LWT - Food Science and Technology 98 (2018) 506–514

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Evaluation of individual lactic acid bacteria for the fermentation of goat milk: Quality parameters

R. Muelas, A. Martí de Olives, G. Romero, J.R. Díaz, M.E. Sayas-Barberá, E. Sendra*

Departamento Tecnología Agroalimentaria, Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández de Elche, Carretera de Beniel, km 3.2, Orihuela, 03312, Spain

ARTICLE INFO

Keywords:
Goat milk
Lactic acid bacteria
Volatiles
Metabolites

ABSTRACT

Nine lactic acid bacteria (LAB) were evaluated as single cultures for goat milk fermentation with the aim to assess their suitability as adjunct cultures. Performance of the cultures was assessed based on technological and product quality parameters and their comparison with a commercial mixed culture. Volatile compounds profile, odor descriptors, acidification rate, microbial counts, gel stability, organic acid and sugar profiles, conjugated linoleic acid (CLA) content, were determined. Most LAB reached counts over 7-log cfu/g, gel stability assessed by centrifugation (average: 64% loss) and whey composition (average: 0.32% protein, 0.24% fat, 4.4% lactose, 3.5% dry matter) and CLA content (1.16% of total fatty acids) were not affected by the assayed cultures. Main differences among fermented milks relied on volatiles (mainly acids and ketones), odor descriptors and sugar metabolism (glucose, galactose and lactic acid). The present study provides useful information for the selection of cultures for goat milk products.

1. Introduction

Goat milk is increasingly getting attention for new product development during the last years due to the uniqueness of goat milk (Clark & Mora García, 2017). Besides, dairy goats are mainly located in low income countries as well as some European countries, and always linked to local breeds and traditional products. One of the main strategies for the development of the dairy goat sector is the diversification of goat milk products and the development of value added dairy products (Pulina, Milán, Lavín, Theodoridis, Morin, Capote, et al., 2018). Fermented dairy foods are positively perceived by consumers as healthy foods due to their digestibility and incorporation of live microorganisms. The first step on product development is the knowledge of the effect of individual bacteria on the properties of the fermented milk (FM) so the strains could be selected based on the best technological and functional properties developed (Li et al., 2017), as even strains from the same species may develop unique sensory notes in the end products.

When defining quality indicators of a starter culture several parameters can be selected, in the present study attention will be focused on the volatile profile and quality of the FMs. Acidification rate is a presumptive indicator of culture viability. High cell growth and acidification rate also reduce fermentation time and enhance strain viability by preventing the growth of undesirable or competitor microorganisms (Marklinder & Lönner, 1992). LAB counts should be at least over 10^7 colony forming units (cfu) per gram in order to fulfill common standards on microbial viability in FMs (Gomes & Malcata, 1999). An important parameter when evaluating a microbial food process is the identification and quantification of metabolic end-products; such as organic acids, sugars and volatile compounds.

Gel stability can be visually perceived and also assessed by water retention ability after centrifugation. The composition of the whey removed by centrifugation (fat and protein losses) may provide information on gel quality. Fatty acids profile of milk has been reported to be affected by fermentation (Trigueros, Barber, & Sendra, 2015), with some strains reported to increase CLA content in milk. Volatile profile and sensory properties of milks fermented by single cultures are not always related to the volatiles and sensory properties developed when the strain is used in combination with others in a mixed culture (Dan et al., 2017), it maybe modified when the balance between species is modified. However, the effect of each individual strain on the volatile profile of FMs provides a deeper knowledge on their flavor development abilities.

A good adjunct culture should not negatively affect sensory and quality attributes of the dairy products, and provide beneficial properties

Abbreviations: lab, lactic acid bacteria; FM, fermented milk; HPLC, high performance liquid chromatography; CECT, Spanish Culture Type Collection; SPME, Solid Phase Micro Extraction; CLA, Conjugated Linoleic Acid

* Corresponding author.
E-mail address: esther.sendra@umh.es (E. Sendra).

https://doi.org/10.1016/j.lwt.2018.09.005
Received 22 May 2018; Received in revised form 31 August 2018; Accepted 4 September 2018
Available online 06 September 2018
0023-6438/

(Champagne, Gomes da Cruz, & Daga, 2018). *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactococcus lactis* are among the most widely used LAB in the food industry, especially in starter cultures for the dairy industry, mixed with other LAB. *Lactobacillus casei* is commonly present in raw and fermented dairy products, and it has been successfully used for the development of functional ice-cream (Balthazar, Silva, Esmerino, Rocha, Moraes, Carmo, et al., 2018) and as a flavor enhancer in low sodium cheese (Silva, Balthazar, Esmerino, Neto, Rocha, Moraes, et al., 2018). *Lactobacillus paracasei,* is closely related to *L. casei. Lactobacillus helveticus* is also commonly present in dairy products. *Lactobacillus plantarum* is largely used in the food industry (meat, dairy, vegetable) and has the ability to produce antimicrobial compounds (Cebeci & Gürakan, 2003). *L. casei, L. paracasei* and *L. plantarum* are considered potential probiotics according to (Hill et al., 2014). Goat milk has been reported as a suitable carrier for probiotics (Ranadheera, Naumovski, & Ajlouni, 2018). *Lactobacillus reuteri* produces folate and B12 vitamin, as well as reuterin, which has antimicrobial properties (Jones & Versalovic, 2009). *Lactobacillus sakei* is frequently used for meat fermentation and plays a role in preserving fresh meats due to its ability to produce antimicrobial peptides (bacteriocins). It is not commonly used for dairy products but it was considered of interest as it is in the same group as *Lactobacillus curvatus,* which is a facultative heterofermentative bacterium commonly present as non-starter LAB in cheeses. Some strains of *L. curvatus* have been reported to be able to produce gut microbial changes associated with obesity reduction (Park et al., 2013). A commercial mixed starter culture commonly used in the dairy industry was taken as a reference culture.

The present work aims to provide new information on fermented goat milk by screening nine selected strains of LAB used as single starter cultures, and a mixed starter for goat milk fermentation based on their: (i) acidification rate, (ii) microbial counts, (iii) gel stability, (iv) fatty acid profile, (v) fermentation metabolites: organic acids, sugars and volatile profiles and (vi) odor.

2. Materials and methods

2.1. Fermented milk manufacture

Bulk tank milk from the experimental Murciano-Granadina dairy goats farm of the Miguel Hernández University was used for the study. Four liters of milk were taken each sampling day. Six independent replicates were run. Milk was pasteurized at 80 °C for 30 min and aseptically distributed into 80 mL sterile flasks for inoculation. Starter cultures were: *Lactobacillus casei* CECT 475*, Lactobacillus curvatus* CECT 5786*, Lactobacillus delbrueckii* subsp. *bulgaricus* CECT 4005*, Lactobacillus helveticus* CECT 541*, Lactobacillus paracasei subs. paracasei* CECT 277*, Lactobacillus plantarum* CECT 5785, *Lactobacillus reuteri* CECT 925, *Lactobacillus sakei* subs. *carnosus* CECT 5964, and *Lactococcus lactis* subsp. *lactis* CECT 4042, (Colección Española de Cultivos Tipo (CECT), Universidad de Valencia, Burjasot, España). The commercial mixed culture MA400 (Danisco; *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis biovar diacetylactis, Streptococcus thermophilus)* was tested as a reference mixed culture for fermentation performance on goats milk and quality characteristics (Morgan et al., 2003). To obtain FM samples 1 mL aliquots of starter culture were added to 80 mL pasteurized milk and the inoculated mixes were incubated at the optimal conditions for each culture as defined by CECT. pH was determined just before incubation and at time intervals until pH around 4.8 was reached. Afterwards, FMs were stored at 4 °C during 24 h before analysis. Most parameters were evaluated on four randomly assigned replicates, whereas sensory evaluation was run on two replicates.

2.2. Milk composition

Milk composition was analyzed using Fourier Transform Infrared Spectrophotometry (FT120 Milko-Scan; Foss Electric, Hillerφd, Denmark) with a commercial calibration from Foss Electric validated according to ISO 21543:2006 (ISO, 2006). The milk samples were previously heated to 40 °C.

2.3. Fermented milks analysis

Organic acids and sugars of FM samples were analyzed by High-Performance Liquid Chromatography (HPLC) (Trigueros, Sayas-Barberá, Pérez-Álvarez, & Sendra, 2012).

MRS agar was used for the count of all lactobacilli which were incubated following indications for optimal growth of each microorganism as defined by CECT. Strains of L. *curvatus, L. paracasei* and *L. plantarum* were incubated at 30 °C under aerobic conditions; *L. casei, L. reuteri* and *L. sakei* were incubated at 37 °C under aerobic conditions; *L. bulgaricus* and *L. helveticus* were incubated at 37 °C in candle jars; *L. lactis* (candle jar) and MA400 (aerobic conditions) were counted on M17 Agar (pH = 7.2 ± 0.2) at 30 °C incubated for 48 h (CECT, 2018).

Gel stability was visually assessed after incubation (signs of syneresis, gas formation, flocculation), and determined by quantifying the volume of whey removed from curd after centrifugation (Morgan et al., 2003). Whey composition was determined using the FT120 Milko-Scan calibrated for whey analysis using a pH-independent calibration by Foss Electric (ISO, 2006). Two replicate determinations were carried for each batch.

Milk fat was extracted (Romeu-Nadal, Morera-Pons, Castellote, & López-Sabater, 2004), and afterwards methylated (Trigueros & Sendra, 2015). Methyl esters were separated on a Shimadzu GC17A gas chromatography unit coupled with a flame ionization detector and a DB-23 capillary column (30 m length, 0.25 mm internal diameter, 0.25 μm film thickness) J&W Scientific, Agilent Technologies. The flow rate of the carrier gas (Helium) was 1.1 mL/min and 35 mL/min at the make-up point, the injector temperature was 240 °C and the detector 260 °C. The injection volume was 0.8 μL (split ratio 1:20). The temperature program was as follows: initial temperature 100 °C held for 1 min, temperature gradient of 3 °C/min until 220 °C, followed by a gradient of 5 °C/min until 245 °C and keeping 245 °C during 1 min. Identification of methylated fatty acids (FAME) peaks was performed by comparing the retention times of the FAME standards. Results were expressed semi-quantitatively as a percentage of the total fatty acid profile. Health-related indexes of fat have been calculated: Atherogenic Index (AI) and Thrombogenic Index (TI) were calculated (Batista et al., 2017) and Desaturase Index (DI) was calculated according to (Lock & Garnsworthy, 2003).

Solid Phase Micro Extraction (SPME) of volatiles was run (Tunick, Iandola, & Van Hekken, 2013, p. 2013). Afterwards, the fiber was desorbed in the injector port of the chromatography unit (Shimadzu CG17A coupled to a GCMS-QP5050) during 3 min at 250 °C. The column used for volatiles separation was a RXi-1301 Sil MS (30 m length, 0.25 mm internal diameter, 1-μm thickness), the mass spectrometer detector of the GC served for the identification of volatile compounds. The carrier gas was Helium (0.8 mL/min) and the temperature program was as follows: initial temperature 40 °C during 2 min, temperature gradient of 10 °C/min until 200 °C and keeping 200 °C during 10 min. Identification of peaks was performed by comparing the retention times of the standards compounds and by Wiley library spectra.

A Quantitative Descriptive Analysis (QDA), which use has been previously reported in dairy foods (Janiaski, Pimentel, Cruz, & Prudencio, 2016), was run for attributes for odor and aroma. The sensory panel used in this study included 9 highly panellists (5 females and 4 males) with ages between 24 and 61 years old, previously trained following the ISO 8586:2012. The preliminary orientation session (30 min) consisted in product evaluation by the sensory panel, who discussed about odor and aroma characteristics. After the orientation session, all members of the panel agreed in describing the sensory odor profile of each FM by 4–5 descriptors. This information was used to establish relationships with the key descriptors of the volatile compounds. Samples were randomly presented to panellists together with a questionnaire. The main objective of the sensory analysis by the expert panel was to establish the presence of off-flavors limiting the potential application of the strains and specific notes of interests for the development of fermented dairy products.

2.4. Statistical analysis

Statistical analysis and comparison among means were carried out using the statistical package SPSS 24.0 (IBM SPSS Statist cs, Chicago, IL, USA). One –way ANOVA test was used (factor: culture). Tukey test was used for means comparison (95% confidence level).

3. Results and discussion

3.1. Physicochemical and microbiological parameters

Overall milk composition was as follows: 4.70 ± 0.16% fat, 3.63 ± 0.06% protein, 4.90 ± 0.03% lactose, and 15 ± 0.22% solids content in milk. Acidification rate varied depending on the strain, the commercial mixed culture achieved final pH within 7 h as expected from the technical note, for all other strains it took between 12 and 27 h to reach a pH near 4.8. *L. reuteri* and *L. plantarum* showed the lowest acidification rates in goat milk (27 h), followed by *L. casei*, *L. paracasei* and *L. curvatus* that needed 24 h. Single cultured FM were expected to have long fermentation times, even the same strain when single shows lower acidification rates than when in mixed cultures (Pinto, Clemente, & De Abreu, 2009). Fermentation times and final pH of the assayed strains were similar to those previously reported on cow milk (Cano-Lamadrid, Trigueros, Wojdyło, Carbonell-Barrachina, & Sendra, 2017).

The highest microbial counts were observed in *L. paracasei*, followed by *L. lactis,* MA-400 and *L. curvatus* FMs (Table 1). Microbial counts in all FM were over 10^7 cfu/g except those of *L. bulgaricus,* which are significantly lower than the others ($p < 0.001$). Counts of this strain on skimmed cows' milk have been reported to be over 7 log-units (Cano-Lamadrid et al., 2017), so goat milk was less favorable to *L. bulgaricus* than cow's. Reported counts of *L. helveticus* were higher on cow milk (1 log unit increase) as compared to goat milk. All other single cultures showed the same behavior in goat as the previously reported in cow milk.

Regarding gel visual appearance: *L. curvatus* FM was flocculated and evidenced gas formation, to a lesser extend the same happened for *L. plantarum*, in both cases it is related to their heterofermentative nature. Milks fermented by *L. sakei, L. bulgaricus* and *L. casei* showed evident signs of syneresis but no gas nor flocculation. Gels obtained with MA400, *L. helveticus*, *L. paracasei*, *L. reuteri* and *Lc. lactis* had set-yogurt appearance with no evident signs of syneresis. Wheying off percentage showed few significant ($P < 0.05$) differences (Table 2), only between *L. helveticus* (highest water retention) and *L. sakei* (lowest water retention) FMs, so being *L. sakei* FM the least stable gel. Regarding whey composition, no significant differences were observed for fat, lactose and dry matter losses in the whey, whereas protein losses in the whey were slightly reduced by the use of the reference mixed culture MA400 (Table 2).

Table 1
Means (± SD) of bacterial counts of fermented goat milk at 24 h refrigerated storage as a function of the microbial culture (n = 16).

Cultures	Bact.count (Log_{10} cfu/g)
Lactobacillus casei CECT 475	8.54 ± 0.4^{bcd}
Lactobacillus curvatus CECT 5786	9.11 ± 0.25^{cd}
Lactobacillus delbrueckii subsp. *bulgaricus* CECT 4005	5.60 ± 0.47^{a}
Lactobacillus helveticus CECT 541	7.77 ± 0.77^{b}
Lactobacillus paracasei subsp. *paracasei* CECT 277	9.56 ± 0.22^{d}
Lactobacillus plantarum CECT 5785	8.74 ± 0.20^{bcd}
Lactobacillus reuteri CECT 925	8.19 ± 1.48^{bc}
Lactobacillus sakei subsp. *carnosus* CECT 5964	8.99 ± 0.58^{cd}
Lactococcus. lactis subsp. lactis CECT 4042	9.47 ± 0.35^{d}
MA-400[a]	9.42 ± 0.32^{d}
SL[b]	***

[a,b,c,d]Means within a column with different superscript letters present significant differences ($p < 0.05$).
CECT: Spanish Colection of Type Cultures.
[a] *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis biovar diacetylactis, Streptococcus thermophilus*.
[b] Significance level: ***$P < 0.001$.

3.2. Organic acids and sugars

Table 3 presents organic acids and sugars concentrations in the FMs. Such compounds contribute to the taste of FMs: sugars providing sweetness and acids providing acidity (from mild acidity to pungent vinegar taste depending on the balance between lactic and acetic acids). Lactose decreased from 4.9% in milk till values between 1.9 and 3.09% in the FMs. Lowest residual lactose was observed for *L bulgaricus* FM, although only significantly lower than those of *L. casei* and *L. para*casei. Regarding glucose, only traces were detected in milk fermented by the mixed culture MA400 and four other cultures, whereas values from 0.21 to 0.35% were observed for *L. helveticus, L. curvatus, L. bulgaricus, L. plantarum* and *L. reuteri* pointing to a lower metabolic activity in such FMs. Regarding galactose levels, the highest concentration was reported for *L. bulgaricus*, as expected, given that this specie of lactobacilli is not able to metabolize galactose. *L. helveticus and L. plantarum* also presented relevant amounts of galactose, although they are able to catabolize galactose. *Lc. lactis* also accumulated galactose as not all strains of this lactococci are able to metabolize it (Hickey, Hillier, & Jago, 1986) and its sugar metabolism is highly dependent on oxygen availability. FM with just traces or reduced amounts of residual monosaccharides point to a higher metabolic rate of the cultures. Lactic acid content observed in FMs is consistent with observed the pH values (Tables 2 and 3). Ethanol and acetic acid were not present in all FM samples. Acetic was mainly present in trace or really low amounts (< 0.02%), whereas ethanol was present only in *L. paracasei, L. reuteri, Lc. lactis* and MA400 FMs in similar amounts, and in *L. plantarum* FM, although in a lower amount.

L. reuteri is a heterofermentative bacterium that produces carbon dioxide, ethanol, acetate and lactic acid from glucose, so compared with homofermentative LAB the expected lactic acid production is lower as confirmed by the present results. *L. plantarum* is a facultative heterofermentative bacterium, able to use a variety of carbon sources and metabolize proteins. *L. casei* is a homofermentative bacterium, so lactic acid is the main metabolite from lactose, which is consistent with observation in Table 3 (no ethanol and few acetic acid content). Overall, sugar profile is highly species specific and acidification rate and sugar metabolism provide information on the future behavior of the strain. When a species accumulates galactose, if used in cheese it may lead to undesirable flavor development. When formulating mixed starters, such galactose accumulators need to be avoided or combined with galactose fermenting strains in the culture (Hickey et al., 1986).

3.3. Total fatty acids profile

The profile of total fatty acids was not affected by the tested LAB, a summary of the results is presented in Table 4. In the present study, neither the evaluated single strains nor the mixed culture enhanced the level of CLA, nor modified health-related indexes compared to milk values. Other authors also reported several LAB not able to enhance CLA levels in FMs (Manzo et al., 2015). Several health-related indexes have been calculated, ATI and TI are similar to that reported by (Ulbricht & Southgate, 1991) in milk, butter and cheese who were the first to define both ATI and TI indexes as being correlated with atherogenicity and thrombogenicity respectively. Observed ATI are about 2–3 points lower than those reported in bovine milk (Batista et al., 2017; Nantapo, Muchenje, & Hugo, 2014), mainly related to the high percentage of unsaturated fatty acids in goats' milk. However, TI is much higher than the reported by Batista et al. (2017), given the larger

Table 2
Overall composition (g/100 mL) of whey, pH and percentage of whey released by centrifugation from fermented goat milk as a function of microbial culture (n = 16).

Cultures	pH	Fat	Crude protein	Lactose	Total solids	Draining (%)
L. casei CECT 475	4.59 ± 0.36[abc]	0.27 ± 0.09[a]	0.61 ± 0.27[bc]	2.65 ± 0.26[a]	3.91 ± 0.31[a]	67.88 ± 5.74[ab]
L. curvatus CECT 5786	4.33 ± 0.27[ab]	0.29 ± 0.06 [a]	0.28 ± 0.05[ab]	2.30 ± 0.04[a]	3.51 ± 0.03[a]	62.67 ± 4.06[ab]
L. delbrueckii subsp. *bulgaricus* CECT 4005	4.24 ± 0.14[a]	0.22 ± 0.10[a]	0.33 ± 0.05[bc]	2.32 ± 0.07[a]	3.51 ± 0.16[a]	66.75 ± 1.69[ab]
L. helveticus CECT 541	4.20 ± 0.12[a]	0.23 ± 0.08[a]	0.31 ± 0.01[b]	2.26 ± 0.04[a]	3.42 ± 0.03[a]	60.90 ± 2.49[a]
L. paracasei subsp. *paracasei* CECT 277	4.35 ± 0.06[ab]	0.25 ± 0.19[a]	0.49 ± 0.36[bc]	2.43 ± 0.25[a]	3.64 ± 0.41[a]	64.82 ± 2.66[ab]
L. plantarum CECT 5785	4.79 ± 0.18[bc]	0.21 ± 0.01[a]	0.51 ± 0.33[bc]	2.49 ± 0.22[a]	3.71 ± 0.25[a]	63.83 ± 2.95[ab]
L. reuteri CECT 925	4.95 ± 0.36[c]	0.19 ± 0.06[a]	0.36 ± 0.03[bc]	2.42 ± 0.06[a]	3.58 ± 0.16[a]	66.18 ± 1.84[ab]
L. sakei subsp. *carnosus* CECT 5964	4.21 ± 0.18[a]	0.28 ± 0.15[a]	0.39 ± 0.02[bc]	2.32 ± 0.10[a]	3.51 ± 0.05[a]	69.71 ± 0.39[b]
Lc. lactis subsp. lactis CECT 4042	4.10 ± 0.03[a]	0.19 ± 0.01[a]	0.31 ± 0.01[b]	2.35 ± 0.01[a]	3.47 ± 0.04[a]	64.82 ± 2.59[ab]
MA-400[a]	4.49 ± 0.08[abc]	0.28 ± 0.09[a]	0.25 ± 0.02[a]	2.35 ± 0.06[a]	3.62 ± 0.15[a]	67.80 ± 1.11[ab]
SL[b]	**	ns	*	ns	ns	*

[abc]Means within a column with different superscript letters present significant differences.
Means within a column with different letters significantly differ.
L.: Lactobacillus; Lc.: Lactococcus.
CECT: Spanish Colection of Type Cultures.
[a] *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis* biovar *diacetylactis, Streptococcus thermophilus*.
[b] Significance level: ** $P < 0.01$; * $P < 0.05$; ns not significant.

Table 3
Means (± SD) of sugars and organic acids in fermented goat milk (g/100 g) as a function of the microbial culture (N = 16).

Microorganism	Lactose	Glucose	Galactose	Ethanol	Lactic acid	Acetic acid
Lactobacillus casei CECT 475	3.03 ± 0.43[bc]	tr	0.11 ± 0.0[a]	tr	0.49 ± 0.13[a]	0.01 ± 0.01[a]
Lactobacillus curvatus CECT 5786	2.43 ± 0.22[abc]	0.25 ± 0.07[ab]	0.14 ± 0.07[ab]	tr	0.62 ± 0.12[ab]	tr
Lactobacillus delbrueckii subsp. *bulgaricus* CECT 4005	1.90 ± 0.36[a]	0.23 ± 0.05[ab]	0.52 ± 0.16[c]	tr	0.73 ± 0.18[ab]	tr
Lactobacillus helveticus CECT 541	2.08 ± 0.46[ab]	0.35 ± 0.20[b]	0.36 ± 0.15[bc]	tr	0.65 ± 0.07[ab]	0.01 ± 0.01[a]
Lactobacillus paracasei subsp. *paracasei* CECT 277	3.09 ± 0.64[c]	tr	tr	0.58 ± 0.08[a]	0.60 ± 0.15[ab]	0.01 ± 0.01[a]
Lactobacillus plantarum CECT 5785	2.51 ± 0.12[abc]	0.21 ± 0.04[a]	0.27 ± 0.11[abc]	0.28 ± 0.21[a]	0.42 ± 0.18[a]	0.02 ± 0.00[a]
Lactobacillus reuteri CECT 925	2.40 ± 0.21[abc]	0.21 ± 0.07[a]	0.18 ± 0.07[ab]	0.56 ± 0.14[a]	0.44 ± 0.13[a]	0.01 ± 0.01[a]
Lactobacillus sakei subsp. *carnosus* CECT 5964	2.75 ± 0.63[abc]	tr	0.04 ± 0.00[a]	tr	0.85 ± 0.08[b]	0.01 ± 0.01[a]
Lc. lactis subsp. lactis CECT 4042	2.37 ± 0.34[abc]	tr	0.32 ± 0.23[ab]	0.54 ± 0.27[a]	0.70 ± 0.10[ab]	0.01 ± 0.01[a]
MA-400[a]	2.40 ± 0.15[abc]	tr	0.04 ± 0.02[a]	0.52 ± 0.17[a]	0.59 ± 0.07[ab]	tr
SL[b]	*	**	**	*	*	*

[abc]Means within a column with different superscript letters present significant differences.
CECT: Spanish Colection of Type Cultures.
tr: trace.
[a] *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis biovar diacetylactis, Streptococcus thermophilus*.
[b] Significance level: * $P < 0.05$; ** $P < 0.01$; ns not significant.

content in myristic acid (C14:0) in goat milk, about 7.5%, compared to cows, milk. DI has been correlated to Δ^9 desaturase activity, indicator of the ability to create a double bond in the 9th position of the fatty acid and it is mainly related to enzymatic activity in the mammary gland (Nantapo et al., 2014), in the present study the strains tested did not caused changes in the calculated health-related indexes (ATI, TI, DI, palmitoleic/palmitic and oleic/estearic) of goat milkfat.

3.4. Volatiles and odor characteristics

Major volatile compounds detected in raw goat's milk were 2-propanone, ethyl acetate, 2-heptanone and octanoic acid. Other compounds detected but in much lower amounts were hexanoic acid, 2-nonanone, nonanal, 2-pentanone, 2-butanone, and the lowest levels of volatiles were for dimethyl-sulphide, valeric acids and ethyl octanoate. Scarce data is available on the volatile compounds of fresh dairy products due to the low concentration of volatiles, and data on goat FMs is even scarcer (Cais-Sokolińska et al., 2015). No data is available in the scientific literature regarding the volatile profile developed by the present cultures on goat milk. Regarding FMs, volatile profiles and concentrations are presented in Table 5, as well as descriptors of the detected compounds reported in the scientific literature (Cheng, 2010; Sfakianakis & Tzia, 2017). A large number of volatile compounds form the overall flavor of FMs, major groups are usually alcohols, aldehydes, ketones, esters, hydrocarbons and organic acids. Ketones contribute to the perception of sweet, buttery and creamy flavors in fermented dairy products, whereas acids contribute to acidic notes (acetic and lactic) or goaty and cheesy (butanoic, hexanoic and octanoic), alcohols mainly provide alcoholic and floral-fruity notes (Table 5). Reported volatile compounds in fresh FMs are mainly limited to those coming from the milk and by-products from glycolysis (Imhof & Bosset, 1994). Most compounds detected were common to all analyzed FM, however relative amounts were quite different depending on the culture used.

FM flavor is a critical factor for quality evaluation and consumer acceptability, so not only volatile profile but also sensory perceptions are essential to describe culture performance. Table 6 includes the descriptors agreed by the evaluators for the odor of each FM. Evaluated FM had mild odor intensity, even plain, so samples were warmed for the evaluation. Such mild intensities are in agreement with the low volatiles concentrations detected (Table 5). Descriptors provided by the sensory panel are in agreement with the compounds detected in milk samples, although not always major compounds are the sole responsible of the main odor, pointing to clear interactions among volatile compounds to build unique sensations, like the reported synergy among methyl ketones and volatile fatty acids (Siek, Albin, Sather, & Lindsay, 1969). As can be seen in Table 6, most individual cultures yielded notes that if high intensity will be reached, non-desirable odor profiles will be developed. It should be taken into account that those same species, when used in mixed cultures yield different volatile profiles (Dan et al., 2017), as an example *Lc. lactis* is also present in the mixed MA400 culture and developed a 'cooked' odor that

Table 4
Means (± SD) of Saturated, Monounsaturated, Polyunsaturated fatty acids (SFA, MUFA, PUFA), total Conjugated Linoleic Acid (CLA) and health-related indexes: Atherogenicity Index (ATI), Thrombogenicity Index (TI), Desaturase Index (DI) and other ratios among fatty acids of goat milk and fermented goat milk (percentage of total fatty acids over total fatty acids) (n = 16) as a function of the starter culture.

Index	MILK	CECT 475[a]	CECT 5786[b]	CECT 4005[c]	CECT 541[d]	CECT 277[e]	CECT 5785[f]	CECT 925[g]	CECT 5964[h]	CECT 4042[i]	CECT MA-400[j]	SL[k]
SFA	59.56 ± 0.77[a]	60.43 ± 0.25[a]	59.90 ± 0.09[a]	60.36 ± 0.02[a]	60.22 ± 0.31[a]	59.82 ± 0.53[a]	60.11 ± 0.01[a]	60.18 ± 0.27[a]	60.55 ± 0.29[a]	59.62 ± 0.50[a]	60.03 ± 0.22[a]	ns
MUFA	31.08 ± 0.37[a]	31.74 ± 0.02[a]	32.02 ± 0.09[a]	31.63 ± 0.17[a]	31.95 ± 0.23[a]	32.37 ± 0.33[a]	32.03 ± 0.21[a]	31.76 ± 0.34[a]	31.69 ± 0.21[a]	32.23 ± 0.52[a]	32.19 ± 0.15[a]	ns
PUFA	5.14 ± 0.58[a]	5.58 ± 0.09[a]	5.72 ± 0.04[a]	5.59 ± 0.01[a]	5.64 ± 0.04[a]	5.71 ± 0.19[a]	5.59 ± 0.04[a]	5.62 ± 0.08[a]	5.56 ± 0.07[a]	5.61 ± 0.38[a]	5.34 ± 0.22[a]	ns
PUFAn-6	3.69 ± 0.06[a]	3.74 ± 0.04[a]	3.87 ± 0.04[a]	3.75 ± 0.01[a]	3.86 ± 0.06[a]	3.92 ± 0.17[a]	3.77 ± 0.01[a]	3.80 ± 0.08[a]	3.75 ± 0.02[a]	4.05 ± 0.22[a]	3.72 ± 0.01[a]	ns
PUFAn-3	0.22 ± 0.01[a]	0.24 ± 0.03[a]	0.23 ± 0.00[a]	0.22 ± 0.00[a]	0.22 ± 0.00[a]	0.23 ± 0.02[a]	0.23 ± 0.00[a]	0.22 ± 0.01[a]	0.22 ± 0.00[a]	0.23 ± 0.01[a]	0.22 ± 0.01[a]	ns
CLA	1.12 ± 0.07[a]	1.16 ± 0.01[a]	1.15 ± 0.01[a]	1.19 ± 0.00[a]	1.16 ± 0.01[a]	1.15 ± 0.01[a]	1.17 ± 0.01[a]	1.18 ± 0.01[a]	1.18 ± 0.01[a]	1.16 ± 0.05[a]	1.16 ± 0.01[a]	ns
PUFA/SFA	0.09 ± 0.01[a]	0.09 ± 0.00[a]	0.10 ± 0.00[a]	0.09 ± 0.00[a]	0.09 ± 0.00[a]	0.10 ± 0.00[a]	0.09 ± 0.21[a]	0.09 ± 0.00[a]	0.09 ± 0.00[a]	0.09 ± 0.01[a]	0.09 ± 0.00[a]	ns
PUFA (n-6/n-3)	16.81 ± 0.59[a]	15.72 ± 1.70[a]	17.06 ± 0.10[a]	17.20 ± 0.13[a]	17.30 ± 0.36[a]	17.31 ± 0.58[a]	16.72 ± 0.33[a]	17.10 ± 0.38[a]	17.39 ± 0.09[a]	17.73 ± 0.53[a]	16.62 ± 0.89[a]	ns
ATI	1.60 ± 0.02[a]	1.62 ± 0.01[a]	1.58 ± 0.00[a]	1.63 ± 0.01[a]	1.61 ± 0.02[a]	1.57 ± 0.04[a]	1.60 ± 0.01[a]	1.62 ± 0.02[a]	1.62 ± 0.00[a]	1.56 ± 0.05[a]	1.60 ± 0.01[a]	ns
TI	2.36 ± 0.03[a]	2.38 ± 0.02[a]	2.35 ± 0.02[a]	2.39 ± 0.00[a]	2.36 ± 0.01[a]	2.33 ± 0.06[a]	2.34 ± 0.00[a]	2.37 ± 0.04[a]	2.39 ± 0.00[a]	2.30 ± 0.06[a]	2.36 ± 0.02[a]	ns
DI	0.02 ± 0.00[a]	0.02 ± 0.00[a]	0.02 ± 0.00[a]	0.02 ± 0.00[a]	0.02 ± 0.00[a]	0.02 ± 0.00[a]	0.02 ± 0.00[a]	0.02 ± 0.00[a]	0.02 ± 0.00[a]	0.02 ± 0.00[a]	0.02 ± 0.00[a]	ns
Palmitoleic/palmitic	0.04 ± 0.00[a]	0.03 ± 0.00[a]	0.04 ± 0.00[a]	0.04 ± 0.00[a]	0.04 ± 0.00[a]	0.04 ± 0.00[a]	0.04 ± 0.00[a]	0.04 ± 0.00[a]	0.03 ± 0.00[a]	0.04 ± 0.00[a]	0.04 ± 0.00[a]	ns
Oleic/estearic	2.19 ± 0.02[a]	2.14 ± 0.02[a]	2.16 ± 0.02[a]	2.13 ± 0.00[a]	2.19 ± 0.00[a]	2.21 ± 0.03[a]	2.21 ± 0.01[a]	2.17 ± 0.03[a]	2.14 ± 0.02[a]	2.25 ± 0.06[a]	2.19 ± 0.03[a]	ns

CECT: Spanish Colection of Type Cultures.
ATI = (C12:0 + 4 × C14:0 + C16:0)/[Σ MUFA + ΣPUFA (n-6) and (n-3)]; TI = (C14:0 + C16:0 + C18:0)/[0.5 × Σ MUFA + 0.5 × Σ PUFA(n-6) + 3 × Σ PUFA(n-3) + (n-3)/(n-6)]; DI = C14:1/C14:0.

[a] *Lactobacillus casei* CECT 475.
[b] *Lactobacillus curvatus* CECT 5786.
[c] *Lactobacillus delbrueckii* subsp. *bulgaricus* CECT 4005.
[d] *Lactobacillus helveticus* CECT 541.
[e] *Lactobacillus paracasei* subsp. *paracasei* CECT 277.
[f] *Lactobacillus plantarum* CECT 5785.
[g] *Lactobacillus reuteri* CECT 925.
[h] *Lactobacillus sakei* subsp. *carnosus* CECT 5964.
[i] *Lactococcus lactis* subsp. *Lactis* CECT 4042.
[j] *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis* biovar *diacetylactis, Streptococcus thermophilus*.
[k] Significance level: ns not significant ($p > 0.05$).

Table 5
Volatile compounds isolated an identified in fermented goat milk by different cultures: Retention time, literature descriptors and average concentration (nL/L) (n = 8).

Volatile Organic Compound	Ret. time	Aromatic descriptor[b]	*L. casei* CECT 475	*L. curvatus* CECT 5786	*L. delbrueckii* subsp. *bulgaricus* CECT 4005	*L. helveticus* CECT 541	*L. paracasei* subsp. *paracasei* CECT 277	*L. plantarum* CECT 5785	*L. reuteri* CECT 925	*L. sakei* subsp. *carnosus* CECT 5964	*Lc. lactis subsp. Lactis* CECT 4042	MA-400[a]
Ethanol	3.012	Mild, ether	24.6 ± 3.4^{c}	56.7 ± 7.2^{d}	3.81 ± 0.95^{a}	1.43 ± 0.32^{a}	114 ± 18^{e}	96.8 ± 27.0^{e}	31.1 ± 2.5^{c}	7.28 ± 1.12^{b}	40.2 ± 0.6^{cd}	133 ± 14^{e}
2-Propanone	3.446	Sweet, fruity	40.0 ± 9.0^{a}	36.0 ± 4.8^{a}	41.4 ± 4.1^{a}	39.3 ± 4.8^{a}	46.5 ± 7.7^{a}	35.8 ± 6.7^{a}	61.4 ± 5.0^{b}	59.1 ± 3.4^{b}	68.8 ± 0.3^{b}	72.1 ± 13^{b}
DMS	3.539	Sulfurous	7.52 ± 0.98^{b}	nd	6.12 ± 1.18^{b}	nd	2.42 ± 0.02^{a}	3.19 ± 0.71^{a}	2.91 ± 0.49^{a}	4.50 ± 0.56^{ab}	nd	7.48 ± 0.09^{b}
Diacetyl	5.584	Buttery, creamy, vanilla	37.7 ± 6.5^{c}	13.6 ± 10.3^{a}	25.8 ± 11.3^{b}	80.6 ± 2.58^{e}	72.6 ± 25.9^{e}	82.4 ± 5.06^{e}	52.1 ± 10.9^{d}	67.8 ± 10.4^{e}	57.2 ± 0.50^{de}	17.2 ± 1.6^{a}
2-Butanone	5.772	Varnish-like, sweet, fruity	4.71 ± 0.61^{b}	1.98 ± 0.44^{a}	3.58 ± 0.20^{ab}	2.72 ± 0.24^{a}	2.93 ± 0.51^{a}	2.12 ± 0.11^{a}	5.06 ± 0.58^{b}	2.81 ± 0.45^{a}	5.41 ± 0.78^{b}	2.58 ± 0.28^{a}
Acetic acid	7.388	Vinegar, pungent, acidic	149 ± 18^{d}	46.9 ± 6.4^{b}	27.3 ± 3.7^{a}	52.6 ± 6.0^{b}	141 ± 21^{d}	113 ± 8^{d}	183 ± 13^{e}	658 ± 46^{f}	69.4 ± 1.7^{b}	117 ± 23^{d}
2-Pentanone	8.976	Fruity, acetone	1.74 ± 0.17^{a}	9.19 ± 0.63^{c}	3.34 ± 0.25^{a}	4.63 ± 0.38^{b}	5.63 ± 0.51^{b}	3.95 ± 0.26^{a}	2.66 ± 0.35^{a}	5.82 ± 0.45^{b}	6.70 ± 0.81^{b}	6.36 ± 0.34^{b}
2-Butanone, 3,3-dimethyl-	9.322	Sweet, butterschotch	nd	0.32 ± 0.3^{a}	76.6 ± 11.0^{d}	165 ± 18^{e}	1.48 ± 0.13^{b}	0.27 ± 0.2^{a}	10.68 ± 1.33^{c}	4.22 ± 0.4^{b}	4.36 ± 0.37^{b}	3.76 ± 0.01^{b}
Acetoin	10.959	Buttery	108 ± 16^{b}	68.1 ± 7.3^{a}	158 ± 24^{c}	184 ± 24^{c}	90.3 ± 14^{b}	608 ± 61^{e}	339 ± 13^{d}	684 ± 620^{e}	113 ± 8^{b}	138 ± 34^{c}
Iso amyl alcohol	11.498	Fruit, banana	1.25 ± 0.03^{b}	nd	nd	nd	nd	1.78 ± 0.2^{b}	0.55 ± 0.3^{a}	nd	1.20 ± 0.1^{b}	nd
Octane	13.219		6.27 ± 0.46^{a}	44.1 ± 3.3^{c}	60.3 ± 7.6^{c}	179 ± 16^{e}	14.3 ± 2.0^{b}	76.3 ± 9.0^{cd}	59.1 ± 1.3^{c}	175 ± 17.1^{e}	46.8 ± 5.7^{c}	99.9 ± 9.5^{d}
Isobutyric acid	13.963	Sweet, mild, rotten apple	22.3 ± 3.8	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pentan-3-ol	15.005		2.22 ± 0.40^{b}	0.50 ± 0.07^{a}	13.9 ± 1.2^{d}	30.8 ± 4.6^{e}	0.46 ± 0.03^{a}	5.68 ± 0.12^{c}	4.08 ± 0.8^{c}	0.96 ± 0.09^{a}	4.15 ± 0.30^{c}	2.21 ± 0.28^{b}
Butanoic acid	15.277	Sweaty,cheesy	26.8 ± 4.3^{f}	0.59 ± 0.2^{a}	1.15 ± 0.15^{b}	1.32 ± 0.21^{b}	4.51 ± 0.49^{d}	3.21 ± 0.41^{c}	5.09 ± 0.69^{d}	7.00 ± 0.97^{e}	2.06 ± 0.58^{b}	9.07 ± 1.02^{e}
2-butanol	15.385	Alcoholic	26.3 ± 2.6^{f}	0.47 ± 0.13^{a}	13.3 ± 1.3^{e}	29.8 ± 4.4^{f}	0.74 ± 0.04^{a}	6.98 ± 0.53^{d}	2.79 ± 0.71^{c}	12.8 ± 0.5^{f}	3.60 ± 0.22^{c}	1.18 ± 0.13^{b}
Isovaleric acid	17.791	Rotten fruit, mild, sweaty, rancid, fecal, putrid, flowery	368 ± 4.21^{d}	0.14 ± 0.10^{a}	0.55 ± 0.14^{a}	20.9 ± 2.3^{c}	0.77 ± 0.26^{a}	22.9 ± 4.7^{c}	12.7 ± 0.8^{b}	16.6 ± 1.23^{b}	0.13 ± 0.30^{a}	nd
Propionic acid	18.250	Vinegar, pungent, sour milk	11.3 ± 1.31^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd
2-heptanone	18.311	Sweet, blue cheese, spicy	8.28 ± 1.68^{a}	7.43 ± 0.86^{a}	15.6 ± 1.6^{b}	27.4 ± 3.5^{c}	14.5 ± 2.9^{b}	22.9 ± 1.4^{c}	13.9 ± 1.3^{b}	26.1 ± 2.5^{c}	20.2 ± 1.5^{c}	25.6 ± 1.7^{c}
Heptanal	18.700	Green, sweet	nd	0.75 ± 0.02^{ab}	0.13 ± 0.03^{a}	0.30 ± 0.01^{a}	0.04 ± 0.1^{a}	1.06 ± 0.21^{ab}	0.83 ± 0.05^{ab}	6.81 ± 0.44^{c}	nd	2.08 ± 0.69^{b}
Alpha.−PINENE	18.942	pine	nd	0.42 ± 0.12^{a}	1.53 ± 0.28^{b}	2.24 ± 0.27^{c}	0.92 ± 0.27^{ab}	1.47 ± 0.13^{b}	0.42 ± 0.04^{a}	2.37 ± 0.30^{c}	1.58 ± 0.18^{b}	1.58 ± 0.34^{b}
Heptane	22.166		nd	0.39 ± 0.4^{a}	0.38 ± 0.09^{a}	nd	0.63 ± 0.08^{a}	1.16 ± 0.14^{b}	0.50 ± 0.3^{a}	nd	0.62 ± 0.08^{a}	2.04 ± 0.2^{c}
Hexanoic acid	24.016	Goat like, fatty. sweat	81.9 ± 13.1^{e}	1.88 ± 0.13^{a}	17.4 ± 1.9^{b}	65.5 ± 4.04^{d}	14.8 ± 1.35^{b}	26.1 ± 1.9^{c}	21.3 ± 2.1^{c}	19.1 ± 1.7^{bc}	18.1 ± 2.17^{bc}	15.9 ± 0.14^{b}
Undecane, 4,7-dimethyl	26.087		25.7 ± 2.6^{bc}	6.68 ± 0.66^{a}	22.8 ± 1.4^{b}	30.7 ± 3.2^{c}	10.3 ± 1.4^{a}	8.55 ± 0.83^{a}	23.4 ± 2.6^{b}	36.8 ± 0.4^{c}	18.3 ± 2.6^{b}	26.1 ± 2.3^{bc}
Nonanal	27.903	Sweet, floral, citrus, grass-like	0.40 ± 0.02^{a}	1.44 ± 0.16^{b}	1.62 ± 0.13^{b}	1.05 ± 0.11^{b}	1.14 ± 0.07^{b}	2.41 ± 0.16^{c}	2.99 ± 0.34^{c}	4.38 ± 0.60^{d}	1.11 ± 0.37^{b}	2.84 ± 0.27^{c}
Octanoic acid	31.974	Wax, soap, goat, musty, rancid, fruity	10.6 ± 0.2^{e}	0.70 ± 0.05^{a}	1.33 ± 0.09^{b}	4.55 ± 0.37^{d}	2.98 ± 0.33^{c}	5.73 ± 0.35^{d}	6.22 ± 0.6^{d}	7.06 ± 0.5^{de}	1.88 ± 0.38^{b}	2.78 ± 0.09^{c}
Tridecane	34.093		1.31 ± 0.13^{a}	2.40 ± 0.07^{b}	8.72 ± 1.18^{e}	4.20 ± 0.78^{c}	4.25 ± 0.55^{c}	7.83 ± 0.44^{de}	6.48 ± 0.59^{d}	4.29 ± 0.67^{c}	2.50 ± 0.21^{b}	2.39 ± 0.47^{b}
Total alcohols			54.4^{c}	57.6^{c}	31.1^{b}	62.0^{c}	116^{d}	111^{d}	38.5^{b}	21.1^{a}	49^{b}	137^{d}
Total aldehydes			0.40^{a}	2.19^{b}	1.75^{b}	1.35^{b}	1.18^{b}	3.47^{c}	3.81^{c}	11.2^{b}	1.11^{b}	4.93^{c}
Total hydrocarbons			32.0^{a}	51.1^{b}	83.6^{c}	210^{e}	25.2^{a}	86.1^{c}	83.0^{c}	212^{e}	65.8^{b}	128^{d}
Total acids			671^{e}	50.3^{a}	47.7^{a}	145^{c}	164^{c}	172^{c}	228^{d}	708^{e}	91.6^{b}	146^{c}
Total ketones			208^{b}	129^{a}	324^{c}	505^{d}	234^{b}	756^{e}	484^{d}	850^{e}	275^{b}	266^{b}
Others			8.82^{b}	2.82^{a}	16.4^{d}	6.44^{b}	7.59^{b}	12.5^{c}	9.81^{bc}	11.2^{c}	4.08^{a}	11.4^{c}

L.: *Lactobacillus*; *Lc.*: *Lactococcus*; nd not detected; DMS dimethyl sulphide. Means within a row with different superscript letters present significant differences ($p < 0.05$).
[a] *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis biovar diacetylactis*, *Streptococcus thermophilus*.
[b] Cheng (2010) and Sfakianakis and Tzia (2017).

was not present in MA400 FM.

According to de Bok et al. (2011) it is possible to obtain volatile compounds fingerprints from single or mixed starter cultures given the differences in microbial metabolism for each species and their interactions. As an example, the authors reported that the single culture of *L. bulgaricus* in cow milk is characterized by higher levels of 2-propanone and 2-heptanone and lower levels of diacetyl as compared with other cultures, in agreement with the present (Table 5) and other studies (Pinto et al., 2009). Also ethanol and acetoin are among the main volatiles in cow milk fermented by *L. bulgaricus* (Imhof & Bosset, 1994).

Main volatiles in *L casei* fermented cow milks include ethanol, acetoin and acetic acid among the main volatiles (Imhof & Bosset, 1994), which is in coincidence with results in Table 5 on goat milk. However, the strain used in the present study on goat milk developed high contents of propionic, isovaleric, hexanoic, butyric, isobutyric and octanoic acids. Propionic acid was solely developed in *L. casei* FM. The observed volatile profile fits the descriptors used by the expert sensory panel as vinegar and cheesy (Table 6). In future studies, when applying these strains to fermented goat milk products, it should be essential to run consumer studies to determine optimal culture dosage (Dantas, Jesus, Silva, Almada, Esmerino, Cappato, et al., 2016) and most valued attributes for consumers on the dairy foods (Janiaski et al., 2016). *L. casei* FM showed a unique volatile pattern, and acids accounted for the major group followed by ketones and total alcohols. For the other cultures, ketones prevailed in all FM, with an equilibrium among acids and ketones for *L. sakei* FM. *L. curvatus* FM followed the same pattern of MA400 FM: ketones, followed by equal amounts of acids, alcohols and hydrocarbons, and just reduced contents of aldehydes and others, however volatile content was much lower. Its odor notes included putrid and animal like. *L. helveticus* FM showed a similar pattern but with reduced proportion of alcohols and enhanced hydrocarbons and developed acidic and cheesy notes.

Lc. lactis has been reported to develop ethanol, diacetyl, acetoin, acetaldehyde and propanone among major volatiles (Imhof & Bosset, 1994) in cow FM. In Table 5 it can be seen that acetoin and diacetyl were the major compounds in goat FM, and the combination of volatiles were responsible for providing a sweet, cooked and lactic odor (Table 6).

L. paracasei has been reported to enhance diacetyl formation when included in bovine milk yogurt (Aunsbjerg et al., 2015) and to provide antifungal protection. In bovine cheese both diacetyl and 2-propanone were enhanced (Pogačić et al., 2015). In the present study main volatile detected was acetic acid and diacetyl was also among major volatiles, the FM showed acid and animal like odors.

Acetoin was the major volatile in *L. sakei* FM, showing the highest content of, not only acetoin, but of total volatile content, and providing sweet, sour and sweaty odor notes. This is consistent with *L. sakei* reported to enhance acetoin in bovine milk cheese (Pogačić et al., 2015).

L. plantarum provides unique acidic tastes to fermented cow milk depending on the balance between volatile acetic acid and lactic acid (Quatravaux, Remize, Bryckaert, Colavizza, & Guzzo, 2006), which is dependent on the access to oxygen. *L. plantarum* strains have been previously evaluated as yogurt adjunct cultures on bovine milk, and although the choice of the specific strain affects the quality of the FM, some compounds are characteristics of their presence such as 2,3-pentanedione, acetaldehyde and acetate (Li et al., 2017; Wang et al., 2017). However, for the present strain acetoin was the prevailing volatile accounting for 60% of the total volatile content of the FM.

L. reuteri FM had acetoin as major volatile compound followed by acetic acid and developed sweaty rancid notes, this species has been reported to be used for cheese making to develop bio-preservation properties (Gómez-Torres, Ávila, Delgado, & Garde, 2016), and although volatile profile of cheese suffered changes, sensory analysis showed only slight modifications: enhanced cheesy notes and reduced milk, yogurt notes.

Table 6
Main sensory descriptors of fermented goats milk odor as a function of the microbial culture (first descriptor being the strongest).

Culture	Descriptors
Lactobacillus casei CECT 475	*Vinegar, lactic acid, blue cheese, cheesy*
Lactobacillus curvatus CECT 5786	*Putrid, cream, sweet, animal-like, lactic acid*
Lactobacillus delbrueckii subsp. *bulgaricus* CECT 4005	*Lactic acid, sour milk, cheese*
Lactobacillus helveticus CECT 541	*Lactic acid, sour milk, cheese, vinegar*
Lactobacillus paracasei subsp. *paracasei* CECT 277	*Sulfurous, lactic acid, unclean, vinegar, animal-like*
Lactobacillus plantarum CECT 5785	*Sulfurous, sweet, animal-like, grass*
Lactobacillus reuteri CECT 925	*Sweaty, rancid, cream, unclean*
Lactobacillus sakei subsp. *carnosus* CECT 5964	*Sweet, sour milk, sweaty, lactic acid*
Lactococcus lactis subsp. *lactis* CECT 4042	*Sweet, cooked, broth, lactic acid*
MA-400[a]	*Buttery, sweet, lactic acid, sour milk*

CECT: Spanish Colection of Type Cultures.
[a] *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis biovar diacetylactis, Streptococcus thermophilus*.

In order to summarize the results of the present study, and assuming the risk of excessive simplification, Table 7 has been prepared scoring only parameters that showed relevant differences among FMs. The high acidification rate, good visual gel stability, high microbial counts, high lactic acid content, non-accumulation of monosaccharides, and odor descriptors of MA400 FM have been taken as reference values. Under such frame, *Lc. lactis*, *L. paracasei* and *L. helveticus* proved to be the strains providing faster acidification, higher microbial counts and fermentation abilities on goats' milk, so pointing to the three most interesting species for further studies and applications for the development and innovation on goat milk products. However, also other tested strains maybe of interest to develop unique odor/volatile profiles. It needs to be taken into account that many consumers are not used to goat milk products and their odor characteristics and given the valuable nutritional and potential interest as probiotic carriers several authors (Costa et al., 2014) have suggested strategies to educate consumers to improve the acceptance of fermented goat milk.

4. Conclusions

The present results may support the dairy industry providing valuable data for new product development. The knowledge of the performance of single strains for the fermentation of goat milk may point to potential uses of the strains in goat milk products either alone or in combination with other LAB as adjunct cultures. The present FMs should not be taken as end products but as an evaluation of the performance of cultures.

All tested LAB reach counts over 10^7 cfu g^{-1} on goat milk, except *L. bulgaricus*. Main differences among the FM rely on acidification rate, gel visual stability, sugar profile, volatile profile and odor descriptors. Gel stability as assessed by draining off. CLA content and overall fatty acid profile are not modified by these LAB. *L. helveticus, L. paracasei* and *Lc. lactis* are the strains with the closest performance to that of a mixed commercial culture, although all tested LAB maybe of interest depending on the required characteristics of the final product.

Further studies may deal with the application of such strains for the development of goat milk products and the study of their consumer acceptance as well as other health related parameters such as the potential development of bioactive peptides and *in vitro* digestion studies.

Table 7
Summary of technological and quality characteristics of fermented goat milk by different cultures.

	L. casei CECT 475	*L. curvatus* CECT 5786	*L. delbrueckii* subsp. *bulgaricus* CECT 4005	*L. helveticus* CECT 541	*L. paracasei* subsp. *paracasei* CECT 277	*L. plantarum* CECT 5785	*L. reuteri* CECT 925	*L. sakei* subsp. *carnosus* CECT 5964	*Lc. lactis subsp. Lactis* CECT 4042	MA-400[a]
Acidification rate	–	–	+	+	–	–	–	+	+	+ +
Gel stability	–	–	–	+	+	–	+	–	+	+
Microbial counts	+	+	–	+	+ +	+	+	+	+ +	+ +
Lactic acid content	–	+	+	+	+	–	–	+	+	+
Sugar metabolism	+	–	–	–	+	–	–	+	–	+
Volatile and odor descriptors	+	–	+	+	–	–	–	–	+	+
Σ positive traits	3	2	3	5	5	1	2	4	6	8

L.: Lactobacillus; Lc.: *Lactococcus.* Sugar metabolism + indicates non accumulation of monosaccharides, Volatile and odor descriptors + indicates similarity to MA400.
[a] *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis, Streptococcus thermophilus.*

Funding

This work was supported by the Spanish Ministerio de Economía y Competitividad and by the European Regional Development Funds FEDER [grant AGL2015-64518-R, MINECO/FEDER, EU].

Acknowledgements

The authors would like to acknowledge Desiré González Verdú and Almudena Martínez Perales for the laboratory assistance.

References

Aunsbjerg, S. D., Honoré, A. H., Marcussen, J., Ebrahimi, P., Vogensen, F. K., Benfeldt, C., et al. (2015). Contribution of volatiles to the antifungal effect of *Lactobacillus paracasei* in defined medium and yogurt. *International Journal of Food Microbiology, 194*, 46–53.

Balthazar, C. F., Silva, H. L. A., Esmerino, E. A., Rocha, R. S., Moraes, J., Carmo, M. A. V., et al. (2018). The addition of inulin and *Lactobacillus casei* 01 in sheep milk ice cream. *Food Chemistry, 246*, 464–472.

Batista, A. L. D., Silva, R., Cappato, L. P., Ferreira, M. V. S., Nascimento, K. O., Schmiele, M., et al. (2017). Developing a synbiotic fermented milk using probiotic bacteria and organic green banana flour. *Journal of Functional Foods, 38*, 242–250.

de Bok, F. A. M., Janssen, P. W. M., Bayjanov, J. R., Sieuwerts, S., Lommen, A., van Hylckama Vlieg, J. E. T., et al. (2011). Volatile compound fingerprinting of mixed-culture fermentations. *Applied and Environmental Microbiology, 77*(17), 6233–6239.

Cais-Sokolińska, D., Wójtowski, J., Pikul, J., Danków, R., Majcher, M., Teichert, J., et al. (2015). Formation of volatile compounds in kefir made of goat and sheep milk with high polyunsaturated fatty acid content. *Journal of Dairy Science, 98*(10), 6692–6705.

Cano-Lamadrid, M., Trigueros, L., Wojdyło, A., Carbonell-Barrachina, Á. A., & Sendra, E. (2017). Anthocyanins decay in pomegranate enriched fermented milks as a function of bacterial strain and processing conditions. *Lebensmittel-Wissenschaft und -Technologie, 80*, 193–199.

Cebeci, A., & Gürakan, C. (2003). Properties of potential probiotic *Lactobacillus plantarum* strains. *Food Microbiology, 20*(5), 511–518.

CECT (2018). *Colección Española de Cultivos Tipo, Vol. 2018*Universidad de Valencia Official National Culture Type Colection.

Champagne, C. P., Gomes da Cruz, A., & Daga, M. (2018). Strategies to improve the functionality of probiotics in supplements and foods. *Current Opinion in Food Science, 22*, 160–166.

Cheng, H. (2010). Volatile flavor compounds in yogurt: A review. *Critical Reviews in Food Science and Nutrition, 50*(10), 938–950.

Clark, S., & Mora García, M. B. (2017). A 100-Year Review: Advances in goat milk research. *Journal of Dairy Science, 100*(12), 10026–10044.

Costa, M. P., Balthazar, C. F., Franco, R. M., Marsico, E. T., Cruz, A. G., & Conte, C. A., Jr. (2014). Changes on expected taste perception of probiotic and conventional yogurts made from goat milk after rapidly repeated exposure. *Journal of Dairy Science, 97*(5), 2610–2618.

Dantas, A. B., Jesus, V. F., Silva, R., Almada, C. N., Esmerino, E. A., Cappato, L. P., et al. (2016). Manufacture of probiotic Minas Frescal cheese with *Lactobacillus casei* Zhang. *Journal of Dairy Science, 99*(1), 18–30.

Dan, T., Wang, D., Jin, R. L., Zhang, H. P., Zhou, T. T., & Sun, T. S. (2017). Characterization of volatile compounds in fermented milk using solid-phase microextraction methods coupled with gas chromatography-mass spectrometry. *Journal of Dairy Science, 100*(4), 2488–2500.

Gomes, A. M. P., & Malcata, F. X. (1999). *Bifidobacterium* spp. and *Lactobacillus acidophilus*: Biological, biochemical, technological and therapeutical properties relevant for use as probiotics. *Trends in Food Science & Technology, 10*(4), 139–157.

Gómez-Torres, N., Ávila, M., Delgado, D., & Garde, S. (2016). Effect of reuterin-producing *Lactobacillus reuteri* coupled with glycerol on the volatile fraction, odour and aroma of semi-hard Ewe milk cheese. *International Journal of Food Microbiology, 232*, 103–110.

Hickey, M. W., Hillier, A. J., & Jago, G. R. (1986). Transport and metabolism of lactose, glucose, and galactose in homofermentative lactobacilli. *Applied and Environmental Microbiology, 51*(4), 825–831.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., et al. (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology, 11*, 506.

Imhof, R., & Bosset, J. O. (1994). Relationships between micro-organisms and formation of aroma compounds in fermented dairy products. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 198*(4), 267–276.

ISO (2006). *ISO 21453:2006. Milk products: Guidelines for the application of near infrared spectrometry.* International Standard Organization.

ISO (2012). *ISO 8586:2012 Sensory analysis – General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors.* International Organization for Standardization.

Janiaski, D. R., Pimentel, T. C., Cruz, A. G., & Prudencio, S. H. (2016). Strawberry-flavored yogurts and whey beverages: What is the sensory profile of the ideal product? *Journal of Dairy Science, 99*(7), 5273–5283.

Jones, S. E., & Versalovic, J. (2009). Probiotic *Lactobacillus reuteri* biofilms produce antimicrobial and anti-inflammatory factors. *BMC Microbiology, 9*(1), 35.

Li, C., Song, J., Kwok, L. Y., Wang, J., Dong, Y., Yu, H., et al. (2017). Influence of *Lactobacillus plantarum* on yogurt fermentation properties and subsequent changes during postfermentation storage. *Journal of Dairy Science, 100*(4), 2512–2525.

Lock, A. L., & Garnsworthy, P. C. (2003). Seasonal variation in milk conjugated linoleic acid and Δ9-desaturase activity in dairy cows. *Livestock Production Science, 79*(1), 47–59.

Manzo, N., Pizzolongo, F., Montefusco, I., Aponte, M., Blaiotta, G., & Romano, R. (2015). The effects of probiotics and prebiotics on the fatty acid profile and conjugated linoleic acid content of fermented cow milk. *International Journal of Food Sciences & Nutrition, 66*(3), 254–259.

Marklinder, I., & Lönner, C. (1992). Fermentation properties of intestinal strains of Lactobacillus, of a sour dough and of a yoghurt starter culture in an oat-based nutritive solution. *Food Microbiology, 9*(3), 197–205.

Morgan, F., Massouras, T., Barbosa, M., Roseiro, L., Ravasco, F., Kandarakis, I., et al. (2003). Characteristics of goat milk collected from small and medium enterprises in Greece, Portugal and France. *Small Ruminant Research, 47*(1), 39–49.

Nantapo, C. T., Muchenje, V., & Hugo, A. (2014). Atherogenicity index and health-related fatty acids in different stages of lactation from Friesian, Jersey and FriesianxJersey cross cow milk under a pasture-based dairy system. *Food Chemistry, 146*, 127–133.

Park, D.-Y., Ahn, Y.-T., Park, S.-H., Huh, C.-S., Yoo, S.-R., Yu, R., et al. (2013). Supplementation of *Lactobacillus curvatus* HY7601 and Lactobacillus plantarum KY1032 in diet-induced obese Mice is associated with gut microbial changes and reduction in obesity. *PLoS One, 8*(3), e59470.

Pinto, S. M., Clemente, M. D. G., & De Abreu, L. R. (2009). Behaviour of volatile compounds during the shelf life of yoghurt. *International Journal of Dairy Technology, 62*(2), 215–223.

Pogačić, T., Maillard, M.-B., Leclerc, A., Hervé, C., Chuat, V., Yee, A. L., et al. (2015). A methodological approach to screen diverse cheese-related bacteria for their ability to produce aroma compounds. *Food Microbiology, 46*, 145–153.

Pulina, G., Milán, M. J., Lavín, M. P., Theodoridis, A., Morin, E., Capote, J., et al. (2018). Invited review: Current production trends, farm structures, and economics of the dairy sheep and goat sectors. *Journal of Dairy Science, 101*(8), 6715–6729.

Quatravaux, S., Remize, F., Bryckaert, E., Colavizza, D., & Guzzo, J. (2006). Examination of *Lactobacillus plantarum* lactate metabolism side effects in relation to the modulation of aeration parameters. *Journal of Applied Microbiology, 101*(4), 903–912.

Ranadheera, C. S., Naumovski, N., & Ajlouni, S. (2018). Non-bovine milk products as emerging probiotic carriers: Recent developments and innovations. *Current Opinion in Food Science, 22*, 109–114.

Romeu-Nadal, M., Morera-Pons, S., Castellote, A. I., & López-Sabater, M. C. (2004). Comparison of two methods for the extraction of fat from human milk. *Analytica Chimica Acta, 513*(2), 457–461.

Sfakianakis, P., & Tzia, C. (2017). Flavour profiling by gas chromatography–mass spectrometry and sensory analysis of yoghurt derived from ultrasonicated and homogenised milk. *International Dairy Journal, 75*, 120–128.
Siek, T. J., Albin, I. A., Sather, L. A., & Lindsay, R. C. (1969). Taste thresholds of butter volatiles in deodorized butteroil medium. *Journal of Food Science, 34*(3) 265-265.
Silva, H. L. A., Balthazar, C. F., Esmerino, E. A., Neto, R. P. C., Rocha, R. S., Moraes, J., et al. (2018). Partial substitution of NaCl by KCl and addition of flavor enhancers on probiotic prato cheese: A study covering manufacturing, ripening and storage time. *Food Chemistry, 248*, 192–200.
Trigueros, L., Barber, X., & Sendra, E. (2015). Conjugated linoleic acid content in fermented goat milk as affected by the starter culture and the presence of free linoleic acid. *International Journal of Dairy Technology, 68*(2), 198–206.
Trigueros, L., Sayas-Barberá, E., Pérez-Álvarez, J. A., & Sendra, E. (2012). Use of date (*Phoenix dactylifera* L.) blanching water for reconstituting milk powder: Yogurt manufacture. *Food and Bioproducts Processing, 90*(3), 506–514.
Trigueros, L., & Sendra, E. (2015). Fatty acid and conjugated linoleic acid (CLA) content in fermented milks as assessed by direct methylation. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 60*(1), 315–319.
Tunick, M., Iandola, S., & Van Hekken, D. (2013). Comparison of SPME methods for determining volatile compounds in milk, cheese, and whey powder. *Foods, 2*(4), 534.
Ulbricht, T. L., & Southgate, D. A. (1991). Coronary heart disease: Seven dietary factors. *Lancet, 338*(8773), 985–992.