Potential impact of dairy yeasts on the typical flavour of traditional ewes' and goats' cheeses

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Abstract

The contribution of *Debaryomyces hansenii*, *Kluyveromyces lactis* and *Kluyveromyces marxianus* strains to the typical flavour of traditional ewes' and goats' cheeses was assessed. Fourteen yeast strains were grown in liquid medium mimicking cheese composition and volatile compounds were identified by GC–MS. Yeasts were able to produce key volatile compounds characteristic of the cheeses from which they were isolated. Inter-species and inter-strain variations were observed. Under the conditions tested *D. hansenii* produced the lowest levels of volatile compounds, with large intra-strain variations. *Kluyveromyces* strains primarily produced esters and alcohols. *K. marxianus* strains were associated with the production of acids, ethyl decanoate, 1-propanol and benzaldehyde, whereas *K. lactis* was correlated with the presence of ketones, ethyl acetate and secondary alcohols. In conclusion, this study shows the heterogeneous potential of dairy yeasts to contribute to final cheese flavour.

1. INTRODUCTION

Yeasts play an important role in proteolysis, lipolysis, fermentation of residual lactose, and assimilation of lactic and citric acid during the ripening of some cheeses, contributing to aroma development and to the rheological properties of the final dairy product (McSweeney, 2004). Moreover yeasts have been recovered from all stages of cheesemaking, as well as from milk, brine and dairy process equipment among others (Corbo, Lanciotti, Albenzio, & Sinigaglia, 2001; Delavenne et al., 2011; Gardini et al., 2006; Seiler & Busse, 1990).

Debaryomyces hansenii is the dominant yeast species found in most cheese varieties (Fleet, 1990; Fox, Guinee, Cogan, & Mc.Sweeny, 2000). *D. hansenii* possesses the ability to grow at high salt concentrations, low pH and low water activity, as well as metabolising lactic and citric acids, which makes cheese a suitable environment for its proliferation (Breuer & Harms, 2006). Lactose-fermenting yeasts *Kluyveromyces lactis* and *Kluyveromyces marxianus* are also regularly found in dairy products and milk. Their lactose-fermenting ability promotes their growth in the cheese, where other yeasts are scarce. Besides these species, cheeses may often contain other yeast species, such as *Yarrowia lipolytica*, *Geotrichum candidum* and *Saccharomyces cerevisiae* (Fleet, 1990).

Cheese flavour is one of the most relevant attributes influencing consumers' acceptance and preference (Arora, Cormier, & Lee, 1995), and is the result of a complex balance between various volatile and non volatile compounds, which individually do not reflect the overall odour and taste (Fox & Wallace, 1997). Many volatile compounds have been implicated in cheese aroma, such as acids, esters, ketones, aldehydes, alcohols or sulphur compounds, and each dairy product has a characteristic and unique composition of volatile components (Plutowska & Wardencki, 2007).

The contribution of yeasts to development of cheese aroma is considered positive in some instances, creating commercial interest in using selected strains as ripening cultures (Frohlich-Wyder, 2003; Romano, Capace, & Jespersen, 2006). Several studies have shown that, in different cheeses, relevant yeast species contribute differently to volatile production. *G. candidum* and *Y. lipolytica* are known to produce considerable amounts of various volatile sulphur compounds; *K. lactis*, *K. marxianus* and *S. cerevisiae* have been found to produce primarily esters; and *D. hansenii* mainly produced branched-chain aldehydes and alcohols (Arfi, Spinnler, Tache, & Bonnarme, 2002; Martin, Berger, Le Du, & Spinnler, 2001; Spinnler, Berger, Lapadatescu, & Bonnarme, 2001; Leclercq-Perlat, Corrieu, & Spinnler, 2004; Sørensen, Gori, Petersen, Jespersen, & Arneborg, 2011). However, these studies emphasised inter-species aroma production, with few surveys focussing on strain variation. Berger, Khan, Molimard, Martin, and Spinnler (1999) reported the production of different yields of sulphur compounds by *G. candidum*, depending on the strain selected, and Gori, Sørensen, Petersen, Jespersen, and Arneborg (2012) recently showed large strain variations in the production of flavour compounds by *D. hansenii.*

Iberian traditional cheeses made from ewes' and goats' milk have high intrinsic value, arising from their unique sensory characteristics, which makes them highly appreciated by consumers (Freitas & Malcata, 2000). In previous studies, yeasts present during the ripening process of ewes' and goats' raw milk cheeses produced in a small traditional dairy in the Mediterranean area of Spain were identified (Padilla, Manzanares, & Belloch, 2014). *D. hansenii* and *K. lactis* were the yeast species most frequently isolated from both kind of cheeses, and the former predominated at the end of ripening period. *K. marxianus*, although less frequent, was present during the first weeks of maturing. Moreover, results demonstrated genetic heterogeneity present in the isolates (Padilla et al., 2014), and their strain-dependent ability to generate bioactive compounds (García-Tejedor, Padilla, Salom, Belloch, & Manzanares, 2013; Padilla et al., 2012). However, there is little knowledge about the impact of the yeast isolates on the final quality of the cheeses.

The objective of the present study was to further characterise both the aforementioned raw milk cheeses and their yeast microbiota, to gain a better understanding of the relationship between yeast ripening strains and cheese flavour. For this purpose, the volatile profile of the cheeses was characterised. Volatile compounds were extracted by Solid Phase Micro Extraction (SPME) and analysed by Gas Chromatography-Mass Spectrometry (CG-MS). Moreover, the ability of 14 yeast strains belonging to *D. hansenii*, *K. lactis* and *K. marxianus* species to grow in a defined medium and produce volatile compounds also present in ripened cheeses was assessed.

2. MATERIALS AND METHODS

2.1 Cheese samples

Commercial semi-hard ewes' and goats' cheeses produced in an artisanal dairy farm sited in the rural Castello province (Spain) were analysed for volatile compounds. The cheeses were made from raw milk coagulated with the addition of mesophilic lactic acid bacteria starters and plant (*Cynara cardunculus*) rennet (Abiasa Company, Pontevedra, Spain). After precipitation of proteins, the curd was cut with vertical and horizontal knives and crumbled manually. The remaining whey was removed first manually and afterwards using a press. After salting, cheeses were air-dried until the rind was formed and ripened in wooden shelves at 10-12ºC and a relative humidity of 85-90% for 60 days.

Three cheeses from the same batch and from ewes' milk and goats' milk were analysed at the end of ripening period (3 batches x 2 cheeses = 6 samples). After the rind was removed, cheeses were cut in pieces and ground with 0.75 mg butylated hydroxytoluene/20 g sample, wrapped in aluminium foil, vacuum-packed and stored at –20ºC until GC analysis.

2.2 Yeast strains

Fourteen yeast strains belonging to the species *K. marxianus* (Km1-Km4), *K. lactis* (Kl1-Kl5) and *D. hansenii* (Dh1-Dh5) isolated during the ripening process from the artisanal cheeses described above and with different genetic characteristics were used in this study (Padilla et al., 2014). Yeast strains were maintained on GPYA medium (2 % glucose, 0.5 % peptone, 0.5 % yeast extract and 2 % agar, pH 5.5).

2.3 Culture conditions and media

Cheese-like medium (CLM; casamino acids 15 g L^{-1}, sodium lactate 19 ml L^{-1}, yeast extract 1 g L^{-1}, $CaCl_2$ g L^{-1}, $MgSO_4$ 0.5 g L^{-1}, KH_2PO_4 6.8 g L^{-1}, NaCl 10 g L^{-1} and lactose 28 g L^{-1}) was prepared according to Kagkli et al. (2006) without addition of L-methionine. Flasks (100-mL) containing 50 mL of CLM were inoculated with 10^6 cells mL^{-1} from overnight pre-cultures grown in GPY medium (GPYA without agar) at 28ºC and 150 rpm. CLM cultures were incubated over 48h at 28ºC and 150 rpm. At the end of the incubation period, samples were taken for OD_{600} measurement. Yeast cells were removed by centrifugation (3220 x g, 10 min) and culture pH was measured. Lactose and L-lactic acid were quantified in the supernatants using Roche enzymatic kits (Darmstadt, Germany). For each strain, three replicate cultures were analysed and a control without yeast inoculation was also included.

2.4 Analysis of headspace volatile compounds by SPME GC–MS

An Agilent HP 7890 series II GC (Hewlett- Packard, Palo Alto, CA, USA) with an HP 5975C mass selective detector (Hewlett-Packard) equipped with Gerstel MPS2 multipurpose sampler (Gerstel, Mülheim an der Ruhr, Germany) was used in all experiments. The volatile components of the samples were extracted by SPME. All extractions were carried out using a DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) fibre of 50/30 mm film thickness (Supelco, Bellefonte, PA, USA). The fiber was conditioned as indicated by the manufacturer prior to use in order to remove any possible contaminants. For cheeses, 5 g of product was placed in a 20 mL headspace vial sealed with a PTFE-faced silicone septum. The vial was maintained at 50ºC for 15 min to equilibrate the headspace, and then the fiber was exposed over 30 min at the same temperature. Before each injection, the fiber was baked at 250ºC for 10 min. Each sample was analysed in triplicate. For CLM yeast cultures, 7 mL of supernatant plus 1.4 g of NaCl were added to a 20 mL headspace vial sealed with a PTFE-faced silicone septum. The vial was kept at 50ºC for 15 min to equilibrate the headspace. The SPME fiber was then exposed to the headspace while maintaining the sample at 30ºC for 15 min. During extraction, the sample was agitated continuously in pulses of 10 sec at 250 rpm. Before and after each injection, the fiber was baked at 250ºC for 10 and 5 min, respectively. Each sample was analysed twice.

After the extraction step, the analytes were thermally desorbed for 5 min from the fiber into the injector port of the GC-MS operating at 240ºC in splitless mode. The compounds were then separated using a DB-624 capillary column J & W Scientific (Agilent Technologies, Santa Clara, CA, USA) (30m, 0.25mm i.d., film thickness 1.4 μm). For volatile analysis, the GC oven temperature program began at 40ºC, where it was held for 5 min, then ramped to 100ºC at 3 ºC min^{-1} and maintained for 5 min, then to 150ºC at 3ºC min^{-1} and to 210ºC at 4ºC min^{-1}, and, finally, held at 210ºC for 5 min. Mass spectra were obtained by electron impact at 70 eV, and data were acquired across the range 29–400 amu (scan mode).

Compounds were identified by comparison with mass spectra from the library database (Nist'05), Kovats retention index (Kovats, 1965) and by comparison with authentic standards. The quantification of volatile compounds was done in SCAN mode using total ion chromatograms (TIC). The results were expressed as abundance units (AU x 10^{-6}). Volatile compounds quantitated from CLM control were subtracted from each yeast-inoculated medium.

2.5 Statistical evaluation

The effect of yeasts on the generation of volatile compounds in CLM was tested by one-way analysis of variance (ANOVA). Differences between sample means were analysed according to Fisher's least significant difference (LSD) test. Principal component analysis (PCA) was used to test relationships among yeast species, pH, lactose and lactate consumption and main volatile compounds. Statistical analysis was performed using the statistic software XLSTAT, 2009.4.03 (Addinsoft, Barcelona, Spain).

3. RESULTS

3.1 Volatile compounds in ewes´and goats´milk cheeses

Sixty-five volatile compounds were quantitied in the headspace of Mediterranean ewes' and goats' cheeses (Table 1). They were classified into acids (14), esters (18), ketones (9), aldehydes (5), alcohols (17), terpenes (1) and sulphur compounds (1). Four of the sixty-five compounds were not present in the ewe's cheese, while eight of them were not present in the headspace of goat's cheese. As expected, most of the volatiles found in these cheeses have been previously reported in other varieties of ewes' and goats' raw milk cheeses (Table 1).

Esters and alcohols were the most abundant chemical families identified in the headspace of the Mediterranean cheeses studied, whereas, quantitatively, carboxylic acids were the most abundant volatiles. Among short and medium-chain carboxylic acids, the most abundant were acetic, butanoic, hexanoic, octanoic and decanoic acids, although branched-chain fatty acids such as 3-methylbutanoic and 2-methylbutanoic acids were also found in both cheeses. Among esters, ethyl esters were the most abundant, although propyl- and branched-chain esters were also identified. Methyl ketones were the most abundant ketones detected in these products while aldehydes were not major components in these cheeses.

3.2 Yeast growth in CLM and production of volatile compounds

Growth and aromatic profile from pure cultures of yeast strains belonging to *D. hansenii*, *K. lactis* and *K. marxianus* were determined. All yeast strains were able to grow in a liquid medium mimicking cheese composition (CLM). Lactose and lactate concentrations and pH values after 48 h of growing in CLM were determined. *Kluyveromyces* strains depleted the available lactose almost completely. *K. lactis* consumed around 5 % lactate, while lactate consumption by *K. marxianus* strains was around 16 %. When grown in CLM, *Kluyveromyces* strains increased the pH from 5 to 5.2-5.8. *D. hansenii* strains grew in CLM, consumed around 25 % of lactose and 5 % of lactate, and the pH value increased to 5.6-6.6.

Volatile compounds detected in the headspace of CLM are summarised in Tables 2 and 3. Only compounds which were also found in the Mediterranean cheeses (Table 1) are listed in Table 2, while Table 3 shows other volatile compounds detected in the headspace of CLM.

As observed in this study, yeasts were able to produce 27 compounds of those compounds found in the cheeses, including 6 acids, 7 esters, 3 ketones, 2 aldehydes and 9 alcohols (Table 2). Interestingly, the volatile composition of the headspace of CLM showed inter-species and inter-strain variations. General variations can be seen in Fig. 1, which shows volatile compounds classified by chemical groups and yeast species. *K. marxianus* and *K. lactis* were the best producers of esters and alcohols, without significant differences between the two species. Similar production of aldehydes was found for *K. marxianus* and *D. hansenii*, while the former was the best acid producer. In general, *D. hansenii* produced the lowest levels of volatile compounds in the conditions tested. Moreover, standard deviations indicated large strain variations for *D. hansenii*.

Among *D. hansenii* strains (Table 2), Dh1 produced the highest levels of total esters and alcohols. *K. marxianus* species was prominent due to the production of acetic acid. Production of octanoic acid was restricted to *K. marxianus* species, with Km3 being the best producer strain. *K. marxianus* Km2 stood out as the leading producer of total esters, due to the high production of 3-methyl-1-butanol acetate, the level of which was almost ten-fold higher than that produced by *K. lactis* strains. Among esters, *K. lactis* strains produced primarily ethyl acetate. Interestingly, ethyl octanoate production was restricted to *K. marxianus* Km1 and Km2 under the conditions tested. With the exception of 2-pentanone production by Dh4, ketone production was restricted to *K. lactis* strains. In contrast, none of the *K. lactis* strains were able to produce aldehydes. Only two aldehydes were detected after yeast growth in CLM: benzaldehyde produced by *K. marxianus* Km3 and Km4 and 3-methylbutanal produced by *D. hansenii* Dh1 and Dh5. Regarding alcohol production, *K. la*ctis produced 9 different volatiles, although *K. marxianus* strains Km1, Km2 and Km3 stood out as the best total alcohol producers, given the production of phenylethyl alcohol. Neither *K. marxianus* nor *D. hansenii* species were able to produce 2-

heptanol and 2-nonanol. Moreover 2,3-butanediol was not detected after growth of *D. hansenii* in CLM, while *K. marxianus* strains were the best producers of such compounds (Table 2).

Apart from these compounds, 23 more volatiles compounds were identified in the headspace of CLM after yeast growth (Table 3). Those compounds mainly comprised esters (16), although 1 ketone, 3 aldehydes, 2 alcohols and methionol were also detected; table 3 also shows the percentage of yeast strains able to produce each compound. Some of these, 3 esters, 2 aldehydes and methyl isobutyl ketone, were only detected in CLM after growth of *D. hansenii*. In contrast, none of the *D. hansenii* strains tested was able to produce propyl and 2-phenylethyl propanoate. It is also worthwhile to note that none of the *K. marxianus* strains were producers of aldehydes or 3-methyl-pentanol. Although these compounds were not detected in the cheeses, most of them have been described as typical cheese volatiles (Curioni & Bosset, 2002).

3.3 Principal component analysis

Finally, a PCA model was developed using a dataset with 14 yeast strains and 30 variables, comprising 27 volatiles (those present in cheeses and CLM, Table 2), lactose and lactate consumption and pH of the medium (Fig. 2). Two principal components were able to explain 70.5% of the total variance observed. Principal component 1 (PC1) accounted for 39.6% of the variance while PC2 accounted for 30.9%. PC1 differentiated the incubations by the yeast genera inoculated. *Kluyveromyces* strains appeared in the positive part of PC1, while *Debaryomyces* was situated in the negative side. *Kluyveromyces* strains were related to the maximum production of volatile compounds and to the highest lactose consumption. On the other hand, growth of *D. hansenii* was associated with the highest increase in pH value, and with the presence of 3-methylbutanal. PC2 differentiated the inoculations within *Kluyveromyces* strains. *K. lactis* was related to the presence of volatiles compounds such as ketones, ethyl acetate and secondary alcohols; on the other side, *K. marxianus* strains were associated with the highest consumption of lactate and with the production of acids (acetic, propanoic and octanoic acids), ethyl decanoate, 1-propanol and benzaldehyde, among others.

4. DISCUSSION

This study provides a characteristic fingerprint of volatiles present in Mediterranean cheeses and indicates the metabolic potential of ripening yeast strains to impact on cheese flavour. The proportion of volatile compounds depends on the extraction method used, and in this case a SPME technique with DVB/CAR/PDMS fibres was employed. The method used allowed comparisons among the different yeast strains, since the volatile compounds were obtained on a semi-quantitative basis.

The present research demonstrates the ability of *K. marxianus* and *K. lactis,* and to a lesser extent *D. hansenii* strains, to produce key volatile compounds characteristic of the cheeses from which they were isolated (Table 1 and 2). All the strains tested in this study were able to grow in a defined cheese-like medium (CLM) containing lactose, lactate and casamino acids and generate volatile compounds. Although these conditions differ from real cheese, this medium has been successfully used for screening purposes of yeast species and strains with potential use in cheese ripening (Kagkli et al., 2006; Spinnler et al., 2001).

As expected, *D. hansenii* strains consumed less lactose than *Kluyveromyces*, and this might account for the lower production of aroma compounds in CLM. The prevalence of *D. hansenii* during ripening in different kind of cheeses has been reported by several authors (Fleet, 1990; Fox & Wallace, 1997; Fox et al., 2000) and it is considered as an obvious candidate for starter cultures (Bockelmann, 2002). Recently, Gori et al. (2012) reported the potential of *D. hansenii* strains to increase the nutty/malty flavour of cheese due to the production of aldehydes, although large strain variations were found. In this study and under the conditions tested, 3 branched-chain aldehydes (2-methylpropanal, 3-methylbutanal and 2-methylbutanal) were only produced by *D. hansenii*, with a large inter-strain variation. 2-Methylpropanal and 2-methylbutanal derived from the catabolism of valine and isoleucine, respectively, were only detected in CLM, whereas 3-methylbutanal derived from leucine was also detected in cheeses characterised here. Aldehydes are potent odorants in several cheese varieties, although they are considered transitory compounds because they are quickly reduced to primary alcohols (Curioni & Bosset, 2002). In fact, the corresponding alcohols derived from the three branched-aldehydes (2-methyl-1-propanol, 3-methyl-1-butanol and 2-methyl-1-butanol) were detected in the cheeses and in *D. hansenii* CLM, as also reported by Sørensen et al. (2011).

Ester formation in cheese is mainly related to yeast metabolism (Molimard & Spinnler, 1996) although some lactic acid bacteria and *Micrococcaceae*, as well as chemical reactions, can be responsible (Gripon, Monnet, Lamberet, & Desmazeaud, 1991). Esters come from a reaction between an alcohol, derived from lactose fermentation or amino-acid catabolism, and a fatty acid or amino acid catabolite intermediate. Most esters detected in cheese are described as having sweet, fruity and floral notes. Although a fruity flavour is traditionally regarded as a defect in cheese varieties such as Cheddar (Horwood, Stark, & Hull, 1987), it is a positive attribute of other cheese varieties such as Parmigiano Reggiano (Meinhart & Schreier, 1986). Ester production by *Kluyveromyces* strains has been reported by several authors (Arfi et al., 2002; Jiang, 1993; Leclercq-Perlat et al., 2004; Martin et al., 2001). Ethyl acetate was the main ester produced, although ethyl propanoate, propyl acetate, butyl acetate, ethyl butanoate and ethyl octanoate were also detected after growth of *Kluyveromyces* (Arfi et al., 2002; Leclercq-Perlat et al., 2004). Moreover, 2-phenylethyl acetate, 3-methylbutyl ethanoate and 2-methylpropyl ethanoate are also produced by *Kluyveromyces* strains (Jiang, 1993; Leclercq-Perlat et al., 2004), but strain-specific variations were not addressed. The present results confirm ethyl acetate as one of the primarily esters formed by *Kluyveromyces* strains, together with the production of 3-methyl-1-butanol acetate by two strains of *K. marxianus*. In total, *K. marxianus* and *K. lactis* strains respectively produced 20 and 16 different kinds of esters, respectively, (Table 2 and 3) highlighting the capability of the genus *Kluyveromyces* for ester production. Production of ethyl octanoate, restricted to *K. marxianus* strains under the conditions tested, was also reported by Leclercq-Perlat et al. (2004). These authors also observed that the ester production efficiency of *K. marxianus* was higher than that of *D. hansenii*, in agreement with the results obtained here. With the exception of two ester compounds, the five *K. lactis* strains tested produced the same ester profile, whereas *K. marxianus* strains differed in seven esters. These results suggest a lower inter-strain variation in *K. lactis* than in *K. marxianus*.

Several of the potential alcohols which may be precursors of the aforementioned esters were also identified in yeast CLM. Those alcohols were also detected after the growth of *D. hansenii* strains, where production of esters was negligible. It has been suggested that a highly hydrolytic activity towards esters in *D. hansenii* strains might be the reason for the limited accumulation of ester compounds (Besancon, Ratomahenina, & Galzy, 1995). The *D. hansenii* strains tested in this study have also been characterised as having hydrolytic activity towards fatty acid esters (Padilla et al., 2014).

Interestingly this study shows that only *K. lactis* strains were able to produce 2-pentanone, 2-heptanone and 2-nonanone, which were characteristic compounds of those cheeses from which they were isolated (Table 1 and 2). A previous study has shown the ability of *K. lactis* to produce other kinds of ketones, such as 3-hydroxy-2-butanone and 1-hydroxy-2-propanone, from a medium containing glucose, yeast extract and vitamins (Jiang, 1993). However, to the best of our knowledge, ketones generation by yeasts in a medium mimicking cheese composition has not been reported. Methyl ketones are associated with fruity, floral and musty notes, and their synthesis has been related to the enzymatic activity of moulds in surface-ripened cheeses (Curioni & Bosset, 2002).

Short-chain free fatty acids, predominant components of the flavour of many cheeses such as those described here, were mainly characteristics of *K. marxianus* CLM. As milk fat was not present in CLM, those acids may originate from the degradation of lactose and free amino acids or by oxidation of ketones, esters and aldehydes (Molimard & Spinnler, 1996). Branched-chain fatty acids, such as 2-methylpropanoic, 2- and 3-methylbutanoic acids, are characteristic compounds of goat and ewe cheeses, and they are probably derived from valine, isoleucine and leucine respectively (Kuzdzal-Savoie, 1980). The potential of selected yeast strains to produce fatty acids when grown in a fat-containing medium deserves further study.

Sulphur compounds were not abundant volatiles either in cheeses or CLM. López del Castillo-Lozano, Delile, Spinnler, Bonnarme and Landaud (2007) reported the necessity of methionine supplementation in culture media for the production of volatile sulphur compounds by yeasts. Since only one sulphur compound was detected in cheeses under the conditions tested, we did not consider supplementing casamino acids present in CLM with methionine. The only sulphur compound generated in CLM was methionol (Table 3), a stable end product of methionine metabolism by yeasts (Liu and Crow, 2010). In the conditions tested, methionol was produced by *Kluyveromyces* strains, and only to a small extent by *Debaryomyces* strains.

5. CONCLUSIONS

This study has confirmed the potential of dairy yeasts to contribute to the final cheese flavour. Moreover, species and strain variations were significant, indicating a heterogeneous contribution to volatile compound production and the feasibility of strain selection to modulate cheese flavour and aroma. However, the development of suitable yeast starters requires further studies, since complex interactions among cheese microbiota should be taken into account. Characterization of enzyme activities involved in flavour formation by dairy yeasts is in progress.

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References

Arfi, K., Spinnler, H., Tache, R., & Bonnarme, P. (2002). Production of volatile compounds by cheese-ripening yeasts: requirement for a methanethiol donor for S-methyl thioacetate synthesis by *Kluyveromyces lactis*. *Applied Microbiology and Biotechnology, 58*, 503-510.

Arora, G., Cormier, F., & Lee, B. (1995). Analysis of odor-active volatiles in Cheddar cheese headspace by multidimensional GC/MS/Sniffing. *Journal of Agricultural and Food Chemistry, 43*, 748-752.

Berger, C., Khan, J. A., Molimard, P., Martin, N., & Spinnler, H. E. (1999). Production of sulfur flavors by ten strains of *Geotrichum candidum*. *Applied and Environmental Microbiology, 65*, 5510-5514.

Besancon, X., Ratomahenina, R., & Galzy, P. (1995). Isolation and partial characterization of an esterase (EC 3.1.1.1) from a *Debaryomyces hansenii* strain. *Netherlands Milk and Dairy Journal, 49*, 97-110.

Bockelmann, W. (2002). Development of defined surface starter cultures for the ripening of smear cheeses. *International Dairy Journal, 12*, 123-131.

Breuer, U., & Harms, H. (2006). *Debaryomyces hansenii* — an extremophilic yeast with biotechnological potential. *Yeast, 23*, 415-437.

Carbonell, M., Núñez, M, & Fernández-García, E. (2002). Evolution of the volatile components of ewe raw milk La Serena cheese during ripening. Correlation with flavour characteristics. *Le Lait, 82*, 683-698.

Condurso, C., Verzera, A., Romeo, V., Ziino, M., & Conte, F. (2008). Solid-phase microextraction and gas chromatography mass spectrometry analysis of dairy product volatiles for the determination of shelf-life. *International Dairy Journal, 18*, 819-825.

Corbo, M. R., Lanciotti, R., Albenzio, M., & Sinigaglia, M. (2001). Occurrence and characterization of yeasts isolated from milks and dairy products of Apulia region. *International Journal of Food Microbiology, 69*, 147-152.

Curioni, P. M. G., & Bosset, J. O. (2002). Key odorants in various cheese types as determined by gas chromatography-olfactometry. *International Dairy Journal, 12*, 959-984.

Delavenne, E., Mounier, J., Asmani, K., Jany, J.-L., Barbier, G., & Le Blay, G. (2011). Fungal diversity in cow, goat and ewe milk. *International Journal of Food Microbiology, 151*, 247-251.

Delgado, F. J., González-Crespo, J., Cava, R., García-Parra, J., & Ramírez, R. (2010). Characterisation by SPME–GC–MS of the volatile profile of a Spanish soft cheese P.D.O. Torta del Casar during ripening. *Food Chemistry, 118*, 182-189.

Delgado, F. J., González-Crespo, J., Cava, R., & Ramírez, R. (2011). Formation of the aroma of a raw goat milk cheese during maturation analysed by SPME-GC-MS. *Food Chemistry, 129*, 1156-1163.

Fernández-García, E., Carbonell, M., Gaya, P., & Nuñez, M. (2004). Evolution of the volatile components of ewes raw milk Zamorano cheese. Seasonal variation. *International Dairy Journal, 14*, 701-711.

Fleet, G. H. (1990). A review: Yeasts in dairy products. *Journal of Applied Bacteriology, 68*, 199-211.

Fox, P. F., & Wallace, J. M. (1997). Formation of misvor compounds in cheese. *Advances in Applied Microbiology*, 45, 17-85.

Fox, P. F., Guinee, T. P., Cogan, T. M., & Mc.Sweeny, P. L. H. (2000). Fundamentals of cheese science. (1st ed.). Gaithersburg: Aspen publuishers, Inc., (Chapter 10).

Freitas, C., & Malcata, X. F. (2000). Microbiology and biochemistry of cheeses with Appélation d'Origine Protegée and manufactured in the

Iberian Peninsula from ovine and caprine milks. *Journal of Dairy Science, 83*, 584-602.

Frohlich-Wyder, M. T. (2003). Yeasts in dairy products. In T. Boekhout & V. Robert (Eds.), *Yeasts in food beneficial and detrimental aspects* (pp. 209–237). Hamburg: Behis Verlag.

García-Tejedor, A., Padilla, B., Salom, J. B., Belloch, C., & Manzanares, P. (2013). Dairy yeasts producemilk protein-derived antihypertensive hydrolysates. *Food Research International, 53*, 203-208.

Gardini, F., Tofalo, R., Belletti, N., Iucci, L., Suzzi, G., Torriani, S., Guerzoni, M. E., & Lanciotti, R. (2006). Characterization of yeasts involved in the ripening of Pecorino Crotonese cheese. *Food Microbiology, 23*, 641-648.

Gori, K., Sørensen, L. M., Petersen, M. A., Jespersen, L., & Arneborg, N. (2012). *Debaryomyces hansenii* strains differ in their production of flavor compounds in a cheese-surface model. *Microbiology Open, 1*, 161-168.

Gripon, J. C., Monnet, V., Lamberet, G., & Desmazeaud, M. J. (1991). Microbial enzymes in cheese ripening. In P. F. Fox (Ed.), *Food enzymes* (pp. 131-168). London: Elsevier Applied Science.

Horwood, J. F., Stark, W., & Hull, R. R. (1987). A "fermented, yeasted" flavour defect in Cheddar cheese. *Australian Journal of Dairy Technology, 42*, 25-26.

Izco, J. M., & Torre, P. (2000). Characterisation of volatile flavour compounds in Roncal cheese extracted by the ‘purge and trap' method and analysed by GC–MS. *Food Chemistry, 70*, 409-417.

Jiang, J. (1993). Identification of flavour volatile compounds produced by *Kluyveromyces lactis*. *Biotechnology Techniques, 7*, 863-866.

Kagkli, D.-M., Tâche, R., Cogan, T., Hill, C., Casaregola, S., & Bonnarme, P. (2006). *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, two potent deacidifying and volatile-sulphur-aroma-producing microorganisms of the cheese ecosystem. *Applied Microbiology and Biotechnology, 73*, 434-442.

Kovats, E. S. (1965). Gas chromatographic characterization of organic substances in the retention index system. In J. C. Giddings & R. A. Keller (Eds.), *Advances in chromatography* (pp. 229-247). New York: Marcel Dekker Inc.

Kuzdzal-Savoie, S. (1980). Determination of free fatty acids in milk and milk products. In Annual Bulletin 118 (pp. 53-66). Brussels: International Dairy Federation.

Larráyoz, P., Addis, M., Gauch, R., & Bosset, J. O. (2001). Comparison of dynamic headspace and simultaneous distillation extraction techniques used for the analysis of the volatile components in three European PDO ewes' milk cheeses. *International Dairy Journal, 11*, 911-926.

Leclercq-Perlat, M. N., Corrieu, G., & Spinnler, H. E. (2004). Comparison of volatile compounds produced in model cheese medium deacidified by *Debaryomyces hansenii* or *Kluyveromyces marxianus*. *Journal of Dairy Science, 87*, 1545-1550.

López del Castillo-Lozano, M., Delile, A., Spinnler, H. E., Bonnarme, P., & Landaud, S. (2007). Comparison of volatile sulphur compound production by cheese-ripening yeasts from methionine and methionine–cysteine mixtures. *Applied Microbiology and Biotechnology, 75*, 1447-1454.

Martin, N., Berger, C., Le Du, C., & Spinnler, H. E. (2001). Aroma compound production in cheese curd by coculturing with selected yeast and bacteria. *Journal of Dairy Science, 84*, 2125-2135.

McSweeney, P. L. H. (2004). Biochemistry of cheese ripening. *International Journal of Dairy Technology, 57*, 127-144.

Meinhart, E., & Schreier, P. (1986). Study of flavour compounds from Parmigiano Reggiano cheese. *Milchwissenschaft, 41*, 689-691.

Molimard, P., & Spinnler, H. E. (1996). Review: Compounds involved in the flavor of surface mold-ripened cheeses: Origins and Properties. *Journal of Dairy Science, 79*, 169-184.

Padilla, B., Ruiz-Matute, A. I., Belloch, C., Cardelle-Cobas, A., Corzo, N., & Manzanares, P. (2012). Evaluation of oligosaccharide synthesis from lactose and lactulose using β-galactosidases from *Kluyveromyces* isolated from artisanal cheeses. *Journal of Agricultural and Food Chemistry, 60*, 5134-5141.

Padilla, B., Manzanares, P., & Belloch, C. (2014). Yeast species and genetic heterogeneity within *Debaryomyces hansenii* along the ripening process of traditional ewes' and goats' cheeses. *Food Microbiology,* 38, 160-166.

Plutowska, B., & Wardencki, W. (2007). Aromagrams – Aromatic profiles in the appreciation of food quality. *Food Chemistry, 101*, 845-872.

Romano, P., Capace, A., & Jespersen, L. (2006). Taxonomic and ecological diversity of food and beverage yeasts. In A. Querol & G. H. Fleet (Eds.), *The yeast handbook-Yeasts in food and beverages* (pp. 13-53). Heidelberg: Springer-Verlag Berlin.

Seiler, H., & Busse, M. (1990). The yeasts of cheese brines. *International Journal of Food Microbiology, 11*, 289-303.

Sørensen, L. M., Gori, K., Petersen, M. A., Jespersen, L., & Arneborg, N. (2011). Flavour compound production by *Yarrowia lipolytica*, *Saccharomyces cerevisiae* and *Debaryomyces hansenii* in a cheese-surface model. *International Dairy Journal, 21*, 970-978.

Spinnler, H. E., Berger, C., Lapadatescu, C., & Bonnarme, P. (2001). Production of sulfur compounds by several yeasts of technological interest for cheese ripening. *International Dairy Journal, 11*, 245-252.

Legends to Figures

Fig. 1. Total volatile compounds abundance by chemical group (expressed as AU x 10^6) in the headspace of CLM supernatants after yeast growth: Km: *Kluyveromyces marxianus*; Kl: *Kluyveromyces lactis*; Dh: *Debaryomyces hansenii*. Data are mean ± SD of levels of volatile compounds produced by the different strains tested. Different letters in the same chemical group indicate significant differences ($p < 0.05$) among yeast species.

Fig. 2. Loadings of the first two principal components (PC1-PC2) of the analysed parameters (pH, percentage of lactose and lactate consumption and volatile compounds) of CLM after growth of different yeast strains: Km1-Km4 (*Kluyveromyces marxianus*), Kl1-Kl5 (*Kluyveromyces lactis*), Dh1-Dh5 (*Debaryomyces hansenii*).

Table 1. Abundance of volatile compounds (expressed as AU×10^6 extracted by SPME) in the headspace of the raw milk cheeses[a].

Compound	LRI[b]	RI[c]	Goats' cheese	Ewes' cheese	Previously reported[d]
Acids					
Acetic acid	709	A	196.2 ± 13.6	224.6 ± 31.6	1-7
Propanoic acid	815	A	3.2 ± 0.1	9.5 ± 1.6	3-7
2-Methylpropanoic acid	852	A	27.3 ± 5.3	12.5 ± 0.1	1,3-6
Butanoic acid	891	A	482.7 ± 15.0	216.3 ± 15.6	1-7
3-Methylbutanoic acid	932	A	30.2 ± 7.1	20.8 ± 1.6	1,3,5-7
2-Methylbutanoic acid	939	A	31.8 ± 7.8	15.1 ± 2.0	7
Pentanoic acid	971	A	2.7 ± 0.3	1.3 ± 0.1	3,4,7
Hexanoic acid	1080	A	676.6 ± 19.5	219.5 ± 9.6	1-4,6,7
Heptanoic acid	1165	A	4.7 ± 0.2	2.0 ± 0.1	3,4
Octanoic acid	1264	A	271.4 ± 7.9	67.1 ± 1.7	2-4
Benzenecarboxylic acid	1283	A	2.0 ± 0.3	1.4 ± 0.1	2
Nonanoic acid	1357	A	2.3 ± 0.1	nd	2,4
Decanoic acid	1453	A	128.2 ± 9.5	33.0 ± 1.8	2-4
Dodecanoic acid	1646	B	3.1 ± 0.4	0.9 ± 0.1	2,3
Esters					
Ethyl acetate	641	A	7.3 ± 2.1	25.4 ± 5.2	1-7
Propyl acetate	743	A	1.1 ± 0.1	4.1 ± 2.0	1,4,5,7
1-Methylpropyl acetate	787	B	nd	10.8 ± 7.4	1,7
Ethyl butanoate	828	A	18.9 ± 6.3	11.2 ± 2.5	1-5,7
Butyl acetate	844	A	nd	0.6 ± 0.1	1,5,7
3-Methyl-1-butanol acetate	907	A	5.6 ± 0.7	3.8 ± 0.3	1,7
Propyl butanoate	923	A	4.6 ± 0.4	2.8 ± 0.7	3-5,7
1-Methylpropyl butanoate	960	B	4.9 ± 0.6	7.5 ± 0.3	-
2-Methylpropyl 2-methyl butanoate	979	B	0.6 ± 0.1	nd	-
Ethyl hexanoate	1027	A	28.9 ± 13.5	15.6 ± 1.0	1-7
3-Methylbutyl butanoate	1084	B	6.2 ± 0.1	nd	-
2-Propenyl hexanoate	1111	B	0.5 ± 0.0	nd	-
Propyl hexanoate	1123	A	6.3 ± 0.1	2.1 ± 0.4	5, 7
1-Methylbutyl butanoate	1175	B	1.4 ± 0.0	0.9 ± 0.1	-
Ethyl 2-methyl-propanoate	1182	B	1.4 ± 0.1	nd	1
Ethyl octanoate	1225	A	16.8 ± 1.4	3.9 ± 0.4	1,3-7
Propyl octanoate	1322	B	1.0 0.0	nd	-
Ethyl decanoate	1425	A	7.5 ± 0.8	1.5 ± 0.1	1,2,4-6
Ketones					
Acetone	529	A	1.2 ± 0.1	1.1 ± 0.3	2
2-Butanone	635	A	43.2 ± 10.7	529.3 ± 26.3	1,3-7
2-Pentanone	729	A	41.7 ± 2.8	14.0 ± 6.2	1,4-7
3-Hydroxy-2-butanone	779	A	3.1 ± 0.6	31.8 ± 7.9	5,6
2-Hexanone	833	A	1.6 ± 0.0	nd	5-7
2-Heptanone	932	A	42.1 ± 3.2	3.7 ± 1.6	1,2,4-7

8-Nonen-2-one	1135	B	3.4 ± 0.5	nd	-
2-Nonanone	1139	A	147.4 ± 22.2	7.8 ± 2.9	1,2,4-7
2-Undecanone	1344	A	3.2 ± 0.6	0.6 ± 0.1	-
Aldehydes					
2-Propenal	519	A	1.4 ± 0.1	0.5 ± 0.1	1, 5, 7
3-Methylbutanal	691	A	1.2 ± 0.2	1.0 ± 0.1	1-7
Hexanal	838	A	0.8 ± 0.1	nd	1, 2, 5, 7
Benzaldehyde	1017	A	1.1 ± 0.1	1.6 ± 0.2	2,3
Benzeneacetaldehyde	1107	A	1.4 ± 0.2	0.8 ± 0.2	3
Alcohols					
Ethyl alcohol	511	A	62.4 ± 9.2	32.5 ± 6.5	1,5-7
Isopropyl alcohol	538	A	3.0 ± 0.5	1.6 ± 0.3	5,7
2-Propen-1-ol	610	B	3.8 ± 0.4	2.7 ± 1.0	1,5-7
1-Propanol	615	A	16.7 ± 1.5	20.6 ± 6.8	1,4-6
2-Butanol	647	A	133.2 ± 13.4	461.4 ± 32.5	1,3-5
2-Methyl-1-propanol	682	A	1.0 ± 0.1	0.4 ± 0.1	1,5,6
1-Methoxy-2-propanol	718	B	1.3 ± 0.2	1.4 ± 0.3	3, 5, 6
1-Butanol	719	A	3.2 ± 0.5	3.8 ± 0.7	1,2,4-7
2-Pentanol	747	A	68.2 ± 5.3	10.7 ± 3.9	1,4-7
3-Methyl-1-butanol	793	A	18.3 ± 0.8	6.6 ± 0.9	1,3-7
2-Methyl-1-butanol	796	A	2.8 ± 0.2	1.2 ± 0.1	7
2,3-Butanediol	879	A	37.9 ± 3.6	59.4 ± 6.4	-
1-Hexanol	920	A	4.9 ± 0.3	2.8 ± 0.7	1,2,5,6
2-Heptanol	944	A	71.2 ± 6.9	14.2 ± 5.9	1,4-7
1-Heptanol	1022	A	nd	0.8 ± 0.1	1,7
2-Nonanol	1147	A	12.1 ± 1.1	2.7 ± 0.7	1, 4
Phenylethyl alcohol	1193	A	5.8 ± 0.6	2.3 ± 0.3	-
Terpenes					
D-Limonene	1043	A	nd	39.2 ± 1.4	1, 2, 4, 5, 7
Sulphur compounds					
Dimethyl sulfone	1057	A	3.1 ± 0.5	1.9 ± 0.3	-

AU: Abundance units, the result of counting the total ion chromatogram (TIC) for each compound.

[a] Values are mean ± SD (n=3).

[b] Linear retention indices (LRI) of the compounds eluted from the GC-MS using a DB-624 capillary column (J&W Scientific 30 m×0.25 mm i.d.×1.4 µm film thickness).

[c] Reliability of identification (RI): A, mass spectrum and retention time identical with an authentic standard; B, tentative identification by mass spectrum.

[d] Compounds previously reported in ewes' and goats' raw milk cheeses. Reference numbers are as follows: (1) Carbonell, Núñez, & Fernández-García, 2002; (2) Condurso, Verzera, Romeo, Ziino, & Conte, 2008; (3) Delgado, González-Crespo, Cava, García-Parra, & Ramírez, 2010; (4) Delgado, González-Crespo, Cava, & Ramírez, 2011; (5) Fernández-García, Carbonell, Gaya, & Nuñez, 2004; (6) Izco & Torre, 2000 and (7) Larráyoz, Addis, Gauch, & Bosset, 2001.

nd: Not detected.

Table 2. Volatile compounds (expressed as AU×10^{6} extracted by HS-SPME) identified in the headspace of CLM after yeast growth[a].

	Kluyveromyces marxianus				*Kluyveromyces lactis*					*Debaryomyces hansenii*				
Compound	Km1	Km2	Km3	Km4	Kl1	Kl2	Kl3	Kl4	Kl5	Dh1	Dh2	Dh3	Dh4	Dh5
Acids														
Acetic acid	214.81^{a}	206.69^{a}	247.29^{a}	97.49^{b}	5.57^{c}	nd	4.19^{c}	49.31^{bc}	17.97^{c}	nd	nd	22.70^{c}	nd	nd
Propanoic acid	nd	nd	nd	6.24^{a}	nd	nd	nd	nd	nd	2.71^{a}	nd	nd	nd	nd
2-Methylpropanoic acid	21.33^{f}	19.76^{f}	39.16^{de}	25.23^{ef}	68.89^{b}	59.98^{bc}	55.01^{bcd}	91.20^{a}	104.68^{a}	23.03^{ef}	0.11^{h}	45.83^{cd}	0.56^{h}	0.93^{gh}
3-Methylbutanoic acid	11.49^{a}	12.52^{a}	1.41^{bc}	2.53^{b}	nd	nd	nd	nd	nd	1.51^{bc}	nd	2.46^{b}	0.60^{c}	0.50^{c}
2-Methylbutanoic acid	52.52^{a}	54.62^{a}	35.17^{c}	29.28^{cd}	38.15^{bc}	34.90^{c}	34.82^{c}	51.31^{a}	50.65^{a}	20.70^{d}	2.93^{e}	46.13^{ab}	0.88^{e}	2.28^{e}
Octanoic acid	1.32^{b}	nd	3.18^{a}	0.50^{b}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total acids	301.47^{a}	293.59^{a}	326.21^{a}	161.27^{b}	112.61^{cd}	94.88^{de}	94.02^{de}	191.82^{b}	173.30^{b}	47.95^{ef}	3.04^{f}	117.12^{cd}	2.04^{f}	3.71^{f}
Esters														
Ethyl acetate	770.82^{d}	914.69^{d}	909.30^{d}	904.95^{d}	1418.81^{c}	1612.86^{abc}	1519.04^{bc}	1702.68^{ab}	1795.43^{a}	185.98^{e}	1.86^{e}	58.67^{e}	10.29^{e}	0.80^{e}
Propyl acetate	15.06cd^{e}	22.94^{a}	19.76^{ab}	12.86^{de}	10.35^{e}	16.25^{bcd}	11.84^{de}	18.41^{abc}	16.00^{bcd}	0.68^{f}	nd	nd	nd	nd
Butyl acetate	nd	nd	11.88^{a}	11.33^{a}	0.53^{b}	0.84^{b}	0.34^{b}	0.55^{b}	0.49^{b}	nd	nd	nd	nd	nd
3-Methyl-1-butanol acetate	1290.58^{b}	1561.42^{a}	545.40^{c}	550.74^{c}	120.26^{de}	148.23^{de}	144.69^{de}	188.40^{d}	163.81^{de}	4.96^{e}	0.30^{e}	0.91^{e}	nd	nd
3-Methylbutyl butanoate	nd	0.79^{a}	nd	0.74^{a}	nd	0.74^{a}	0.68^{a}	0.65^{a}	0.79^{a}	nd	nd	nd	nd	nd
Ethyl octanoate	2.23^{a}	3.19^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ethyl decanoate	2.00^{bc}	1.38^{cd}	2.85 ^{b}	5.78^{a}	nd	nd	nd	nd	nd	2.13^{bc}	nd	0.91^{d}	nd	nd
Total esters	2080.69^{b}	2504.41^{a}	1489.20^{d}	1486.40^{d}	1549.95^{d}	1778.92^{bcd}	1676.59^{cd}	1910.69^{bc}	1976.52^{bc}	193.75^{e}	2.16^{e}	60.49^{e}	10.29^{e}	0.80^{e}
Ketones														
2-Pentanone	nd	nd	nd	nd	1.24^{b}	0.95b^{c}	0.52^{de}	0.76^{cd}	1.95^{a}	nd	nd	nd	0.24^{e}	nd
2-Heptanone	nd	nd	nd	nd	8.22^{d}	11.28^{c}	10.97^{c}	15.90^{a}	13.51^{b}	nd	nd	nd	nd	nd
2-Nonanone	nd	nd	nd	nd	4.54^{c}	6.36^{c}	5.26^{c}	11.02^{a}	8.80^{b}	nd	nd	nd	nd	nd
Total ketones	nd	nd	nd	nd	14.00^{d}	18.59^{c}	16.75^{c}	27.68^{a}	24.26^{b}	nd	nd	nd	0.24^{e}	nd
Aldehydes														
3-Methylbutanal	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.62^{a}	nd	nd	nd	2.13^{a}
Benzaldehyde	nd	nd	21.59^{b}	38.96^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total aldehydes	nd	nd	21.59^{b}	38.96^{a}	nd	nd	nd	nd	nd	1.62^{c}	nd	nd	nd	2.13^{c}
Alcohols														
Ethyl alcohol	1075.15^{cde}	1054.34^{de}	1273.58^{a}	1134.08^{bcd}	1215.53^{ab}	1011.79^{e}	994.41^{e}	1157.85^{bc}	1140.75^{bcd}	543.92^{f}	nd	252.55^{g}	10.85^{h}	12.37^{h}

1-Propanol	29.90^{c}	26.35^{c}	55.70^{b}	66.05^{a}	16.34^{d}	14.92^{de}	16.13^{d}	12.09^{de}	10.85^{ef}	$7.15f^{g}$	0.29^{h}	$2.95g^{h}$	0.52^{h}	0.33^{h}
2-Methyl-1-propanol	193.25^{d}	193.23^{d}	187.82^{d}	156.24^{e}	283.99^{b}	277.63^{bc}	256.03^{c}	285.64^{b}	337.74^{a}	193.06^{d}	10.53^{g}	66.77^{f}	9.97^{g}	3.02^{g}
3-Methyl-1-butanol	1570.08^{cd}	1517.17^{cd}	1619.72^{bc}	1440.63^{d}	1748.11^{ab}	1658.89^{abc}	1633.48^{abc}	1578.36^{cd}	1789.07^{a}	591.30^{ef}	702.24^{e}	458.81^{f}	87.48^{g}	31.85^{g}
2-Methyl-1-butanol	942.22^{e}	932.17^{e}	1228.13^{a}	1181.01^{abc}	1144.75^{bcd}	1130.80^{cd}	1110.76^{d}	1111.02^{d}	1110.01^{d}	1213.12^{ab}	191.44^{g}	825.22^{f}	28.01^{h}	22.46^{h}
2,3-Butanediol	13.98^{b}	11.58^{bc}	31.72^{a}	14.40^{b}	8.91^{cd}	2.03^{e}	4.06^{e}	7.66^{d}	7.88^{d}	nd	nd	nd	nd	nd
2-Heptanol	nd	nd	nd	nd	6.11^{b}	6.61^{b}	5.78^{b}	7.64^{a}	8.44^{a}	nd	nd	nd	nd	nd
2-Nonanol	nd	nd	nd	nd	8.37^{b}	8.34^{b}	7.65^{b}	11.72^{a}	13.13^{a}	nd	nd	nd	nd	nd
Phenylethyl alcohol	1301.50^{a}	1291.65^{a}	745.37^{b}	578.62^{c}	282.76^{de}	251.70^{de}	286.85^{d}	230.59^{de}	218.21^{e}	47.08^{f}	230.40^{de}	49.93^{f}	15.12^{f}	28.15^{f}
Total alcohols	5126.08^{a}	5026.49^{ab}	5142.04^{a}	4571.03^{cd}	4714.87^{bc}	4362.71^{d}	4315.15^{d}	4402.59^{cd}	4636.08^{cd}	2595.63^{e}	1134.90^{g}	1656.23^{f}	151.95^{h}	98.18^{h}

AU: Abundance units, the result of counting the total ion chromatogram (TIC) for each compound.

[a] Values are mean from n=3. Volatile compounds from CLM control were subtracted to each yeast CLM. Means followed by different letters in the same row indicate significant differences among yeast strains ($p<0.05$; one-way ANOVA with Fisher's LSD test)

nd: Not detected.

Table 3. Generation of volatile compounds (not found in the Mediterranean cheeses) in the headspace of CLM inoculated with yeasts.

			Yeast[a]		
Compound	LRI[b]	RI[c]	*K. marxianus*	*K. lactis*	*D. hansenii*
Esters					
Ethyl propanoate	738	A	100[C]	100	80
Ethyl 2-methyl-propanoate	785	A	100	100	40
2-Methylpropyl acetate	804	A	100	100	20
Propyl propanoate	837	A	50	100	0
Ethyl 2-methyl-butanoate	876	A	100	100	40
Ethyl 3-methyl-butanoate	879	A	0	0	40
2-Methylpropyl propanoate	895	A	75	100	20
2-Methyl-1-butanol acetate	909	A	100	100	40
3-Methyl-1-butanol propanoate	995	A	100	100	20
2-Methyl-1-butanol propanoate	999	A	100	80	20
2-Methylbutyl 2-methyl-propanoate	1044	A	50	100	40
3-Methylbutyl 2-methyl-butanoate	1128	A	0	0	40
2-Methylbutyl 2-methyl-butanoate	1133	A	0	0	40
2-Phenylethyl acetate	1317	A	25	100	20
2-Phenylethyl propanoate	1407	A	100	100	0
Phenylethyl butyrate	1451	A	100	100	20
Ketones					
Methyl isobutyl ketone	781	B	0	0	80
Aldehydes					
Acetaldehyde	469	A	0	100	60
2-Methylpropanal	595	A	0	0	60
2-Methylbutanal	700	A	0	0	60
Alcohols					
3-Methyl-pentanol	899	A	0	100	80
3,7-Dimethyl-6-octen-1-ol	1285	B	100	100	20
Sulphur compounds					
Methionol	1060	A	100	100	20

[a] Percentage of strains producing volatile compound in CLM media.
[b] Refer to foodnote [a] in Table 1.
[c] Refer to foodnote [b] in Table 1.

Figure 1 Padilla et al., 2013

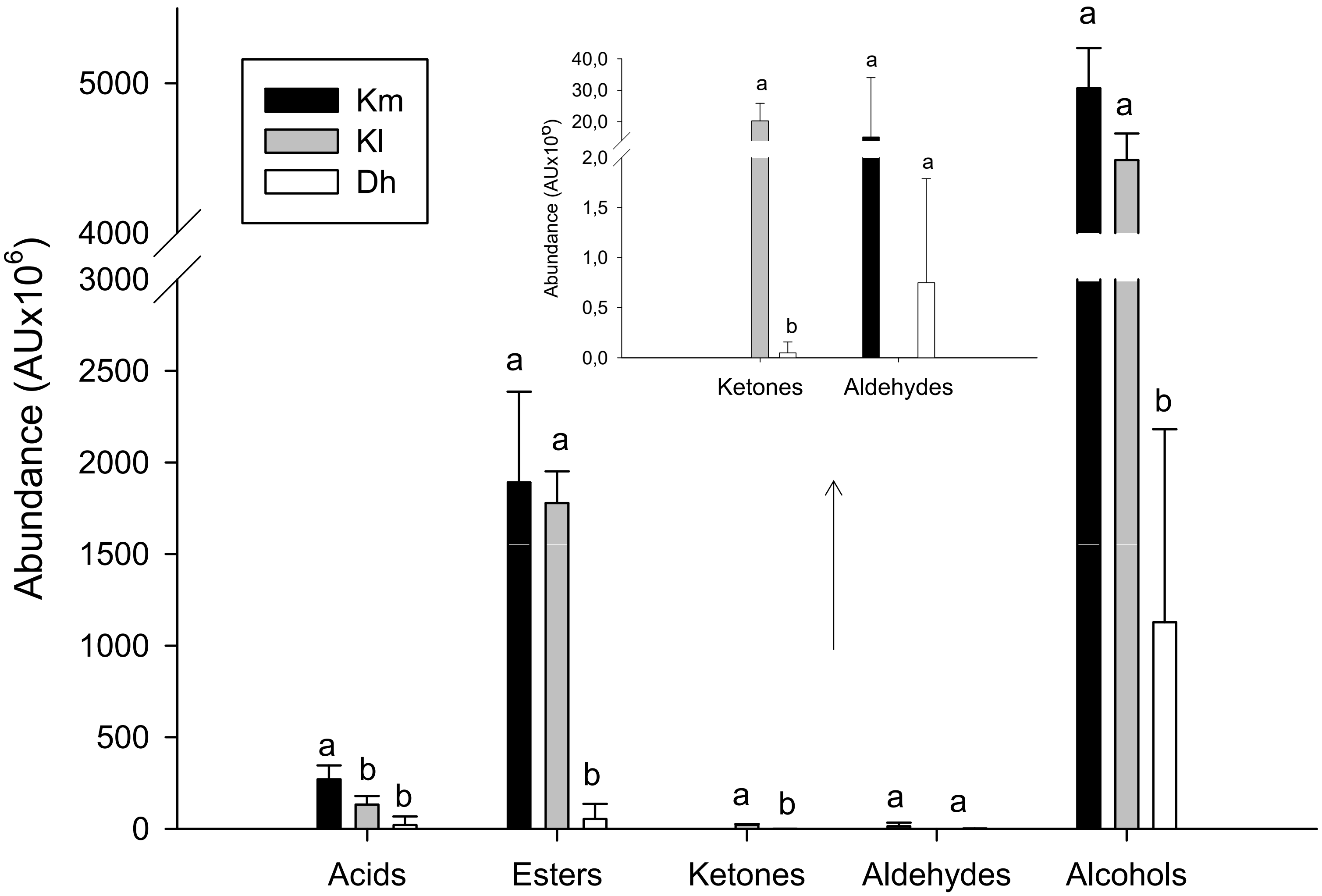

Figure 2 Padilla et al., 2013