growth in the optimal conditions, the strains were centrifuged at 5000 × rpm for 5 min, washed twice in Ringer's solution (Oxoid), re-suspended in the same solution until reaching an optical density at 600 nm (OD_{600}), measured with a 6400 Spectrophotometer (Jenway Ltd., Felsted, Dunmow, UK), of ca. 1.00 which approximately corresponds to a cell density of 10^8 - 10^9 CFU/mL (depending on the cell morphology) of each LAB (Guarcello et al., 2016).

A cheese based medium (CBM) was prepared following the protocol described by (Neviani et al., 2009) with some modifications. A 4-month ripened Caciocavallo Palermitano cheese, purchased from a local market, was used to prepare CBM. One hundred and twenty grams of grated cheese were dissolved in 1 L of sodium citrate solution (0.07 mol/L, pH 7.5) and heated at 45°C for 50 min. The cheese suspension was centrifuged at 8000 rpm × 15 min and filtered through sterile cotton gauze to remove surfaced fat layer and, after that, the resulting solution was sterilized for 15 min at 121°C. Cell suspensions were inoculated (1% v/v) in vials (20 mL volume) containing 5 mL of CBM (Sgarbi et al., 2013). The vials were sealed with parafilm to avoid the dispersion of VOCs and incubated anaerobically in hermetic jars added with the AnaeroGen AN25 system (Oxoid) for 4 d at 30 and 44°C for NSLAB and SLAB, respectively. CBM without inoculums was used for comparison in order to evaluate the VOCs produced by the indigenous cheese microbiota. Furthermore, a curd based medium (CuBM) was included as a non fermented control medium to compare the spectra of the VOCs generated by the selected strains. CuBM was prepared as described per CBM using 120 g of grated curd in place of cheese. Each trial was carried out in duplicate.

The levels of LAB soon after inoculation and after 4-d fermentation in CBM were determined by plate count on MRS (NSLAB and SLAB rods) and M17 (SLAB cocci) incubated at the optimal temperatures for 48 h under anaerobiosis conditions. Analyses were carried out in triplicate.

2.3. Analysis of volatile organic compounds of fermented CBM and ripened cheeses

The characterization of VOCs produced by the fermented CBMs was carried out through a gas chromatographer with a single quadrupole mass spectrometer detector (GC-MS) (QP2010, Shimadzu), coupled with a fused-silica capillary column coated with a polar stationary phase (OMEGAWAX ^{TM250}, 30m x 0.25 mm x 0.25μm, Supelco, USA).

The traditional Caciocavallo Palermitano cheeses object of this study were previously produced according to Settanni et al. (2012) and collected at 120 d of ripening. The cheeses were brought to room temperature and the rind removed before being grated. Samples of 4 g (Randazzo et al., 2008) of grated Caciocavallo Palermitano cheeses were placed in 20 mL vials, as described above for the tests carried out with CBM.

The headspace solid phase micro extraction (HS-SPME) was applied as sampling technique, using a carboxen/polydimethylsiloxane (CAR/PDMS) fiber (Supelco, USA). The CAR/PDMS fiber was exposed to the headspace for 30 min, after preheat of 5 min at 45°C in a water bath. The absorbed molecules were desorbed into the injection port for 5 min at 250°C. Volatile molecules were separated with following temperature program: held 5 min at 40°C , 5°C min^{-1} to 185 °C, held 1 min, 5°C min^{-1} to 200 °C, held 10 min, 10 °C min^{-1} to 240 °C. Helium was used as the carrier gas. The MS interface and the ion source were kept at 250°C and 230°C respectively. Acquisition was performed in electron impact mode (70 eV); the mass range used was m/z 40-350. Compounds were identified by matching mass spectra with the NIST library (GCMS Solution Library, Shimadzu) and/or spectra published in literature. The mass spectra observed were compared with mass spectra from the NIST databank taken in consideration spectra with similarity major of 95%. The peak areas were used directly to give the percentage volatile composition by dividing the area of each peak to the total area under all of the peaks. Three replicates (vials) were analyzed for each sample.

2.4. Statistical and explorative multivariate analyses

Data were statistically analyzed using SAS 9.2 software. The VOCs identified in CBM inoculated with each of the 11 LAB strains were analyzed by the GLM procedure with a monofactorial model in which the effect of LAB strain was considered at 12 levels (non-inoculated CBM plus 11 LAB strains in CBM). The comparisons were made exclusively between CBM *versus* the 11 LAB strains used; the differences were tested with the Tukey's test and significance was at $P \leq 0.05$.

A multivariate statistical approach was applied with the canonical discriminant analysis according to the CANDISC procedure, in order to correlate the VOCs generated by Caciocavallo Palermitano cheeses with those generated by individual LAB in CBM.

3. Results and discussion

3.1. Growth of LAB in synthetic medium

The growth of the 11 LAB strains in CBM is reported in Table 1. The initial levels of the strains ranged between 5.24 and 6.73 Log CFU/mL. All strains showed a clear growth in CBM indicating the suitability of this synthetic medium to perform the evaluation of the VOCs profile of single strains confirming the previous observations of Sgarbi et al. (2013). In general, the most consistent development was registered for NSLAB strains with *L. rhamnosus* FMAC240, *P. pentosaceus* FMAC67, *E. durans* FMAC134B and *E. faecalis* FMA721 showing an increase of about 2 orders of magnitude. As expected, SLAB were not able to develop at high numbers in a cheese model, since their increase was barely of 0.45 – 0.69 Log CFU/mL. This observation is due to the absence of lactose in CBM (Neviani et al., 2009) resulting from the activity of the SLAB (Dìaz-Muñiz et al., 2006) that fermented the cheese used for the preparation of the synthetic medium. No growth was estimated in the control trials, confirming that no contaminating microorganisms were present in CBM.

3.2. Composition of VOCs from fermented CBM

After CBM fermentation, the total profile of the VOCs produced by the 11 strains included 43 compounds, almost the same number of compounds detected by Sgarbi et al. (2013) who used a similar CBM system. In a previous work carried out by Carpino et al. (2017) on the contribution of LAB to the cheese VOCs, the authors analyzed the composition of the VOCs generated after UHT milk fermentation and found a lower number of molecules in comparison to our work. The difference among the number of VOCs found might be attributable to the higher complexity of the VOCs of CBM in comparison to those of fermented milk. Thus, CBM seems to be a useful medium to evaluate the production of cheese VOC, supporting the finding of Sgarbi et al. (2013). However, the non-inoculated CBM showed a quite similar VOC spectrum, in terms of compound composition rather than relative abundances, of the CBM inoculated with the selected LAB strains. Thus, the control CuBM allowed the comparison of the molecules attributable to the LAB activity.

The composition of VOCs registered for each LAB in CBM, the native cheese microbiota (non-inoculated CBM) and the unfermented matrix (CuBM) is reported in Table 2. Seven chemical classes were recognized for inoculated and non-inoculated CBM: alcohols, aldehydes, ketones, esters, aromatic hydrocarbons, organic acids and hydrocarbons. A lower diversity of VOCs was displayed by CuBM, for which alcohols, organic acids and hydrocarbons were below the detection limits. The VOCs identified in CBM have importance on the sensory characteristics of cheeses and some of them are related to specific sensory descriptors (Acree and Arn, 2004; Fuchsmann et al., 2015; Morales et al., 2003; Singh et al., 2003).

The class of ketones represented a consistent percentage of the VOCs for almost all bacteria (from 52.31 at 81.26 relative abundance %) and were also registered in CuBM (14.21 %). Due to the high differences among the relative abundance % of CuBM and CBM, it is clear that this class of chemical, with the exception of *P. acidilactici* (12.30 %) is generated by LAB. The highest production of ketones was registered for *P. pentosaceus* (81.26 %) indicating an opposite behavior of the two strains of *Pediococcus* genus in CBM. Ketones are generated from the β-oxidation of fatty acids (Guillen et al., 2004) and are commonly present in dairy products. These compounds have a characteristic odor and a low perception thresholds. Among the ketones identified, 2,3-butanedione (diacetyl), detected at high levels for *L. casei* and *L. rhamnosus*, and its reduction product 2-butanone, 3-hydroxy- (acetoin), significantly associated to *P. pentosaceus*, are mainly associated to pyruvate, lactose or citrate metabolism by some LAB starters (Irmler et al., 2013). In fact, acetoin was not detected in CuBM confirming that this compound is only generated during fermentation. Diacetyl was detected at low levels in CuBM showing that, except for *P. acidilactici*, it was produced during fermentation.

The classes of alcohols and esters followed ketones in terms of relative abundance percentage of VOCs. Alcohols were not detected in CuBM since they derive from fermentation processes (mainly linked to reducing NADH-dependent enzymes). In our work, the highest relative abundances % of these compounds were generated during the development of *P. acidilactici*, *L. delbrueckii* and *L. paracasei* (27.74, 21.08 and 17.66 % respectively, P<0.0001). 2-Butanol was the major alcohol detected in all inoculated CBM. However, with the exception of *P. acidilactici*, *L. delbrueckii* and *L. paracasei*, the relative abundance % registered for non-inoculated CBM was comparable or slightly higher than that registered for the other strains. Although the alcohols have limited influence on the flavor of cheese, due to their high sensory thresholds, they represent an index of the fermentation process (Langler et al., 1967). On the other hand, esters are important contributors to the flavor of many cheeses (Urbach, 1995) even at low concentrations, due to their high volatility at room temperatures (Bontinis et al., 2012). Most esters have floral, fruity and yeasty notes. They are formed during the esterification of alcohols and fatty acids either by microorganisms or by chemical reactions (Majcher et al., 2011). The last phenomenon might explain the presence of esters, mainly 1-propen-2-ol acetate and methyl salicylate, in the unfermented CuBM. Both compounds were registered at lower levels after fermentation. In our study, the largest contribution to the production of esters was given by *P. acidilactici* and *L. delbrueckii* (20.25 and 18.53 % respectively, P≤0.0001). Within these odor compounds, formed mainly by the same two LAB (*P. acidilactici* and *L. delbrueckii),* it is worth noting the presence of butanoic acid ethyl ester and hexanoic acid ethyl esters, considered compounds who give fruity notes (Bontinis et al., 2012).

P. acidilactici generated a significantly higher (P≤0.002) relative abundance % of aldehydes than those registered for CBM, and the other strains. Except, benzaldehyde, 3-methyl that was the only aldehyde detected in CuBM, all other compounds within this class were produced during fermentation. Aldehydes are the main products of the autoxidation of unsatured fatty acids (Gioacchini et al., 2010) and are responsible of herbaceous aroma (Engels et al., 1997). Generally, some aldehydes may derive from the catabolism of amino acids, such as butanal-3-methyl from leucine and butanal-2-methyl form isoleucine (Urbach, 1995). These compounds, with a malt note, are usually produced by thermophilic LAB (Barbieri et al., 1994; Neeter et al., 1996). Thus, these results shows an unknown characteristic of *P. acidilactici*. However, aldehydes do not accumulate in cheese because they are rapidly converted into alcohols or into the corresponding acids.

Aromatic hydrocarbons can derive from the degradation of amino acids. Most of these compounds tend to increase during ripening (Gioacchini et al., 2010). In particular, *S. thermophilus* FM854 showed the highest production of these chemicals (1.14%). Among NSLAB, the relative abundances % of aromatic hydrocarbons produced by *Enterococcus* and *Pediococcus* strains were higher than those found in CBM and those generated by *Lactobacillus* strains. Interestingly, the highest relative abundance % of m-di-tert-butylbenzene was registered for CuBM. Enzymes with lipolytic activity (esterases, lipases) cause the release of linear-chain acids from fatty acids (Curioni and Bosset, 2002; Tavaria et al., 2004). These molecules are typical in cheese productions; in our study, *P. acidilactici* and *L. delbrueckii* produced consistent levels of these organic acids (26.89 and 18.30 %, $P \leq 0{,}0001$), in particular, butanoic acid (rancid, cheese, sweat notes) and hexanoic acid (sweat note). These two organic acids are usually important component of cheese flavor (Toso et al., 2002).

Hydrocarbons are a family of secondary products deriving from forage fed to animals or produced during ripening as a result of lipid autoxidation (Barbieri et al., 1994). In our work, these compounds were detected only in the CBM fermented by three *Enterococcus* strains with the exclusion of *E. gallinarum*. Due to their high sensory threshold values, hydrocarbons themselves probably do not make a major contribution to the final aroma of cheeses, but serve as precursors for the formation of other aromatic compounds (Bontinis et al., 2012).

3.3. Correlation between VOCs produced by the individual strains and VOCs of LAB origin detected in Caciocavallo Palermitano cheese

In order to correlate the VOCs detected in 4-month ripened cheeses with those of LAB origin, the compounds detected in fermented CBMs were used to filter the results from the head space of the Caciocavallo Palermitano cheese samples (Table S1). Thus, only the VOCs in common with LAB were retained from cheeses (Table 3) and used for the statistical analysis. Candisc analysis revealed that 1-hexanol, o-xylene and m-xylene were the cheese VOCs that showed the highest positive correlations with LAB, while hexanal, 2-butanone and ethyl acetate were those inversely highly correlated.

1-hexanol, related to green notes (Poveda et al., 2008), is one of the most abundant alcohols detected in aged cheeses and is formed during ripening (Ocak et al., 2015), In general, raw milk cheeses contain greater amounts of alcohols than pasteurized milk cheeses (Ortigosa et al. 2001; Rodriguez-Alonso et al. 2009) and this is due to their higher microbial diversity. Furthermore, the production of hexanol has been reported to be influenced by the high temperatures which impact also the metabolism of certain species such as *L. casei* (De Filippis et al., 2016) and Caciocavallo Palermitano cheese is produced applying the stretching technology that includes a treatment of the acidified curd at 85 – 90°C. o-Xylene and m-xylene are aromatic hydrocarbons responsible for geranium and roasted/fatty/old oil/bread notes, respectively (Acree and Arn, 2004; Fuchsmann et al., 2015), not very common in cheeses. In particular, m-xylene has been detected in red Swiss Tilsit cheese (Fuchsmann et al., 2015) and unspecified isomers of xylene have been identified as volatile components of Beaufort cheese from the French Alps (Dumont and Adda 1978).

The plot resulting from the multivariate analysis (Fig. 1) indicated that the VOCs of LAB origin that characterized the ripened cheeses were closed to those of CBM, confirming the suitability of this synthetic medium to study the *in vitro* generation of VOCs, and those of the following species: *E. gallinarum*, *L. paracasei*, *L. delbrueckii*, *L. rhamnosus* and *L. casei*. The relevant role of *L. casei*, *L. rhamnosus* and *L. paracasei* on the final cheese volatile chemical fraction might be easily explained with the fact that these three species are typical NSLAB of several ripened cheeses (Beresford et al., 2001; De Angelis et al., 2001; Gardiner et al., 1998) and that they dominated the LAB populations of Caciocavallo Palermitano cheese at four months of ripening (Di Grigoli et al., 2015). On the contrary, the influence of the VOCs from the starter LAB strain *L. delbrueckii* FMAC8 to the aroma profile of cheeses deserves a deepen attention. This finding could be due to the high persistence of *L. delbrueckii*, found in Caciocavallo Palermitano until 60 d of ripening (Di Grigoli et al., 2015). Thus, the volatile metabolites generated by the starter species that dominate the microbial community during the first phases of Caciocavallo Palermitano cheese production (Settanni et al., 2012) are still defining at this stage. The VOCs from *S. thermophilus* were quite distant from those of the cheeses, probably because this species was no more detected in cheese after 30 d of ripening (Di Grigoli et al., 2015) and cannot influence strongly the profile of the ripened cheeses.

Regarding *Enterococcus* population, only *E. gallinarum* FMA288 showed a VOC pattern contributing strongly to the final cheese profile. *E. gallinarum* has been isolated from different fresh (Nassib et al., 2013) or short-term (2-month) ripened cheeses (Suzzi et al., 2000) and also Di Grigoli et al. (2015) detected this species for no longer than 60 d indicating that this species does not exert the same role of *E. casseliflavus, E. faecalis* and *E. durans* that are generally associated with cheeses ripened for more than two months (Arizcun et al., 1997; Gelsomino et al., 2001; Todaro et al., 2011). The results of this work confirm that enterococci contribute consistently to the development of the organoleptic characteristics of typical cheeses (Foulquié Moreno et al., 2006), although concerning Caciocavallo Palermitano cheese this finding is mainly due to *E. gallinarum* until four months of ripening, that can be considered an intermediate ripening in comparison with other hard cheeses ripened for more than 12 months.

4. Conclusions

Among the sensorial characteristics of cheeses, the volatile chemical compounds are defining for the consumers' acceptance. VOCs have different odor activity and the differences in their composition determine differences in the sensory characteristics of cheeses. In the present study, the ability of SLAB and NSLAB to produce VOCs in synthetic medium was evaluated and all strains tested were able to grow in CBM, making the comparison of the VOCs patterns possible. The strains of *L. delbrueckii*, *L. casei*, *L. paracasei*, *L. rhamnosus* and *E. gallinarum* influenced the development of characteristic cheese aromatic compounds. The aromatic profile generated by *L. casei* and *L. rhamnosus,* the LAB species mostly isolated from different Caciocavallo cheeses, was different from that of the other strains and control CBM and was able to produce typical flavor compounds as 2,3-butanedione and 2-butanone, 3-hydroxy. The high chemical complexity found in a ripened cheese is the result of very complex relationships and mutual influence of several microorganisms; with this study, the individual contribution of the starter (*L. delbrueckii* and *S. thermophilus*) and non starter (*E. casselliflavus, E. faecalis, E. durans, E. gallinarum, L. casei, L. paracasei, L. rhamnosus, P. acidilactici* and *P. pentosaceus*) LAB to the volatile profile of Caciocavallo Palermitano cheese was clarified.

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References

Acree, T., Arn, H., 2004. Flavornet and human odor space: Gas chromatography–olfactometry (GCO) of natural products.

Arizcun, C., Barcina, Y., Torre, P., 1997. Identification and characterization of proteolytic activity of *Enterococcus* spp. isolated from milk and Roncal and Idiazabal cheese. Int. J. Food Microbiol. 38, 17–24.

Barbieri, G., Bolzoni, L., Careri, M., Mangia, A., Parolari, G., Spagnoli, S., Virgili, R., 1994. Study of the volatile fraction of Parmesan cheese. J. Agric. Food Chem. 42, 1170–1176.

Beresford, T.P., Fitzsimons, N.A., Brennan, N.L., Cogan, T. M., 2001. Recent advances in cheese microbiology. Int. Dairy J. 11, 259–274.

Bezerra, T.K.A., de Oliveira Arcanjo, N.M., de Araújo, A.R.R., de Queiroz, A.L.M., de Oliveira, M.E.G., Gomes, A.M.P., Madruga, M.S., 2016. Volatile profile in goat coalho cheese supplemented with probiotic lactic acid bacteria. LWT-Food Scie. Technol. 76, 209–215.

Bonanno, A., Tornambè, G., Bellina, V., De Pasquale, C., Mazza, F., Maniaci, G., Di Grigoli, A., 2013. Effect of farming system and cheesemaking technology on the physicochemical characteristics, fatty acid profile, and sensory properties of Caciocavallo Palermitano cheese. J. Dairy Sci. 96, 710–724.

Bontinis, T.G., Mallatou, H., Pappa, E.C., Massouras, T., Alichanidis, E., 2012. Study of proteolysis, lipolysis and volatile profile of a traditional Greek goat cheese (Xinotyri) during ripening. Small Rumin. Res. 105, 193–201.

Broadbent, J.R., Steele, J.L., 2005. Cheese flavor and the genomics of lactic acid bacteria-genomics and molecular biology are valuable in helping to define how these bacteria contribute to the flavor and texture of cheeses. ASM News-Am. Soc. Microbiol. 71, 121–128.

Carpino, S., Randazzo, C. L., Pino, A., Russo, N., Rapisarda, T., Belvedere, G., Caggia, C., 2017. Influence of PDO Ragusano cheese biofilm microbiota on flavour compounds formation. Food Microbiol. 61, 126–135.

Chaves-López, C., De Angelis, M., Martuscelli, M., Serio, A., Paparella, A., Suzzi, G., 2006. Characterization of the Enterobacteriaceae isolated from an artisanal Italian ewe's cheese (Pecorino Abruzzese). J. Appl. Microbiol. 101, 353–360.

Cruciata, M., Sannino, C., Ercolini, D., Scatassa, M. L., De Filippis, F., Mancuso, I., La Storia, A., Moschetti, G., Settanni, L., 2014. Animal rennets as sources of dairy lactic acid bacteria. Appl. Environ. Microbiol. 80, 2050–2061.

Curioni, P., Bosset, J., 2002. Key odorants in various cheese types as determined by gas chromatography-olfactometry. Int. Dairy J. 12, 959–984.

De Angelis, M., Corsetti, A., Tosti, N., Rossi, J., Corbo, M.R., Gobbetti, M., 2001. Characterization of non-starter lactic acid bacteria from Italian ewe cheeses based on phenotypic, genotypic, and cell wall protein analyses. Appl. Environ. Microbiol. 67, 2011–2020.

De Filippis, F., Genovese, A., Ferranti, P., Gilbert, J. A., Ercolini, D., 2016. Metatranscriptomics reveals temperature-driven functional changes in microbiome impacting cheese maturation rate. Sci. Rep. 6.

Di Grigoli, A., Francesca, N., Gaglio, R., Guarrasi, V., Moschetti, M., Scatassa, M. L., Settanni, L., Bonanno, A., 2015. The influence of the wooden equipment employed for cheese manufacture on the characteristics of a traditional stretched cheese during ripening. Food Microbiol. 46, 81–91.

Dìaz-Muñiz, I., Banavara, D.S., Budinich, M.F., Rankin, S.A., Dudley, E.G., Steele, J.L., 2006. Lactobacillus casei metabolic potential to utilize citrate as an energy source in ripening cheese: a bioinformatics approach. J. Appl. Microbiol. 101, 872–882.

Dumont, J.P., Adda, J., 1978. Occurence of sesquiterpenes in mountain cheese volatiles. J. Agric. Food Chem. 26, 364–367.

Engels, W., Dekker, R., De Jong, C., Neeter, R., Visser, S., 1997. A comparative study of volatile compounds in the water-soluble fraction of various types of ripened cheese. Int. Dairy J. 7, 255–263.

Foulquié Moreno, M.R., Sarantinopoulos, P., Tsakalidou, E., De Vuyst, L., 2006. The role and application of enterococci in food and health. Int. J. Food Microbiol. 106, 1–24.

Franciosi, E., Settanni, L., Cavazza, A., Poznanski, E., 2009. Presence of enterococci in raw cow's milk and “Puzzone di Moena” cheese. J. Food Proc. Pres. 33, 204–217.

Fuchsmann, P., Stern, M.T., Brügger, Y.-A., Breme, K., 2015. Olfactometry profiles and quantitation of volatile sulfur compounds of Swiss tilsit cheeses. J. Agric. Food Chem. 63, 7511–7521.

Gardiner, G., Ross, R.P., Collins, J.K., Fitzgerald, G., Stanton, C., 1998. Development of probiotic Cheddar cheese containing human-derived *Lactobacillus paracasei* strains. Appl. Environm. Microbiol. 64, 2192–2199.

Gatti, M., Bottari, B., Lazzi, C., Neviani, E., Mucchetti, G., 2014. Invited review: Microbial evolution in raw-milk, long-ripened cheeses produced using undefined natural whey starters. J. Dairy Sci. 97, 573–591.

Gelsomino, R., Vancanneyt, M., Condon, S., Swings, J., Cogan, T.M., 2001. Enterococcal diversity in the environment of an Irish Cheddar-type cheesemaking factory. Int. J. Food Microbiol. 71, 177–188.

Gioacchini, A.M., De Santi, M., Guescini, M., Brandi, G., Stocchi, V., 2010. Characterization of the volatile organic compounds of Italian 'Fossa' cheese by solid-phase microextraction gas chromatography/mass spectrometry. Rapid Commun. Mass Spectrom. 24, 3405–3412.

Gobbetti, M., De Angelis, M., Di Cagno, R., Mancini, L., Fox, P.F., 2015. Pros and cons for using non-starter lactic acid bacteria (NSLAB) as secondary/adjunct starters for cheese ripening. Trends Food Sci. Technol. 45, 167–178.

Guarcello, R., Carpino, S., Gaglio, R., Pino, A., Rapisarda, T., Caggia, C., Marino, G., Randazzo, C.L., Settanni, L., Todaro, M., 2016. A large factory-scale application of selected autochthonous lactic acid bacteria for PDO Pecorino Siciliano cheese production. Food Microbiol. 59, 66–75.

Guillén, M., Ibargoitia, M., Sopelana, P., Palencia, G., Fresno, M., 2004. Components detected by means of solid-phase microextraction and gas chromatography/mass spectrometry in the headspace of artisan fresh goat cheese smoked by traditional methods. J. Dairy Sci. 87, 284–299.

Irmler, S., Bavan, T., Oberli, A., Roetschi, A., Badertscher, R., Guggenbühl, B., Berthoud, H., 2013. Catabolism of serine by *Pediococcus acidilactici* and *Pediococcus pentosaceus*. Appl. Environ. Microbiol. 79, 1309–1315.

Langler, J.E., Libbey, L.M., Day, E.A., 1967. Identification and evaluation of selected compounds in Swiss cheese flavor. J. Agric. Food Chem. 15, 386–391.

Lazzi, C., Povolo, M., Locci, F., Bernini, V., Neviani, E., Gatti, M., 2016. Can the development and autolysis of lactic acid bacteria influence the cheese volatile fraction? The case of Grana Padano. Int. J. Food Microbiol. 233, 20–28.

Licitra, G., Ogier, J.C., Parayre, S., Pediliggieri, C., Carnemolla, T.M., Falentin, H., Madec, M.N., Carpino, S., Lortal, S., 2007. Variability of the bacterial biofilms of the “tina” wood vat used in the Ragusano cheese making process. Appl. Environ. Microb. 73, 6980–6987.

Majcher, M.A., Goderska, K., Pikul, J., Jelen, H.H., 2011. Changes in volatile, sensory and microbial profiles during preparation of smoked ewe cheese. J. Sci. Food Agric., 91, 1416–1423.

Marilley, L., Casey, M., 2004. Flavours of cheese products: metabolic pathways, analytical tools and identification of producing strains. Int. J. Food Microbiol. 90, 139–159.

McSweeney, P.L., Sousa, M.J., 2000. Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. Le Lait 80, 293–324.

Morales, P., Fernández-García, E., Gaya, P., Nuñez, M., 2003. Formation of volatile compounds by wild *Lactococcus lactis* strains isolated from raw ewes’ milk cheese. Int. Dairy J. 13, 201–209.

Morea, M., Matarante, A., Di Cagno, R., Baruzzi, F., Minervini, F., 2007. Contribution of autochthonous non-starter lactobacilli to proteolysis in Caciocavallo Pugliese cheese. Int. Dairy J. 17, 525–534.

Nassib, T.A., El-Sharoud, W.A., Shalabi, O.M.A., 2013. Prevalence of *Enterococcus* in Egyptian dairy products. J. Food Dairy Sci. - Mansoura Univ., 4, 487–496.

Neeter, R., De Jong, C., Teisman, H., Ellen, G., 1996. Determination of volatile components in cheese using dynamic headspace techniques. Special Publication-Royal Soc. Chem. 197, 293–296.

Neviani, E., De Dea Lindner, J., Bernini, V., Gatti, M., 2009. Recovery and differentiation of long ripened cheese microflora through a new cheese-based cultural medium. Food Microbiol. 26, 240–245.

Ocak, E., Javidipour, I., Tuncturk, Y., 2015. Volatile compounds of Van Herby cheeses produced with raw and pasteurized milks from different species. J. Food Sci. Technol. 52, 4315–4323.

Oliszewski, R., Wolf, I.V., Bergamini, C.V., Candioti, M., Perotti, M.C., 2013. Influence of autochthonous adjunct cultures on ripening parameters of Argentinean goat's milk cheeses. J. Sci. Food Agric. 93, 2730–2742.

Ortigosa, M., Torre, P., Izco, J.M., 2001. Effect of pasteurization of ewe’s milk and use of a native starter culture on the volatile components and sensory characteristics of Roncal cheese. J. Dairy Sci. 84, 1320–1330.

Poveda, J.M., Sánchez-Palomo, E., Pérez-Coello, M.S., Cabezas, L., 2008. Volatile composition, olfactometry profile and sensory evaluation of semi-hard Spanish goat cheeses. Dairy Sci. Technol. 88, 355–367.

Randazzo, C.L., Pitino, I., De Luca, S., Scifo, G.O., Caggia, C., 2008. Effect of wild strains used as starter cultures and adjunct cultures on the volatile compounds of the Pecorino Siciliano cheese. Int. J. Food Microbiol. 122, 269–278.

Rodriguez-Alonso, P., Centeno, J.A., Grabal, J.I., 2009. Comparison of the volatile profiles of Arzua-Ulloa and Tetilla cheeses manufactured from raw and pasteurized milk. LWT–Food Sci. Technol. 42, 1722–1728.

Scatassa, M.L., Gaglio, R., Macaluso, G., Francesca, N., Randazzo, W., Cardamone, C., Di Grigoli, A., Moschetti, G., Settanni, L., 2015. Transfer, composition and technological characterization of the lactic acid bacterial populations of the wooden vats used to produce traditional stretched cheeses. Food Microbiol. 52, 31–41.

Settanni, L., Di Grigoli, A., Tornambé, G., Bellina, V., Francesca, N., Moschetti, G., Bonanno, A., 2012. Persistence of wild Streptococcus thermophilus strains on wooden vat and during the manufacture of a traditional Caciocavallo type cheese. Int. J. Food Microbiol. 155, 73–81.

Sgarbi, E., Lazzi, C., Tabanelli, G., Gatti, M., Neviani, E., Gardini, F., 2013. Nonstarter lactic acid bacteria volatilomes produced using cheese components. J. Dairy Sci. 96, 4223–4234.

Singh, T., Drake, M., Cadwallader, K., 2003. Flavor of Cheddar cheese: A chemical and sensory perspective. Compr. Rev. Food Sci. Food Saf. 2, 166–189.

Suzzi, G., Caruso, M., Gardini, F., Lombardi, A., Vannini, L., Guerzoni, M., Andrighetto, C., Lanorte, M.T., 2000. A survey of the enterococci isolated from an artisanal Italian goat's cheese (semicotto caprino). J. Appl. Microbiol. 89, 267–274.

Tavaria, F., Ferreira, A.S., Malcata, F.X., 2004. Volatile free fatty acids as ripening indicators for Serra da Estrela cheese. J. Dairy Sci. 87, 4064–4072.

Todaro, M., Francesca, N., Reale, S., Moschetti, G., Vitale, F., Settanni, L., 2011. Effect of different salting technologies on the chemical and microbiological characteristics of PDO Pecorino Siciliano cheese. Eur. Food Res. Technol. 233, 931–940.

Toso, B., Procida, G., Stefanon, B., 2002. Determination of volatile compounds in cows' milk using headspace GC-MS. *J. Dairy Res. 69*, 569–577.

Urbach, G., 1995. Contribution of lactic acid bacteria to flavour compound formation in dairy products. Int. Dairy J. 5, 877–903.

Table 1. Microbial loads of individual LAB in CBM (log cfu/mL).

Species	Strain	Sample and time of isolation (reference)	Inoculation level	Growth level
E. casselliflavus	FMAC163	Cheese 120 d (Di Grigoli et al., 2015)	6.73 ± 0.04	7.44 ± 0.03
E. faecalis	FMA721	Wooden vat biofilm (Settanni et al., 2012)	5.56 ± 0.01	7.67 ± 0.14
E. durans	FMAC134B	Cheese 30 d (Di Grigoli et al., 2015)	5.66 ± 0.06	7.56 ± 0.01
E. gallinarum	FMA288	Wooden vat biofilm (Settanni et al., 2012)	6.20 ± 0.05	7.71 ± 0.03
P. acidilactici	FMAC31	Cheese 120 d (Di Grigoli et al., 2015)	6.22 ± 0.10	7.59 ± 0.05
P. pentosaceus	FMAC67	Cheese 120 d (Di Grigoli et al., 2015)	5.53 ± 0.06	7.41 ± 0.57
L. casei	FMAC16	Cheese 120 d (Di Grigoli et al., 2015)	6.23 ± 0.32	7.43 ± 0.01
L. paracasei	FMAC21	Cheese 30 d (Di Grigoli et al., 2015)	6.39 ± 0.07	7.34 ± 0.01
L. rhamnosus	FMAC240	Cheese 120 d (Di Grigoli et al., 2015)	5.24 ± 0.04	7.86 ± 0.18
L. delbrueckii	FMAC8	Cheese 60 d (Di Grigoli et al., 2015)	6.66 ± 0.22	7.11 ± 0.14
S. thermophilus	FMA854	Wooden vat biofilm (Settanni et al., 2012)	6.36 ± 0.03	7.05 ± 0.02
Negative control			0	0

Abbreviations: *E.*, *Enterococcus*; *L.*, *Lactobacillus*; *P.*, *Pediococcus*; *S. Streptococcus*.
Results indicate mean values ± Standard deviation of three plate counts. The counts of cocci SLAB were performed on M17 broth, while those of rods SLAB and NSLAB on MRS broth.

Table 2. VOCs generated in CBM after the growth of the different LAB strains. Data are expressed as relative abundance percentages and are reported as the mean of triplicate experiments.

Compound[a]	RT[b]	CuBM	CBM	*E. casseliflavus* FMAC163	*E. faecalis* FMA721	*E. durans* FMAC134B	*E. gallinarum* FMA288	*P. acidilactici* FMAC31	*P. pentosaceus* FMAC67	*L. delbrueckii* FMAC8	*L. casei* FMAC16	*L. paracasei* FMAC21	*L. rhamnosus* FMAC240	*S. thermophilus* FM854	SEM	Significance
Alcohol		**-**	**10.77**	**9.92**	**10.00**	**8.59**	**9.74**	**27.80***	**6.27**	**21.12***	**7.66**	**17.66***	**8.18**	**9.92**	1.807	<0.0001
Ethanol	3,85	-	0.41	0.22	0.29	0.35	0.23	0.75	0.20	0.70	0.26	0.65	0.34	0.27	0.196	ns
2-Butanol	6,12	-	4.18	3.08	3.31	2.76	3.47	11.30*	2.10*	8.66*	2.87	4.74	3.19	3.79	0.542	<0.0001
1-Propanol	6,56	-	0.64	0.52	0.42	0.64	0.41	2.12*	0.46	1.95*	0.39	0.97	0.51	0.57	0.220	0.0006
2-Pentanol	9,26	-	1.36	0.83*	0.78*	0.72*	1.21	3.23*	0.58*	2.29*	1.02	1.61	0.80*	0.99	0.166	<0.0001
1-Butanol	10,06	-	2.52	2.54	2.62	1.59	2.44	6.18*	1.64	4.50*	1.84	5.02*	1.99	2.46	0.531	0.0006
1-Butanol, 3-methyl-	12,09	-	0.67	0.88	0.43	0.61	0.52	1.21	0.24	1.00	0.43	0.79	0.54	0.36	0.333	ns
2-Heptanol	15,54	-	0.43	0.55	0.59	0.58	0.42	1.03*	0.38	0.69	0.57	0.52	0.53	0.54	0.176	ns
1-Octanol, 2-butyl-	15,42	-	-	0.46	0.32	0.50	-	-	-	-	-	-	-	-	0.114	ns
1-Decanol, 2-methyl-	15,66	-	-	0.3	0.15	0.19	-	-	-	-	-	2.06	-	-	0.777	ns
1-Hexanol	16,50	-	0.45	0.44	0.93*	0.47	0.90*	1.75*	0.54	1.14*	0.21*	1.09*	0.19*	0.78*	0.0762	<0.0001
2-Nonanol	20,99	-	0.11	0.09	0.1	0.13	0.06	0.17	0.09	0.15	0.06	0.13	0.08	0.08	0.0226	ns
1-Octanol	21,95	-	-	-	0.07	0.04	0.07	0.05	0.03	0.05	-	0.08	-	0.08	0.0047	<0.0001
Aldehydes		**3.67**	**3.15**	**3.08**	**2.39**	**0.95**	**3.07**	**12.20*§**	**1.13**	**7.42**	**3.41**	**6.65**	**4.97**	**1.22**	1.753	0.0014
Butanal	2,97	-	-	-	-	-	-	1.01	-	0.11	1.84	-	-	-	0.0406	<0.0001
Butanal, 2-methyl-	3,43	-	0.38	0.47	0.15	-	0.29	1.01	0.07	3.00	0.16	0.77	0.36	0.09	0.518	ns
Butanal, 3-methyl-	3,50	-	1.33	0.61	0.28	-	1.39	7.69*	0.17	2.96	1.35	4.6	0.37	0.3	2.16	0.0383
Hexanal	7,91	-	0.12	0.35	0.33	0.15	-	0.65*	-	0.14	-	-	0.1	_	0.0782	0.0012
Benzaldehyde	21,12	-	1.24	1.11	1.11	0.44*	1.26	1.66*	0.81*	1.14	-	1.14	4.04*	0.71*	0.120	<0.0001
Benzaldehyde, 3-methyl	24,14	3.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Benzaldehyde, 2,4-dimethyl-	27,96	-	0.08	0.54	0.52	0.36	0.13	0.19	0.09	0.08	0.06	0.14	0.11	0.12	0.200	0.0080
Ketones		**14.21***	**70.86§**	**67.35§**	**69.77§**	**78.43§**	**78.27§**	**12.30***	**81.26§**	**34.15*§**	**79.70§**	**52.31*§**	**72.98§**	**71.97§**	4.555	<0.0001
2-Butanone	3,24	3.16*	1.52§	0.47*§	0.51*§	0.47*§	1.01*	3.36*	0.39*§	2.93*	0.89*§	2.32*§	1.14*§	0.59*§	0.118	<0.0001
2-Butanone, 3-methyl-	4,70	-	0.60	0.81	0.43	0.51	0.67	2.02*	0.24	1.60*	0.6	1.12*	0.54	0.39	0.148	<0.0001
2,3-Butanedione	4,83	5.10*	11.48§	17.83*§	11.27§	10.07	19.07*§	2.07*	12.55§	11.69§	34.65*§	16.33*§	29.2*§	6.78*	1.462	<0.0001
2-Heptanone	11,19	3.60*	1.00§	1.07§	0.75§	0.77§	0.74§	2.20*§	0.57§	1.70*§	0.72§	1.38§	0.83§	0.73§	0.110	<0.0001
2-Butanone, 3-hydroxy-	14,54	-	55.00	45.46	55.71	65.42	55.91	0.88*	66.67*	14.87*	41.75*	30.02*	40.2*	62.58	3.56	<0.0001
2-Nonanone	17,49	1.42*	1.00§	1.34*	0.75*§	0.90§	0.74*§	1.32	0.54*§	1.05§	0.87§	0.70*§	0.87§	0.54*§	0.085	<0.0001
2-Undecanone	22,97	0.93*	0.25§	0.37§	0.36§	0.29§	0.12§	0.44§	0.29§	0.31§	0.22§	0.44§	0.20§	0.36§	0.066	<0.0001
Esters		**29.10***	**8.68§**	**12.57*§**	**11.17§**	**8.95§**	**5.57§**	**20.26*§**	**8.64§**	**18.54*§**	**6.18§**	**13.72*§**	**7.77§**	**13.22*§**	1.449	<0.0001
1-Propen-2-ol, acetate	2,39	18.06*	7.23§	11.13*§	10.31*§	8.05§	4.71*§	17.32*	7.90§	15.44*	5.13§	11.59*§	5.87§	12.27*§	1.002	<0.0001
Butanoic acid, ethyl ester	6,45	-	0.6	0.46	0.21*	0.10*	0.38	1.48*	0.27	1.47*	0.47	0.64	0.67	0.46	0.123	<0.0001
Acetic ac.,2-methylpropyl ester	7,58	-	0.27	0.43	0.32	0.56	0.17	0.47	0.14	0.57	0.21	0.71*	0.66	-	0.139	0.0312
Hexanoic acid, ethyl ester	12,89	-	0.36	0.41	0.17*	0.10*	0.16*	0.55	0.22	0.66*	0.22	0.46	0.32	0.31	0.0634	0.0005
Methyl salicylate	27,15	11.04*	0.12§	0.14§	0.16§	0.14§	0.08§	0.18§	0.11§	0.18§	0.09§	0.16§	0.10§	0.18§	0.0132	<0.0001
Ethyl Acetate	3,06	-	0.09	-	-	-	0.07	0.25*	-	0.21*	0.06	0.17	0.14	-	0.0176	<0.0001
Aromatic hydrocarbons		**3.38***	**0.19§**	**0.91*§**	**0.95*§**	**0.83*§**	**0.05§**	**0.55§**	**0.87*§**	**0.32§**	**0.12§**	**0.22§**	**0.04§**	**1.14*§**	0.228	<0.0001
o-Xylene	9,50	-	0.12	0.27	0.3	0.24	-	0.33*	0.40*	0.16	0.06	0.09	0.02	0.54*	0.0619	0.0005
m-Xylene	9,78	-	0.07	0.2	0.2	0.14	-	0.22*	0.36*	0.17	0.06	0.13	0.02	0.43*	0.0485	0.0005
m-di-tert-butylbenzene	18,52	3.38	-	0.45§	0.44§	0.45§	0.05§	-	0.11§	-	-	-	-	0.16§	0.1022	<0.0001
Organic acid		**-**	**6.25**	**3.80**	**3.33**	**1.02***	**3.26**	**26.89***	**1.82***	**18.30***	**2.91**	**8.46**	**6.06**	**2.53***	1.162	<0.0001
Butanoic acid	25,03	-	3.89	2.16	1.94	0.54*	1.95	12.95*	0.97*	10.33*	1.68*	5.00	3.74	1.01*	0.658	<0.0001
Hexanoic acid	29,84	-	2.36	1.64	1.39	0.48*	1.31	11.64*	0.85*	7.25*	1.23	3.46	2.31	1.52	0.481	<0.0001
Octanoic Acid	34,23	-	-	-	-	-	-	2.31	-	0.72	-	-	-	-	0.145	<0.0001
Hydrocarbones		-	-	**1.83**	**1.86**	**0.85**	-	-	-	-	-	-	-	-	0.678	ns
Nonane, 2,6-dimethyl	5,12	-	-	0.92	0.97	0.34	-	-	-	-	-	-	-	-	0.0936	0.006
Decane, 4-methyl	5,24	-	-	0.76	0.71	0.40	-	-	-	-	-	-	-	-	0.103	ns
Decane, 3,7-dimethyl	12,38	-	-	0.10	0.12	0.08	-	-	-	-	-	-	-	-	0.0091	0.001
Hexadecane	12,56	-	-	0.05	0.06	0.03	-	-	-	-	-	-	-	-	0.0092	ns
Others		**49.64***	**0.08§**	**0.54§**	**0.54§**	**0.38§**	**0.05§**	-	-	**0.14§**	**0.04§**	**0.99§**	-	-	1.186	<0.0001
Furan 2-methyl	2,86	4.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-Decene, 2,4-dimethyl-	8,58	-	-	0.25	0.3	0.17	0.02	-	-	-	0.04	0.87	-	-	0.244	ns
1-Undecene, 7-methyl-	8,72	-	0.08	0.29*	0.24*	0.21*	0.03	-	-	0.14	-	0.12	-	-	0.0405	0.0009
Prehnitene	18,61	1.53	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Furfural	19,61	2.35	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2-Furanmethanol	24,50	41.66	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: CBM, cheese based medium; CuBM, curd based medium; *E., Enterococcus*; *L., Lactobacillus*; *P., Pediococcus*; *S. Streptococcus*.

Symbols: -, not detected; *, significance (P<0.05) of the comparison with CBM; significance (P<0.05) of the comparison with CuBM.

[a] Identification: MS (NIST, Library), Similarity > 95%

[b] RT: Retention Time (min)

Table 3. Canonical discriminant analysis: correlation coefficients for VOCs in common between Caciocavallo Palermitano cheese and CBM fermented by LAB strains with the canonical variables 1 and 2.

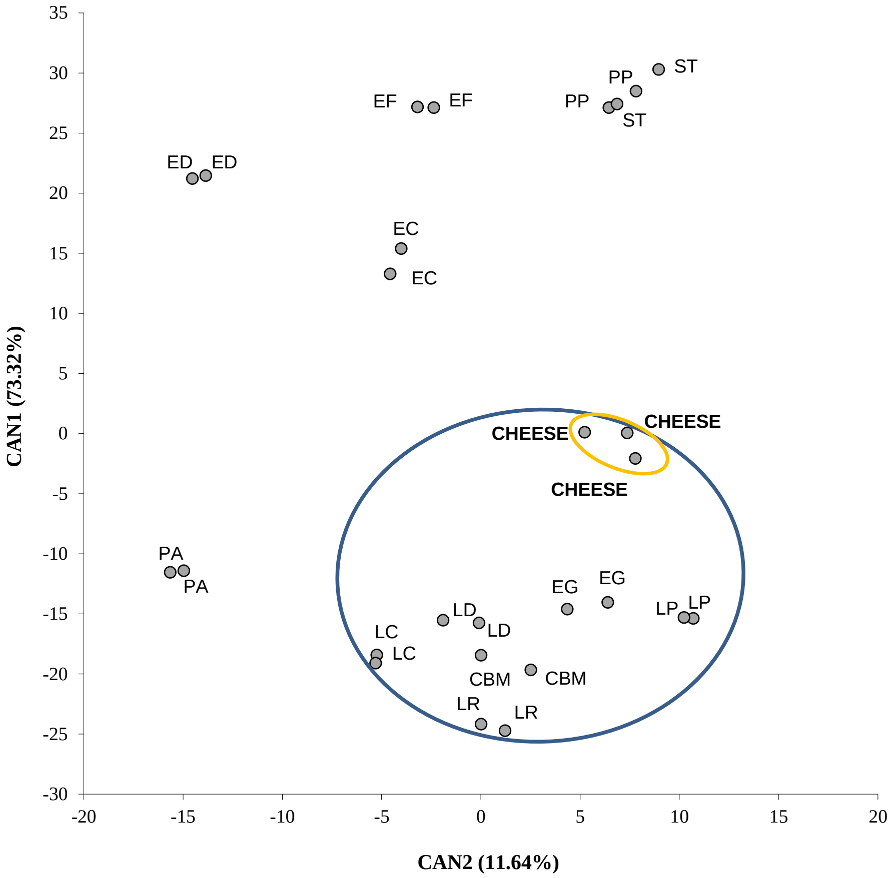
Compound[a]	Relative abundance % in cheese	Canonical V1 Variance %: 73.32	Canonical V2 Variance %: 11.64
Ethanol	2.01	0.218364	-0.108000
2-Butanol	2.45	0.207920	-0.308471
1-Butanol	0.19	0.274967	-0.047738
2-Heptanol	0.52	0.381865	-0.330127
1-Hexanol	0.57	**0.560646**	0.078972
Hexanal	0.35	0.333622	**-0.511534**
2-Butanone	1.25	**-0.621729**	-0.020801
2-Nonanone	2.59	0.294526	-0.392939
Hexanoic acid, ethyl ester	2.75	0.120034	0.468177
Ethyl Acetate	0.56	**-0.899220**	0.184209
o-Xylene	0.17	**0.859064**	0.049584
m-Xylene	0.51	**0.814294**	0.203808
Butanoic acid	51.59	-0.373260	0.247021
Hexanoic acid	32.07	-0.285883	0.191259
Octanoic Acid	2.43	-0.222745	-0.304033

Data are reported as the mean of three measures for each replicate.
[a] Identification: MS (NIST, Library), Similarity > 95%

Figure captions

Fig. 1. Plot from canonical discriminant analysis showing the distribution of cheeses and LAB as function of canonical variables 1 and 2 based on VOCs. Abbreviations: CBM, cheese-based medium; EC, *E. caselliflavous*; EF, *E. faecalis*; ED, *E. durans*; EG, *E. gallinarum*; PA, *P. acidilactici*; PP, *P. pentosaceus*; LD, *L. delbrueckii*; LC, *L. casei*; LR, *L. rhamnosus*; ST, *S. thermophilus*.

Fig. 1.



35
30
25
20
15
10
5
0
-5
-10
-15
-20
-25
-30
-20
-15
-10
-5
0
5
10
15
20
CAN1 (73.32%)
CAN2 (11.64%)
EF
EF
PP
PP
ST
ST
ED
ED
EC
EC
CHEESE
CHEESE
CHEESE
PA
PA
EG
EG
LP
LP
LD
LD
LC
LC
CBM
CBM
LR
LR