Effects of selected mesophilic *Lactobacillus* strains obtained from camel milk on the volatile and sensory profiles of a model short-ripened pressed cow's milk cheese

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HIGHLIGHTS

- *Lb. plantarum* and *Lb. brevis* strains from camel milk showing technological interest
- *Lactobacillus* adjuncts modify the volatile profile of short-ripened cow cheeses
- *Lactobacillus* adjuncts differentiate the sensory quality of short-ripened cheeses
- Best score for the combination of both *Lb. plantarum* and *Lb. brevis* with DL-starter

Abstract

Four Tetilla-type cheeses were made in duplicate from pasteurized cow's milk. A control cheese was manufactured with a mesophilic commercial DL-starter only, and the other three cheeses were made with the same starter plus (i) an adjunct culture of a high-diacetyl producer *Lactobacillus plantarum* strain obtained from camel milk, (ii) an adjunct culture of a peptidolytic *Lb. brevis* strain isolated from the same source, or (iii) a combination of both adjuncts. After 28 days of ripening, the abundances of the volatiles acetic acid, hexanoic acid, ethyl butanoate and ethyl hexanoate, as well as the scores for flavour preference were significantly ($P < 0.05$) higher in the cheeses made with the two adjuncts than in the control cheeses. The results suggest that the use of selected mesophilic *Lactobacillus* strains obtained from camel milk as adjunct cultures could differentiate the volatile profile and the sensory quality of short-ripened pressed cow's milk cheeses.

1. Introduction

One of the main challenges of modern cheese industry is the need for product diversification to fulfill the requests and tastes of the various sectors of an increasingly demanding population. In this sense, the use of bacterial strains with original and attractive flavour-forming abilities represents a promising strategy to diversify the current range of cheeses with new or improved sensory properties (Van Hoorde et al., 2010). Camel (*Camelus dromedarius*) milk produced in traditional low-tech systems has a high bacterial diversity and is a fertile ground for the isolation of novel lactic acid bacteria (LAB) strains of technological and functional interest, with a view to the development of novel dairy starters and adjunct LAB cultures (Shori, 2017). From a compositional point of view, camel milk has a relatively low fat content with a high amount of unsaturated fatty acids, and contains more free amino acids and peptides than cow's milk (Ashmaig, Hasan, & El Gaali, 2018; Konuspayeva, Faye, & Loiseau, 2009). Camel milk is obtained at a higher temperature and with a lower pH (about 6.5) than the milk from other mammals, and has a greater amount of natural antimicrobial agents such as ascorbic acid, lysozyme, lactoperoxydase, lactoferrin, and immunoglobulins (Farah, 1993; Konuspayeva et al., 2008), thus it has been described as an excellent source of potential probiotic LAB strains (Ashmaig et al., 2018; Shori, 2017).

In fact, many LAB strains isolated from raw camel milk have shown interesting and peculiar technological properties that reflect a diversity hitherto unknown in conventional dairy starters. Drici, Gilbert, Kihal, and Atlan (2010) obtained atypical lactococcal strains from dromedary's milk that combine interesting technological traits such as tolerance to 50 ºC, good acidifying and proteolytic activities, and citrate-utilizing abilities. These poperties would be of special interest in the manufacture of

cooked-curd cheeses and to generate high amounts of diacetyl and other flavour compounds. On the other hand, different mesophilic *Lactobacillus* strains isolated from raw camel milk or its fermented products have shown strong proteolytic activities related to the formation of bioactive peptides, or production of exopolysaccharides (Abushelaibi, Al-Mahadin, El-Tarabily, Shah, & Ayyash, 2017; Shori, 2017). Starter LAB and non-starter LAB interact since the early stages of manufacture to the cheese ripening process, and combinations of starter bacteria with mesophilic *Lactobacillus* spp. are common tools used to accelerate and conduct cheese ripening (Gobbetti et al., 2018). These strategies represent an interesting approach to enhance flavour intensity or impart atypical but desirable flavour notes to cheese (Banks & Williams, 2004; De Angelis et al., 2001; Gobbetti, De Angelis, Di Cagno, Mancini, & Fox, 2015). *Lactobacillus plantarum* and *Lb. brevis* are among the main microbiota of many Mediterranean and Middle-Eastern cheese varieties produced from ewe's and goat's milk (De Angelis et al., 2001; Hassanzadazar, Mardani, Yousefi, & Ehsani, 2017; Pino et al., 2018). *Lactobacillus plantarum* has been used as an adjunct culture to enhance and accelerate flavour development of many cheese varieties (Banks & Williams, 2004; Mugampoza, Gkatzionis, Linforth, & Dodd, 2019). On the other hand, *Lb. brevis* has been hypothesized to impact cheese ripening because of its potential to metabolise amino acids by non-transaminating reactions and endogenous transamination (Liu, Holland, & Crow, 2003). A number of strains belonging to *Lb. plantarum* and *Lb. brevis* have also been characterised as probiotics due to their capacity to generate bioactive compounds such as conjugated linoleic acid or gamma-amino-butyric acid (Renes et al., 2017; Ribeiro, Domingos-Lopes, Stanton, Ross, & Silva, 2018).

Recently, Fguiri et al. (2016) selected two *Lb. plantarum* and one *Lb. brevis* strains isolated from camel milk to study their potential use in the production of fermented Tunisian products from goat and camel milk, and Ayyash, Abu-Jdayil, Hamed, and Shaker (2018) studied the effect of an exopolysaccharide-producing *Lb. plantarum* strain obtained from camel milk on the texture and sensory properties of a low-fat cow's milk cheese. In a previous work of our group (Belkheir, Centeno, Zadi-Karam, Karam, & Carballo, 2016), it was concluded that a high-diacetyl producer *Lb. plantarum* strain and a peptidolytic *Lb. brevis* strain producing volatile sulphur compounds, both peculiar strains obtained from the predominant microbiota of camel milk samples collected in South-West Algeria, may have a potential interest in cheese making. Thus, the aims of the present study were (i) to investigate the effects of adjunct cultures of the aforementioned *Lb. plantarum* and *Lb. brevis* strains on the volatile profile and sensory characteristics of a model short-ripened pressed cheese made from pasteurized cow's milk (Tetilla-type); and (ii) to compare the chemical, volatile and sensory profiles of the experimental cheeses with those previously described for Tetilla cheese with Protected Designation of Origin (PDO).

2. Materials and methods

2.1. Bacterial strains and culture media

The selected LAB strains used as adjunct cultures were *Lactobacillus plantarum* C22P and *Lactobacillus brevis* C21B. Both strains (deposited in the Laboratory of Biology of Microorganisms and Biotechnology of the Ahmed Ben Bella University of Oran, Algeria) had been obtained from Algerian camel milk, and investigated for their

technological interest aptitudes in a previous study (Belkheir et al., 2016). The *Lb. plantarum* C22P strain was able to metabolize citrate on calcium citrate agar and produced high quantities of diacetyl-acetoin in skim milk (see supplementary file S1). The strain was responsible for intense butter and vanilla flavours in pasteurized whole milk, which is not a common feature in *Lb. plantarum* strains isolated from milk or cheese from other mammals. The *Lb. brevis* C21B strain was selected on the basis of its high aminopeptidase activities. This strain was responsible for sulphury and garlic flavours in pasteurized milk, related to high abundances of volatile sulphur compounds. To our knowledge, this characteristic has never been described in dairy strains of *Lb. brevis*. Frozen cultures (–80 ºC) of the strains *Lb. plantarum* C22P and *Lb. brevis* C21B were grown in MRS broth (Oxoid, Basingstoke, UK) at 30 ºC for 24 h, and then subcultured at 2% (v:v) in 100 mL sterile (110 ºC, 15 min) reconstituted skim milk (Oxoid) and incubated at 30 ºC for 48 h.

The mesophilic freeze-dried CHN19 DL-starter (Chr. Hansen, Hørsholm, Denmark) was used in the manufacture of all cheeses. The absence of antimicrobial activity of the C22P and C21B strains against the CHN19 starter and against each other was verified by the well diffusion agar test.

2.2. Experimental cheese making

The model of short-ripened pressed cheese variety chosen to assay the selected mesophilic lactobacilli was Tetilla cheese. This cheese variety together with the very similar Arzúa-Ulloa represent about 60% (4.9 million kg in 2018) of the total annual production of PDO (unmixed) cow's milk cheeses manufactured in Spain (Anon., 2020). Forty litres of whole milk (4.0% fat content) produced in spring from cows fed

144 with high proportions of pasture, were pasteurized (74 ºC, 15 s) and distributed equally
145 in four automatic 10 L vats (Perinox, Albacete, Spain) to make four different cheeses.
146 Calcium chloride (0.1 g L^{-1}) was added to milk after pasteurization. A control cheese
147 was manufactured by adding 0.5 direct culture units (U) of the CHN19 commercial
148 starter to the cheese milk previously cooled to 32 ºC. To make the remaining three
149 cheeses, 0.25 U (lower inoculation level to avoid over acidification) of the CHN19
150 starter were added along with (i) the *Lb. plantarum* C22P adjunct (skim-milk) culture,
151 (ii) the *Lb. brevis* C21B adjunct culture, or (iii) the mixture of both adjuncts
152 (C22P+C21B). The *Lactobacillus* adjunct cultures were added at a 1% (v:v) ratio (0.5%
153 of each strain when combined), in order to attain an inoculation ratio of about 6 log
154 cfu/mL. After an acclimatization period of 20 minutes at the curdling temperature (32
155 °C), a chymosin liquid rennet produced by fermentation (CHY-MAX® Plus, Chr.
156 Hansen, 200 international milk-clotting units mL^{-1}) was added at a rate of 0.25 mL L^{-1}
157 of milk.
158 Cheeses were made following the guidelines of the Tetilla PDO Standard Procedures, as
159 previously described (Centeno, Garabal, Docampo, Lorenzo, & Carballo, 2017). A fresh
160 cheese of about 1.1 kg was obtained from each of the vats. All cheeses were ripened at
161 7±1 ºC and 85±2% relative humidity for 28 days. Two cheese making trials were
162 performed using milk from two milkings acquired from the same farm in two different
163 days and maintained at 4 ºC to a maximum of 16 h.
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165 *2.3. Cheese sampling and physicochemical analysis of cheeses*
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167 All cheeses were sampled on day 28 of ripening. Physicochemical, biochemical and
168 volatile compounds analyses were carried out on one half-cheese per treatment and trial,

the other half being used for sensory analysis. Samples for chemical and volatile compound analyses were taken after removing an outer layer of depth 2–3 mm. Physicochemical and biochemical analyses were carried out on samples stored at 4 ºC for a maximum of 24 h. For the analysis of volatile compounds, cheese sections of approximately 100 g were cut, wrapped in aluminum foil and vacuum packed in plastic bags that were kept frozen (–30 ºC). The compositional parameters (dry extract, fat, protein and ash content) and pH were determined as previously described (Centeno, Rodríguez-Alonso, Carballo, & Garabal, 2015). All analyses were carried out in duplicate and the results averaged for each cheese making trial.

2.4. Determination of proteolysis index and aminopeptidase activities

Cheese overall proteolysis was determined on duplicate samples by the *o*-phthaldialdehyde (OPA) test (Church, Swaisgood, Porter, & Catignani, 1983), as described by Picon et al. (2010). Results were expressed as absorbance at 340 nm. Aminopeptidase activity released into the cheese was measured on duplicate samples of an extract obtained by homogenizing 10 g of cheese with 20 mL of 10 mM pH 7.0 sodium phosphate buffer, followed by centrifuging (10,000 × *g*, 15 min, 4 ºC) and filtering through Whatman No. 2 paper, as described by Alonso, Picon, Gaya, Fernández-García, and Nuñez (2012). Leucine *p*-nitroanilide (Leu-*p*-NA) and lysine *p*-nitroanilide (Lys-*p*-NA) (Sigma-Aldrich, St. Louis, MO, USA), were used as substrates. Results were expressed as activity units (AU), one AU corresponding to the activity of enzyme(s) producing 1 nmol of *p*-nitroaniline/min per gram of cheese.

2.5. Analysis of volatile compounds

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195 Volatile compounds were analyzed using the headspace solid phase microextraction–
196 gas chromatography–mass spectrometry (SPME–GC–MS) methodology with a
197 quadrupole GC–MS system. One g of cheese was transferred into a 10 mL headspace
198 vial, which was immediately sealed with a polytetrafluoroethylene-faced silicone
199 septum (Supelco, Bellefonte, PA, USA). Samples were heated at 35 ºC and allowed to
200 equilibrate for 15 min in a thermo block (Memmert model 100-800, Schwabach,
201 Germany). A carboxen/polydimethylsiloxane 75 μm (Supelco) fibre was then exposed
202 into the headspace for 30 min. The SPME adsorbed compounds were injected into the
203 chromatograph for 8 min using the splitless technique. The temperature of the injection
204 port was 260 ºC. The separation of volatile compounds was performed on a Hewlett-
205 Packard 6890N (Agilent Technologies, Santa Clara, CA, USA) gas chromatography
206 system with a DB-624 capillary column (J&W Scientific, Folsom, CA, USA), as
207 previously described (Belkheir et al., 2016). Identification of volatiles was performed
208 by comparison of their retention times with those of authentic standards (Supelco), and
209 by mass spectra matches with those published in the National Institute of Standards and
210 Technology mass spectral library (NIST05, Gaithersburg, MD, USA). The abundances
211 of the identified compounds were expressed as peak area units/10^6. Two independent
212 analytical replicates were prepared for all cheeses and the results were averaged for
213 each of the trials.

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215 *2.6. Sensory analysis*

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217 Sensory evaluation was performed in a tasting room that complied with International
218 Dairy Federation Standard 99C (IDF, 1997), by a panel of eight previously trained

219 assessors which were connoisseurs and experienced in evaluation of pasteurized-milk
220 PDO Galician (NW Spain) cheeses. The panel composition showed an equal
221 distribution of males and females aged between 25 and 50 years. The evaluation was
222 carried out during two different work sessions (one session per experimental trial) on
223 one half-cheese per treatment. The cheese samples were blind-tested and coded with
224 random numbers with selected three-digit code. All half-cheeses were allowed to reach
225 room temperature (20±2 ºC) for 2 h prior to evaluation, and then cut into eight sections
226 of the same approximate size (50-70 g). After removing the rind, the cheese sections
227 were coded with the identifying numbers and presented in random order to the panelists.
228 Panelists were given unsalted crackers and water to cleanse their palates between testing
229 different samples.
230 The following attributes or sensory parameters were scored: firmness, elasticity, and
231 texture preference (appreciated by finger pressure, and by chewing in the mouth), and
232 acid, bitter, buttery and sulphury flavours, flavour intensity and flavour preference
233 (evaluated by chewing, salivating and swallowing). The panelists marked the attributes
234 using whole numbers on a quantitative scale ranging from 0 to 7, where 0 represented
235 the lowest or weakest value and 7 the highest or most intense grade. The results were
236 expressed as a mean score assigned by the entire panel for each attribute and cheese. An
237 overall preference score was also obtained by adding the mean scores for texture
238 preference and those for flavour preference with twice the value, and referring the
239 results to 100. An “observations” section in which the panelists noted down the
240 descriptors they considered useful for defining the attributes of the cheeses, was
241 included in the sensory testing sheets.
242
243 *2.7. Statistical analysis*

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245 All statistical tests were performed using the SPSS Statistics software package version
246 23 (IBM SPSS Inc., Chicago, IL, USA). The variables studied were previously tested
247 for the assumption of normality (Kolmogorov-Smirnov test) and homogeneity of
248 variance (Levene's test). The data obtained for physicochemical, biochemical, volatile
249 compounds and sensory parameters were subsequently assessed by one-way analysis of
250 variance (ANOVA) and significant differences were considered at $P < 0.05$ level using
251 Duncan's Multiple Range test. All variables were also subjected to a correlation
252 analysis using a bivariate correlation test, and Pearson's coefficients were calculated
253 with differences declared significant at a 5% significance level. Finally, Principal
254 Component Analysis (PCA; Varimax rotation with Kaiser normalization, two
255 components extracted) was applied to reduce the dimensionality of the data set
256 consisting of the sensory parameters together with the chemical traits and main volatile
257 compounds (those with abundances higher than 1.0×10^6 peak area units in at least one
258 cheese) showing significant differences between cheeses.
259

260 **3. Results and Discussion**
261

262 *3.1. Compositional analysis and pH of the cheeses*
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264 The cheeses made in the present study fulfill the compositional and pH criteria specified
265 by Tetilla PDO Regulations (45-50% dry matter; ≥45% fat/dry matter; ≥40%
266 protein/dry matter; and pH between 5.0 and 5.5). The results were similar to those
267 determined in Tetilla PDO cheeses previously analysed (Centeno et al., 2015), except
268 for the lower pH values in the cheeses made with the only *Lb. brevis* C21B adjunct

(C21B cheeses). The mean pH values ranged between 5.04 for the C21B cheeses and 5.47 for the cheeses manufactured with the sole *Lb. plantarum* C22P adjunct (C22P cheeses) (Table 1). The pH values of the cheeses made with the *Lb. brevis* strain were significantly ($P < 0.05$) lower than those of the C22P cheeses and the control cheeses. Adjunct cultures of lactobacilli should not interfere with the acidifying activity of the starter during cheese making (Bancalari et al., 2017). Slow acid production has been described for strains of *Lb. plantarum* isolated from ewe's milk and cheese (Medina, Katz, Gonzalez, & Oliver, 2001). As regards *Lb. brevis*, some strains are not able to acidify milk at 37 ºC (Rönkä et al., 2003); this behaviour must be expected as a consequence of the obligately heterofermentative nature of this species. The lowest pH values of the cheeses made with the *Lb. brevis* C21B adjunct might be related to the proteolytic activity of this strain and the release of peptides and free amino acids that stimulate the growth of the lactococcal starter.

3.2. Determination of proteolytic indices and aminopeptidase activities

The highest OPA values (mean of 0.376) were found in the C21B cheeses. These values were significantly ($P < 0.05$) higher than those of the cheeses made with the two adjunct cultures (Table 1). Contrary to the results of the present study, Puchades, Lemieux, and Simard (1989) reported greater proteolytic action in matured Cheddar cheeses made with a *Lb. plantarum* adjunct than in cheeses made with a *Lb. brevis* adjunct, which scored the lowest ripening index value related to total free amino acids.

The aminopeptidase activities on Leu-*p*-NA released in the cheeses made with the *Lb. brevis* adjunct, and the activities on Lys-*p*-NA in the C21B cheeses were significantly ($P < 0.05$) higher than in the other cheeses (Table 1). Aminopeptidase activities on Lys-

294 *p*-NA in the C21B cheeses (mean value of 2.11 AU) are higher than those determined
295 by Alonso et al. (2012) and Picon et al. (2010) for 30-days mixed-milk cheeses (0.98
296 and 1.47 AU, respectively).
297
298 *3.3. Analysis of volatile compounds*
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300 A total of 23 volatile compounds were detected in the headspace of the cheese samples
301 analyzed by the methodology used in the present study. Significant ($P < 0.05$)
302 differences between the different cheeses were found for 16 compounds, including two
303 aldehydes, three alcohols, four ketones, two fatty acids, three esters and two sulphur
304 compounds (Table 2).
305 Two branched-chain compounds were identified in all cheeses: the aldehyde 3-
306 methylbutanal and the alcohol 3-methylbutanol. The contents of the branched-chain
307 alcohol 3-methylbutanol were significantly ($P < 0.05$) higher in the C21B cheeses than
308 in the C22P cheeses and the control cheeses. Branched-chain aldehydes and alcohols
309 may contribute positively to the overall flavour when properly balanced (Engels,
310 Dekker, de Jong, Neeter, & Visser, 1997), imparting a fruity, green or nutty note
311 (Bancalari et al., 2017). It has been reported that non-starter mesophilic lactobacilli
312 individually or mixed with commercial strains, are able to produce 3-methylbutanal and
313 3-methylbutanol (Bancalari et al., 2017; Mugampoza et al., 2019; Randazzo, Pitino, De
314 Luca, Scifò, & Caggia, 2008). Puchades et al. (1989) observed a marked increase of the
315 amino acid leucine, the precursor of these branched compounds, in matured Cheddar
316 cheeses made with added *Lb. brevis*.
317 The highest abundances of 2-heptanol were found in the C22P cheeses, with
318 significantly ($P < 0.05$) higher values than the C21B cheeses. This compound,

319 associated to herbaceous and fruity aromas, has been considered as a key odourant in
320 many cheese varieties (Curioni & Bosset, 2002). Significant positive correlations
321 between the occurrence of *Lb. plantarum* in cheese and the volatile 2-heptanol have
322 been reported (Pino et al., 2018; Randazzo et al., 2008).
323 The abundances of the ketones 2,3-butanedione (diacetyl), 3-hydroxy 2-butanone
324 (acetoin), and 2-butanone were significantly ($P < 0.05$) higher in the C22P cheeses than
325 in the C21B cheeses. The use of citrate by some species of non-starter lactobacilli
326 favours an excess of pyruvate, which is converted into diacetyl, acetoin and butanediol
327 (Gobbetti et al., 2015). Drici et al. (2010) suggested the possibility of transfer of the cit^+
328 plasmid from *Leuconostoc* species to lactococcal strains isolated from dromedary's
329 milk. The contents of the ketone 2- nonanone were significantly ($P < 0.05$) higher in the
330 cheeses made with the *Lb. plantarum* adjunct than in the other cheeses. 2-Nonanone and
331 other methyl ketones such as 2-heptanone have been associated with cheesy and mouldy
332 odours, and blue cheese aroma; these ketones may derive from the metabolism of non-
333 starter mesophilic lactobacilli (Rothe, Engst, & Erhardt, 1982).
334 The abundances of acetic and hexanoic acids, and those of the ethyl butanoate ester
335 were significantly ($P < 0.05$) higher in the cheeses made with the two adjuncts than in
336 the control cheeses (Table 2). Ethyl hexanoate and ethyl octanoate esters were identified
337 in the cheeses made with the *Lb. brevis* adjunct, reaching their highest contents in the
338 cheeses made with the two adjunct cultures. Short-chain hexanoic and octanoic acids
339 could probably be released from triglycerides through the action of non-specific
340 bacterial esterases and lipases. The presence of lipase and esterase activities on
341 mesophilic lactobacilli has been demonstrated in screening studies (Medina et al., 2001;
342 Ribeiro et al., 2018), and intracellular esterases from *Lb. plantarum* have been purified
343 and characterised (Gobbetti et al., 2015). Ethyl ester compounds in cheeses have been

positively correlated to the species *Lb. plantarum* and *Lb. brevis* (Pino et al., 2018; Randazzo et al., 2008; Van Hoorde et al., 2010).

As regards sulphur compounds, the contents of dimethyl disulphide (DMDS) and dimethyl trisulphide (DMTS) were significantly ($P < 0.05$) higher in the cheeses made with the *Lb. brevis* C21B adjunct than in the other cheeses. The thioesters DMDS and DMTS are expected to be formed mainly from the amino acid methionine by the metabolism of LAB and secondary microbiota (Curioni & Bosset, 2002; Engels et al., 1997). It has been reported that a number of *Lactobacillus* strains, including *Lb. brevis* are able to degrade methionine via an aminotransferase (Banks & Williams, 2004; Engels et al., 1997).

The volatiles 2-heptanol, 2-nonanone, hexanoic acid, ethyl-hexanoate, ethyl octanoate and DMTS, together with the presumed contaminants phenol and methylbenzene, had not been previously detected in pasteurized-milk Tetilla PDO cheeses (Centeno et al., 2015; Rodríguez-Alonso, Centeno, & Garabal, 2009). Like the control cheeses made in the present study, the previously investigated Tetilla PDO cheeses showed a simple volatile profile related to the non-specific (D- or DL-) starters used in the manufacturing process. This profile was characterised by low amounts of branched-chain aldehydes and alcohols (mainly 3-methyl-1-butanal and 3-methyl-1-butanol), low amounts of fatty (acetic and butanoic) acids and esters, and of the ketones 2-butanone and 2-heptanone, and high abundances of the ketones diacetyl and acetoin, together with ethanol and isopropyl alcohol.

3.4. Sensory analysis

Results of sensory analysis are shown in Table 3. Firmness, elasticity and texture

preference were significantly ($P < 0.05$) lower in the C21B cheeses than in the other cheese groups. The texture (in the mouth) of the C21B cheeses was perceived as granular and friable by most panelists, whereas the texture of the cheeses made with the two adjunct cultures was described as the smoothest and creamiest (see supplementary file S2), which is a desirable characteristic for Tetilla PDO cheese. Although it has been reported that the use of *Lb. brevis* in cheese manufacture resulted in gassy cheeses (Puchades et al., 1989), the formation of eyes in the cheese mass was very similar in all the cheeses made in the present study, with 10-20 small (≤ 3 mm in diameter) rounded eyes per dm^2 appreciated in the cut in half of the cheeses (data not shown). Most of the eyes could have been generated by the commercial aromatic DL-starter.

Acid flavour was significantly ($P < 0.05$) higher in the cheeses made with the *Lb. brevis* adjunct than in the other cheeses, possibly related to the lower pH of the former. For their part, the C22P cheeses showed significantly ($P < 0.05$) higher buttery flavour values than the other cheeses. This fact may be attributed to the higher contents of the volatiles diacetyl and acetoin, which are responsible for buttery and nutty flavours in cheese (Curioni & Bosset, 2002). According to Tetilla PDO Regulations, cheeses should have a milky, slightly acidic, buttery, and mild flavour.

Bitter flavour was significantly ($P < 0.05$) higher in the C21B cheeses than in the other cheese groups. Puchades et al. (1989) related the off-flavours detected in matured Cheddar cheeses made with a *Lb. brevis* adjunct to large quantities of free arginine, an amino acid responsible for bitter-sweet tastes. Sulphury flavour was only detected in the C21B cheeses by six panelists. A slight garlic nuance was also perceived in these cheeses by four panelists. It has been reported that sulphur compounds, such as DMDS and DMTS, can provide particular flavour notes in cheese such as onion and garlic-like odours (Curioni & Bosset, 2002). As a final point, a slight rancid note was also detected

394 by five panelists in the cheeses made with the two *Lactobacillus* adjuncts, possibly
395 related to the higher contents of short-chain fatty acids.
396 Flavour preference was significantly ($P < 0.05$) higher in the cheeses made with the two
397 adjunct cultures than in the other cheeses, and overall preference was significantly ($P <$
398 0.05) lower in the C21B cheeses than in the other cheese groups (Table 3). Rönkä et al.
399 (2003) observed that supplementation of yoghurt with probiotic *Lb. brevis* strains had
400 no negative effects on yoghurt appearance or taste. Nevertheless, Puchades et al. (1989)
401 reported unpleasant off-flavours in Cheddar cheeeses made with a *Lb. brevis* adjunct
402 culture, and an onion-like off-flavour in those made with a *Lb. plantarum* adjunct.
403 Finally, Terzić-Vidojević et al. (2015) suggested the suitability of selected *Lb.*
404 *plantarum* strains for the manufacture of fresh soft cheese from cow's milk, and that of
405 *Lb. plantarum* and *Lb. brevis* for white pickled cheese production. According to these
406 authors, it could be necessary to combine non-starter LAB strains with commercial
407 starters containing faster producers of diacetyl (DL-starters) in order to obtain
408 satisfactory sensory properties.
409 Regarding the main bivariate correlations between sensory parameters and chemical and
410 volatile compounds, texture preference was positively correlated ($P < 0.01$) with pH and
411 elasticity, flavour intensity was positively correlated ($P < 0.05$) with the volatiles 3-
412 methylbutanol, ethyl butanoate and DMTS, and flavour preference was negatively
413 correlated ($P < 0.01$) with bitter and sulphury flavours (Table 4). The sensory
414 parameters together with the chemical traits and main volatile compounds showing
415 differences between cheeses were extracted in two components by the PCA method
416 (Figure 1). Component 1 explained 57.8% of the total variance and was highly
417 positively correlated ($r > 0.800$) with the parameters: pH, firmness, elasticity and overall
418 preference, and highly negatively correlated ($r < –0.800$) with OPA index, lysine

aminopeptidase, DMTS, acid flavour and bitter flavour (see supplementary file S3). Component 2 explained 17.6% of the total variance, and was highly positively correlated with the parameters hexanoic acid and ethyl butanoate. The variables pH and texture preference had negative loadings on the second component.

4. Conclusions

The particular characteristics of camel milk regarding its composition and the environmental conditions in which it is obtained probably constitute the reason for the isolation of peculiar LAB strains physiologically and metabolically differentiated from those obtained from the milk of other mammals. The use of adjunct cultures of selected singular strains of *Lb. plantarum* and *Lb. brevis* isolated from camel milk, either alone or in combination, seems to enhance secondary proteolysis and the formation of volatile compounds, particularly ketones, fatty acids, esters and sulphur compounds, and consequently modify the flavour profile of pasteurized-milk Tetilla-type cheese. The use of the sole *Lb. brevis* adjunct culture also appears to modify the pH and texture, with the additional formation of atypical flavours giving rise to the most differentiated cheeses; however, these products are not well scored according to the Tetilla PDO sensory standards that demand creamy textures and milder aromas with predominant buttery notes. On the contrary, when both adjuncts are used in combination, the cheeses obtained are preferred to the control cheeses made with only commercial DL-starters. Although new research efforts should be made to confirm the results of the present study, it seems that the use of adjunct cultures of peculiar strains of mesophilic lactobacilli obtained from raw camel milk would differentiate the sensory properties and contribute to the quality of short-ripened cheeses such as Tetilla cheese.

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594

595 **Table 1**. Physicochemical parameters, proteolysis index and aminopeptidase activities
596 (mean±standard error of the averages of two replicates for two cheese making trials)
597 determined in 28-day-old Tetilla-type cheeses made with *Lb. plantarum* and *Lb. brevis*
598 adjunct cultures
599

	CONTROL	+ *Lb. plantarum* C22P	+ *Lb. brevis* C21B	+ C22P + C21B	*P* value
Dry matter (%, w/w)	49.6±0.30	49.5±0.23	49.8±0.16	49.6±0.24	0.689
Fat/dry matter (%, w/w)	55.3±0.46	55.1±0.49	56.0±0.57	54.7±1.05	0.387
Protein/dry matter (%, w/w)	42.0±0.32	42.7±1.39	42.0±0.82	43.2±0.25	0.437
Ash (%, w/w)	1.88±0.23	1.94±0.13	1.55±0.08	1.74±0.11	0.188
Moisture in fat free basis (%)	69.5±0.03	69.4±0.04	69.6±0.41	69.1±0.70	0.716
pH	5.45±0.040^{A}	5.47±0.039^{A}	5.04±0.034^{C}	5.25±0.025^{B}	0.000
OPA proteolysis index[1]	0.351±0.018^{AB}	0.350±0.021^{AB}	0.376±0.014^{A}	0.343±0.016^{B}	0.088
Aminopeptidase activities[2]					
Leu-*p*-NA	1.30±0.25^{B}	1.30±0.28^{B}	1.96±0.32^{A}	2.02±0.37^{A}	0.007
Lys-*p*-NA	1.28±0.27^{B}	1.29±0.24^{B}	2.11±0.30^{A}	1.63±0.29^{B}	0.004

600

601 $^{A–C}$Mean values within a row with different superscripts are significantly different ($P < 0.05$; Duncan's
602 test).

603 [1]Expressed as absorbance at 340 nm.

604 [2]Expressed as activity units (nmol *p*-nitroaniline min^{-1} g^{-1}).

605

606 **Table 2**. Abundances (mean±standard error of the averages of two replicates for two
607 cheese making trials; results expressed as peak area units/10^6) of the volatile
608 compounds determined in 28-day-old Tetilla-type cheeses made with *Lb. plantarum* and
609 *Lb. brevis* adjunct cultures
610

	CONTROL	+ *Lb. plantarum* C22P	+ *Lb. brevis* C21B	+ C22P + C21B	*P* value
ALDEHYDES AND ALCOHOLS					
Hexanal	11.06±2.41^{A}	7.33±1.83^{AB}	4.73±1.28^{B}	7.09±1.85^{AB}	0.113
Heptanal	12.03±5.59^{A}	6.49±1.72^{AB}	nd^{aB}	nd^{B}	0.037
3-Methyl-1-butanal	7.21±2.04	5.88±1.66	10.42±2.95	13.79±3.90	0.143
3-Methyl-1-butanol	2.71±0.23^{B}	2.19±0.81^{B}	6.02±1.41^{A}	4.99±1.40^{AB}	0.063
2,3-Butanediol	8.65±3.70	9.18±4.09	4.16±0.95	5.91±2,22	0.407
Hexanol	0.86±0.25^{A}	nd^{B}	nd^{B}	0.47±0.31^{AB}	0.031
2-Heptanol	nd^{C}	7.00±1.73^{A}	2.61±0.74^{BC}	5.39±1.52^{AB}	0.016
Phenol	0.12±0.16	0.42±0.23	nd	0.16±0.10	0.174
KETONES					
2,3-Butanedione	260.39±93.80^{AB}	274.41±65.68^{A}	83.07±44.99^{B}	176.63±40.99^{AB}	0.119
3-Hydroxy-2-butanone	77.49±25.56^{AB}	131.69±43.07^{A}	34.52±10.22^{BC}	65.71±23.68^{AB}	0.102
2-Butanone	9.65±5.23^{AB}	10.36±5.03^{A}	nd^{B}	nd^{B}	0.075
2-Heptanone	0.87±0.39	1.42±0.44	0.72±0.29	1.22±0.40	0.370
2-Nonanone	nd^{B}	1.56±0.44^{A}	0.41±0.12^{B}	1.38±0.39^{A}	0.017
FATTY ACIDS AND ESTERS					
Acetic acid	20.44±6.55^{B}	55.82±13.43^{AB}	76.64±26.76^{AB}	99.71±29.10^{A}	0.075

Butanoic acid	21.58±5.27	28.44±7.76	32.09±9.92	43.18±15.38	0.334
Hexanoic acid	nd^{B}	3.48±2.40^{AB}	6.44±2.61^{AB}	6.95±3.34^{A}	0.131
Ethyl butanoate	15.22±4.13^{B}	25.69±9.66^{AB}	41.93±6.94^{A}	39.89±11.66^{A}	0.096
Ethyl hexanoate	nd^{B}	nd^{B}	4.04±2.10^{AB}	4.46±1.98^{A}	0.061
Ethyl octanoate	nd^{B}	nd^{B}	0.61±0.17^{A}	0.97±0.31^{A}	0.009
SULPHUR COMPOUNDS					
Dimethyl disulphide	nd^{C}	nd^{C}	7.16±2.07^{A}	3.92±0.97^{B}	0.008
Dimethyl trisulphide	nd^{B}	nd^{B}	4.96±1.61^{A}	2.80±1.08^{A}	0.017
HYDROCARBONS					
Alpha-pinene	0.82±0.31	1.12±0.37	0.66±0.21	0.86±0.25	0.539
Methylbenzene	1.96±0.97	0.85±1.20	1.67±0.56	1.96±0.48	0.577

611

612 $^{A–C}$Mean values within a row with different superscripts are significantly different ($P < 0.05$; Duncan's
613 test).

614 [a]nd: Compounds not detected (considered as 0.0 values for statistical analysis).

615

616 **Table 3**. Sensory parameters (mean±standard error of the scores obtained for two
617 cheese making trials; 0-7 point scale) determined in 28-day-old Tetilla-type cheeses
618 made with *Lb. plantarum* and *Lb. brevis* adjunct cultures
619

	CONTROL	+ *Lb. plantarum* C22P	+ *Lb. brevis* C21B	+ C22P + C21B	*P* value
Texture					
Firmness	4.8±0.71^{A}	5.3±0.71^{A}	3.6±0.74^{B}	4.5±0.76^{A}	0.001
Elasticity	6.0±0.76^{A}	6.1±0.64^{A}	3.8±0.71^{B}	5.6±0.52^{A}	0.000
Preference	5.9±0.64^{A}	5.8±0.89^{A}	3.9±0.83^{B}	5.5±0.93^{A}	0.000
Flavour					
Acid	3.1±0.35^{B}	3.3±0.71^{B}	4.9±0.83^{A}	4.3±0.89^{A}	0.000
Buttery	3.9±0.83^{B}	4.9±0.83^{A}	3.3±0.89^{B}	3.9±0.64^{B}	0.021
Bitter	2.4±0.52^{B}	2.0±0.93^{B}	4.8±0.89^{A}	2.8±0.46^{B}	0.000
Sulphury	0.0±0.00^{B}	0.0±0.00^{B}	1.3±1.04^{A}	0.0±0.00^{B}	0.000
Intensity	5.6±0.52	5.6±0.74	6.1±0.83	5.8±0.71	0.459
Preference	5.3±0.70^{B}	5.6±0.74^{B}	3.6±0.52^{C}	6.5±0.53^{A}	0.000
Overall preference[1]	80±9.4^{B}	82±7.6^{AB}	53±5.9^{C}	88±5.7^{A}	0.000

620

621 $^{A–C}$Mean values within a row with different superscripts are significantly different ($P < 0.05$; Duncan's
622 test).

623 [1]Determined by adding the values of "texture preference", and those of "flavor preference" multiplied by
624 2 and referring the results to 100.

625

626 **Table 4**. Main bivariate correlations between sensory parameters, chemical parameters
627 and volatile compounds determined for the experimental Tetilla-type cheeses made in the
628 present study
629

PARAMETERS	Pearson's correlation coefficients[a]		
	Texture preference	Flavour intensity	Flavour preference
pH	0.733**	NS	NS
3-Methyl-1-butanol	–0.708*	0.810*	NS
Ethyl butanoate	NS	0.801*	NS
Dimethyl trisulphide	–0.855**	0.753*	NS
Firmness	0.396*	NS	0.514**
Elasticity	0.614**	NS	0.526**
Bitter flavor	–0.637**	NS	–0.573**
Sulphury flavor	–0.481**	NS	–0.485**

630
631 [a] NS: Not significant; *: Significant correlation at $P < 0.05$; **: Significant correlation at $P < 0.01$.
632

633 FIGURE CAPTIONS

634

635 **Figure 1**. Component plot in rotated space showing the variables subjected to Principal

636 Component Analysis (the full names of some variables are shown in Tables 1 to 3)

637

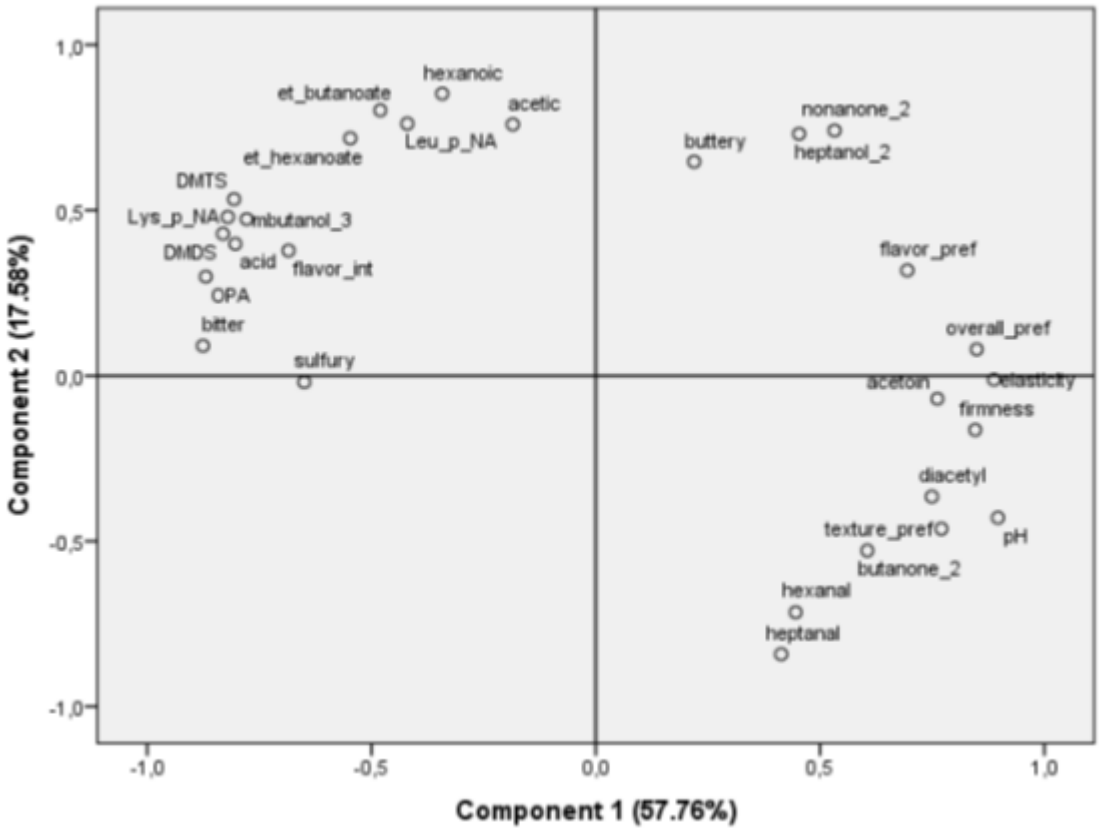
SUPPLEMENTARY MATERIAL CAPTIONS

File S1. Main physiological, biochemical and technological characteristics of the mesophilic Lactobacillus strains obtained from Algerian camel milk used as adjunct cultures

File S2. Main descriptors used to define the sensory attributes of the cheeses

File S3. Rotated factor loadings and total variance explained in the Principal Component Analysis

649 (FIGURE 1)



650

651 **SUPPLEMETARY MATERIAL**

652 **S1**. Main physiological, biochemical and technological characteristics of the mesophilic

653 *Lactobacillus* strains obtained from Algerian camel milk used as adjunct cultures

	Lb. plantarum C22P	*Lb. brevis* C21B
Growth in skim milk at:		
10 ºC	+	+
40 ºC	+	+
45 ºC	-	+
50 ºC	-	-
pH 5.5 (30 ºC)	+	+
pH 5.0 (30 ºC)	+	+
Gas production (MRS, 30 ºC)	-	+
Utilization of citrate on calcium citrate agar	+	-
Fermentation of:		
D-galactose	+	+
D-glucose	+	+
D-lactose	+	+
Enzymatic activities (nmol hydrolysed substrate; 30 ºC, 4 h; API ZYM, bioMérieux):		
Acid phosphatase	≥40	≥40
Alkaline phosphatase	20	10
Phosphohidrolase	30	10
Leucine aminopeptidase	≥40	≥40
Valine aminopeptidase	≥40	≥40
Cysteine aminopeptidase	30	20
Trypsin	5	0
Chymotrypsin	5	5
Esterase	10	20
Esterase-lipase	10	10
Lipase	20	10
α-Galactosidase	10	30
β-Galactosidase	≥40	≥40
α-Glucosidase	30	≥40
β-Glucosidase	≥40	≥40
Acidifying activity (pH skim milk; 30 ºC, 6 h; IDF Bulletin 306, 1995)	6.48	6.42
Aminopeptidase activities (specific activity; 30 ºC; El Soda & Desmazeaud, 1982; Can J Microbiol 28)		
Leu-AP	10.9	20.0
Lys-AP	12.9	53.0
Producton of diacetyl-acetoin (skim milk; 30 ºC, 24 h; mg diacetyl L^{-1}; IDF Standard 149A, 1997)	120	0.0

Main flavour descriptors of fermented whole cow's milk (30 ºC, 24 h)	buttery, vanilla	sulphury, garlic
Main volatiles produced in fermented whole cow's milk (30 ºC, 24 h)	acetic acid, acetoin, diacetyl, 2-heptanone	acetic acid, butanoic acid, dimethyl disulphide

654

SUPPLEMENTARY MATERIAL

S2. Main descriptors used by the panelists to define the sensory attributes of the cheeses (the number of panelists who used each of the descriptors in at least one session is included in brackets)

Attributes	Control	+ *Lb. plantarum* C22P	+ *Lb. brevis* C21B	+ C22P + C21B
Texture (fingers)	Highly elastic (5) Highly deformable (4)	Highly elastic (6) Highly firm (5)	Slightly elastic (4) Slightly firm (6)	Elastic (5) Soft / spreadable (4)
Texture (mouth)	Smooth (5) Creamy (4) Melting (4) Rubbery (3)	Floury (4) Greasy / soft (3) Melting (4) Doughy (5)	Granular (6) Friable (7) Sticky (4) Doughy (3)	Highly smooth (5) Highly creamy (6) Melting (5) Doughy (4)
Flavour	Buttery (5) Nutty (3) Lactic (4)	Highly buttery (6) Nutty (5) Non acidic / flat (3)	Bitter (7) Slightly sulphury (6) Slight garlic (4) Slightly metallic (3) Highly acidic / sour (5)	Buttery (5) Slightly bitter (2) Slightly rancid (5) Piquant (3) Slightly acidic (4)

SUPPLEMENTARY MATERIAL

S3. Rotated factor loadings and total variance explained in the Principal Component Analysis

Rotated Component Matrix (a)

	Component	
	1	2
pH	0,897	-0,429
proteolysis OPA	-0,869	
leucyl aminopeptidase	-0,42	0,762
lysine aminopeptidase	-0,82	0,48
1-hexanal	0,446	-0,715
1-heptanal	0,413	-0,842
3-methyl-1-butanol	-0,778	0,473
2-heptanol	0,453	0,731
diacetyl	0,749	-0,366
acetoin	0,762	
2-butanone	0,605	-0,529
2-nonanone	0,532	0,741
acetic acid		0,76
hexanoic acid	-0,342	0,852
ethyl butanoate	-0,48	0,802
ethyl hexanoate	-0,547	0,718
dimethyl disulfide	-0,831	0,429
dimethyl trisulphide	-0,806	0,533
firmness in mouth	0,846	
elasticity in mouth	0,888	
preference in mouth	0,77	-0,463
acid taste	-0,804	0,398
bitter taste	-0,876	
buttery flavor		0,647
sulphury flavor	-0,65	
flavor intensity	-0,685	0,378
flavor preference	0,694	
overall preference	0,849	

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.a
(a) Rotation converged in 3 iterations

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	16,172	57,757	57,757	16,172	57,757	57,757	13,058	46,635	46,635
2	4,923	17,582	75,34	4,923	17,582	75,34	8,037	28,705	75,34
3	2,322	8,291	83,631						
4	1,426	5,093	88,724						
5	1,382	4,934	93,658						
6	1,175	4,197	97,855						
7	0,601	2,145	100						
8	9,60E-16	3,43E-15	100						
9	6,93E-16	2,48E-15	100						
10	5,53E-16	1,98E-15	100						
11	4,97E-16	1,78E-15	100						
12	3,78E-16	1,35E-15	100						
13	3,16E-16	1,13E-15	100						
14	2,69E-16	9,60E-16	100						
15	2,03E-16	7,24E-16	100						
16	1,56E-16	5,58E-16	100						
17	8,81E-17	3,15E-16	100						
18	4,77E-17	1,70E-16	100						
19	-1,22E-17	-4,36E-17	100						
20	-6,19E-17	-2,21E-16	100						
21	-1,60E-16	-5,73E-16	100						
22	-3,20E-16	-1,14E-15	100						
23	-3,43E-16	-1,23E-15	100						
24	-4,74E-16	-1,69E-15	100						
25	-6,19E-16	-2,21E-15	100						
26	-9,94E-16	-3,55E-15	100						
27	-1,20E-15	-4,30E-15	100						
28	-3,66E-15	-1,31E-14	100						

Extraction Method: Principal Component Analysis.

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